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TITLE **BENEFICAL CONSEQUENCES OF A SELECTIVE GLUTAMINE SYNTHETASE INHIBITIOR IN OATS AND LEGUMES**

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Beneficial Consequences of a Selective Glutamine Synthetase Inhibitor in Oats and Legumes

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We have been examining the effects of administering a unique glutamine synthetase inhibitor to cereals and N_2-fixing legumes. We have used a bacterium (*Pseudomonas syringae* pv. *tabaci*) to deliver this inhibitor to provide extended treatment periods. We inoculated the root systems of oat and legume plants with *P. tabaci* to provide for delivery of the inhibitor to the plant vascular systems. Inoculation of legumes is accompanied by increased plant growth, total plant nitrogen, nodulation, and nitrogen fixation activity. Inoculation of the oats is accompanied by either of two results depending upon the genotype of the oat plant. One result is inhibition of plant growth followed by plant death as consequences of the loss of all of the glutamine synthetase activities in the plant and the subsequent accumulation of ammonia and cessation of nitrate uptake. The second and opposite result is observed in a small population of oats screened from a commercial cultivar and includes increased plant growth and seed protein. Thus the effects of this inhibitor can be beneficial when applied to appropriate plant material. In an attempt to effectively communicate these findings to the reader, we first introduce the inhibitor (a novel amino acid) and its bacterial delivery system, the target of the inhibitor (glutamine synthetase catalyzed ammonia assimilation), and the two different nitrogen economies in the legume and cereal plants used experimentally. The physiological, biochemical, and molecular genetic consequences of the inhibitor action in cereals and legumes, as we presently understand them, are then presented.

Certain isolates of *Pseudomonas syringae* produce and release a novel amino acid toxin, tabtoxinine-ß-lactam (TßL) ([2-amino-4-(3-hydroxy-2-oxoazacyclobutan-3-yl) butanoic acid] (Figure 1) (Stewart, 1971). These bacteria are leaf pathogens of various host plants and in this capacity produce chlorosis. We have used the most well characterized of these pathogens, *P. syringae* pv. *tabaci,* a tobacco leaf pathogen. This bacterium effectively colonizes the root surface of many plants but infects the leaves of only its normal host, tobacco (Valleau *et al.*, 1942, Knight *et al.*, 1986, Knight *et al.*, 1988). While growing in the rhizosphere, pathovar *tabaci* releases TßL which is then taken up by the plant (Knight *et al.*, 1986; Knight *et al.*, 1988; Knight & Langston-Unkefer, 1988). TßL enters plant cells through the amino acid transport system and can thus be effectively accumulated against a concentration gradient (Bush & Langston-Unkefer, 1987). Because of its growth habit and release of TßL, pv. *tabaci* was selected as a natural bacterial delivery system for TßL that effectively delivers TßL over extended periods of plant growth and experimentation. We have used sterile sand cultures for the plants to assure that no extraneous bacteria complicate the experiments and their interpretation. [We caution others who wish to work with pv. *tabaci* to get to know pv. *tabaci* very well; it is difficult to work with because it is an inconsistent producer of the toxin and therefore, constant, quantitative monitoring of the toxin production must be done in order to achieve reliable results.]

The physiological target of this toxin, TßL, is glutamine synthetase (EC 6.3.2.1) (Knight *et al.*, 1986; Knight *et al.*, 1988; Turner, 1981). We have shown that radiolabeled toxin provided to plant roots is recovered from tissue extracts bound with one-to-one stoichiometry to inactivated glutamine synthetase subunits (Knight *et al.*, 1986; 1988); thus direct evidence has been obtained demonstrating that TßL targets glutamine synthetase directly *in vivo*. TßL is an irreversible inhibitor of glutamine synthetase; enzyme activity is not recovered by extensive dialysis and is lost in a time-dependent manner

(Langston-Unkefer *et al.*, 1987). TßL is an active site-directed inhibitor and, as such, binds at the same part of the active site as the substrate, glutamate (Langston-Unkefer *et al.*, 1987). One molecule of TßL is bound per subunit in completely inactivated glutamine synthetase; this TßL remains bound to the enzyme, and no release of enzyme-bound TßL can be detected over a period of several days (Langston-Unkefer *et al.*, 1987). Hydrolysis of ATP is required for this irreversible inactivation of glutamine synthetase, and because of this, the inactivation of glutamine synthetase by TßL is similar to the inactivation of glutamine synthetase by the commonly used inhibitor, methionine sulfoximine. No fully functional glutamine synthetase enzymes have been found to possess any resistance to methionine sulfoximine; in this important property, these two inhibitors are strikingly and importantly different. Tabtoxinine-ß-lactam is a selective inhibitor of only certain glutamine synthetase isozymes. We will see [illegible] that this selectivity is critical [illegible] observed in these plants and our use of this toxin [illegible] as a research tool.

Glutamine synthetase acts in concert with glutamate synthase to catalyze the primary pathway for ammonia assimilation in higher plants (Lea & Miflin, 1974; Miflin *et al.*, 1980; Wallsgrove *et al.*, 1983). Glutamine synthetase is responsible for assimilating ammonia produced by reduction of nitrate (or nitrite), protein catabolism and nitrogen fixation, and for reassimilating the ammonia released by photorespiration (Keys *et al.*, 1978). In this last function, glutamine synthetase can be considered as an ammonia detoxification mechanism (Givan, 1979). It is the loss of this detoxification function in *P. syringae* pv. *tabaci* infected leaves that results in the accumulation of millimolar concentrations of ammonia. These ammonia concentrations are sufficient to cause the observed disruption of the chloroplast membranes which would generate the chlorosis symptomatic of the disease state (Frantz *et al.*, 1982; Goodman, 1972; Turner & Debbage, 1982). In its role as an ammonia assimilatory enzyme, glutamine synthetase acts in the roots and leaves to catalyze the assimilation of ammonia derived from nitrate or nitrite. In the nodules glutamine synthetase catalyzes the assimilation of ammonia produced by nitrogen fixation.

Glutamine synthetase, the target of TßL, is encoded by several different genes in plants (Tingey *et al.*, 1987 & ref's therein). In order to be able to interpret the results of treatment of plants with TßL, we must know the location of these different forms of glutamine synthetase in these plants. In cereals multiple genes are expressed and encode for three different forms of glutamine synthetase. These forms are GS1, which is synthesized and found in the cytoplasm of the leaves; GS2 which is synthesized in the leaf cytoplasm and moved to the chloroplast where it functions; and GSr, which is synthesized and found in the root tissue. In legumes, at least one additional glutamine synthetase gene is expressed to provide legumes with at least four forms of glutamine synthetase. These forms are GS1 (leaf cytoplasm), GS2 (leaf chloroplast), GSr (root and nodule), and a nodule specific glutamine synthetase, GSn, which is encoded for by a genome expressed specifically in root nodules formed by the symbiosis between the legume and specific bacteria. In the legume nodule, both the GSr and GSn are present and active in the plant tissues of these nodules; this property is particularly important in the beneficial interaction between legumes and pv. *tabaci* (Figure 2).

How oat plants respond to the TßL is somewhat less complex than how legume plants respond to TßL; for this reason we will discuss the oat's response first and utilize this discussion as a general introduction to the more complex legume system. The basic ammonia assimilatory mechanisms are similar in these two types of plants. In cereals such as oats, nitrate or nitrite is taken up by the plant roots and reduced to ammonia via the catalytic activities of nitrate reductase and nitrite reductase enzymes; the product ammonia is assimilated by the action of glutamine synthetase which produces glutamine from glutamate, ATP and ammonia. The substrate glutamate is regenerated by the action of glutamate synthase which catalyzes the transfer of the amide nitrogen of glutamine to carbon 2 of α ketoglutarate to form two molecules of glutamate. This action commonly takes place in the plant roots, although reduction and assimilation can occur in the leaf tissues. Glutamine can be exported to aerial parts of the plant

where the nitrogen is distributed to needed biosynthetic pathways via the action of various transferase enzymes. The exact nature of the normal role of the leaf cytosolic form of glutamine synthetase is somewhat unclear regarding the extent of its participation in primary ammonia assimilation or its possible role in reassimilating ammonia released by photorespiration. As some plants mature, the assimilation of ammonia becomes primarily a function of leaves and therefore the roots must derive assimilated nitrogen from the leaves. The leaf chloroplast form of glutamine synthetase presumably plays a significant role in reassimilating the photorespiratory ammonia.

Our initial studies with bacterial delivery of TßL used oat plants to examine the effects of TßL delivered to the root systems (Knight *et al.*, 1986). Oats are not hosts for infection of their leaves, and thus the infestation of the [illegible]. The majority of oat plants inoculated with pv. *tabaci* died within one week of the initial treatment. These dying plants supported a significant rhizosphere population of pv. *tabaci*, contained no active glutamine synthetase, and ceased to take up nitrate. These responses were observed only in plants inoculated with a toxin-producing isolate of pv. *tabaci*; plants inoculated with a nontoxin-producing isolate did not display these responses, appearing to be completely normal in growth and glutamine synthetase activity (Knight *et al.*, 1986). These findings indicate that the TßL toxin, by pv. *tabaci*, is the cause of the plants' death.

In sharp contrast to the rapid death of most of the oat plants whose rhizosphere was infested with pv. *tabaci* was the response of a small population of oat plants within this cultivar (1 / 175 plants) (*Lodi*, U. of Wisconsin); these oats did not die, but rather thrived on the infestation (Knight *et al.*, 1988). This small population of oats grew well, were darker green, and contained elevated total leaf protein, these plants also had significantly greater leaf glutamine synthetase activity than did the control oat plants (Knight *et al.*, 1988 & Table I). These unusual oat plants contained no measurable glutamine synthetase activity in their roots. Representative, *tabaci* tolerant oat plants

were selfed for four generations and all offspring were tolerant of the infestation; all offspring thrived on infestation of their rhizosphere with pv. *tabaci* just as the parentals thrived on the infestation. Detailed examination of these tolerant plants demonstrated that these plants did not protect themselves from pv. *tabaci* by inhibiting the growth of the pathogen in their rhizosphere nor did they inhibit TßL release by pv. *tabaci* nor did they detoxify TßL. These plants did, however, contain leaf glutamine synthetases that were insensitive to TßL (Knight *et al.*, 1988). These tolerant plant leaf glutamine synthetases were purified and tested *in vitro* for inactivation by TßL and were much less sensitive to TßL than the enzyme in their roots or in the tissues of their TßL-intolerant counterparts. Active TßL was recovered from the leaves of the tolerant plants, indicating that the inhibitor and the active glutamine synthetases were present together in the cell; this observation provides evidence that the TßL insensitive enzymes are effectively isolated in the plant cells. The mechanism of this resistance to TßL by the leaf glutamine synthetases is being investigated using a combination of biochemical and molecular biological approaches.

The increase in total leaf protein almost certainly implies that other proteins, in addition to glutamine synthetase, have been increased in these leaf cells. We are examining this possibility by using antibodies prepared to various leaf proteins to identify proteins whose concentrations are significantly increased in the challenged tolerant oat leaves.

If the selective retention of the leaf glutamine synthetase can also be accomplished in other grains, such as rice, we may be able to achieve an increase in the leaf protein and total plant fresh weight in these grains also. The biochemical and molecular consequences resulting from shifting the assimilation of nitrogen completely away from the roots are unclear; presumably this shift is of significance to the altered nitrogen economy in these challenged tolerant plants. An important question is which form(s) of nitrogen is transported to the leaves; nitrate or ammonia or a combination of these two compounds are likely

candidates. We also need to understand the role (if any) of the ammonia present (1-2 mM) in these leaves and how the roots of these plants meet their nitrogen requirements without functional glutamine synthetase to provide assimilated nitrogen.

The tolerant oat plants showed higher glutamine synthetase activity in their leaves compared to the sensitive oat plants. The increased glutamine activity in the leaves of the tolerant oats can be ascribed to an increase in the activity of the glutamine synthetase genes as determined by Northern blot hybridization. Hybridization of fractionated RNA from the leaves of sensitive and tolerant oats with a ^{32}P-labeled cDNA clone of glutamine synthetase from corn showed on the order of a magnitude higher level of hybridization signal in the tolerant oats than in the sensitive oats. Analysis of glutamine synthetase proteins in the leaves by immunological techniques showed the presence of a novel glutamine synthetase polypeptide in the tolerant oat. This novel glutamine synthetase polypeptide is probably tolerant to TßL.

Our studies with TßL-treated legumes have been focused primarily on alfalfa. Nodulating alfalfa plants whose rhizosphere is infested with *P. syringae* pv. *tabaci* are larger, contain more assimilated nitrogen, have a higher specific activity of nitrogen fixation per gram of nodule, and have greater numbers of nodules than their noninfested counterpart control plants. As we stated earlier in this chapter, both cereals and legumes utilize glutamine synthetase to catalyze the initial step of ammonia assimilation, and both systems utilize glutamate synthase to regenerate the substrate glutamate needed for the initial assimilatory step. After these two enzymes have acted to generate glutamate and glutamine, the two pathways diverge. In cereals the initial assimilation is complete and glutamine is the form in which assimilated nitrogen is exported from the roots to the aerial parts. In legumes the assimilatory pathway continues to produce aspartate, alanine, and finally asparagine. Asparagine is exported to the aerial parts of the plants and is the major form of exported nitrogen (Figure 3).

Legume nodules are composed of both plant and bacterial fractions; each fraction has distinct contributions in the symbiotic relationship (Figure 2). The bacterial fraction fixes nitrogen to ammonia, and the plant fraction assimilates this ammonia and scavenges oxygen. The bacterial genome encodes for nitrogenase and its associated proteins; the plant genome encodes for the ammonia assimilatory enzymes and the oxygen scavenger, leghemoglobin. The development of these nitrogen-fixing structures involves significant biochemical and molecular genetic changes in both the host plant and its particular symbiotic partner bacteria (*Rhizobium meliloti* for alfalfa). It is generally thought that the plant is more or less in charge of the relationship; the bacterial fraction is heavily suppressed in its normal basic metabolism while the plant continues its normal functions and derives the benefit of the fixed nitrogen. The regulation of the developmental changes is not well understood but must involve signaling between the plant and the bacterium during formation of the symbiosis and during the period of active nitrogen fixation and assimilation. It is biochemically, intuitively reasonable that the metabolic activities of nitrogen fixation and assimilation are not operating independently but are inter-related and biochemical communication occurs between them.

Our studies on the effects of infestation of the legume rhizosphere with pv. *tabaci* included several experimental approaches that show that the observed changes were the result of the action of the bacterially delivered inhibitor, Tbl. Oat plants responded only to infestation by the toxin-producing isolate and not to the nontoxin-producing isolate. Likewise, alfalfa plants respond only to infestation by the toxin-producing isolate and no changes in nitrogen metabolism or plant growth were observed in plants infested with a nontoxin-producing isolate (Knight & Langston-Unkefer, 1988). We also tested several naturally occurring Tox$^-$ mutants of the toxin-producing isolate and found no detectable changes in plant growth or nitrogen fixation or nitrogen metabolism (Knight & Langston-Unkefer, 1988). In separate experiments, we allowed a small number of plants to take up pure toxin through their roots and observed significant changes in glutamine synthetase activity and amino acid pools (data not shown);

these are the expected consequences of the administration and action of a glutamine synthetase inhibitor. As we mentioned earlier in this chapter, the plants were grown in an acid-washed sterile sand culture; these sterile culture conditions exclude any possible beneficial effects by pv. *tabaci* that may arise by virtue of any antibiotic action toward other, deleterious bacteria in the plants' rhizosphere (Knight & Langston-Unkefer, 1988).

Our initial results of increased plant growth, root nodule formation, nitrogen fixation capability, and altered ammonia assimilation with pv. *tabaci*-infested alfalfa were described previously (Knight & Langston-Unkefer, 1988). These infested alfalfa plants contained approximately twice the amount of total assimilated nitrogen that the control plants contained. These infested alfalfa plants also contained no glutamine synthetase activity in their roots and had approximately one half of the control level of glutamine synthetase activity in the plant fraction of the nodule. The glutamine synthetase activity in the bacteroids was normal, expressed as per gram nodule tissue. The impairment of glutamine synthetase activity in the plant fraction of the nodule arises from the selective inactivation of the root form of glutamine synthetase that is present in the nodule tissue (Figure 2, Knight & Langston-Unkefer, 1988). The nodule-specific form the glutamine synthetase provides the glutamine synthetase activity that remains in these nodules; this form of glutamine synthetase is much less sensitive to inactivation by TßL than is the root form of the enzyme (Knight & Langston-Unkefer, 1988). This selective impairment of glutamine synthetase is accompanied by changes in the amino acid and ammonia pools in the nodule (Knight & Langston-Unkefer, 1988), reflecting the importance of glutamine synthetase activity in ammonia assimilation. Given the primary role that glutamine synthetase plays in ammonia assimilation, it is possible that nitrogenous compounds other than asparagine are being exported from these nodules with impaired glutamine synthetase activity, to in order to provide these plants with their abundant supply of nitrogen. Subsequent investigations have shown that asparagine is the major ninhydrin positive compound being exported

from these nodules. How these plants have synthesized this asparagine is being investigated.

Our studies of alfalfa plants whose rhizosphere was infested with pv. *tabaci* have usually used plants that were 30-45 days old (Knight & Langston-Unkefer, 1988); however, we have also examined plants that were considerably older and that had also been inoculated within the first two weeks of their growth. These plants continued to outgrow their counterpart controls.

Nodules are necessary for alfalfa to survive infestation of its rhizosphere by pv. *tabaci*. Plants without nodules were inoculated with pv. *tabaci* and all non-nodulated plants died within a few days of the inoculation (Knight & Langston-Unkefer, 1988).

We have examined the reversibility of the effects of infestation of with pv. *tabaci*. The pathogen population declines sharply after growing the plants in temperatures near 10C for several days (which does not allow for effective growth of pv. *tabaci*). Plants from which the pathogen has been effectively removed (the pathogen population was monitored as described by Knight et al., 1986), quickly returned to a normal nitrogen economy as judged by examining the amino acid pools in the plants and measuring glutamine synthetase activity in their roots and nodules. The altered nitrogen economy was rapidly re-established in these plants when pv. *tabaci* was re-introduced to their rhizosphere, and the plants were allowed to grow in temperatures that allow the pathogen to grow (25C). These findings demonstrate that many of the changes occurring in these plants are rather readily reversible.

In order to begin to understand the effects of nitrogen source on the system, we also examined the consequences of treatment of the pv. *tabaci* infested plants with nitrate (Table II). Nitrate was supplied as potassium nitrate (10 mM) to the plants beginning at about 4 days after inoculation with pv. *tabaci*. Control plants without pv. *tabaci* infestation were also used and the

results were compared. As expected, the plants grown with nitrate supplement were much larger than the plants dependent only on nitrogen fixation for nitrogen. However, the pv. *tabaci* -infested plants had about twice the number of nodules per plant compared with the uninfested control plants. Furthermore, these infested plants had significantly greater specific activity of nitrogenase, as measured by acetylene reduction, than did the control plants. As expected, the *tabaci* infested alfalfa had impaired glutamine synthetase activity in the plant fraction of their nodules. The root glutamine synthetase was completely absent in these plants.

We inoculated soybean plants with pv. *tabaci* to determine if the increases in growth and nitrogen fixation observed so consistently in alfalfa would be observed in another legume. Soybeans were chosen because they represent another major class of legumes, the ureide exporters. As seen in Table III, the soybeans also thrived on the infestation of their rhizosphere by pv. *tabaci*. Fresh weights increased, as well as nodules per plant and total nodule weight. The nitrogenase activity per gram nodule is increased but this increase is not as dramatic as the increase observed in alfalfa (Knight & Langston-Unkefer, 1988). Likewise, the decrease in glutamine synthetase activity in the soybean nodules in this experiment is not as great as the decrease observed in alfalfa. Examination of the amino acid pools of the soybeans revealed unusual pool sizes of amino acids; this change is also similar to the changes in the amino acid pools in the infested alfalfa nodules. The observation of the differing degrees of change in metabolism and decreases in glutamine synthetase in these two legumes suggests that the impact on fixation is dependent upon the extent of perturbation of glutamine synthetase function.

As a first approach toward understanding the molecular basis of TßL-induced biochemical changes in alfalfa nodules involved analysis of RNA populations of control and pathogen-infested nodules. Two dimensional gel profiles of the translation products of poly(A)RNA from control and pv. *tabaci* treated nodules are almost identical except for a few minor changes (Figure 4).

Northern blot hybridization of RNA from control and pv. *tabaci*- treated nodules using a few alfalfa nodulin (structural) cDNA probes showed no significant difference. The level of leghemoglobin mRNA and protein also do not show any differences between treated and control nodules. All these results taken together would suggest that TßL-induced biochemical changes in the nodule did not alter the expression of the nodulins that are present in high concentrations, which are likely to be those with structural, not catalytic, functions. However, preliminary experiments suggest that there are changes in the level of expression of glutamine synthetase and phosphoenolpyruvate carboxylase genes accompanying TßL treatment.

Our studies of this system continue on both biochemical and molecular tracks. We have intentionally refrained from using a single working hypothesis to interpret our data or to guide our investigations. We are beginning at the point of known action in this system, that is to say, the site of action of the glutamine synthetase-inhibiting toxin released by the pathogen. One conclusion that can be drawn from both the legume and cereal studies is that the prolonged use of inhibitors with selective action can be of significant benefit in metabolic studies. The findings suggest that selective perturbations of metabolism are potentially beneficial for improved crop production.

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Table I. Effects on plant growth and glutamine synthetase activity of rhizosphere infestation with *P. syringae* pv. *tabaci* in oat plants tolerant of the infestation.*

Parameters	Unchallenged Sensitive	Unchallenged Tolerant	Challenged Tolerant
Fresh Weight (grams/plant)	6.5 ±0.6 (100%)	6.2 ± 0.5 (95%)	9.1 ± 0.8 (140%)
Glutamine Synthetase* (Units / mg protein)			
Leaf	1.8 ±0.1 (100%)	1.8 ±0.1 (100%)	3.0 ± 0.1 (167%)
Root	1.9 ±0.1 (100%)	1.8 ± 0.0 (95%)	0.3 ± 0.0 (16%)

* Plants (15) were germinated and inoculated (challenged) with *P. syringae* pv. *tabaci* at 7 days post-germination. At 34 days after germination, these plants were compared with 15 uninoculated plants of sensitive genotype and 15 plants of tolerant genotype. Plants were grown under the same conditions as described previously (Knight *et al.* 1988). Glutamine synthetase activity was measured using an ADP-dependent transferase activity as described in Knight *et al.* (1988).

Table II. Effects of nitrate on plant growth, nitrogenase, and glutamine synthetase in plants infested with *P. syringae* pv. tabaci (Tox-) (% control) (Data collected at 45 days growth.)*

Inoculation with *P. syringae*	Plant Growth Parameters: Fresh weight, foliar (mg / plant)	Plant Growth Parameters: Nodules / plant	Nitrogenase activity (Units / g nodule /h)	Glutamine synthetase activity Nodule, plant fraction (Units / mg protein)
None	267 ± 99 (100%)	2.3 ± 1.2 (100%)	Not detected	1.3 (100%)
pv. tabaci (Tox-)	425 ± 152 (159%)	5.6 ± 1.4 (243%)	2.2 ± 0.5	0.75 (58%)

* Plants were grown as described previously (Knight & Langston-Unkefer, 1988) except they were supplied 10 mM KNO_3 twice weekly. All measurements were done as described previously (Knight & Langston-Unkefer, 1988).

Table III. Effects on growth, nitrogenase, and glutamine synthetase of infestation of soybean plants with Tox+ strain of *P. syringae* pv. *tabaci* (% of control) (35 days of growth)*

Inoculation with *P. syringae*	Plant Growth Parameters: Fresh wt. Foliar (mg plant)	Nodules /plant	Nodule weight (mg / plant)	Nitrogenase activity (Units / g nodule/h)	Glutamine synthetase activity: Nodule plant fract'n (Units/mg protein)	Root
None	537 (100%)	75 (100%)	236 (100%)	7.4 (100%)	2.4 (100%)	2.5 (100%)
pv. *tabaci*	690 (128%)	138 (184%)	380 (161%)	8.4 (114%)	1.7 (71%)	2.0 (80%)

* Soybean cultivar McCall was grown in a sand/vermiculite mixture (1:1) under the same light periods as the alfalfa and were provided a mineral solution without nitrogen. Seedlings were inoculated with *Bradyrhizobium japonicum* at 7 days of growth and inoculated with pv. *tabaci* at 14 days. All measurements were done as described previously (Knight & Langston-Unkefer, 1988).

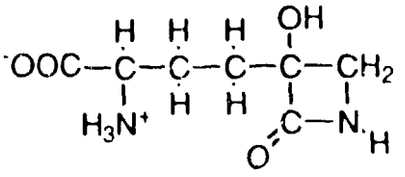
FIGURE LEGENDS

Figure 1. Tabtoxinine-ß-lactam (TßL). The exocellular glutamine synthetase inhibitor released by *Pseudomonas syringae* pv. *tabaci* (Tox$^+$).

Figure 2. Nitrogen Metabolism in Legume Root Nodules. The tissue location is given for specific nitrogen metabolism activities within the nodule and for the various forms of glutamine synthetase: GSn, the nodule-specific form; GSr, the root form; GS, the two *Rhizobium* glutamine synthetases. The arrow indicates the form and tissue location of the glutamine synthetase targeted by TßL in the alfalfa roots and nodules.

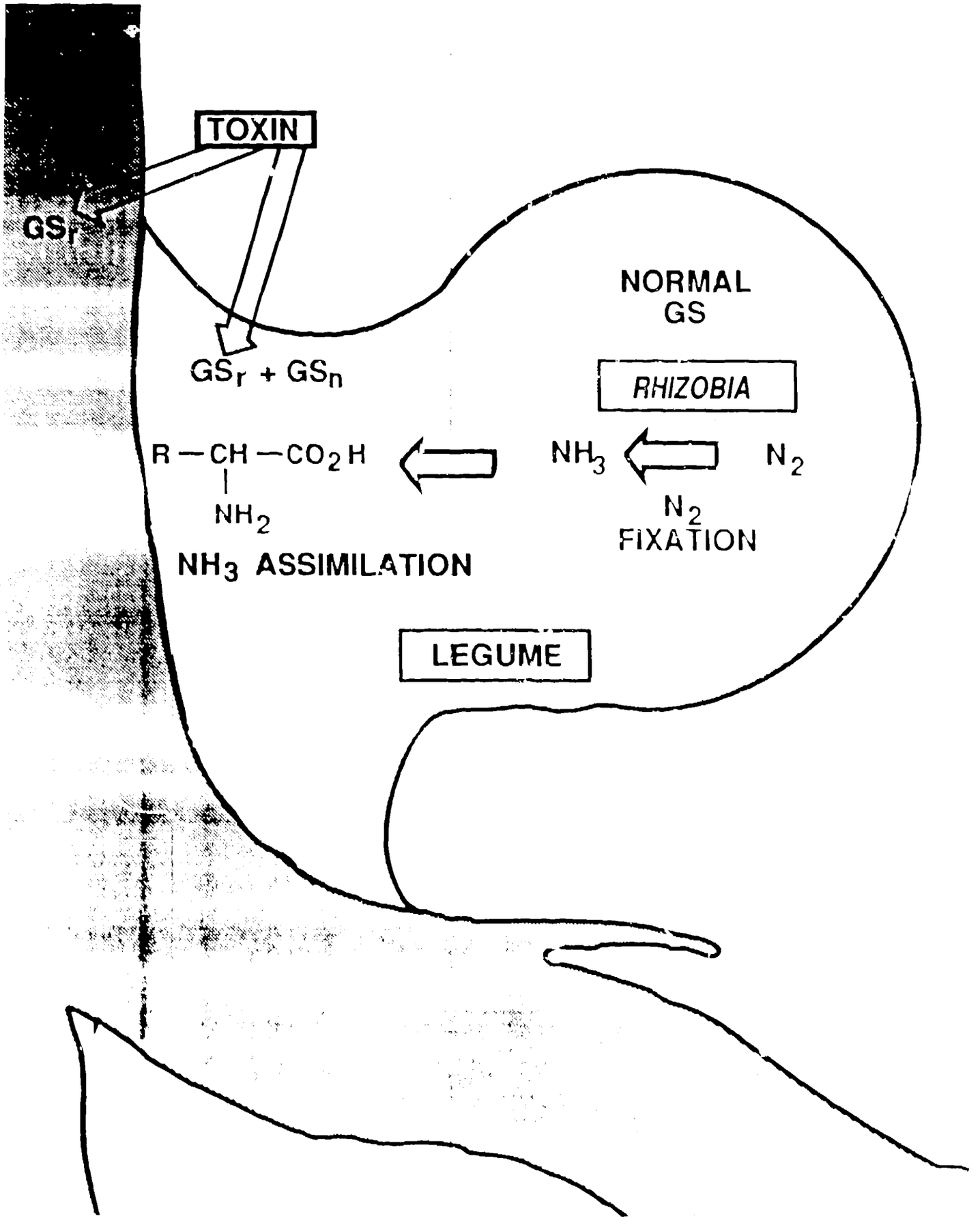
Figure 3. Ammonia Assimilation in Alfalfa Nodules. A general scheme for ammonia assimilation is presented. The solid lines depict reactions with probable roles in normal ammonia assimilation; the dashed lines depict reactions with possible roles in alternative routes of ammonia assimilation in nodules. AD, alanine dehydrogenase; ASN, asparagine synthetase; GDH, glutamate dehydrogenase; GOGAT, glutamate synthase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; GS, glutamine synthetase; NDH, malate dehydrogenase; NIT, nitrogenase; PEPC, phosphoenolpyruvate carboxylase.

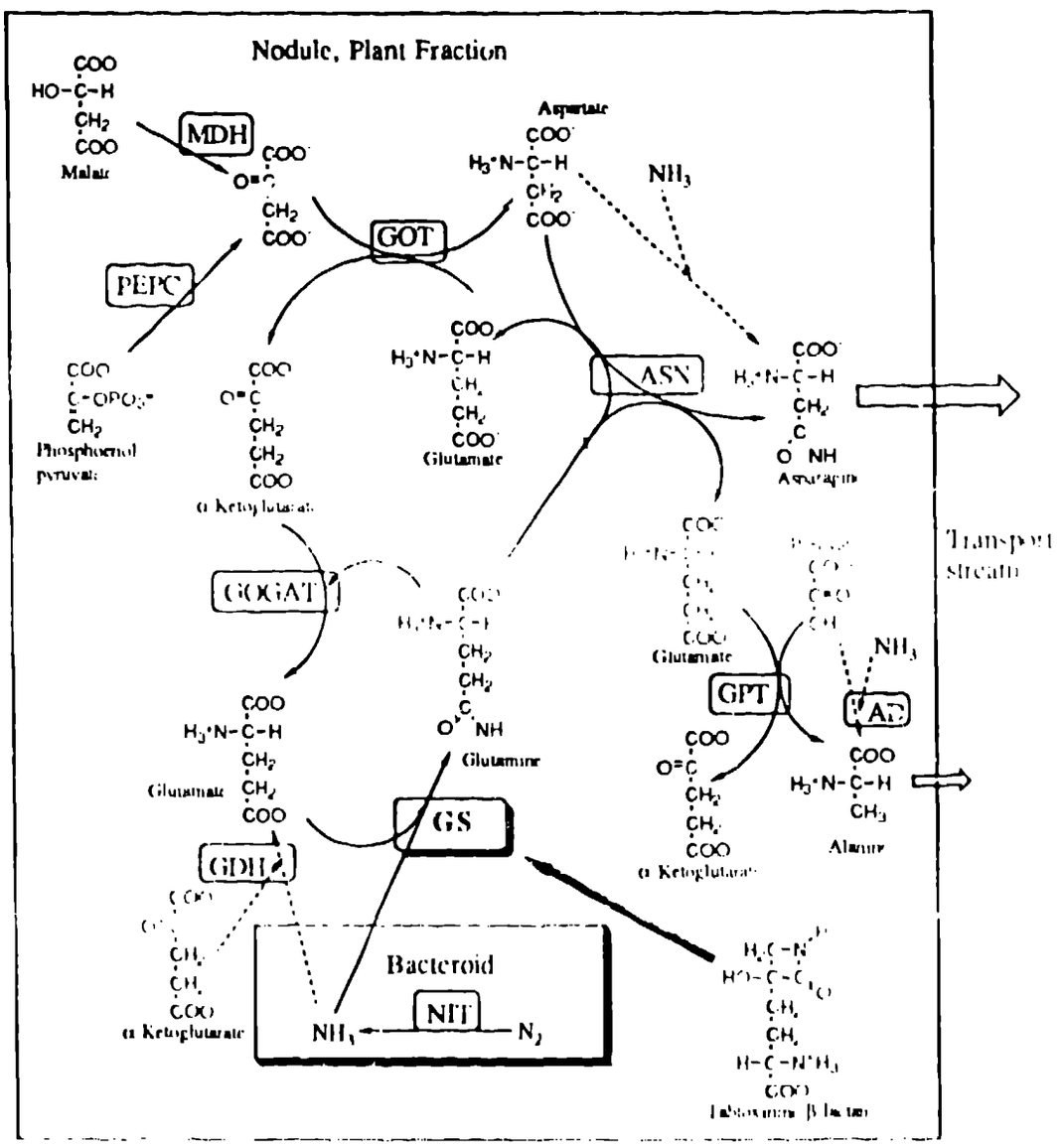
Figure 4. *In vitro* Translation Products Subjected to IEF followed by SDS PAGE. RNA from 45-day old nodules was translated using rabbit reticulocyte lysate and ^{35}S methionine. Small arrows point to translation products that appear to differ between Tox$^+$ and control nodules. Black and white arrows point to nodulin translation products that also appear to be different in Tox$^+$ and control nodules. The overall appearance of the gels is similar, indicating that changes in gene expression are fairly subtle. This is to be expected because the nodules appear normal in morphology and gross changes in major nodulins with structural roles are therefore not predicted.



Tabtoxinine-β-lactam

NITROGEN METABOLISM




Nodule, Plant Fraction
Malate
MDH
Aspartate
NH3
GOT
PEPC
Phosphoenol pyruvate
α Ketoglutarate
Glutamate
ASN
Asparagine
Transport stream
GOGAT
Glutamine
Glutamate
GPT
AT
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α Ketoglutarate
GS
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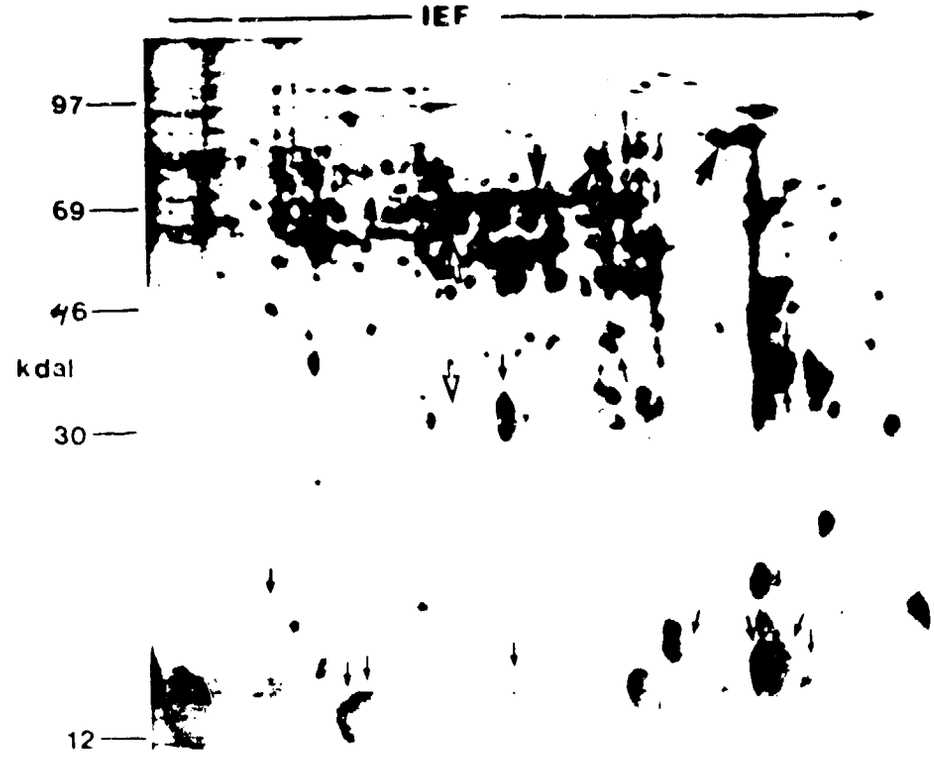
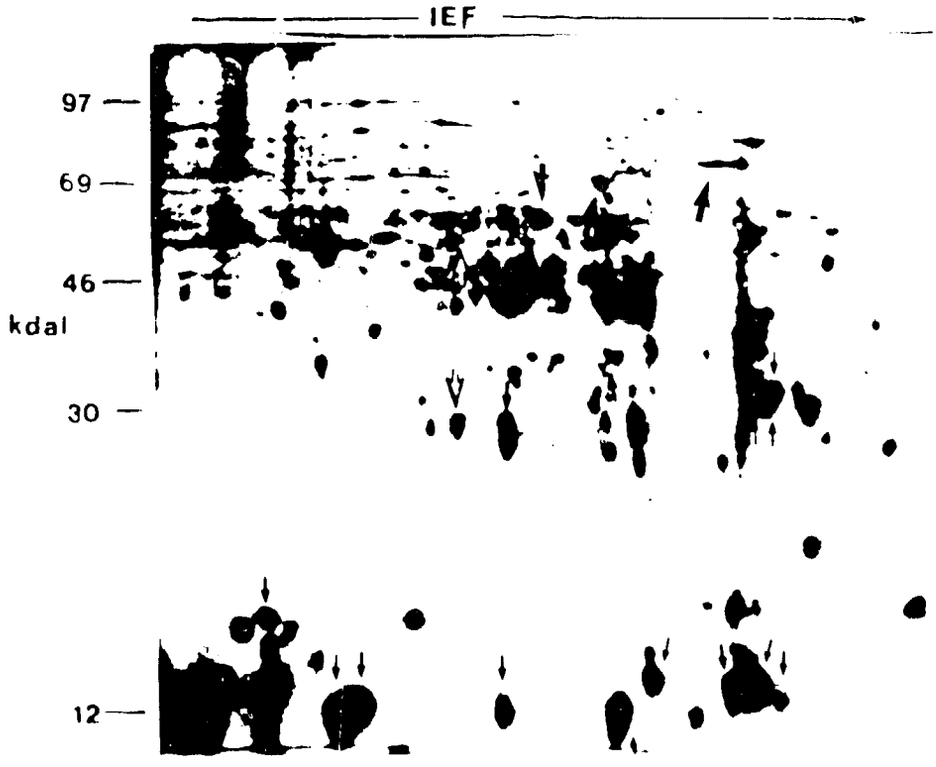
α Ketoglutarate
Bacteroid
NIT
NH3
N2



TOX+



CONTROL