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Two Indirect Methods for Detecting Ureide Synthesis by Nodulated Legumes[1]

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ERIC W. TRIPLETT
Department of Plant Pathology, University of California, Riverside, California 92521

ABSTRACT

Two methods were developed for the detection of altered ureide metabolism in legume nodules. Both techniques are based on the positive correlation between the presence of high xanthine dehydrogenase (EC 1.2.1.37) specific activity in nodules and the ability of those nodules to produce the ureides, allantoin and allantoic acid. In the first method, nodulated legumes are treated for 2 weeks with a soil drench of allopurinol. After allopurinol treatment, leaves of N_2-fed, ureide-producing legumes, soybean, cowpea, and lima bean, became very chlorotic. Leaves of KNO_3^- or NH_4Cl-fed ureide-producing legumes were unaffected by the allopurinol treatment. Leaves of the amide-producing legumes, alfalfa, clover, peak, and lupin, were unaffected by the allopurinol treatment with N_2, KNO_3, or NH_4Cl as nitrogen source. These experiments showed that long-term allopurinol treatments are useful in differentiating between ureide- and amide-producing legumes when effectively nodulated. A second method was developed for the rapid, qualitative estimation of xanthine dehydrogenase activity in legume nodules. This method utilizes pterin, an alternate substrate for xanthine dehydrogenase. Xanthine dehydrogenase hydroxylates pterin in the presence of NAD^+ to produce isoxanthopterin. When exposed to long wave ultraviolet light (365 nanometers), isoxanthopterin emits blue fluorescence. When nodules of ureide-producing legumes were sliced in half and placed in microtiter plate wells containing NAD^+ and pterin, isoxanthopterin was observed after 6 hours of incubation at room temperature. Allopurinol prevented isoxanthopterin production. When slices of amide-producing legume nodules were placed in wells with pterin and NAD^+, no blue fluorescence was observed. The production of NADH by xanthine dehydrogenase does not interfere with the fluorescence of isoxanthopterin. These observations agree with the high specific activity of xanthine dehydrogenase in nodules of ureide-producing legumes and the low activity measured in amide-producing nodules. The wild soybean, *Glycine soja* Sieb. and Zucc., was examined for ureide synthesis. Stems of wild soybean plants had a high ureide abundance with N_2 as sole nitrogen source when nodulated with either *Rhizobium fredii* or *Bradyrhizobium japonicum*. Ureide abundance declined when nitrate or ammonium was added to the nutrient solution. Nodule slices of these plants produced isoxanthopterin when incubated with pterin. Nodule crude extracts of *G. soja* had high levels of xanthine dehydrogenase activity. Both *Glycine max* and *G. soja* plants were found to produce ureides when plants were inoculated with fast-growing *R. fredii*. The two methods described here can be used to discriminate ureide producers from amide producers as well as detect nitrogen-fixing legumes which have altered ureide metabolism. A nodulated legume that lacks xanthine dehydrogenase activity as demonstrated by the pterin assay cannot produce ureides since ureide synthesis has been shown to require xanthine dehydrogenase activity both *in vivo* and *in vitro*. A nodulated legume that remains green during allopurinol treatment also lacks ureide synthesis since the leaves of ureide-producing legumes are very chlorotic following allopurinol treatment.

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Nodulated leguminous plants can be classified into two groups with respect to the assimilation of fixed N for subsequent transport to the stems and leaves of the plant. In temperate legumes, such as alfalfa and lupin, fixed N is assimilated mainly into the amides, asparagine and glutamine, while in legumes of tropical origin, fixed N is assimilated into the ureides, allantoin and allantoic acid (19). Allantoin and allantoic acid represent up to 90% of the total nitrogen transported to the above-ground parts of soybean and cowpea from the nodule (9, 10, 12–15, 20). In both types of legumes, assimilation of fixed N into transport forms occurs in the plant cell cytoplasm (17, 18, 22). Ureide synthesis occurs via *de novo* purine synthesis followed by purine oxidation (18) and usually occurs only when tropical legumes have nodules which are actively fixing N (10, 12, 13, 19).

If ureide synthesis is beneficial to crop productivity, ureide production may have been increased during the domestication of the soybean. To investigate this possibility, the extent of ureide production of the wild soybean, *Glycine soja*, is presented in this manuscript. However, among the nearly 300 accessions of *G. soja* and 8000 lines of *Glycine max* which are present in the United States Department of Agriculture collection, only 32 cultivars of *G. max* and one accession of *G. soja* have been examined for ureide synthesis (14, 16, this manuscript). Mutants or lines of soybean which lack ureide synthesis when effectively nodulated would be useful for the study of the biochemical regulation of the pathway as well as for the examination of the effect of ureide synthesis on crop productivity. Such plants could be studied for the biochemical defect which prevents ureide synthesis and compared with wild-type plants for crop productivity. The inheritance of those factors which control ureide synthesis could also be studied in these plants.

Selection of such mutants or lines of soybean requires rapid, simple, and reliable screening methods for ureide synthesis. At present, the only methods for screening legumes for ureide production require the direct measurement of ureides in stem tissue or xylem sap (8, 14). Both of these methods are laborious in that they require either the collection of xylem sap or the extraction of water-soluble compounds from stem tissue and are difficult for a large number of samples. Following collection or preparation of samples, these methods require the analysis of ureides and either measurement of total N or nitrate. This manuscript describes the development of rapid, simple, and reliable screens for ureide synthesis by a large number of nodulated legume plants. These procedures are based on the essential role of xanthine dehydrogenase in nodule ureide synthesis.

MATERIALS AND METHODS

Plant and Rhizobium Culture. Plants were cultured on a N-free nutrient solution in the greenhouse and inoculated with the appropriate strain of *Rhizobium* or *Bradyrhizobium* as described previously (21, 24). Along with the plant species used in previous experiments (21), white clover (*Trifolium repens* cv New Zealand

White) inoculated with *Rhizobium leguminosarum* bv. *trifolii* strain USDA 2046 and wild soybean (*G. soja* Sieb. and Zucc. accession PI 163.453) inoculated with *Bradyrhizobium japonicum* strain 122DES, *R. fredii* USDA strains 191 or 205 were also used in the experiments described here. The domestic soybean cultivar Pella was used in this experiment. In the experiments designed to quantitate ureide synthesis by *G. soja*, soybean and wild soybean seeds were sown in 2 L pots. Six seeds were sown in each pot. Treatments included plants dependent upon N_2 as sole source of N as well as plants fertilized three times a week with 50 ml of either 10 mM KNO_3 or 10 mM NH_4Cl. Plants fed nitrate or ammonium were also inoculated with *B. japonicum* strain 122DES. After 6 weeks, stem and nodule tissue from all treatments harvested and stored at −80°C for future analysis of ureide abundance in stems and XDH[2] activity in nodules. *Rhizobium* and *Bradyrhizobium* strains were maintained on the minimal medium described by Bergersen (3).

Source of Reagents. The salts used in the plant nutrient solution, NAD^+, allopurinol, and pterin were obtained from Sigma Chemical Co., St Louis, MO. Isoxanthopterin was purchased from Aldrich Chemical Co., Milwaukee, WI.

Purification of XDH from Cowpea and Soybean Nodules. XDH genase from soybean and cowpea nodules was purified as described by Triplett *et al.* (25). A 30 to 45% $(NH_4)_2SO_4$ fraction from 200 g nodules was prepared as described by Triplett (21). This fraction was placed over a column containing a monoclonal antibody specific for XDH bound to Sepharose. Active XDH was eluted from the column with 25 mM glycine (pH 11) with 1 mM DTE, concentrated to a volume of 1 ml on an Amicon YM-5 column, and dialyzed against 10 mM K-phosphate (pH 7.8) with 1 mM DTE. The monoclonal antibody 2B5 was used to purify the soybean enzyme while antibody 2C1 was used to purify the cowpea enzyme.

XDH Assays. The XDH assay with xanthine as substrate was performed as described previously by following NADH production at 340 nm (23). With pterin as substrate, 0.2 ml of 0.1 mM pterin, 0.2 ml of 5 mM NAD^+, and 0.2 ml containing 2 μg of purified soybean or cowpea XDH was added to a microcuvette. All assay components were dissolved in 50 mM K-phosphate (pH 7.8) with 1 mM DTE. After 10 min at room temperature, the formation of product, isoxanthopterin, was observed under long wave (365 nm) UV light by the emission of blue fluorescence. Crude nodule extracts of alfalfa, lupin, clover, pea, lima bean, cowpea, wild soybean, and soybean were prepared and assayed for XDH activity as described previously (21, 23). Protein was measured according to the method of Bradford (4).

Determination of Ureide Abundance. The definition of ureide abundance ([ureide-N/ureide-N plus nitrate-N] × 100) described by Herridge (8) was used. Stem tissue was extracted according to the method of Herridge (8). The ureide content of stem tissue was determined using the method of Young and Conway (28). Nitrate was measured by the method of Cataldo *et al.* (5).

Allopurinol Screen. *Experiment 1.* Soybean seeds were sown in 30 cm × 3 cm cylindrical tubes. Two weeks after inoculation with *B. japonicum* strain 122DES, 5 ml of 0 to 400 μM allopurinol were applied as a soil drench to the plants. After the treatment, plants and leaves were photographed.

Experiment 2. Soybean plants were cultured in 2 L pots containing five plants each. Two weeks following inoculation with *B. japonicum* strain 122DES, plants were treated with 50 ml of one of the following solutions for 14 consecutive days: (a) 10 mM K_2SO_4; (b) 10 mM K_2SO_4 plus 400 μM allopurinol; (c) 20 mM KNO_3; or (d) 20 mM KNO_3 plus 400 μM allopurinol. Potassium sulfate was included in N_2-dependent plants as a control to differentiate K and NO_3 effects. After 14 d, leaves of plants from each treatment were photographed.

Experiment 3. Soybean, cowpea, lima bean, lupine, pea, clover, and alfalfa plants were cultured as described for experiment 1. Two weeks after inoculation with the appropriate bacterial strain, plants were treated with 50 ml of one of the four treatments described in experiment 2 as well as (e) 20 mM NH_4Cl; or (f) 20 mM NH_4Cl plus 400 μM allopurinol. After 14 consecutive d of treatment, the leaves were photographed and their appearance was recorded.

Pterin Assay. The pterin assay is based on an alternate XDH reaction first described by Forrest *et al.* (7) to be involved in pteridine metabolism in *Drosophila.* In this reaction, pterin is hydroxylated to isoxanthopterin with NAD^+ as electron acceptor (Fig. 1).

Nodules of alfalfa, clover, lupin, pea, cowpea, lima bean, and soybean were sliced in half with a razor blade. One nodule slice was placed per well of a polyvinyl microtiter plate. Each well contained 50 μl of 5 mM NAD^+ in buffer and 100 μl of one of the following solutions: (a) buffer alone, (b) 0.1 mM pterin in buffer, or (c) 0.1 mM pterin plus 0.1 mM allopurinol. The buffer contained 50 mM K-phosphate (pH 7.8) and 1 mM DTE. After 6 h at room temperature, the appearance of isoxanthopterin in the wells was observed with a long wave (365 nm) UV light source. The presence of XDH in nodules was shown by the emission of blue fluorescence caused by isoxanthopterin production. Wells which contain nodule slices that lack XDH activity showed the light green fluorescence characteristic of the substrate of this XDH reaction, pterin. Both frozen and fresh nodules worked well with this screen. In performing the pterin screen, controls containing allopurinol plus pterin were included so that the difference in fluorescence between pterin and isoxanthopterin was detected easily with each sample.

RESULTS

Ureide Production by *Glycine soja*. The ureide abundance ([ureide-N/ureide-N plus nitrate-N] × 100) of stems of *Glycine max* and *G. soja* was determined with three sources of N including dinitrogen fixation by either slow-growing bradyrhizobia or fast-growing rhizobia (Table I). *Glycine soja* stems had a high ureide abundance with N_2 as sole N source regardless of the strain used to inoculate the plants. Urieide abundance decreased in both species with nitrate or ammonium treatment when compared to the N_2-fed plants. Nitrate and ammonium treatments decreased ureide abundance to a lesser extent in *G. max* than *G. soja* (Table I). This was probably owing to the fact that the *G. max* plants were far larger than the *G. soja* plants while both species were fed an equal amount of ammonium or nitrate on a per plant basis. Both soybean species produced ureides when inoculated with fast-growing *R. fredii* strains.

Nodules of *G. soja* had high *in vitro* levels of XDH which were comparable to those measured in *G. max* nodules (Table I),

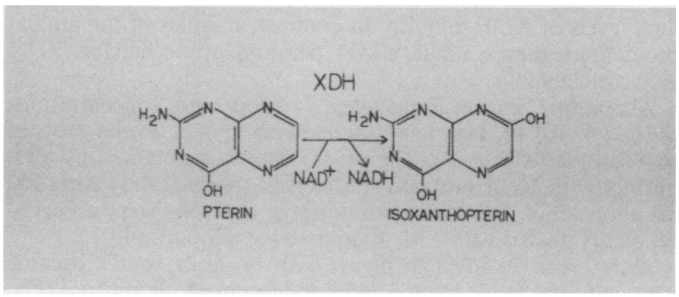

FIG. 1. A nonphysiological reaction catalyzed by purified XDH from soybean and cowpea nodules. The product of this reaction emits blue fluorescence upon irradiation with long wave (365 nm) UV light.

[2] Abbreviation: XDH, xanthine dehydrogenase.

Table I. *Ureide Abundance and XDH Specific Activity of Domestic and Wild Soybean Plants Supplied with Nitrate, Ammonium, or N_2 as Nitrogen Source*

Nodulated plants were inoculated with *B. japonicum* strain 122DES, *R. fredii* strain 191, or *R. fredii* strain 205. Each value represents the mean of three replicates.

Nitrogen source	Ureide Abundance[a]		XDH Activity	
	G. max	*G. soja*	*G. max*	*G. soja*
			nmol NADH produced/ min·mg protein	
KNO_3	77.8	46.5	NT[b]	NT
NH_4Cl	87.3	53.2	NT	NT
N_2				
B. japonicum 122DES	94.0	95.9	13.2	9.8
R. fredii 191	99.0	85.9	13.4	12.4
R. fredii 205	96.3	96.3	ND[c]	17.8

[a] As defined by Herridge (8). [b] Not tested. [c] Not determined since no nodules were formed in this treatment.

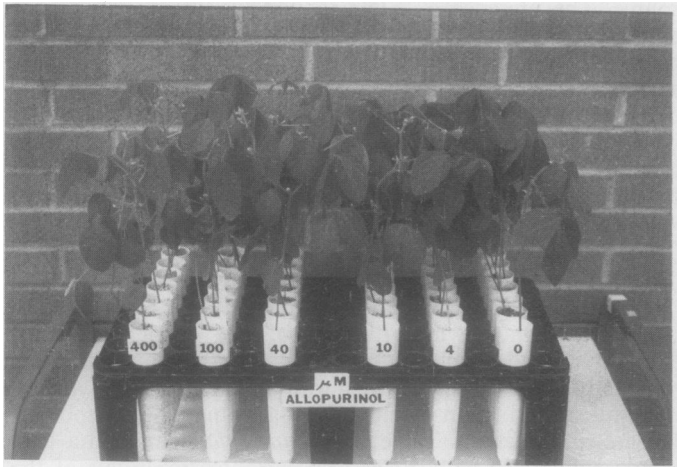

FIG. 2. Soybean plants treated with a concentration series of allopurinol. To each tube containing a soybean root system was applied 50 ml of one of six concentrations of allopurinol (0–400 μM) for 14 consecutive days. Treatment of plants began 2 weeks after inoculation.

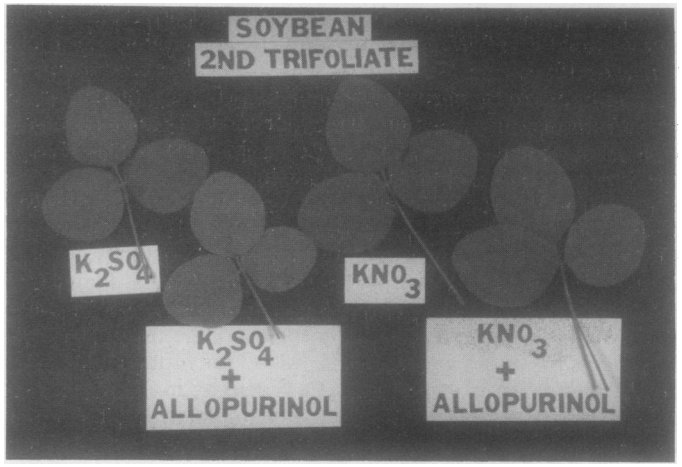

FIG. 3. Leaves of soybean plants after soil drench treatment with one of the following four treatments: (a) 10 mM K_2SO_4, (b) 10 mM K_2SO_4 plus 0.4 mM allopurinol, (c) 20 mM KNO_3, and (d) 20 mM KNO_3 plus 0.4 mM allopurinol. Each 2 L pot contained five plants and was treated with 50 ml of one of the above treatments for 14 consecutive d.

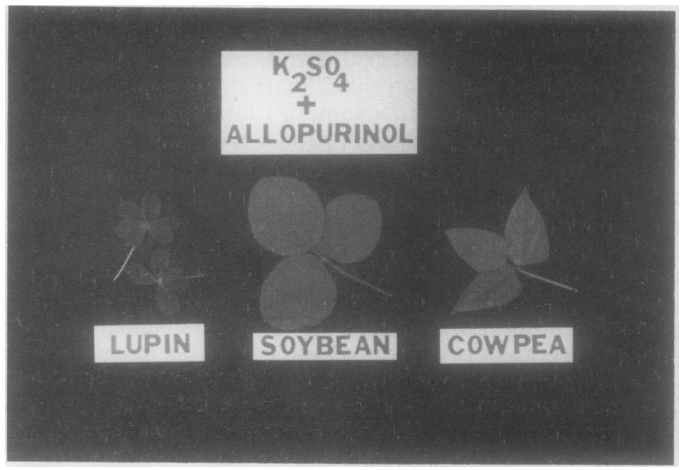

FIG. 4. With N_2 as sole N source, soybean and soybean leaves become severely chlorotic following allopurinol treatment. Lupine leaves are unaffected by the allopurinol treatment.

whether the plants were inoculated with the fast-growing *R. fredii* or the slow-growing *B. japonicum* (Table I).

XDH Specific Activity in Nodules of Ureide- and Amide-Producing Legumes. To confirm previous studies (6, 16, 20), showing that ureide production is positively correlated with XDH activity, the XDH specific activity of nodule crude extracts from 8 legume species was determined. Nodules of the ureide producing legumes, soybean, wild soybean, cowpea, and lima bean had high levels of XDH activity. In contrast, nodules of the amide-producing legumes, alfalfa, clover, pea, and lupine, had low XDH activity (Table II).

Allopurinol Screen. *Experiment 1.* Allopurinol concentrations of 0, 1, 4, 10, 40, 100, and 400 μM were applied to the roots of soybean plants (Fig. 2). The severe chlorosis obtained by allopurinol with N_2 as nitrogen source was observed only with 400 μM allopurinol. Decreasing amounts of chlorosis were observed on plants treated with 100, 40 and 10 μM allopurinol.

Experiment 2. Soybean plants with N_2 as N source became very chlorotic following a 2 week treatment of allopurinol (Fig. 3). Under the conditions of this treatment, the soybean leaves became chlorotic after 5 d. With nitrate as nitrogen source, the leaves remained green although the plants did become slightly stunted as a result of the allopurinol treatment. Similar to soybean, leaves of cowpea and lima bean plants were affected in all treatments in the same manner as leaves of soybean plants (data not shown).

Experiment 3. The treatments described in experiment 2 were repeated in experiment 3 for soybean, cowpea, and lima bean as well as alfalfa, pea, lupine, and clover. Other treatments were added to determine the effects of allopurinol on legumes with ammonium as sole N source. The ureide-producing legumes, soybean, cowpea, and lima bean, showed severe chlorosis of the leaves with allopurinol treatment and N_2 as sole N source (Fig. 4). With nitrate or ammonium as sole N source, allopurinol treatment did not cause leaf chlorosis in soybean, cowpea, or lima bean. Leaves of amide-producing legumes, alfalfa, clover, pea, and lupine, were not affected by allopurinol regardless of N source. Leaves of soybean, cowpea, and lupine are shown in Figure 4 following allopurinol treatment with N_2 as sole N source.

Pterin Assay. As a preliminary experiment to determine the ability of nodule XDH to hydroxylate pterin, 2 μg of purified soybean XDH was added to each of three cuvettes containing either no substrate, pterin, or pterin plus allopurinol. Purified soybean nodule XDH catalyzed this reaction as illustrated by

the appearance of the blue fluorescent product, isoxanthopterin, under long wave UV light in the cuvette containing pterin but no allopurinol (Fig. 5). Allopurinol inhibits the production of isoxanthopterin by purified nodule XDH (Fig. 5). Since allopurinol inhibited this reaction, the production of isoxanthopterin was catalyzed by XDH. The same results were obtained with purifed cowpea XDH (data not shown).

Along with isoxanthopterin, another product of this reaction is NADH which does not interfere with the assay. Although NADH absorbs strongly at 340 nm, this compound appears dark green at a concentration of 1 mM when exposed to long wave UV light and does not interfere with the appearance of isoxanthopterin.

A rapid screen was developed for nodule XDH activity using pterin as a substrate for the enzyme. Slices of alfalfa, clover, lupine, pea, soybean, lima bean, and cowpea nodules were placed in microtiter wells containing NAD^+ and either buffer alone, pterin, or pterin plus allopurinol. Nodulated soybean, cowpea, and lima bean plants have been shown to synthesize ureides as the major products of the assimilation of fixed N in the nodule (1, 2, 9, 10, 12, 20). Nodules of alfalfa, pea, clover, and lupine do not produce significant quantities of ureides (1, 2, 26). Blue fluorescence appeared only in wells with pterin containing nodule slices of ureide-producing species (Fig. 6). Allopurinol inhibited the formation of isoxanthopterin in this nodule assay (Fig. 6) as well as with purified XDH (Fig. 5). While isoxanthopterin was produced with pterin as substrate by nodule slices of cowpea, lima bean, and soybean, no isoxanthopterin was observed in wells containing nodule slices of the amide-producers, alfalfa, clover, pea, and lupine (Fig. 6).

DISCUSSION

Xanthine dehydrogenase, which catalyzes the hydroxylations of hypoxanthine and xanthine prior to allanotic acid synthesis, has been shown to be involved in nodule ureide synthesis both *in vivo* and *in vitro* (2, 22). The specific activity of XDH is very low in legume nodules that lack ureide synthesis (6, 17). The methods described in this paper take advantage of the role of XDH in ureide synthesis. One method utilizes allopurinol, a specific inhibitor of XDH, while the second method employs an alternate substrate for the enzyme, pterin. Neither method requires xylem sap collection, stem extraction, or measurements of ureides, total N, or nitrate. These two methods also do not require any immunochemical or radiochemical assays or expensive equipment. Although antibodies specific to XDH are available in this laboratory, an immunological screen for XDH was not developed since such assays are often lengthy, requiring several steps and would require nodule tissue extraction. Also, a method which did not require XDH antibodies was thought to be of more general use.

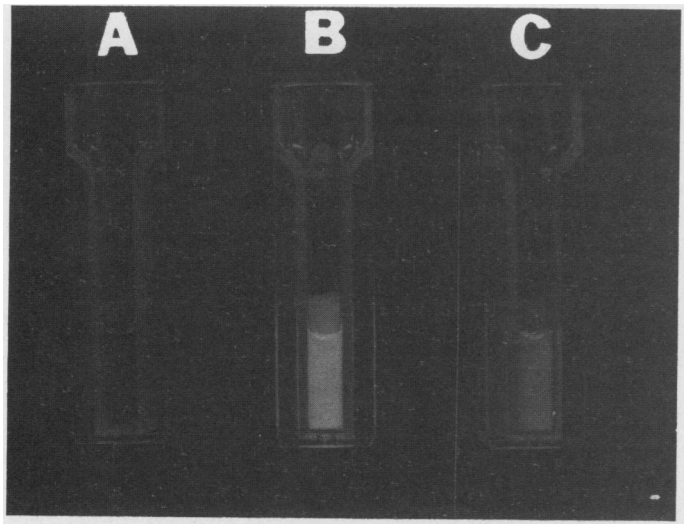

FIG. 5. The production of isoxanthopterin by purified soybean XDH with pterin as substrate. The cuvettes contain no substrate (A), pterin only (B), and pterin plus allpurinol (C).

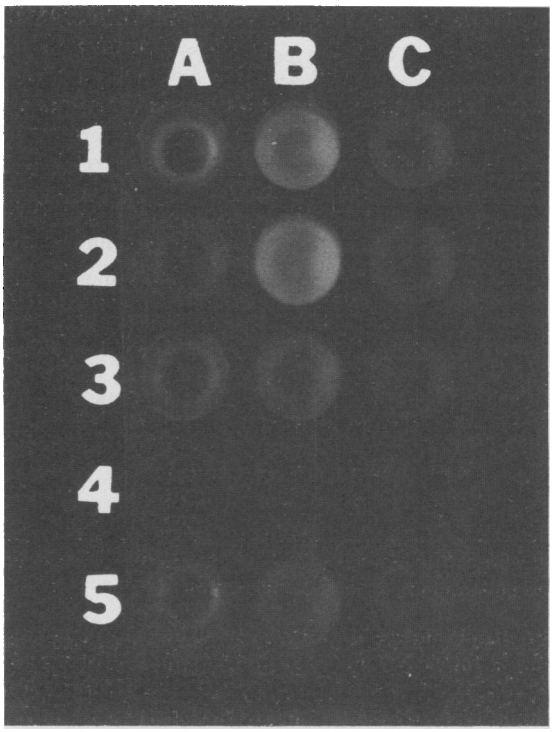

FIG. 6. The production of isoxanthopterin was observed in a microtiter plate with wells containing nodule slices of soybean (1), cowpea (2), pea (3), lupin (4), and clover (5). In each well was placed 50 μl 5 mM NAD^+ and 100 μl of buffer alone (A), 0.1 mM pterin (B), or 0.1 mM pterin plus 0.1 mM allopurinol (C). After 8 h at room temperature, the microtiter plate was photographed.

The pterin assay represents the third report of a rapid screen of legume nodules for a specific enzymic activity. Wacek and Brill (27) described a simple method for the culture of large numbers of soybeans which could then be assayed rapidly for nitrogenase activity by the acetylene reduction technique. Lambert *et al.* (11) recently developed a method for rapidly screening soybean nodules for hydrogenase activity using the methylene blue reduction assay.

Two of the objectives of this laboratory are to determine the contribution of allantoic acid synthesis toward crop productivity and the molecular mechanism regulating induction of ureide synthesis following nodule initiation. In an effort to accomplish these objectives, soybean genotypes which lack ureide synthesis would be useful. Efforts to find such genotypes include the screening of effectively nodulated accessions of *G. soja*, lines of the domestic soybean, and induced mutations of the soybean cultivar Pella for those plants that lack ureide synthesis. Data is presented here describing ureide synthesis by one accession line of *G. soja*. As is the case with *G. max*, ureide production by *G. soja* is significant and is inhibited by the addition of nitrate or ammonium to the nutrient solution (Table I). These data suggest that ureide synthesis is not a trait that was enhanced during the domestication of the soybean plant.

To screen all lines of *G. max* and accessions of *G. soja* in the United States Department of Agriculture collection as well as screen plants with induced mutations, new methods for the

Table II. *Summary of the Results of the Two Screen Methods Described in this Manuscript*

The results reported here for the allopurinol screen refer to the appearance of the leaves following 14 d of allopurinol treatment with N_2 as nitrogen source. A (+) sign in the pterin screen column means that isoxanthopterin was produced with nodule slices of a particular species. A (−) sign in the pterin screen column refers to the absence of isoxanthopterin in those wells. Similarly, a (+) sign in the ureide production column indicates that a particular species assimilates most of its fixed N in the form of ureides. A (−) sign in that column refers to the absence of significant ureide synthesis by that species. Specific activity of XDH is defined as nmol of NADH produced/min·mg protein at 30°C with xanthine as substrate and was measured in nodules of plants which were not treated with allopurinol.

Species	Allopurinol Screen	Pterin Screen	XDH Specific Activity	Ureide Production (Ref.)
Soybean	Chlorotic	+	9.47	+ (12, 13, 20)
Wild soybean	NT[a]	+	9.80	+ (This paper)
Cowpea	Chlorotic	+	2.09	+ (9, 15)
Lima bean	Chlorotic	+	7.25	+ (1)
Pea	Green	−	0.50	− (1)
Lupine	Green	−	0.33	− (1)
Alfalfa	Green	−	0.20	− (1, 26)
Clover	Green	−	0.00	− (1)

[a] Not tested.

detection of ureide synthesis are needed. Two screens for such a purpose are presented here. Both methods are inexpensive. The pterin screen is rapid, not labor intensive, and can be performed in test tubes, disposable cuvettes, or microtiter plates and requires only the removal of a single nodule from a plant. However, although the pterin assay is useful as a screen for XDH activity, this assay is not useful for the detection of any other biochemical defects in ureide synthesis. A soybean mutant that lacks XDH activity will also lack ureide synthesis since XDH has been shown to be required for ureide synthesis *in vivo* and *in vitro* (2, 22). The pterin assay will not detect a soybean mutant which has XDH activity but lacks ureide synthesis owing to mutation in another step of ureide synthesis. In summary, the pterin assay will detect soybean plants which lack nodule XDH activity and, as a result, lack ureide synthesis as well.

Utilization of the allopurinol screen, although labor intensive, would detect defects in nodule ureide synthesis other than in XDH activity. Any nodulated soybean plant which is incapable of ureide synthesis would be unaffected by treatment with allopurinol even if XDH activity is present in the nodule of such a plant. A soybean plant which fails to produce ureides when effectively nodulated is expected to assimilate N_2 via some other pathway and hence be unaffected by treatment with allopurinol. This is demonstrated by the observations that the assimilation of exogenously supplied nitrate or ammonium by nonnodulated soybeans is not inhibited by allopurinol and that amide-producing, effectively nodulated legumes are also unaffected by allopurinol. Thus far, 1500 lines of soybean from the United States Department of Agriculture collection have been screened using allopurinol. None of these lines were unaffected by allopurinol treatment when nodulated. The screening of these lines suggests that most soybean lines are incapable of redirecting the pathway of dinitrogen assimilation from ureide to amide synthesis when the XDH reaction is inhibited. The pterin screen is a recent development of this laboratory and has only been used to screen 45 cowpea lines. Nodules of all of the cowpea lines had XDH activity.

The allopurinol experiments suggest that soybean and cowpea nodules cannot produce amides when the pathway of ureide synthesis is blocked at the XDH reaction. Nonureide producing legumes, whether they be nitrate-fed, ammonium-fed, or N_2-fixing amide producers, are unaffected by allopurinol inhibition of the XDH reaction. These observations suggest that the regulatory step for turning off ureide synthesis and turning on amide synthesis in the presence of exogenously supplied ammonium in soybean or cowpea nodules is not the XDH reaction but is some step prior to purine hydroxylation.

In summary, two methods for the detection of ureide production by nodulated legumes have been developed. Both techniques are based on the positive correlation between XDH activity and ureide synthesis by nodules of tropical legumes (6, 17, 21, this manuscript). A summary of the results of the two methods is listed in Table II. The pterin assay utilizes a nonphysiological substrate of XDH, pterin, which is hydroxylated by XDH to a blue fluorescent compound, isoxanthopterin. Only nodules of ureide-producing legumes produced isoxanthopterin in the presence of pterin. The pterin assay is useful for detecting XDH activity in large numbers of nodules rapidly. A second screen utilizes a specific inhibitor of XDH, allopurinol, which when fed as a soil drench to ureide-producing, N_2-fixing legumes for 2 weeks, causes severe leaf chlorosis presumably owing to inhibition of nodule dinitrogen assimilation. This screen is labor intensive but is useful for detecting any effectively nodulated legume which lacks ureide synthesis.

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