SOYBEAN SEED GALACTINOL SYNTHASE ACTIVITY AS DETERMINED BY A NOVEL COLORIMETRIC ASSAY

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ABSTRACT - Galactinol synthase (GS) is a key enzyme for the biosynthesis of raffinose oligosaccharides (RO) which are the flatulence factors present in soybean seeds and several other legumes. Understanding of soybean seed GS properties is, therefore, of biotechnological interest. The GS enzyme catalyses formation of galactinol and UDP from UDP-gal and *myo*-inositol. This enzyme is currently assayed by an isotopic method. We have then idealized a more convenient method for GS assay based on the indirect colorimetric determination of the UDP formed which is then hydrolyzed by exogenous apyrase and the resulting Pi quantified by a modification of the colorimetric method of Fiske & SubbaRow. The color developed is stable, and the method is suitable for detection of very low GS activity. The GS activity profiles of developing soybean seeds determined by the isotopic and the colorimetric methods are closely related. The GS enzyme was partially purified (46-fold) by treatment of seed extract with $MnCl_2$, sequential chromatographies on DEAE-Sepharose, Phenyl-Sepharose CL-4B and Q-Sepharose columns. The crude and the partially purified enzyme showed maximum activity at pH 7.0 and 50 ºC. Dithiothreitol and $MnCl_2$ enhanced considerably the activity of the partially purified enzyme. While UDP-glc could be hydrolyzed by the enzyme at a reative activity corresponding to 49% of that calculated for UDP-gal, UDP-man and sucrose were completely ineffective as alternative substrates.

ADDITIONAL INDEX TERMS: Flatulence, galactinol synthase, colorimetric assay, raffinose oligosaccharides.

ABBREVIATIONS - GS, Galactinol synthase; DTT, Dithiothreitol; EDTA, Ethylenediaminetetraacetic acid; HEPES, (N-[2-Hydroxy-ethylpiperazine-N'-[2-ethanesulfonic acid]}; PMSF, Phenylmethylsulfonyl Fluoride; RO, Raffinose oligosaccharides; SD, Standard deviation; TCA, Trichloroacetic acid; TRIS, Tris(hidroxymethyl) aminomethane; UDP- [U-^{14}C]gal, Uridine 5'diphospho-[U-^{14}C]galactose; UDP-glc, Uridine diphospho-glucose; UDP-man, Uridine diphospho-manose.

ATIVIDADE DE GALACTINOL SINTASE DE SEMENTE DE SOJA DETERMINADA POR UM NOVO ENSAIO CALORIMÉTRICO

RESUMO - Galactinol sintase (GS) é a enzima-chave para a biossíntese de oligosacarídeos de rafinose (RO), que são os fatores antinutricionais causadores de flatulência, os quais estão presentes em sementes de soja e em outros legumes**.** A GS catalisa a formação de galactinol e UDP a partir de UDP-gal e

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*mio*inositol. A atividade dessa enzima é determinada atualmente pelo método radioisotópico que, apesar de adequado tecnicamente, apresenta vários inconvenientes, tais como a necessidade de substrato de alto custo, bem como de cuidados adicionais e serviços especializados para descarte dos resíduos radioativos. Assim, desenvolveu-se um método colorimétrico alternativo ao método radioisotópico, baseado na determinação colorimétrica indireta do UDP formado pela hidrólise enzimática (apirase) desse nucleotídeo e determinação do Pi resultante pelo método de Fiske & SubbaRow, com modificações. A cor desenvolvida é estável e o método é sensível para detecção de quantidades nanomolares de Pi.

Os perfis de atividade da GS em sementes de soja em diferentes fases de desenvolvimento, determinados pelos métodos colorimétrico e radioisotópico, são semelhantes. Adicionalmente, a GS de sementes de soja foi purificada (46-vezes) por tratamento do extrato das sementes com $MnCl_2$, e uma seqüência de cromatografias em colunas de DEAE-Sepharose, Phenyl-Sepharose CL-4B e Q-Sepharose. As atividades de GS no extrato bruto e na amostra parcialmente purificada foram máximas em pH 7.0 e 50 ºC. Ditiotreitol e $MnCl_2$ aumentaram consideravelmente a atividade da enzima parcialmente purificada. Enquanto UDP-glc pode ser hidrolisado pela enzima com uma atividade relativa correspondendo a 49% da atividade contra UDP-gal, UDP-man e sacarose foram completamente ineficazes como substratos alternativos. Os valores de K_M para conversão de UDP-gal e *mio*-inositol foram de 2,0 mM e 2,93 mM, respectivamente, determinados pelo método de Lineaweaver-Burk.

TERMOS ADICIONAIS PARA INDEXAÇÃO: Flatulência, galactinol sintase, ensaio colorimétrico, oligosacarídeos de rafinose.

INTRODUCTION

The raffinose oligosaccharides (RO) are present in various higher plants (Lee *et al*., 1970). They are second only to sucrose in abundance in legume seeds (Dey, 1985). Their primary role is thought to be reserve carbohydrates for short- and long-term purposes and for transport (Chatterton *et al*., 1990, Dini *et al.*, 1989, Pharr and Sox, 1984), and protection of plants against cold and seeds against desiccation (Kandler and Hopf, 1980; Saravitz *et al*., 1987). Nevertheless, the RO have been implicated as causative factors for flatulence after ingestion of food containing soybean meals (Rackis *et al*., 1970).

The biosynthesis of RO [(α-D-galatosyl-1,6)$_n$-sucrose] occurs via sequential transfers of galactosyl units to sucrose mediated by specific transferase enzymes. The galactosyl donor is galactinol (1L-1-O-α-D-galactopyranosyl-*myo*-inositol) which is synthesized in the reaction UDP-gal + *myo*-inositol → UDP + galactinol, catalyzed by galactinol synthase (UDP-α-D-gal:1L-*myo*-inositol-1-O-α-D-galactopyranosyltransferase; EC 2.4.1.123). The galactinol synthase (GS) is believed to be a key enzyme in the biosynthetic pathway of RO. It is the first enzyme to commit sucrose to biosynthesis of RO and is, therefore, a metabolic control point in carbon partitioning between sucrose and RO. GS activity in soybean seeds increases immediately prior to the beginning of the accumulation of RO, and shows a positive correlation with levels of these oligosaccharides (Saravitz *et al*., 1987; Handley *et al*., 1983; Lowell and Kuo**,** 1989). Thus, manipulation of GS *in vivo* may lead to understanding of the physiological role of the RO in plants and reduction of flatulence factors in legumes.

GS is currently assayed by a radioisotopic method originally described by Handley and Pharr (1982), and modified by Liu *et al.*, (1995). However, this radioisotopic method is expensive and requires special handling and disposal mehtods for radioactive wastes. To facilitate the assay of GS, we have developed a colorimetric method which is more convenient for routine use. This assay is based on the indirect quantification of the UDP formed from *myo*-inositol and UDP-gal by the action of GS. The resulting UDP is then hydrolyzed to UMP and Pi by exogenous insect or potato apyrase, with subsequent quantification of Pi by the colorimetric method of Fiske and SubbaRow (1925) with modifications. Here, we report on the reliability of

this method, as well as on the GS activity profile of soybean seeds during development in the field, as determined by both the radioisotopic method and the proposed colorimetric method.

Although GS has been purified from *Cucurbita pepo* (Webb, 1982; Smith *et al*., 1991; Liu *et al*., 1995) and kidney bean cotyledons (Liu *et al*., 1995), purification of soybean seed GS has not been reported yet. A partial purification protocol for soybean seed GS and some of the its kinetic properties are therefore reported here.

MATERIALS AND METHODS

Chemicals – Potato apyrase (grade V), UDP, UDP-gal, *myo*-inositol, HEPES, DTT, Fiske and SubbaRow reducer, Dowex-1(1X4-100 chloride strongly basic anion exchange resin), hydrophilic scintillation solution, TCA, $MnCl_2$, and PMSF were purchased from Sigma. UDP-[U-^{14}C]gal was purchased from Amersham. All other chemicals were of analytical grade.

Insect apyrase – Insect apyrase was prepared using 200 pairs of pink salivary glands from the 5th instar larvae of *Rhodnius prolixus* (laboratory stock). The glands were excised from the heads, homogenized in 200 µL of water and then centrifuged at 3,000 x *g* for 10 min at 4 ^{o}C. The supernatant (crude insect apyrase extract) was assayed for its apyrase activity (Sarkis *et al*., 1986) and stored at –20 ^{o}C until use. One unit of apyrase activity corresponds to the amount of protein necessary to hydrolyze one µmol of UDP per minute at pH 7.5 and 37 ºC.

Extraction - Soybean plants (*Glycine max* L. Merril, var. FT CRISTALINA RCH) were grown under field conditions from mature seeds. Plants were watered daily. After beginning of flowering, the seeds were harvested at 5-day intervals for 30 days and used either for determination of fresh and dry weights, or stored at –20 ºC until use. For preparation of GS extracts the seedcoats and axes were removed, and the cotyledons (5g) were ground in a mortar with 10 mL of cold extraction buffer (50 mM HEPES, pH 7.0, containing 1 mM DTT and 1 mM PMSF). The homogenate was then centrifuged at 28,000 x *g* for 30 min at 4 ^{o}C and the supernatant (GS extract) used for GS assays.

Dry weight determination. Seeds were weighed and dried in a closed chamber at 70^{o}C until constant weight.

GS Assay

Radioisotopic method - The radioisotopic assay was performed as described by Handley and Pharr (1982), with slight modifications. The reaction mixture contained 50 µL of galactinol synthase extract, 60 mM *myo*-inositol, 2 mM DTT, 50 mM HEPES buffer (pH 7.0), 4 mM $MnCl_2$, 20 µg of bovine serum albumin and 4 mM UDP-[U-^{14}C]gal (0.25 µCi µmol^{-1}) in a total volume of 100 µL. The reaction was conducted for 30 min at 32 ºC and terminated by the addition of 400 µL of 100% ethanol. Unreacted UDP-[U-^{14}C]gal was removed by adding 400 µL of a Dowex-1 anion exchange resin slurry (0.6 g mL^{-1}) to each tube and incubation for 50 min under agitation (200 rpm). The tubes were then centrifuged for 10 min at 12.000 rpm and the supernatant collected. A 50 µL aliquot of this supernatant was pipetted into 1 cm^2 filter paper discs (Whatman n° 2) which were then dried and placed in scintillation counter flasks containing 6 mL of a hydrophilic scintillation solution. The radioactivity was determined in a Beckman liquid scintillation counter with an efficiency of 75%. Reaction mixtures containing no *myo*-inositol were used as controls. Each assay was done in triplicates. Control counts were subtracted from test counts and enzyme activity expressed as µmol of galactinol formed, considering the specific activity of the UDP-[U-^{14}C]gal. According to Handley and Pharr (1982), the limit of detection of this assay is 100 dpm above background or 0.5 nmoles galactinol mL enzyme^{-1} min^{-1}.

Colorimetric method - The colorimetric assay was performed using the same reaction mixture for the isotopic method of Handley and Pharr (1982) as described above, using UDP-gal instead of UDP-[U-^{14}C]gal, with modifications. The reaction was allowed to proceed for 30 min (except when stated) at 32 ºC, and terminated by placing the tubes in boiling water for 2 min. To each tube were added 500 µL of water, 10 µL (0.3 U) of insect or potato apyrase solution, and 150 µL of apyrase reaction mixture (Tris-HCl buffer, 250 mM, pH 7.5, containing 25 mM KCl, 7.5 mM $CaCl_2$, 0.5mM EDTA-Na, and 50 mM glucose) (Sarkis *et al.*, 1986). After incubation for 10 min at 37 ºC, the apyrase reaction was stopped by addition of 60 µL of 75% TCA. The tubes were then cooled on ice for 10 min, centrifuged at 3,000 x *g* for 10 min and the amount of Pi in the supernatant was determined by a modified Fiske and SubbaRow (1925) protocol. To each tube were added 100 µL of 2.5% ammonium molybdate dissolved in 2 N HCl and 40 µL of Fiske and SubbaRow reducer. After 2 min at room temperature, 40 µL of a 34% sodium citrate.$2H_2O$ solution were added to the tubes, and absorbance was immediately measured at 660 nm. The amount of UDP formed by the GS was determined using a standard curve constructed with UDP hydrolyzed by the apyrase, and correlated to the amount of UDP produced by the galactinol synthase. One unit (U) of GS activity corresponds to the amount of protein necessary to produce one µmol of UDP per minute. Each assay was done in triplicate. Control tests were performed by omitting *myo*-inositol from the galactinol synthase reaction mixture.

GS purification - Cotyledons (150 g) from seeds harvested 70 days after beginning of flowering were pre-incubated with 600 mL of 20% hexane for 30 min and washed immediately with 4 L of cold water. The cotyledons were then ground in a mortar with 300 mL of cold extraction buffer (50 mM HEPES, pH 7.0, containing 1 mM DTT and 1 mM PMSF). The homogenate was centrifuged at 28,000 x *g* for 30 min at 4 ºC. $MnCl_2$ (1 M solution) was added to the resulting supernatant (GS extract) to a final concentration of 25 mM. The mixture was then centrifuged at 28,000 x *g* for 30 min and the supernatant dialyzed overnight against water and centrifuged again at 28,000 x *g* for 20 min. This last supernatant was applied into a DEAE-Sepharose column (2.6 x 14 cm) previously equilibrated with running buffer (50 mM MOPS – Na, pH 7.0, containing 2 mM DTT). Bound proteins were initially eluted with 300 mL of the MOPS running buffer followed by elution with a linear gradient formed with 180 mL of the running buffer and 180 mL of the same buffer containing 0.5 M NaCl. Samples of 6 mL were collected at a flow rate of 90 mL h^{-1}. Fractions containing GS activity were pooled, supplemented with solid $(NH_4)_2SO_4$ to a final concentration of 1 M and applied to a Phenyl-Sepharose 4B column (2.6 x 14 cm) pre-equilibrated with 1 M $(NH_4)_2SO_4$ dissolved in MOPS running buffer. Elution was carried out with 150 mL of 1 M $(NH_4)_2SO_4$ dissolved in MOPS running buffer, followed by a linear negative gradient formed with 150 mL of MOPS running buffer containing 1 M $(NH_4)_2SO_4$ and 150 mL of the same buffer. The column was further eluted with 150 mL of MOPS running buffer. Elution was performed at a flow rate of 120 mL h^{-1}. Samples of 6 mL were collected. Active fractions were pooled, dialyzed overnight against water and applied into a Q-Sepharose column (2.6 x 14 cm) previously equilibrated with running buffer. Proteins were eluted with 110 mL of running buffer followed by a linear gradient formed with 150 mL of the running buffer and 150 mL of the same buffer containing 0.5 M of NaCl. Samples of 6 mL were collected at a flow rate of 90 mL h^{-1}. Active fractions were pooled, dialyzed against water for 6 h at 4 ºC, and used for kinetic studies or stored at –20 ºC until use. All purification steps were carried out on ice or at 4 ºC. GS activity was determined by the colorimetric method.

Electrophoresis

Electrophoresis was carried out in 12% sodium dodecyl sulphate polyacrylamide gel (SDS-PAGE) according to Laemmli (1970). Proteins were stained with silver reagent (Blum *et al*,. 1987).

Kinetics

Initial rates of substrate hydrolysis were determined by the colorimetric method. The buffers (50 mM) used for optimal pH determination were Na-acetate buffer (pH 4-6), BES-Na (pH 6.5-7), BICINE-Na (pH 7.6-8.9). K_M values for UDP-galactose hydrolysis and *myo*-inositol utilization were determined by the Lineweaver-Burk plot, and by an algorithm for least squares estimation of non-linear parameters (Marquardt, 1979).

RESULTS AND DISCUSSION

GS catalyzes the reaction UDP-gal + *myo*-inositol → galactinol + UDP. Although this enzyme is considered of biotechnological interest, there is no chemical method available for its specific assay. The isotopic method (Handley and Pharr, 1982) requires ^{14}C-labeled UDP-gal as substrate; although extremely sensitive, it is expensive and the radioactive wastes require special handling and disposal methods. We have, therefore, developed a novel GS assay based on the colorimetric quantification (Fiske and SubbaRow, 1925) of the Pi produced by the enzymatic hydrolysis of the UDP formed by the GS enzyme. As shown in Figure 1 absorbance values at 660 nm are proportional to the amount of Pi up to 600 μM. Phosphate ions linked to UDP and UDP-gal do not react with the Fiske and SubbaRow reagent even at concentrations as high as 800 μM (Figure 1). However, UDP is promptly hydrolyzed by insect or potato apyrase (ATP-diphosphohydrolase; ATPase; EC 3.6.1.5) which catalyzes the hydrolysis of the *g*- and *b*-phosphate residues from triphospho- and diphosphonucleosides (Meyerhof, 1945). The *Rhodnius prolixus* crude saliva is devoid of 5'nucleotidase, inorganic pyrophosphatase, phosphatase and adenylate kinase activities but hydrolyzes ATP and ADP to the corresponding diphospho- and monophosphatonucleotide (Sarkis *et al.*, 1986), and therefore insect saliva apyrase was used throughout this work. As shown in Figure 1 the absorbance curve obtained with UDP hydrolyzed with apyrase matches exactly the standard curve constructed with KH_2PO_4. However, no color is developed when the UDP is replaced by UDP-gal (Figure 1) or mononucleotide phosphates (not shown). The standard deviation values determined for the GS assay were always less than 10% of the mean values, reflecting the reliability of our colorimetric assay method. Additional experiments have shown that the insect enzyme may be successfully replaced by potato apyrase but not by alkaline phosphatase which was shown to be much less effective than the apyrases (data not shown).

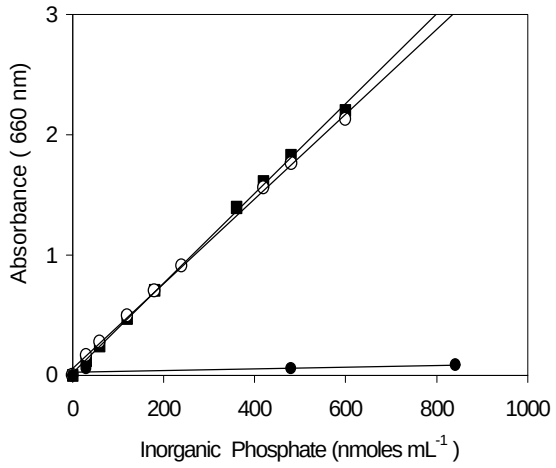

FIGURE 1 - Color intensity (absorbance at 660nm) of the reaction mixture containing different concentrations (nM) of inorganic phosphate from KH_2PO_4 . (■), or resulting from hydrolysis of UDP (O) or UDP-gal with insect apyrase(●).

The GS-apyrase coupled reaction was shown to be of first order with variable volumes (0-50L) of GS extract, and to be linear over a 60 min pereiod of time (data not shown). The possibility that sucrose phosphate synthase or sucrose synthase may account for the observed color development can be ruled out as no color developed above the background levels when UDP-gal was omitted from the reaction mixture, or sucrose was added as substrate. UDP-glc acts as substrate but with low specificity. However, as UDP is the product of reactions catalyzed by sucrose phosphate synthase and sucrose synthase

our colorimetric assay may be also adapted and used for assay of these enzymes in the presence of the corresponding substrates.

Quantification of the Pi formed by enzymatic hydrolysis of the UDP produced in the GS reaction mixture required minor modifications of the method of Fiske and SubbaRow (1925) which describes the dissolution of the ammonium molybdate in 5 N HCl. Under our assay conditions, HCl at the concentration of 5 N induced color development resulting from the acid hydrolysis of organophosphate compounds. However, at the concentration of 2 N, the HCl used to prepare the ammonium molybdate acidic solution did not induce additional unspecific color development. The color developed was only fairly stable, but addition of Na-citrate as recommended by Lanzetta *et al.* (1979) for determination of Pi considerably improved stability of the dye-phosphomolybdate complex. Additionallly, it has the advantage of being insensitive to any newly released phosphate by acid hydrolysis of organophosphate compounds.

The profiles of GS activity determined for soybean seeds of different ages by both the isotopic method (Handley and Pharr, 1982) and the novel colorimetric method are closely related (Figure 2), indicating that the colorimetric method is reliable for quantification of GS activity. The activity levels in developing seeds varied with the amount of dry matter present in the seeds. Highest activity was observed for the yellow colored seeds showing 42% dry matter, corresponding to the 6.5 reproductive growth stage (Fehr *et al.*, 1971). These results are in agreement with those previously reported by Lowell and Kuo (1989). GS has been considered an important regulator of carbon partitioning between sucrose and raffinose and stachyose in developing soybean seeds (Saravitz *et al.*, 1987).

Soybean seed GS was partially purified by treatment with $MnCl_2$ and a sequence of chromatographic procedures. The elution profile of the GS on a Q-Sepharose column, the last column of the sequence, is shown in Figure 3. A summary of the purification steps is shown in Table 1. The

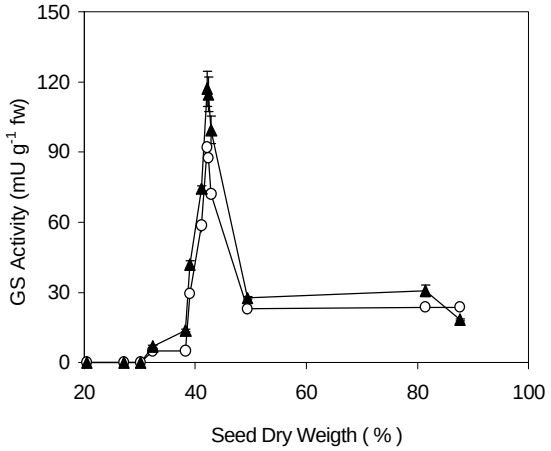

FIGURE 2 - Galactinol synthase activity in soybean seed at different developmental stages, as determined by the colorimetric method (▲), and the isotopic method (O).

enzyme was purified 46.3-fold to a specific activity of 66.35 mU mg^{-1} protein. SDS-PAGE analysis showed that the resulting GS sample contained several proteins with molecular masses ranging from 18 to 66 kDa (not shown). The activity of the partially purified GS was maximal at pH 7.0 (Figure 4A) and 50 ^{o}C (Figure 4B), but substantial activities were detected at extremes of pH (5-6 and 7.6-8.9). The optimal temperature is in agreement with the data reported for the *Ajuga reptans* GS enzyme which showed increasing activity with increasing temperature up to a maximum of 50 ºC (Bachmann *et al.*, 1994). The pH and temperature profiles obtained for the partially purified enzyme match exactly that obtained with the enzyme sample resulting from the treatment of the seed extract with $MnCl_2$ (Figure 4A,B). The optimal pH value is comparable to that reported for the GS enzyme from kidney bean cotyledons, which had an optimal pH of 7.0 (Liu *et al.*, 1995), and to that reported for the enzyme purified from zucchini squash leaves, which had a broad pH optima from 7.0 to 8.0, with activity dramatically decreasing below 6.5 and above 8.0 (Smith *et al.*, 1991).

TABLE 1 - Summary of the purification steps of soybean seed galactinol synthase. Enzyeme activity was assayed by the colorimentric method.

Purification Step	Total Protein	Total Activity	Specific Activity	Purification	Yield
	mg	MU	mU mg^{-1}	Fold	%
Crude extract	16808	24122	1.44	1	100
$MnCl_2$	3864	29102	7.53	5.2	121
DEAE-Sepharose	1245	14647	11.76	8.2	61
Phenyl-Sepharose	228	11208	49.16	34.3	47
Q-Sepharose	109	7232	66.35	46.3	30

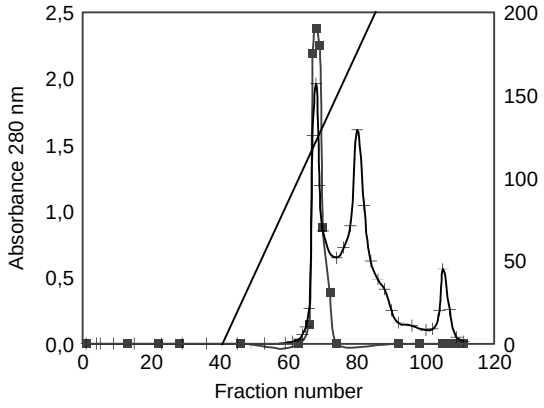

FIGURE 3 -Elution profile of soybean galactinol synthase on a Q-Sepharose column. Absorbance at 280 nm (+); enzyme activity (■) assayed by the colorimentric method; 0 - 0.5 M aCl gradient (—).

The activity of the partially purified GS enzyme was enhanced by $MnCl_2$ (Figure 5) and DTT (not shown). These results are in agreement with those reported for the GS enzymes from *Cucurbita pepo* (Webb, 1982; Smith *et al*., 1991; Liu *et al*., 1995) and kidney bean cotyledons (Liu *et al*., 1995). Cations were effective in inhibiting the GS enzyme, and $CuSO_4$ (10 mM) and $CoCl_2$ (1 mM) completely abolished the activity. On the other hand, however, HgCl (1 mM) did not affect the GS activity (Table 2). Inhibition of *Cucurbita pepo* (Webb, 1982) and zucchini squash leaves (Smith *et al*., 1991) by divalent cations has been previously reported.

Although the GS was fully active with the substrate UDP-gal it also hydrolyzed UDP-Glc, but not UDP-man and sucrose. K_M and V_{max} values calculated for UDG-gal using the colorimetric method were 5.2 mM and 195 nmoles min^{-1}, respectively, as determined by the algorithm for least squares estimation of non-linear parameters (Marquardt, 1979), or 2.0 mM and 195 nmoles min^{-1}, respectively, as determined by the Lineweaver-Burk plot. For *myo*–inositol, K_M and V_{max} values of 3.5 mM and 53.4 nmoles min^{-1}, respectively, were determined by the algorithm mentioned above (Marquardt, 1979), and of 2.93 mM and 53.4 nmoles min^{-1}, respectively, as determined by the Lineweaver-Burk plot. These

values are comparable to the K_M values of 1.8 mM and 6.5 mM reported for the conversion of UDP-gal and *myo*-inositol, respectively, by GS purified from zucchini squash leaves (Smith *et al*., 1991). Nevertheless, these K_M values are larger than those reported for conversion of UDP-gal (0.4 mM) and *myo*-inositol (4.5 mM) by a GS purified from kidney bean cotyledons (Liu *et al*., 1995).

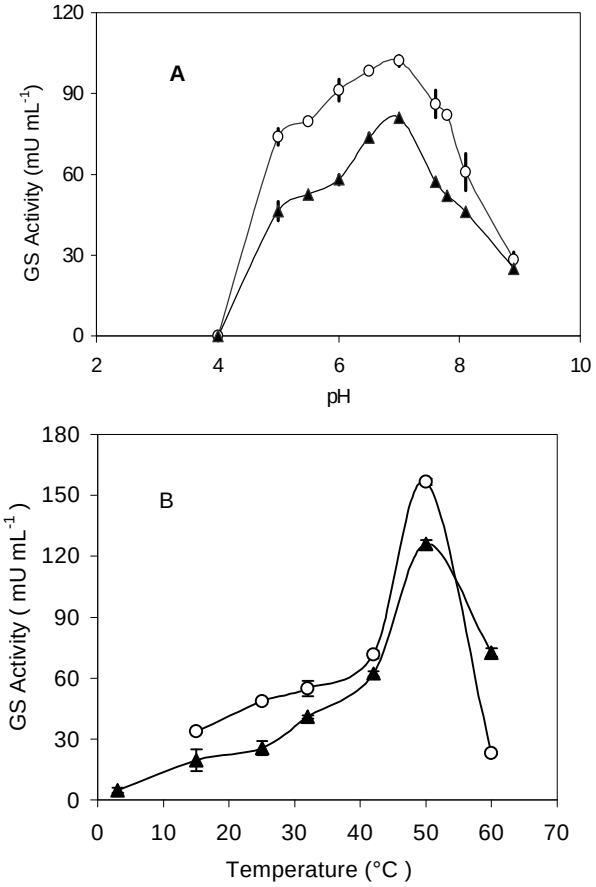

FIGURE 4 - Effect of pH (A) and temperature (B) on the activity of soybean galactinol synthase assayed by the colorimentric method. crude extract (▲); partially purified enzyme (O).

Further kinetic and molecular characterization of soybean seed GS may be helpful for the understanding of its role in seed development. In addition, the gene encoding the GS enzyme may be useful for production of transgenic soybean plant with low capacity to produce the raffinose oligosaccharides, the causal agents of flatulence following leguminous seed ingestion. Additional work aiming at further purification and characterization of GS is being carried out.

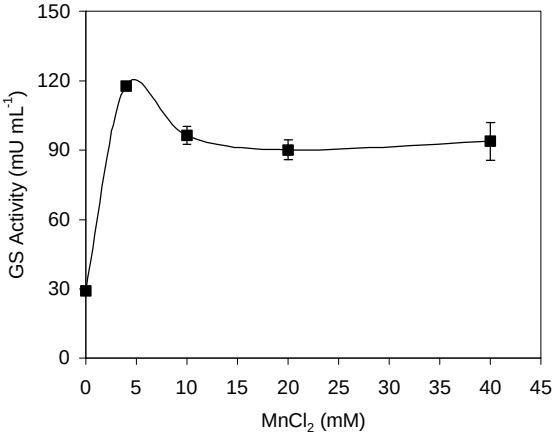

FIGURE 5 - Effect of $MnCl_2$ on the activity of soybean seed partially purified galactinol synthase assayed by the colorimentric method

TABLE 2 - Effect of ions on the activity of soybean seed partially purified galactinol synthase assayed by the colorimentric method.

Reagent	mM	GS activity (mU mL^{-1})	GS relative activity (%)
Control	—	116.3 ± 2.62	100
$ZnSO_4$	1	101.6 ± 5.69	87
	10	36.8 ± 3.52	32
$CuSO_4$	1	113.4 ± 8.16	97
	10	0	0
$AlCl_3$	1	120.4 ± 11.59	104
	10	96.1 ± 4.70	83
$FeCl_3$	1	119.0 ± 6.33	102
	10	80.9 ± 5.15	70
$CoCl_2$	1	0	0
HgCl	1	117.4 ± 4.29	101

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