Enhancement of β-Glucosidase and β-Galactosidase of *Trigonella foenum-graecum* by Exposure to the Allelochemical Mimosine

T. R. Santosh, K. K. Balasubramanian, and K. Lalitha*

Department of Chemistry, Indian Institute of Technology, Madras, Chennai 600 036, India

Glycohydrolases assume significance in the metabolism of biological systems and have important industrial applications in the areas of pharmaceuticals, food, and medicine. Glycosidases were screened in germinating seeds, and attempts were made to enhance their levels. Screening of glycosidases in the seedlings during a 72 h germination period revealed higher levels of β-glucosidase and β-galactosidase in *Trigonella foenum-graecum* compared to *Cicer arietinum* and *Vigna radiata*. Activity of β-galactosidase was in general higher than that of β-glucosidase in all the seedlings tested. During growth, exposure of the seedlings to an allelochemical, mimosine, at 0.1 mM resulted in the enhancement of enzyme levels by 50% in the seedlings of *T. foenum-graecum*, whereas the addition of mimosine to the assay medium in vitro did not affect the enzyme activities. Hydrolytic activity was enhanced by addition of glycerol in the medium up to 0.1 M in the case of β-glucosidase and with 0.05 M in the case of β-galactosidase. In general, the hydrolytic rate was higher by about 30% in the seedlings exposed to mimosine compared to that of the control. Concomitant enhancement in the rates of transgalactosidation by 51% and transglucosidation by 23% was also noted, underscoring the relevance of plant glycohydrolases for appropriate applications.

Keywords: *β-Glucosidase; β-galactosidase; transglycosidation; mimosine; allelochemical; Trigonella foenum-graecum; seed germination*

INTRODUCTION

Research in the area of glycosidases has gained momentum in recent times owing to the important role of many glycoproteins and glycolipids in biological systems and their potential biotechnological applications related to food and medicine (Young et al., 1993; Kotz et al., 1994; Ponce et al., 1997). Glycosidases acting as either endo- or exoglucanases catalyze the hydrolysis of glycosidic bonds in both simple and complex carbohydrates, yielding low molecular weight monosaccharides and oligosaccharides. Glycosidases also catalyze appropriate transglycosylation reactions (Sinnott, 1990; Mori et al., 1997) in the presence of an alcohol or another sugar, resulting in the synthesis of new saccharides and the release of an aglycone moiety. Glycosyl hydrolases have been indispensable in the study of complex structural and functional carbohydrates (Flowers and Sharon, 1979).

β-Glucosidases (EC 3.2.1.21) and β-galactosidases (EC 3.2.1.23) were studied widely in prokaryotes (Deschavanne et al., 1978; Withers and Street, 1988) and eukaryotes (Peralta et al., 1990; Beattie et al., 1994; Hall et al., 1997). Detailed investigations on these enzymes have focused on the mechanism of catalysis (Sinnott, 1990; Gebler et al., 1992; He and Withers, 1997), sequence comparison and structure (Henrissat, 1991), and classification into 48 families (Henrissat and Bairoch, 1993) to form a gene superfamily (Jenkins et al., 1995). Multiple forms of β-glucosidases in plants were linked to at least three important biological functions: (i) biomass conversion (Dey and del Campillo, 1982); (ii) chemical defense against pathogens and herbivores by the process of cyanogenesis (Hughes et al., 1992); and (iii) regulation of the biological activity of plant phytohormones such as cytokinin, gibberellin, and auxins (Brozobohaty et al., 1993; Leah et al., 1995). β-Galactosidases from microbial and animal sources have been studied in detail (Roth and Huber, 1994; Hall et al., 1997; Matern et al., 1997), and in plants, they were reported in both membrane bound and soluble forms (Klis et al., 1974). β-Galactosidases were suggested to have a variety of functions in different locations, including degradation of polysaccharides in germinating seeds (Matheson and Saimi, 1977; Ross et al., 1994); ripening of fruits (Ali et al., 1995; Carey et al., 1995); and changes in membrane galactolipids (Dey and del Campillo, 1982).

Owing to the immense potential for the use of β-glycosidases in clinical and food biotechnology (Young et al., 1993; Kotz et al., 1994), attempts were made in the present study to screen the enzymes in selected seeds and to explore the possibility of enhancing the levels of plant glycosidases through exposure to the allelochemical, mimosine. Mimosine is a non-protein amino acid, occurring exclusively in the leaves of *Leucaena leucocephala* and *Mimosa pudica*, constituting about 2−5% of the dry matter (Vasanthy et al., 1986). *L. leucocephala*, a nitrogen fixing perennial, has been a prominent choice in agroforestry schemes owing to its multiple uses as fuel, timber, and as a leaf mulch with ability to suppress weeds (Rama Devi et al., 1997). Mimosine ingestion resulted in toxicity to cattle to varying extent and was also shown to be thyrotoxic. (Hallengrein et al., 1987).

* Address correspondence to this author at Professor of Biochemistry, HSB 264, Department of Chemistry, Indian Institute of Technology, Madras, Chennai 600 036, India (phone 91-044-235-1365, ext 3129; e-mail klalitha@acer.iitm.ernet.in; fax 91-044-235-0509 or 91-044-235-2545).

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In earlier investigations in our laboratory, *L. leucocephala* was identified among many different plant species tested as the unique primary biomass source toward high-rate biomethanation, suitable for large scale energy production (Vasanthy et al., 1986). Mimosine was found to be effectively degraded during the biomethanation process (Vargheese, 1995) as estimated by a specific method (Lalitha et al., 1993), and the resultant sludge was devoid of mimosine toxicity as assessed by a specific bioassay procedure using the insect *Corcyra cephalonica*. Interestingly, the detoxified sludge was found to be growth promoting to the insect (Vargheese, 1995). It was therefore interesting to consider if mimosine exerted similar beneficial influence on the growth of a plant species as well.

In the present study, attempts were made to study the effect of low concentrations of mimosine up to a level of about 2 mM on the general growth parameters of germinating *Trigonella foenum-graecum* seedlings. The present study was also aimed to assess the level of β-glycosidases of germinating seeds and to explore the possible enhancement of enzyme activities from a suitable source by exposure to mimosine. In contrast to the reports on the toxicity of mimosine (Hegarty et al., 1976; Jones and Megarrity, 1983), the results of the present study reveal no detrimental effects of mimosine on the germinating seeds of *T. foenum-graecum* when exposed to the allelochemical in concentrations up to 0.4 mM with significant enhancement in the activities of β-glycosidases of the plant.

MATERIALS AND METHODS

Seeds used in the present study were obtained from a seed dealer in Madras, India. Mimosine, bovine serum albumin (BSA), poly(vinylpyrrolidone) (PVP, K-30 mol wt = 40 000), phenazine methosulfate (PMS), and 2,6-dichlorophenol indophenol (DCPIP) were purchased from Sigma Chemicals (St. Louis, MO). *p*-Nitrophenyl β-D-glucopyranoside (PNPG) and *o*-nitrophenyl β-D-galactopyranoside (ONPG) were gifts from Dr. D. Loganathan, Department of Chemistry, Indian Institute of Technology, Madras. All other chemicals were of analytical reagent grade from E. Merck, (Bombay, India). Deionized water was used throughout the study unless otherwise specified.

Studies with Germinating Seeds. For initial screening studies, the seeds of *Vigna radiata*, *Cicer arietinum* and *T. foenum-graecum*, each in 10 g batches, were washed well with water, treated with 0.1% (w/v) mercuric chloride for 1 min, and rinsed with deionized water. The seeds were soaked for 15 h prior to germination for 72 h in the dark in Petri dishes and uniformly irrigated with deionized water. For studies on the effect of mimosine, washed seeds of *T. foenum-graecum* were treated with 0.1% (w/v) mercuric chloride, and the thoroughly washed seeds were subjected to germination as described above. Periodically the seeds were irrigated uniformly with deionized water for the controls, while for studies on the effect of mimosine, individual batches of seeds in duplicates were irrigated with solutions containing mimosine in the range of concentrations from 0.1 to 2 mM. The period of germination and volume of water used for irrigation remained the same for all batches within an experiment.

Germinated seedlings from 10 g of dry seeds were ground manually using a mortar and pestle in a medium containing 0.22 M mannitol, 0.07 M sucrose, 1 mM EDTA, 0.3% (w/v) PVP, and 0.05% (w/v) BSA. The ratio of medium to wet weight of the seedlings was maintained at 2:1, and the pH was maintained at 7.2 by the addition of 0.1 N KOH. The homogenates were filtered through layers of cheesecloth and centrifuged for 20 min at 10000*g* in a Beckman model J2-21 centrifuge (Beckman Instruments Inc., U.K.) to sediment cell debris and mitochondria. The supernatant fractions were further centrifuged at 30000*g* for 1 h, and the resultant soluble fractions were used for enzyme assays.

For studies on the influence of mimosine on germinating *T. foenum-graecum*, the soluble fraction thus obtained from a typical batch was subjected to 80% saturation with solid ammonium sulfate (w/v), and the solution was stirred at 4 °C for 30 min. The precipitated protein was collected by centrifugation at 10000*g* for 20 min. The resultant pellet was suspended in 0.2 M citrate−phosphate buffer (pH 5.0) and dialyzed overnight at 4 °C against three changes of 0.002 M citrate-phosphate buffer (pH 5.0). This fraction was used for the estimation of glucose and enzyme assays. All operations were carried out at 4 °C, and each experiment was repeated three times in independent batches.

Enzyme Assays. Succinate dehydrogenase (SDH) activity was estimated by following the reduction of 2,6-DCPIP based on the method of Hatefi (1978). β-Glucosidase and β-galactosidase activities were assayed as per the method of Weber and Fink (1980) using PNPG and ONPG, respectively, as substrates. The reaction mixtures were incubated with the enzyme extracts for 15 min for all reactions except transglycosidation experiments, where the incubation period was 2 h. Units of enzyme activity for experiments excluding transglycosidation studies are expressed as micromoles of aglycone released in 15 min, at 37 °C, and measured at 420 nm for *p*-nitrophenol (PNP) and at 400 nm for *o*-nitrophenol (ONP) using a Hitachi model 220A dual beam spectrophotometer. For transglycosidation experiments, both aglycone released and the respective sugar levels were measured after incubation for 2 h. Levels of reducing sugars were measured by the method of Miller (1959). Protein determinations were made by the method of Lowry et al. (1951) using BSA as the standard. All analyses were done in duplicate for three independent experiments and values presented as the mean ± SD. Statistical analysis was done using a Student's *t*-test and significance appropriately indicated for comparisons with *P* values less than 0.05.

RESULTS AND DISCUSSION

Activities of β-Glycosidases during Germination of Legume Seeds. During the present study on the β-glycosidases of germinating seeds, the enzyme assays were carried out using *o*- and *p*-substituted phenyl glycosides which are routinely used for estimations of glycosidases by other researchers. Activities of β-glucosidase and β-galactosidase were initially assessed during germination of the legume seeds, *V. radiata*, *C. arietinum*, and *T. foenum-graecum*, and the results (Table 1) indicated a general increase in the specific activities of these enzymes in all the seedlings during germination from 24 to 72 h except for β-galactosidase of *V. radiata*. During germination, β-glucosidase activity increased by 45% in *C. arietinum*, whereas a 3-fold increase resulted in *V. radiata* in 72 h compared to the activities measured at 24 h. A maximal specific activity of about 300 units was noted in seedling fractions of both *V. radiata* and *C. arietinum* in 72 h, whereas the same level of activity was attained in *T. foenum-graecum* seedlings within 24 h and a further 3-fold increase was seen at 72 h with specific activity reaching 960 units.

The activity of β-galactosidase in *T. foenum-graecum* also was maximal among the seeds tested. When compared to the activity at 24 h (1020 U), there was a 3-fold increase (3750 U) at 72 h. Seedlings of *C. arietinum* with 740 units at 24 h increased by 55% to 1150 units in the same period. Specific activity in *V. radiata*, in contrast, was 931 units at 24 h, decreasing by almost 80% at 72 h. Such a change is indicative of a probable fast turnover of carbohydrates in the seedlings.

Table 1. Changes in Activities of β-Glucosidase and β-Galactosidase in Seeds during Germination[a]

	glycosidase activity (units/mg of protein)[b]					
	β-glucosidase			β-galactosidase		
	period of germination (h)[c]					
source	24	48	72	24	48	72
Vigna radiata	95 ± 2.85	106 ± 3.6	287 ± 3.61[a]	931 ± 15.2	640 ± 8	201 ± 5.3[a]
Cicer arietinum	210 ± 4.35	240 ± 4.4	300 ± 15.4[a]	740 ± 12.1	960 ± 9	1150 ± 12[a]
Trigonella foenum-graecum	312 ± 8.21	748 ± 13	960 ± 12[a]	1020 ± 18	2350 ± 22	3750 ± 25[a]

[a] Significance for values in the horizontal row for seedlings germinated for 72 h compared to 24 h with $P < 0.01$ are indicated by the superscript a. [b] Unit of activity for β-glucosidase refers to nmol of PNP and for β-galactosidase, nmol of ONP released in 15 min per milligram of protein at 37 °C. [c] Seeds germinated in the dark at 30 °C, and enzyme activities measured in the homogenates of the seedlings harvested after the indicated period of germination.

Table 2. Effect of Mimosine on the Growth of *T. foenum-graecum*[a]

	protein (mg/g)		succinate dehydrogenase (units)[c]	
system[b]	mitochondria	supernatant	per g tissue	per mg protein
control	3.8 ± 0.092	45.6 ± 3.34	28.5 ± 1.52	7.4 ± 0.611
mimosine added (μM)[c]				
50	3.8 ± 0.101	49.6 ± 4.08	28.5 ± 1.61	7.4 ± 0.562
100	4.2 ± 0.148	44.8 ± 3.09[c]	34.2 ± 2.32[c]	8.0 ± 0.613
200	4.1 ± 0.157	52.8 ± 3.86	39.0 ± 2.82[a]	10.4 ± 1.251[c]
400	3.7 ± 0.112	48.8 ± 3.72	18.0 ± 1.10[a]	4.3 ± 0.433[a]

[a] Significance for batches exposed to mimosine compared to control, within the same column, indicated by superscripts: a, $P < 0.01$; c, $P < 0.05$. [b] Seeds germinated in the dark for 72 h in control batch and with added mimosine in individual batches at the indicated levels. [c] Succinate dehydrogenase activity expressed as nmoles of DCPIP reduced/min.

In general, in all the seedlings, β-galactosidase activity was higher than that of β-glucosidase. During germination, the specific activities of both the enzymes were found to be maximal in *T. foenum-graecum* followed by *C. arietinum* in 72 h.

The total activity profile also indicated a similar trend of higher activity of β-galactosidase compared to β-glucosidase in all the seedlings, though with wide variations in the actual levels. β-Galactosidase activity in *V. radiata* was fairly high at 24 and 48 h (332 U), decreased by 50% in 72 h. In contrast, in *C. arietinum* a 2-fold increase from 24 to 48 h resulted in 370 units and remained at this level even at 72 h. In *T. foenum-graecum*, the activity of β-galactosidase remained relatively low up to 48 h (91−141 units), but increased at 72 h (465 units), attaining the highest level of activity measured among the seeds. A general increase in the levels of β-glucosidase was seen during 72 h germination of all the seeds, and maximal activity was present in *C. arietinum* at 72 h of germination (169 units).

β-Galactosidase is involved in the degradation of plant cell wall polysaccharides containing arabinogalactan side chains with a core structure of β-(1,4) linked D-galactopyranoside units (Matheson and Saimi, 1977; Ross et al., 1994). Changes in the level of enzyme activity were reported in germinating seeds of mung bean, lupin, and rice (Dey and del Campillo, 1982). During the ripening of fruits, both the soluble and cell bound forms of the enzyme were found to increase (Ali et al., 1995; Carey et al., 1995). The degradation of galactolipids involving β-galactosidases was linked to the changes arising in the structure of the membranes and related functions (Dey and del Campillo, 1982). Similarly, the presence of another membrane bound β-glucosidase in mustard seedlings specific for cholesteryl glucoside indicates its importance in lipid metabolism and related membrane function. With steryl β-glucosides as important components of plant membrane structures, glycosylation or deglycosylation of sterols in membranes was related to changes in lipid−lipid and lipid−protein interactions and related membrane organization and function (Kalinowska and Wojciechowski, 1978). In addition, β-glucosidase catalyzed hydrolysis of cinnamyl alcohol glucosides was reported to be involved in lignin biosynthesis (Hosel et al., 1978).

Effect of Mimosine on the Growth of *T. foenum-graecum*. The effect of the allelochemical mimosine on the germination of *T. foenum-graecum* was studied by exposing seedlings to mimosine in concentrations from 0.1 to 2 mM. Though mimosine, exclusively endogenous to the leaves of legumes *L. leucocephala* and *M. pudica*, is widely considered to be toxic to grazing cattle (Hegarty et al., 1976; Jones and Megarrity, 1983) and its metabolite 3,4-dihydroxypyridone was also found to be thyrotoxic to rats (Hallengrein et al., 1987), detailed studies on the metabolism of mimosine are still lacking. In the legume *T. foenum-graecum*, mimosine exposure in concentration from 50 to 200 μM, revealed no toxic effect on the seedlings germinated for 72 h. The seedlings grown in the presence of mimosine (100 μM) for a period of 72 h exhibited a visible enhancement in growth with an increase in the length of shoots (20%) accompanied by a 30% increase in the length of the roots as well as the fresh weight of the seedlings. The dry weight was increased by almost 50% (data not presented). Results on the levels of protein and the mitochondrial SDH are given in Table 2. Protein levels in the supernatant increased by about 20% whereas SDH was enhanced significantly by about 35−45% in terms of both specific activity and total activity measured. But when mimosine was present at a concentration of 400 μM during germination, the activity of SDH declined by about 35%, reflecting the apparent toxicity beyond this level of exposure.

Despite some reports on the degradation of mimosine to varying extent in the rumen of cattle by both protozoa and bacteria, Kudo et al. (1990) concluded that the presence and extent of tissue metabolism of mimosine still remains an open question. Present study indicates that the physiological action of mimosine is beneficial, promoting growth when tested in concentrations up to 0.2 mM in the legume plant *T. foenum-graecum*. Fur-

Table 3. β-Glucosidase and β-Galactosidase Activity in Seedlings of *T. foenum-graecum* As Influenced by Mimosine during Germination[a]

system[b]	enzyme activity (U/g of seeds)[c]	
	β-glucosidase	β-galactosidase
control	19.5 ± 0.81	65.0 ± 1.15
mimosine added (mM)		
0.1	29.5 ± 1.05[a]	86.8 ± 2.7[a]
0.2	26.1 ± 1.11[b]	78.7 ± 1.53[a]
0.4	21.5 ± 0.43[c]	64.3 ± 0.45
1	17.0 ± 0.62[c]	42.7 ± 2.66[a]
2	16.0 ± 0.26[b]	41.0 ± 1.93[a]

[a] Significance within the column for systems when compared to control indicated by superscripts: a, $P < 0.001$; b, $P < 0.01$; c, $P < 0.05$ [b] Seeds germinated in the dark for 72 h in the presence of mimosine at the indicated levels. [c] Unit of activity refers to micromoles of PNP released for β-glucosidase and micromoles of ONP released for β-galactosidase, in 15 min, per gram of dry weight seeds.

ther metabolic studies need to be performed with properly radiolabeled mimosine to understand the mechanism of its action.

Enhancement of Activities of β-Glycosidases in *T. foenum-graecum* on Exposure to Mimosine. The effect of mimosine exposure on the glycosidase activities of *T. foenum-graecum* during germination was studied. Activities of enzymes in the seedlings grown up to 72 h of germination in the presence of mimosine added at various concentrations are given in Table 3. Significant enhancement in the β-glucosidase resulted upon exposure to mimosine up to 54% at 0.1 mM and 34% at 0.2 mM. When mimosine was added during germination, in concentrations of 0.4 mM, the activity was almost the same as that of the control. Subsequently, addition of mimosine at 1 and 2 mM, lowered the enzyme activity significantly by 18% of that of the control seedlings. It should be noted that addition of mimosine at similar ranges of concentration in vitro did not affect the enzyme activity in any manner.

The measurement of β-galactosidase activity in seedlings exposed to mimosine during germination also revealed similar responses. β-Galactosidase activity increased by 35% at 0.1 mM mimosine compared to 22% at 0.2 mM mimosine. Upon exposure to mimosine, the extent of increase in β-galactosidase activity observed was less than that noted for β-glucosidase. Electrophoretic studies (PAGE) revealed that the ammonium sulfate fractions of the soluble extracts of the seedlings contained only one major zone of activity each for these two enzymes. This indicates only one major type of enzyme protein for each of the two glycosidases tested in the plant during the early germination phase. In contrast to the decrease of β-glucosidase by about 20% at 2 mM, the β-galactosidase activity declined significantly by 35% from 1 to 2 mM mimosine exposure. Activity of β-galactosidase was also unaffected when mimosine was added in vitro in the range of concentrations from 0.1 mM to 2 mM in the assay medium. Another study indicated that bacterial interaction with *Cyamopsis tetragonoloba*, a legume, resulted in the induction of α-galactosidase activity (Overbeeke et al., 1990).

Influence of Glycerol in the Medium on the Hydrolytic and Transglycosidating Activities of β-Glycosidases of *T. foenum-graecum*. Glycosidases transfer glycosyl residues to low molecular weight alcohols and water in a kinetically controlled reaction while hydrolysis is favored thermodynamically. Transfer

Table 4. Effect of Glycerol in the Medium on the Hydrolysis and Transglycosidation Activities of β-Glucosidase of Germinated *T. foenum-graecum*[a]

glycerol in the medium (M)	β-glucosidase activity (units/g of dry seeds)[b]			
	PNP released[c]		glucose released[d]	
	control	mimosine[e]	control	mimosine[e]
0	96 ± 2.11	115 ± 2.5[a]	89.6 ± 4.13	99.2 ± 4.82
0.05	118 ± 2.8[d]	131 ± 3.5[c]	89.6 ± 4.32	99.2 ± 5.14
0.1	128 ± 3.4[d]	147 ± 3.9[b]	99.2 ± 5.19	112.0 ± 5.4

[a] Significance within the horizontal rows for systems with mimosine added in vivo when compared to corresponding controls indicated by superscripts: a, $P < 0.001$; b, $P < 0.01$; c, $P < 0.02$. Significance within the vertical columns with glycerol in the reaction medium when compared to medium without glycerol indicated by superscripts: b and d, $P < 0.001$; c, $P < 0.01$. [b] Units expressed as μmoles of aglycone released in 2 h at 37 °C per gram of dry weight seeds. [c] Hydrolytic activity of β-glucosidase expressed as μmoles of PNP released per gram of dry seeds. [d] Micromoles of glucose released in 2 h at 37 °C per gram of dry seeds. [e] Seeds germinated in 0.1 mM mimosine for 72 h in the dark, and enzyme activity measured using crude enzyme protein.

reactions occur when substrate and acceptor concentrations are above operational K_M values. Alcohols act as better nucleophiles than water, resulting in an enhanced rate of glycoside cleavage with the formation of new glycosidic bonds (Sinnott, 1990). Since glycerol was found to be a better glucosyl acceptor among the various alcohols for transglycosylation studies (Umezurike, 1987), glycerol was chosen as the acceptor in the present study to measure the transglycosylation activities of the partially purified enzymes. The effect of glycerol in the assay medium on the hydrolytic activity of β-glucosidase was measured in mimosine-grown and control seedlings. Hydrolytic rate was enhanced by 23% at 0.05 M and 33% at 0.1 M glycerol (Table 4). The rate of hydrolysis was, in general, 15−20% higher in mimosine-grown seedlings compared to control seedlings irrespective of the levels of glycerol in the medium. Seedlings exposed to mimosine showed a significant enhancement by 30% in the hydrolytic rate at 0.1 M glycerol. In effect, in the presence of 0.1 M glycerol, seedlings with mimosine exposure contained 15% more activity than the control seedlings. Thus, exposure of seedlings to mimosine during growth resulted in an enhancement of β-glucosidase activity. Simultaneously, the transglycosidation rates were 24% at both 0.05 and 0.1 M glycerol in both fractions of the control and mimosine-exposed seedlings.

In the case of β-galactosidase activity, it was noted that the hydrolytic rate was, in general, 15−30% higher in mimosine-exposed groups compared to the control, an effect also noted with β-glucosidase activity (Table 5). The maximum hydrolytic rate was seen in the mimosine-grown seedlings compared to the control in the absence of glycerol. In the control seedlings, the rate of hydrolysis increased by 20% with added glycerol, while there was no pronounced effect in the fractions of seedlings exposed to mimosine. It is likely that mimosine exposure elicits maximal total activity of the β-galactosidase in the seedlings, which was observable in the medium without added glycerol, and therefore no further effect was brought about by added glycerol. This could be attributed to an increase due either to some effect on enhanced synthesis of protein or to enhanced catalytic activity by structural alterations of the enzyme. It is interesting to note that even in the absence of glycerol 24% transglycosidation activity was observed in the mimosine-grown seedlings. In both the control and mimosine-exposed seedlings, the extent of

Table 5. Effect of Glycerol in the Medium on the Hydrolysis and Transglycosidation Activities of β-Galactosidase of Germinated *T. foenum-graecum*[a]

glycerol in the medium (M)	β-galactosidase activity (units/g of dry seeds)[b]			
	ONP released[c]		galactose released[d]	
	control	mimosine[e]	control	mimosine[e]
0	239.2 ± 12.2	307.2 ± 16.7[a]	224.0 ± 11.3	234 ± 10.1
0.05	280.4 ± 13.6[e]	323.2 ± 16.6[c]	169.6 ± 10.6[d]	211 ± 10.0[b]
0.1	285.8 ± 14.1[e]	323.2 ± 17.1	140.8 ± 13.1[d]	157 ± 9.2[d]

[a] Significance within the horizontal rows for systems with mimosine added in vivo when compared to corresponding controls indicated by superscripts: a, $P < 0.01$; b, $P < 0.02$; c, $P < 0.05$. Significance within the vertical columns with glycerol in the reaction medium when compared to medium without glycerol indicated by superscripts: d, $P < 0.01$; e, $P < 0.05$. [b] Units expressed as μmoles of aglycone released in 2 h at 37 °C per g dry seeds. [c] Hydrolytic activity of β-galactosidase expressed as μmol of ONP released per g dry seeds. [d] μmol of galactose released in 2 h at 37 °C per g dry seeds. [e] Seeds germinated in the presence of 0.1 mM mimosine for 72 h in the dark and enzyme activity measured using crude enzyme protein.

Table 6. Effect of Mimosine on the Extent of Transglycosidation by Glycosidases of Germinated *T. foenum-graecum*

system	% transglycosidation		
	glycerol concentration (M)		
	0	0.05	0.1
β-glucosidase			
control seedlings	6.0	24.0	23.0
added mimosine during germination[a]	13.0	24.0	24.0
β-galactosidase			
control seedlings	6.0	39.5	50.7
added mimosine during germination[a]	23.0	34.7	51.4

[a] Seeds were germinated in the dark for 72 h in the presence of 0.1 mM mimosine.

transglycosidation varied from 35 to 40% at 0.05 M glycerol and to 50% at 0.1 M glycerol (Table 6). Enzymatic transglycosidation occurred with enhanced efficiency in the presence of methanol, glycerol, sucrose, and fructose (Umezurike, 1987). With increasing glycerol concentration in the presence of high concentrations of donor (>0.5 mM), a concomitant increase in the velocity was reported with β-glucosidase. Any glucosyl acceptor which is more nucleophilic than water and which can thus react with the solvent separated ion-pair would compete effectively with ion-pair collapse and thus favor transglycosidation at the expense of hydrolysis.

The present study demonstrated reasonably high levels of both β-glucosidase and β-galactosidase among various germinating seeds tested. Activity of β-galactosidase was higher than that of β-glucosidase in all the seeds. The allelochemical mimosine, contrary to its reported toxicity (Hegarty et al., 1976; Jones and Megarrity, 1983), was actually found to be beneficial to the growth of select species since the results reveal no adverse effects of mimosine on the growth of the germinating seeds of *T. foenum-graecum* when exposed to the allelochemical in concentrations up to 0.4 mM. Exposure to mimosine during germination of *T. foenum-graecum* resulted in enhancement of glycosidases. Our work indicates that the hydrolytic activity is enhanced upon mimosine exposure along with a concomitant increase in the extent of transglycosidation as well. Increase in the levels of both hydrolytic and transglycosidation activities of the glycosidases is attained by appropriate choice of the medium. More studies with purified enzymes and their activities toward different acceptors are required to assess further the nature of these glycosidases. Developments in the purification of glycosidases and their biotechnological applications in food industry and enzyme therapy of metabolic disorders have immense potential, and further studies in this direction are in progress.

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