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The β-glucosidase null phenotype in maize is due to a jacalin-related chimeric lectin and its lectin domain is responsible for β-glucosidase aggregation

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β-glucosidases catalyze hydrolysis of alkyl and aryl-β-D-glucosides as well as β-linked oligosaccharides**.** In maize, two isozymes of β-glucosidase (Glu1 and Glu2) have been identified and are thought to be involved in the defense against pathogens and herbivores by releasing toxic aglycones, such as hydroxamic acids, from their glucosides. The predominant hydroxamic acid glucoside in maize is 2-glucopyranosyl-4-hydroxy-7-methoxy-1, 4-benzoxazin-3-one (DIMBOAGlc), whose aglycone DIMBOA is the primary defense chemical against aphids and the European corn borer (*Ostrinia nubilalis*).

Certain maize inbred lines were reported to be devoid of β-glucosidases (Biochem. Genet. 15:383-394, 1977). These inbreds were originally thought to be homozygous for a null allele at the *glu1* locus. However, we found that they have β-glucosidase activity but the enzyme in them occurs as large insoluble complexes (Biochem. Genet. 28:319-336, 1990). Furthermore, we identified a 32 kD protein, referred to as β-glucosidase aggregating factor (BGAF), which specifically interacts with maize β-glucosidases to form insoluble complexes (Plant Physiol. 122:563-572, 2000). BGAF is a chimeric protein consisting of an N-terminal dirigent domain and a C-terminal Jacalin-related lectin (JRL) domain (J. Biol. Chem. 276:11895-11901, 2001; J. Biol. Chem. 282:7299-7311, 2007).

To gain an insight into lectin and β-glucosidase aggregating activities of BGAF, native BGAF free of β-glucosidase was isolated from maize null-line H95 and its recombinant version was produced in *E. coli*. Both native and recombinant BGAF agglutinated rabbit erythrocytes, and their hemagglutinating activity, was inhibited preferentially by galactose, lactose and glycoproteins containing N-acetyl-D-galactose- amine and N-acetyllactosamine residues (Table 1). BGAF binds to maize Glu1 even in the presence of saturating concentrations of galactose, indicating that the sugar and the β-glucosidase binding sites are distinct (Fig. 1). When kinetic constants (*K*m and *k*cat) for Glu1 were determined (using *para*-nitrophenyl-β-D-glucopyranoside as substrate) in the absence and presence of BGAF, no differences in the *K*m and *k*cat values were observed, suggesting that BGAF does not have any effect on β-glucosidase activity. Of the two domains in BGAF (expressed separately in *E. coli*), only the JRL domain was able to retard the mobility of Glu1 on the native gel (Fig. 2), clearly indicating that the binding site(s) for β-glucosidase are in the JRL domain. Replacing the JRL domain of a BGAF homolog from sorghum (non-binder) with the JRL domain of maize BGAF (binder) resulted in a chimera with high affinity for maize Glu1 (Fig. 3),

Table 1. Inhibition of hemagglutination activity[a] of native BGAF, recombinant BGAF and BGAF-Glu1 complex by saccharides and glycoproteins.

	Native BGAF			
Saccharides	MIC mM	rP[b]	rP rBGAF	rP BGAF-Glu1 complex[c]
Galactose	7.8	1	0.50	0.25
Methyl-α-D-galactopyranoside	3.9	2	1	1
Methyl-β-D-galactopyranoside	31.2	0.25	0.25	0.12
Galactosamine	15.6	0.50	0.50	0.12
N-acetyl-D-galactosamine	31.2	0.25	0.25	0.62
N-acetylneuraminic acid	31.2	0.25	0.25	NI
Mannose	15.6	0.50	0.50	NI
Methyl-α-D-mannopyranoside	7.8	1	1	NI
Lactose	1.9	4.1	4.1	1
N-acetyllactosamine	3.9	2	2	1
Raffinose	1.9	4.1	4.1	2
Stachyose	7.8	1	1	ND[e]
Glycoproteins				
Ovalbumin	0.0013	6000	6000	6000
Horseradish peroxidase	NI	-	NI	NI
Asialofetuin	<0.001	>7800	>7800	>7800
PSM	0.001	7800	7800	7800

[a]Inhibition of hemagglutination was assayed by serially diluting saccharide and glycoprotein solutions in the microtiter wells, followed by the addition of 4 units of the lectin, and then the addition of a 2% suspension of trypsinized rabbit erythrocytes after 30 min. The lowest concentration of saccharides or glycoproteins that visibly decreased the extent of agglutination was defined as the minimum inhibitory concentration (MIC). The MIC values were obtained from two independent measurements.

[b]Relative potency, relative to D-galactose with native BGAF.

[c]Isolated from the maize H95 "null" line.

[d]NI, No detectable inhibition.

[e]ND, not determined.

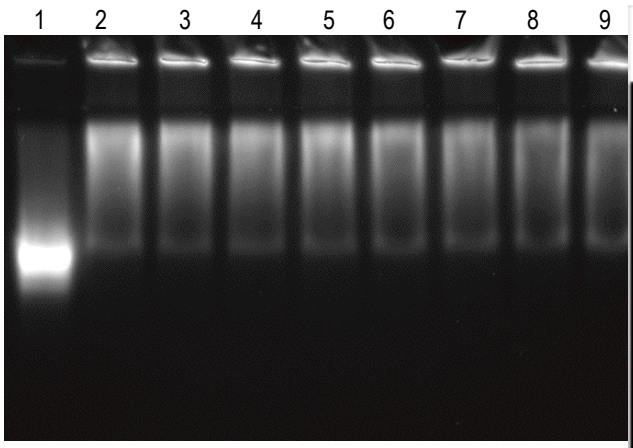

Figure 1. Gel-shift assay to detect binding of BGAF to maize Glu1 in the presence of increasing concentrations of galactose. rBGAF (14 nM) was incubated with increasing concentrations of galactose (0-125 mM) at room temperature for 1 h in PBS, pH 7.4. Following addition of Glu1 (58 nM) and incubation for 2 h, aliquots were withdrawn and electrophoresed on an 8% native gel. β-glucosidase activity was detected by staining with 4-methylumbelliferyl-β-D-glucopyranoside (4-MUG). Lane 1, Glu1; lane 2, BGAF + Glu1 with no sugar, lanes 3-9, rBGAF plus Glu1 in the presence of 1.9-125 mM galactose. In the presence of BGAF, β-glucosidase activity zones (smearing) detected with 4-MUGlc are retarded in a region extending from the top of the resolving gel to the sample well in the stacking gel (lane 2). Note the same pattern is observed even in the presence of saturating concentrations of galactose (lanes 3-9), indicating that carbohydrate and β-glucosidase binding sites are distinct.

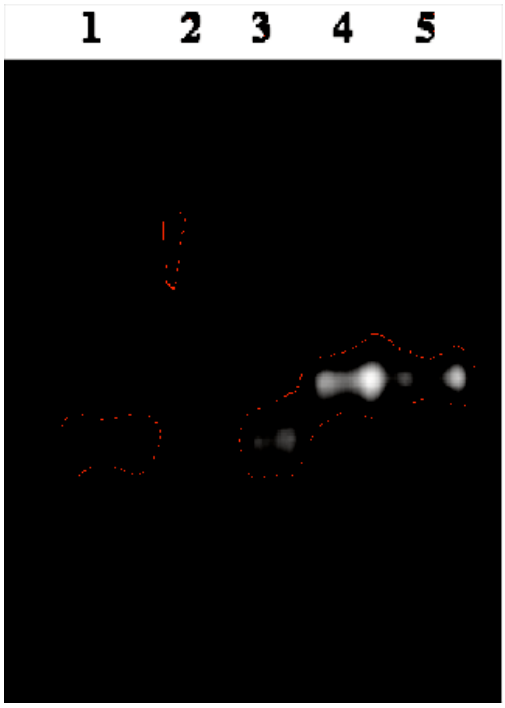

Figure 2. Gel-shift assay to detect binding of dirigent and the JRL domain to maize Glu1. Glu1 (58 nM; lane 1) was incubated with rBGAF (14 nM; lane 2), dirigent (100 nM; lane 3), JRL (100 nM; lane 4) and dirigent plus JRL (100 nM each; lane 5) at room temperature for 2 h in PBS, pH 7.4. Electrophoresis and staining was done as described in the legend for Figure 1. Note that only the JRL domain retards the mobility of Glu1 (lanes 4 and 5).

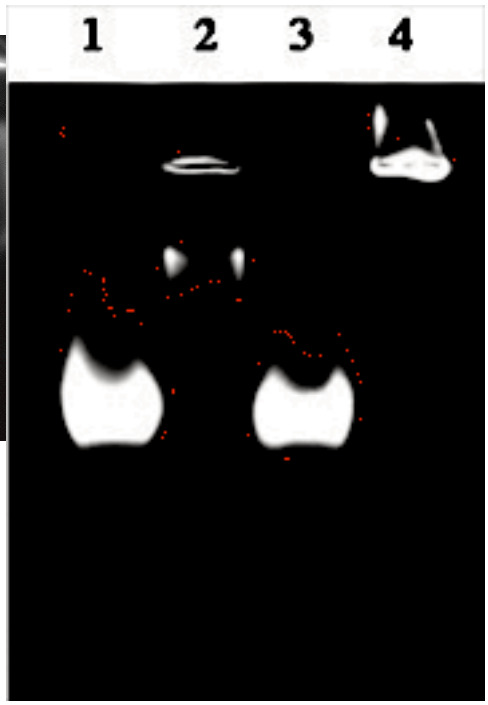

Figure 3. Gel-shift assay to detect binding of chimeric BGAF (consisting of the dirigent domain of the BGAF homolog from sorghum and the JRL domain of maize BGAF) to maize Glu1. Glu1 (58 nM, lane 1) was incubated with 200 nM maize rBGAF (lane 2), sorghum rBGAF (lane 3) and chimeric BGAF (lane 4) at room temperature in PBS, pH 7.4. Electrophoresis and staining was done as described in the legend for Figure 1. Sorghum rBGAF itself does not bind Glu1 (lane 3), whereas chimeric BGAF formed complexes with Glu1, which are retained in the sample well (lane 4).

indicating that the JRL domain is responsible for β-glucosidase aggregation and hence the null-phenotype in maize. The facts that BGAF had no adverse effect on β-glucosidase activity and that the sugar-binding site is free in the complex to interact with sugars suggest that the BGAF-β-glucosidase complex might protect maize against pests by binding to glycoproteins and producing a local burst of DIMBOA in the oral cavity or by damaging the peritrophic membrane lining the midgut of insect larvae.