COMPARATIVE SUSCEPTIBILITY OF STARCH GRANULES OF DOUBLE- AND TRIPLE-MUTANTS CONTAINING *AMYLOSE-EXTENDER*, *WAXY*, *SUGARY-1*, *SUGARY-2* AND *DULL* GENES OF MAIZE INBRED OH43 (*ZEA MAYS* L.) TO AMYLASE¹

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Summary Starch granules were prepared from 14 double- and 26 triple-mutants containing *amylose-extender* (*ae*), 14 double- and 18 triple-mutants containing *waxy* (*wx*), 15 double- and 20 triple-mutants containing *sugary-1* (*su*₁), 13 double- and 23 triple-mutants containing *sugary-2* (*su*₂), and 14 double- and 19 triple-mutants containing *dull* (*du*) of maize inbred Oh43 (*Zea mays* L.). The relative susceptibilities of these starch granules to fungal glucoamylase were determined and the starch granules were examined by scanning electron microscopy. A commercial normal maize starch was used as a control. Starch granules of the double- and triple-mutants containing *su*₁ and *su*₂ were digested two to eight times faster than normal. The *ae* gene reduced susceptibility and seems to be epistatic to *su*₁ and *su*₂. Starch granules of the double- and triple-mutants containing *wx* were digested about two times faster than normal and those containing *shrunk-2* (*sh*₂) were digested 1.2 to eight times faster than normal. Starch granules of triple-mutant combinations with *opaque-2* (*o*₂) showed digestion properties which were comparable to those of their respective nonopaque double-mutant counterpart.

Keywords maize starch granules, susceptibility to fungal amylase, maize mutants

Starch granules of normal maize are more susceptible to the action of amylases than those of tubers and roots (1, 2). Maize endosperm mutants, however, induce exceedingly wide variations in the relative susceptibility of starch granules to

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amylases (2-4). We prepared starch granules from kernels of maize inbred Oh43 (*Zea mays* L.) (2) and from four inbred lines of maize B37, C103, W64A and Oh43 (5, 6) and showed that: 1) Starch granules of the *amylose-extender* (*ae*) mutant were more resistant to the action of amylases than those of the normal counterpart. 2) Starch granules of the *sugary-1* (*su*₁) and *sugary-2* (*su*₂) mutants were digested by amylases two to three times faster than normal. 3) Starch granules of *brittle-1* (*bt*₁), *brittle-2* (*bt*₂), *opaque-1* (*o*₁), *shrunk-2* (*sh*₂) and *waxy* (*wx*) mutants tended to be digested faster than normal. 4) The *opaque-2* (*o*₂) mutant, which improves the nutritional quality of endosperm protein, in general, did not change the relative susceptibility of starch granules to digestion by amylase. 5) Starch granules of double-mutant combinations of starch-modifying genes with *o*₂ exhibited digestion properties similar to their respective nonopaque single-mutant counterpart.

This paper presents the relative susceptibility and extent of enzyme degradation in yet other endosperm mutant combinations which have been synthesized in the maize inbred Oh43, but not reported, and confirms the results of a few genetic effects and interactions by these combinations on the relative susceptibility and extent of enzyme degradation. We prepared starch granules from kernels of near isogenic conversions of 14 double- and 26 triple-mutants containing *ae*, 14 double- and 18 triple-mutants containing *wx*, 15 double- and 20 triple-mutants containing *su*₁, 13 double- and 23 triple-mutants containing *su*₂ and 14 double- and 19 triple-mutants containing the *dull* (*du*) gene and compared the relative susceptibility of these starch granules to fungal glucoamylase.

MATERIALS AND METHODS

Maize mutants. Mature kernels of several endosperm mutants (116 genotypes) containing 2- and 3-way combinations of the following endosperm mutants in the inbred Oh43 background were used: *ae*, *du*, *wx*, *su*₁, *su*₁st, *su*₂, *bt*₁, *bt*₂, *floury-1* (*fl*₁), *floury-2* (*fl*₂), *soft starch* (*h*), *o*₂, *shrunk-1* (*sh*₁), *sh*₂ and *shrunk-4* (*sh*₄) (see Table 1). The materials were grown at the Purdue Agronomy Farm. Kernels of a commercial dent-maize were kindly supplied by Mr. T. Miwa, Central Research Laboratories, Nihon Shokuhin Kako, Co., Ltd., Mishima, Japan.

Starch granules. Starch granules were prepared by a method reported previously (2).

Sources of enzymes. Sources of *Rhizopus* glucoamylase, glucose oxidase and peroxidase were described earlier (2).

Analytical methods. Susceptibility of starch granules to glucoamylase was studied by a method reported previously (2). Percent degradation shown in Figs. 1, 2, 3 and 6 were the mean values of two determinations; however, those in Figs. 4 and 5 were the values of a single run. Owing to the wide variations in susceptibility to the amylase of starch granules of each one of the double- and triple-mutants containing *ae*, *wx*, *su*₂, *su*₁ and *du*, the duration of enzymatic reactions were 2 hr for Figs. 1 and 2, 1 hr for Figs. 3, 5 (shaded bars) and 6 and 0.5 hr for Figs. 4 and 5 (open bars),

respectively. The data (Table 1) were expressed on the basis of the relative percentage of the commercial normal control enzyme degradation determined as a percentage glucose equivalent.

Preparation of starch granules attacked by glucoamylase, specimen mounting and scanning electron microscopy. Starch granules were attacked by glucoamylase under the conditions reported previously (2). Procedures for specimen mounting and scanning electron microscopic observations were followed as described earlier (5) except that starch granules were coated with 100–200 Å of gold layers by ion sputtering by the use of an Ion Coater IB-3 of Eiko Engineering Co., Ltd.

RESULTS

*Starch granule susceptibility to glucoamylase of double- and triple-mutants containing *ae* gene of the Oh43 maize*

Figure 1 and Table 1 show that among the starch granules of 14 double-mutants containing *ae*, those of *ae sh₂* and *ae wx* tended to be digested faster than normal, while those granules of the remaining mutant combinations were digested at a similar rate to, or slower than normal.

Starch granules of seven triple-mutants containing *ae*, which were digested faster than normal, showed that *ae du wx* and *ae su₂ sh₂* were fastest followed by *ae*

Fig. 1. Digestion of starch granules of double-mutants containing *amylose-extender* (*ae*) of maize inbred Oh43 and a commercial normal maize attacked by *Rhizopus* glucoamylase. Dotted bar, normal maize; open bars, endosperm mutants.

Table 1. Relative susceptibility^a to glucoamylase of starch granules of several double- and triple-mutant combinations with *amylose-extender*, *dull*, *waxy*, *sugary-1* or *sugary-2* gene of inbred maize Oh43.

	<i>ae</i>	<i>du</i>	<i>wx</i>	<i>su</i> ₁	<i>su</i> ₂
<i>du</i>	82.7	—	—	—	—
<i>wx</i>	125.0	273	—	—	—
<i>su</i> ₁	65.9	321	232	—	—
<i>su</i> ₁ st	51.4	215	151	—	—
<i>su</i> ₂	39.4	227	344	312	—
<i>bt</i> ₁	89.0	108	213	627	431
<i>bt</i> ₂	100.0	97	236	—	563
<i>fl</i> ₁	26.9	91	168	304	342
<i>fl</i> ₂	23.3	101	195	437	277
<i>h</i>	32.9	106	156	778	352
<i>o</i> ₂	35.3	89	234	373	353
<i>sh</i> ₁	44.2	107	199	390	401
<i>sh</i> ₂	119.0	122	250	—	514
<i>sh</i> ₄	71.9	142	217	455	328
<i>du fl</i> ₂	79.1	—	—	—	—
<i>du o</i> ₂	91.6	—	—	—	—
<i>du sh</i> ₁	58.6	—	—	—	—
<i>du sh</i> ₂	113.0	—	—	—	—
<i>du su</i> ₁	183.0	—	—	—	—
<i>du su</i> ₂	54.9	—	—	—	—
<i>du wx</i>	267.0	—	—	—	—
<i>o</i> ₂ <i>fl</i> ₂	23.1	—	193	368	—
<i>o</i> ₂ <i>sh</i> ₂	163.0	—	—	504	433
<i>sh</i> ₁ <i>fl</i> ₂	52.2	—	254	600	516
<i>sh</i> ₁ <i>o</i> ₂	69.5	113	255	—	487
<i>su</i> ₁ <i>fl</i> ₂	72.7	292	—	—	—
<i>su</i> ₁ <i>o</i> ₂	78.7	—	—	—	—
<i>su</i> ₁ <i>sh</i> ₁	73.1	318	—	—	—
<i>su</i> ₁ <i>sh</i> ₂	169.0	—	—	—	—
<i>su</i> ₁ <i>su</i> ₂	69.9	299	—	—	—
<i>su</i> ₁ <i>wx</i>	169.0	—	—	—	—
<i>su</i> ₂ <i>fl</i> ₂	41.4	303	—	545	—
<i>su</i> ₂ <i>o</i> ₂	41.4	229	—	573	—
<i>su</i> ₂ <i>sh</i> ₁	77.1	280	—	788	—
<i>su</i> ₂ <i>sh</i> ₂	258.0	275	—	788	—
<i>su</i> ₂ <i>wx</i>	81.1	517	—	—	—
<i>wx fl</i> ₂	34.7	—	—	541	—
<i>wx o</i> ₂	81.1	321	—	296	602
<i>wx sh</i> ₁	95.6	238	—	433	835
<i>wx sh</i> ₂	198.0	227	—	—	839
(normal)	24.9 ± 7 ^b	14.4 ^c , 14.2 ^c	14.3 ± 0.2 ^b	8.3 ^c	5.1 ^c

^a Susceptibility of normal maize starch granules=100; ^b % Degradation, mean ± standard deviation, *n*=3; ^c % Degradation, single determination.

Fig. 2.

Fig. 3.

Fig. 2. Digestion of starch granules of triple-mutants containing *ae* of the Oh43 and the normal maize attacked by *Rhizopus* glucoamylase. See the legend to Fig. 1.

Fig. 3. Digestion of starch granules of double- and triple-mutants containing *waxy* (*wx*) of the Oh43 and the normal maize attacked by *Rhizopus* glucoamylase. See the legend to Fig. 1.

wx sh₂, *ae du su₁*, *ae su₁ wx*, *ae su₁ sh₂* and *ae o₂ sh₂* (Fig. 2 and Table 1). Granules of the *ae du wx* and *ae su₂ sh₂* mutants were digested 2.7 and 2.6 times faster, respectively, than normal. Starch granules of 19 triple-mutants containing *ae* were digested at a similar rate to, or slower than normal.

Starch granule susceptibility to glucoamylase of double- and triple-mutants containing *wx* of the Oh43

Starch granules of 14 double- and three triple-mutants containing *wx* were

Fig. 4. Digestion of starch granules of double- and triple-mutants containing *sugary-1* (*su*₁) of the Oh43 and the normal maize attacked by *Rhizopus* glucoamylase. See the legend to Fig. 1.

digested 1.3 to 3.4 times faster than normal (Fig. 3 and Table 1). The *wx su*₂ mutant granules were 3 to 4 times more susceptible than normal.

*Starch granule susceptibility to glucoamylase of double- and triple-mutants containing su*₁ *of the Oh43*

Figure 4 and Table 1 show that starch granules of 10 of the double- and 10 of the triple-mutants containing *su*₁ were digested 3.0 to 7.9 times faster than normal. Those starch granules of *su*₁ *su*₂ *sh*₁, *su*₁ *su*₂ *sh*₂ and *su*₁ *h* were digested nearly eight times more rapidly than normal.

*Starch granule susceptibility to glucoamylase of double- and triple-mutants containing su*₂ *of the Oh43*

Starch granules of 11 double- and 16 triple-mutants containing *su*₂ were digested 2.8 to 8.4 times faster than normal (Fig. 5 and Table 1). Those granules of the *su*₂ *wx sh*₂, *su*₂ *wx sh*₁, *su*₁ *su*₂ *sh*₁ and *su*₁ *su*₂ *sh*₂ triple-mutants were digested the

Fig. 5. Digestion of starch granules of double- and triple-mutants containing *sugary-2* (*su*₂) of the Oh43 and the normal maize attacked by *Rhizopus* glucoamylase. Dotted bars, normal maize; open bars, endosperm mutants (duration of reaction, 0.5 hr), shaded bars: endosperm mutants (duration of reaction, 1 hr).

most rapidly, while *su*₂ *bt*₂ and *su*₂ *sh*₂ were the most rapidly digested of the *su*₂ double-mutants.

Starch granule susceptibility to glucoamylase of double- and triple-mutants containing du of the Oh43

Figure 6 and Table 1 show that starch granules of six double- and 13 triple-mutants containing *du* were digested faster than normal. Granules of *du su*₂ *wx* mutant were digested 5.2 times faster than normal and the *du su*₁ double-mutant was digested 3.2 times faster than normal. Granules of seven double- and one triple-mutant containing *du* were digested at a similar rate to those of normal maize.

SEM observations of starch granules attacked by glucoamylase

SEM observation of starch granules of the double- and triple-mutants containing *wx* and *du* with the exception of *ae wx*, *ae du*, *ae wx fl*₂, *ae du sh*₁, *ae su*₁ *wx*, *ae du su*₁, *du su*₁, *du su*₁ *fl*₂, *du su*₂, *du su*₂ *o*₂, *du su*₂ *sh*₁, *du su*₂ *sh*₂, *su*₂ *wx o*₂, *su*₂ *wx sh*₁ and *su*₂ *wx sh*₂ showed, in general, numerous pinholes on the surface layers

Fig. 6. Digestion of starch granules of double- and triple-mutants containing *dull* (*du*) of the Oh43 and the normal maize attacked by *Rhizopus* glucoamylase. See the legend to Fig. 1.

after enzymatic reaction and pores that penetrated into the inner layers of the granules during enzymatic attack (Figs. 7b–7g). On the other hand, starch granules of the double- and triple-mutants containing *ae*, which had a lower susceptibility to amylase, showed shapes and surfaces similar to those of the native undegraded granules after enzymatic reaction (Fig. 7h). However, starch granules of the double- and triple-mutants containing *ae*, which had a similar or greater susceptibility to

Fig. 7. Scanning electron photomicrographs of starch granules of several endosperm mutants of the Oh43 and the normal maize attacked by *Rhizopus* glucoamylase.

a, normal (14.6); b, wx o₂ (34.1); c, wx bt₂ (34.5); d, wx su₁ (33.9); e, wx su₁st (22.0); f, wx su₂ (50.2); g, du o₂ (12.7); h, ae o₂ (8.8); i, ae bt₁ (22.3); j, ae bt₂ (25.0); k, ae sh₂ (29.6); l, ae o₂ sh₂ (40.7); m, ae su₂ sh₁ (19.2); n, su₁ o₂ (19.0) and o, su₂ o₂ (29.3). Numbers in parentheses are % degradation.

Fig. 7. (continued)

Fig. 7. (to be continued)

amylase compared to normal (Figs. 1 and 2), showed pinholes on the surface layers after enzymatic attack (Figs. 7i–7m). There was no clear relationship between the relative susceptibility of starch granules to glucoamylase and the enzymatic erosion observed by SEM on the surface and the inner portions of granules of either the *su*₁ or *su*₂ double- and triple-mutant combinations (Figs. 7d–7f and 7m–7o).

DISCUSSION

As stated by SMITH and LINEBACK (7), it is apparent that the extents and patterns of degradation of native starch granules are functions of the source of the starch and type of amylase (1, 2, 8). This is true for starch granules of endosperm mutants of maize (2, 5, 6).

Among single endosperm mutants, starch granules of the *su*₁ and *su*₂ mutants were digested by amylases two to three times faster than normal. Starch granules of the *wx*, *sh*₂, *bt*₁ and *bt*₂ mutants tended to be digested faster than normal (2–6).

Starch granules of the double- and triple-mutants containing *su*₁ and *su*₂ were digested two to eight times faster than normal (Figs. 5 and 6 and Table 1). The *su*₂, *bt*₁ and *h* and combinations of *sh*₁ *fl*₂, *su*₂ *fl*₂, *su*₂ *o*₂, *su*₂ *sh*₁, *su*₂ *sh*₂, *wx* *fl*₂ and *o*₂ *sh*₂ further enhanced the starch granule susceptibility of the double- and triple-mutants containing *su*₁. The *bt*₂ and *sh*₂ and combinations of *wx* *o*₂, *wx* *sh*₁, *wx* *sh*₂ and *sh*₁ *fl*₂ enhanced the effect of *su*₂. However, the *ae* gene (2) seems to be epistatic to *su*₁ and *su*₂, which resulted in starch granules that were less susceptible to glucoamylase in the double-mutants, *ae su*₁ and *ae su*₂ (Fig. 1) and the triple-mutants, *ae su*₂ *fl*₂, *ae su*₂ *o*₂ and *ae du su*₂ (Fig. 2).

Starch granules of the double- and triple-mutants containing *wx* were digested about two times faster than normal (Fig. 3 and Table 1). Recently, YAMADA and TAKI (9) and BOYER *et al.* (10) reported that *ae wx* starch consists of nearly 100% amylopectin with an anomalous fine structure. In this connection, elucidation of the relationship between the relative susceptibility to glucoamylase of starch granules of the *ae du wx*, *ae wx sh*₂, *ae su*₁ *wx*, *ae wx*, *ae wx sh*₁, *ae wx o*₂, *ae su*₂ *wx* and *ae wx fl*₂ mutants (Table 1) and the structural characteristics of these starches is needed. Further studies are in progress.

Starch granules of the double- and triple-mutants containing *sh*₂ were digested 1.2 to 8 times faster than normal (Table 1). The interactions of *sh*₂ with *wx*, *su*₂, *su*₁ and *du* genes enhanced the effect of *sh*₂ on starch granule susceptibility of the double- and triple-mutants containing *sh*₂.

Starch granules of double-mutant combinations with *o*₂ showed digestion properties that were comparable to their respective nonopaque single-mutant counterpart (2, 5, 6). The same general situation was observed for triple-mutant combinations with *o*₂ and their respective nonopaque double-mutant counterparts (Table 1).

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