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 AMYLASE

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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 (su1), 13 double- and 23 triple-mutants containing sugary-2 (su2), 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 su1 and su2 were digested two to eight times faster than normal. The ae gene reduced susceptibility and seems to be epistatic to su1 and su2. Starch granules of the double- and triple-mutants containing wx were digested about two times faster than normal and those containing shrunken-2 (sh2) were digested 1.2 to eight times faster than normal. Starch granules of triple-mutant combinations with opaque-2 (O2) 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

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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 amylase-extender (ae) mutant were more resistant to the action of amylases than those of the normal counterpart. 2) Starch granules of the sugary-1 (su1) and sugary-2 (su2) mutants were digested by amylases two to three times faster than normal. 3) Starch granules of brittle-1 (bt1), brittle-2 (bt2), opaque-1 (o1), shrunken-2 (sh2) and waxy (wx) mutants tended to be digested faster than normal. 4) The opaque-2 (o2) 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 o2 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 su1, 13 double- and 23 triple-mutants containing su2 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, su1, su1st, su2, bt1, bt2, floury-1 (fl1), floury-2 (fl2), soft starch (h), o2, shrunken-1 (sh1), sh2 and shrunken-4 (sh4) (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, su2, su1 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 spattering 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](image-url)

*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* 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.

|                | ae  | du  | wx  | su₁ | su₂ |
|----------------|-----|-----|-----|-----|-----|
| du             | 82.7| —   | —   | —   | —   |
| wx             | 125.0| 273 | —   | —   | —   |
| su₁            | 65.9| 321 | 232 | —   | —   |
| su₂⁻           | 51.4| 215 | 151 | —   | —   |
| su₃            | 39.4| 227 | 344 | 312 | —   |
| bt₁            | 89.0| 108 | 213 | 627 | 431 |
| bt₂            | 100.0| 97  | 236 | —   | 563 |
| fl₁            | 26.9| 91  | 168 | 304 | 342 |
| fl₂            | 23.3| 101 | 195 | 437 | 277 |
| h              | 32.9| 106 | 156 | 778 | 352 |
| o₂             | 35.3| 89  | 234 | 373 | 353 |
| sh₁            | 44.2| 107 | 199 | 390 | 401 |
| sh₂            | 119.0| 122 | 250 | —   | 514 |
| sh₃            | 71.9| 142 | 217 | 455 | 328 |
| du fl₁         | 79.1| —   | —   | —   | —   |
| du o₂          | 91.6| —   | —   | —   | —   |
| du sh₁         | 58.6| —   | —   | —   | —   |
| du sh₂         | 113.0| —   | —   | —   | —   |
| du su₁         | 183.0| —   | —   | —   | —   |
| du su₂         | 54.9| —   | —   | —   | —   |
| du wx          | 267.0| —   | —   | —   | —   |
| o₂ fl₂         | 23.1| —   | 193 | 368 | —   |
| o₂ sh₁         | 163.0| —   | —   | 504 | 433 |
| sh₁ fl₂        | 52.2| —   | 254 | 600 | 516 |
| sh₁ o₂         | 69.5| 113 | 255 | —   | 487 |
| su₁ fl₂        | 72.7| 292 | —   | —   | —   |
| su₁ o₂         | 78.7| —   | —   | —   | —   |
| su₁ sh₁        | 73.1| 318 | —   | —   | —   |
| su₁ su₂        | 169.0| —   | —   | —   | —   |
| su₁ wx         | 69.9| 299 | —   | —   | —   |
| su₂ fl₁        | 41.4| 303 | —   | 545 | —   |
| su₂ o₂         | 41.4| 229 | —   | 573 | —   |
| su₂ sh₁        | 77.1| 280 | —   | 788 | —   |
| su₂ sh₂        | 258.0| 275 | —   | 788 | —   |
| su₂ wx         | 81.1| 517 | —   | —   | —   |
| wx fl₂         | 34.7| —   | —   | 541 | —   |
| wx o₂          | 81.1| 321 | —   | 296 | 602 |
| wx sh₁         | 95.6| 238 | —   | 433 | 835 |
| wx sh₂         | 198.0| 227 | —   | —   | 839 |

(normal 24.9 ± 7b 14.4c 14.2c 14.3 ± 0.2b 8.3c 5.1c)

* Susceptibility of normal maize starch granules = 100; b % Degradation, mean ± standard deviation, n = 3; c % Degradation, single determination.
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
digested 1.3 to 3.4 times faster than normal (Fig. 3 and Table 1). The wx su2 mutant granules were 3 to 4 times more susceptible than normal.

Starch granule susceptibility to glucoamylase of double- and triple-mutants containing su1 of the Oh43

Figure 4 and Table 1 show that starch granules of 10 of the double- and 10 of the triple-mutants containing su1 were digested 3.0 to 7.9 times faster than normal. Those starch granules of su1 su2 sh1, su1 su2 sh2 and su1 h were digested nearly eight times more rapidly than normal.

Starch granule susceptibility to glucoamylase of double- and triple-mutants containing su2 of the Oh43

Starch granules of 11 double- and 16 triple-mutants containing su2 were digested 2.8 to 8.4 times faster than normal (Fig. 5 and Table 1). Those granules of the su2 wx sh2, su2 wx sh1, su1 su2 sh1 and su1 su2 sh2 triple-mutants were digested the
most rapidly, while \textit{su}_2 \textit{bt}_2 and \textit{su}_2 \textit{sh}_2 were the most rapidly digested of the \textit{su}_2 double-mutants.

**Starch granule susceptibility to glucoamylase of double- and triple-mutants containing \textit{du} of the \textit{Oh43}**

Figure 6 and Table 1 show that starch granules of six double- and 13 triple-mutants containing \textit{du} were digested faster than normal. Granules of \textit{du} \textit{su}_2 \textit{wx} mutant were digested 5.2 times faster than normal and the \textit{du} \textit{su}_1 double-mutant was digested 3.2 times faster than normal. Granules of seven double- and one triple-mutant containing \textit{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 \textit{wx} and \textit{du} with the exception of \textit{ae \textit{wx}}, \textit{ae \textit{du}}, \textit{ae \textit{wx} fl}_2, \textit{ae \textit{du} sh}_1, \textit{ae \textit{su}_1 \textit{wx}}, \textit{ae \textit{du} \textit{su}_1}, \textit{du \textit{su}_1}, \textit{du \textit{su}_1 fl}_2, \textit{du \textit{su}_2}, \textit{du \textit{su}_2 o}_2, \textit{du \textit{su}_2 sh}_1, \textit{du \textit{su}_2 sh}_2, \textit{su}_2 \textit{wx o}_2, \textit{su}_2 \textit{wx sh}_1 and \textit{su}_2 \textit{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.

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

| Mutant | Degradation |
|--------|-------------|
| *dum*  |             |
| *dumt* |             |
| *dum*  |             |
| *duh*  |             |
| *dush* |             |
| *duh*  |             |
| *dush* |             |
| *dusn* |             |
| *dusu* |             |
| *dusw* |             |
| *dusx* |             |
| *dusu* |             |
| *dusw* |             |

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. (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 su1 or su2 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 su1 and su2 mutants were digested by amylases two to three times faster than normal. Starch granules of the wx, sh2, bt1 and bt2 mutants tended to be digested faster than normal (2–6).

Starch granules of the double- and triple-mutants containing su1 and su2 were digested two to eight times faster than normal (Figs. 5 and 6 and Table 1). The su2, bt1 and h and combinations of sh1 fl2, su2 fl2, su2 o2, su2 sh1, su2 sh2, wx fl2 and o2 sh2 further enhanced the starch granule susceptibility of the double- and triple-mutants containing su1. The bt2 and sh2 and combinations of wx o2, wx sh1, wx sh2 and sh1 fl2 enhanced the effect of su2. However, the ae gene (2) seems to be epistatic to su1 and su2, which resulted in starch granules that were less susceptible to glucoamylase in the double-mutants, ae su1 and ae su2 (Fig. 1) and the triple-mutants, ae su2 fl2, ae su2 o2 and ae du su2 (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, YAMAUDA 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 sh2, ae su1 wx, ae wx, ae wx sh1, ae wx o2, ae su2 wx and ae wx fl2 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 sh2 were digested 1.2 to 8 times faster than normal (Table 1). The interactions of sh2 with wx, su2, su1 and du genes enhanced the effect of sh2 on starch granule susceptibility of the double- and triple-mutants containing sh2.

Starch granules of double-mutant combinations with o2 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 o2 and their respective nonopaque double-mutant counterparts (Table 1).
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