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Dependence of Seed Vigor during Germination on Carbohydrate Source in Endosperm Mutants of Maize[1]

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ABSTRACT

Differences in seed vigor of four genotypes of maize (*Zea mays* L.), *brittle-1* (*bt1*), *shrunken-2* (*sh2*), *sugary* (*su*), and *normal*, in an isogenic background, were investigated. Excised whole embryos and axes were grown on Murashige and Skoog (MS) media containing various carbohydrate sources. Of the four genotypes examined, *sh2* seeds had the lowest vigor, especially under germination stress conditions. Embryo dry weights of *sh2* were less than *su* and *normal* but equal to *bt1* and made up nearly 25% of the whole seed weight. The *sh2* seeds and whole embryos had low starch levels compared with the other three genotypes. Sugar levels were comparable in the three endosperm mutants, which were two times higher than *normal*. Optimum growth of excised embryos of all genotypes was obtained on MS medium containing 5% sucrose. However, this concentration did not totally overcome poor germination and growth of *sh2* embryos and axes. Axes of *su* and *normal* had greater growth rates than *sh2* and *bt1* on sucrose-free medium, although the difference between genotypes decreased when whole embryos were used. When ground endosperm was employed as the carbohydrate source, *sh2* embryos germinated and grew poorly, particularly on *normal* endosperm. With a commercial corn starch as the carbohydrate source, *sh2* germlings were shorter in length and displayed a greater loss in dry weight than the other genotypes. The poor growth of *sh2* embryos on ground endosperm and starch media may indicate a dysfunction of the scutellum or axis in relation to carbohydrate metabolism and utilization.

The incorporation of endosperm mutants into maize breeding programs in recent years has dramatically altered the carbohydrate composition of resultant hybrids. The *brittle-1* (*bt1*) gene causes a 2-fold increase in sucrose (12) in comparison to *sugary* (*su*) and a 50% reduction in starch (3) in comparison to normal at 22 d post-pollination. With the *shrunken-2* (*sh2*) gene, total sugars increase 10-fold over *normal* and 4-fold over *su* at the denting stage with most of this increase due to sucrose (14). Starch levels in *sh2* are greatly reduced throughout development compared to *su* and *normal* (4). Starch synthesis is reduced in *sh2* due to low amounts of ADPG pyrophosphorylase (6, 23). These high-sugar mutants demonstrate superior postharvest sugar retention (10, 22).

A major limiting factor to the acceptance and success of *sh2* hybrids is their poor seed and seedling vigor. Germination and seedling vigor measurements of *sh2* are significantly lower in both laboratory and field tests in comparison to *su*, *bt1*, and *normal* (20). The seeds of *sh2* are smaller, lighter, have a lower endosperm to embryo dry weight ratio than *su* or *normal* (24), and are very susceptible to fungal rots during germination in the field (2).

The cause of low seed and seedling vigor in the *sh2* genotypes may be due to several factors, such as a smaller endosperm, susceptibility to pathogens, or smaller embryo. Selection among lines containing the *sh2* genotype is effective for improving seed quality (1). Genetic susceptibility of *sh2* to infection by *Fusarium moniliforme* reduces seed vigor to some extent (19). Respiration rates (24) and ATP levels (20) of germinating seeds of *sh2*, *su*, and *normal* do not account for the growth differences among the genotypes. Wann (24) obtained significant differences in seedling growth between these genotypes in a standard germination test but not when excised embryos were grown on a nutrient medium. He concluded that the small endosperm of high-sugar genotypes is primarily responsible for their poor seed and seedling vigor. The purpose of this work was to further investigate the embryo-endosperm relationship and its effect on seed vigor of four isogenic maize genotypes.

MATERIALS AND METHODS

Production of Plant Material. Isogenic parental lines developed by Wolf (25) and Hannah (12) of maize (*Zea mays* L.) homozygous for *su*, *sh2*, *bt1*, and *normal* were planted in the field and greenhouse in Gainesville, FL. Hybrid materials were then obtained by the hand cross-pollination of the inbred parents of *sh2*, Fa56, and Fa32 ('Florida Sweet'), or the *su*, *bt1*, and *normal* inbred versions of Iowa 2132 and Iowa 2256 (the parents of 'Iobelle'). Ears were harvested when mature (46 d postpollination), dried slowly at 30°C and 30% RH, then stored at 10°C and 45% RH until used.

Rolled Towel and Cold Soil Tests. Viability and seedling vigor measurements were obtained by placing four replicates of 25 seeds of each genotype in moist rolled towels at 25°C. After 7 d, the germinated seeds were counted and total seedling lengths

Table I. *Viability and Seedling Vigor of Four Genotypes of Maize as Determined by Rolled Towel Test (RTT) and Cold Soil Test (CST)*

Genotype	Germination		Seedling Length	
	RTT	CST	RTT	CST
	%		cm	
bt1	89 ± 4[a]	20 ± 2	13.3 ± 1.0	12.7 ± 1.2
sh2	70 ± 2	9 ± 2	12.0 ± 1.1	17.2 ± 2.5
su	97 ± 1	78 ± 5	13.1 ± 0.7	26.9 ± 1.0
normal	100 ± 0	94 ± 3	21.4 ± 1.6	18.7 ± 0.6

[a] Each value is the mean of four replicates of 25 seeds ± SE.

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Table II. *Dry Weight Relationships of Various Seed Parts of Four Genotypes of Maize*

Genotype	Axis	Scutellum	Axis + Scutellum[a]	Endosperm	Pericarp	Total[b]
			mg/seed			
bt1	1.9 ± 0.1[c]	19.1 ± 1.5	21.0 ± 1.4	86 ± 4	10.9 ± 0	118 ± 5
sh2	1.8 ± 0.1	17.4 ± 0.5	19.2 ± 0.6	53 ± 1	9.7 ± 0.3	82 ± 2
su	2.2 ± 0.2	27.0 ± 1.5	29.2 ± 1.7	122 ± 6	12.6 ± 0.1	164 ± 8
normal	4.1 ± 0.2	36.5 ± 0.5	40.6 ± 0.5	196 ± 2	17.2 ± 0.2	254 ± 3
			% total seed			
bt1	1.6 ± 0.1	16.1 ± 0.7	17.7 ± 0.6	73.0 ± 0.5	9.3 ± 0.3	
sh2	2.2 ± 0.1	21.4 ± 0.4	23.6 ± 0.4	64.6 ± 0.9	11.9 ± 0.4	
su	1.4 ± 0.1	16.6 ± 0.2	17.9 ± 0.2	74.4 ± 0.2	7.7 ± 0.4	
normal	1.6 ± 0	14.4 ± 0.2	16.1 ± 0.1	77.2 ± 0.2	6.8 ± 0	

[a] Sum of axis and scutellum dry weights.
[b] Sum of axis, scutellum, endosperm, and pericarp dry weights.
[c] Each value is the mean of three replicates of 10 seeds ± SE.

Table III. *Sugar and Starch Contents of Whole Seeds and Embryos (Embryonic Axis plus Scutellum) of Four Genotypes of Maize*

Genotype	Fructose	Glucose	Sucrose	Maltose	Total Sugar[a]	Starch
			mg/g dry wt seed			
Whole seed						
bt1	1.32 ± 0.24[b]	0.93 ± 0.13	19.2 ± 0.4	0.28 ± 0.28	21.7 ± 1.0	472 ± 15
sh2	1.10 ± 0.04	0.59 ± 0.08	23.4 ± 0.2	ND[c]	25.1 ± 0.3	306 ± 3
su	0.70 ± 0.03	1.49 ± 0.23	20.9 ± 1.4	0.31 ± 0.17	23.4 ± 1.2	560 ± 8
normal	0.05 ± 0.05	0.05 ± 0.05	9.9 ± 0.6	1.94 ± 0.29	11.9 ± 0.8	551 ± 17
			mg/g dry wt embryo			
Whole embryo						
bt1	1.70 ± 0.04	0.78 ± 0.07	33.8 ± 9.5	2.02 ± 2.02	38.2 ± 11.4	116 ± 3
sh2	0.86 ± 0.40	0.39 ± 0.20	45.0 ± 0.3	ND	46.2 ± 0.8	59 ± 2
su	11.13 ± 3.34	9.86 ± 3.15	41.9 ± 3.0	6.61 ± 4.10	69.4 ± 13.5	169 ± 2
normal	1.27 ± 0.03	0.67 ± 0.04	29.4 ± 7.9	0.28 ± 0.28	31.6 ± 7.6	89 ± 6

[a] Sum of fructose, glucose, sucrose, and maltose contents.
[b] Each value is the mean of four replicates ± SE.
[c] ND, not detected.

were measured. To determine viability and vigor under stress conditions, four replicates of 25 seeds of each genotype were subjected to a cold soil test (26). Seeds were planted in plastic boxes (18 × 12.5 × 9 cm) containing measured amounts of field soil and water, sealed, and incubated at 10°C for 7 d, then transferred to 25°C for 4 d. The germinated seeds were counted, and total seedling lengths were recorded.

Dry Weights of Seed Parts. Three replicates of 10 seeds of each genotype were allowed to imbibe water in Petri dishes for approximately 4 h at 25°C. The pericarp, endosperm, scutellum, and embryonic axis were then dissected from each seed and dried at 70°C for 48 h. The dry weights were expressed as mg/seed and percentage of total seed.

Sugars and Starch Analyses. Sugars were extracted by mixing 500 mg of dry seeds finely ground in a coffee grinder or excised embryos with 25 ml of 95% ethanol and incubating at 80°C for 60 min. Samples were cooled and filtered through a Büchner funnel. The filtrate was adjusted to 50 ml. Sugars were measured by GLC as described by Ferguson *et al.* (9) as modified by Styer and Cantliffe (18).

The residue from the ethanol extraction was dried at 70°C for 24 h, weighed, and used for starch determination. Starches were extracted by mixing 100 mg residue with 15 ml of 0.5 N NaOH and incubating at 100°C for 30 min (5). The samples were cooled, neutralized with 15 ml of 0.5 N acetic acid, and filtered through a Büchner funnel. A 2-ml aliquot of each extract was incubated with 2 ml of an amyloglucosidase solution (10 mg amyloglucosidase from *Rhizopus* mold dissolved in 1 ml of 0.1 M sodium acetate buffer, pH 4.5) at 55°C for 60 min. After cooling to room temperature, a 25-μl aliquot was injected into a YSI model 27 Industrial Analyzer. The amount of glucose recovered was converted to starch on a dry weight basis.

Preparation of Culture Media. For all culture experiments, a modified Murashige and Skoog (16) medium containing 2% (w/v) agar and adjusted to pH 6.5 was used. The carbohydrate source was altered in the following ways: (a) sucrose added at 0, 0.1, 1, 5, or 10% (w/v); (b) commercial corn starch (Sigma Chemical Company) autoclaved separately and mixed to a 5% (w/v) final concentration; and (c) ground endosperm of each of the genotypes.

Ground endosperm was obtained by surface sterilizing seeds of each genotype for 10 min in 1.75% NaOCl, rinsing three times in sterile distilled H_2O, and removing the embryos. The endosperm tissue was then surface sterilized for 5 min as above and finely ground in a sterilized grinder. Endosperm and culture media were mixed and poured into 9-cm Petri plates to give a final quantity of approximately 1 g endosperm/plate. The plates were then incubated at 25°C for 3 d. Contaminated plates were discarded.

Isolation and Culture of Whole Embryos and Embryonic Axes. Seeds of each genotype were surface sterilized for 10 min in 1.75% NaOCl and rinsed three times in sterile distilled H_2O.

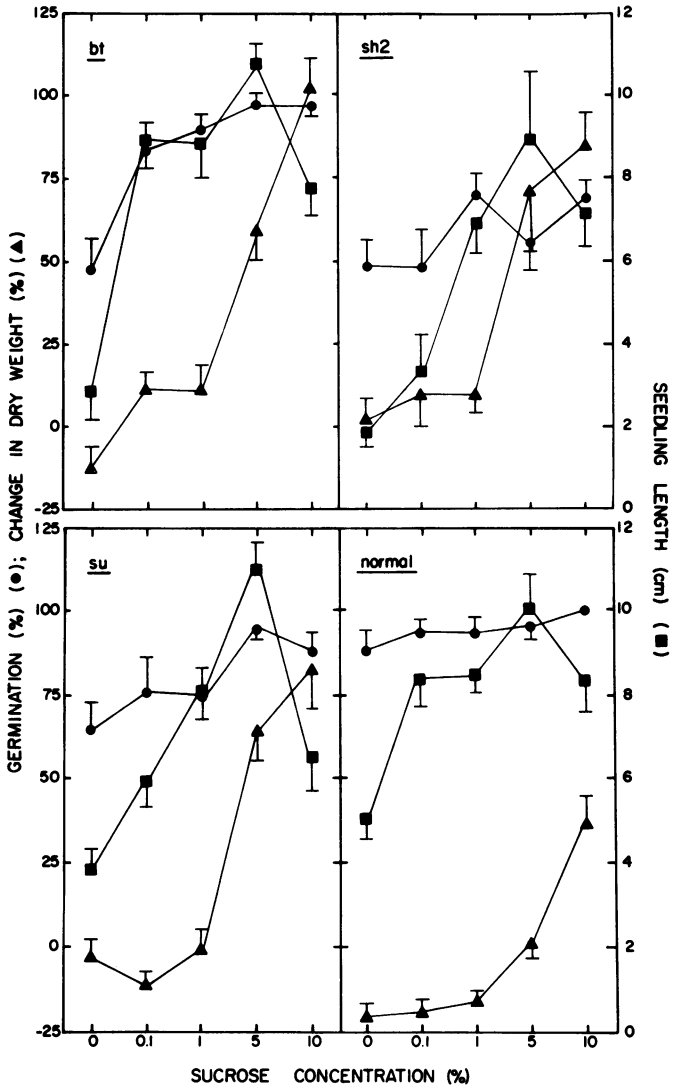

FIG. 1. Growth of whole embryos (embryonic axis + scutellum) of four genotypes of maize cultured on media containing various sucrose concentrations. The change in dry weight was calculated as in Table IV. Each value is the mean of 10 replicates of four embryos/dish ± SE.

Embryonic axes or whole embryos (axis plus scutellum) were removed and surface sterilized as described above; axes for 1 min and whole embryos for 5 min. Four axes or embryos were embedded per plate and incubated upright at 25°C for 5 d. After this period, germination, seedling lengths, and seedling dry weights were determined.

RESULTS

Germination was lower in *sh2* than the other three genotypes in the rolled towel test, whereas both *bt1* and *sh2* germinated poorly in the cold soil test (Table I). Germination of *su* declined slightly under stress conditions but *normal* germinated at higher than 90% in both tests. Seedling lengths did not reflect growth rate (vigor) differences between genotypes.

Seeds of *normal* had greater total seed weights primarily resulting from a heavier endosperm than the other genotypes (Table II). *Shrunken-2* seeds were lightest in weight, mainly due to the lack of endosperm. Embryos of *sh2* weighed half as much as embryos of *normal*. Based on percentage of the total seed, the embryo and pericarp make up a larger portion of the *sh2* seed than they did of the other genotypes. Differences in endosperm as a percentage of total seed weight were less obvious, 65% for *sh2* and 77% *normal*.

The lowest starch and highest sugar levels were measured in *sh2* seeds (Table III). Starch contents in *su* and *normal* were similar whereas total sugars in the latter genotype were half as much as any of the others. Whole embryos (axis + scutellum) of *sh2* also contained the least amount of starch but had less sugars than *su* embryos. The highest levels of starch and of all measured sugars except sucrose were found in *su* embryos, whereas *bt1* was

Table IV. *Growth of Embryonic Axes of Four Genotypes of Maize Cultured on Media Containing Various Sucrose Concentrations*

Genotype	Sucrose Concn.	Germination	Seedling Length	Change in Dry Wt[a]
		%	*cm*	%
bt1	0	75 ± 9[b]	0.77 ± 0.04	21 ± 18
	5	89 ± 5	1.71 ± 0.32	240 ± 35
	10	75 ± 10	1.09 ± 0.22	217 ± 18
sh2	0	45 ± 8	0.72 ± 0.10	19 ± 9
	5	57 ± 12	1.16 ± 0.30	180 ± 38
	10	64 ± 6	1.04 ± 0.15	166 ± 24
su	0	92 ± 4	1.06 ± 0.07	44 ± 10
	5	86 ± 7	1.84 ± 0.33	221 ± 40
	10	98 ± 3	1.39 ± 0.15	215 ± 25
normal	0	80 ± 5	1.13 ± 0.09	−16 ± 4
	5	94 ± 4	2.53 ± 0.60	84 ± 15
	10	100 ± 0	1.48 ± 0.17	110 ± 13

[a] Calculated as $\frac{\text{seedling dry wt} - \text{avg. embryonic axis dry wt}}{\text{avg. embryonic axis dry wt}} \times 100.$

[b] Each value is the mean of at least seven replicates of four axes/dish ± SE.

Table V. *Growth of Whole Embryos (Embryonic Axis plus Scutellum) of Four Genotypes of Maize Cultured on Media Containing Ground Endosperm of Each of the Genotypes as a Carbohydrate Source*

Genotype		Germination	Seedling Length	Change in Dry Wt[a]
Embryo	Endosperm			
		%	*cm*	%
bt1	*bt1*	75 ± 6[b]	4.7 ± 0.3	−9 ± 4
	sh2	92 ± 4	5.3 ± 0.4	1 ± 7
	su	92 ± 5	5.7 ± 0.3	−1 ± 6
	normal	60 ± 8	5.0 ± 0.8	7 ± 6
sh2	*bt1*	63 ± 13	1.9 ± 0.3	−10 ± 12
	sh2	50 ± 18	1.7 ± 0.7	−13 ± 7
	su	45 ± 15	2.1 ± 0.6	−5 ± 7
	normal	38 ± 7	1.4 ± 0.2	−21 ± 4
su	*bt1*	83 ± 8	5.4 ± 0.6	6 ± 6
	sh2	92 ± 5	3.6 ± 0.5	−7 ± 6
	su	92 ± 5	6.6 ± 0.9	−4 ± 4
	normal	65 ± 11	5.2 ± 0.8	1 ± 7
normal	*bt1*	60 ± 15	3.9 ± 0.7	−21 ± 5
	sh2	30 ± 12	1.0 ± 0.3	−27 ± 8
	su	85 ± 10	6.3 ± 0.6	−21 ± 2
	normal	83 ± 5	5.1 ± 0.7	−17 ± 2

[a] Calculated as $\frac{\text{seedling dry wt} - \text{avg. whole embryo dry wt}}{\text{avg. whole embryo dry wt}} \times 100.$

[b] Each value is the mean of at least six replicates of four embryos/dish ± SE.

Table VI. *Growth of Whole Embryos (Embryonic Axis plus Scutellum) of Four Genotypes of Maize Cultured on Media Containing 5% Corn Starch as a Carbohydrate Source*

Genotype	Germination	Seedling Length	Change in Dry Wt[a]
	%	*cm*	%
bt1	72 ± 12[b]	5.7 ± 0.6	−10.2 ± 8.0
sh2	38 ± 8	2.2 ± 0.5	−23.4 ± 8.7
su	53 ± 9	4.6 ± 0.7	−18.5 ± 4.8
normal	95 ± 5	7.4 ± 0.8	−19.3 ± 2.3

[a] Calculated as $\frac{\text{seedling dry wt} - \text{avg. whole embryo dry wt}}{\text{avg. whole embryo dry wt}} \times 100.$

[b] Each value is the mean of eight replicates of four embryos/dish ± SE.

intermediate to the other genotypes in embryo carbohydrate levels while *normal* was lowest. Sucrose was the predominant sugar found in both seeds and embryos of all four genotypes.

When whole embryos were cultured on MS media with or without sucrose, only *bt1* embryos exhibited an increase in all three growth parameters at the lowest concentration of sucrose (Fig. 1). *Shrunken-2* embryos had the lowest germination of all genotypes, while *normal* embryos exhibited the highest germination. Seedling lengths of all four genotypes increased over the range of 0 to 5% sucrose. At 10%, however, a sharp decrease in seedling length was noted, possibly due to an osmotic effect of sucrose. Seedling dry weights of *sh2* did not increase until 5% sucrose was added to the media. *Normal* and *su* seedlings had no increase in dry weight until 5% sucrose was incorporated into the media. *Normal* embryos had the highest germination and seedling lengths of all genotypes when grown on a sucrose-free medium.

Embryonic axes (no scutellum attached) of each genotype were able to germinate and grow to some extent when placed on a sucrose-free medium (Table IV). Axes of high-sugar mutants (*sh2* and *bt1*) produced shorter seedlings than did axes of *su* and *normal* on media without sucrose. The lowest germination occurred in *sh2* axes. Additional sucrose improved germination in *sh2* and *normal*. Seedling lengths of all genotypes were increased by the addition of 5% sucrose in the medium, but were somewhat shorter at 10% sucrose than at 5%. Dry weights of all seedlings were increased dramatically in the presence of sucrose. However, without sucrose in the medium, axes of *bt1*, *sh2*, and *su* had a net increase in dry weight, while *normal* axes had a net loss.

Ground endosperm was incorporated into the media to evaluate the effectiveness of the mutant endosperm to support embryo growth (Table V). Regardless of the type of endosperm used, *sh2* embryos germinated and grew poorly. In particular, *sh2* embryos appeared to utilize *normal* endosperm the least of any endosperm tested. The *sh2* endosperm was not able to support the growth of *su* and *normal* embryos, although *bt1* embryos grew well on this endosperm. *Normal* embryos were also unable to effectively use *bt1* endosperm, thus indicating a general inability of embryos from seeds containing relatively high levels of starch to utilize high-sugar mutant endosperms.

On a commercial corn starch media, germination of *sh2* embryos was extremely low (Table VI). Seedling lengths of this genotype were 30% to 48% of those derived from the other genotypes. The *sh2* germlings had a greater loss in dry weight than the other genotypes, indicating a possible inability to use this carbohydrate source as efficiently as sucrose (Fig. 1), especially when compared to *normal* embryos.

DISCUSSION

Previous work has demonstrated the weak seed vigor of high-sugar endosperm mutants, such as *sh2* (17, 20, 24). Fungal pathogens are known to reduce germination and plant stand of *sh2* hybrids (2). Increased amount of leaching from the seed (18) may stimulate fungi located in the soil or on the seed to grow and attack the germinating seed. Increased susceptibility due to higher sugar and moisture contents of *sh2* kernels during development can lead to infection by *Fusarium moniliforme* and can reduce seed vigor (19). However, noninfected *sh2* seeds germinated significantly less than noninfected *su* seeds under cold soil conditions (19), indicating that some other factor(s) might also contribute to poor seed vigor.

Possibly, the poor seed vigor of *sh2* is directly related to a starch deficient endosperm which cannot sustain early seedling growth (24). The lower starch content results in a collapsed, shrunken seed with a low endosperm to embryo ratio. Yet, seeds of *sh2* imbibe water at a faster rate (18), respire at a greater rate up to 4 d after initiation of imbibition (Styer, unpublished data; 24), and produce more ATP during this period than do *su*, *bt1*, or *normal* (20).

Carbohydrate hydrolysis in the endosperm is not initiated for at least the first 48 h of germination (13); soluble sugars in the embryo may be quickly used (24) as may be lipids (7) and proteins (15). This demonstrates the importance of stored reserves in the embryo for early seedling growth. When axes were cultured on MS media without sucrose, *su* and *normal* grew better than *sh2* and *bt1*; however, differences between genotypes decreased when whole embryos were similarly cultured. In any event, growth of embryos on culture media was vastly improved by the addition of sucrose, regardless of genotype. Total germination was generally also improved by sucrose addition. Yet, regardless of sucrose concentration, germination and vigor (growth) of *sh2* whole embryos or isolated axes never matched that of the other three genotypes.

When embryos were excised and cultured on media containing ground endosperm of each genotype, *sh2* had reduced seedling lengths and germination compared to the other genotypes, regardless of the source of endosperm used in the culture. This difference was further amplified when sterilized commerical corn starch was utilized as the carbohydrate source in the media. Seedling growth of *sh2* embryos was one-half to one-third that of the other genotypes. Tilton (21) suggests that the scutellum synthesizes or provides a growth factor which is the initial stimulus for hypocotyl and root growth during germination. The scutellum is important not only in storing reserves but in producing and secreting enzymes and translocating metabolites to the growing axis (8). Even though the embryo may not be necessary for the development of α-amylase activity in the endosperm (11), considerable quantities of this and other carbohydrases must be released from the scutellum, as evidenced by the growth of *normal* embryos on starchy media. Also, carbohydrate conversions of sugars take place in the scutellum as the products of starch breakdown are moved to the embryo for utilization and growth.

The poor growth of *sh2* embryos on ground endosperm and starch media may indicate some type of dysfunction of the scutellum or axis in relation to carbohydrate metabolism and/or utilization. This, in part, with a starch deficient endosperm and, in many cases, with an association with pathogens during seed development and germination, may lead to the poor seed vigor observed in *sh2* endosperm maize mutants.

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