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Differential Seed and Seedling Vigor in *Shrunken-2* Compared to Three Other Genotypes of Corn at Various Stages of Development[1]

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Abstract. **New hybrids of sweet corn (*Zea mays* L.) utilize genes which allow the build-up of high levels of sugar but which also lead to problems with seed and seedling vigor. In this study, ATP and various seedling vigor measurements were compared with seed germination and seedling vigor in *normal* and 3 corn endosperm mutants harvested at 16 to 42 days post-pollination. Germination and seedling vigor measurements (germination rate, radicle length, fresh and dry weight) showed that a *shrunken-2 (sh2)* corn was significantly lower in both laboratory and field tests than *sugary (su), brittle (bt),* and *normal.* The latter 3 genotypes were nearly equal in seedling vigor. Sugar levels of all mutants and *normal* were similar at 42 days post-pollination. Total polysaccharides in *sh2* were 50% or more below the other 3 genotypes. ATP levels in seeds imbibed 4 hours, were generally similar in *sh2* as in the other genotypes. In a time course study of 0 to 96 hr imbibition using 42 day-old seeds, the ATP content of *sh2* seeds, was generally as high or higher than in the other 3 genotypes. It did not appear that ATP level was related to poor vigor during the early stages of germination of *sh2* corn seeds.**

Mutations conditioning carbohydrate alterations, principally higher sugar, in maize endosperm have been incorporated into sweet corn breeding programs (2, 9). The mutant *sugary (su)* has long been the principal gene of present sweet corn hybrids. Yet, use of the *shrunken-2 (sh2)* gene for *su* will increase sucrose 2 to 3 times, with a corresponding decrease in starch (7, 10). Tsai and Nelson (14) indicated that this sugar increase was due to a block in ADPG production resulting in reduced starch synthesis. Creech (6) investigated many single, double, and triple endosperm mutants and found that total sugar and sucrose were higher early in kernel development (20 days post-pollination) than during later stages of development (28 days post-pollination) whereas starch increased as development progressed.

The higher sugar and lower starch content of these endosperm mutants may lead to production problems. The seeds of *sh2* hybrids, for instance, are smaller, lighter, more easily damaged and have poorer germination and seedling vigor than *su* sweet corn seeds (6). The higher sugar content of the kernel during seed development has been associated with an increase in rot by pathogens (1, 3).

The low germination and seedling vigor of mutants such as *sh2* may also be due to the low total carbohydrate content of the endosperm (6). Since carbohydrates are the main storage components in corn endosperm, breakdown of starch produces energy components such as adenosine triphosphate (ATP) which is used for synthesis of protein and nucleic acids. If endosperm carbohydrate is low, then the supply of ATP may be reduced. Thus, germination and subsequent seedling vigor may be adversely affected.

The following experiments were designed to determine changes in seed and seedling vigor of various corn genotypes during seed development and to relate these to percent germination and to ATP.

Materials and Methods

Corn genotypes homozygous for *brittle (bt), sugary (su),* and *shrunken-2 (sh2)* were planted in a randomized complete block design in the field along with *normal* corn. The *bt-A* allele (9), subsequently referred to as *bt,* was used. Seeds for analysis were obtained by self-pollination of *bt* and F_1 normal (W64A × 182E) plants whereas *su* seeds (Iowa 2256 × Iowa 2132) and *sh2* seeds (FA56A × FA32) were the result of cross-pollination. Flowering of all lines occurred within 7 days of each other. The corn was grown according to Florida recommendations (11).

At least 4 ears per replication from 4 replications were harvested at different developmental stages from 16 to 42 days post-pollination, husked, and placed in a forced air drying oven at 35°C until dry. The dried kernels were stored in a dessicator at room temperatures.

Germination and seedling vigor measurements were obtained by treating 10 seeds from each of the dried ears with the fungicide Spergon then placing them on moist paper towels. The towels were rolled up with a wax paper backing and placed in a germinator at 25°C. Germination counts were made after 1, 3, 5, and 7 days to determine rate and total percent emergence. Germination rate was calculated according to the formula of Shmueli and Goldberg (12). After 7 days radicle lengths were measured on germinated seedlings only, then fresh weights were recorded and dry weights were obtained by drying seedlings in an oven at 70°C for 48 hr. Fresh and dry weights of the germinated seedlings were calculated on an individual seedling basis.

To determine emergence under field conditions, seeds 24 to 42 days post-pollination were planted in a fine sandy soil at Gainesville. Twenty-five seeds were planted 2.5 cm deep in rows 3 m long and 1 m apart. Four replications of each harvest date from each genotype were planted in a randomized complete block design. The emergence rate, total emergence percentage, and seedling height 2 weeks after planting were determined. Fresh and dry weights were determined on 10 randomly selected seedlings per replicate.

ATP was extracted from 10 kernels from each ear according to the procedure of Ching (4) and Ching and Danielson (5). The seeds were imbibed with distilled water 4 hr at 25°C before extraction. In a separate experiment 42 day-old seeds were imbibed at 25°C for periods of 0 to 96 hr and periodically assayed for ATP. After imbibition, the kernels were crushed and extracted with 10 ml of boiling distilled water. After

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cooling, 5 ml of extract were diluted with 5 ml of buffer containing 0.05 M N-2-hydroxyethyl-piperazine-N'-2-ethanesulfonic acid (HEPES) and 0.05 M magnesium acetate at pH 7.5 Recovery of an internal ATP standard was 95% or better by using this method. The ATP assay used was that previously described by St. John (13). Freeze-dried firefly extract containing luciferin-luciferase was reconstituted by adding 5 ml of ice-cold distilled water which resulted in an enzyme preparation containing 0.05 M potassium arsenate and 0.02 M magnesium sulfate, pH 7.4. Light emission from the ATP-enzyme preparation was recorded using an Aminco Chem-Glo Photometer. The ATP concentration was determined by comparison of the unknown peak height to a standard curve.

The methods of Gonzales et al. (8) were used to extract and analyze total sugars and total polysaccharides from frozen 42 day-old material, except that starch and water soluble polysaccharides were not separated.

Results

Laboratory seedling vigor tests indicated that *sh2* was lower in vigor than *bt, su,* or *normal* (Table 1). Averaged over all developmental stages *sh2* seeds had a significantly slower rate of germination, lower total percent germination, shorter radicle length (except for normal), and lower fresh and dry weights than the other genotypes. Although the average radicle length of *normal* was less than *sh2, normal* appeared to be as vigorous as *bt* or *su.* Averaged over all genotypes both germination percentage and rate were high for immature seeds (20 to 32 days old), but decreased slightly in seeds 36 to 42 days post-pollination. Generally, seedling weights (fresh and dry) of all genotypes increased with seed maturity. The corresponding increase in radicle lengths with maturity was probably related to increased seedling weight. Radicle lengths almost doubled between 16 and 20 days and again between 18 and 26 days post-pollination. A slight decrease in overall radicle length occurred after 36 days. This decrease was probably related to an increase in radicle thickness in seedlings grown from the older seeds since fresh weights were unaffected.

All lines were sufficiently mature so that seeds germinated in every lot at 16 days post-pollination (Table 2). Germination percentage and rate were extremely high in *bt* seeds at all stages of seed maturity beyond 18 days. Vigor indices (radicle length and seedling weights) progressively increased until the 36 day harvest date. Generally *sh2* had less viability, slower germination and smaller seedlings than *bt*. All of the 16 day-old *su* seeds were viable, and viability remained high until the 32 day harvest date. Both viability and germination rates were significantly reduced in 36 and 42 day-old seeds. Vigor of those seeds that did germinate was as high or higher than from less mature seeds. After 20 days post-pollination, germination percentages and rates were optimum for normal. However, the other vigor traits improved as the seeds matured. Seeds matured 36 to 42 days prior to harvest produced seedlings having shorter radicle lengths but without a concomitant decrease in fresh weight. Also, the radicles were thicker.

Table 1. Main effects of laboratory seedling vigor measurements on different corn genotypes at different stages of development.

Line	Germination (%)	Germination rate index	Avg radicle length (cm)	Seedling wt (mg) Fresh	Dry
bt	96a[z,y]	2.8a	13.2b	745a	74b
sh2	77c	1.9d	11.6c	517b	57c
su	92ab	2.1c	18.9a	765a	89a
normal	90b	2.6b	9.5d	816a	97a
Days post-pollination					
16	76d[x]	2.2cde	6.1f	179g	15h
18	85cd	2.5abc	8.6e	325f	30g
20	91bc	2.6ab	11.8d	522e	48f
22	90bc	2.5abc	10.8de	570e	47ef
24	96ab	2.5abc	14.1c	676d	69de
26	95ab	2.3bcd	15.7abc	794c	82d
28	100a	2.8a	15.4bc	846c	97c
32	90bc	2.1def	17.4a	980b	111c
36	80d	1.9ef	16.6ab	1144a	139b
42	79d	1.8f	14.5c	1179a	163a

[z]Mean separation in columns by Duncan's multiple range test, 5% level.
[y]Data averaged over all days post-pollination.
[x]Data averaged over all genotypes.

Table 2. Laboratory seedling vigor measurements from dried seeds of 4 corn genotypes harvested at various stages of development.

Days post-pollination	Germination (%)	Germination rate index	Avg radicle length (cm)	Seedling wt (mg) Fresh	Dry
		brittle			
16	70b[z]	2.0c	3.5e	93h	9f
18	100a	3.0a	9.4d	447g	37e
20	95a	2.9a	12.2c	547f	44de
22	95a	2.9a	9.8d	616e	52d
24	100a	2.9a	10.2d	734d	70c
26	100a	2.9a	16.2b	846c	74c
28	100a	2.9a	15.2b	930b	93b
32	100a	3.0a	19.7a	971b	97b
36	95a	2.5b	15.8b	1033a	124a
42	100a	2.9a	20.2a	1075a	132a
		shrunken-2			
16	75c	2.1bc	7.1e	227g	17e
18	95a	2.6a	8.8de	235g	21e
20	70cd	1.8cd	11.4c	424f	43d
22	75c	1.8cd	7.9e	477e	52d
24	85b	2.2d	12.9bc	460ef	50d
26	85b	1.8cd	13.1b	662c	77c
28	100a	2.7a	14.2ab	531d	56d
32	65d	1.4e	15.5a	848a	95ab
36	70cd	1.5de	15.3a	824a	94b
42	45e	0.9f	9.7d	718b	109a
		sugary			
16	100a	3.0a	12.2f	271h	22h
18	100a	3.0a	14.7e	358g	32h
20	100a	2.8ab	17.6d	490f	49g
22	100a	2.6b	17.2d	560e	56f
24	100a	2.1c	17.6d	693d	72e
26	95a	1.5d	21.1ab	875c	91d
28	100a	2.6b	21.7a	941b	110c
32	95a	1.3d	19.7bc	971b	115b
36	55c	0.7e	19.9bc	1700a	227a
42	70b	1.2d	19.3c	1486a	236a
		normal			
16	60c	1.8b	1.6f	68i	10f
18	45d	1.4c	1.4f	167h	22f
20	100a	3.0a	6.0e	596g	57e
22	90b	2.7a	8.2d	612f	66de
24	100a	3.0a	15.8a	778e	80cd
26	100a	2.9a	12.3b	778e	89c
28	100a	2.9a	10.4c	984d	130b
32	100a	2.9a	14.5a	1084c	132b
36	100a	2.9a	15.6a	1168b	136b
42	100a	2.1b	8.8cd	1286a	170a

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

Table 3. Field seedling vigor measurements of 4 genotypes of corn harvested at various stages of development.

Days post-pollination	Emergence (%)	Emergence rate index	Height (cm)	Seedling wt (mg) Fresh	Dry
		brittle			
24	89b[z]	3.8c	8.7d	520d	79d
26	97a	4.6b	9.6c	805c	111c
28	98a	4.6b	10.6b	947b	127b
32	98a	4.9a	12.2a	1280a	171a
36	92b	4.0c	10.3b	863c	122bc
42	99a	4.8ab	11.6a	1284a	178a
		shrunken-2			
24	27e	1.1e	7.1b	267b	48b
26	44c	1.7d	7.4b	281b	49b
28	38d	1.3e	6.1c	161b	31c
32	67b	2.6c	7.1b	289b	50b
36	78a	3.6a	9.4a	525a	81a
42	70b	3.2b	9.5a	600a	93a
		sugary			
24	99a	4.7ab	9.4c	592d	90d
26	97a	4.5bc	10.4b	740c	110c
28	95a	4.4c	10.3b	768c	115c
32	99a	4.6abc	10.7ab	1027b	150b
36	99a	4.8a	11.3a	1238a	176a
42	98a	4.6abc	10.6b	1106b	161b
		normal			
24	90c	4.1c	9.7cd	833b	119c
28	91c	4.2c	9.8bcd	763b	110c
28	96ab	4.6ab	9.3d	745b	107c
32	97a	4.8a	10.6a	1123a	155a
36	92bc	4.5b	10.3abc	1038a	139b
42	98a	4.6ab	10.4ab	1148a	162a

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

Since laboratory tests indicated that *sh2* seeds would germinate at immature stages of development, seeds from all 4 genotypes, ranging in age from 24 to 42 days post-pollination, were planted in the field to test for vigor under stress. Lower seedling vigor was recorded for *sh2* in the field test than in the laboratory test whereas the *bt, su,* and *normal* seeds performed about the same except there was no reduction in *su* germination at 36 and 42 days (Table 3). Emergence percentage and rate, and seedling fresh and dry weight more than doubled for *sh2* seeds from 24 to 42 days post-pollination, yet those values were below those of the other 3 genotypes. Seedling height of *sh2* also significantly increased due to the more mature kernels. Germination rate and seedling size, as indicated by height and fresh and dry weight, also increased for the other 3 genotypes as seed maturity progressed. Although vigor was lower, 24 day-old seeds from *su, bt* and *normal* genotypes generally germinated as well as 42 day-old seeds.

Table 4. Total sugars and polysaccharides (fresh weight bases) in kernels of 4 corn genotypes at 42 days post-pollination.

Genotype	Sugar (%)	Polysaccharide (%)
sh2	1.8a[z]	12c
bt	1.3ab	25f
su	1.7a	39a
normal	0.8b	35ab

[z]Mean separation in columns by Duncan's multiple range test, 5% level.

Table 5. ATP content in the kernels of 4 corn genotypes harvested at various stages of development and oven-dried at 35° C. Data taken after 4 hours of imbibition.

Days post-pollination	ATP content (nmoles/seed) *bt*	*sh2*	*su*	*normal*	Mean
16	12.6c[z]	2.6a	1.6c	4.3b	5.3bc
18	18.9b	1.6a	1.5c	15.9a	9.4a
20	27.3a	3.8a	1.4c	3.3b	8.9ab
22	6.5de	1.2a	1.6c	3.8b	3.2c
24	6.6de	1.6a	2.0c	4.1b	3.6c
26	9.0cd	1.9a	2.2c	2.0b	3.8c
28	8.3cd	2.1a	10.2ab	1.5b	5.5bc
32	7.8de	3.3a	6.8b	1.4b	4.8bc
36	4.1e	1.8a	6.7b	1.6b	3.5c
42	4.2e	4.1a	10.9a	1.8b	5.2bc
Mean	10.5a[y]	2.4b	4.5b	4.0b	

[z]Mean separation in columns by Duncan's multiple range test, 5% level.
[y]Mean separation in rows.

All 4 genotypes had relatively low amounts of sugar at 42 days (less than 2%), yet all the mutant genotypes had slightly more sugar than *normal* (Table 4). At 42 days post-pollination, *sh2* only contained ½ to ¼ of the total polysaccharides found in the other 3 genotypes.

Breakdown of starch to sugar during germination supplies the young embryo with energy for synthesis of new compounds. Since the total polysaccharide level in mature *sh2* seeds was much less than in the other genotypes, then possibly the poor germination of *sh2* is due to inadequate sources of energy. Averaged over all corn genotypes, ATP in imbibed seeds increased from 16 to 18 days post-pollination and exhibited a drop at 22 days (Table 5). At later maturity dates, ATP levels tended to increase but remained below the concentrations observed at 18 to 20 days maturity. The ATP content of imbibed *bt* seed averaged over all harvest dates was significantly greater than the other 3 genotypes. *Brittle* seeds had greater levels of ATP at 16 to 20 days post-pollination than at later stages whereas in *sh2* kernels there were no such differences. *Normal* seeds contained the highest ATP level at 18 days, while no differences were found among the other stages of development. Mature 42 day-old *su* produced more ATP after 4 hr imbibition than did *sh2* and *bt*.

Thus with imbibed seed, ATP levels were higher during early than later maturity. Since larger, and more mature seeds did not produce the higher levels of ATP after 4 hr imbibition that might have been expected from the larger endosperms, a second experiment was conducted with imbibition time increased to 96 hr to see if genotypes *su, bt* and *normal* with more carbohydrate reserves would produce more ATP than *sh2* with less reserves. The results showed that ATP levels for all genotypes sharply increased beginning at 48 hr when radicle emergence occurred with *sh2* showing the greatest increase (Table 6).

Discussion

Laboratory and field seed germination and seedling vigor measurements conducted on *normal, bt,* and *su* seeds at different maturities showed that as the seed matured germination and vigor increased. However, *sh2* germinated better from 16 to 36 days post-pollination than at 42 days. Seedling vigor of *sh2* appeared to be highest at or before 36 days post-pollination and not at 42. Because of its high sugar content it was felt that pathogens might have preferentially attacked *sh2* seed (1) and that the longer the seed was in the field the more the pathogens

Table 6. ATP content of 42 day-old seed of 4 corn genotypes after various imbibition times.

Imbibition time (hrs)	ATP content (nmoles/seed)			
	bt	*sh2*	*su*	*normal*
0	4.0	12.6	6.0	1.3
2	1.7	13.0	1.7	1.4
4	2.1	1.8	1.8	1.2
8	1.8	3.6	1.8	.8
16	17.2	36.5	12.8	7.5
24	10.6	7.1	4.7	9.3
48	136.0	138.0	21.6	100.0
72	246.0	1200.0	394.0	986.0
96	87.0	194.0	152.0	68.0

adversely affected seed viability and vigor. Seeds that were harvested 42 days post-pollination were larger than those harvested on the 16th day. Large seeds probably contain more endosperm and therefore should have the capacity to produce more ATP initially or at least over a longer period of time than small seeds. However, averaged over all genotypes seeds imbibed for 4 hr had higher ATP concentrations during early maturity (18-20 days). ATP then dropped off at 22 days, and finally increased slightly at maturity (42 days). Thus, larger and more mature seeds did not produce the higher levels of ATP after 4 hr imbibition that may have been expected from the larger endosperms. When imbibition time was increased to 96 hr all lines had a similar pattern of ATP production. Since the ATP content was highest after 72 and 96 hr imbibition for the smallest endosperm genotype, *sh2,* it does not appear that the lack of ATP during early germination was a limiting factor leading to poor vigor in the *sh2* line.

In the present work, no determinations were made on the total adenylate pool (ATP, ADP, AMP) which would give an indication of the energy charge. The turnover rate of ATP may play an important role in distinguishing the vigor characteristics between *normal* and *sh2*. A higher turnover rate of ATP for *normal* as compared to *sh2* may account for the lower ATP levels found in *normal* in this research.

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Effect of Harvest Duration on Yield and on Depletion of Storage Carbohydrates in Asparagus Roots[1]

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Abstract. **Harvesting a young planting of asparagus (*Asparagus officinalis* L.) for 4 or 6 weeks the second year after transplanting 1-year-old crowns, followed by harvesting for 8 or 10 weeks the third year, reduced yields significantly the fourth year. Carbohydrate levels in asparagus storage roots decreased during harvest and continued to decrease after harvest during fern production. Carbohydrate levels increased in storage roots after stalks had matured, and were restored to preharvest levels by mid- to late summer. All treatments possessed comparable levels of storage carbohydrates by the end of the season. Asparagus storage carbohydrates were identified as fructose-oligosaccharides, which varied considerably in size, mobility, and percent fructose and glucose. The largest oligosaccharides were composed of ∿ 90% fructose, ∿ 10% glucose; molecular weights did not exceed 4,000.**

Asparagus yields have experienced a general decline in Michigan since 1970, although total production has increased because of expanded area planted (6). The reasons for the decline in yield are not well understood, but are probably due to environmental and biological stresses. The effects of

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