Respiration and Carbohydrate Metabolism during Germination of sh2 and Sh2 Sweet Corn Seed

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Abstract. Sweet corn (Zea mays var. rugosa L.) seed with the endosperm mutant shrunken-2 (sh2) often exhibit low seed vigor and poor field emergence. Seed respiration and carbohydrate metabolism during germination of supersweet ‘Jubilee’ (sh2) and sugary sweet ‘Jubilee’ (Sh2) were studied. There were no significant vigor differences expressed by isolated embryos from sh2 and sugary (Sh2) seeds, indicating similar embryo physiology. Respiration rates were higher in the sh2 genotype during early stages of germination (24 hours) while they declined later. The available sucrose originating from the endosperm reserves was depleted by day 4. This insufficiency of a sustained energy source due to rapid consumption and minimal stored reserves may limit subsequent seedling growth in the sh2 genotype.

Use of the endosperm mutant sh2 has successfully improved sweet corn fresh-market and processing quality. However, low seed vigor and poor field emergence are major problems associated with this mutant. Reduced seedling vigor in seed of endosperm mutants is usually correlated with increased levels of sugar in the kernel (Crane, 1964). It is still not clear, however, whether the low seed vigor is due to inadequate stored endosperm reserves, genetically inferior embryo, or differential carbohydrate metabolism during seed germination. Some disfunction in carbohydrate metabolism within the scutellum or axis may also exist in the sh2 genotype (Styer and Cantliffe, 1984). Starch degradation and mobilization during early germination in sh2 and su corn genotypes could adversely affect seedling vigor (Harts and DeMason, 1989). The importance of competent respiratory metabolism in the expression of seedling vigor in field crops such as barley (Hordeum vulgare L.) and dent corn has been widely reported (McDaniel, 1969; McDonald, 1975; Woodstock, 1973; Woodstock and Grabe, 1967). Respiration rates of germinating seed did not, however, account for differences in seedling growth between the sh2 genotype and its normal counterparts (Wann, 1980). ATP levels from 0 to 96 h of imbibition were generally similar and even slightly higher in sh2 than in other genotypes (Styer et al., 1980). The research we report here focused on the basic carbohydrate metabolism occurring during germination of sh2 and su sweet corn seed. The objectives were to 1) clarify whether the low seed vigor of sh2 sweet corn is due to an inferior embryo or endosperm effects, and 2) study respiratory and carbohydrate metabolism in the sh2:Sh2 genotypes during seed germination.

For these studies we used four seed lots of supersweet sweet corn (sh2) hybrid ‘Jubilee’ (Sh2), and three seed lots of normal sweet corn (su) hybrid ‘Jubilee’ as control. Seeds were provided by Rogers Brothers Seed Co., Boise, Idaho. A completely randomized design was used with four replications for the sh2 genotype and three replications for control.

Embryo culture. Embryos were excised from dry kernels, surface sterilized for 3 min with 2% sodium hypochlorite, and rinsed with sterile water before being planted on a Murashige and Skoog (MS) shoot multiplication cell culture medium (Sigma, St Louis). Twenty-five embryos were randomly selected from each seed lot and cultured on medium at 27C for 7 days. Shoots and roots were separated and their dry weights determined. An alternative embryo-culture method using moistened Kimpak (Kimberly-Clark, Neenah, Wis.) as the culture medium was also used to evaluate embryo vigor without additional nutrients. Embryos were surface sterilized, washed with sterile water, and grown in Kimpak-lined petri plates for 7 days at 27C.

Respiration. Respiration rates of germinating seeds were measured manometrically in a Gilson differential respirometer (Burris et al., 1971). Four replications of five kernels per seed lot were surface sterilized, washed in distilled water, and planted on moist Kimpak in the bottom of a Gilson flask; measurements were made at 0, 4, 8, 12, 24, 36, and 48 h. With potassium hydroxide (20% w/v) and a paper wick in the flask center well and after a 30-min equilibration, O2 uptake (microliters per hour per kernel) was measured at 28C. Respiration of excised embryos was measured as O2 depletion from a cuvette containing each embryo immersed in 5 ml of distilled water, using a Clark-type (YSI Model 53, Biological Oxygen Monitor, Yellow Springs, Ohio) electrode calibrated with an air-saturated, distilled-water at 28C. Embryos were cultured in moist Kimpak at 28C for 0, 3, 6, 9, 12, and 24 h. Eight randomly selected embryos of each seed lot were used to measure the O2 uptake at different stages of embryo germination.

Sugar analysis. Seeds were germinated on...
moist Kimpak at 28°C in the dark for 0, 1, 2, 3, 4, 6, and 8 days. Embryo and endosperm were separated for sugar analysis. Excised embryos were also germinated on moist Kimpak under the same conditions for sugar analysis. Sugars were extracted by homogenizing 10 embryos or endosperms in 10 ml of 85% ethanol with a Brinkmann homogenizer and centrifuging at 16,000 × g for 10 min. The ethanol supernatant was vacuum evaporated. Water was added to a final volume of 3.5 ml/extract. The extract was cleared by centrifuging 10 embryos or endosperms in 10 ml of 0.2-µm filter. Sugars were separated by high performance liquid chromatography with a LC-NH2 column, a mobile phase of 75 acetonitrile : 25 water, a flow rate of 1 ml·min⁻¹, and a refractive index detector (Chen and Burris, 1990).

The sh2 seeds had only half the kernel dry weight of control seed, while embryo dry weights were similar in both genotypes (data not shown). Standard warm germination (Assn. Official Seed Analysts, 1985) rates were high in both genotypes, indicating that the seeds were viable and capable of germinating under optimum conditions; however, seeds of the sh2 genotype germinated poorly in the cold germination test (Table 1).

Seedling vigor, determined by shoot and root dry weights, showed that the sh2 genotype had only one-third the seedling dry weight of the control in cold and warm germination tests (Table 1). Results of embryo culture in tissue culture medium and in sterile water (Kimpak as medium) showed no significant difference in embryo vigor, as indicated by shoot and root dry weight, between the two genotypes (data not shown). These results suggest that poor seedling vigor in the sh2 genotype may not be due to an inferior embryo. Embryos grew well, without fungal contamination, in nutrient-free moist Kimpak, further suggesting that embryos are vigorous. Growth of embryos on MS cell culture medium was not as good as on moist Kimpak. Further, fungal contamination was observed in the growth medium, and development of roots was suppressed. Therefore, all the seeds or embryos used for respiration and carbohydrate studies were surface sterilized after being removed from the fungi-free Kimpak culture.

The soluble sugar contents differed between tissues; sucrose and raffinose were found in the embryo and glucose and sucrose in the endosperm. Sucrose was the major sugar reserve in both tissues (Table 2). Glucose was mainly found in the endosperm of the control. Sugar composition and content of the embryo in both genotypes were similar, further confirming the effect of endosperm tissue on seedling vigor. Endosperm tissue of the sh2 genotype had three times more sucrose than that of control seed, which may result in a relatively slower initial respiration (Table 2). Respiration rates increased in control seed as degradation of endosperm reserves provided energy for new seedling growth (Fig. 1).

Use of carbohydrates during germination was studied by measuring levels of simple carbohydrates in both embryo and endosperm during dark germination. In embryo culture, sucrose decreased rapidly during the first day of germination, then remained generally low during the next 5 days in both genotypes. Similar sucrose levels in cultured embryos of sh2 and Sh2 correlate with the similarities in embryo vigor. Glucose concentrations within the embryo during culture were similar for sh2 and Sh2 genotypes (Fig. 3). Glucose and sucrose concentrations were much lower in excised embryos (Fig. 3) than in embryos of intact kernels during germination (Fig. 4). Thus, endosperm reserves appear to be critical to seed germination and seedling growth.

The concentration of sucrose in germinating seed decreased during the first 24 h of imbibition in both genotypes (Fig. 4), reflecting a rapid use of soluble sugar as a respiratory substrate by the embryo. Since rapid respiration starts earlier than endosperm degradation, preexisting mitochondria must function coincident with hydration of the embryo. Later, sucrose levels in the embryo of the control increased rapidly and reached a maximum following 6 days of germination. Rapid increases in sucrose in control seed embryos likely resulted from hydrolysis of reserves in the endosperm that were transported to the growing embryo. However, in the sh2 embryo, no further increase in sucrose content was found throughout 8 days of germination. Sucrose levels in
the endosperm of sh2 decreased dramatically after the initiation of germination (Fig. 5). Between days 4 and 5 of germination, sucrose was depleted to an undetectable level. Lack of energy after 4 days of germination resulted in a depressed respiration rate retarding the synthetic metabolism needed for normal seedling growth, as expressed by reduced seedling dry weight (Table 1). Levels of glucose were low in the endosperm of the sh2 genotype throughout 8 days of germination (Fig. 5). In the endosperm of the control, glucose increased dramatically following the start of germination and reached a maximum after 6 days, at which time the amount of glucose in endosperm of control was 10 times that of the sh2 genotype. This change was similar to the change in sucrose resulting from degradation of endosperm reserves (Fig. 5). Major soluble reserves (sucrose and glucose) may be limiting in the sh2 genotype during subsequent seedling growth when the seedling is still in a heterotrophic stage. Therefore, rapid respiration and depletion of embryo reserves coupled with a lack of endosperm reserves to provide a sustained energy supply would appear to be responsible for the low vigor of sh2 seed.

Literature Cited