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Sucrose Metabolizing Genes are Critical for Growth and Development of Maize Seed

By

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Summary

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Seed development is intimately dependent upon the metabolic utilization of sucrose. Our long-term studies have shown that two seed mutants in maize, miniature1 (mn1) and shrunken1 (sh1), are caused by mutations in seed-specific genes of sucrose metabolism. A deficiency of the Mn1-encoded cell wall invertase (CWI) enzyme leads to a drastic reduction in seed size identified as the mn1 seed mutation. We suggest that CWI plays a critical role in providing hexose sugars for mitotic divisions in the early stages of endosperm development. The CWI also controls endosperm sink strength and developmental stability of the maternal cells in pedicel. The sh1 seed is characterized by a collapsed crown of the mutant endosperm. The causal basis for the sh1 phenotype is a loss of the Sh1-encoded endosperm-specific sucrose synthase-1 (SS1) which cleaves sucrose to yield precursors for both cellulose and starch biosynthesis. Our recent studies show that the SS1 enzyme plays a predominant role of providing the substrate for cellulose biosynthesis; whereas, the second enzyme, SS2, appears to yield precursors for starch biosynthesis in a developing endosperm.

Introduction

Sucrose is the principal and preferred form of photosynthate for long distance transport to terminal storage sink tissues such as developing seeds in maize and other cereals. In addition to being a sole source of carbon for numerous metabolic processes and storage as starch, sucrose also serves as a signal molecule in regulating gene expression and normal development in plants (JANG & SHEEN 1997, SMEEKENS & ROOK 1997, WEBER & al. 1997). Despite such a global role of

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sucrose in metabolic and developmental biology, there are large gaps in our knowledge on specific genes and physiological processes that may be critical to the unloading and utilization of sucrose in economically important tissues such as developing seeds. In this regard, maize is a valuable resource due to a large repertoire of seed mutants generally categorized as shrunken, shriveled or miniature phenotypes. This report is focused on two seed mutants, *shrunken1* (*sh1*) and *miniature1* (*mn1*), that are clearly caused by impaired utilization of sucrose in the endosperms of developing seeds.

Deficiency of Cell Wall Invertase (CWI) causes Miniature1 (*mn1*) Seed Phenotype

The *mn1* seed mutation, first described by LOWE & NELSON 1946, is one of the most drastic nonlethal single gene seed mutations in maize. The homozygous recessive *mn1* mutant phenotype is specific to the seeds that show a loss of nearly 80% of the seed weight as compared to the wild type. Histological studies show that the development of the *mn1* kernels is normal up-to nine days after pollination (DAP); soon thereafter there is a withdrawal of maternal cells, producing a gap between the pedicel and the basal region of the endosperm. The loss of chalazal bridge in *mn1* kernels leads to near arrest of endosperm development at ~14 DAP after pollination (LOWE & NELSON 1946). Thus, the *mn1* seed mutation is developmentally unique in plants where a single gene, *Mn1*, affects both filial (the seed) and maternal (the pedicel) generations in a developing seed.

MILLER & CHOUREY 1992 have shown that the *mn1* seeds are deficient for a CWI that is specific to developing endosperm. Subsequent analyses, including the isolation of new *mn1* mutants and the cloning of endosperm-specific CWI gene, *Incw2*, have yielded critical evidence that the *Mn1* gene is a structural gene for the CWI protein (CHENG & al. 1996). These genetic studies document for the first time that CWI is critical for endosperm development. Remarkably, WEBER & al. 1996 have shown in *Vicia faba* that greater CWI activity and high hexose levels in cotyledonary cells are correlated with extended mitotic activity and ultimately, greater sink activity. Similarly, an increased tuber size in transgenic potato is associated with an increase in the apoplastic expression of yeast invertase (SONNEWALD & al. 1997). In maize, the *Mn1* encoded CWI is temporally the first enzyme to metabolize the incoming sucrose, and its highest levels are seen at 12 DAP (CHENG & al. 1996), a stage that coincides with the cell division phase in maize endosperm (KISSELBACH 1949).

The endosperm CWI activity also has a critical role in the developmental stability of placento-chalazal cells that determine the anatomical continuity between endosperm and the pedicel (CHENG & al. 1996). Why the invertase-deficiency in an endosperm causes such anatomical abnormality in the pedicel is unclear. It has been suggested that a lack of sucrose utilization in endosperm may cause sucrose accumulation and a transient osmotic imbalance in the pedicel, and consequently the degeneration of maternal cells in the early stages of seed

development (MILLER & CHOUREY 1992). Thus, the role of CWI in seed development can be regarded as a major metabolic switch which controls both the appropriate partitioning of carbon in the developing endosperm and also the developmental fate of the maternal placento-chalazal cells in the pedicel.

Deficiency of Sucrose Synthase (SS) causes the Shrunken1 (sh1) Seed Phenotype

The enzyme sucrose synthase catalyzes a reversible conversion of sucrose and UDP to UDP-Glucose and fructose. It also plays a major role in energy metabolism by mobilizing sucrose into diverse pathways relating to metabolic, structural, and storage functions of plant cells. The enzyme is well analyzed in many plant species; however, the most detailed analyses have been done in maize. Briefly, the two biochemically similar isozymes, SS1 and SS2, are encoded by molecularly homologous genes *Sh1* and sucrose synthase1 (*Sus1*) loci, respectively (see: CHOUREY & al. 1998, and references therein). The *Sh1*-encoded SS1 protein is present in most abundant levels in developing endosperm and a mutational loss of this isozyme is the causal basis of endosperm-specific *shrunken1* seed phenotype (CHOUREY & NELSON 1976). The *Sus1*-encoded SS2 protein is also detected in both developing endosperm and embryo. Unlike the large number of *sh1* mutants, there is only a single mutant of the *Sus1* gene (CHOUREY & al. 1988).

The two SS Isozymes have Distinctive Roles in Cellulose and Starch Biosynthesis in a Developing Endosperm

The availability of mutants for the two SS loci and three possible genotypes, *Sh1Sus1*, *sh1Sus1*, and *sh1sus1-1*, have allowed us to assess the relative contributions of each isozyme in developing endosperm. Recent determinations of starch levels in lineage-related genotypes have shown reductions of only ~25% in the *sh1* mutant as compared to the wild type endosperm (SINGLETERY & al. 1997, CHOUREY & al. 1998). Several lines of evidence now suggest that starch deficiency per se is not the causal basis of the *sh1* seed phenotype. Instead, the recent data suggest that SS1 is critical for generating substrates for cellulose biosynthesis in developing endosperm (CHOUREY & al. 1998). In particular, the anatomical data on kernel sections show cell degeneration unique to the *sh1* mutant at 12 DAP. No such cellular loss is seen in the normal, *Sh1Sus1* kernels or in the *sh2* mutant which accumulates only ~20 to 30% of the wild type levels of starch (CHOUREY & al. 1998). Cell degeneration is restricted to the centrally located starch storage cells, and it occurred prior to the onset of the rapid phase of starch biosynthesis which is known to initiate at ~12 DAP. The 12 DAP stage is also marked by the end of mitosis and the beginning of cell elongation (KISSELBACH 1949) and

endoreduplication or polytenization of chromosomes in endosperm cells (SCHWEIZER & al. 1995).

Although the SS catalyzes a readily reversible reaction, the sucrose breakdown reaction leading to UDP-Glucose synthesis is considered to be the more important *in vivo* function in endosperm. Because UDP-Glucose is a common precursor for starch and cellulose biosynthesis, the drastic reductions of SS activity, respectively >90 and 99% in *sh1Sus1* and *sh1sus1-1* genotypes as compared to the *Sh1Sus1* genotype, affects both the pathways. Such reductions in the SS activity, coincident with increased demands of cellulose during the cell elongation phase and the onset of starch biosynthesis, must create competing demands for UDP-Glucose. We have suggested that a rate limiting flux of UDP-Glucose in cellulose biosynthesis during cell elongation is the causal basis of the cell degeneration process, and ultimately the *sh1* seed phenotype (CHOUREY & al. 1998).

Reduced levels of endosperm starch content in the double mutant, *sh1sus1-1*, as compared to the single gene mutant, *sh1Sus1*, (53 and 78%, respectively, and 100% in the *Sh1Sus1*) suggests that the SS2 protein may perform a rate-limiting role in starch biosynthesis (CHOUREY & al. 1998). In addition, the SS1 must yield precursors for starch biosynthesis as evidenced by the reduced size of endosperm starch grains in the *sh1* mutant relative to the wild type (CHOUREY & al. 1998). An overall role of the SS enzyme in starch biosynthesis is also observed in transgenic potato tubers. Antisense inhibition of the SS genes that show a loss of up to ~98% of the SS activity is associated with a reduction of ~66% starch content as compared to the control tubers (ZRENNER & al. 1995). Whether or not there is any effect on cellular stability in such transgenic tubers is not known.

A c k n o w l e d g e m e n t s

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