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Maize *SUT1* functions in phloem loading

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The functions of dicot sucrose transporters (SUTs) in apoplastic phloem loading of sucrose are well established; however, whether SUTs similarly function in monocots was unresolved. To address this question, we recently provided genetic evidence that *ZmSUT1* from maize (*Zea mays*) is required for efficient phloem loading. *sut1-m1* mutant plants hyperaccumulate carbohydrates in leaves, are defective in loading sucrose into the phloem, and have altered biomass partitioning. Presumably due to the hyperaccumulation of soluble sugars in leaves, mutations in *ZmSUT1* lead to downregulation of chlorophyll accumulation, photosynthesis and stomatal conductance. However, because we had identified only a single mutant allele, we were not able to exclude the possibility that the mutant phenotypes were instead caused by a closely linked mutation. Based on a novel aspect of the *sut1* mutant phenotype, secretion of a concentrated sugar solution from leaf hydathodes, we identified an additional mutant allele, *sut1-m4*. This confirms that the mutation of *SUT1* is responsible for the impairment in phloem loading. In addition, the *sut1-m4* mutant does not accumulate transcripts, supporting the findings reported previously that the original mutant allele is also a null mutation. Collectively, these data demonstrate that *ZmSUT1* functions to phloem load sucrose in maize leaves.

Key words: carbohydrate partitioning, guttation, hydathode, maize, phloem loading, *sucrose transporter1, sut1-m4*

Abbreviations: SUT, sucrose transporter; *Mu*, *mutator*; UTR, untranslated region; *Ds*, *dissociation*

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Introduction

Most plant species translocate sucrose as the reduced form of carbon from source leaves to nonphotosynthetic sink tissues.[1,2] In apoplastic phloem loading species, sucrose transporters (SUTs) import sucrose from the apoplast into the companion cells and/or sieve elements.[3-5] In dicot plants, genetic and biochemical evidence has shown that SUTs function to phloem load sucrose in leaves.[6-10] However, it was unclear which SUTs perform this role within monocots. We recently obtained genetic evidence suggesting that maize *SUT1* functions in phloem loading in leaves.[11]

Using reverse genetics, we obtained a *Mutator* (*Mu*) transposable element insertion into the 5' UTR of the *SUT1* gene (referred to as *sut1-m1*).[11] Plants homozygous for the *sut1-m1* mutation have greatly diminished growth, altered biomass partitioning, reduced fertility, hyperaccumulate carbohydrates within mature leaves, and fail to load ^{14}C-labeled sucrose into the veins.[11] Here we describe further aspects of the mutant phenotype directly related to the inability to phloem load sucrose in source leaves, and we report the characterization of additional mutant alleles, which confirm the phenotypes result from the loss of *SUT1* function.

Inhibition of Phloem Loading Leads to Secretion of Soluble Sugars from Maize Leaves

Under normal growing conditions, wild-type maize plants secrete droplets of water from specialized structures, called hydathodes,[12] when water potential in roots is greater than that in leaves (**Fig. 1A**). This process is known as guttation.[13-15] Typically, guttation fluids contain only trace amounts of reduced carbon compounds and rapidly evaporate

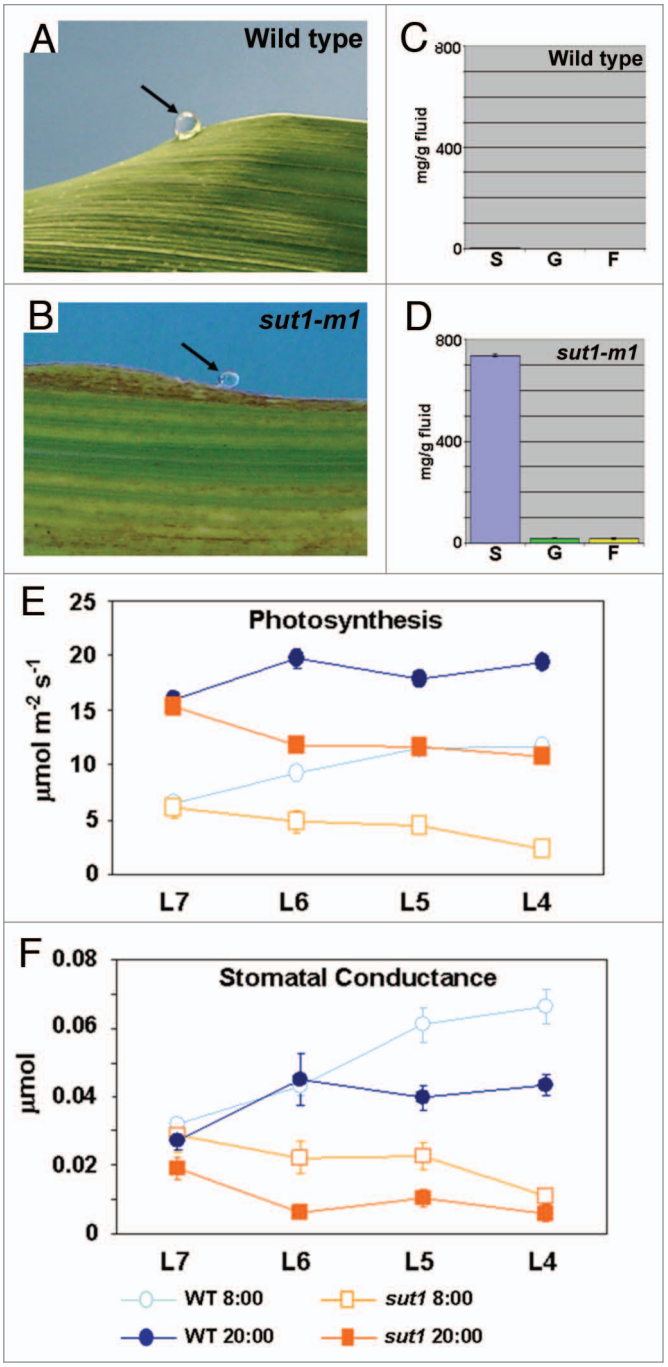

Figure 1. Additional phenotypes of *sut1* mutant plants. (A and B) Photographs of guttation fluid on leaf margins. (C and D) Graphs of quantifications of sucrose (S), glucose (G) and fructose (F) in guttation fluid. (A and C) Wild type. (B and D) *sut1-m1* mutant. (E and F) Measurements of photosynthesis (E) and stomatal conductance (F) rates of wild-type and *sut1-m1* mutant leaves. L7-L4 indicate leaves seven through four on three-week-old plants. Circles represent wild type and squares represent *sut1* mutants. Open symbols indicate data collected at 8 AM and filled symbols indicate data collected at 8 PM (described in ref. 11).

from the leaf surface shortly after dawn. Interestingly, we observed that *sut1-m1* mutant leaves retained guttation fluids on their surface (**Fig. 1B**). This was also seen in wild-type plants that had been cold-girdled,[11] a process that results in inhibition of phloem transport (data not shown). This novel phenotype was not observed for the many other maize leaf carbohydrate hyperaccumulation mutants.[16-25] Guttation fluid was collected from untreated wild-type sibling and *sut1-m1* mutant plants and assayed for soluble sugar concentrations. Wild-type guttation fluid contained very low sugar concentrations, whereas guttation fluid from *sut1-m1* leaves contained over 700 mg of sucrose/g of fluid and 20–25 mg each of glucose and fructose/g of fluid (**Fig. 1C and D**). We infer that both the loss of *SUT1* function and the inhibition of phloem transport by girdling result in the build-up of apoplastic sucrose that subsequently migrates into the xylem transpiration stream. The presence of the relatively equal amounts of glucose and fructose in the guttation fluid is likely explained by cell wall invertases that cleave sucrose into the two monosaccharides.[26]

Accumulation of Carbohydrates in *sut1* Mutant Leaves is Associated with Decreased Photosynthetic and Stomatal Conductance Rates

High levels of photoassimilates are known to feedback and negatively regulate photosynthesis.[27,28] In *sut1-m1* mutants, photosynthetic and stomatal conductance rates are comparable to wild-type plants only in leaves emerging from the whorl (leaf 7 in **Fig. 1E and F**), which are in the process of transitioning to source tissue.[11] Once leaves become sources, photosynthesis and stomatal conductance rates drastically decline in the *sut1-m1* mutant relative to wild type (leaves 4–6 in **Fig. 1E and F**), presumably due to the excess accumulation of carbohydrates downregulating photosynthesis. However, even with greatly elevated carbohydrate levels, *sut1* mutant leaves perform limited photosynthesis and gas exchange.

Phenotypes Observed in the *sut1* Mutant Plants are Representative of Null Mutations in the *SUT1* Gene

Based on the novel phenotype of the concentrated sucrose solution being secreted from leaf margins (**Fig. 1B**), an additional mutant allele of *ZmSUT1* (*sut1-m4*) was independently isolated. The *sut1-m4* allele was recovered from a *Ds* transposable element remobilization

screen in the W22 inbred background. The new mutant had an identical phenotype to that reported for the *sut1-m1* mutant, including leaf chlorosis and carbohydrate hyperaccumulation (**Fig. 2A–D**). PCR and DNA sequence analysis revealed that the *Ds* transposon inserted into the 5' UTR of *ZmSUT1*, 11 basepairs downstream of the *Mu* insertion site in the *sut1-m1* allele.[11] By RT-PCR analyses on RNA isolated from mature leaves of *sut1-m4* plants, no *SUT1* RNA was detected, indicating that the *sut1-m4* allele is an RNA null (**Fig. 2E and F**). These data are in agreement with both the *sut1-m1* and *sut1-m4* transposable element insertion alleles being nulls. Furthermore, these data demonstrate that the loss of *SUT1* function is responsible for the phenotypes observed, and that SUT1 functions to load sucrose into the phloem in maize leaves. Finally, we note that two other *SUT1 Ds* insertion alleles are available (www.plantgdb.org/prj/AcDsTagging/), one located in the promoter that confers no obvious phenotype [*sut1-m2*, #I.S06.0327_JSR05], and a second in the 5' UTR 66 basepairs downstream of the *Mu* insertion in the *-m1* allele, which has an identical mutant phenotype to the *sut1-m1* and *sut1-m4* plants [*sut1-m3*, #I.S06.1500_JSR05].

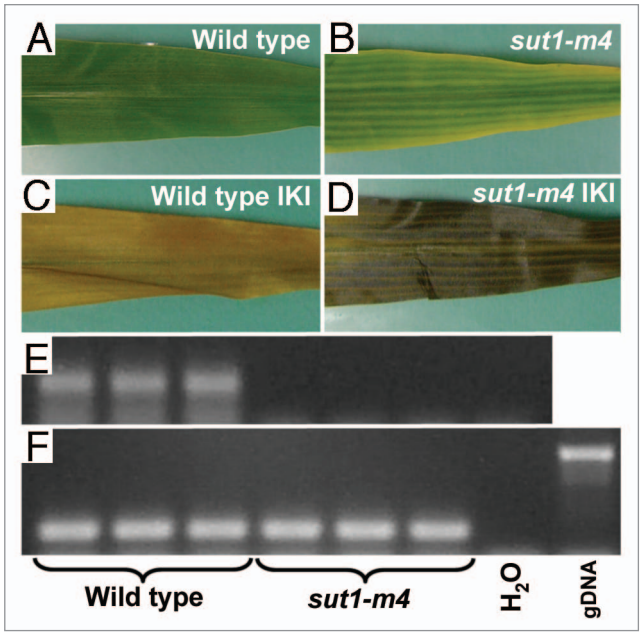

Figure 2. *sut1-m4* mutant leaves contain excess starch and do not accumulate transcripts. (A and B) Photographs of leaves. (C and D) Same leaves cleared of photosynthetic pigments and starch (IKI) stained. (A and C) Wild type. (B and D) *sut1-m4* mutant. (E and F) RT-PCR on three biological replicates isolated from wild-type and *sut1-m4* mutant leaves. (E) RT-PCR with *SUT1*-specific primers. (F) RT-PCR with *ubiquitin* primers as a cDNA normalization control (described in ref. 11).

Implications and Future Work

Though debilitated, *sut1* mutant plants are viable. This suggests that there may be partial genetic redundancy, and that one of the other six *SUT* genes in maize may be able to load sucrose into the phloem, albeit not as efficiently as SUT1. Alternatively, in the absence of *SUT1* function, sucrose may enter the phloem via another route, such as symplastically.[2,5,11] Plasmodesmata are present at all cellular interfaces between mesophyll cells and sieve elements, although their numbers were reported as "scarce" between vascular parenchyma cells and companion cells.[29] A similar hypothesis was recently proposed for *Arabidopsis thaliana* plants mutated in the principal *SUT* responsible for phloem loading.[9] Indeed, symplastic phloem loading of sucrose appears to be more widespread in plants than previously appreciated.[1,2] Future work examining compensatory changes in the expression of *SUT* family members in maize *sut1* mutants, and by analyzing double and higher order *sut* mutant combinations will address these possibilities.

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