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RESEARCH ARTICLE

Genome-wide identification, characterization, and expression analysis of the *SWEET* gene family in cucumber

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Abstract

SWEETs (sugars will eventually be exported transporters) are a novel class of recently identified sugar transporters that play important roles in diverse physiological processes. However, only a few species of the plant *SWEET* gene family have been functionally identified. Up till now, there has been no systematic analysis of the *SWEET* gene family in Cucurbitaceae crops. Here, a genome-wide characterization of this family was conducted in cucumber (*Cucumis sativus* L.). A total of 17 *CsSWEET* genes were identified, which are not evenly distributed over the seven cucumber chromosomes. Cucumber SWEET protein sequences possess seven conserved domains and two putative serine phosphorylation sites. The phylogenetic tree of the *SWEET* genes in cucumber, *Arabidopsis thaliana*, and *Oryza sativa* was constructed, and all the *SWEET* genes were divided into four clades. In addition, a number of putative *cis*-elements were identified in the promoter regions of these *CsSWEET* genes: nine types involved in phytohormone responses and eight types involved in stress responses. Moreover, the transcript levels of *CsSWEET* genes were analyzed in various tissues using quantitative real-time polymerase chain reaction. A majority (70.58%) of the *CsSWEET* genes were confined to reproductive tissue development. Finally, 18 putative watermelon *ClaSWEET* genes and 18 melon *CmSWEET* genes were identified that showed a high degree of similarity with *CsSWEET* genes. The results from this study provided a basic understanding of the *CsSWEET* genes and may also facilitate future research to elucidate the function of *SWEET* genes in cucumber and other Cucurbitaceae crops.

Keywords: cucumber, gene expression, phylogenetic analysis, sugar transporter, *SWEET*, watermelon

1. Introduction

Soluble sugars, which are the primary products of photosynthesis, can be used as sources of carbon skeletons for the biosynthesis of many other cellular compounds, signals, osmolytes, and transport molecules, and they can be used as transient energy storage (Chen *et al.* 2015a). In plants, sugars are synthesized in source leaves during photosynthesis and are eventually delivered to various heterotrophic tissues such as roots, seeds, and fruits to enable their

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growth and development (Lalonde *et al.* 2004; Chen *et al.* 2014; Ruan 2014). Not surprisingly, this cellular exchange of sugars requires transport proteins to mediate uptake or release from cells or subcellular compartments (Chen *et al.* 2015a). Thus far, three families of eukaryotic sugar transporters have been identified as key players, namely, monosaccharide transporters (MSTs), sucrose transporters (SUTs), and *SWEETs* (sugars will eventually be exported transporters) (Eom *et al.* 2015). It is now widely accepted that these sugar transporters play key roles in the entire plant sugar translocation and allocation, and they significantly determine the crop output and quality (Kühn and Grof 2010; Ayre 2011; Slewinski 2011; Chen *et al.* 2015a; Eom *et al.* 2015).

MSTs and SUTs belong to the major facilitator superfamily and are characterized by 12 transmembrane domains (Kühn and Grof 2010; Ayre 2011; Slewinski 2011). *SWEETs* are a recently identified family of sugar efflux transporters that selectively transport monosaccharides or disaccharides across intracellular or plasma membranes, and they belong to the MtN3/saliva family (Pfam code PF03083) (Chen *et al.* 2010; Xuan *et al.* 2013; Chen 2014; Eom *et al.* 2015). *SWEETs* are widely distributed in various organisms, including prokaryotes, animals, and plants (Yuan and Wang 2013; Patil *et al.* 2015). It has been predicted that eukaryotic SWEET proteins typically comprise seven α-helical transmembrane (TM) domains that are organized as tandem repeats of two 3-TM domains (containing two conserved MtN3/saliva motifs) that are separated by a single TM that is less conserved (Xuan *et al.* 2013). The resulting structure has been described as the 3-1-3 TM SWEET structure (Chen *et al.* 2010). In contrast to eukaryotic SWEET proteins, the prokaryotic SWEET proteins, which are known as SemiSWEETs, contain only one 3-TM, possibly indicating that eukaryotic SWEETs have evolved *via* duplication and fusion of the basic 3-TM unit that is present in prokaryotic SemiSWEETs (Xuan *et al.* 2013).

In plants, a growing body of evidence suggests that *SWEETs* are involved in many significant physiological and biochemical processes where sugar efflux plays a vital role, such as phloem loading for long-distance sugar transport, nectar secretion, pollen nutriture, seed filling, plant-pathogen interaction, and biotic and abiotic stress regulation (Chen 2014; Chandran 2015; Chen *et al.* 2015a; Eom *et al.* 2015). In *Arabidopsis* (*Arabidopsis thaliana*), *AtSWEET11* and *AtSWEET12*, which localize to the plasma membrane of phloem parenchyma cells and export sucrose from these cells into the apoplast, are responsible for the first step of phloem loading of sucrose in long-distance transport (Chen *et al.* 2012). The nectar-specific *AtSWEET9* is essential for nectar production and can function as a sucrose efflux transporter (Lin *et al.* 2014). Similarly, the *NEC1* gene isolated from *Petunia* is thought to play a role in nectar secretion because it is highly expressed in nectar cells (Ge *et al.* 2000, 2001). Additionally, many *SWEET* genes, such as *AtSWEET5* (also called *VEX1*), which is expressed at different stages of pollen development (Engel *et al.* 2005), *AtSWEET8* (also called *RPG1*), which is strongly expressed in microspores and tapetum during male meiosis (Guan *et al.* 2008), and *AtSWEET13* (also called *RPG2*), which is highly expressed in anthers (Sun *et al.* 2013), play a broader role in pollen nutriture. In rice (*Oryza sativa*), *OsSWEET5* is also expressed in anthers (Zhou *et al.* 2014). *SWEETs* were also shown to be involved in seed filling. *AtSWEET11*, *AtSWEET12*, and *AtSWEET15* (also called *SAG29*) exhibit specific spatiotemporal expression patterns in developing seeds of *Arabidopsis*, but only the triple knockout mutant showed severe seed defects, including retarded embryo development, reduced seed weight, and lower starch and lipid content, causing a wrinkled seed phenotype at maturity (Chen *et al.* 2015b). In contrast with *Arabidopsis*, seed filling in domesticated maize (*Zea mays*) and rice depends on hexose transport across the basal endosperm transfer layer. This progress is controlled by *ZmSWEET4c* in maize and *OsSWEET4* in rice (Sosso *et al.* 2015). Moreover, *SWEETs* are the targets of extracellular pathogens that modify the expression of *SWEETs* to gain sugars to fuel their own growth and reproduction (Yuan *et al.* 2009; Chen *et al.* 2010; Streubel *et al.* 2013; Chong *et al.* 2014; Cohn *et al.* 2014; Hu *et al.* 2014; Chen *et al.* 2015). *SWEETs* may also play important roles in biotic and abiotic stress tolerance. For example, *AtSWEET15* is obviously induced during leaf senescence and osmotic stresses, including high salinity, cold, and drought, *via* an abscisic acid-dependent pathway (Seo *et al.* 2011). The *AtSWEET15*-overexpressing transgenic plants display an accelerated leaf senescence and are hypersensitive to high salinity stress, whereas the *atsweet15*-deficient mutant lines are less sensitive to high salinity (Seo *et al.* 2011). Studies have revealed that *AtSWEET16* and *AtSWEET17* are the primary factors in controlling glucose or fructose content in the tonoplasts of *Arabidopsis* leaves and roots, especially under cold or low nitrogen supply conditions (Chardon *et al.* 2013; Klemens *et al.* 2013; Guo *et al.* 2014). More recently, studies have shown that *AtSWEET11* and *AtSWEET12* are also involved in cold stress or water deficit conditions (Hir *et al.* 2015; Durand *et al.* 2016). The *AtSWEET11;12* double mutant exhibited greater freezing tolerance than the wild-type and both single mutants (Hir *et al.* 2015). Additionally, the transcript levels of *AtSWEET11*, *AtSWEET12*, and *AtSUC2*, which are all involved in sucrose phloem loading, are largely up-regulated in source leaves under water deficit condition (Durand *et al.* 2016). This is in agreement with the