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Molecular and functional characterization of *Bemisia tabaci* aquaporins reveals the water channel diversity of hemipteran insects

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ABSTRACT

The Middle East-Asia Minor 1 (MEAM1) whitefly, *Bemisia tabaci* (Gennadius) is an economically important pest of food, fiber, and ornamental crops. This pest has evolved a number of adaptations to overcome physiological challenges, including 1) the ability to regulate osmotic stress between gut lumen and hemolymph after imbibing large quantities of a low nitrogen, sugar-rich liquid diet; 2) the ability to avoid or prevent dehydration and desiccation, particularly during egg hatching and molting; and 3) to be adapted for survival at elevated temperatures. One superfamily of proteins involved in the maintenance of fluid homeostasis in many organisms includes the aquaporins, which are integral membrane channel proteins that aid in the rapid flux of water and other small solutes across biological membranes. Here, we show that *B. tabaci* has eight aquaporins (BtAqps), of which seven belong to the classical aquaporin 4-related grade of channels, including Bib, Drip, Prip, and Eglps and one that belongs to the unorthodox grade of aquaporin 12-like channels. *B. tabaci* has further expanded its repertoire of water channels through the expression of three BtDrip2 amino-terminal splice variants, while other hemipteran species express amino- or carboxyl-terminal isoforms of Drip, Prip, and Eglps. Each BtAqp has unique transcript expression profiles, cellular localization, and/or substrate preference. Our phylogenetic and functional data reveal that hemipteran insects lost the classical *glp* genes, but have compensated for this by duplicating the *eglp* genes early in their evolution to comprise at least three separate clades of glycerol transporters.

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1. Introduction

Aquaporins belong to the major intrinsic protein family and function as bidirectional transport channels of water or other small solutes across biological membranes (Benga, 2009; Campbell et al., 2008; Murata et al., 2000; King et al., 2004; Gomes et al., 2009). Aquaporins are widely distributed in all kingdoms of life (Abascal et al., 2014; Finn and Cerdà, 2015) and play numerous essential physiological roles, particularly in regulating cell water balance. Generally, aquaporins consist of six transmembrane (TM) alpha helices connected through five loops (A-E) in an "hourglass" form (Jung et al., 1994). The amino- and carboxyl-termini are located on the cytoplasmic side of the membrane and each channel consists of two similar halves formed by a tandem repeat. Loops B

Abbreviations: Aqp, aquaporin; ar/R, aromatic arginine; Bib, big brain; BtAqp, *Bemisia tabaci* aquaporin; cDNA, complementary DNA; CDS, coding sequence; cRNA, complementary RNA; Drip, *Drosophila* integral protein; EGFP, enhanced green fluorescent protein; Eglp, entomoglyceroporin; ER, endoplasmic reticulum; EST, expressed sequence tag; Glp, aquaglyceroporin; hAQP, human aquaporin; MEAM1, Middle East-Asia Minor 1; MBS, modified Barth's media; MW, molecular weight; NPA motif, asparagine-proline-alanine motif; ORF, open reading frame; PCR, polymerase chain reaction; pI, isoelectric point; PMSF, phenylmethylsulfonyl fluoride; Prip, *Pyrocoelia rufa* integral protein; RACE, rapid amplification of cDNA ends; RT-PCR, reverse transcriptase polymerase chain reaction; SRA, Short Read Archive; TM, transmembrane.

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(cytoplasmic) and E (extracellular) both contain the signature Asn, Pro, Ala (NPA) motif, and these two loops fold back into the channel from opposite sides of the membrane to form a seventh "broken" half membrane helix with the NPA motif at the center of the channel (Agre et al., 1993; Finn and Cerdà, 2015; Sui et al., 2001; Törnroth-Horsefield et al., 2010; Zardoya, 2005). Solute selectivity is determined by the constriction region at the extracellular side of the channel formed by an aromatic Arg (ar/R) selectivity filter (Beitz et al., 2006; Gonen and Walz, 2006; Jung et al., 1994). While each monomeric aquaporin can independently function as an individual water pore, they normally oligomerize as tetramers in the cell membrane forming a central pore, which in some cases can function as an ion channel (Agre et al., 1993; Gomes et al., 2009; Yool and Weinstein, 2002).

Eukaryotic aquaporins are divided into four major grades, including the classical aquaporins, the aquaporin 8-type aquaamoniaporins, the aquaporin 11/aquaporin 12-type unorthodox channels, and the aquaglyceroporins (Glps) (Finn and Cerdà, 2015). Previous attempts to classify the increasing number of arthropod aquaporins have provided several somewhat ambiguous groupings, including the identification of three subfamilies (Campbell et al., 2008), four groups (Kambara et al., 2009; Goto et al., 2011), or two to five clades (Wallace et al., 2012; Fabrick et al., 2014; Jing et al., 2016). More recent classification suggests that insects have aquaporins belonging to three major grades, including the classical aquaporins, the Glps, and the unorthodox aquaporin 12-like (Aqp12L) proteins (Finn et al., 2015; Stavang et al., 2015). The classical arthropod aquaporins include genes previously characterized as Clade A of the classical insect aquaporins (Wallace et al., 2012; Fabrick et al., 2014; Jing et al., 2016) and encompass four major subfamilies, including the *Drosophila* integral protein (Drip)-like aquaporins (Kaufmann et al., 2005), the *Pyrocoelia rufa* integral protein (Prip)-like aquaporins (Lee et al., 2001), the big brain proteins (Bib) (Campbell et al., 2008) and the recently described entomoglyceroporins (Eglps) (Finn et al., 2015). These Eglps, which can transport water, urea, and a range of polyols including glycerol, evolved through mutation of the conserved His in the ar/R selectivity filter of water-selective channels and are phylogenetically more closely related to the classical aquaporin 4-type channels than to the Glps (Finn et al., 2015). Furthermore, while the Glps are prevalent in the older lineages of hexapods, several modern lineages, including the holometabolous insects, appear to have lost Glps and alternatively possess expanded clusters of *eglp* genes (Finn et al., 2015).

Due to increased availability of sequencing data from insects, a number of unorthodox Aqp12L aquaporins are now being identified (Fabrick et al., 2014; Finn et al., 2015; Stavang et al., 2015). The Aqp12L channels are distinguished by the fact that they are not transported to the plasma membrane when expressed in *Xenopus laevis* oocytes, contain a non-canonical NPC motif, and the Arg in the ar/R selectivity filter is replaced by a Leu (Morishita et al., 2005; Gorelick et al., 2006; Finn and Cerdà, 2015). The actual function of such Aqp12L channels in insects and other arthropods remains unknown.

Insect aquaporins are involved in many physiological processes, including some that enable insects to process large volumes of liquid diet and overcome extreme conditions such as temperature, osmotic pressure due to sugar-rich diets, and desiccation (Campbell et al., 2008; Cohen, 2012, 2013; Spring et al., 2009). Therefore, they have potential as novel targets for pest control (Douglas, 2006; Cohen, 2013). One of the most common and costliest pests of the agricultural world is the Middle East-Asia Minor 1 (MEAM1) whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) (hereafter denoted as *B. tabaci*). This pest harms crops by feeding on large quantities of phloem sap and excreting copious amounts of honeydew, which reduces postharvest quality (e.g. "sticky cotton") and promotes growth of sooty mold fungi, and by transmitting pathogenic viruses, such as the agriculturally important Begomoviruses (Byrne and Bellows, 1991; Navas-Castillo et al., 2011).

Because *B. tabaci* ingests large volumes of sugar-rich, amino acid deficient phloem sap (Douglas, 2006), the high simple sugar content of phloem results in osmotic stress placed on the tissues of the alimentary system. Whiteflies possess both biochemical and physiological mechanisms to help alleviate the problem of both large quantities of liquid diet and osmotic stress. Enzymatic transformation of sugars from high to lower osmotic potential is thought to reduce osmotic potential in the whitefly gut (Salvucci, 2003). Whiteflies also use a filter chamber to transfer water between the proximal and distal gut regions (Goodchild, 1966; Cicero et al., 1995; Douglas, 2006; Hubert et al., 1989) that likely enables rapid removal of excess fluid and helps decrease osmotic potential. We previously demonstrated that a *B. tabaci* aquaporin (referred to as BtAQP1) functions as a water-specific Drip channel that is highly expressed in the filter chamber (Mathew et al., 2011). Here, we provide molecular and functional characterization of eight *B. tabaci* aquaporins (including the previously described BtDrip1).

Using the classification system of Finn and Cerdà (2015), we show that whiteflies and other hemipterans lack classical Glps and have evolved multiple *eglp* genes. *B. tabaci* has an extended repertoire of three Eglps, which together with two Drips, a Prip, and a Bib comprise the *B. tabaci* classical aquaporins. *B. tabaci* also contains one unorthodox Aqp12L. Functional oocyte swelling assays show specificity of the BtAqps for water and glycerol, and together with expression profiling and cellular localization, the potential importance of the superfamily for nutrient and water homeostasis.

2. Materials and methods

2.1. Insects

A *B. tabaci* colony was maintained on broccoli (*Brassica oleracea*, Italica Group) inside a 0.9 × 0.9 × 2 m^3 cage in a greenhouse (maintained between 21 and 32 °C with ambient photoperiod).

2.2. Molecular cloning of BtAqps

Both full-length and partial BtAqp sequences were identified by direct query searches and local BLAST searches of the University of Arizona *B. tabaci* sequence database (maintained by Judith Brown) and from the Short Read Archive (SRA) databases SRX022878 and SRA036954 published by Wang et al. (2010) and Xie et al. (2012), respectively. The full-length *Btdrip1* cDNA (EU127479.1), previously referred to as BtAQP1, was obtained from an earlier study (Mathew et al., 2011). We verified variant forms of *Btdrip2* present in SRAs using primers 28BtAQP3, 29BtAQP3, and 61BtAQP3 in 5′-RACE as previously described by Mathew et al. (2011). Sequences with full-length open reading frames (ORFs) corresponding to BtPrip, BtEglpA, BtEglpB1, BtEglpB2, and BtAqp12L were identified from either the University of Arizona *B. tabaci* sequence database or SRAs and were verified by cloning from cDNA. A partial fragment corresponding to *Btbib* was present in the SRAs. Whereas 3′-RACE with nested primers 31BtAQP5 and 32BtAQP5 provided the 3′-end of *Btbib*, several attempts using 5′-RACE failed to provide a complete cDNA 5′-end.

Full-length *Btaqp* coding sequences (CDS) corresponding to ORFs were confirmed by PCR amplification from *B. tabaci* adult cDNA using primer pairs (Table 1). All products corresponding to full-length CDS were PCR amplified using ExTaq DNA polymerase (Takara-Clontech, Mountain View, CA) and products were electrophoresed on a 1% agarose gel and visualized using SYBR Safe (Life Technologies, Carlsbad, CA). Bands were gel-purified using