The Roles of Cotton (*Gossypium hirsutum*) Aquaporins in Cell Expansion

by

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DECLARATION

Statement of Originality

The thesis contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968.

Mr. Youmin Zhu

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ABBREVIATIONS

Ψg	Gravitational force
Ψp	Hydrostatic pressure (turgor)
Ψs	Osmotic (solute) potential
Ψw	Water potential
ABA	Abscisic acid
AQP	Aquaporin
AUX1	Auxin transporter protein 1
C	Cotyledon
CBF3	CRT–binding factor 3
cRNA	Complementary RNA
CTAB	Cetyl Trimethyl Ammonium Bromide
DEPC	Diethyl parocarbonate
DAA	Days after anthesis
DNA	Deoxyribose nucleic acid
dNTP	DeoxynucleoSide Triphosphate
DTT	Dithiothreitol
EDTA	Ethylene diaminetetraacetic acid
EST	Expressed Sequence Tags
ETR1	Ethylene receptor 1
ETO1	Ethylene–overproduction protein 1
GFP	Green fluorescent protein
GUS	β–Glucuronidase
ICAC	International cotton advisory committee
KEA5	K^+ efflux antiporter 5
KUP5	K^+ uptake permease 5
LB	lysogeny broth culture medium
L_p	Hydraulic conductivity
MIP	Major Intrinsic Protein
mRNA	Massager RNA
NCBI	National Center for Biotechnology Information
NIP	NOD26–like Intrinsic Protein
OD	Optical density
ORF	Open Reading Frame
PCR	Polymerase Chain Reaction
PD	Plasmodesmata
PFN1	Profilin 1
PIP	Plasma membrane intrinsic protein
PRP3	Proline–rich protein 3
PVP	Polyvinylpyrrolidone
Q–PCR	Quantitative PCR
Rd29A	Responsive to desiccation 29A
RHD6	Protein ROOT HAIR DEFECTIVE6
RNA	RiboNucleic Acid
SAT32	Protein salt tolerance 32
SDS	Sodium dodecyl sulphate
STP1	Sugar transporter 1
SUT	Sucrose transporter
Taq	Thermus Polymerase
T–DNA	Transfer DNA
TE	Tris–EDTA–NaCl buffer
TIP	Tonoplast intrinsic protein
Tris	Tris （hydroxymethyl） aminomethane
UTR	Untranslantion region
X–gal	5–Bromo–4–chloro–3–indoyl–β–D–galactoside
YEP	Yest–extract peptone
β–Me	β–Mercaptoethanol
bp	Base pair
g	Gram
h	H(s)
L	Litre
M	Molar
min	Minute(s)
rpm	revolutions per minute
k	kilo 10^3
c	centi 10^{-2}
m	milli 10^{-3}
μ	micro 10^{-6}
n	nano 10^{-9}

ABSTRACT

Aquaporins (AQPs), with a major function as water channels, may play important roles in cell expansion. However, the roles of GhAQPs (*Gh*, *Gossypium hirsutum*) in cell expansion have not been clearly clarified. To address the roles of GhAQPs in plant cell expansion, this study aimed at identifying candidate *GhAQPs* involved in cotton fibre expansion by exploring their expression patterns and sub–cellular localization, examining their physiological roles *in vivo*, and exploring the cellular and molecular basis of the observed *GhAQPs*–mediated phenotypes.

EST contigs were analysed by searching *GhAQPs* with high EST numbers in the cotton EST database (www.cottondb.com) to generate a preliminary selection of eight candidate *GhAQPs* possibly involved in cotton fibre elongation. These were four *GhPIPs* (*GhPIP1;2*, *GhPIP1;3*, *GhPIP2;3*, *GhPIP2;4*) and four *GhTIPs* (*GhTIP1;1*, *GhTIP1;2*, *GhTIP1;4*, *GhTIP2;1*). Semi–quantitative transcript analyses reviewed that *GhAQPs* were highly expressed during fibre expansion (5–15 days after anthesis, DAA) and relatively lower during the post expansion phase (~20 DAA onwards), which was generally consistent with temporal changes in rates of fibre elongation. These *GhAQPs* were also highly expressed in other expanding organs and more lowly expressed in expanded organs. *GhPIP2;3* and *GhTIP1;2* exhibited the highest transcript levels of all candidates, suggesting that *GhPIP2;3* and *GhTIP1;2* played a major role in cell expansion of cotton fibre and other organs, and thus were targeted as fibre expansion representatives for more detailed study. *GhAQPs* fused with green fluorescent protein (GFP) constructs were transformed into WT Arabidopsis by floral dipping to determine the intracellular localization of GhAQPs. In addition, a parallel experiment was designed in which *GhAQPs* fused with red fluorescent protein (RFP) constructs were transformed into WT Arabidopsis and Arabidopsis transformed by a tonoplast marker *ShMTP* (magnesium transporter proteins)–*GFP*. For GhPIP2;3 and GhTIP1;2, a putative plasma membrane or tonoplast intracellular localization was discovered, respectively. There is a strong link between AQP localization and their functions. The cell membrane localization of GhPIP2;3 and GhTIP1;2 implied that these water channels would increase the permeability of the cell membranes, which reinforces their role in cell expansion.

To examine the physiological role of identified *GhAQPs in vivo*, a complementation and an overexpression experiments were performed by transforming *GhPIP2;3*/*GhTIP1;2* into Arabidopsis (a time–saving model to study GhAQPs' function) T–DNA insertion lines *atpip2;3–1*/*attip1;3* and WT respectively. The mutants *atpip2;3–1* and *attip1;3* exhibit reduced root length compared to WT when grown in the standard ½ MS medium or a medium containing 100 mM NaCl and reduced pollen tube length when grown in the standard medium or a medium without nitrogen (NO_3^-) respectively. In this study, overexpression of *GhPIP2;3* in *pip2;3–1* and overexpression of *GhTIP1;2* in *tip1;3* respectively complemented the mutants' short root and pollen tube lengths respectively in the standard medium or in other media (osmotic/salt stress and without nitrogen), which demonstrated that GhPIP2;3 and GhTIP1;2 really functioned as a PIP and a TIP respectively *in planta* (translated into functional proteins after transformation). Overexpression of *GhPIP2;3* and *GhTIP1;2* in WT Arabidopsis exhibited phenotypes of increased root/root cortex cell and pollen tube lengths respectively in ½ MS medium alone as well as modified ½ MS media causing osmotic/salt stress and nitrogen deficiency, suggesting that GhPIP2;3 and GhTIP1;2 played important roles in cell expansion. During cell expansion, GhAQPs were found to confer salt tolerance by increasing water transport and diluting salt ions. It was also observed that the Arabidopsis root length didn't show significant differences between their growth under NaCl and KCl stresses except the mutant showed shorter root length in 100 mM NaCl medium compared with in 100 mM KCl medium, suggesting Cl^- might be the major ion performing toxicity and leading to the root reduction while Na^+ toxicity also played some roles in root reduction. Three lines overexpressing *GhPIP2;3* in WT showed significantly less reduced root length compared with WT in ½ MS + 100 mM NaCl medium compared with in ½ MS + 200 mM sorbitol medium, suggesting overexpression of *GhPIP2;3* increased salt tolerance.

In exploring the molecular basis of the observed increased root and pollen tube elongation in the lines overexpressing *GhPIP2;3* and *GhTIP1;2* respectively, a new phenomenon was found that overexpression of *GhPIP2;3* and *GhTIP1;2* could increase the expression of some sugar transporters (*ERD6* for *GhPIP2;3*, *ERDL6* and *STP11* for

GhTIP1;2) and K^+ transporters (*KC1* and *SKOR* for *GhPIP2;3*) (except that *SWEET17* was decreased in overexpression of *GhPIP2;3* lines, as decreasing the expression of *SWEET17* might help to maintain cytoplasmic sugar homeostasis or maintain the solutes concentration in the vacuole during cell expansion). The roles of GhPIP2;3 and GhTIP1;2 in cell expansion might be due to their water transport activity combined with their impact on elevating sugar and K^+ transporter expression that lowered cell water potentials with the overall effect of increasing turgor pressure to drive cell expansion. Another new discovery was that root hair length of the lines overexpressing *GhPIP2;3* in WT were significantly increased compared with WT grown on MS medium containing 100 mM NaCl (½ MS + 100 mM NaCl). Meanwhile, three lines overexpressing *GhPIP2;3* in *pip2;3–1* background complemented the short root hair phenotype in the mutant in the ½ MS medium, and three lines overexpressing *GhPIP2;3* in WT increased root hair length compared with WT in the ½ MS medium. At a molecular level overexpression of *GhPIP2;3* was found to increase expression of root hair elongation related genes including *AUX1* (auxin transport), *ETR1* (ethylene receptor), *Myosin XIK* (Myosin XI) and *EPC1* (Glycosyltransferase), and some hair density related genes such as *IAA17* (Repressor of auxin–responsive transcription), *PRP3* (Proline–rich cell wall protein), *RHD6* (Protein ROOT HAIR DEFECTIVE6) and *RHL1* (Topoisomerase subunits). These findings suggested that overexpression of *GhPIP2;3* might increase root hair elongation and hair density by inducing expression of these root hair elongation related genes (in addition to increasing cell turgor for hair elongation by enhancing water transport) and root hair density related genes. The molecular basis for the role of *GhPIP2;3* on salt tolerance was also explored. Among the salt tolerance genes chosen from NCBI and published papers, three salt tolerance genes, *Rd29A* (Responsive to desiccation 29A), *SAT32* (Protein salt tolerance 32) and *SOS1* (Sodium/hydrogen exchanger 7) were found to be higher expressed in the lines overexpressing *GhPIP2;3* than in WT. It was proposed that overexpression of *GhPIP2;3* increased salt tolerance probably by a combination of increased water transport, increased root hair length and density, overlaid by enhancing expression of salt tolerance genes. Furthermore, one interesting discovery was that overexpression of *GhPIP2;3* increased stomatal density, which might increase CO_2 assimilation, then contributed to transpiration and photosynthesis.