

Summary of PhD Thesis

**Genome-wide identification of tomato ABC transporters and functional analysis of
SlABCB4 involved in auxin transport**

Laboratory of Horticulture Science, Department of Biological Mechanisms and Functions,
Graduate School of Bioagricultural Sciences, Nagoya University

PETER AMOAKO OFORI

Tomato, a member of *Solanaceae* family is an important vegetable in human diet and a valuable model crop in fleshy fruit physiology studies. In recent years, research in tomato has increased resulting from the availability of its high quality genome sequence information, expressed sequence tag (EST) database, full-length cDNA resources, transcriptome and metabolome databases. Micro-Tom the dwarf tomato variety is an excellent research material for basic science studies in fruits because of its small size and short lifecycle. Fruit quality is an important fruit characteristics which is mostly affected by compound composition including sugars, organic acids and secondary metabolites, such as phenolic and terpenoids accumulated in the fruit. Thus, these compounds accumulated at varied concentrations determines fruit taste, flavor, smell and color. The accumulation of these compounds are facilitated by plant transporters including aquaporins, sugar, organic acid and ATP-binding cassette (ABC) transporters. Hence these transporters must play indispensable roles in accumulating metabolites in developing fruits. ABC transporters are ATP-driven proteins that actively transport wide range of molecules, such as organic acids, metal ions, phytohormones and other secondary metabolites. Therefore, in this study, a genome-wide identification of genes encoding ABC transporters in tomato genome was performed to provide fundamental information such as genes expression analysis, gene names, protein topology etc. important for future studies. In addition, one candidate of tomato ABC proteins was selected to clarify its possible roles in tomato fruit development.

In the first study, a genome-wide analysis of ABC transporters in tomato genome was performed. A total of 154 genes putatively encoding ABC transporters were identified. Phylogenetic analysis classified the identified ABC transporters into 8 subfamilies which includes; 9 ABCAs, 29 ABCBs, 26 ABCCs, 2 ABCDs, 2 ABCEs, 6 ABCFs, 70 ABCGs and 10 ABCIs. The Pfam web server revealed the structural orientation and conserved domains for each protein. Among the 154 ABC proteins, 47 proteins are soluble ABC proteins lacking transmembrane domain (TMD) and 107 ABC proteins with TMD. In addition, 54 full-size ABC proteins with (TMD-NBD)x2, 53 half size ABC proteins with (TMD-NBD)x1 or (NBD-TMD)x1 and 47 quarter-size ABC proteins with (NBD-NBD) or NBD topologies were identified.

The gene expression profiles from the tomato eFP browser for *SIACB20*, *SIABCC10*, *SIABCC11*, *SIABCC21*, *SIABCF6*, *SIABCG17*, *SIABC17* etc. showed distinctive gene expression profiles in developing fruit tissues or organs suggesting their potential responsibilities in fruit development. Some tomato ABCs such as *SIACB25*, *SIABCC22*, *SIABCD1*, *SIABCI3* etc. showed ubiquitous gene expression patterns suggesting their responsibilities for basic cellular maintenance. Real-time-semi-quantitative PCR gene expression analysis further revealed the gene expression patterns of selected ABCs in various organs of 'MicroTom'. The selected genes included; *SIACB4*, *SIABCC11*, *SIABCG7*, *SIABCG8*, *SIABCG9*, *SIABCG12*, *SIABCG13*, *SIABCG17*, *SIABCG22*, *SIABCG28* and *SIABCG36*. These candidates were selected because the full length cDNA sequences were available on TOMATOMICs database. *SIACB4*, a closest orthologue of Arabidopsis AtACB19, an auxin transporter, showed ubiquitous gene expression, but lower gene expression was revealed in mature fruits suggesting a possible auxin transport role in various organs of tomato. *SIABCC11* showed high expression level detections in mature leaf and fruits after 21 DAP. Although *SIABCC11* have no close orthologue in Arabidopsis, it may play essential roles in the later part of tomato fruit development. The gene expression patterns of *SIABCG9*, *SIABCG13*, *SIABCG17*, *SIABCG22* and *SIABCG28* in fruit suggested a

possible roles in fruit development and/or ripening. *SIABCG36* showed ubiquitous gene expression and is likely to transport metabolites involved in cuticle formation since its closest orthologue of Arabidopsis, *AtABCG32* is responsible for cuticle formation.

In the second study, *SIABCB4* was selected to clarify its functional roles in fruit development. The phylogenetic tree of full-size members of the ABCB subfamily showed at least 6 Arabidopsis ABCB proteins characterized as auxin transporters. Among the 6 Arabidopsis ABCB proteins implicated as auxin transporters, *AtABCB19* showed high evolutionary relationship with *SIABCB4*. Transient expression of *SIABCB4*-GFP fusion proteins in epidermal cells of *Nicotiana benthamiana* leaf by agro-infiltration showed plasma membrane localization. Transport activity of *SIABCB4* was performed to determine its transport direction as well as transport substrate by transient expression of *SIABCB4* proteins in mesophyll protoplast of *Nicotiana benthamiana* leaf. *SIABCB4* displayed indole-3-acetic acid (IAA) export from the cytosol to the apoplastic space. Similarly, *SIABCB4* catalyzed IAA transport in heterologous yeast system. In MicroTom, quantitative reverse transcription gene expression analysis of *SIABCB4* showed different gene expression patterns in various organs and tissues i.e. leaf, root, flower and developing fruits. Gene expression level increased at the early stage of fruit development and the highest transcript level was detected at 14 days after pollination (DAP). However, the gene expression levels started to decline after 21 DAP, suggesting that transport activity of *SIABCB4* is higher at the early stages of fruit development. Spatiotemporal transcriptome analysis of tomato fruit further revealed high gene expression levels of *SIABCB4* in all fruit tissues including seed, columella, placenta, locular tissue, septum and pericarp tissues of developing fruit (<http://tea.solgenomics.net/>). This suggests that *SIABCB4* may facilitate transport of auxin from the seed through the placenta to other fruit tissues constitutively in developing fruit of tomato. In addition, moderate transcript levels were detected in young leaf and stem suggesting that *SIABCB4* distribution of auxin is not limited to reproductive tissues but also in vegetative tissues.