Functional analyses of lipocalin proteins in tomato

（課程博士・様式７）（Doctoral qualification by coursework, Form 7）

学 位 論 文 要 旨

Abstract of Doctoral Thesis

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論文題目：

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論文要旨：

Abstract : Tomato is an economically important food worldwide and highly accumulated lycopene and ß-carotene in ripe stage of tomato fruit. ‘Micro-Tom’ is a cultivar that was originally bred for home gardening. The tomato cultivar ‘Micro-Tom’, which was produced by crossing Florida Basket and Ohio 4013-3 cultivars, is not only an ideal house plant for home gardening, but also a good model cultivar for tomato research with some advantages, such as small size, short life cycle, easy fruit setting, easy to grow and capacity to grow under fluorescent lights at a high density (Scott and Harbaugh 1989). Mutant’s library derived from an ethyl methanesulfonate (EMS) mutagenesis has been produced for genetic studies in ‘Micro-Tom’ (Watanabe et al. 2013). To better understand the mechanism of plastid differentiation from chloroplast to chromoplast, In my laboratory by Suzuki et al. (2015) analyzed and compared plastid proteome and plastid morphologies with ‘Micro-Tom’ and two other varieties, ‘Black’ and ‘White Beauty’. The result of study showed that compared plastid proteome of ‘Micro-Tom’ with ‘Black’ and ‘White Beauty’ using the two-dimensional gel electrophoresis, the differences of spot number and isoelectric points of TIL (temperature-induced lipocalin) and decreasing CHRC (plastid-lipid-associated protein) and HrBP1 (hairpin binding protein-1) in ‘Black’ and ‘White Beauty’ were detected (Suzuki et al. 2015). Plastid proteomic data showed that lipocalins are related to differentiation of chromoplasts and ripening in tomato fruits. Lipocalins are a group of proteins and distributed in bacteria, invertebrate, and vertebrate animals. Plant lipocalins are divided into three families: temperature-induced lipocalins (TIL), chloroplastic lipocalins (CHL), and lipocalin-like protein. Lipocalin has various functions including those related to environmental stress response, apoptosis induction, membrane formation and fixation, regulation of immune response, cell growth, and metabolism adjustment. However, very little is known about plant lipocalins. To better understand the function of lipocalins, I made over-expressed *SlTIL1*, *SlTIL2* and *SlCHL*

and gene silenced tomato plants. In addition, to elucidate the roles of *SlTIL1, SlTIL2*, and *SlCHL* in light and heat response, the changes of phenotypes and gene expression were investigated in over-expressed plants and gene silenced plants under light condition 1 (405 μmol m^{-2} s^{-1}) and a light condition 2 (105 μmol m^{-2} s^{-1}) and under normal condition (24 °C) and heat condition (37 °C; 5 days). The over-expressed *SlTILs* and *SlCHL* plants compared with wild-type plants under 405 μmol m^{-2} s^{-1} showed their leaves with curling, longer terminal leaflet, bullwhip phenotype in leaves, early flowering, increasing number of flowers and inflorescences, and bigger peduncle and fruits. The over-expressed *SlTIL1* showed longer of leaves with curling, over-expressed *SlTIL2* showed earlier flowering, and over-expressed *SlCHL* showed dark greening in seedling and mesocarp of mature green fruit and earlier ripening compared others. Moreover pericarp transversal cell structures were expanded in over-expressed *SlTIL1, SlTIL2* and *SlCHL* fruits. I also suppressed *lipocalins* expression in tomato using virus-induced gene silencing (VIGS) to observe their phenotype. The suppressed expression of *SlTILs* induced aberrant shapes of leaves, such as yellowing and curling, and yellowing in pericarps and mesocarps of fruits. The suppressed expression of *SlCHL* induced yellowing in pericarps and mesocarps, and greening in endocarps of fruits. Moreover subcellular localization analyses of SlTILs and SlCHL using a particle bombardment showed that the fusion proteins of SlTILs-sGFP and sGFP-SlTILs were located around the plasma membrane, plastid, nuclear and in reticulate structure. SlCHL-sGFP was localized in the chloroplast. Their promoters that 1000 bp upstream from the start codons of lipocalins are included many light-responsive *cis*-elements in promoters. *SlSOD*s (*SlSOD1*, *SlSOD3* and *SlSOD6*) were highly expressed in seedling, leaf, flower and fruit of over-expressed *SlTIL1, SlTIL2* and *SlCHL* plants, on the other hand very low expression of them in their gene silenced plants. I found that O_2^- and H_2O_2 also participates in over-expressed *SlTIL1, SlTIL2* and *SlCHL* and gene silenced plants under light stress (405 μmol m^{-2} s^{-1}) and heat stress (37 °C; 5 days) tolerance. Over-expressed *SlTIL1, SlTIL2* and *SlCHL* under light stress (405 μmol photon m^{-2} s^{-1}) and heat stress (37 °C; 5 days) showed lower oxidative damage (O_2^- and H_2O_2) in normal and stress condition compared control (wild-type) leaf plants and increased level activities of *SlSODs*. Furthermore, I used pTRV-based VIGS-system in these study to silence *PDS, SlTIL1, SlTIL2*, *SlCHL* and *CHRC*. Under light stress (405 μmol m^{-2} s^{-1}) and heat stress (37 °C; 5 days) showed increased oxidative damage (O_2^- and H_2O_2) and decreased level activities of *SlSODs*. The changes in the expression of *SlSOD*s were consistent with the accumulations of ROS, which indicated that lipocalins might have an important role in abiotic oxidative stress tolerance in tomato plants. Especially SlTIL1 and SlTIL2 are localized around their membranes and protect them from ROS. The results will contribute to elucidating the functions of lipocalin in plants, and provide new strategies to improve the tolerance to abiotic stress in tomato plants.