Genetic and Physiological Characterization of Tomato F-box Gene *HAWAIIAN SKIRT* Mutants

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ABSTRACT

A comprehensive tomato mutagenized populations had been developed using γ-ray irradiation and *ethyl methanesulfonate* (EMS) mutagenesis in cv. Micro-Tom genetic background. Among these tomato mutant populations, many of them have been exploited for functional genomic and breeding studies. However, the number of mutants that have not been characterized are still numerous. TOMJPE8986 has been identified as a parthenocarpy mutant and also showed higher TSS compared to cv. Micro-Tom (WT). The most distinct phenotypes of the mutant are leaflet fusion and reduction in leaflet serration.

In Chapter 2, the identification of the responsible gene for the TOMJPE8986 phenotypes was carried out using genetic rough mapping and whole genome sequencing technology. It was identified that the F-box encoding gene *Solyc01g095370* was found to carry an amino acid change mutation. Based on the protein alignment analysis, it was found out that *Solyc01g095370* is the putative ortholog of *AtHWS* with respective proteins showing more than 60% homology. TOMJPE8986 exhibits fused leaflet and reduction in leaflet serration, which were similar to the ones has been reported in the *athws-1* mutant. By TILLING platform, it was identified three additional mutants lines which also mutated in the F-box gene *Solyc01g095370*, which were TOMJPW283, TOMJPW3299, and TOMJPW3583. Based on the results of this study, it was strongly suggested that *Solyc01g095370* is the putative ortholog of *AtHWS*. Therefore, from now on we referred *Solyc01g095370* as *SlHWS* and TOMJPE8986 as an *slhws-1* mutant.

In Chapter 3, *slhws-1* and the additional mutant lines were evaluated for their potential parthenocarpy phenotype. To evaluate the stability of the trait, the evaluations were carried out in three environmental conditions. The results revealed that all of the evaluated mutants showed high parthenocarpic fruit formation from the emasculated flowers. In accordance with their potential parthenocarpy phenotype, these mutants also showed a high degree of seedless-fruit

formation from the pollinated flower. However, the parthenocarpy level was largely influenced by the environmental condition. Occasionally, these mutants were able to produce a low number of seeds. The results from crossing evaluation revealed that the male sexual organ was the major determinant for low seed number in *slhws-1*. To investigate the allelism between *slhws-1* and three additional mutant lines, allelism tests was performed. The results showed that TOMJPW283 and TOMJPW3299 are allelic to *slhws-1* based on their leaf morphology and seed number observation. Therefore, hereinafter we referred them as *slhws-2* and *slhws-3,* respectively. Based on the results in this study, it was also concluded that *SlHWS* is involved in fruit development of tomato.

In Chapter 4, BC_3 generation of *slhws-1* was developed and characterized for their plant and fruit quality-related traits. The BC_3 plants showed an increase in plant height, longer internode, less lateral shoot, fused leaflet, and reduction in leaflet serration, which lead to global alteration in plant architecture. Fruit weight and shape of seeded-fruit and fruit firmness of the BC_3 *slhws-1* were similar to the ones observed in the WT. BC_3 *slhws-1* showed that fructose and glucose contents were significantly higher in the BC_3 seeded-fruits, while no significant difference was observed in lycopene and β-carotene levels between BC_3 fruit and its WT in a spectrophotometer analysis. BC_3 generation showed high expression of parthenocarpic fruit and seedless-fruit formation, and their potential parthenocarpy could be seen from their rapid ovaries growth in both emasculated and non-emasculated flowers. Severe reduction in seed formation that was observed in the backcross population has been shown as the results of several factors, *i.e.,* a low number of pollen grain per flower, low pollen viability and less anther dehiscence. Accordingly, histological analysis in the flower of BC_3 mutant generation revealed that the alteration in tapetum morphology, as well as early release of the microspores from the tetrad, lead to low male fertility. In addition, BC_3 *slhws-1* also showed flowering delay compared to the WT. From this study, it was concluded that mutation in *slhws-*

1 caused some alteration in plant and fruit quality-related traits and defect on flower organ development that leads to the reduction in seed formation.

In Chapter 5, Near Isogenic Lines (NILs) of *slhws-1* were generated in cv. Aichi First and cv. Ueleie 106 WP genetic backgrounds. The population was phenotyped for several traits that related to the mutation of *slhws-1* in cv. Micro-Tom genetic background. BC_2 NIL independent lines exhibited similar defect in leaf morphology, less lateral shoot formation, reduction in seed formation, and some degree of seedless fruit formation. However, flowering delay and TSS phenotypes were influenced by the genetic background of the evaluated NILs. Based on these results, it was concluded that the effect of the *slhws-1* mutation on plant and fruit quality-related phenotypes depends on the trait observed as well as the genetic background.

In Chapter 6, the transcriptomic analysis was performed to gain insights into molecular mechanisms underlying the key phenotypes in the *slhws-1* mutant. Recently, *AtHWS* has been reported to be involved in miRNA biogenesis and function. The RT-qPCR analysis was carried out to investigate the levels of miRNAs in the *slhws-1* mutant. It was found out that the expression level of miRNAs was altered in stage 9 of *slhws-1* flower bud and the elevated levels of miR164 was hypothesized to be responsible for the observed leaf and flower phenotypes. The transcriptomic profiles and RT-qPCR analysis revealed differential expression in male function-related genes. Differentially expression of some gene related to anther development strongly suggested might be responsible in low fertility observed in *slhws-1*.