Complementation of the tomato HWS gene with its Arabidopsis counterpart demonstrates conservation of the gene function between both species

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Abstract  The HAWAIIAN SKIRT (HWS) gene was originally described in Arabidopsis for the characteristic fusion of sepals in the mutant. A tomato line mutated in the putative ortholog gene was isolated in a previous study. The tomato hws-1 mutant showed facultative parthenocarpy and produced fruits with elevated Brix, revealing the gene as a hopeful resource for crop improvement. To confirm the orthology relationship between the Arabidopsis and tomato HWS genes, the hws-1 mutant was complemented with either the tomato wild-type genomic fragment or the Arabidopsis sequence of the gene. In both complementation experiments, defective phenotypes of hws-1 are rescued, albeit to different extents. Recovery of these phenotypes, which include parthenocarpic fruit production, increased Brix, loss of leaflet serration, alteration of bud and petal shape, firmly establishes SlHWS as an ortholog of the originally described HWS in Arabidopsis. This work indicates that the function of HWS is likely to be conserved in a wide range of plant species.

Key words: F-box gene, HAWAIIAN SKIRT, parthenocarpy, tomato.

Parthenocarpy can be defined as the production of plant fruits in the absence of ovule fertilization. The male reproductive function being particularly sensitive to heat stress (Peet et al. 1998), parthenocarpic crops are expected to be more resilient against unfavorably high temperatures with regards to fruit production. A tomato (Solanum lycopersicum) parthenocarpic line was previously isolated from a genetic screening of an ethyl-methanesulfonate-mutagenized population of the Micro-Tom cultivar (Saito et al. 2011; Shikata et al. 2016). The causal gene was identified as Solyc01g095370, which encodes an F-box protein showing more than 60% identical amino acids with the Arabidopsis (Arabidopsis thaliana) HAWAIIAN SKIRT (HWS) protein (Damayanti et al. 2019). The mutant was therefore tentatively designated as slhws-1. In addition to the parthenocarpic trait, slhws-1 fruits showed an increased Brix level. As Brix level usually corresponds to sugar content, this finding further deepened the interest in the mutant in the context of crop improvement.

Although several pieces of evidence pointed towards the fact that both genes are orthologous (Damayanti et al. 2019), the phylogenetic distance between both species and the absence of fleshy fruits in Arabidopsis weakened any conclusions that could have been drawn from comparative phenotypic studies alone. To generate the lacking genetic evidence, two types of complementation experiments of the slhws-1 mutant were performed. In a first experiment, Solyc01g095370 is demonstrated to be the causal gene of the slhws-1 phenotype; a second experiment establishes SlHWS as the tomato ortholog of AtHWS. These are described in the following.

In a previous study, sequencing results in the tomato hws-1 line revealed the presence of a cytosine-to-adenosine transversion located towards the 3' end of the CDS of the Solyc01g095370 gene (Damayanti et al. 2019). To firmly demonstrate that the mutation was responsible for the mutant phenotype, the full genomic sequence of Solyc01g095370 consisting of a 5,361bp promoter and 1,218bp CDS fragment (Figure 1A) was introduced into the slhws-1 following the standard transformation method (Sun et al. 2006). A minimum of nine transformants of the T2 generation, in parallel with a minimum of nine wild type (WT) individuals, was grown on rockwool blocks periodically supplied with Ohtsuka House 1 and 2 nutrient solutions (OAT Agrio Co., Ltd., Japan) in standard cultivation conditions (22°C, under fluorescent lighting with a 16h/8h light/dark cycle). All observations were done on 3-month-old plants with...
HWS function is conserved in Arabidopsis and tomato

fruits at the red ripe stage. Statistical significance in our different measurements was determined from an ANOVA test (type II, \( p < 0.05 \)) which was eventually followed by a Tukey’s test.

In addition to the parthenocarpy and high sugar traits, several phenotypic features were associated with the presence of the mutation at the recessive state. Plant overall architecture was markedly altered in \( \text{slhws-1} \), contrasting with the short and compact Micro-Tom WT. Mutants were about ten centimeters taller on average; the number of lateral shoots was halved, and stem diameter was also noticeably larger in \( \text{hws-1} \) (Figure 2A; Table 1). In all analyzed individuals complemented with the genomic fragment construct, these traits were partially rescued to the exception of the stem diameter, which was even smaller than the WT one. Brix was measured in triplicates using the pericarp of individual fruits after removal of seeds and jelly part using a refractometer PAL-J (Atago Co., Ltd., Tokyo, Japan). The fruits of \( \text{hws-1} \) were of comparable diameter, however they were fewer and characterized by an elevated Brix (Table 1). Complementation with the WT genomic fragment allowed producing an intermediate number of fruits and completely restored WT Brix levels. Parthenocarpic fruit formation and seedless-fruit formation were counted from fruits more than 2 g of fresh weight and

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Figure 1. Design of transformation vectors. A. Full genomic fragment of the SlHWS gene in the pPZP212 vector backbone. B. AtHWS coding sequence fused to the tomato native promoter in the pPZP212 backbone.

Figure 2. Representative visual features of the \( \text{slhws-1} \) mutant compared with the WT control as well as with tomato and Arabidopsis complementation lines. A. Expanded overall plant architecture. Scale bar=5 cm. B. Reduction in leaflet serration. Scale bar=2 cm. C. Narrow-ended petal shape. Scale bar=1 cm. D. Barrel-shaped buds. Scale bars=1 cm.
the number of seeds was counted from 15 fruits in each line. The very low fertility associated with the formation of parthenocarpic fruits in *slhws-1* was shown in an earlier study to result mainly from a defective male function (Damayanti et al. 2019). This trait was also rescued in the complemented lines (Table 1). Reduction in leaf serration has been associated with the *hws* phenotype in both Arabidopsis (Lang et al. 2018) and tomato (Damayanti et al. 2019). Serrations were restored on the leaflets of the *slhws-1*/*SlHWS* individuals, almost to the extent of the WT (Figure 2B). Similarly, the petals on the complemented individuals could barely be distinguished from the WT ones, whereas *hws-1* petals were strikingly narrow at their extremities (Figure 2C). Another distinctive feature of the mutant is the barrel shape of the flower buds associated with short sepals, leaving the tip distinctive of the mutant one (Table 1). Flower bud shape and leaflet serrations were barely distinguishable from the WT ones. Collectively, these results indicate that *Soya0g095370* is the causal gene for the *HWS* phenotype.

To assess whether *AtHWS* could complement *slhws-1*, the CDS of *SIHWS* in the complementation construct described earlier was swapped with the 1,236-bp-long CDS of *AtHWS*, leaving the rest of the vector unchanged (Figure 1B). Observation of a minimum of 12 transformatants of the T1 generation revealed that reproductive traits were either fully rescued or rescued to the same extent of the tomato WT genomic complementation, with the notable exception of fruit number, which was not significantly different from the mutant line (Table 1). Fruit diameter (mm) 19.3 ± 1.2, 15.9 ± 0.6, 17.0 ± 0.4

| Trait                      | WT              | slhws-1         | slhws-1/SlHWS   | slhws-1/AtHWS   |
|----------------------------|-----------------|-----------------|-----------------|-----------------|
| Plant height (cm)          | 20.7 ± 2.1 b    | 33.0 ± 2.3 a    | 28.9 ± 2.5 ab   | 28.3 ± 1.5 ab   |
| Stem diameter (mm)         | 4.91 ± 0.13 b   | 5.56 ± 0.10 a   | 3.98 ± 0.09 a   | 4.75 ± 0.18 b   |
| Lateral shoot number       | 2.5 ± 0.2 b     | 1.3 ± 0.2 b     | 2.0 ± 0.4 ab    | 2.2 ± 0.1 ab    |
| Fruit number               | 34.6 ± 3.3 b    | 13.5 ± 1.9 b    | 24±3.2 ab       | 18.4 ± 2.8 b    |
| Fruit diameter (mm)        | 19.3 ± 1.2      | 17.4 ± 1.2      | 15.9 ± 0.6      | 17.0 ± 0.4      |
| Total soluble solids (Brix) | 4.8 ± 0.1 b     | 8.6 ± 0.6 a     | 5.8 ± 0.4 b     | 5.2 ± 0.2 b     |
| Ratio of seeded fruits (%) | 100 ± 0.0 a     | 7.5 ± 5.4 b     | 83.3 ± 8.3 a    | 90 ± 10.0 a     |
| Seed number                | 21.1 ± 1.9 a    | 4.0 ± 2.7 b     | 17.7 ± 2.0 a    | 20.2 ± 2.3 a    |

Values represent mean ± SE (n=9 plants); statistically different groups as indicated with superscript letters; flowers were mechanical pollinated as in Damayanti et al. 2019.

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