Note

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; González-Carranza et al. 2007). The mutant was therefore tentatively designated as *slhws-1*. In addition to the parthenocarpy 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 5,361 bp promoter and 1,218 bp 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

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

Figure 2. Representative visual features of the *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.

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 *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 *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 *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

Table 1. Evaluation of the phenotypic rescue of *hws-1* in the complementation lines.

Trait	WT	<i>slhws-1</i>	<i>slhws-1/SlHWS</i>	<i>slhws-1/AtHWS</i>
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 ^c	4.75±0.18 ^b
Lateral shoot number	2.5±0.2 ^a	1.3±0.2 ^b	2±0.4 ^{ab}	2.2±0.1 ^{ab}
Fruit number	34.6±3.3 ^a	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.

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 of the anther cone uncovered (Figure 2D). Here again, complemented mutants with the genomic *HWS* fragment showed buds comparable to the WT ones. Collectively, these results indicate that *Solyc01g095370* is the causal gene for the *HWS* phenotype.

To assess whether *AtHWS* could complement *slhws-1*, the CDS of *SlHWS* 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 transformants of the T₂ 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 one (Table 1). Flower bud shape and leaflet serrations were barely distinguishable from the WT ones (Figure 2C, D). The overall plant aspect as well as the petal shape were intermediate between the mutant and the WT lines, indicating a partial rescue for these traits. Based on these results and results in the first complementation experiment data, it is concluded that the *Arabidopsis* *HWS* protein can rescue the *slhws-1* phenotype, substantiating the claim that both genes are orthologs.

In rice, both the *EP3* and *FBK1* genes share a high homology with *SlHWS* (Damayanti et al. 2019;

Lombardo et al. 2021). The *ep3* mutant shows an erect panicle shape, increase in the number of vascular bundles in specific regions of the panicle and an increase in grain yield (Li et al. 2011; Piao et al. 2009). *FBK1* has been described for its role in determining cell wall thickening by targeting for degradation a reductase involved in lignin synthesis (Borah and Khurana 2018). Although the functions of these genes do not seem to overlap, both *EP3* and *FBK1* can complement the *Arabidopsis* *hws-1* mutant (González-Carranza, unpublished; Yu et al. 2015). Interestingly, grain yield is increased in the *ep3* mutant (Li et al. 2011). Most likely, the poplar *PtaHWS* is another ortholog (Dash et al. 2015), although to our knowledge there are no complementation experiments to confirm this hypothesis. In this work, complementation of the tomato mutant with the *Arabidopsis* sequence brings another piece of evidence supporting the idea that the gene function is remarkably conserved across unrelated species. It is expected that in a wide variety of plants, orthologs of *HWS* control aspects of fruit development. In tomato, *Arabidopsis* and poplar, functional disruption of the gene has been shown known to affect the microRNA metabolism and it is hypothesized that the mutant phenotypes are resulting from the accumulation of specific microRNAs species (Damayanti et al. 2019; Dash et al. 2015; Lang et al. 2018; Zhang et al. 2017). It would be interesting to confirm whether the microRNA metabolism is also altered in the rice mutants. Moreover, protein sequence analysis of the different orthologs would provide clues towards the identification of a putative target for *HWS* in the microRNA pathway.

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