Formation of the SCF complex with the F-box proteins encoded by genes linked to the *S* locus in *Prunus*

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Introduction

Many species in the Rosaceae and Solanaceae exhibit the S-RNase-based self-incompatibility (SI). In this system, F-box protein(s) encoded in the *S* locus determines the pollen specificities. An F-box protein generally forms the SCF complex via Skp1 for the degradation of its substrate protein (Fig. 1). The pollen *S* F-box proteins in the Pyrinae and Solananceae were reported to form the canonical SCF complex, while they were also shown to form non-canonical one via S-RNase binding protein 1 (SBP1), to degrade non-self S-RNases.

In *Prunus*, the pollen *S* F-box protein (SFB) and three other pollen-expressed F-box proteins (SLFL1-3) are encoded in the region linked to the *S* locus, all of which were speculated to be involved in SI. Although the biochemical functions of SLFLs remain to be elucidated, we previously identified the Skp1-like protein (SSK1) interacting with SFB and SLFLs, and the *Prunus* SBP1 homolog (Matsumoto et al., 2012; Matsumoto and Tao, 2012). Here, we report that SFB and SLFLs may form both canonical and non-canonical SCF complexes with SSK1 and SBP1, respectively.

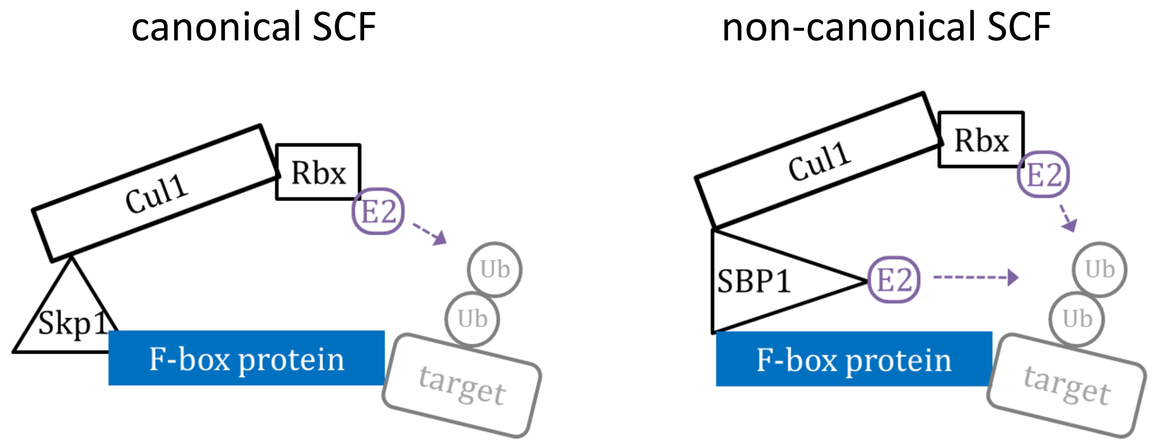

Fig.1 The schematic diagram of the canonical and non-canonical SCF complex.
In the SCF complex, an F-box protein recognizes a substrate protein and the other components contribute to the ubiquitinylating activity by recruiting the ubiquitin-conjugating E2 enzyme .
The canonical SCF complex is consisted of F-box protein, Skp1, Cul1 and Rbx1, the latter two of which are the highly conserved proteins (left). On the other hand, the non-canonical SCF complex is formed by F-box protein and SBP1, or by those with Cul1 and Rbx1 (right). Rbx1 and SBP1 interact directly with E2.

PavSFB and PavSLFLs could form the canonical and non-canonical SCF complexes.

All of GST-fused *P. avium* SFB and SLFLs interacted with the pollen-expressed Cul1 homologs (PavCul1A, PavCul1B) along with PavSSK1, indicating that they would form canonical SCF complex with either of the Cul1 homologs (Fig. 2A, B). Furthermore, it appeared that PavSFB and PavSLFLs also interacted with PavSBP1 (Fig. 2C).

These results indicate *Prunus* SFB and SLFLs could form both the canonical SCF complex via SSK1 and the non-canonical one via SBP1, as reported for pollen *S* F-box proteins of other plant families (Kao et al., 2006; Zhao et al., 2010; Yuan et al., 2014; Minamikawa et al., 2014).

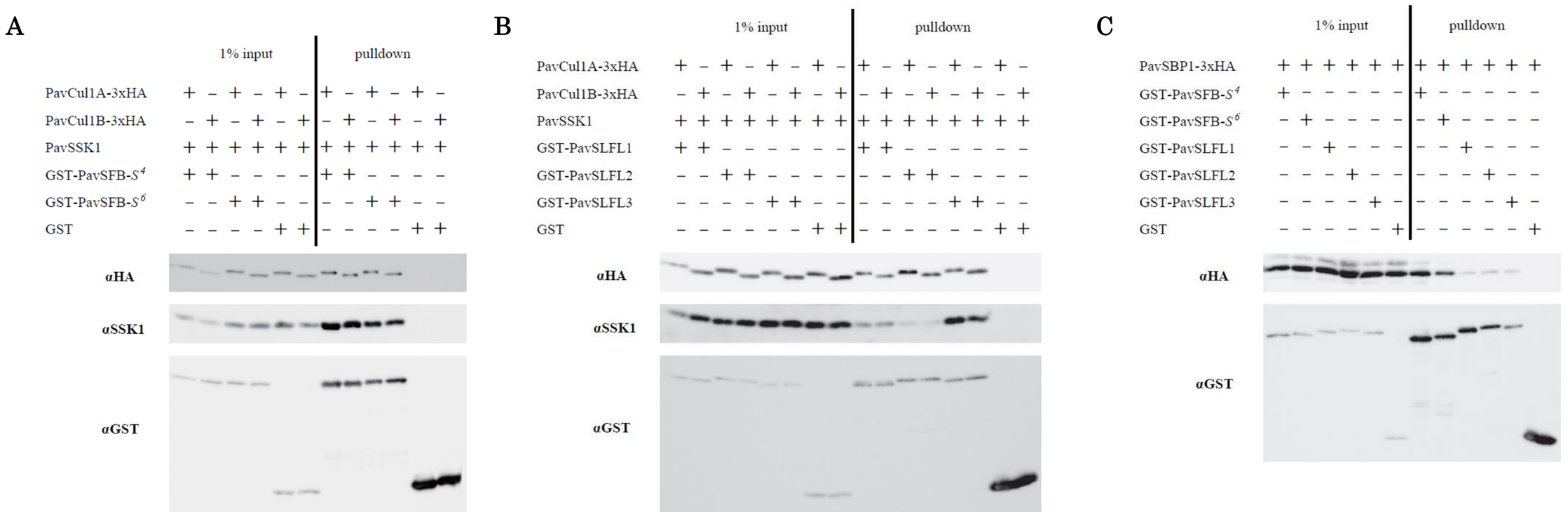

Fig.2 in vitro binding assays of recombinant F-box proteins with the canonical SCF components.
GST-fused PavSFB/PavSLFL were co-expressed in the cell free system with PavSSK1 and 3xHA-tagged PavCul1A/B (A, B) or with PavSBP1 (C). The pulled down proteins with the glutathione sepharose were detected by the immunoblot.

PavSSK1 and *PavCul1A* were more abundantly expressed.

qRT-PCR was conducted to estimate the abundance of the transcripts for SCF components in pollen (Fig. 3). The highest expression was observed with *PavSSK1*, with its expression level being 500 – 1000 times as much as *PavSBP1*. *PavCul1A* expression was the second highest, about 150 times as much as that of *PavCul1B*. These results indicate that PavSSK1 and PavCul1A would be more abundantly available for SFB and SLFLs to form the SCF complex.

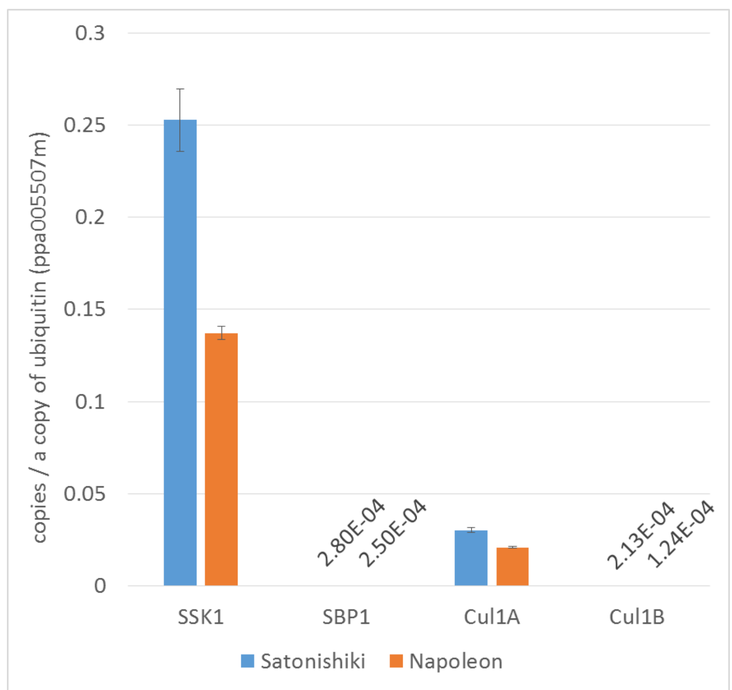

Fig.3 Expression level comparison of the SCF components for SFB and SLFLs in sweet cherry pollen.
Transcripts abundance in pollen of two sweet cherry cultivars ('Satonishiki' and 'Napoleon') was quantified by qRT-PCR. The cloned PCR products were used to establish the standard curves. The data represents the mean of three biological replicates with two technical replicates. Error bars indicate SE.

Conclusion

This study showed that F-box proteins encoded in the regions linked to *Prunus S* locus, PavSFB and PavSLFLs, could form both the canonical and the non-canonical SCF complexes. The *PavSSK1* and *PavCul1A*, the components of the former complex, were confirmed to be more abundantly expressed in sweet cherry pollen.

These results may indicate that PavSFB and PavSLFLs would mainly form the canonical SCF complex with PavSSK1 and PavCul1A, to ubiquitinylate their specific substrate proteins in pollen. Further studies to identify their substrates are required for full elucidation of the *Prunus* SI mechanism.