

# Tomato (*Solanum lycopersicum*) homologs of TRIPTYCHON (SITRY) and GLABRA3 (SIGL3) are involved in anthocyanin accumulation

Rumi Tominaga-Wada,\* Yuka Nukumizu and Takuji Wada

Interdisciplinary Research Organization; University of Miyazaki; Miyazaki, Japan

**Keywords:** tomato, *Arabidopsis*, R3 MYB, *CPC*, *SITRY*, anthocyanin

In *Arabidopsis thaliana* the CPC-like MYB transcription factors [CAPRICE (CPC), TRIPTYCHON (TRY), ENHANCER OF TRY AND CPC 1, 2, 3/CPC-LIKE MYB 3 (ETC1, ETC2, ETC3/CPL3), TRICHOMELESS 1, 2/CPC-LIKE MYB 4 (TCL1, TCL2/CPL4)] and the bHLH transcription factors [GLABRA3 (GL3) and ENHANCER OF GLABRA 3 (EGL3)] are central regulators of trichome initiation and root-hair differentiation. By transforming the tomato orthologous genes *SITRY* (*TRY*) and *SIGL3* (*GL3*) into *Arabidopsis*, we demonstrated that *SITRY* inhibited trichome initiation and enhanced root-hair differentiation. These results suggest that tomato and *Arabidopsis* partially use similar transcription factors for epidermal cell differentiation, and that a CPC-like R3 MYB may be a key common regulator of plant trichome and root-hair development. CPC and GL3 are also known to be involved in anthocyanin biosynthesis. Here, we show that anthocyanin accumulation was repressed in the *CPC::SITRY* and *GL3::SIGL3* transgenic plants, suggesting that *SITRY* and *SIGL3* can influence anthocyanin pigment synthesis.

The differentiation of plant epidermal cells into trichomes and root hairs has proven to be a very useful paradigm for investigating early events in plant cell development. In *Arabidopsis thaliana*, the *CAPRICE* (*CPC*) gene, a member of the R3-type MYB transcription factor family, is an important regulatory factor for trichome initiation and root hair formation.<sup>1</sup> Genes closely related to *CPC* in the *Arabidopsis* genome include: *TRYPTICHON*, *TRY*; *ENHANCER OF TRY AND CPC1*, *ETC1*; *ENHANCER OF TRY AND CPC2*, *ETC2*; *ENHANCER OF TRY AND CPC3*/*CPC-LIKE MYB3*, *ETC3/CPL3*; *TRICHOMELESS1*, *TCL1* and *TRICHOMELESS2/CPC-LIKE MYB4*, *TCL2/CPL4*.<sup>2-10</sup> The *TRY* protein functions primarily during trichome initiation.<sup>2,11</sup> Another type of transcription factor in *Arabidopsis*, the bHLH transcription factor *GLABRA3* (*GL3*), also controls trichome initiation and root hair formation.<sup>12</sup> Previously, we reported that the *CPC*, *TRY*, *ETC1*, *ETC2* and *CPL3* proteins interact with *GL3* in yeast and might function together as a transcription factor complex.<sup>7</sup>

Recently, we identified tomato *SITRY* and *SIGL3* genes as orthologs of the *Arabidopsis* *TRY* and *GL3* genes, respectively.<sup>13</sup> The *CPCp::SITRY* transgenic plants produced more root hairs and had no trichomes, a phenotype analogous to that of the *35S::CPC* transgenic plants.<sup>13</sup> In contrast, *GL3p::SIGL3* transgenic plants closely resembled wild-type. These results suggest that *Arabidopsis* and tomato have orthologous transcription factors for epidermal cell differentiation including trichome initiation and root-hair differentiation. Furthermore, *SITRY*-like R3

MYB appears to function as an important common transcription factor for both trichome initiation and root-hair differentiation.<sup>13</sup>

In addition to epidermal cell differentiation, *Arabidopsis* *CPC* and *GL3* genes are known to regulate anthocyanin biosynthesis.<sup>14,15</sup> To verify that tomato *SITRY* and *SIGL3* also play roles in anthocyanin accumulation, we measured the anthocyanin content of *SITRY* and *SIGL3* transgenic plants. Total plant pigments were extracted from leaves, and anthocyanin levels were measured according to previously reported protocols.<sup>16,17</sup> Compared with the wild-type Col-0, the *CPC::SITRY* transgenic plants had significantly reduced levels of anthocyanin accumulation (Fig. 1A). Compared with the *cpc-2* mutant, *cpc-2* transgenic plants transformed with *CPC::SITRY* also accumulated significantly less anthocyanin (Fig. 1A). Anthocyanin accumulation was somewhat reduced in the *GL3::SIGL3* transgenic plants compared with wild type Col-0 (Fig. 1B). In comparison with *gl3-7454* mutants, *gl3-7454* transgenic plants transformed with *GL3::SIGL3* also had significantly lower levels of anthocyanin accumulation (Fig. 1B). Therefore, the tomato homologs to the *TRY* and *GL3* genes, *SITRY* and *SIGL3*, also function as repressors of anthocyanin accumulation.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

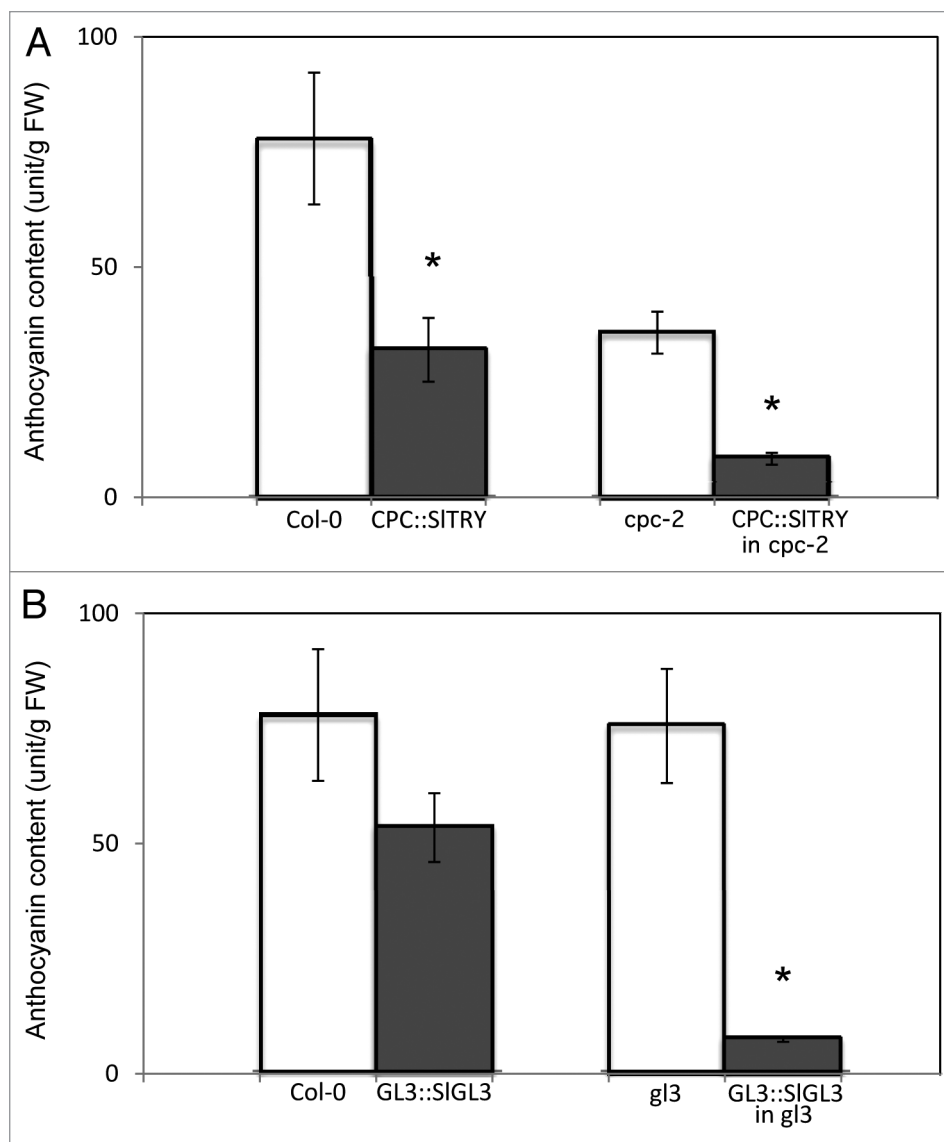
#### Acknowledgments

This work was financially supported by JSPS KAKENHI Grant Numbers 24658032, 23570057 and by the program

\*Correspondence to: Rumi Tominaga-Wada; Email: rtominaga@cc.miyazaki-u.ac.jp

Submitted: 03/04/13; Revised: 04/05/13; Accepted: 04/05/13

Citation: Tominaga-Wada R, Nukumizu Y, Wada T. Tomato (*Solanum lycopersicum*) homologs of TRIPTYCHON (*SITRY*) and GLABRA3 (*SIGL3*) are involved in anthocyanin accumulation. *Plant Signal Behav* 2013; 8: e24575; <http://dx.doi.org/10.4161/psb.24575>



**Figure 1.** Anthocyanin accumulation is inhibited by *SITRY* and *SIGL3*. **(A)** Anthocyanin content in wild-type Col-0, *CPC::SITRY*, *cpc-2* and *CPC::SITRY* in *cpc-2*. Error bars indicate the standard deviations. Bars marked with asterisks indicate a significant difference between the wild-type Col-0 and the *CPC::SITRY* transgenic line, or the *cpc-2* mutant and the *CPC::SITRY* in the *cpc-2* transgenic line by Student's t-test ( $p < 0.050$ ). **(B)** Anthocyanin content in wild-type Col-0, *GL3::SIGL3*, *gl3-7454* and *GL3::SIGL3* in *gl3-7454*. Error bars indicate the standard deviations. Bars marked with asterisks indicate a significant difference between the wild-type Col-0 and the *GL3::SIGL3* transgenic line, or the *gl3-7454* mutant and the *GL3::SIGL3* in *gl3-7454* transgenic line by Student's t-test ( $p < 0.050$ ).

“Improvement of Research Environment for Young Researchers” Technology, a grant for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science and from the University of Miyazaki.

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