

## Degradation of class E MADS-domain transcription factors in *Arabidopsis* by a phytoplasmal effector, phyllogen

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**M**embers of the *SEPALLATA* (*SEP*) gene sub-family encode class E floral homeotic MADS-domain transcription factors (MADS TFs) that specify the identity of floral organs. The *Arabidopsis thaliana* genome contains 4 ancestrally duplicated and functionally redundant *SEP* genes, *SEP1–4*. Recently, a gene family of unique effectors, phyllogens, was identified as an inducer of leaf-like floral organs in phytoplasmas (plant pathogenic bacteria). While it was shown that phyllogens target some MADS TFs, including *SEP3* for degradation, it is unknown whether the other *SEPs* (*SEP1*, *SEP2*, and *SEP4*) of *Arabidopsis* are also degraded by them. In this study, we found that all 4 *SEP* proteins of *Arabidopsis* are degraded by a phyllogen using a transient co-expression assay in *Nicotiana benthamiana*. This finding indicates that phyllogens may broadly target class E MADS TFs of plants.

Many angiosperms have flowers composed of 4 whorls of floral organs: sepals, petals, stamens, and carpels. The identity of these floral organs is regulated by a specific combination of floral homeotic MADS-domain transcription factors (MADS TFs) constituting the ABCE model.<sup>1–3</sup> Among these MADS TF genes, class E genes of the *SEPALLATA* (*SEP*) gene sub-family are essential for the development of all 4 floral organs as well as for floral meristem determinacy<sup>1,4</sup> because they function as the “glue” for MADS TF tetrameric complex formation.<sup>5,6</sup> The *Arabidopsis thaliana* genome contains 4 functionally redundant *SEP* genes,

*SEP1–4*. This is suggested by the findings that the triple mutant (*sep1/2/3*) and the quadruple mutant (*sep1/2/3/4*) exhibit abnormal flowers with green sepal- or leaf-like organs in all 4 whorls, and also exhibit a loss of floral meristem determinacy,<sup>1,4</sup> however, a mutant of one of *SEP* genes produces only subtle phenotypes, consistent with the possibility of functional redundancy.<sup>1</sup>

Phytoplasmas are obligate plant pathogenic bacteria with highly reduced genomes.<sup>7,8</sup> They inhabit both plant phloem sieve cells and insect cells altering their own gene expression,<sup>9</sup> and cause drastic morphological changes in infected plants, including witches’ broom (proliferation of small branches resulting in a characteristic bushy growth) and phyllody (a loss of floral meristem determinacy and the transformation of floral organs into leaves).<sup>10,11</sup> In recent publications, witches’ broom and phyllody symptoms of plants were shown to be associated with aberrant expression patterns of auxin-related genes and the genes encoding MADS TFs, respectively.<sup>12,13</sup> Moreover, an inducer of witches’ broom, designated TENGU, and a gene family of phyllody-inducing effectors, designated phyllogens, were identified from phytoplasmas.<sup>12,14,15</sup>

Phyllogens interact with and degrade some MADS TFs, including *SEP3* of *Arabidopsis*.<sup>15,16</sup> However, it is unknown whether the other 3 class E proteins (*SEP1*, *SEP2*, and *SEP4*) of *Arabidopsis* are also degraded by phyllogens, though their interactions with a phyllogen were shown in a yeast 2-hybrid assay.<sup>16</sup> It is also interesting from the perspective of the phylogenetic differences between *SEP*

**Keywords:** *Arabidopsis*, floral development, MADS transcription factors, phyllody, phyllogen, phytoplasma, *SEPALLATA*

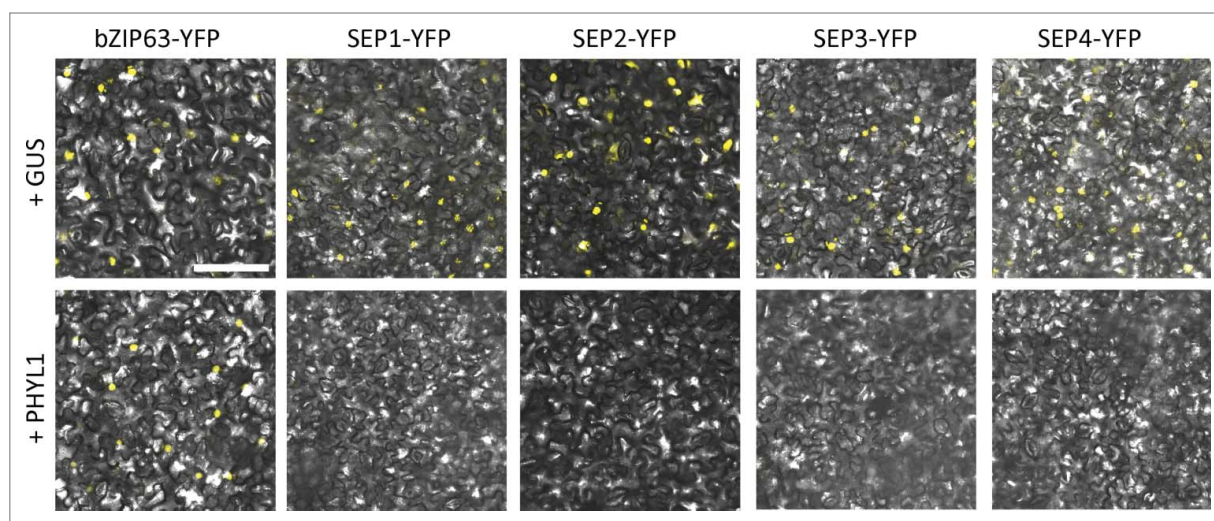
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Submitted: 02/18/2015

Revised: 04/03/2015

Accepted: 04/10/2015

<http://dx.doi.org/10.1080/15592324.2015.1042635>



**Figure 1.** PHYL1 induces the degradation of SEP1–4 proteins. *Agrobacterium* cultures ( $OD_{600} = 1.0$ ) for transient expression of YFP-fused proteins (bZIP63 and SEP1–4) and GUS or PHYL1 protein were mixed at a ratio of 1:10 and infiltrated into *Nicotiana benthamiana* leaves. The accumulation and subcellular localization of the transiently expressed YFP-fused proteins were observed 50 h after infiltration. Scale bar = 100  $\mu$ m. The *Agrobacterium* strain and plasmids were the same as in the previous study, respectively.<sup>15</sup> The composition of the infiltration buffer is as follows: 10 mM morpholinepropanesulfonic acid (MES, pH 5.6), 10 mM  $MgCl_2$ , and 150  $\mu$ M acetosyringone. Nuclear localization of the transcription factors used in this study was confirmed by DAPI staining (data not shown).

genes: in an evolutionary analysis of *SEP* gene sub-family, *SEP3* and the other 3 *SEP* genes of *Arabidopsis* were divided into 2 different clades.<sup>17</sup>

In this study, we examined whether a phylogen degrades SEP1, SEP2, and SEP4 of *Arabidopsis*. We transiently expressed YFP-fused SEP1, SEP2, SEP3, SEP4, or bZIP63, a basic leucine zipper transcription factor involved in the glucose-abcisic acid interaction network of *Arabidopsis*,<sup>18</sup> in *Nicotiana benthamiana* leaves by agroinfiltration in combination with either GUS protein (control) or a phylogen (PHYL1) of OY-W phytoplasma,<sup>19</sup> and monitored their accumulation and subcellular localization by confocal microscopy. As expected, all of the YFP-fused transcription factors localized to the nucleus when GUS was co-expressed (Fig. 1, upper panels). While co-expression with PHYL1 did not affect the accumulation or nuclear localization of YFP-fused bZIP63, it reduced the fluorescence derived from YFP-fused SEP1, SEP2, and SEP4 as well as SEP3-YFP (Fig. 1, lower panels), indicating that all 4 *SEP* proteins of *Arabidopsis* can be degraded in the presence of phylogen *in vivo*. This result agrees well with the fact that transgenic *Arabidopsis* plants

expressing phylogen show floral homeotic phenotypes similar to those of triple or quadruple mutants of *sep* genes.<sup>1,4,14,15</sup> Moreover, *SEP3*- and *SEP1/2/4*-like genes are highly conserved among various angiosperms as genes of the *SEP* sub-family encoding class E MADS TFs.<sup>17</sup> Therefore, it is likely that phylogen broadly target class E proteins of angiosperms, including eudicots, monocots, and other taxa, for degradation. Future studies investigating the target specificity of phylogen will shed further light on the relationships between phyllody caused by phytoplasmas and the function of class E genes in angiosperms.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Funding

This work was supported by Grants-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (category “S” of Scientific Research Grant 25221201), by the Funding Program for Next Generation World-Leading Researchers (project: GS005) initiated by

the Council for Science and Technology Policy (CSTP), and by the Program for Promotion of Basic Research Activities for Innovative Bioscience (PROBRAIN).

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