

# Petaloidy and floral organ identity genes in *Hydrangea chinensis* (Hydrangeaceae)

## 華八仙(八仙花科)類花瓣構造與花部器官決定基因之研究



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### Abstract

The inflorescence of Hydrangeaceae plants is often aggregated as a pseudanthium, which contains enlarged sepals on peripheral flowers of the inflorescence. The flowers with these petaloid sepals are generally sterile and may play important roles in attracting pollinators. In this study, *Hydrangea chinensis* was used to examine the underlying mechanism of the petaloidy formation. One A-class (*HycFL*), three B-class (*HycPI*, *HycAP3*, *HycTM6*), one C-class (*HycAG*) and one E-class (*HycMADS1*) floral homeotic genes were identified from *H. chinensis*, and verified by their conserved C-terminal motifs and the results of phylogenetic analysis. RT-PCR results show that the A class homolog *HycFL*, one B class homolog, *HycTM6*, and the E-class gene homolog *HycMADS1* have ubiquitous expression in all floral parts. Another B-class homolog, *HycAP3*, is expressed in petals, stamens, styles, and lower part of carpels, which is fused to part of normal sepals. In contrast, *HycPI* is highly expressed in petals and stamens, and weakly in lower part of carpels. The C-class gene homolog, *HycAG*, is expressed in the inner three whorls of flowers but the expression is higher in stamens and carpels.

Under Scanning Electromicroscope examination, the epidermal surfaces of cells on examined floral parts all have cuticular striation, while only adaxial epidermal cells of enlarged sepals show conical shape, a feature commonly found in the epidermal cells of a petal. Such conical cell formation has been demonstrated to be controlled by a transcription factor, *MIXTA*, in *Antirrhinum majus*. We have also successfully cloned *HycMYB1*, a homolog of *MIXTA*. The RT-PCR result shows that *HycMYB1* is highly expressed in enlarged sepals and much less in normal sepals and petals. Since *HycFL*, *HycTM6*, *HycMADS1* and *HycMYB1* are all expressed in enlarged sepals, they are the candidate genes involved in the formation of petaloid sepals of *Hydrangea chinensis*.

### Results

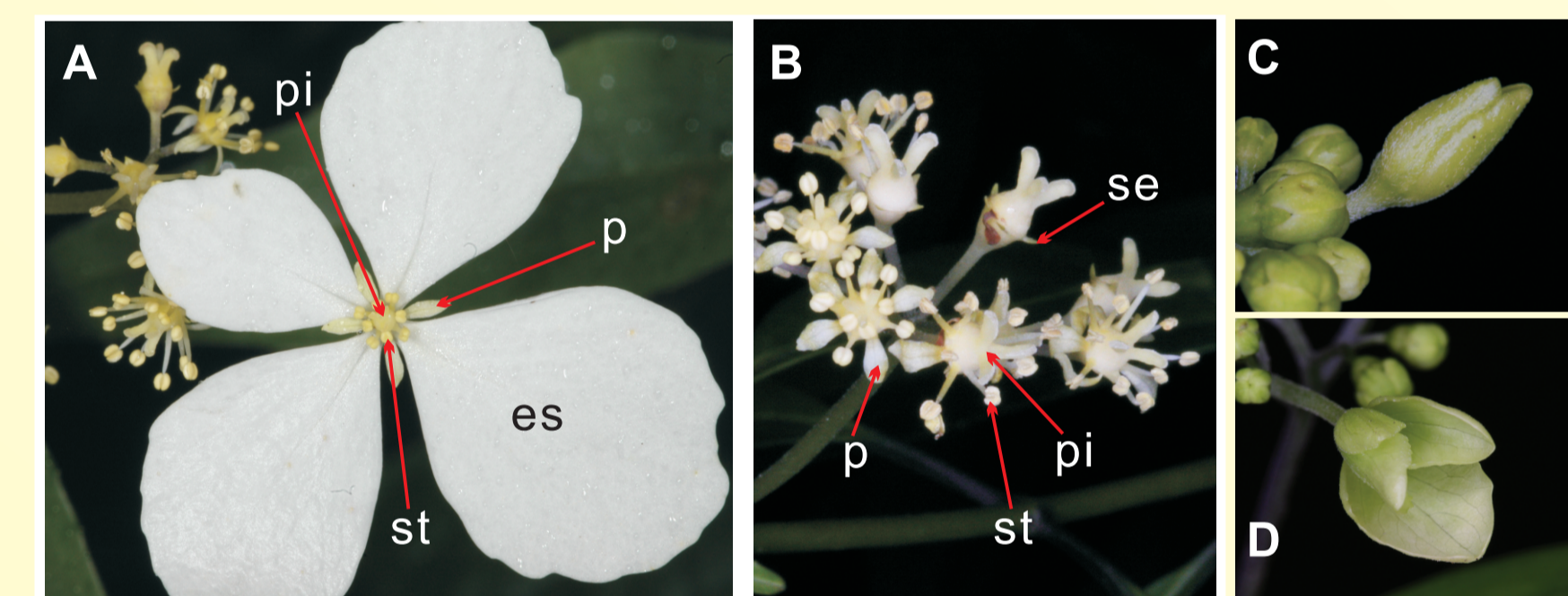


Figure 1. Flowers of *Hydrangea chinensis*. (A) The peripheral flowers with enlarged sepals. (B) Inner flowers of an inflorescence. es, enlarged sepal; p, petal; pi, pistil; se, normal sepal; st, stamen. (C)&(D) Early stage of the peripheral flower. The sepals start to enlarge and become white gradually. (Xinsanmenghu and Yang-Ming Mountain, Taipei, photos from J.-M. Hu and T.-H. Chang)

### Morphological study

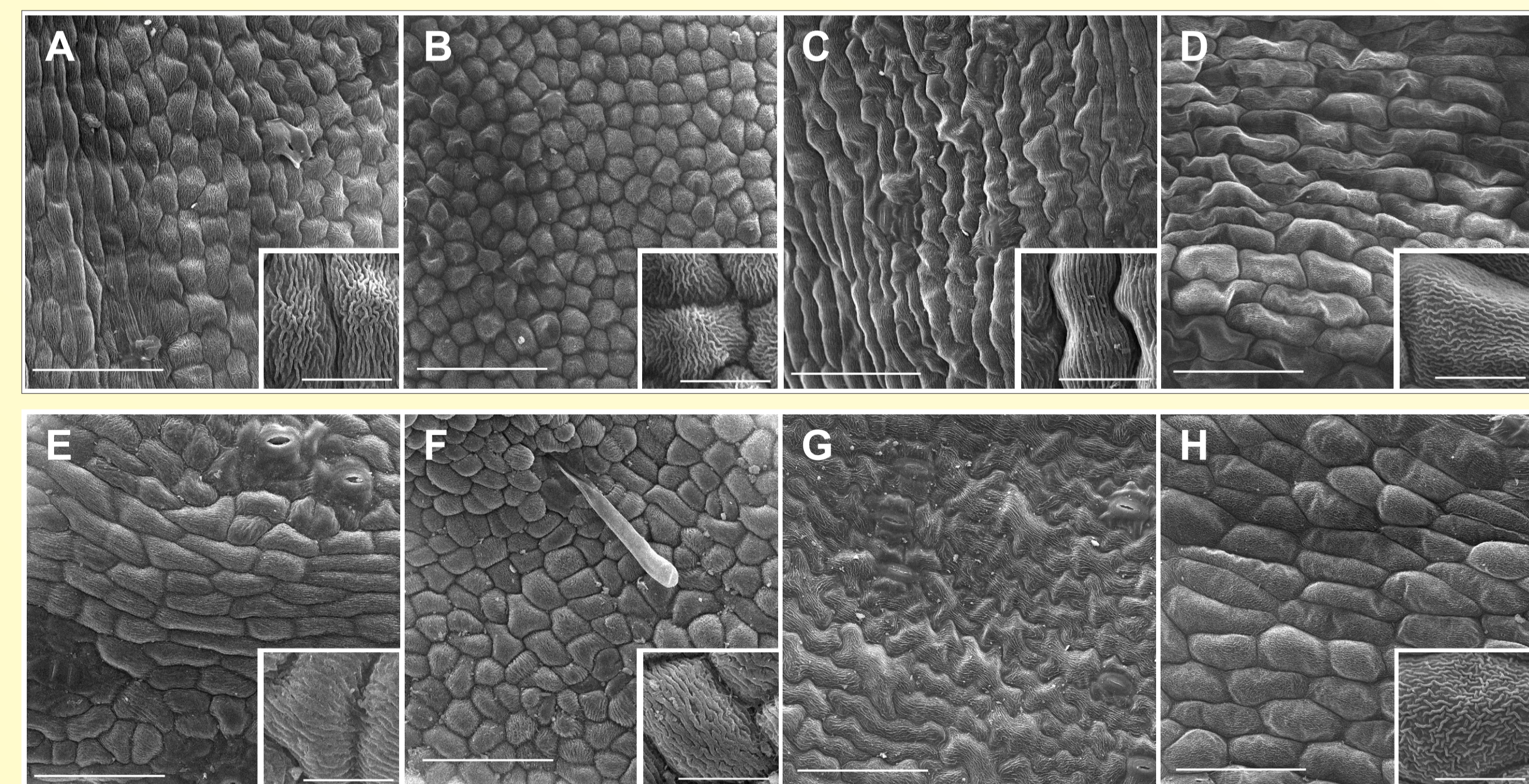


Figure 2. Morphology of epidermal cells of petals and sepals of *H. chinensis*. (A)-(D), SEM of perianth epidermal cells on peripheral flowers with enlarged sepals. (A) Abaxial side of petals, the shape of cells is irregular and stomata are present. (B) Adaxial side of petals, the cells are long and oval. (C) Abaxial side of enlarged sepals, with more regular and oval shape of cells. (D) Adaxial side of enlarged sepals, with distinct conical cells. (E)-(H), SEM of perianth epidermal cells on central fertile flowers. (E) Abaxial side of sepals, with oval but slightly irregular cells. (F) Adaxial side of sepals, with short oval cells. (G) Abaxial side of petals, with considerably irregular and flat cells. (H) Adaxial side of petals, with cells similar to the ones in (B). Cuticular striation are observed on all epidermal surfaces with slightly variation. The insets of the figures are close-up images. In (A)-(H), Scale bars equal to 100µm, and 20µm in the insets.

### Gene identification and RT-PCR expression pattern

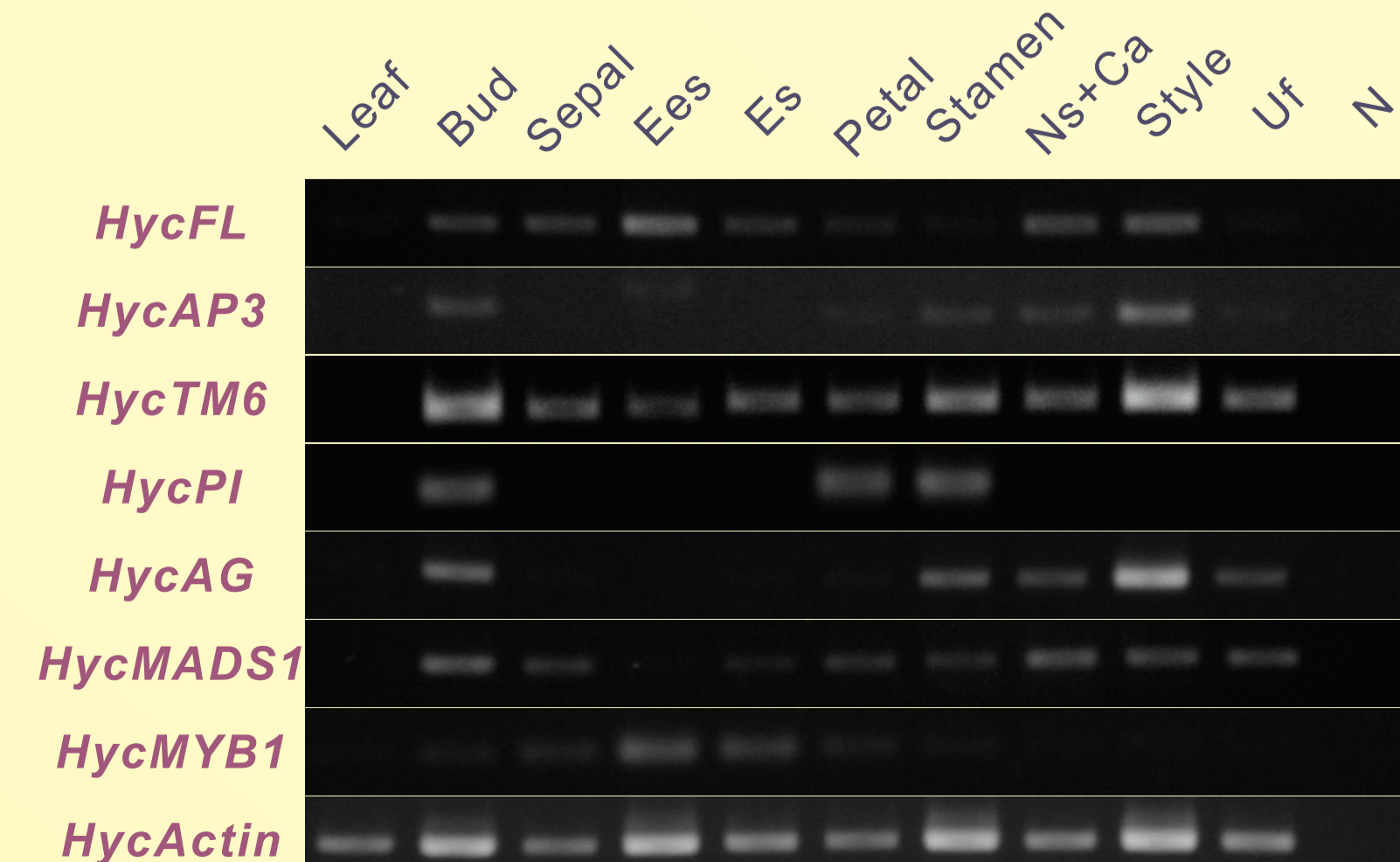


Figure 3. Six floral homeotic genes and one homolog of *MIXTA* are identified from *H. chinensis*. 25 cycles RT-PCR results of floral organ identity genes and a *MIXTA* homologue from *H. chinensis*, showing expression among different organs. *HycActin* gene, an *Actin* homolog from *H. chinensis*, is used as an internal control. Ees, early stage enlarged sepals; Es, enlarged sepals; Ns+Ca, lower part of carpels, which is fused to part of normal sepals; Uf, upper parts of fruits; N, negative control.

### Phylogenetic analysis

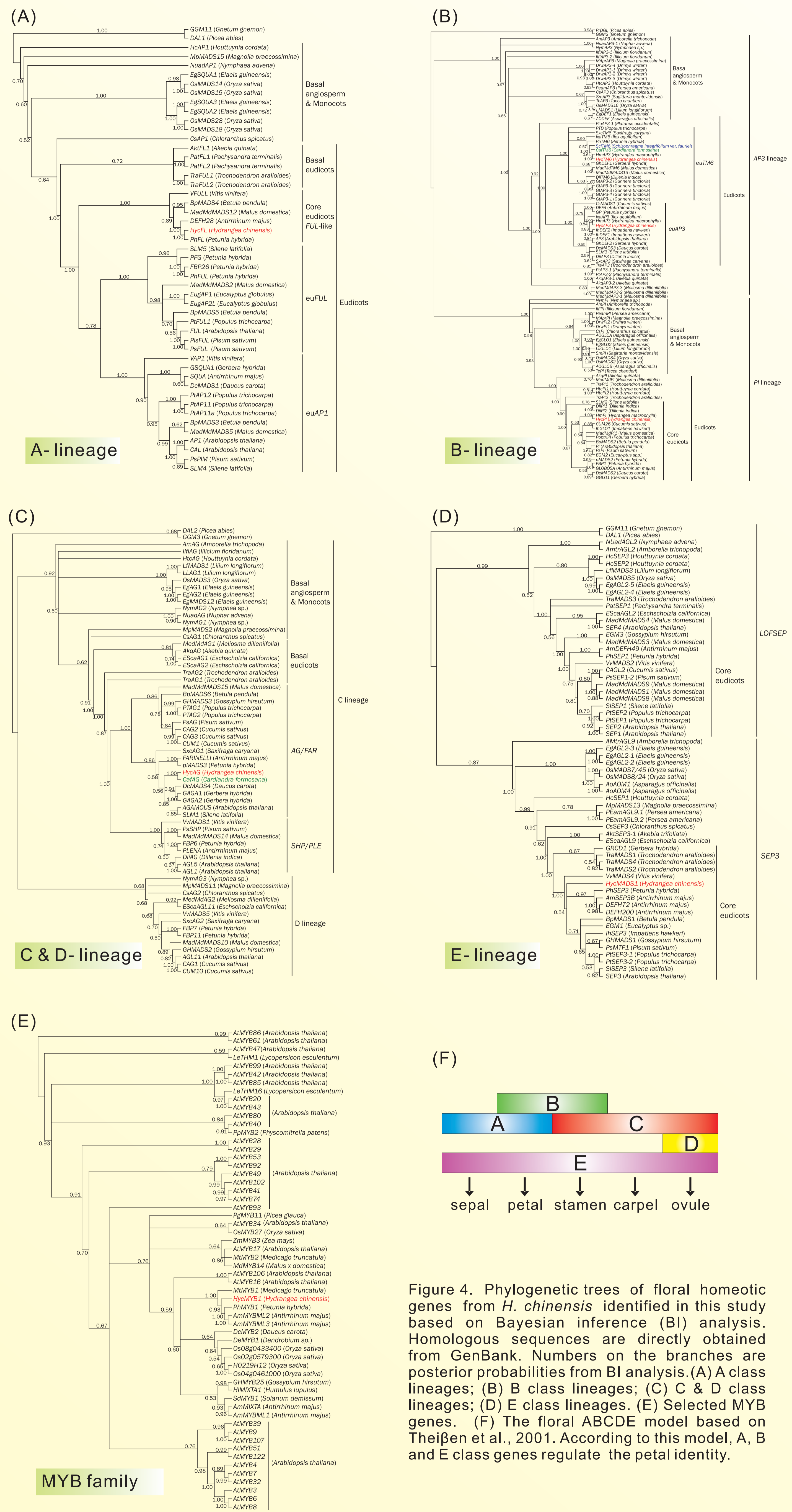


Figure 4. Phylogenetic trees of floral homeotic genes from *H. chinensis* identified in this study based on Bayesian inference (BI) analysis. Homologous sequences are directly obtained from GenBank. Numbers on the branches are posterior probabilities from BI analysis. (A) A class lineages; (B) B class lineages; (C) C & D class lineages; (D) E class lineages. (E) Selected MYB genes. (F) The floral ABCDE model based on Theißen et al., 2001. According to this model, A, B and E class genes regulate the petal identity.

### Conclusion

- Six floral homeotic genes and one homolog of *MIXTA* are identified from *H. chinensis*.
- HycFL*, *HycTM6*, *HycMADS1* and *HycMYB1*, but not others, are expressed in enlarged sepals, therefore, likely involved in the formation of enlarged sepals. The expression patterns of *HycPI* and *HycAG* consist with the prediction of the floral ABCDE model.
- Conical cells are only observed on the adaxial epidermis of enlarged sepals and cuticular striation on cell surfaces are present in all perianth parts.