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Comparative analysis of B-class gene expression from *Habenaria radiata* Spreng. and its petaloid-sepal mutant (Orchidaceae)

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Objectives

To understand the molecular mechanisms of floral development in *Habenaria radiata* (Orchidaceae), we have tried to isolate and characterize of the B class gene from *H. radiata* and to compare the expression patterns between wild-type and its petaloid-sepal mutant.

Materials and Methods

1. Material:

Plant - wild type *Habenaria radiata* cv. 'Ginga', petaloid sepal mutant *Habenaria radiata* cv. 'Hishou'

2. Methods:

For anatomical analysis, the fully expanded flowers in ethanol and acetic acid (FAA) were transparentized and examined using a light microscope. For molecular biological analyses, B-class genes were isolated by RACE method from *Habenaria radiata*. Phylogenetic analysis is performed by neighbor-joining method. Gene expressions were analyzed by the methods of RT-PCR and Northern hybridization.

Results and Discussion

The B-class of MADS box genes plays an important role in controlling petal development in whorl 2. The result of morphological approach indicated that the wild-type of *H. radiata* 'Ginga' has sepal in whorl 1 and petal in whorl 2 and that in petaloid-sepal mutant 'Hishou' had alteration from sepals to petals in whorl 1. We isolated and characterized three B-class genes, *HrGLO1*, *HrGLO2* and *HrDEF*. The result of phylogenetic analysis of the B-class genes was indicated that these genes in *H. radiata* were placed in the monophyletic group of monocots. Northern hybridization analyses using the dissected floral organs of wild-type *H. radiata* revealed that *HrGLO1* and *HrGLO2* were expressed in all floral organs, sepal, petal, and column. With regard to *HrDEF*, signals were detected in the petal and column but not in the sepal. While in petaloid-sepal mutant, all of three genes were expressed in all floral organs. This indicates that distinctive expression of *HrDEF* gene correlates with the differentiation of sepal and petal in *H. radiata*.