CHAPTER I

INTRODUCTION

Statement of purpose

Orchid is an important economic flower of Thailand. The annual export value of fresh orchid flowers are approximately 2,500 million Baht (Data from Department of Export Promotion, Thailand, 2012). The major import markets of Thai orchids are Japan, USA, Italy, the Netherlands, China, Taiwan and South Korea. Thai cut-flower orchids have a variety of colors, odors, sizes, forms and also are longer vase life than other cut flowers. Thailand is the world largest exporter of tropical orchids and the export value of Thai orchids continues to rise steadily.

Dendrobium is one of the most popular tropical orchids. Thailand produces cut-flower orchids more than 45,000 tons each year and most of them are *Dendrobium* hybrids. Other orchid hybrids include *Mokara, Aranda, Oncidium,* and *Vanda.* However, Thai orchids are facing competition increased in the international market. The major competitors of Thailand's export orchids are Taiwan, the Netherlands, New Zealand, Australia and Singapore. Therefore, to maintain current markets and expand into new markets, Thailand should accelerate the development of new varieties of orchid hybrids, especially in new colors and forms.

Dendrobium Sonia cv. Earsakul is one of the important tropical orchids of Thailand. As an exotic flower, this orchid is popular and exported to many countries around the world. *D.* Sonia cv. Earsakul has only a purple flower with a white base of sepals and petals. Development of a new flower color with no change of the flower form is impossible by conventional breeding. Advances in plant molecular biotechnology have great potential to contribute to the breeding of a novel color flower using recombinant DNA technology or genetic engineering. The advantage of molecular breeding is that the desirable flower color can be created without interfering with other characteristics via metabolic engineering of the anthocyanin biosynthetic pathway. However, to generate a transgenic plant, stable transformation and plant tissue culture are required. In orchid, the process of transformation and regeneration

from tissue culture is lengthy and costly. It will take at least 3 - 4 years from transformation to flowering. If the phenotype of transformed orchid is not as expected, it will waste money, time and labor. Therefore, the development of transient transformation to test the effect of genetic modification on flower color of orchid within a few days or weeks will help before making a decision on stable transformation.

Transient transformation can be both transient expression and suppression. Transient expression is aimed to temporarily express a protein produced from a transgene at local transformed regions or tissues, whereas transient suppression is aimed to knockout a host target gene in order to decrease the level of its protein product. RNA interference (RNAi) technology is a powerful tool for gene silencing and used to study gene function and develop new traits in plants. RNAi has been extensively used for metabolic engineering of flower color by modification of the anthocyanin biosynthesis pathway.

In this project we will use RNAi technology to temporarily suppress the anthocyanin biosynthetic gene, *dihydroflavonol 4-reductase* (*DFR*), in the flowers of *D.* Sonia cv. Earsakul.

Objective of the study

To develop transient RNAi system via agroinfiltration for determining effect of anthocyanin biosynthetic gene silencing on flower color of *D.* Sonia cv. Earsakul by using the *DFR* silencing model.

Hypothesis of the study

Transient *DFR* RNAi system can silent the *DFR* gene and inhibit anthocyanin synthesis causing white color at the transient transformed site on flowers of *D.* Sonia cv. Earsakul.

Expected outputs of the study

1. The expression profiles of the *DFR* gene in sepals and petals of *D.* Sonia cv. Earsakul flowers at different developmental stages.

2. The model of transient RNAi system for transient gene silencing in the anthocyanin biosynthesis pathway of *D.* Sonia cv. Earsakul.