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Differential gene expression analysis by cDNA-AFLP between flower buds of *Phalaenopsis* Hsiang Fei cv. H. F. and its somaclonal variant

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ABSTRACT

Somaclonal variation occurs during plant tissue culture and introduces changes that can result in the development of desirable traits. Using cDNA-amplified restriction fragment length polymorphism (cDNA-AFLP) analysis, we compared gene expression patterns between flower buds from wild type (donor) plants of *Phalaenopsis* Hsiang Fei cv. H. F., whose flowers are bronze in color, and from its somaclonal variants, whose flowers have a mosaic yellowish color, and sometimes an aberrant shape. Using 128 fluorescently labeled primer sets, a total of 2269 transcript-derived fragments (TDFs) were analyzed. Among them, 25 TDFs were differentially expressed between the wild type plant and its variant. After cloning and sequencing these differentially expressed TDFs, we found that they contained 27 distinct sequences. Further confirmation of the differential expression of these sequences was carried out by using semi-quantitative RT-PCR. We found that five sequences showed higher expression levels in the wild type plant compared to those in the variant plant. These corresponded to sequences that encoded casein kinase, isocitrate dehydrogenase, cytochrome P450, *EMF2*, and a no hits found protein. In contrast, two other sequences, whose roles were unknown, were expressed to a higher level in the variant plant compared to those in the wild type plant. The differential expression of these genes may lead to the mosaic color patterns as well as the aberrant flower shapes in the somaclonal variants of *Phalaenopsis* Hsiang Fei cv. H. F.

1. Introduction

Somaclonal variation describes the variation observed among plants that have been propagated from a single donor clone [1]. It has been recognized that plant tissue culture methods, which introduce changes into crop plants, could be used to develop new breeding lines [2]. Advantageous new cultivars have already been obtained for many important crops and ornamental plants [3], such as wheat [4], rice [5], potatoes [6], carnations [7], chrysanthemums [8], and harlequin-type flowers of *Phalaenopsis* orchid [9].

The molecular mechanisms of somaclonal variation have been characterized in different plants [10–12]. Two different levels of variation have been proposed [13]. One type is meiotically heritable and usually irreversible. For example, chromosomal changes, which include changes in ploidy and chromosomal breakages, have been found among tissue culture variants [13]. Sequence variation of *alcohol dehydrogenase 1* (*Adh1*) alleles in maize somaclonal variants has also been described [14]. The other source of somaclonal variation is of epigenetic origin [13]. These changes are heritable, but potentially reversible. Variations in both global methylation levels and the methylation of specific sites indicate that epigenetic mechanisms contribute to the process of somaclonal variation [10,15–18]. It has been suggested that both the activation of transposable elements and retrotransposons play important roles in somaclonal variation [19–21].

Several different approaches have been used to study somaclonal variation in a variety of plants. Mutations in somaclonal variants of maize have been analyzed by random genome scanning using restriction fragment length polymorphisms (RFLPs), and the

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results showed that most of the somaclonal variants are genetically similar to the controls [15]. In oil palm, the rate of global DNA methylation was investigated using HPLC, which showed that there were significant differences between wild type and variant plants [17]. Both methylation-sensitive RFLPs and methylation-sensitive amplification polymorphisms (MSAPs) were used to study DNA methylation polymorphisms that correlated with the mantled somaclonal variation in somatic embryo-derived oil palms [10,18]. This enabled the identification of two genes that showed somaclonal variation-related polymorphisms. Another potentially useful technique is cDNA-amplified fragment length polymorphism (cDNA-AFLP) analysis, which is highly reproducible and can be used to screen systematically a large number of differentially expressed cDNAs [22–26].

Phalaenopsis spp. are important ornamental plants that have a wide (large) market both in Taiwan and world-wide. Mass production of *Phalaenopsis* plants is achieved by the tissue culture of axillary buds from flower stalks. Somaclonal variation occurs during the proliferation of both adventitious buds and/or protocorn-like bodies [27]. Efforts in understanding somaclonal variation in *Phalaenopsis* have concentrated on variations at the level of transcription [9] or on differences in proteins and enzymes [28].

The flowers of *Phalaenopsis* Hsiang Fei cv. H. F. are normal in type and bronze in color (Fig. 1A). A somaclonal variant with flowers that have a mosaic color pattern was selected by the Taiwan Sugar Corporation (TSC) in 1992 and named as *Phalaenopsis* Hsiang Fei cv. TSC18 (Fig. 1B). This cultivar was awarded the third prize at the Taiwan International Orchid Show in 1993. Different types of somaclonal variants were obtained from the micropropagated plantlets of *Phalaenopsis* Hsiang Fei cv. H. F. (Fig. 1C). In this analysis, the gene expression profiles of wild type *Phalaenopsis* Hsiang Fei cv. H. F. plant and variant plant were compared using

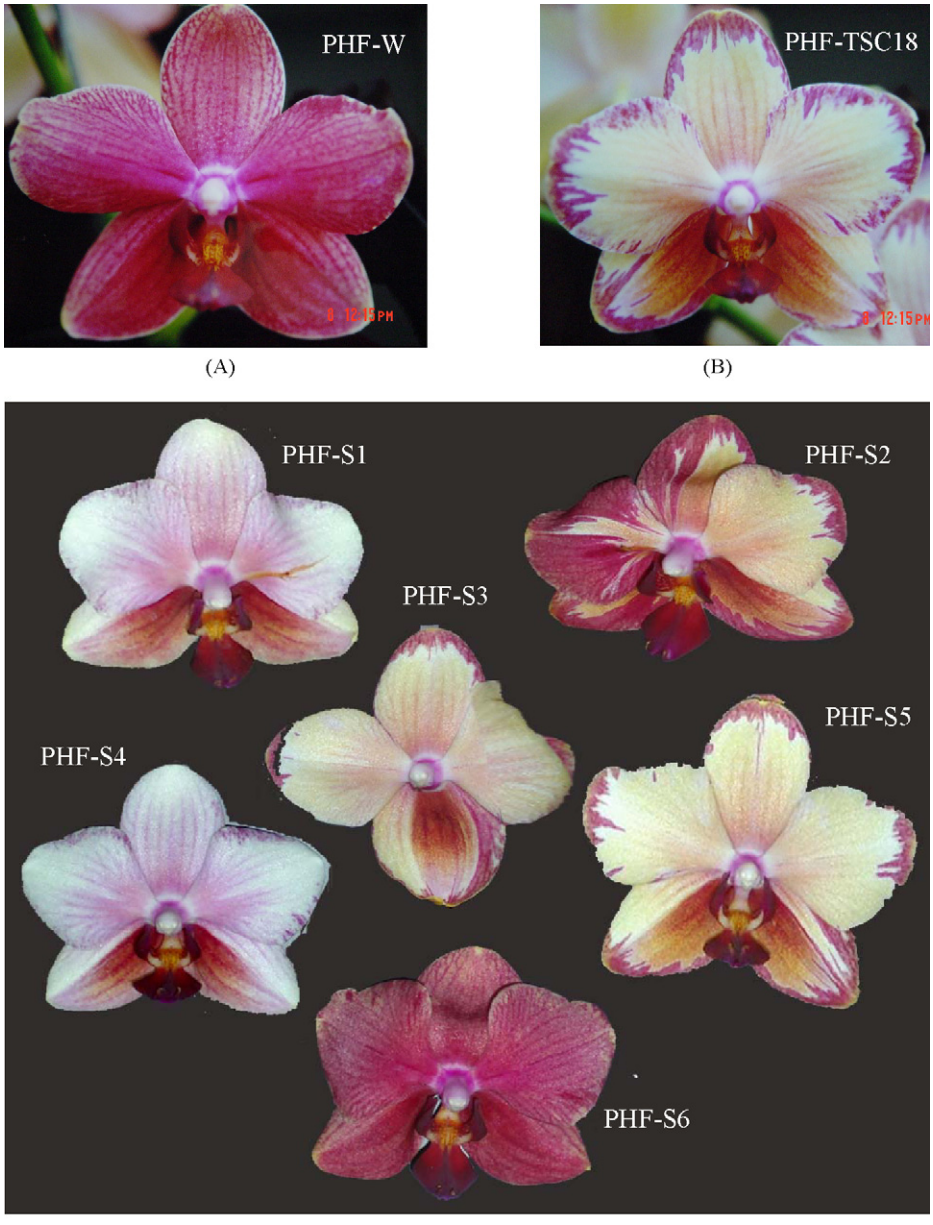

Fig. 1. Flower of *Phalaenopsis* Hsiang Fei cv. H. F. from a wild type plant (bronze color; A) and its somaclonal variant with either mosaic yellow color (B) and/or with an aberrant morphology (C). The clone IDs of both wild type and somaclonal variants are shown. PHF-W: wild type *Phalaenopsis* Hsiang Fei cv. H. F.; PHF-TSC18: the original somaclonal variant of *Phalaenopsis* Hsiang Fei cv. H. F.; PHF-S1–S6: six somaclonal variants of *Phalaenopsis* Hsiang Fei cv. H. F.