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# Transcriptional profile of differentially expressed genes related to abortive flower buds under short light period stress in petunia

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ABSTRACT

The long-day plant *Petunia* × *hybrida* 'Fantasy Red' was found to produce a considerable number of premature abortive flower buds when placed under short light period stress. Suppression subtractive hybridization (SSH) was performed to compare the gene expression profiles between normal and abortive petunia flower buds as induced by short light periods. Using reverse northern blotting, a total of 912 positive clones were successfully identified and sequenced, and 289 non-redundant, differentially expressed transcripts were obtained. According to the Blast2GO and KEGG pathway analyses, the significant distinct cellular component was identified as mitochondrion, and the notable different metabolic pathways were ascribed to carbohydrate metabolism and lipid metabolism. The quality of the SSH libraries was verified through quantitative real-time reverse transcription-PCR (qRT-PCR) analysis of 14 genes. In addition, six of these genes were demonstrated to be of temporal and/or spatial specificity using qRT-PCR, implying that they might be responsible for the premature flower abortion. Furthermore, *Phms2* (KC465381), a sequence sharing a high degree of similarity with male sterility 2 gene was characterized, and its inconsistent expression pattern predicted a novel role for *Phms2* in flower bud abortion. The data presented here provide insight into the molecular mechanisms of premature flower abortion under short light periods and, hence, may be of value for assessing candidate genes with potential for creating novel germplasms with enhanced photoperiod stress tolerance.



## 1. Introduction

Premature abortion of reproductive organs at various developmental stages can cause serious reductions in agricultural production worldwide under various abiotic stress conditions (Kinet et al., 1985). Blind shoots and flower buds, and premature flower or fruit abscission have been reported in many species when grown under conditions of low light intensity (Roar, 1971; Wien et al., 1989; Marcelis et al., 2004), low temperature (Roar, 1971; Nayyar et al., 2005; Thakur et al., 2010), high temperature (Turner and Wien, 1994a; Guilioni et al., 1997), and salinity (Vadez et al., 2012).

Abiotic stresses are generally considered to affect reproductive development via their effects on photosynthesis (i.e. decreased) and/or dark respiration (i.e. increased), thus resulting in decreased source strength demand (Turner and Wien, 1994b; Marcelis et al., 2004). The flower and fruit organs are the most susceptible to abortion when source strength is decreased, and the rate of abortion increases linearly (Marcelis et al., 2004). In addition, an abiotic stress, such as heat stress, may specifically reduce the metabolic activity of the flower/flower bud (Aloni et al., 1991). Short light period stress is an abiotic stress in which the availability of light is restricted through a reduced photoperiod, and has been known to be a limiting factor in winter crop production since the implementation of protected agriculture. Nevertheless, little is known about the molecular mechanism of its impact on plant reproductive development.

Assimilate allocation is thought to account for a variety of abortions (Kinet et al., 1985). Prior to anthesis, the inflorescence may abort, in the main, due to competition for assimilates between the vegetative apex and the last initiated inflorescence (Bertin, 1995). By contrast, the reproductive success of basal organs at the expense of those more distally placed is a common pattern in many crop plants. The different developmental potential has been attributed, in part, to unequal allocation of nutrients among reproductive structures, as evidenced by the measurement of carbohydrates

*Abbreviations:* DIG, digoxygenin; ESTs, expressed sequence tags; qRT-PCR, quantitative real-time reverse transcription-PCR; sqRT-PCR, semi-quantitative reverse transcription-PCR; SSH, suppression subtractive hybridization; ORF, open reading frame; Bl, bud length.

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between retained and aborted flowers (Weis and Webster, 1990; Turner and Wien, 1994a, 1994b; Guilioni et al., 1997; Nayyar et al., 2005).

Many authors claim that abscission of organs is primarily regulated by hormones, and the hormonal control of abscission of reproductive structures in many species can primarily be ascribed to the combined action of auxin and ethylene (Wien et al., 1989; Huberman et al., 1997; Marcelis et al., 2004). Furthermore, the ABA content in aborted flowers was found to be higher than in retained flowers (Nayyar et al., 2005). However, such studies have been largely restricted to morphological, physiological or biochemical analyses, and, to date, there is little molecular evidence regarding the phenomenon.

Petunia (*Petunia* × *hybrida* 'Fantasy Red') was found to be a long-day plant in a photoperiodic study (not shown) and it would generate both abortive and normal flower buds on the same plant when placed under short light period stress (Fig. 1). It often happens to any flowering plants that a part of flower should abort during the flower development, and usually hide behind the leaves. However, the extent of abortion in 'Fantasy Red' could be extremely high under the short light period stress, as at the first time, it was discovered that all flower buds above the plants were abortive. As an ornamental plant, premature flower abortion would seriously reduce the economic value of petunia 'Fantasy Red', especially when it is a multiflora type which produces smaller but more prolific flowers than grandifloras.

The abortive buds of 'Fantasy Red' exhibit the same morphological traits as normal buds during the early stages of flower development (Fig. 1a–c), but prematurely terminate growth during the maturation of the corolla (Fig. 1e), and subsequently undergo senescence (Fig. 1g). This particular phenotype means that the cultivar represents an exceptional material with which to elucidate the mechanism associated with flower bud abortion. Firstly, unlike typical abortion which is generally followed by abscission of the organ (also known as drop, fall, shed) (Sage and Webster, 1987; Wien et al., 1989; Weis and Webster, 1990; Turner and Wien, 1994a; Bertin, 1995), the abortive buds of short light period stressed *Petunia* remain on the plant together with the normal flowers. Secondly, initial stage of the abortive flower buds appear as fresh green flower buds which display identifiable malformation traits (Fig. 1e). Lastly, flowers of 'Fantasy Red' are small in size and large in quantity, so they are suitable for the treatment in the growth chambers and easy to form enough abortive flower buds for this study. Hence, this material is ideal for detecting the early changes involved in directing the subsequent development of the abortive flower bud type.

Suppression subtractive hybridization (SSH) is a powerful technique for the isolation of differentially expressed genes from two distinct mRNA populations (Diatchenko et al., 1996). In addition, this method has been successfully used to explore the differentially expressed genes of flower buds (Zhang et al., 2009; Ma et al., 2010), and also the genes involved in the abiotic stress responses of plants (Nguyen et al., 2009; Zhang et al., 2012). In this study, SSH was adopted, together with macroarray and RT-PCR analyses, to reveal the molecular mechanisms of abortive petunia flower buds induced under short light periods. Two SSH cDNA libraries were constructed from the abortive and normal flower buds of petunia. A total of 289 non-redundant differentially expressed transcripts were successfully obtained from both libraries and some of the genes were further studied. The results showed that mitochondrion maybe play more important role than other cellular components, and carbohydrate metabolism and lipid metabolism could be most likely the pathways involved in development of the abortive flower buds. Based on these findings, the roles of the putative translational products of some key genes are discussed with a view to providing new insights into the mechanisms of flower bud abortion under abiotic stress. In particular, a novel gene encoding a male sterile-like protein was implicated in the abortive bud trait.

## 2. Materials and methods

### 2.1. Plant materials, growth conditions and stress treatment

Seeds of petunia (*Petunia* × *hybrida* 'Fantasy Red') were sown into plug-trays (55 cm × 28 cm × 5.5 cm, 128 cells) containing a mix of peat moss, perlite and vermiculite at a volume ratio of 1–1–1, respectively. When the 6th true leaf expanded, single plants were transferred to 9 cm pots (volume 370 mL) containing a mix with an increased peat moss content (i.e. 2–1–1). The plants were maintained under short light periods (9 h $d^{-1}$ photoperiod) and irradiance of 240 ± 20 μmol $m^{-2}$ $s^{-1}$ delivered by a mixture of cool white fluorescent and incandescent lamps (approximately 210 μmol $m^{-2}$ $s^{-1}$ from fluorescent lamps and 30 μmol $m^{-2}$ $s^{-1}$ from incandescent lamps) within a growth chamber maintained at 23 °C day/18 °C night and 60 ± 10% relative humidity, until abortive flower buds appeared which were colorless and unfurled exposing the flowers center. Upon harvesting, materials were immediately frozen in liquid nitrogen and stored at −80 °C until used for RNA extraction.

### 2.2. Construction of suppression subtractive hybridization libraries

Abortive buds (ab) (bud length excluding sepal [Bl] = 3 ± 0.5 mm) and normal flower buds (nb1) (Bl = 3 ± 0.5 mm), developing on the same plants as one other, were collected to construct the SSH libraries (Fig. 1). The materials (floral organs and other tissues) for RNA extraction were sampled from at least 6 individual plants, and mixed randomly. Total RNA was isolated using RNAiso Reagent (TaKaRa, Japan) according to previously described methods (Ma et al., 2010). Double-stranded cDNA pools for suppression subtractive hybridization were pre-amplified using the Super Smart cDNA Synthesis kit (Clontech, USA). The ab- (ab as the tester and nb1 as the driver) and nb1- (nb1 as the tester and ab as the driver) SSH libraries were constructed from the PCR-Select cDNA Subtraction kit (Clontech, USA) according to the manufacturer's instructions. A *Petunia* housekeeping gene (*Beta-Actin*) was used to assess the subtraction efficiency of the cDNA libraries, according to manufacturer's instructions. The products from the second round of PCR were purified using an AxyPrep™ DNA Gel Extraction kit (Axygen Scientific, USA), subsequently cloned into the pMD18-T vector (TaKaRa, Japan) and transformed into *Escherichia coli* DH5α competent cells (TransGen Biotech, Beijing). Finally, the individual clones were randomly selected and cultured in 96-well plates containing LB medium with 15% glycerol, at 37 °C overnight. The constructed subtractive cDNA libraries were stored at −80 °C.

### 2.3. Differential screening of SSH libraries via reverse northern blotting

One microliter of each bacterial culture was used for the PCR amplification with nested PCR primers: 1 (5′-TCGAGCGG CCGCCCGGGCAGGT-3′) and 2R (5′-AGCGTGGTCGCGGCCGAGGT-3′). Subsequently, 5 μL of each PCR product was mixed with 5 μL NaOH (0.6 mol/L), and 1 μL of this mixture was imprinted onto Nylon$^+$ membranes (Roche, Germany). After neutralization through immersion in 0.5 M Tris–HCl (pH 7.5) for 5 min, the membranes were washed with sterile distilled water and baked at 80 °C for 2 h. The blots were stored at 4 °C until further use. *Beta-Actin* cDNA was also printed on each membrane as an internal control and