



IDENTIFICATION AND EVALUATION OF REFERENCE GENES FOR qPCR AND EXPRESSION OF FLOWERING LOCUS T (FT) GENE IN SWEET CHERRY BUDS OVER A GROWING SEASON.



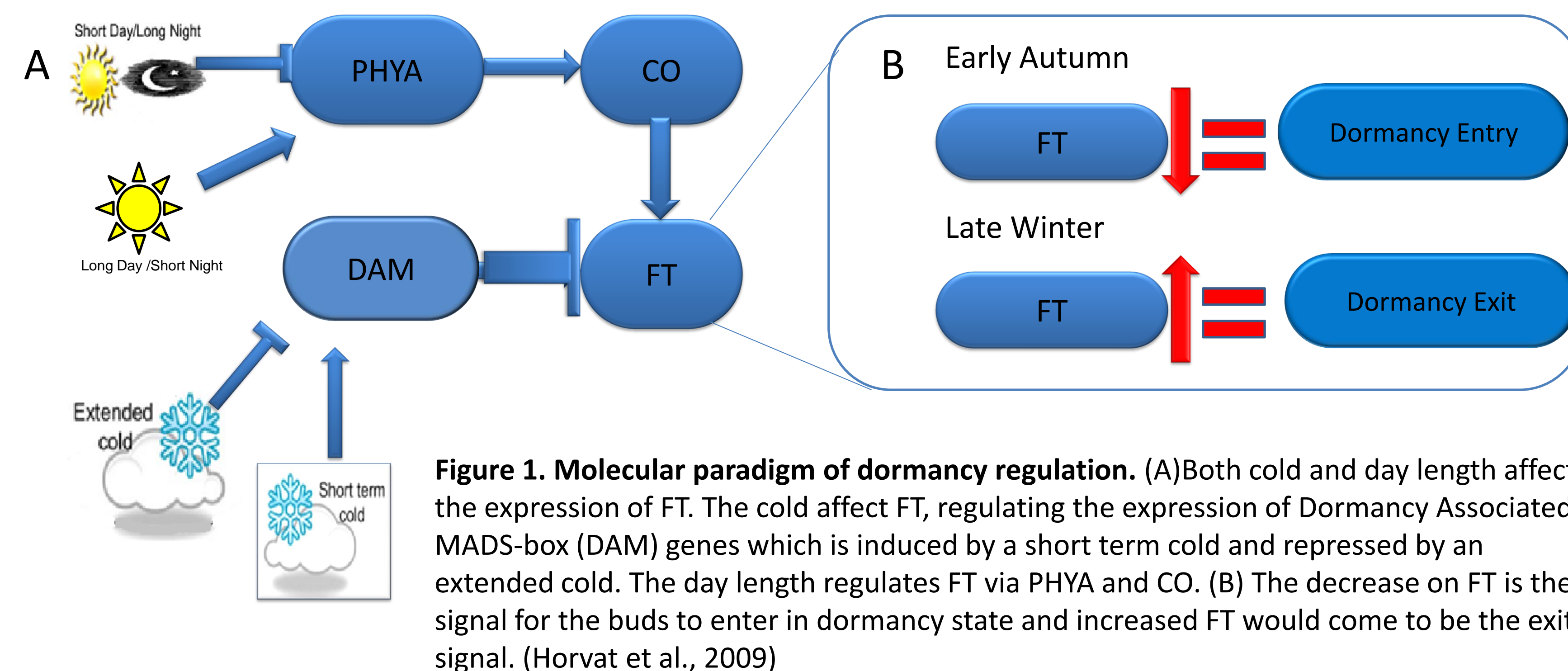
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INTRODUCTION

In perennial trees such as sweet cherry, when buds appeared in late spring, they have to wait the end of winter to bloom. This process is called dormancy. Dormancy is a state of growth cease to withstand the cold of winter. There are environmental clues that trigger a differential expression of genes that control this process. It has been postulated a possible paradigm of genes that would control this process (Fig. 1) (Horvat 2009, modified). In early fall, temperatures drop and triggers the expression of Dormancy Associated MADS-Box (DAM) genes (Hisayo et al., 2011) that inhibit the expression of Flowering Locus T (FT), and this decrease is the starting signal for dormancy. Besides cold, there is another pathway that converges in FT. The length of the day controls the expression of Phytochrome A (PHYA), which controls the expression of Constans (CO) that activates FT (Henrik et al., 2009). In fall, the shortening of day length finally leads to a decrease in the expression of FT. Extended periods of cold and day length increase in late winter repressing DAM genes leading to an increase in FT expression. The increase in FT is the signal for dormancy release.

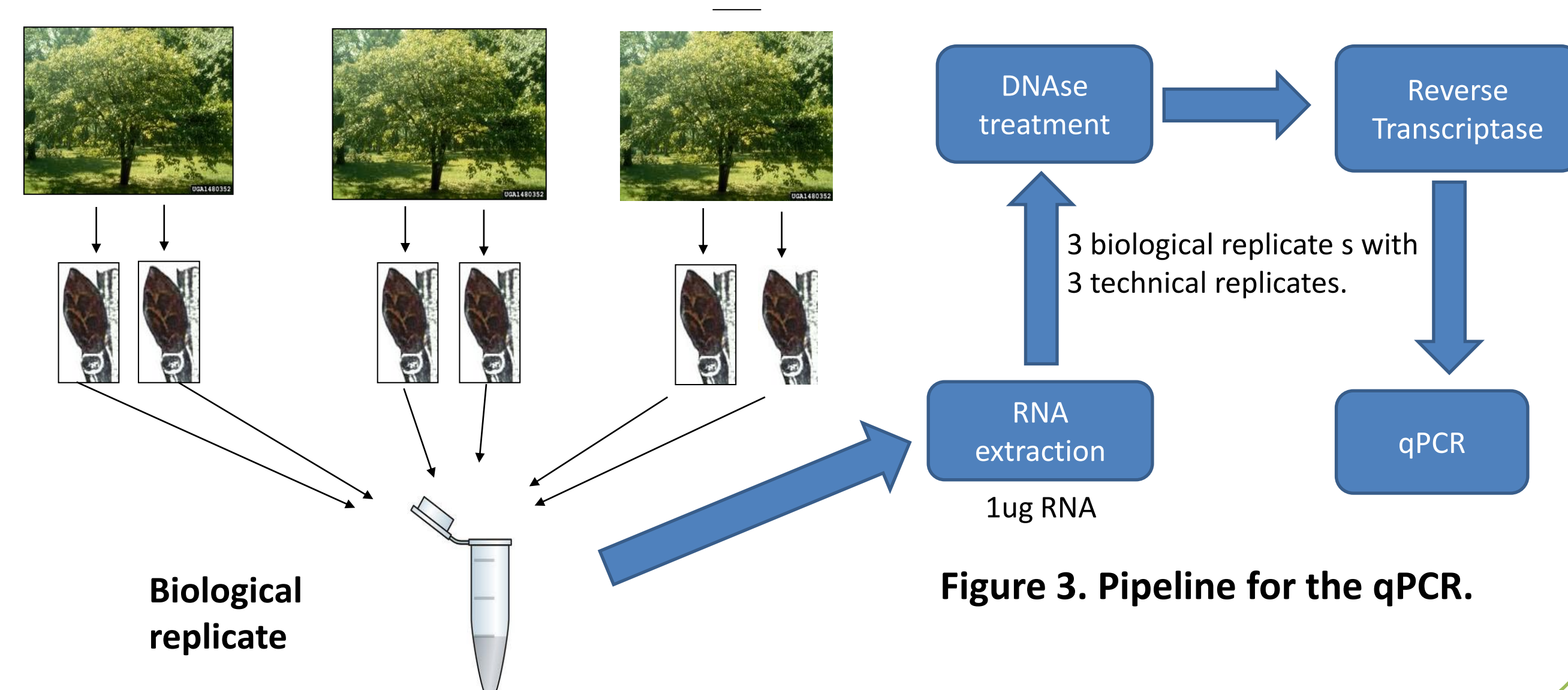
Our goal in this work was to analyze FT expression by qRT-PCR at different times of the growing season in sweet cherry.



EXPERIMENTAL DESIGN

Figure 2. Experimental design.

Buds and leaves from three individuals of the sweet cherry variety Bing were sampled to gather a pool considered as a biological replicate.



RESULTS

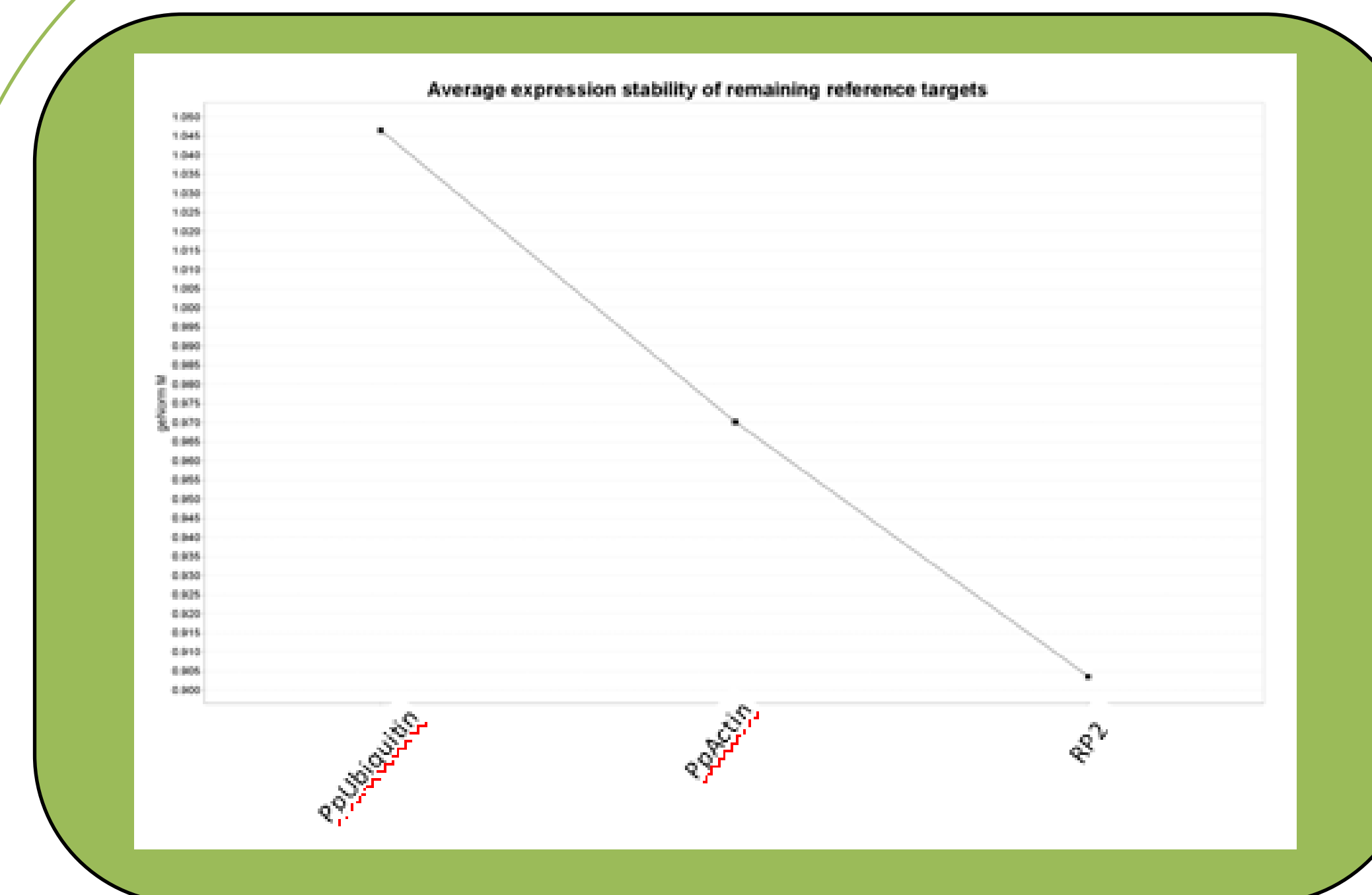


Figure 4. Average stability of reference genes. The stability of three commonly used peach qPCR reference genes was assessed for application in sweet cherry. PpUbiquitin (ppa010595m), RNA polymerase II (RP2) (ppa008812m) and PpActin (ppa007211m) genes were selected. M value was calculated with the GeNorm software, a value of M> 1 is considered stable. The genes were amplified from cDNA obtained from leaves from three different dates, and buds at two different developmental stages.

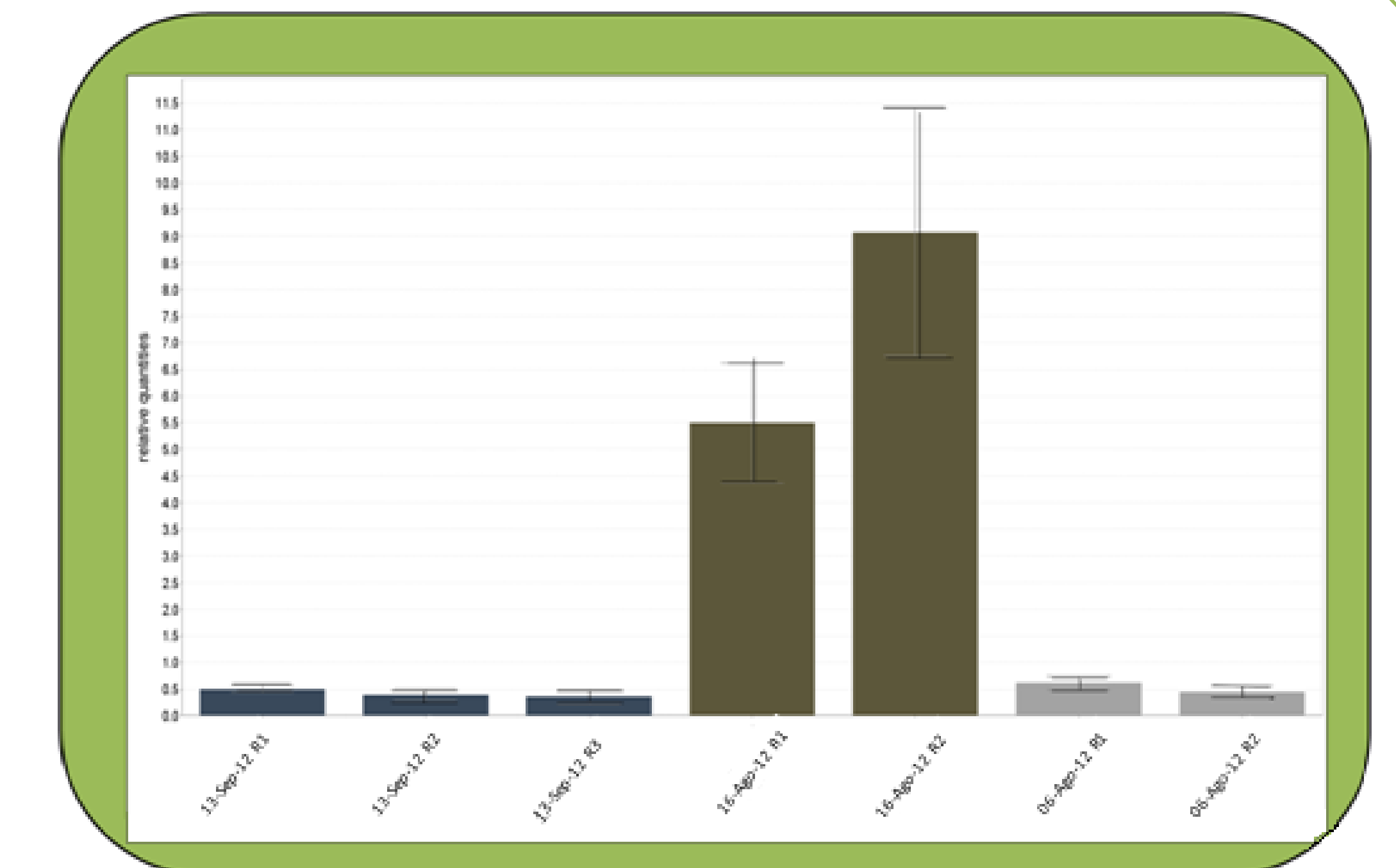


Figure 6. Relative expression of FT in sweet cherry buds during 2012 growing season. Relative expression of FT gene was normalized with PpUbiquitin as reference gene. Date of sampling is indicated in x-axis. It was observed an increase in FT expression at 16th August then returns to a baseline expression. SD is indicated in each bar.

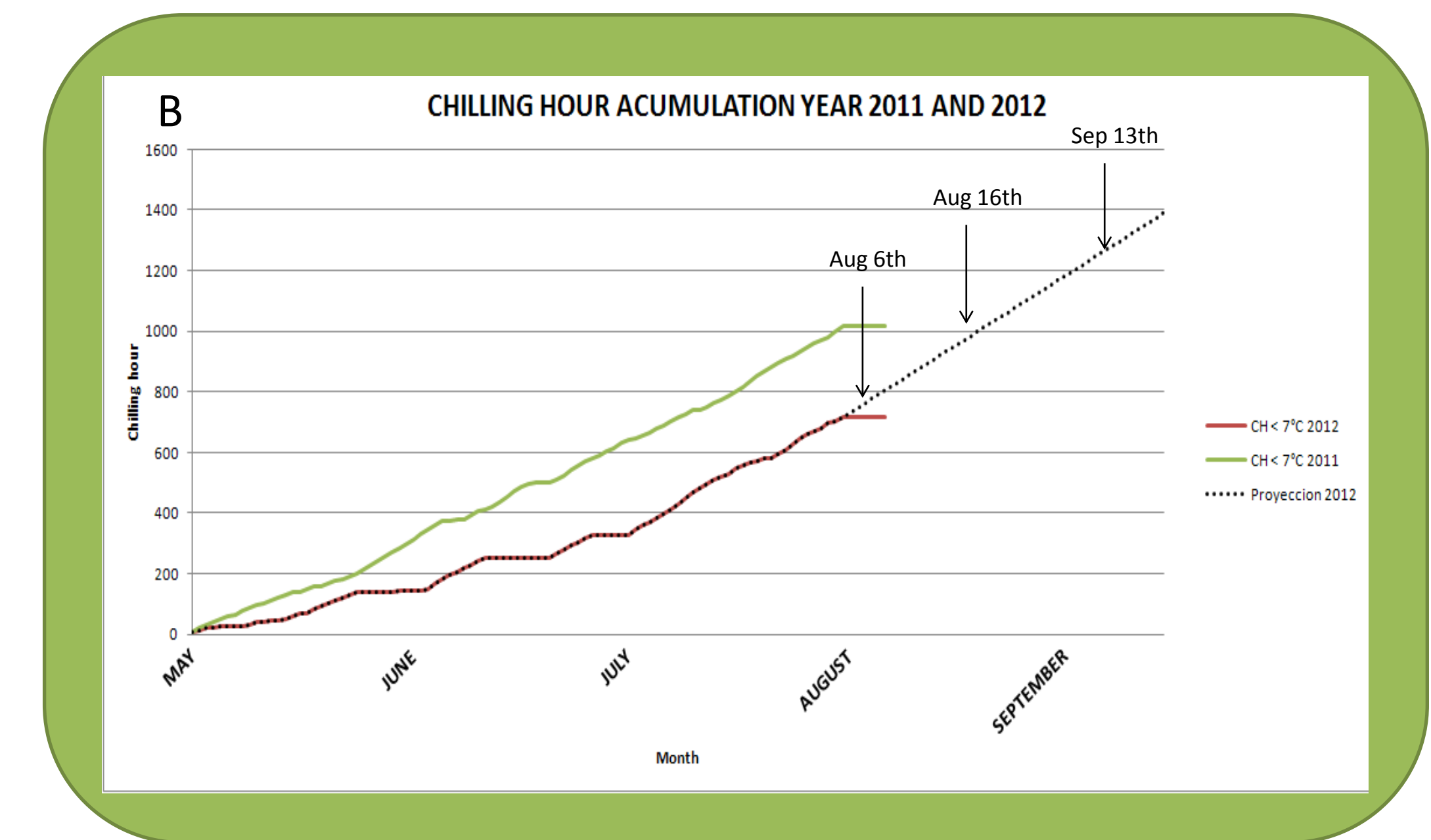
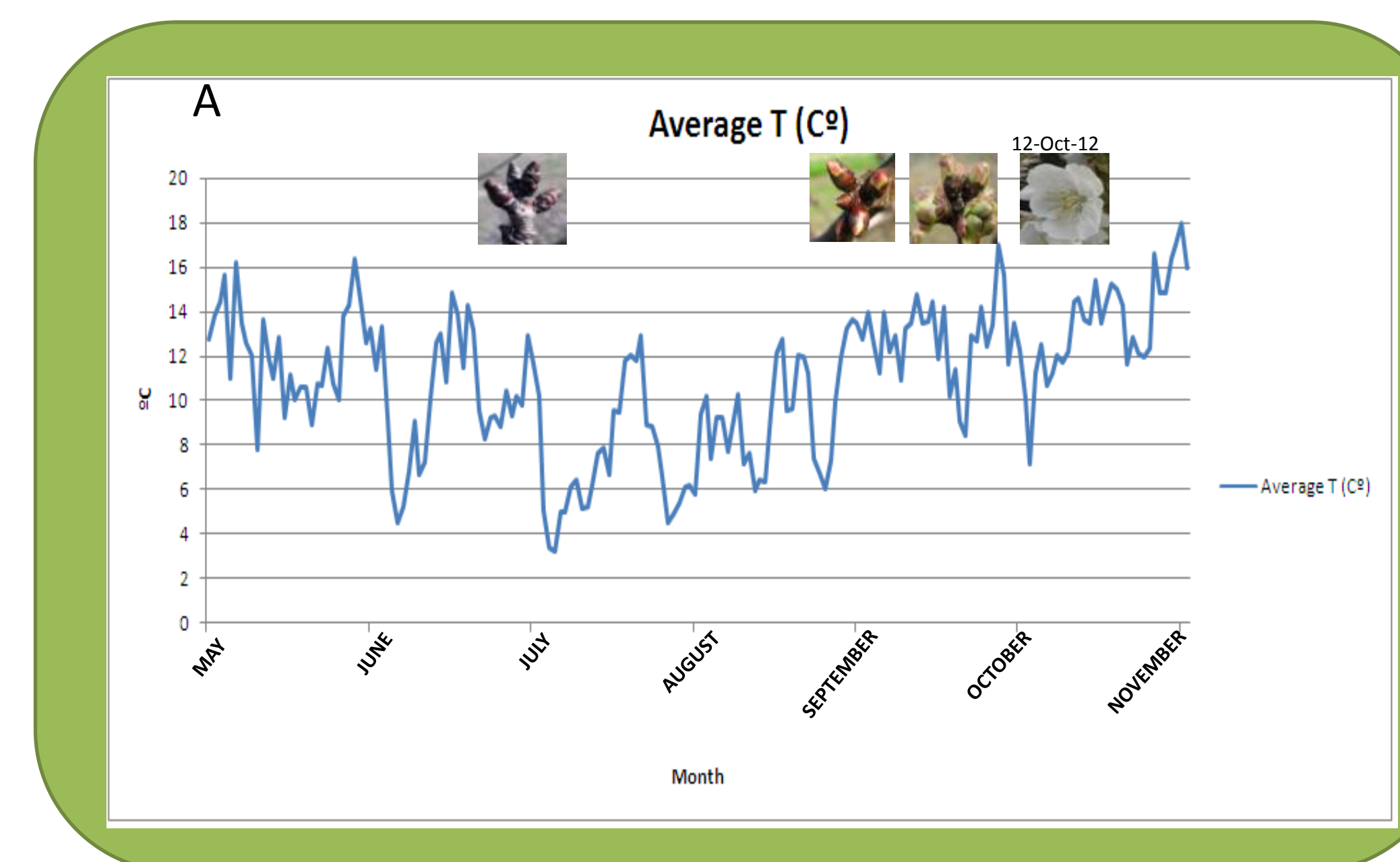


Figure 5. Average day temperature in 2012 and accumulation chilling hours in 2011 and 2012 season. (A) Average daily temperature during 2012 growing season. Flowering date (50% of flower) is indicated. Picture of the different developmental stages of the buds are shown through the months. (B) Chilling hours data were collected on www.agroclima.cl from 1st May until 31st July. The remaining period were projected. Green line: year 2011, red line: year 2012, dotted line: 2012 projection. Sampling dates are indicated with black arrows.

CONCLUSIONS

- Three candidates of potential reference genes were successfully tested in *Prunus avium*. PpUbiquitin was the one that showed greater stability.
- It was observed an increase in FT expression in the samples collected the 16th August 2012, which coincides with approximately 1000 chilling hours accumulated.
- This increase of FT could be the exit signal of dormancy.
- After the ending of dormancy, buds must wait until temperature rise, to bloom. From mid August 2012, temperature began to rise and flowering date was in 12th October 2012.

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