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Flower Bud Development and Chilling Requirements in Sweet Cherry cv 'Bing'

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Abstract

In order to survive to low temperatures, fruit trees in temperate regions stop their growth and enter dormancy. This is not just a survival strategy; chilling is also a prerequisite for adequate flowering. Chilling requirements are specific for each genotype and determine the possible geographical distribution of the different cultivars. In spite of the relevance of dormancy for fruit production, very little is known on the biological events during this time in flower buds, in which no visible changes can be observed until bud burst. In this work, flower bud development has been sequentially examined in sweet cherry (*Prunus avium* L.) cv "Bing" grafted on Santa Lucia 64 rootstock, in relation to dormancy and chilling accumulation. The work was performed over two consecutive years, paying attention to the possible anatomical and cytochemical changes accompanying dormancy. Results showed that, while no anatomical variations occurred along dormancy, conspicuous cytochemical changes could be tracked along this period that may help to understand the requirement for chilling.

INTRODUCTION

Fruit trees in temperate regions stop their growth and enter dormancy to survive low temperatures during winter. As occurs in other woody perennials, growth and dormancy cycles coordinate with the seasons (Rohde and Bhalerao, 2007). Blooming in sweet cherry, as in other temperate fruit trees, lasts for a few days only, but flower buds develop over months (Mounzer et al., 2008; Koutinas et al., 2010; Andreini et al., 2012). Flower differentiation takes place at the end of the previous summer. During the autumn flower bud development arrests and enters dormancy. This is not just a survival strategy to low temperatures, since chilling accumulation is also a prerequisite to resume growth (Coville, 1920; Knight, 1801) and adequate flowering (Perry, 1971). Chilling requirements are genetically regulated (Jansson and Douglas, 2007), and determine the possible geographical distribution of cultivars. In spite of the relevance of dormancy for fruit production, very little is known of the biological events during this process in reproductive buds, in which no visible changes can be observed until bud burst.

Chilling requirements have been empirically calculated by estimating the end of dormancy when the flower buds recovered their capacity to grow under favorable conditions in controlled chambers (Brown and Kotob, 1957; Ruiz et al., 2007; Alburquerque et al., 2008). A number of models have been developed to count the number of chilling hours (CH), due to the fact that not all temperatures affect the plant in the same way (Guak and Neilsen, 2013). Early work based the records on the number

of hours under 7.2ºC (45ºF) (Weinberger, 1950). Other models were later developed, to better fit different climatic regions, establishing a range of temperatures as the "UTAH Model" (Richardson et al., 1974), or the "Dynamic Model" (Fishman et al., 1987). Recently, research has focused on the genetic control of dormancy (Jimenez et al., 2010; Leida et al., 2012; Saito et al., 2013), but the interpretation of these data is hampered by the lack of knowledge of the biological processes underneath (Arora et al., 2003; Charrier and Améglio, 2011).

In sweet cherry, as it occurs in other histerant species, flower development took place in absence of leaves until blooming, so bud growth has to be supported by previously accumulated reserves in the tree (Ayala and Lang, 2015). Work in other *Prunus* species show the key role that starch accumulated in the pistil upon flower opening plays supporting the reproductive process (Rodrigo and Herrero, 1998; Rodrigo et al., 2000). In order to provide a frame to understand dormancy, the aim of this work was to characterize cherry flower bud development in relation to dormancy, paying attention to any possible changes in starch distribution in the flower along dormancy.

MATERIALS AND METHODS

Flower buds from three trees of sweet cherry (*Prunus avium* L.) cultivar "Bing" grafted on Santa Lucia 64 (*Prunus mahaleb* L.) rootstock were sampled weekly from middle September, before dormancy, to bloom, over two years (2011-2012 and 2012-2013). Trees were selected from a cultivar collection located at the Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA) in Montañana (Zaragoza) at a latitude 41º44´30" N, longitude 0º47´00" W and 220 m altitude.

To characterize flower development, three fresh flower buds were monitored weekly under a stereomicroscope Leica MZ16 (Leica, Cambridge, UK) connected to a digital camera DFC320 (Leica, Cambridge, UK). In another set of three flower buds at phenological stage A (Baggiolini, 1952) or 50 by the BBCH code (Fadón et al., 2015), the starch content in the ovary was evaluated. For this purpose, the flower buds without scales were weekly fixed in ethanol: acetic acid 3:1 (v/v) (Williams et al., 1999), embedded in paraffin wax, and sectioned at 10 µm with a Leica Jung 2045 rotatory microtome (Leica, Cambridge, UK). Starch content in the ovary was evaluated in two flowers per bud, resulting in six flowers per collecting day from the establishment of dormancy, when flower buds were closed and covered by dark brown scales at phenological stage A (Baggiolini, 1952) or BBCH code 50 (Fadón et al., 2015), to bud burst. Starch were quantified by using an image analysis system fitted to the microscope (Rodrigo et al., 1997). For this purpose, the sections were stained with I_2KI (Johansen, 1940) for 5-10 min and observed under a DM 2500 microscope (Leica, Cambridge, UK). Images were taken with a DC-300 camera (Leica, Cambridge, UK) connected to the microscope. Image processing was done using an Image Analysis System Quantiment 550 (Leica, Cambridge, UK). Data of starch content were analyzed with one-way ANOVA, followed by Duncan's multiple-range test. Statistical analysis was performed with the SPSS software (SPSS Inc., Chicago, IL, USA).

The date of chilling fulfillment was estimated by counting the number of CH from 1st October (Weinberger, 1950) until reaching 1000-1100 CH, which are considered the chilling requirements of cv. "Bing" in our conditions (Tabuenca, 1983). This occurred approximately 3-4 weeks before full bloom. Daily records of temperature were registered in a meteorological station placed at the research center.

RESULTS AND DISCUSSION

Dormancy Stage

Flower induction took place during the summer and flower buds kept on growing during the autumn when flower buds were at phenological stage A (Baggiolini, 1952) or BBCH code 50 (Fadón et al., 2015), consistently with previous observations in sour cherry (Guimond et al., 1998). Flowers remained in the same developmental stage from the autumn until budbreak. This developmental stage was characterized by all floral whorls differentiated, sepals, petals, stamens and pistil. The pistil presented a characteristic green color, and it was possible to distinguish the incipient parts of the pistil: ovary, style and stigma. Anthers were translucent and with no filament (Fig. 1A).

Subsequent histological work allowed characterizing anther and pistil development during dormancy. Anthers presented the four locules, and the different anther layers were already differentiated: sporogenus tissue, tapetum, middle layers and epidermis (Fig. 1B). Ovules could not be observed, but it was possible to distinguish the precursor tissue of the obturator (Fig. 1C). This characterization of flower buds during dormancy seems to be consistent with dormant flower buds of apricot (*Prunus armeniaca* L.) (Julian et al., 2011; Andreini et al., 2012) and sour cherry (*Prunus cerasus* L.) (Felker et al., 1983). However, in gymnosperms, as *Tsuga heterophylla* and *Chamaeccyparis nootkatensis*, different stages of stamen development could be observed during dormancy (Sedgley and Griffin, 1989; Mirgorodskaya et al., 2015).

Starch in the Flower Bud

During dormancy no anatomic differences could be observed in flower buds. However, starch accumulated in the different bud structures in this period. Variations in starch content of flower buds have been also detected in sour cherry (Felker et al., 1983) and apricot (Julian, 2008). Starch quantification in cherry flower buds showed that starch accumulation was not constant during dormancy, but followed a particular pattern in which important differences were detected in each phase of dormancy. As flower buds entered dormancy, just starch traces were detected (Fig. 2A). A progressive starch accumulation was observed in the next weeks until mid-January, concomitantly with the accumulation of CH (Fig. 2B, 2C). Then, starch decreased gradually until bloom (Fig. 2D). Thus, an important accumulation of starch in the pistil took place during dormancy. Maximum starch accumulation in January was some thirty times more than the one recorded at the end of September, when flower buds were closed and covered by dark brown scales at phenological stage A (Baggiolini, 1952) or BBCH code 50 (Fadón et al., 2015). Evolution of starch content during dormancy could be a conserved process in other *Prunus* sp., since a similar pattern has been described in apricot (Julian, 2008). These changes observed in starch content support the idea that while no growth took place, flower buds were physiologically active (Luna et al., 1991; Rohde and Bhalerao, 2007; Sedgley and Griffith, 1989).

These results show clearly that the pattern of flower bud development was similar for the two years studied: flower buds entered dormancy and remained dormant at the same stage of development, with all the floral whorls differentiated. Although no anatomical or morphological changes were apparent during dormancy, flower buds were physiologically active, and starch gradually accumulated in the ovary, reaching a maximum value at the end of dormancy. These results set a framework that contributes to understanding the biological processes that take place in flower buds during dormancy.

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Figures

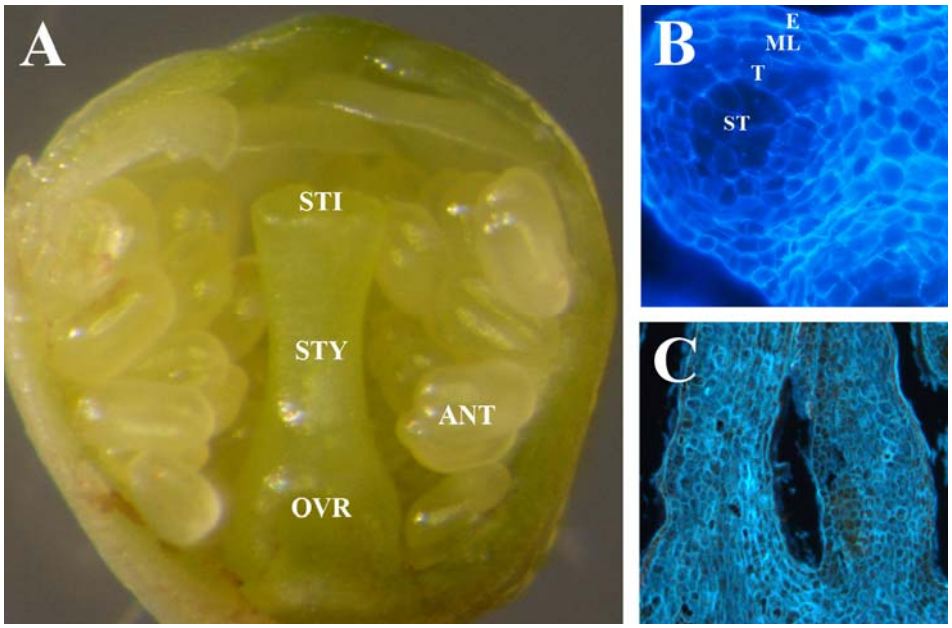
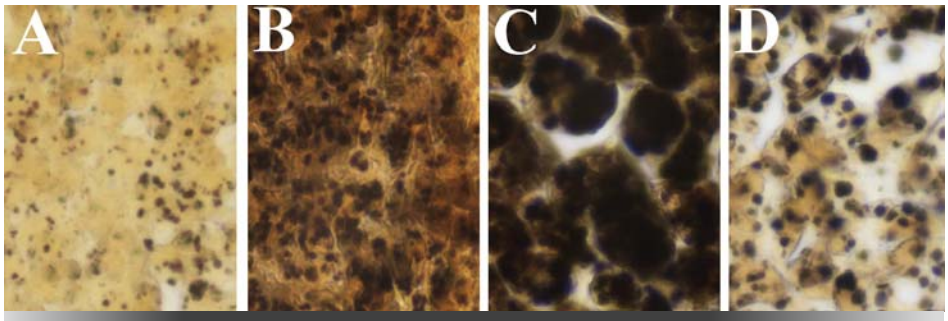


Fig. 1. Flower bud stage of development during dormancy in sweet cherry cv. “Bing” (A). Transverse sections of anther (B) and ovary section (C).
STI, stigma; STY, style; OVR, ovary; ANT, anthers; ST, sporogenous tissue; T, tapetum; ML, middle layers; E, epidermis.

STARCH IN THE OVARY				
DATE	**23 SEP 2010**	**18 NOV 2010**	**13 JAN 2011**	**9 MAR 2011**

CHILLING HOURS	**0**	**178**	**1073 (Chilling fulfilment)**	
STARCH CONTENT (∑Optical density)	**3639±1223[a]**	**17286±7385[ab]**	**140928±15932[b]**	**26390±3711[c]**

Fig. 2. Starch content in the ovary during dormancy in flowers of sweet cherry cv. "Bing" and number of chilling hours accumulated at sample dates. Traces of starch at dormancy establishment (A). Progressive starch accumulation during dormancy (B, C). Starch content at bud burst (D). Mean ± SE of the average values. Different letters indicate significant differences ($P < 0.05$) using Duncan's multiple range test.