Flower bud development and winter dormancy in sweet cherry (*Prunus avium* L.)

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Abstract

In temperate fruit trees, chilling accumulation during winter dormancy is required for proper flowering and is one of the main drawbacks of growing temperate trees in warmer latitudes. Chilling requirements are specific for each cultivar, suggesting a tight genetic control. The importance of chilling requirements, for cultivar adaptation to particular areas, has resulted in the development of different empirical models to quantify low temperatures. However, the biological processes behind these requirements remain unveiled. In *Prunus* species flower development requires several months from differentiation, at the end of the previous summer, until bloom the following spring. Development is halted during the winter, when the flower buds enter a dormant stage. To evaluate any possible changes behind the requirements for chilling, in this work flower development has been examined in sweet cherry (*Prunus avium* L.) and related to temperature, light, and chilling requirements. Anatomical characterization of flower buds, from the autumn to the spring, showed that the flower buds survive the winter at a particular stage of flower development. While no anatomical changes were detected during the theoretical chilling fulfillment, further cytochemical characterization revealed that conspicuous changes occurred in the pistil. Quantification of these changes with an image analysis system fitted to the microscope sheds light on the biological basis for chilling requirements.

Keywords: chilling requirements, microscopy, image analysis, pistil, cytochemistry.

INTRODUCTION

Temperate fruit trees adapt to the fluctuation of the seasons entering into a dormant stage during winter. Dormancy allowed surviving to low winter temperatures, but chilling accumulation is also required for proper flowering. This fact is one of the main drawbacks of growing temperate trees in warmer latitudes. In *Prunus* species, while flowering just lasts few days in spring, flower bud develops over months. Flower differentiation starts at the end of the previous summer, but flower development is halted in the autumn by entering into a dormant stage to survive winter (Perry, 1971). During dormancy, growth is ceased and meristems acclimate to cold and remain unresponsive to growth signals (Cooke et al., 2012). For growth, to be resumed, flower buds firstly require to pass a certain period under low temperatures (Coville, 1920; Rohde and Bhalerao, 2007), and resume growth once temperature increased (Perry, 1971; Sedgley and Griffin, 1989). Chilling requirements are specific for each cultivar, suggesting a tight genetic control (Jansson and Douglas, 2007).

The importance of chilling requirements for cultivar adaptation to particular areas has resulted in the development of different empirical models to quantify low temperatures (Fishman et al., 1987; Richardson et al., 1974; Weinberger, 1950). Chilling requirements for cultivars are empirically calculated (Brown and Kotob, 1957; Weinberger, 1950), since there is a lack of knowledge on the physiological bases of dormancy and the environmental factors for dormancy release (Campoy et al., 2011; Luedeling, 2012).

In histerant species as sweet cherry (*Prunus avium* L.), and other *Prunus* species, blooming occurs before leaves emergency (Fadón et al., 2015a), so flower bud development and growth are supported by reserves accumulated during the previous season (Rodrigo et

al., 2000). Thus, the reproductive process is supported by the starch accumulated in the pistil upon flower opening (Rodrigo and Herrero, 1998; Rodrigo et al., 2000).

To evaluate any possible changes behind the requirements for chilling, in this work flower development has been examined in sweet cherry and related to temperature, light, and chilling requirements before, during and after dormancy, paying special attention to the pattern of starch distribution into the bud and of chilling accumulation.

MATERIAL AND METHODS

Three trees of the sweet cherry cv. Burlat were selected from an experimental orchard located at Centro de Investigación y tecnología Agroalimentaria de Aragón (CITA) in Montañana (Zaragoza) at 41°44'30" N, 0°47'00" W and 220 m above sea level. Seven flower buds were randomly collected, over two years, at early dormancy (21st and 25th October), during dormancy (13th and 17th January) and after dormancy (9th and 13th March).

For dormancy prediction, chilling fulfillment was calculated over two years by counting the number of Chilling Hours [CH, hours under 7.2°C (45°F)] (Weinberger, 1950) from 1st October until reaching 900-1000 CH, which are considered the chilling requirements of 'Burlat' (Tabuenca, 1983).

The phenology of the flower buds was characterized in the field. To characterize flower development, each sampling date two flower buds - each containing three flowers - were dissected under a stereoscopic microscope MZ-16 (Leica, Cambridge, UK), and photographed with a digital camera DC- 300 (Leica, Cambridge, UK).

For histochemical characterization, two further flower buds were fixed in glutaraldehyde (Sabatini et al., 1963), and embedded in Historesin Embedding Kit (Leica, Heidelberg, Germany). Sections 2 µm thick were cut on an EM UC6 ultramicrotome (Leica, Heidelberg, Germany) and subsequently stained with PAS and Toluidine Blue for insoluble carbohydrates and general structure (Feder and O'Brien, 1968).

To evaluate the starch content in the ovary, another set of three flower buds was fixed in ethanol: acetic acid 3:1 (v/v) (Williams et al., 1999) and embedded in paraffin wax to be sectioned at 10 µm (Rodrigo and Herrero, 1998). Middle ovary sections of two flowers per bud, thus six flowers per sample date, were selected and stained using the potassium iodide: iodine reaction (I₂KI) (Johansen, 1940) for 5 - 10 min and mounted with Histomount (National diagnostics, Atlanta, USA). Sections were photographed with a DC-300 digital camera (Leica, Cambridge, UK) connected to a DM 250 microscope (Leica, Cambridge, UK), and then each image was processed using a Quantiment Q550 Image Analysis System (Leica, Cambridge, UK), using a modification of the method for starch quantification described by Rodrigo et al. (1997).

RESULTS AND DISCUSSION

The phenological stage of the flower buds in mid-October were the same in both years and corresponded with the stage 93 of the BBCH code (Fadón et al., 2015b). At this time leaves had faded color and leaf fall started (Fig. 1 A). Inside each flower bud, there were three to four flower primordia protected by external scales. In each flower primordium, it was possible to distinguish the different whorls: sepals, petals, anthers and pistil. The pistil presented the suture line, and the anthers had reached their characteristic shape but the filaments of the stamen were not developed (Fig. 1 B). Histological analysis of the ovary showed cells with irregular shapes and vacuoles, without starch (Fig. 1 C).

Figure 1. Flower bud characterization of sweet cherry cultivar Burlat before, during and after dormancy. (A) Flower buds external appearance at dormancy establishment. (B) Flower primordium developmental stage at dormancy establishment. (C) Ovary tissue at the onset of dormancy, showing no starch. (D) Flower buds external appearance during dormancy, at chilling fulfillment. (E) Flower primordium developmental stage during dormancy. (F) Ovary tissue during dormancy, with clear starch grains. (G) Flower buds external appearance after dormancy, at bud burst. (H) Flower primordium developmental stage after dormancy, at bud burst. (I) Ovary tissue after dormancy, at bud burst, without starch.

In the first year (cold year), chilling requirements were fulfilled between 30th December and 5th of January, while for the second year (mild year) this occurred a month later, between the 27th January and 2nd February.. The external appearance of the flower buds corresponded with the stage 50 of the sweet cherry BBCH code (Fadón et al., 2015b) and stage A (Baggiolini, 1952) (Fig. 1 D). Inside the buds, flower primordia presented a slighter growth than in the autumn. In the pistil, the incipient ovary, style and stigma could be distinguished. The anthers presented a translucent appearance, but without filaments (Fig. 1 E). This stage corresponded with those observed in cv. 'Bing' (Fadón et al., 2015c). This is also consistent with previous observations in sour cherry (Guimond et al., 1998) and peach (Reinoso et al., 2002). During dormancy, the ovary cells were larger and rounded with small vacuoles and numerous starch grains (Fig. 1 F).

At bud burst, in mid-March, flower buds presented the phenological stage 51 of the BBCH code (Fadón et al., 2015b) or stage B (Baggiolini, 1952) (Fig. 1 G). Flowers had significantly increased in size. In the stigma, the papilla started to develop (Fig. 1 H). In the ovary cells, the starch grains had vanished as the cells presented a big central vacuole.

Slight anatomic differences were observed in flower development before, during and after dormancy. However, important variations in starch content were detected in the ovary cells before, during and after dormancy, differences which were conserved over the two years studied. In mid-autumn, when few chilling hours had accumulated (about 20 CH the first year and 30 the second), no starch was detected in the ovary (Fig. 2). In mid-January, when about 1000 CH were accumulated in the cold year and about 800 CH in the mild year, the amount of starch was significantly higher, reaching values between 90 and 100 x 10³ (Fig. 2).

Figure 2. Starch content in the ovary tissue of sweet cherry flower primordium in cultivar Burlat before, during and after dormancy over two years.

This means that during endo-dormancy, which corresponds with the chilling accumulation period (Lang et al., 1987), starch accumulated in the ovary cells. Measures at bud burst, in mid-March, presented approximately half the starch content than in January, around 50 x 10³ (Fig. 2). This period after chilling fulfillment until growth resumption is

considered as ecodormancy (Lang et al., 1987) and corresponds with a period of starch decrease in the ovary. Differences in starch content were not significant between years, although less starch was accumulated in the mild year than in the cold year. However, differences in starch content were highly significant between before, during and after dormancy.

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