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Corresponding Author:
Anton Milyaev
Dep. of Crop Physiology of Specialty Crops (340f)
University of Hohenheim
Emil-Wolff-Str. 25
70599 Stuttgart
Germany
Email: anton.milyaev@uni-hohenheim.de

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Apple bud histology: A tool to study floral bud development in relation to biennial bearing

Anton Milyaev[1], Julian Kofler[1], Henryk Flachowsky[2], Magda-Viola Hanke[2], Jens-Norbert Wünsche[1]

[1]Fg. Ertragsphysiologie der Sonderkulturen (340f), Institut für Kulturpflanzenwissenschaften, Universität Hohenheim, Emil-Wolff-Str. 25, 70599 Stuttgart
[2]Institut für Züchtungsforschung an Obst, Julius Kühn-Institut (JKI), Bundesforschungsinstitut für Kulturpflanzen, Pillnitzer Platz 3a, 01326 Dresden

1. Introduction, Knowledge, Objectives

Apple is an important horticultural crop of and the most frequently planted fruit tree in temperate regions. However, it is well known that apple exhibits biennial bearing behavior, which can be described as yield fluctuations with a heavy crop load in one year (‘on’) and little or no crop load in the subsequent (‘off’) year (Wünsche and Ferguson 2010). Commercial production of strongly biennial apple varieties, such as ‘Fuji’, without additional crop load regulation, is economically unsustainable. The overall research goal of the current project is to reveal the largely unknown physiological and molecular mechanisms of biennial bearing (Monselise and Goldschmidt 1982, Rivero et al. 2017) and thereby to understand why 'Fuji' and other biennial apple varieties exhibit poor flower bud formation following a year with a high crop load. The process of floral bud formation can be conveniently divided into three phases, induction, initiation and differentiation. At bud induction, a fate change occurs and buds form either leaf primordia and remain vegetative or both leaf and floral primordia to enter the reproductive cycle (Foster et al. 2003). At this earliest developmental stage it is impossible to distinguish vegetative and floral buds (Abbott 1977). Flower initiation marks the transition from a vegetative to floral meristem, a time point at which structural changes of the apical bud meristem, a tissue consisting of undifferentiated cells, can be studied histologically. The first morphological sign of reproductive bud development is the doming of the apical meristem (Hoover et al. 2004). Once the time frame of the meristem transition is known, it is then of particular interest to study specific physiological and molecular mechanisms leading up to this event which might explain the differential bud developmental process on apple trees with high and no crop load. The publication proposes a histological method of apple bud examination to identify flower initiation for the following studies on revealing the biennial bearing mechanism.

2. Material and Methods

The experiment was designed in a high-density apple orchard located at the Centre of Competence for Fruit Cultivation (KOB, Ravensburg, Germany, 47°46'2.89"N 9°33'21.21"E, altitude 490m). The trial included 130 trees on M9 rootstock from each of the following apple cultivars: 'Fuji', which is considered as a biennial variety, and 'Royal Gala', a non-biennial variety. All trees from each cultivar were divided into 'on' and 'off' treatments, where 'on' trees were not thinned and maintained the natural crop load and 'off' trees were completely deflowered by hand at full bloom on April 28th 2015 ('off' trees) and carried no fruit. Both treatments were allocated randomly and a new set of four trees per variety and treatment was sampled weekly, starting 30 days after full bloom. Throughout 15 weeks, apple buds from two-year-old spurs were collected (two buds per tree, 480 in total) for histological analysis. After cutting, samples were placed immediately into safe-lock tubes filled with FAA fixative solution (5 ml 37% formaldehyde, 5 ml 5% acetic acid, 90 ml 50% ethanol) and stored at 5°C. Prior to microscopy, apple buds were embedded into paraffin blocks, cut into one hundred 5 µm thick tissue sections using a microtome, placed on glass microscope slides and stained (Wacker`s trichromatic W-3A botanical stain). Thereafter, all 100 specimens were microscopically screened to identify the most representative section based on the parallel alignment of cells in the outer three tunica cell layers of the meristematic tissue. Apical meristem's height and width were measured and classified according to Hanke (1981). Consistent with this classification, apple bud development can be divided into five stages (Figure 1). Bud microscopy has been conducted in the fourth quarter of 2016 and 180 apple buds (15 dates, 2 varieties, 2 treatments, 3 trees, 1 bud per tree) were evaluated.

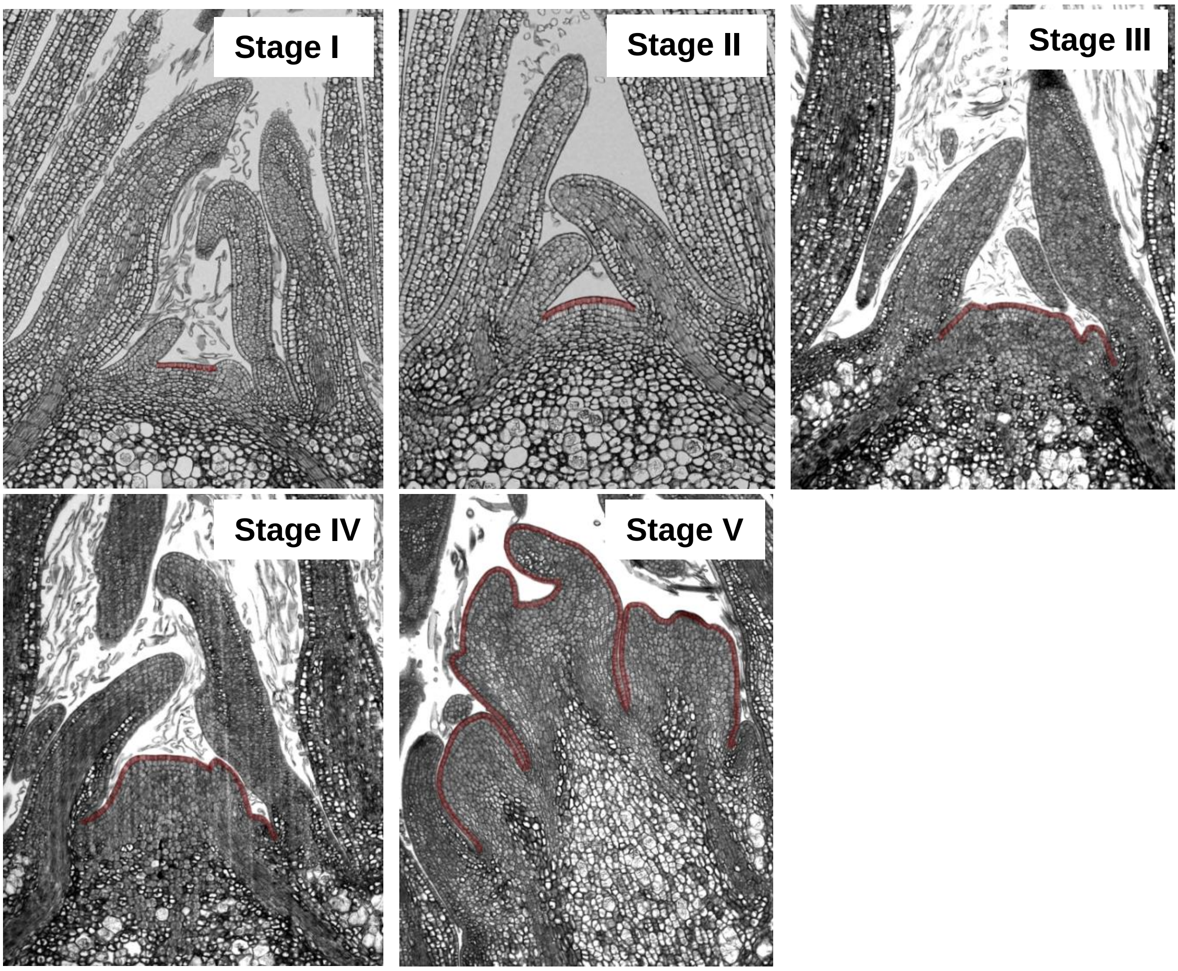

Fig. 1 Histological sections of ‘Fuji’ apple buds, representing five developmental stages according to the classification of Hanke (1981). Vegetative bud: Stage I and II; generative bud: Stage III, IV and V.

At the first stage (I), the apical bud meristem is flat and narrow, the leaf primordia are produced. At the second stage (II) a little swelling of the slightly broadened meristem appears, apex continues to produce the leaf primordia. The third stage (III) can be characterized by doming and expansion of the meristem and the commencement of flower primordia development. The fourth stage (IV) is recognizable by the formation of inflorescence primordia. At the fifth stage (V), the floral organs are differentiating, floral primordia are clearly distinct from leaf primordia. It should be noted that the first two stages of apple bud development are considered vegetative as it is not yet clear whether the bud will become floral or remain vegetative. It has been proposed that the first sign of the meristem transition towards floral development can be observed not earlier than stage III (Hanke *et al.* 2007).

3. Results

Each of the evaluated 180 buds could be linked to a certain developmental stage. The primary task of the current study was to identify the time point at which both varieties initiated floral buds and whether there are any differences between ‘on’ and ‘off’ treatments. Applying a distinct separation of stages I and II as vegetative, ‘not initiated’, and stages III, IV and V as floral, ‘initiated’, we assorted buds from each sampling date into two categories. Figure 2 shows the percentage of initiated buds at days after full bloom (DAFB) for each variety and treatment.

The transition time from vegetative to floral meristem in apple buds differed markedly depending on variety and treatment. The first signs of floral bud initiation were detected for ‘Fuji’ at 70 and 120 DAFB for ‘off’ and ‘on’ trees, respectively, and for ‘Royal Gala’ at 99 DAFB for both treatments. Consequently, buds from ‘Fuji-off’ trees committed to flowering about 1 month earlier than those from ‘Royal Gala-off’ trees. In contrast, flower initiation of buds from ‘Royal Gala-on’ trees occurred 21 days earlier when compared to those from ‘Fuji-on’ trees.

4. Discussion

The data clearly suggest that floral bud initiation in apple cultivars ‘Fuji’ and ‘Royal Gala’ was related to crop load, with ‘on’ trees having fewer floral buds than ‘off’ trees. This result was similar for both varieties (Figure 2). It is, however, noticeable that ‘Fuji’ was more responsive to crop load than ‘Royal Gala’ with distinct differences in percent bud initiation between ‘on’ and ‘off’ treatments thus confirming its strong biennial bearing habit. Further evidence is provided by having only one initiated bud observed at 120 DAFB (Figure 2).

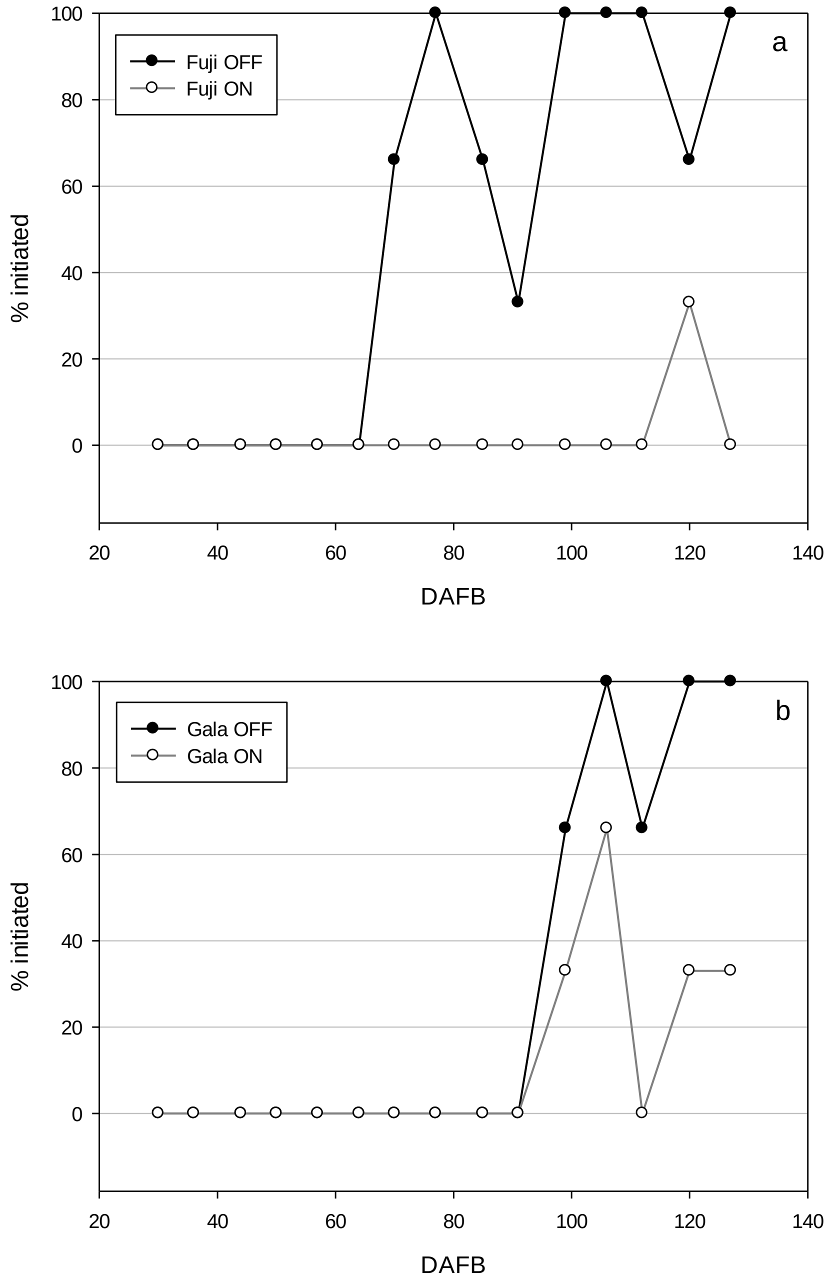

Fig. 2 Floral bud initiation time of the apple cultivars 'Fuji' (a) and 'Royal Gala' (b) within two treatments in 2015: with natural crop load ('on') and after flower removal at full bloom ('off')

5. Conclusions

Histological analysis renders the possibility to identify the onset of floral bud initiation in order to study prior to this event physiological signals (e.g. hormones, proteins, carbohydrates) and gene expression that might be associated with biennial bearing in apple.

6. Literature

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