Flowering Development in 'Algerie' Loquat under Scanning Electron Microscopy

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Abstract

Loquat blooms in late fall forming terminal panicles on shoots mostly formed during spring and after a period of summer rest. Macroscopic evaluations reveal that panicle formation initiates in Spain during September; however, flower induction and initiation are not, so far, properly dated. Modifications of the flowering dates require of a more profound knowledge of the evolution taking place within the reproductive buds. With the aim to document the anatomical changes leading to the formation of the terminal panicles, a developmental study was initiated using scanning electron microscopy. Sequential sampling of terminal buds has revealed that the first indications of flower commitment in 'Algerie' loquat occur on late July. Trimerous anisomerous structures corresponding to two different sized floral bracteoles and a centered flower dome were often seen the first week of August. Not until mid-August, the outermost flower whorl containing the sepals could be clearly recognized, 4-5 weeks before the large size of the apical buds reveals their flowering nature. Sepals develop sequentially in a 2/5 pattern of phyllotaxy during August. Petals rose simultaneously alternating to sepals at the end of August. Stamens formation was initiated on September 9th, and anthers could be distinguished on September 23rd. Gynoecium elevates forming five stigmas on mid-September, a week after stamens initiation. Two anatropous bitegmic and crassinucelate ovules per carpel could be seen developing below hypanthium at the end of September. Trees subjected to postharvest water stress initiate flower formation 2-3 weeks earlier. Water deficits extending beyond July likely affect flower quality and their reproductive potential.

INTRODUCTION

Loquat (*Eriobotrya japonica* Lindl.) blooms in late fall, after a brief period of summer rest, forming terminal panicles on the new growth of shoots developed during previous spring. Macroscopic evaluations have revealed that in Spain panicle formation of 'Algerie' loquat initiates during September; however, loquat flower induction and initiation are not, so far, properly dated. Hueso and Cuevas (2004) have shown that a period of water stress from mid-May to August advances significantly loquat bloom and harvest dates, making its cultivation more profitable. However, work in progress proves that water stress extending during August is detrimental, since diminishes flower size and delays last steps of flower development, losing part of the phenological advances previously achieved. Modifications of loquat flowering dates, without compromising flower fertility, require of a more profound knowledge of the evolution taking place within the reproductive bud. With the aim to document the anatomical changes leading to the formation of the panicles, and within them, the arising of the individual flower buds, a developmental study was initiated using Scanning Electron Microscopy. The objectives of this work are 1) date flower initiation in 'Algerie' loquat grown in South East Spain; 2) describe the process of flower differentiation under optimal growing conditions; 3) determine alterations in reproductive phenology due to the implementation of a regulated deficit irrigation (RDI) regime.

MATERIAL AND METHODS

Twenty four terminal buds of 'Algerie' loquat were sampled every week from the end of vegetative growth (mid-July) to bloom (mid-November). Collections were carried out at random from main spring shoots of six adult full-irrigated (≈7650 m^3 of water per year) and well-maintained trees growing in the Experimental Station of Cajamar "Las Palmerillas" (latitude 36º48'N, longitude 2º43'W). With the aim to process the most representative and uniform samples for each date, the buds once collected were taken to the lab and ordered by size. Then, the four intermediate-sized buds were selected and immediately fixed in 3% glutaraldehyde in phosphate buffer, pH 7.2. Before observation the buds were partially dissected removing most external bracts under binocular, and subsequently dehydrated, critical point dried, sputter-coated with 20 nm gold, and finally observed under a Hitachi S-3000N Scanning Electron Microscopy, mostly operated at 15-20 kV. During the last steps of flower development and when the floral nature of the buds was deduced from their large size and appearance, we chose to process the individual floral buds arising from the basal parts of the developing panicles, given their most advanced phonological stage. To determine the effects of regulated deficit irrigation on reproductive phenology samples were taken following exactly the same procedure. RDI trees were irrigated as controls except during a period of six weeks (from mid-June to the end of July) when watering was suspended (for more details, see Cuevas et al., this volume). Precipitation in the area reaches an average of 231 mm, mostly during fall and winter. No rain occurred during water stress period.

RESULTS AND DISCUSSION

Sequential sampling of terminal buds from the end of shoot growth to anthesis has revealed that the first indications of flower initiation in 'Algerie' loquat may be dated at the end of July. Vegetative buds characterized for a narrow conic shaped meristem predominate until mid-July (Fig. 1a). In the last week of July, three months and a half before bloom, a transitional stage in which the base of the apical meristem enlarges was recognized (Fig. 1b). This stage can be labeled as panicle initiation. Rodriguez (1983) marks, also in Spain, the end of July as the beginning of bud differentiation in spring shoots. Formation of new nodes in shoots of 'Algerie' grown in SE Spain ends in mid-July, although elongation of preformed nodes may extend shoot length until summer rest Cuevas et al. (1997). End of shoot growth is needed before the terminal bud differentiate into panicle. Lin et al. (1999) inform that in Zhejiang (China) inflorescences begin to differentiate main axes in the beginning of August, while secondary axes are formed in the middle or the end of August.

Unequivocal floral structures were first observed during the first days of August. In these days, trimerous anisomerous structures, corresponding to two different-sized floral bracteoles and a centred flower dome, were often seen (Fig. 1c). Enlargement of a progressive more complex panicle bearing multiple trimerous structures spirally disposed along the inflorescence axis continued during August (Fig. 1d). At this time this bud is still macroscopically labeled as undifferentiated and quiescent. One bracteole develops to a larger size than the opposite, differences that often perdure in time (Fig. 1c and d). Late forming flowers at the base of more advanced meristem show, however, that the very early steps of bracteole formation is characterized for similar small lateral elevations. Both bracteoles are extremely hairy.

The outermost flower whorl containing sepals could not be clearly recognized until mid-August (Fig. 2a), 5-6 weeks before the large size of the apical buds reveals macroscopically their flowering nature. In Zhejiang (China) sepals and petals form at the beginning of September (Lin et al., 1999). Sepals form sequentially in a 2/5 pattern of phyllotaxy. Initially, the sepals kept large differences in size that correspond to the order of formation. All sepals are initiated before any petals. During the next two weeks, the most evident anatomical changes were the growth of the five sepals and their curvature enclosing incipient petal initiation (Fig. 2b). Mature sepals developed abundant hairs abaxially but not in their inner surface. Five petals start to grow simultaneously at the end of August alternating in position with the sepals. At the end of their formation petals, mostly glabrous, became bifid. Differential development was often seen among individual flower buds of the panicle, some of then bearing incipient stamens, while others only showed floral bracteoles formed. During first samples dates the apex of the panicle seemed to be more advanced, although as flower development progresses basal flowers of the panicle superseded the apical flowers.

Stamens were initiated on September 9th, as small protuberances within limits marked by a ring of still very small petals (Fig. 2c). Anthers could be clearly distinguished on September 23rd, more or less when such developmental stage occurs in China (Lin et al., 1999). Although anthers could be recognized in mid-September, filament's rising occurs late in development (Fig 2d). Stamens of equal height arise from the same distance to the center of the meristem, but as they grow some anthers displace others and become alternant in two whorls, so first ring partially overlaps the second one. Filaments remain adnate in a single whorl. Anthers are dorsifixed and introrse and split longitudinally. Individual pollen grains were occasionally seen in broken anthers on October 21st, 27 days before anthesis.

Gynoecium elevated forming five called-to-be stigmas after mid-September, a week after stamens initiation. When styles rise, they leave the center of the meristem empty, what can be perceived as a depression (Fig 3a). Pistils finish their growth below anthers level, which partially cover stigmas. Six pistils per flowers were rarely detected. Two ovules per carpel could be seen at the end of September developing below a shortly campanulate hypanthium following axial placentation (Fig. 3b and c). Loquat ovules are anatropous, bitegmic and crassinucellate. Inner tegument forming a ring around nucellus is seen in immature ovules the first week of October, more than one month before bloom (Fig. 3c). Ovules seemed to be completely formed before petals aperture (Fig. 3d). A well-developed common obturator for each two ovules was clearly visible some days before bloom (Fig 3e). The ten ovules arrange radially (Fig. 3f).

Trees subjected to postharvest water stress initiate flower formation about three weeks earlier. Flower initiation anticipation under deficit irrigation was maintained along flower development process until bloom, without consistent differences. Water-stressed trees bloomed 26 days before than controls (Cuevas et al., this volume). Water deficits extending beyond July likely affect flower quality and their reproductive potential.

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Figures

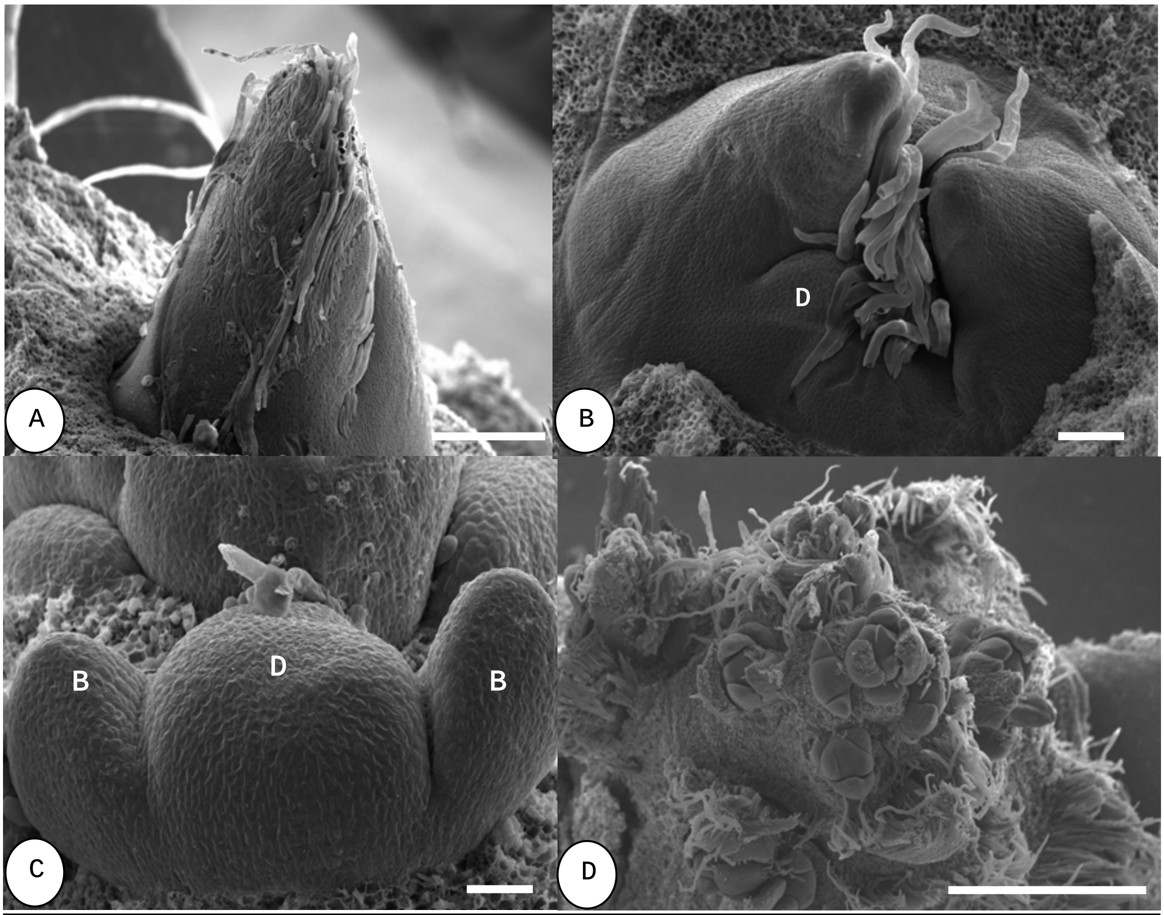

Fig. 1. Flower initiation in "Algerie" loquat. A) Vegetative meristem. B) Transitional stage. C) Trimerous forms corresponding to first unequivocal floral structures. D) Panicle in formation. B: Bracteole. D: Flower dome. Bar A=500 µm; B-C=100 µm; D=1 mm.

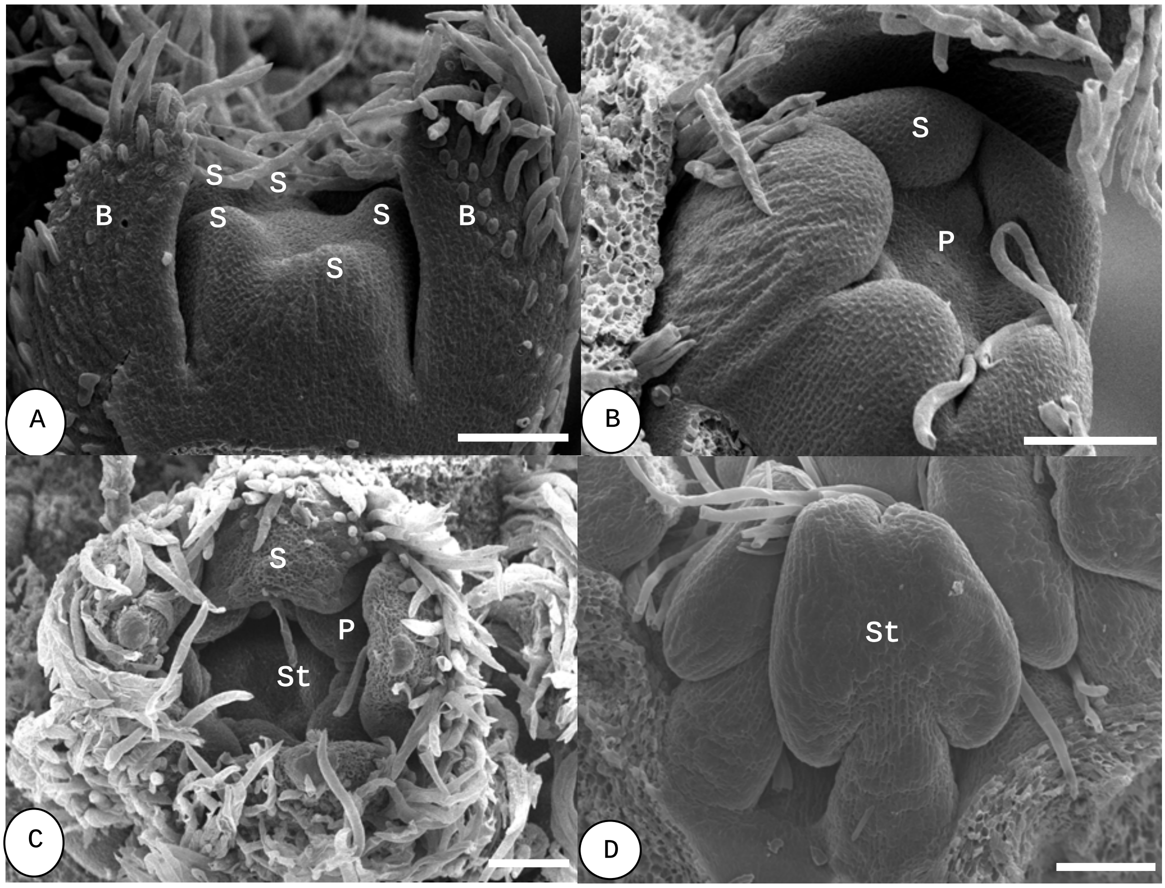

Fig. 2. Flower development in 'Algerie' loquat. A) Sepals formation. B) Petals inception. C) Stamens initiation. D) Anther and filament. B: Bracteole. S: Sepal. P: Petal. St: stamen. Bar=100 µm.

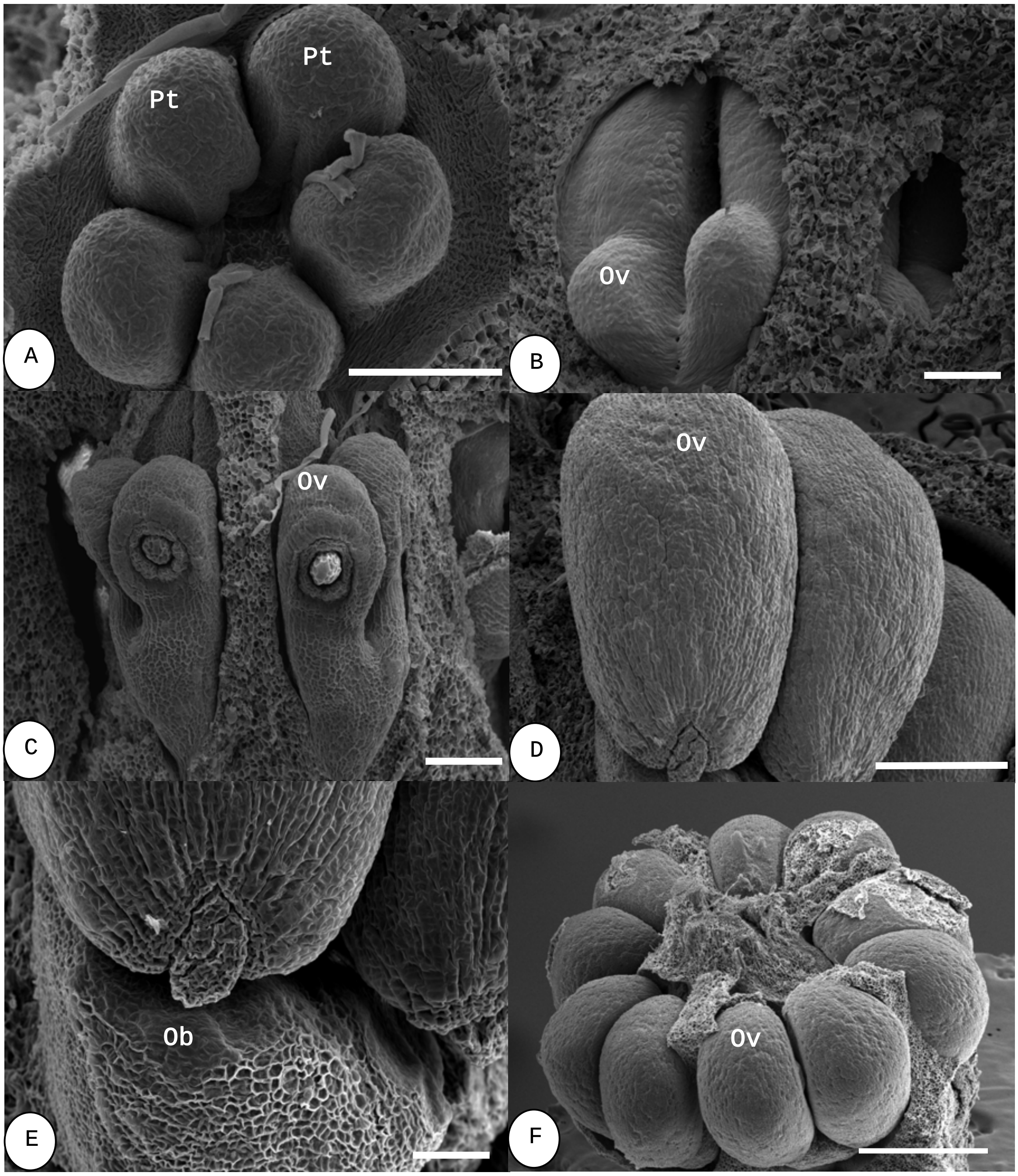

Fig. 3. Gynoecium development in “Algerie” loquat. A) Stigmas elevation. B) Ovule primordia inception. C) Immature ovules showing integuments and nucellus. D) Mature ovule; micropyle is visible. E) Obturator. F) Ovules arrangement. Pi: Pistils. Ov: Ovules. Ob: Obturator. Some organs were removed to allow vision of inner structures. Bar=100 µm, except F where bar=500 µm.