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ORIGINAL PAPER

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FLOWER INITIATION AND DEVELOPMENT IN ENDEMIC IRANIAN LILY (*Lilium ledebourii* Boiss.)

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ABSTRACT

This study was performed to depict the anatomical changes in apical meristems in order to determine the flower initiation and development of Iranian lily (*Lilium ledebourii*). Observations carried out by scanning electron microscope (SEM) on groups of bulbs with different age and size showed that only large (≥55 g and ≥6 cm in diameter) 9-year-old and older bulbs expressed flowering transition, abandoning the juvenile condition. The switch from vegetative to reproductive in the apical meristem was characterized by flattening its dome. Flower initiation started between 10 and 20 days after planting, once the bulbs have passed a period of vernalization of two months at 3°C. The first hint of floral organ definition was noted 30 to 50 days after planting when the outer perianths started to grow followed by inner perianths in a pattern of 3−3. After complementation of flower formation by stamen and pistil appearance and development, flower abortion occurred in some bulbs 30 days after flower initiation. Flower bud abortion could not be linked to the bulb size. Identification of the exact time of flower initiation will be useful to provide proper management of Iranian lily in the process of domestication of this endemic endangered lily.

Key words: *Lilium ledebourii*, flower development, bulb size, SEM

INTRODUCTION

Iranian lily (*Lilium ledebourii* Boiss.), also known as Susan-e-Chelcheragh, is an endangered and well-favored ornamental plant growing voluntarily only in scanty parts of Hyrcanian forests, north of Iran at altitudes between 1750−2300 m. The genus *Lilium* contains ~110 species that are distributed in the Northern Hemisphere, mainly Asia, Europe and North America, and have more than 7,000 cultivars [Okubo and Sochacki 2012]. Iranian lily is a perennial geophyte with yellowish scales and a thick stem. The first aboveground stem appears 4–5 years after germination of seeds. Leaves are erect and linear-lanceolate. Flowers are white, grouped in a large raceme with 2−15 flowes, with a pedicel up to 13 cm long rised up, or spreading-reflexed (Fig. 1A and B). It blooms commonly from June to July [Mirmasoumi et al. 2013]. The annual cycle of the plant is as follows: bulbs sprout in spring, mid of April, then they grow, and flower usually two to three months later, from June to July. The natural habitat of the plant has severe winter and moderate temperatures during spring and sum-

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mer. Iranian lily has not yet been domesticated despite its beauty and valuable appearance.

During quiescence period, bulbous plants do not exhibit any visible external growth. In fact, it is well known that the processes of organogenesis in many geophytes occur in underground buds during quiescence period [Kamenetsky and Rabinowitch 2002]. Different lily species vary extensively regarding timing of flower initiation and can be divided accordingly into different subclasses [Wilkins and Dole 1997]. Some of them, like *L. martagon* and *L. dauricum*, initiate their flowers in August, the year before bloom, and once aerial leaves start to whiter. On the contrary, *L. regale* and *L. amabile* start their floral initiation just before shoot emergence in spring. Other lilies start to initiate their flowers well after shoot emergence in June with blooming occurring late in September. On the other hand, in *L. longiflorum*, it has been shown that bulb size conditions its fate (either remain vegetative in a juvenile stage or turn into reproductive entering into the adult phase) [Lazare and Zaccai 2016]. The knowledge of the precise influence of bulb size and age on the formation of flowers is essential in the process of domestication and future commercialization of Iranian lily. Unfortunately, there is no report revealing the morpho-physiological (weight, diameter, and age) traits of *L. ledebourii* bulbs, through which the plant acquires competence to undergo flowering transition.

This transition from vegetative (juvenile stage) to generative (adult phase) condition is a dramatic and remarkable event in the life cycle of a plant [Araki 2001, Poethig 2003]. This phase transition is controlled through a series of signal transduction pathways that regulate the developmental stage and age, at which the plant becomes competent for flowering [Amasino 2010, Srikanth and Schmid 2011]. The anatomical changes taking place during the transition to the reproductive phase (flower initiation) have been extensively documented in various plant taxa. The exact time of the flower initiation is of great importance, because it considerably affects the blooming synchronization and appropriate pollination [Ramzan et al. 2014]. Actually, plant growth and size act in many species as developmental landmarks. On the other hand, the knowledge of the timing of transition phase is necessary for ensuring the best conditions for correct flower development. For example, premature flowering results in smaller inflorescences of a lower market value, while a prolonged vegetative phase enhances the biomass of plant, but reduces the percentage of plants undergoing flowering [Demura and Ye 2010]. The identification of the exact time of flower initiation will be important for providing the growth requirements needed to the bulbs such as water, nutrients, good environmental conditions, and pest control to ensure the flower quality that future market will demand.

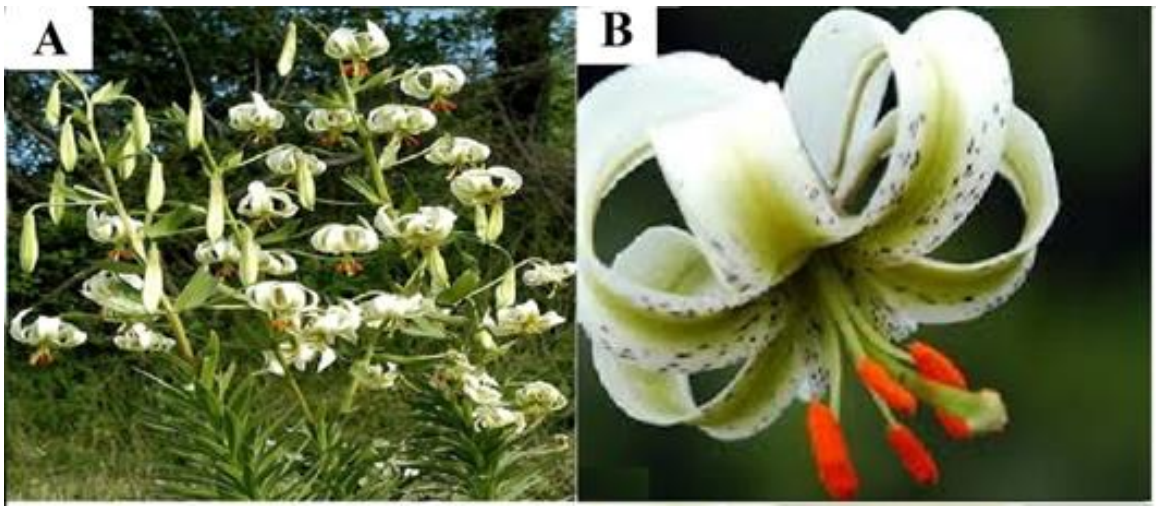


Fig. 1. (A) Feature of Iranian lily (*L. ledebourii*) plants in natural habitat, and (B) a single flower of the plant

The aims of the present work are: 1) to identify the exact time, at which flower initiation occurs in Iranian lily, 2) to depict and characterize the anatomical stages of its florogenesis process and 3) to determine the morphological and physiological characteristics of its bulbs when they become mature and competent for flowering. In order to reach these objectives, we sampled vernalized bulbs every 10 days from cold storage to blooming and observed the stem apical meristems (SAM) of bulbs of different size and age under Scanning Electron Microscopy.

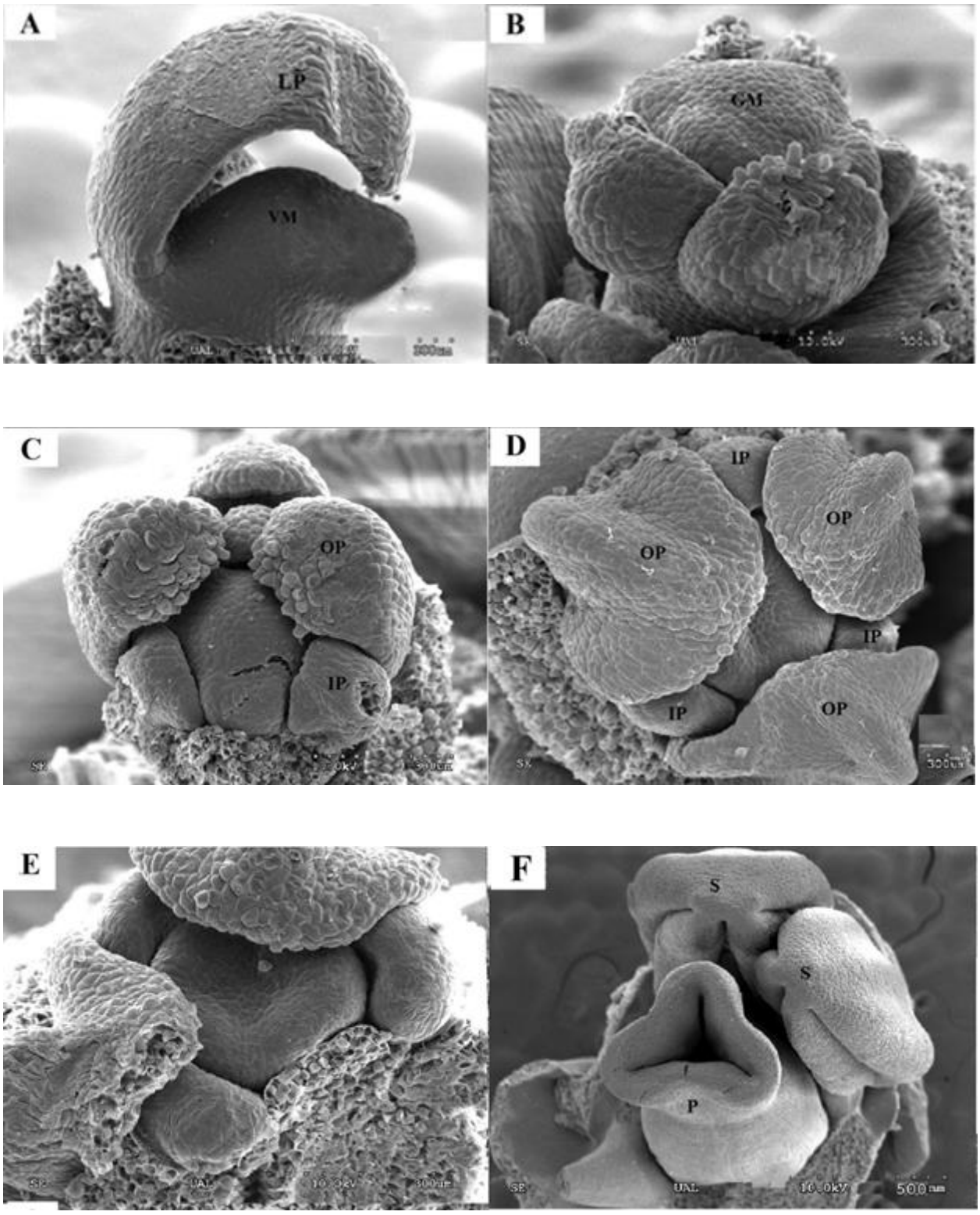
MATERIAL AND METHODS

Plant material manipulation. Bulbs of *L. ledebourii* grown in nature in the mountain district of Kelardasht, in Mazandaran province (36°32'14.3"N; 51°3'27.53"E, and 2270 m above sea level), were harvested at the end of August 2016, after leaf withering and well before exposition to cold temperatures. The 5.8S ribosomal DNA sequence of the plant sample was registered by our group in Genbank with accession number KX495217.1, and named as *L. ledebourii* strain Kelardasht Salehi 1. The sampled bulbs were subdivided into three groups, 100 bulbs each, based on their weight and diameter as follows: first: 55–70 g with 6–7 cm in diameter; second: 45–55 g with diameter 4–6 cm, and third: 35–45 g with 3–4 cm. All bulbs were then placed in a light and well drained medium consisting of perlite:coco peat in a ratio 1 : 1 and stored for a month in a chamber at constant temperature of 17°C, resembling the natural condition of their habitat. Subsequently, they were transferred to a cold storage for 2 months at 3°C for vernalization and breaking dormancy processes. During cold storage, the bulbs media were always kept humid. Finally, the bulbs were individually planted into pots (25 cm diameter and 35 cm height) in a greenhouse under 20/17°C day/night temperatures, where they received full Hoagland nutritive solution every week. Stem apical meristems (SAM) samples constituting of 6 bulbs each were collected every 10 days starting at the beginning of cold storage and until visual flower bud appearance was obvious. The number of old shoot-residual growth points (old shoots remnants) that indicate the age of the plant in years, was counted for each single bulb prior to their processing. Scales during cold storage, and leaves after bulb sprouting samplings, were removed to facilitate the handling of the SAM samples.

Scanning electron microscopy observations. Scanning electron microscopy (SEM) was used to identify the changes in SAM and for monitoring the florogenesis process of *L. ledebourii*. Samples were handled according to Fukai and Goi (2001) with minor modifications. SAM were collected from each group of bulbs and brought to the laboratory for dissection under a light microscope (CKX41, Olympus, Tokyo, Japan). After dissection, SAM samples were fixed in FAA solution [formaldehyde : acetic acid : 70% ethanol, 10 : 5 : 85] at room temperature for 1 week, then washed 5 times, 10 min each, in phosphate buffer 0.1 M, pH 7.2. Afterwards, the samples were dehydrated by washing in a series of ethanol (2 × 50% ethanol (30 min), 75% ethanol (30 min), 90% ethanol (30 min), 95% ethanol (30 min) and 2 × 100% ethanol (30 min)). Then, the samples were stored in 100% ethanol and critical point dried in an HCP-2, Hitachi (Tokyo, Japan) equipment, wherein they were placed first in a 50–80% liquid carbon dioxide (L-CO_2) for 20 min at 10°C and then for 5 min at 40°C. For coating with 10 nm of gold, the dried samples were put in a metal stubs, and placed in an ion sputter (E-1030, Hitachi, Tokyo, Japan). A scanning electron microscope (S-4300, Hitachi, Tokyo, Japan) was used for observations.

RESULTS AND DISCUSSION

This study was performed to identify the precise time of flower bud initiation stage in Iranian lily with the final aim of enhancing its flower quality by providing the nutritional and environmental factors needed during flower development. The observations carried out on sequentially sampled bulbs of different ages and sizes allowed to identify the first changes in the SAM and the morphological and physiological characteristics of the bulbs that make them competent for flowering.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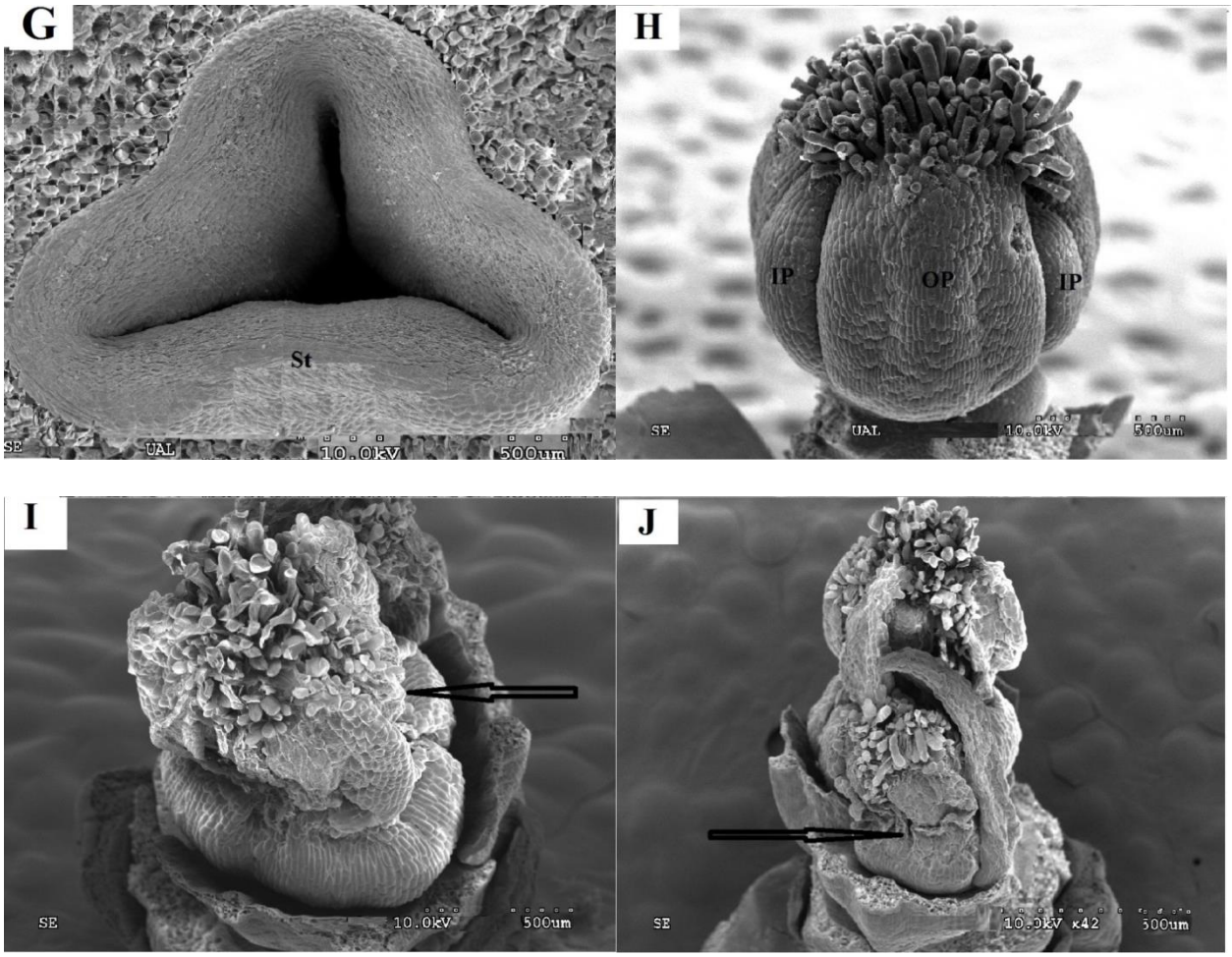

Fig. 2. (A) SAM at vegetative stage. (B to H) Different stages of flower development in Iranian lily as displayed by SEM. (I and J) Flower bud abortion (arrows). Abbreviations: LP − leaf primordia; VM − vegetative meristem; GM − generative meristem; OP − outer perianth; IP − inner perianth; St − stigma; P − pistil; S − stamen

The vegetative shoot apex was characterized by the presence of an apical dome with a large number of leaf primordia (Fig. 2A). Based on our results, SAM of the first group of bulbs (those with a weight of 55–70 g and 6–7 cm in diameter), remained vegetative 10–20 days after planting, when new roots were emerged. After new roots were formed and the shoot emerged, the SAM was transitioned from vegetative phase to reproductive phase as shown in Fig. 2B. During this transition, the SAM became flatted. Fukai and Goi (2001) described floral initiation in *L. longiflorum* and marked the first sign of floral initiation as the swelling of the axillary buds, which results in an uneven shoot apex.

Since the morphology and function of petals (corolla) and sepals (calyx) are similar in *Lilium* species, they are called inner and outer perianth, respectively [Fukai and Goi 2001]. After formation of the dome in the SAM, the outer verticil of the perianth started to grow followed by the inner perianth (Fig. 2C and D). Then, the edges of the SAM started to rise up and the center sank gradually (Fig. 2E) to form stamen and pistil, respectively (Fig. 2F and G).

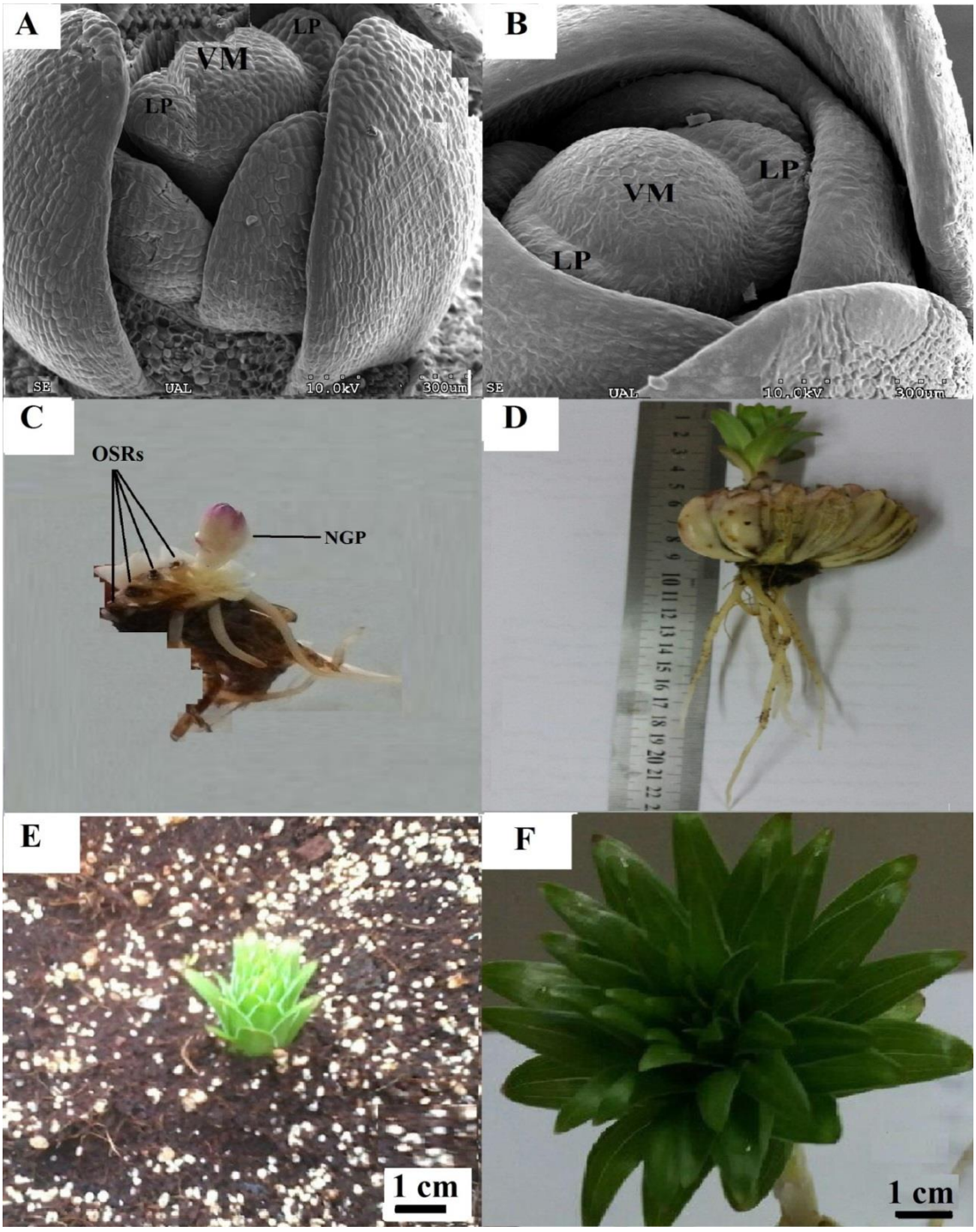

Fig. 3. (A and B) SAM at 10 and 40 days after planting in Iranian lily for bulbs with weight ligther than 55 g and diameter lower than 6 cm as displayed by SEM. (C) Bulb after removing scales. (D and E) The growing stage during the occurrence of flower initiation. (F) The feature of plant when an entire flower has been microscopically formed. Abbreviations: LP − leaf primordia; VM − vegetative meristem; OSRs − old shoots remnants; NGP − new growth point

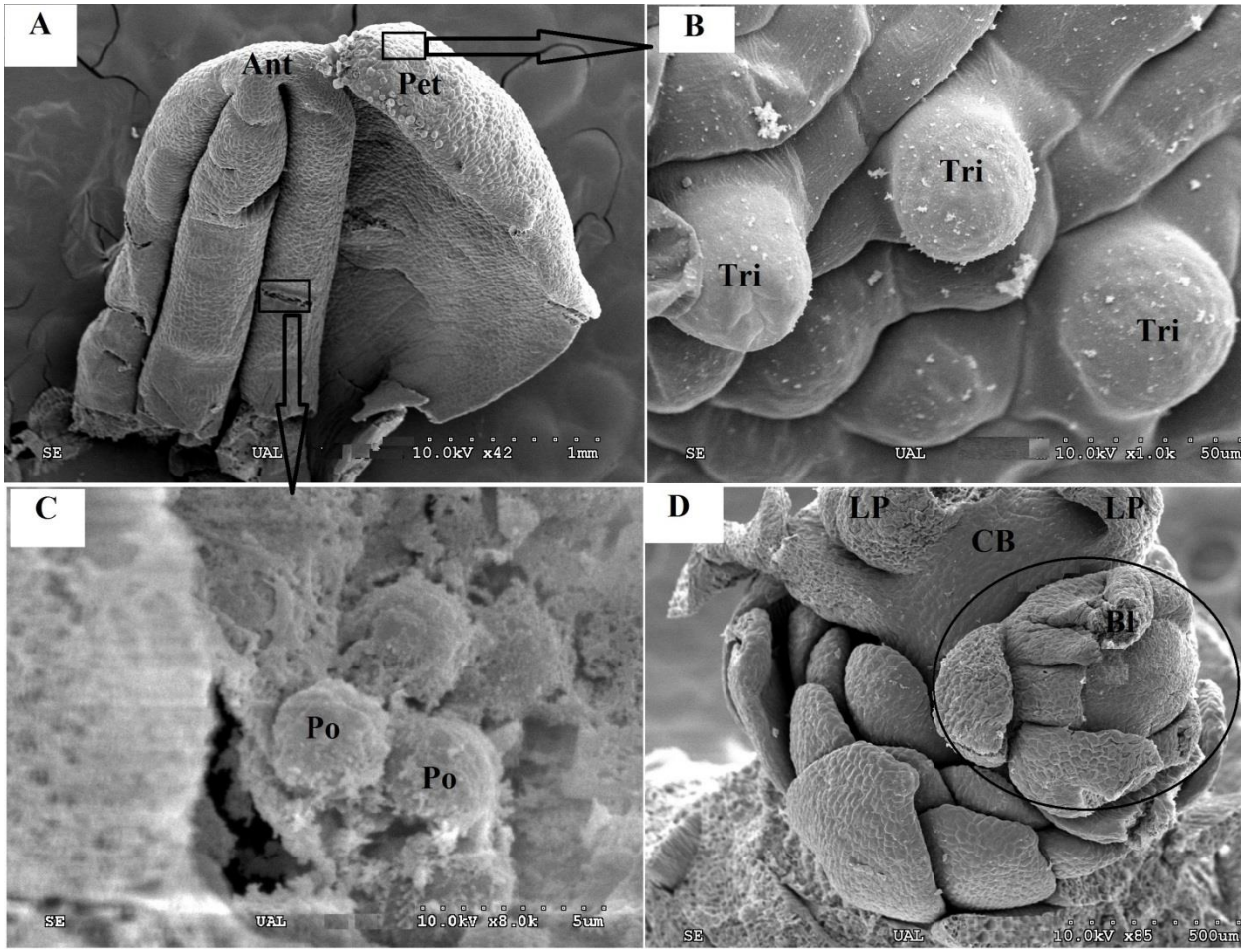

Fig. 4. (A) Perianth and anther feature. (B) The formation of trichomes in the adaxial surface of enlarging outer perianth and (C) pollen grain inside the anther 40–50 days after planting. (D) Appearance of new bulblet from the side of current year's bulb as displayed by SEM. Abbreviations: Ant − anther; Pet − perianth; Tri − trichome; Po − pollen; LP − leaf primordia; CB − current bulb; Bl − bulblet

Our results show that, in *L. ledebourii*, the first hint of flower bud can be identified 20 to 30 days after the flat dome initiation (Fig. 2H). In some plants, flower abortion occurred at this moment, 30 days after flower initiation (Fig. 2I and G). It seems that the most sensitive stage in the plant development occurs 10–20 days after planting (flower initiation), and then, from flower initiation to the mid-stage of flower development (30 days after flower initiation).

In this study, only bulbs of the first group with a weight heavier than 55 g and a diameter above 6 cm formed flowers. This coincides with observations carried out in *L. longiflorum* where bulb size regulates the flowering pathway [Lazare and Zaccai 2016]. Apical meristems of bulbs lighter than 55 g (bulbs of the second and third group) remained vegetative throughout their growth cycle (Fig. 3A and B). It seems that the size of bulbs is important in supplying growth regulators for flowering [Manimaran et al. 2017]. The counting of old growing points (shoots remnants, Fig. 3C) on the basal plate of the bulbs accurately reflects the bulb age in this genus. Iranian lily bulb age could be determined as the sum of the number of old shoots remnants and

the number of years, four to five, a seed gets to produce the first shoot [Dhyani et al. 2012]. The number of shoot remnants counted indicated that the bulbs of the first group (those with a weight of 55–70 g and 6–7 cm in diameter) were 9-year-old or older. This late abandoning of a juvenile condition is probably influenced by the limited resources available in the wild at the sampling location in the forests of Kelardasht mountains. Hopefully, the cultivation of Iranian lily bulbs seems to bring forward the acquisition of flowering capacity (work in progress).

Externally, an observer could identify flower initiation period in Iranian lily when its shoot has emerged and reached 3–4 cm above ground (Fig. 3D and E). Shoot growth slowed down in the next 20 to 30 days (Fig. 3F), when the development of the outer perianth could be microscopically observed. Stamen formation occurred after appearance of outer and inner perianths. In a later step, pollen grains were formed inside anthers 40–50 days after planting (Fig. 4A and C). In this stage, trichomes were also formed in the adaxial surface of the perianths (Fig. 4B). Below ground, new bulblets were formed and started to grow (Fig. 4D).

The process of flowering includes five successive stages begining from flower induction, followed by initiation, organogenesis that involves floral parts differentiation, growth and maturation of floral organs, and finally blooming or flower anthesis. The successive stages are more or less easy to separate, but the knowledge on the factors that control them and the determination of the period of the growth cycle, during which they happen in the bulb, are necessary [De Hertogh and Le Nard 1993] for the domestication of a plant. Anderson et al. [2010] have reported that, in *Lilium*, flower formation starts during or towards the end of the cold storage period, but it is only completed after planting. *Lilium* species were divided into four groups depending on their time of flower initiation by Ohkawa [1977]. So that, in the first group, the initiation of flower buds begins in early fall, before bulb sprouting, and it is completed after emergence of shoot. Asiatic hybrid lilies belong to this group, wherein some Asiatic hybrids start their flower initiation as early as during bulb cold storage [Ohkawa 1977]. The second group, such as *L. hansonii*, starts flower bud initiation 5–10 days after the shoot emergence and completes their flower maturation 20–30 days after planting. The third group includes Oriental hybrid lilies that start flower initiation and development a little bit later 10–15 days after planting. Finally, the fourth group, in which *Longiflorum* hybrids is classified, has flower initiation and development commencement 20–30 days after the bulb planting. The results of our experiments illustrated that Iranian lily (*L. ledebourii*) could be categorized in the third group, which starts its flower initiation about 10–20 days after planting. Our results highlight critical periods of flowering of Iranian lily (*L. ledebourii*) to get its maximum potential in terms of dormancy breaking, minimize flower bud abortion and enhance flower quality. The improvement of the size, quality and vase life of the flowers of *L. ledebourii* by proper cultivation are still needed to fully develop the potential of the beautiful but fragile *L. ledebourii* in horticulture and in floriculture industry in general.

CONCLUSIONS

According to our results, Iranian lily (*L. ledebourii*) starts floral initiation 10–20 days after bulb planting if proper cold requirements are provided. Like *L. longiflorum*, the switch from vegetative to reproductive in the apical meristem of *L. ledebourii* was characterized by being flatted its dome. In our study, flower initiation occurred only in those bulbs that had experienced at least 9 growing seasons and reached a size of 55–70 g and 6–7 cm in diameter. Flower abortion occurred in some plants 30 days after flower initiation.

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