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Morphological Studies of Flower Bud Initiation and Development in Bulbous Iris Stored at Various Temperatures^{1, 3}

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Abstract. Flower buds are absent in dormant iris bulbs, but floral initiation occurs after subjecting them to a high temp, heat curing treatment, followed by a holding period at a moderate temp, and then a low temp pre-cooling period. The effects of cultivars, digging dates, ethylene gas treatment, and different storage temp on earliness and uniformity of flowering were studied in microscopic sections of bulb growing points of samples collected at intervals from the different treatment lots. The results indicated that variations in field growing conditions produced bulbs with varying degrees of maturity, of which some would respond properly to curing treatments and others would not. Properly matured bulbs grown in the Pacific Northwest can be heat cured by exposing them to a temp of 32.2°C for 10 days. Holding temp lower than 15.5°C delayed subsequent flower bud initiation. Ethylene gas treatment prior to heat curing appeared to stimulate floral initiation.

The variations in flowering time noted from year to year in flowering trials of bulbous iris suggest that several factors must affect their time of floral initiation and development sequence leading to flowering as influenced by storage and ethylene treatments.

Blaauw (1934) showed that a specific storage temp was required for floral initiation in bulbous iris (5). Hartsema et al. (4) reported that bulbs could be held in the vegetative state (retarded condition) for 12 months at a temp of 25°C. Pereira (5) reported that floral initiation could begin at temp between 20-25°C, but abortion occurred if these temp continued. Kamerbeek (4) pointed out that the bulb was more retarded at 30°C than at 25°C as determined by measurement of the length of the first foliar leaf after 1 year of storage. He concluded this measurement to be a good indicator of shoot growth in dry bulbs without roots. Storage of dry bulbs at temp above 40°C resulted in dehydration and death (4). Stuart et al. (8) recommended a min heat curing of 10 days at 32°C. Ethylene treated bulbs bloomed earlier and within a shorter period of time (Stuart et al. 6). In their experiment, 99% of the treated bulbs bloomed while nearly one third of the untreated plants failed to flower.

Materials and Methods

Bulbs of the hybrid group of Dutch Iris, 'Wedgwood' and 'Ideal', which are derivatives from crosses of *Iris xiphium* L. var. *Praecox* x *I. tingitana* Boiss and Reut. and x *I. lusitanica*

Ker-Gawl. were grown at Sumner, Washington. Bulbs dug in July and August were heat cured at the Western Washington Research and Extension Center, Puyallup, before shipment to Beltsville. These bulbs were then cold stored for various periods of time at various temp and forced in the greenhouses during 3 different years. Specific treatments are described in the results section. The 9 to 10-cm diam graded bulbs were grown in flats and were discarded at the end of each forcing season. Small samples were removed from the various bulb treatments at periodic intervals for microscopic determination of flower bud initiation. The growing point was removed with a min of extraneous tissue to facilitate penetration of the fixing solutions and then processed through infiltration and embedding in paraffin. Longitudinal median sections were cut at a thickness of 10 to 12 microns. The sections were stained with either iron-hematoxylin or satranin and fast green (3). The ethylene gas treatment consisted of exposing the bulbs in 1-gal glass containers at 21°C. Bulbs were forced in 35.6 X 50.8 cm wood flats of steamed soil at a night temp of 12.7°C.

Results and Discussion

The vegetative growing point is situated very close to the base of the bulb (Fig. 1 and 2) up to the time of pre-cooling. At this dormant stage, it is not readily distinguishable by macroscopic observation because of its small size and lack of color contrast. Early stages of floral initiation can be identified by low power microscopic observation (x 80), without staining the paraffin sections.

According to Pereira (5), floral initiation can occur after the bulbs have been pre-cooled at 13°C for approx 4 weeks. The first indication of transition from the vegetative to the reproductive state is an increase in the rate of cell division in the rib meristem about 10 cell layers below the tunica. This increased mitotic activity gradually occurs over the entire apical dome region resulting in a change of the vegetative zonation pattern. According to Blaauw cited by Hartsema (1), the development of the flower occurs as follows: Three primordia

¹Received for publication June 9, 1972.

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³Grateful acknowledgement is made to Neil W. Stuart for treating and forcing the bulbs and for his assistance in preparing this manuscript. I also thank Charles J. Gould for supplying the partially treated bulbs and for his helpful comments. Thanks are due also to B. E. Struckmeyer for her extensive review of this manuscript.



Fig. 1. Vegetative iris bud. X37.

differentiate from the rising growing point. Stamens differentiate from the upper part of these primordia, while the first set of tepals appears from the base. A second whorl of tepals develops subsequently and this whorl alternates with the first tepals formed. Finally, the 3 carpels develop in positions opposite the stamens.

The growing point becomes enlarged before the formation of floral primordia is apparent. The enlargement consists of an increase in width of the apex and an increase in ht of the dome (Fig. 3). A comparison of Fig. 3 with Fig. 2 illustrates the enlargement phase. Subsequently, the first lobes of the staminal primordia appear on the upper sides of the dome thus producing

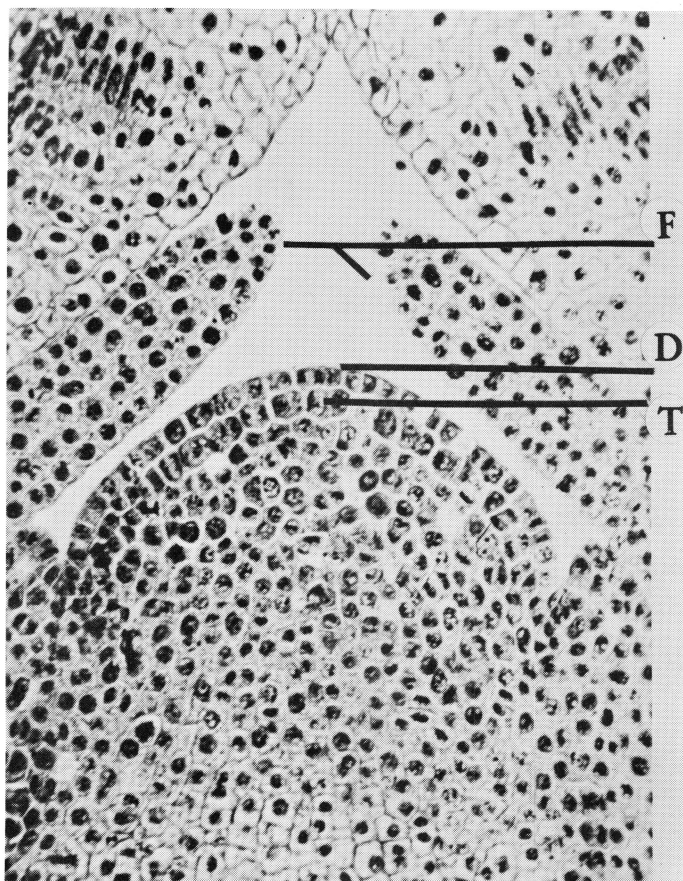


Fig. 2. Longitudinal median section of a vegetative iris bud in a dormant bulb; young foliar leaves (F) on either side of apical dome (D); tunica (T) X200.

an appearance of a flattening of the top of the original dome surface. This is followed by an indentation of the central region which results from its retarded upward growth as the staminal primordia begin differentiation into 3 separate lobes (Fig. 4). Both the inner and outer sets of spathe leaves are present at this stage in the bulb (Fig. 4). The indentation of the dome surface deepens gradually into an elongated opening as the staminal primordia develop into individual structures (Fig. 5 and 6). According to Pereira (5), the approx stage of development attained at the end of the pre-cooling period of 4 to 6 weeks is illustrated in Figs. 3 through 5. However, all bulb samples that I examined at planting time had vegetative buds. An outer whorl of tepals is differentiated and they alternate with the first tepals. Thus, the tepals are formed in a position between the stamens and the sets of spathe leaves. Protuberances appear on the walls, down near the base of the cavity between the developing stamens. These are pistillate primordia (Fig. 7) from the sample 3 weeks after planting.

As flower development continues, stigmas emerge from the pistillate primordia (Fig. 8). The 3 stigmas differentiate as distinct organs resulting in a basipetal extension of the central opening or channel. Styler primordia differentiate styler wall tissue, producing a styler canal (Fig. 8). Growth results in an upward extension of the floral parts in the bulb. At this developmental stage, floral organs are located in the emerging shoot on top of the bulb. The stage shown in Fig. 8 can be attained by the fifth week of forcing if bulbs were not retarded. In the advanced stage of flower bud development, initiation of pollen mother cells becomes evident in the anther (Fig. 9 and 10). This stage occurs after approx 6 weeks of forcing. The

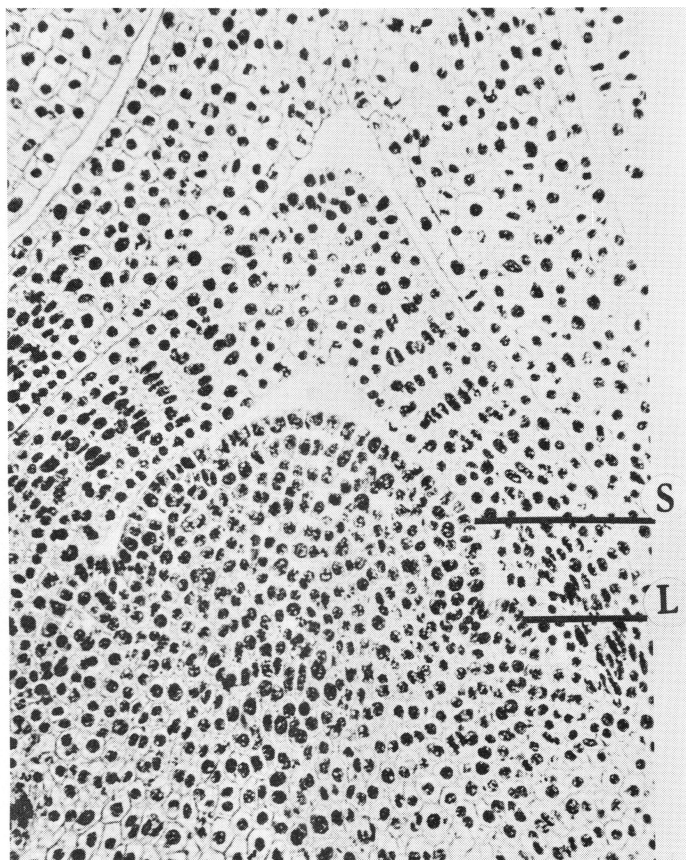


Fig. 3. Transitional stage of iris bud growth. Increase in ht and width of apical dome followed by differentiation of staminal primordium (S) spathe leaf primordium (L). X80.

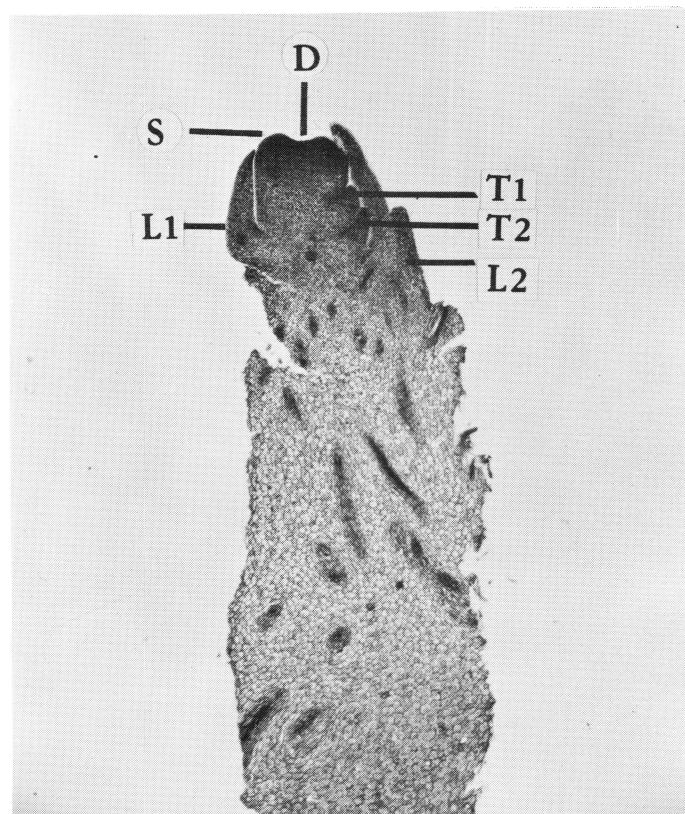


Fig. 4. Depression at apex (D) is early indication of flower bud initiation in iris. Lobes (S) become staminal primordia adjoined by inner tepal (T₁) and outer tepal (T₂) primordia. First (L₁) and second (L₂) spathe leaves are present. X37.

single style becomes greatly elongated (Fig. 9). In the center of the flower, carpel differentiation has begun as evidenced by cavity development (Fig. 9). A carpel is formed below each stamen at this stage of differentiation (1). As development of these 2 organs progresses, the stamens show attachment above the ovary, thus resulting in an inferior position of the ovary (Fig. 11). Formation of ovular primordia within the carpel (Fig. 12) occurs after that of pollen primordia differentiation in the anther (Fig. 10). A comparison of the difference in the stages of development within the same flower is illustrated by the tetrads of pollen grains (Fig. 13) and the young ovule stage prior to the appearance of the embryo sac (Fig. 14). Tetrads of pollen grains are formed by meiotic cell divisions of each pollen mother cell in the anther. During the meiotic process, the chromosome number of pollen grains and egg cells is reduced to one-half the

chromosome complement in the vegetative cells. The megaspore mother cell is differentiated in the nucellus tissue which becomes covered gradually by the growth of the integument layer to form an ovule (2). After the megaspore mother cell has undergone meiotic cell divisions, a mature embryo sac is produced in each ovule. No mature embryo sacs were observed in the bulbs sampled. The flower is now mature and ready for pollination at the flowering stage. Stuart et al. (7) reported that blooming can occur after 8 to 11 weeks of forcing.

Secondary buds emerge in the axil of the second spathe leaf and their differentiation into floral parts is apparent when development of the first flower has reached the approx stage shown in Fig. 8.

Heat Curing Experiment. Five heat curing treatments with a

Table 1. Stages and development of flower buds from bulbs given different treatments.

Tr. #	Heat curing temp/days F	Types of bulb storage		No. weeks between planting and examining		
		Holding temp/days F	Precooling temp/days F	6 Flower bud ^z stage	7 Flower bud ^z stage	8 Flower bud ^z stage
1	None (Shed)	Shed ^y	50°/42	Fig. 2	Fig. 6	Fig. 8
2	90°/10	Shed	50°/42	Fig. 3	Fig. 4	Fig. 9
3	90°/10	Shed	55°/42	Fig. 4	Fig. 5	Fig. 8
4	90°/10	60°/21	50°/42	Fig. 5	Fig. 6	Fig. 9
5	95°/14+104°/3	65°/21	50°/21	Fig. 5	Fig. 4	Fig. 10
6	104°/10	65°/21	50°/42	Fig. 5	Fig. 3	Fig. 7

^zStages of approximate bud development illustrated by photomicrographs in the Fig. indicated

^yShed (natural fluctuating temp)

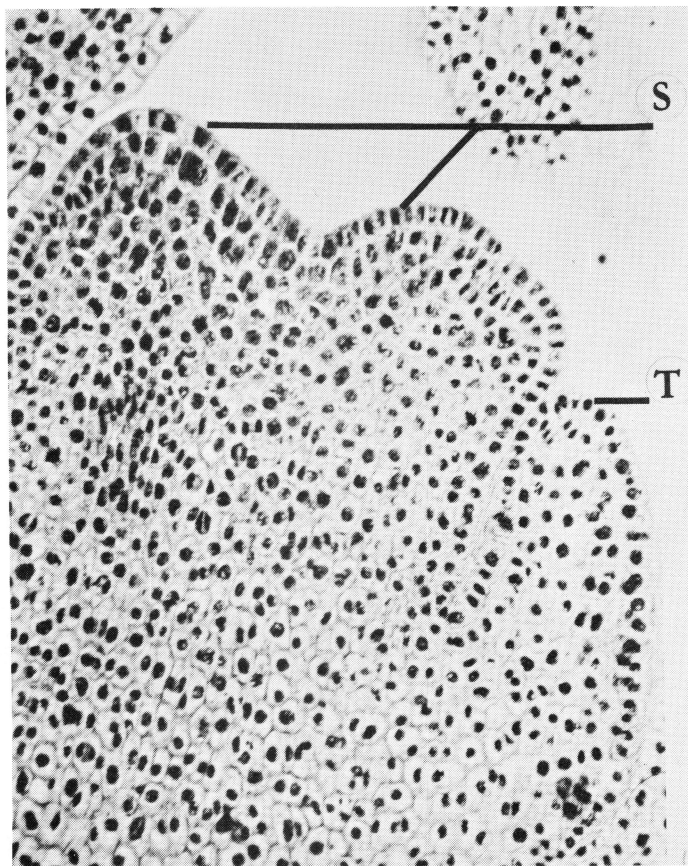


Fig. 5. Higher magnification of very young iris flower bud showing staminal primordia (S); tepal primordium (T). X200.

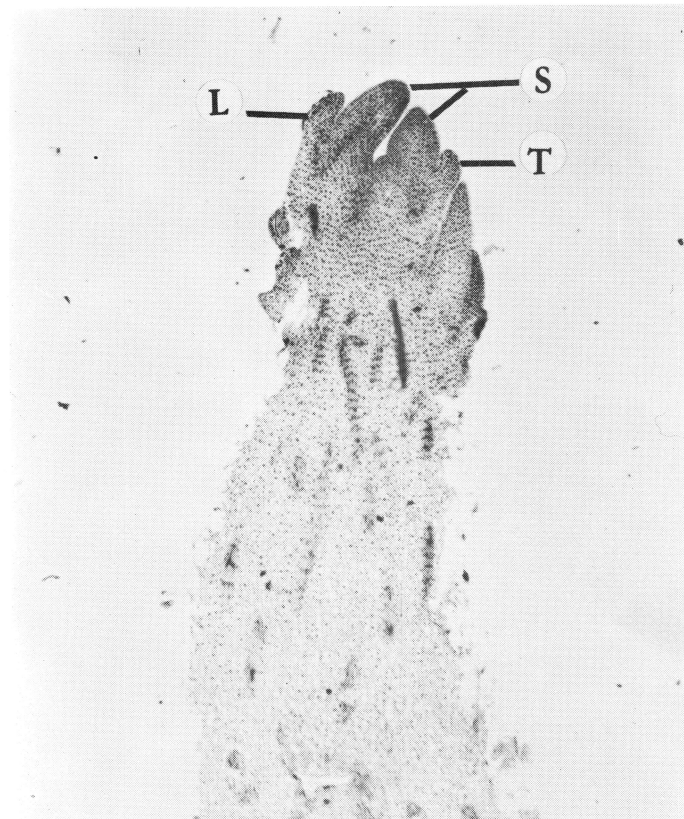


Fig. 6. Iris floral bud primordia elongate causing separation into 3 staminal primordia 2 of which are shown (S). Tepal (T) is adjacent and is enclosed by spathe leaf (L). X37.

control lot were tested (Table 1). Each treatment consisted of 120 bulbs from which 2 bulbs were removed at intervals of 6, 7, and 8 weeks after planting. Both bulbs from the control sample designated Lot #1 were vegetative in the first collection. Based on the 2 bulb samples, Treatment Lot #5 was the most effective in promoting floral development while Treatment Lot #6 showed the least cellular activity. Treatment Lot #5 is a standard treatment utilized by Dutch growers. Bulbs grown in the Pacific Northwest usually do not require as much heat curing as those grown in Holland. Therefore, heat curing is accomplished by storage at 32.2°C for 10 days (Table 1). The additional heat curing in Treatment Lot #6 was inhibitory.

Holding Temperature Experiment. In the second forcing season an experiment consisted of 3 holding temp treatments

and a control lot held at common storage during the holding temp period. The bulbs were dug July 22 and storage was started 5 days later. Each treatment consisted of 120 bulbs and 5 bulbs were removed for examination 3, 4, and 5 weeks after planting (Table 2). In Treatment Lot #2, normal initiation and development of the flower occurred. This is a standard holding temp. However, in Treatment Lot #3, all 3 of the bulbs 4 and 5 weeks after planting were still vegetative (Fig. 1 and 2). In Treatment Lot #4 bulbs 5 weeks after planting were vegetative (Table 2). The largest specimen in each paraffin block from a given Treatment Lot was sectioned, and if floral initiation had not occurred, the second largest specimen was sectioned. If floral initiation had not occurred in the 3 largest bulbs, examination was discontinued.

The wide range of bud development shown in Table 2 suggests that differences in size of flower buds or the lack of floral initiation were not the result of bulb storage treatment, but were due to the variability of environmental conditions under

Table 2. Determination of flower bud initiation after bulb treatment at various holding temp.

Tr. #	Heat curing temp/days F	Holding temp/days F	Precooling temp/days F	No. weeks between planting and examining		
				3	4	5
				Flower bud ² stage	Flower bud ² stage	Flower bud ² stage
1	90°/10	Common storage	50°/42	Fig. 7	Fig. 9	Fig. 11
2	90°/10	65°/35	50°/42	Fig. 8	Fig. 11	Fig. 11
3	90°/10	60°/35	50°/42	Fig. 8	Fig. 1	Fig. 1
4	90°/10	55°/35	50°/42	Fig. 7	Fig. 11	Fig. 1

²Stages of approximate bud development illustrated by photomicrographs in the Figs. indicated.

which the bulbs had been grown. For example, the degree of flower bud development in Treatment Lot #4 (4 weeks) and illustrated in Fig. 11 when compared with its counterpart collected a week earlier (Fig. 7) cannot be explained by treatment differences. However, the complete failure of all bulbs collected 5 weeks after planting in Treatment Lot #3 (Fig.

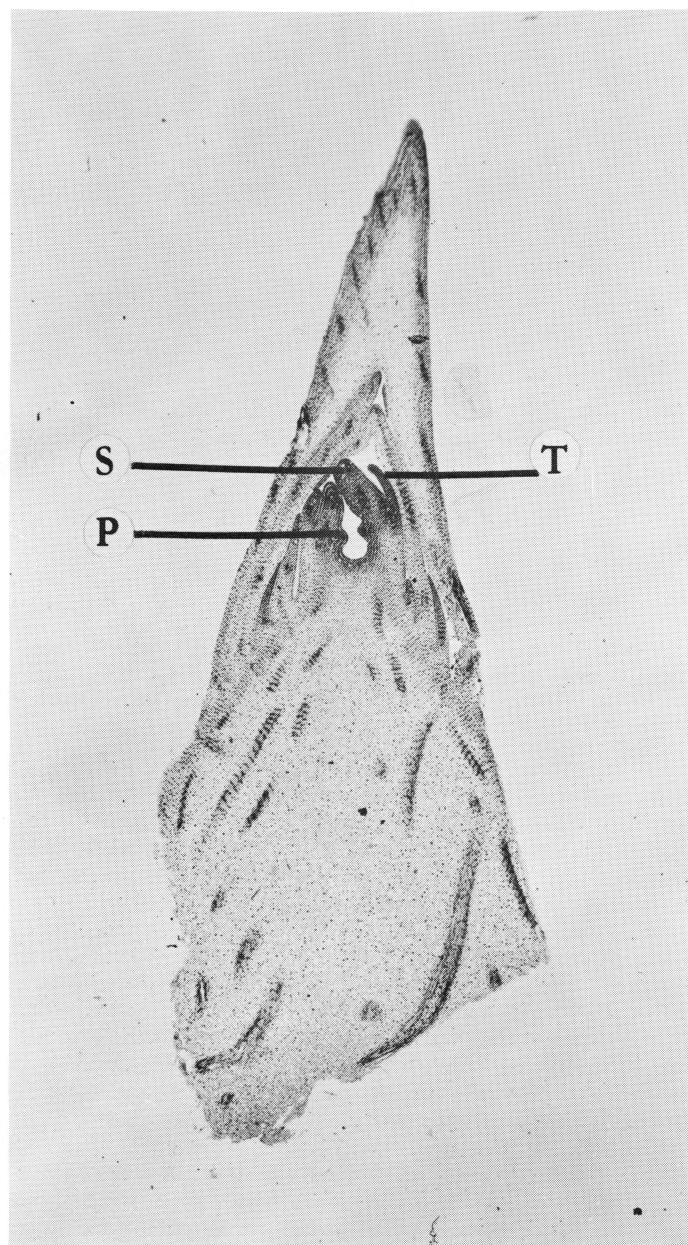


Fig. 7. Pistillate primordia (P) are differentiated as the stamens (S) and tepals (T) continue growth above the slow growing center of the original apex of the floral iris bud. X37.

1 and 2) indicates this to be a result of unfavorable holding temp. Heat curing and a holding temp of 18.3°C maintained for 3 weeks promoted flower bud initiation. Low holding temp of 12.7°C interrupted this phase of the floral initiation process in Treatment Lot #4 sampled 5 weeks after planting. Floral initiation was retarded or flowers aborted and the plants produced only 3 leaves. A holding temp of 15.5°C is apparently a borderline temp, as in Treatment Lot #3 the fourth and fifth weeks' collections remained vegetative. But the explanation as to why Treatment Lot #4 taken at intervals of 3 and 4 weeks

showed floral initiation while those taken in the fifth week remained vegetative (Fig. 1) can be made only on the basis of bulb variability in the third and fourth weeks' collections that permitted floral initiation to occur in spite of the low holding temp.

Variable Factors Experiments. During the third forcing season 2 tests were undertaken to study whether floral initiation was promoted by digging dates, holding temp, pre-cooling treatments, or the interactions of these treatments. Treatment with ethylene gas and varietal influence were tested in the second of those tests. A total of 43 lots of 120 bulbs each, was utilized. Microscopic examination of dissected bulb samples of every lot revealed such a high degree of irregularity that I could not ascertain the effects of the treatments. However, an

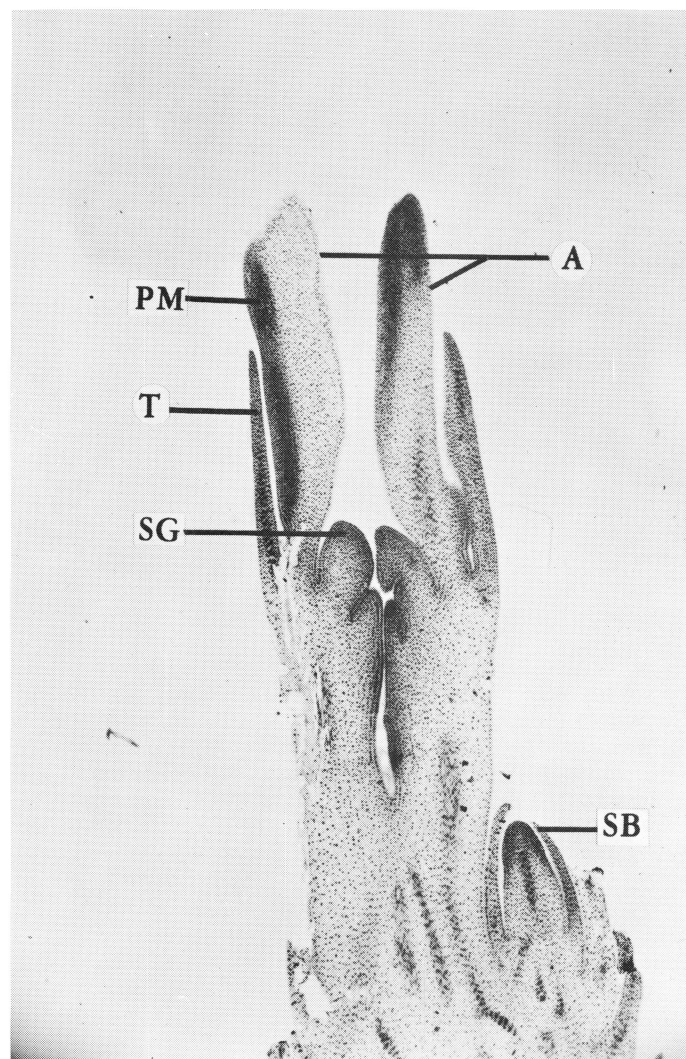


Fig. 8. Continuing iris floral bud development shows tepals (T), primordia for pollen mother cells (PM) in anthers (A), and stigma primordia (SG); secondary bud has developed in lower right (SB). X37.

interesting result in the second experiment was the development of an advanced stage of floral bud from a single treatment with 25 ppm of ethylene gas applied to bulbs in the shed prior to heat curing. This development was stimulated by the ethylene application rather than a chance selection of the most highly developed bulbs. Stuart et al. (6) used concn of 1, 5, and 10 ppm of ethylene to obtain significantly earlier and more uniform flowering. They suggested that ethylene might play a role in flower bud initiation and development by augmenting the accelerating effects of the bulb temp treatments.

Conclusions

Essentially, the artificially applied temp treatments are a duplication of the naturally occurring field conditions of the Mediterranean Region, the native home of the genus, *Iris*. At maturity the bulbs are subjected to the late summer heat which induces curing. This is followed by moderating temp of the autumn season which is equivalent to the holding temp period. With the advent of winter temp, the pre-cooling temp phase is completed and the iris bulbs sprout naturally in the spring.

are initiated after vegetative growth has started. Bulbs which are not sufficiently heat cured and are subsequently exposed to cool temp too soon develop only to the 3 leaf stage and fail to flower. The heat curing process apparently triggers some mechanism which permits a succession of chemical reactions necessary for normal vegetative growth and flower bud initiation.

When bulbs of a given cultivar attain a max degree of maturity, they can be expected to flower uniformly after

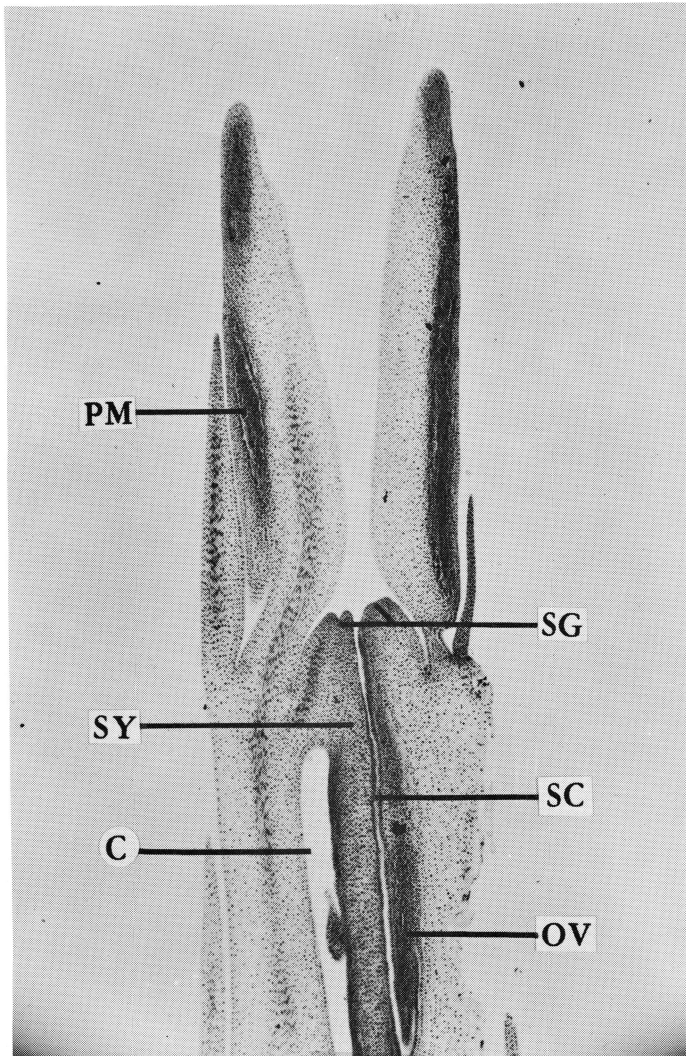


Fig. 9. Advanced iris floral development shows pollen mother cell development in anther (PM), differentiation of stigma (SG) and style (SY), greatly elongated styler canal (SC), ovular primordia (OV), and cavity in carpels (C). X37.

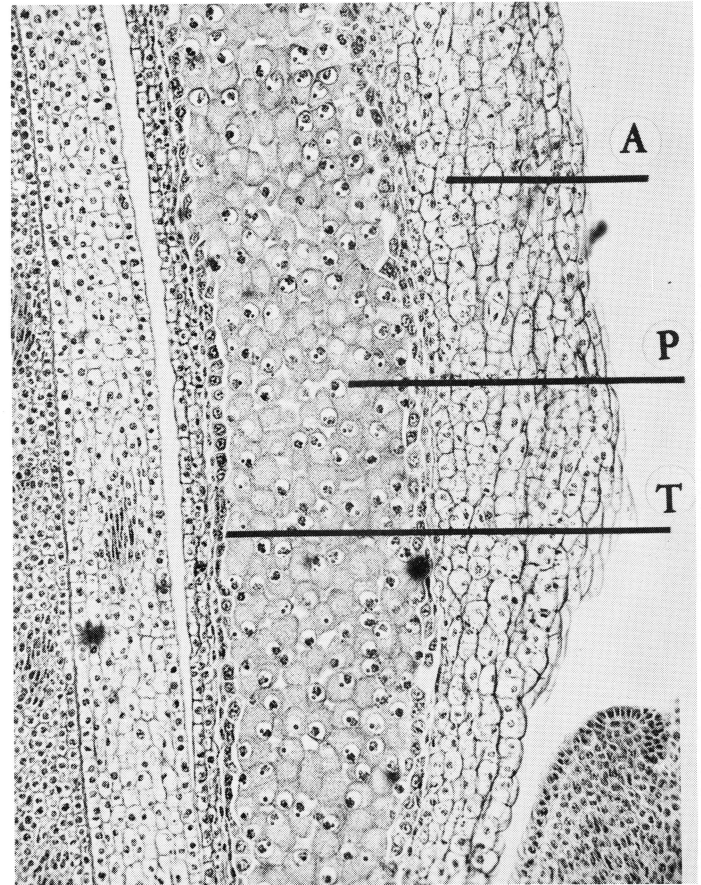


Fig. 10. Longitudinal view of anther (A) containing pollen mother cells (P) and remains of tapetum layer of cells (T) of flower bud stage in Fig. 9. Each cell (P) via meiosis forms a tetrad cell containing 4 separate pollen grains as in Fig. 13. X100.

In order to obtain early, uniform flowering, a series of variable temp treatments for commercial forcing of bulbous iris has been devised. Heat curing at 32.2°C for 10 days is followed by a holding temp of 18.3°C for a period of 3 weeks. The bulbs are then pre-cooled at 10.°C for 6 weeks or approx 1,000 hr after which they are ready for planting in October. Flower buds

receiving the temp treatments just described. Bulbs which have been grown under highly variable environmental conditions, however, will not have reached the desirable stage of maturity in a high percentage of bulbs. Lack of uniformity of flowering, therefore, results from immature bulbs incapable of responding uniformly to the standard temp treatments.

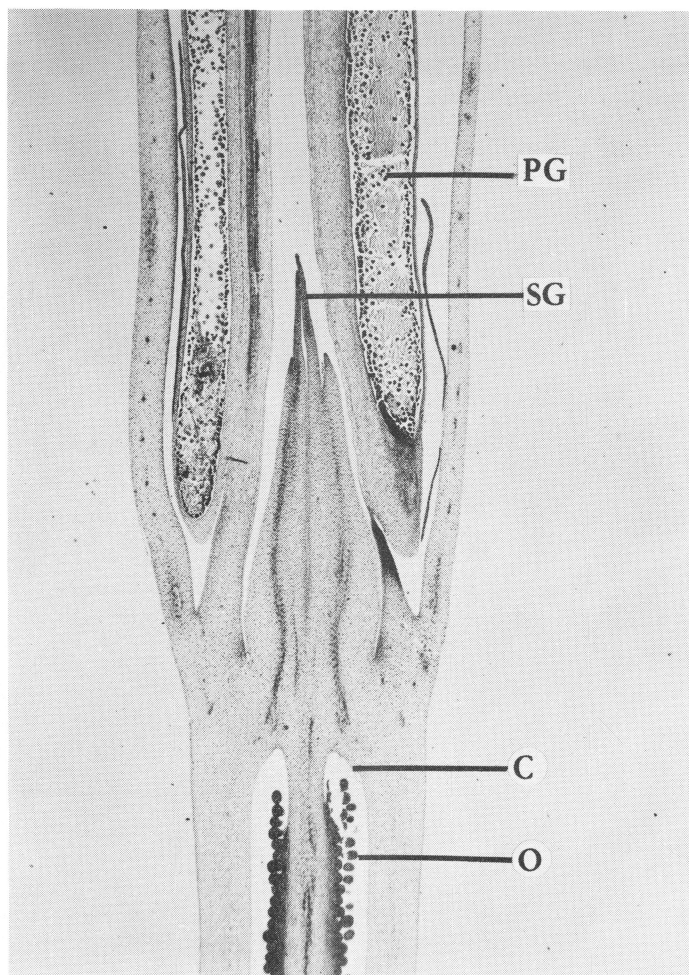


Fig. 11. Fully mature flower contains pollen grains (PG), stigmatic surface of pistil (SG), and ovules (O) in carpel (C). X37.

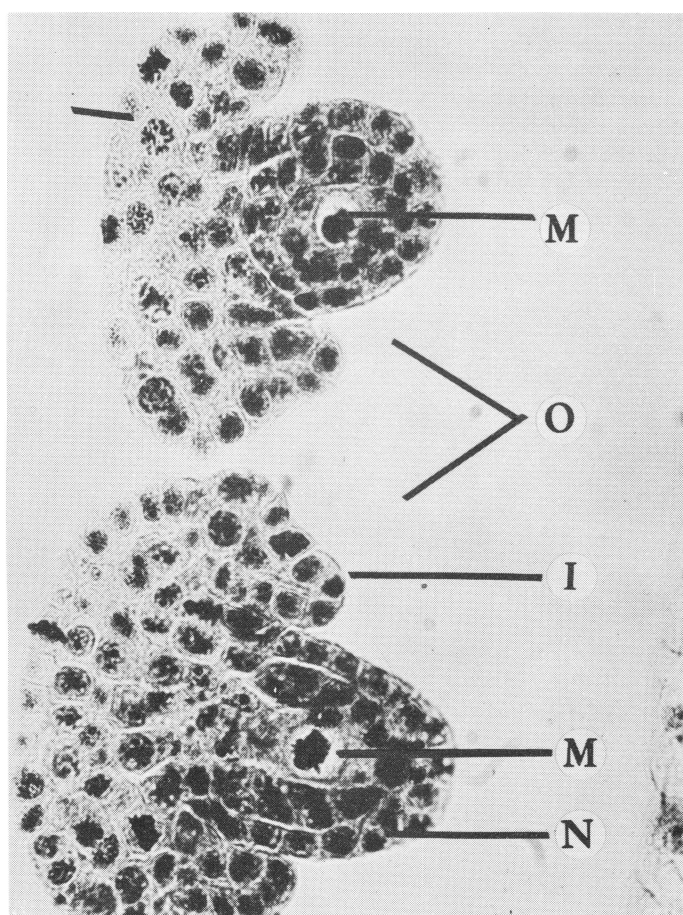


Fig. 12. Megaspore mother cells (M) present in 2 ovules (O) just prior to stage reached in Fig. 11. Prominent cell (M) embedded in nucellus tissue (N) enclosed by integument layer (I). X400.

Seasonal variation is a big factor in uniform flowering response. Excessive variation in soil nutrients, moisture, and soil type results in the production of bulbs possessing varying degrees of maturity. Digging of immature bulbs following a late season can result in much variability and in an excessive number of blind plants unless adequate temp treatments are applied. Bulb size is not necessarily a criterion of bulb maturity.

Highly erratic responses observed for the 3 forcing seasons were due to the lack of the optimum stage of bulb maturity in sufficiently large percentages in the bulb lots. Where feasible, the use of larger test samples ranging up to 25% of the bulbs in an experiment, would probably reduce the effects of bulb variability to an acceptable level.

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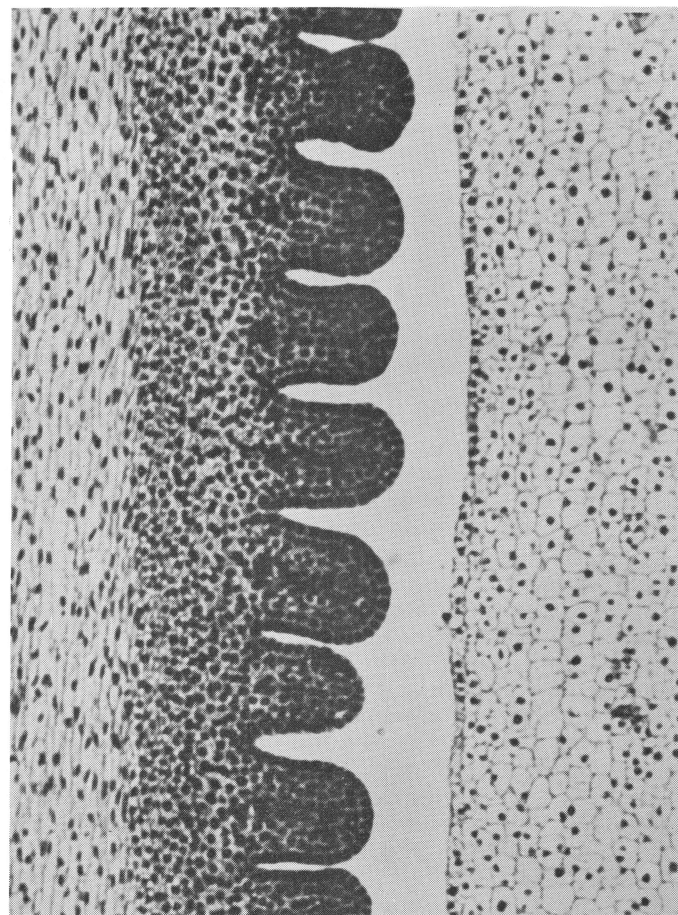
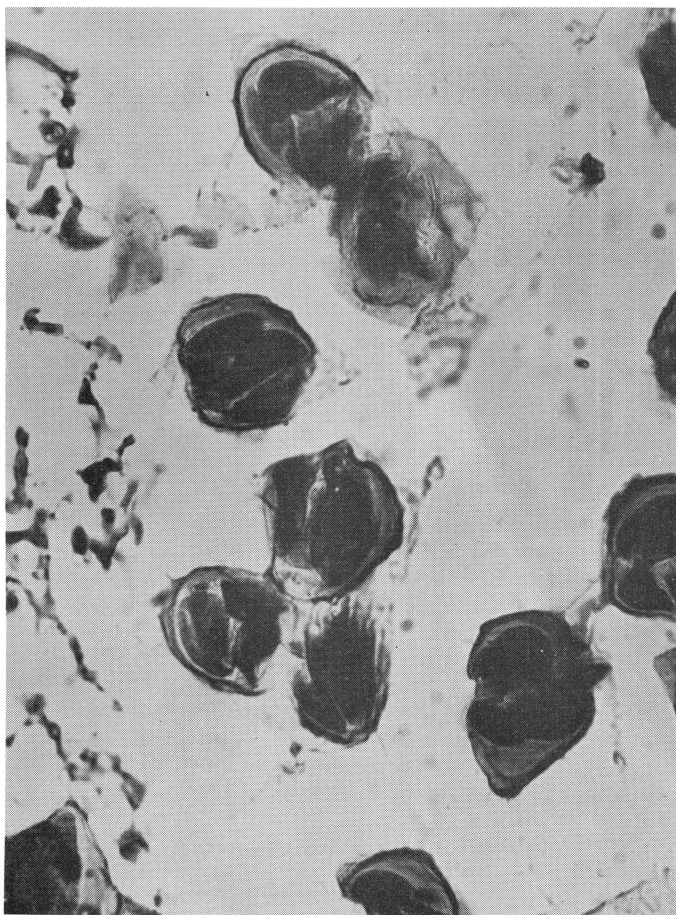


Fig. 13. Tetrad of pollen grains in same flower shoot as shown in Fig. 14 shows advanced stage of development compared with ovules. Anthesis can occur in 5 to 10 days. X200.

Fig. 14. Longitudinal view of ovules in carpel of same floral bud specimen in Fig. 13 shows young ovules prior to development of megaspore mother cell stage in Fig. 12. X80.

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