Flower Formation in the Saffron Crocus (*Crocus sativus* L). The Role of Temperature

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Keywords: dormancy, flower initiation, flowering, saffron spice, shoot growth

Abstract

The freshly formed replacement corms of saffron (*Crocus sativus* L.) had no chilling requirements, but sprouting only occurred after a period of after-ripening. Sprouting could be accelerated by a short curing at 30 ºC. Shoot growth occurred at any temperature between 1 and 30 ºC. The optimal temperature for shoot growth (23-25 ºC) proved also optimal for flower initiation. No flower primordia were present in the resting buds. Flower organogenesis occurred during the early summer growth. The optimal temperature for flower emergence (17 ºC) was markedly lower than for organogenesis. The differences in air temperature explain the different calendar time for saffron flower initiation in different locations. Water availability plays a minor role, if any, on flower formation. Storing the corms at an appropriate temperature will allow to control and extend the period of saffron flowering.

INTRODUCTION

Crocus sativus is an autumn flowering geophyte with subhysteranthous behaviour. Leaf emergence coincides or occurs shortly after flowering. The photosynthetic activity of the leaves during the winter and the early spring months contributes to the formation of the replacement corms at the base of the shoots. The leaves wither at the onset of the dry season, and during late spring and most of summer the plant shows no aboveground organs or roots. This condition is usually described in horticultural sense as dormant (Res, 1992), a term which may be misleading as cell division occurs at this time in the apical meristem of the apparently resting buds. Flower initiation occurs during this period. In the corms formed at the base of a non-flowering shoot, flower formation is usually restricted to the apical, and dominant, bud. In those corms formed at the base of a flowering shoot, flower formation may occur in two or three of the buds closest to the apex (Molina et al., 2004d). The few published descriptions of the early stages of flower initiation showed wide differences in its timing. It occurred during March in Azerbaijan (Milyaeva and Azizbekoba, 1978) and in Israel (Greenberg-Kaslasi, 1991), and during July in Kashmir (Koul and Farook, 1984). Greenberg-Kaslasi (1991) suggested that corm size or seasonal variations determined the difference in transition dates, but the role of these factors have not been tested critically. Particularly streaking is the lack of information on the role of temperature on flower formation and flowering (Negbi et al., 1989; Chrungoo, 1992).

In the present report, we summarize our recent work on the effect of temperature on flower initiation and flowering in saffron. Also, we present a detailed description of flower ontogenesis and the characterization of the different stages. A more detailed account of this work is being published elsewhere (Molina et al., 2004a, b, c).

MATERIALS AND METHODS

The experiments were performed using saffron corms (*Crocus sativus* L) raised at Quero, Toledo, Spain. The characterization of flower ontogenesis was performed lifting periodically corms during the summer. Corms of a uniform size (ca. 35 mm in diameter) were selected. After the removal of the protective cataphylls, the shoot apex was examined under the dissecting microscope. A further characterization of flower initiation

was performed viewing longitudinal sections through the central part of the apex with the light microscope, and observing the naked shoot apex (i.e. after the removal of the protecting sheath of cataphylls) with the scanning electron microscope. The changes in the apex of these corms were compared to changes in corms 15 mm in diameter, which were not going to flower.

The handling of the corms in the experiments performed to determine the influence of temperature on flower formation was as described previously (Molina et al., 2004b). In brief, the corms were lifted shortly after leaf senescence (early June), the mother corm parts were removed, and the recently formed replacement corms were separated and graded. The clean replacement corms were dipped in a 0.1% prochloraz solution to prevent mould infestations, surface-dried immediately using a forced drought, and stored for 3-4 days under shelter at ambient temperature. Flower initiation was studied using corms 30 mm in diameter. To determine the influence of temperature on flower initiation, the corms were placed on a mat of dry rock in plastic trays and incubated in the dark at the desired temperature in an atmosphere with 85% relative humidity. After the desired period of incubation, the corms were watered and placed to flower in the light at 17 ºC.

To determine the influence of location on plant ontogenesis, corms of the same batch were planted in four locations with contrasting climate.

RESULTS AND DISCUSSION

Shoot Growth

During the last stages of formation of the replacement corms and shortly after leaf withering, the apical meristem of the buds made no visible growth. These non-growing buds had the apex protected by a sheath of cataphylls (Figure 1). Both the size of the shoot apex (which at this time was a naked meristem without leaf primordia) and that of the sheath of cataphylls, were larger in the big-size (and potentially- flowering) corms than in the smaller corms (Figure 2).

In the big sized corms, cell division at the meristem and the subsequent shoot growth slightly preceded the growth of the cataphylls (Figure 2). However, the subsequent faster growth of the cataphylls ensured the formation of a protected and unobstructed path for flower emergence up to the soil surface. Both cell division and cataphyll growth occurred earlier in the bigger size corms than in the smaller ones (Figure 2). By July 17, the sheath of cataphylls of the big corms reached 2 mm in length. A significant increase in the height of the shoot apex was already measurable in these corms two weeks earlier (by July 3). In the small corms, no growth of the cataphylls or the shoot was detectable by the end of July. They only were evident in the samplings performed by mid August (Figure 2).

The quiescent buds showed no chilling requirements, and were able to sprout over a wide range of temperatures. The corms stored at temperatures just above freezing made very little growth (1-2 ºC; Molina et al., 2004c). Shoot growth increased with temperature up to 23 ºC (Molina et al., 2004d). Shoot growth in the range 23-25 º C was much faster than at 30ºC. (Molina et al., 2004d).

The time course of shoot growth for corms incubated in a growth room at a constant 25º C temperature is shown in Figure 3. There was a lag period of one-month duration before the increase in length of the cataphylls was detectable. Molina et al. (2004a; 2004b) demonstrated that this lag period lasted for two months when the corms were lifted and forced to grow at an earlier date. Also, these authors found that a 20-day-long heat curing at 30 ºC shortened significantly the duration of this lag period and accelerated shoot growth and flowering.

When incubated at a constant temperature (at 25 ºC), cataphyll length increased linearly with the time of incubation (Figure 3). The growth rate under these environmental conditions for corms lifted in late June was similar to that recorded under field conditions in the traditional saffron growing areas of Spain (Molina et al., 2004d).

This is not surprising since the mean soil temperature from June to mid September was in the range 23-25 ºC in the years we performed the experiments.

Flower Initiation

No flower organs were present in the resting meristem. Flower initiation occurred in early summer during the early stages of shoot growth, and was modulated by temperature. Incubation of the corms in the range of 17-30 ºC allowed flower formation.

Optimal temperature for flower formation was in the range 23-25 ºC. At this temperature the rate of flower initiation was highest, and the number of flowers initiated was maximal (Molina et al., 2004d). At least 50 days of incubation at this temperature was needed for the formation of the maximum number of flowers (Molina et al., 2004b). Corms incubated at 17 ºC formed at most one flower per corm. In most of our experiments we obtained 2.5 to 3.0 flowers per corm. In corms much bigger than those used in our work we have observed the formation of +7 flowers in a single corm.

The main stages of flower differentiation for corms grown under field conditions at Albacete, Spain, are shown in figure 4. The duration of the process of flower initiation was similar in these corms to that depicted in figure 3 for corms incubated in a growth room at 25 ºC. A 30-day interval elapsed from the initiation of the leaves (early July) to the initiation of the gynoecium (end of August).

The sequence of organ initiation at the shoot apex was as follows:

1. Leaf initiation (flower score 2).
2. Bract initiation (flower score 4)
3. Stamen initiation (flower score 6)
4. Tepal initiation (flower score 8). Three of the tepals formed at the outer edge of the stamen primordia. The additional three tepals formed in the gaps in the whorl of stamen primordia (Figure 4F)
5. Gynoecium formation (flower score 10).

In the buds forming several flowers, the first forming (and emerging) flower formed at the top of the shoot apex, and caused the cessation of meristematic activity. The additional flowers formed at more basal positions. These flowers arose apparently at the axil of a leaf (Figure 5). Each of these flowers formed bracts (Figure 5).

Flower Emergence

Incubating the corms at high temperature for a long time resulted in flower abortion. This was also true for corms incubated at the optimal temperature for flower initiation (23-25 ºC). Incubation at these temperatures lasting more than 150 days resulted in a reduction in flower number and some of the flower primordia withered (Molina et al., 2004b). Optimal temperature for flower emergence was close to 17 ºC.

At any time from the filling of the replacement corms, shoot growth seemed independent on water availability. In corms kept in an atmosphere with 85% relative humidity, shoot growth proceeded beyond flower emergence. We only could demonstrate a marginal and indirect effect of water availability on flower emergence. Root formation, which depends strictly on water availability, increased significantly flower size but had no effect on the time of flowering (Molina et al., 2004b). On the other hand, water availability determined leaf growth. In agreement with Plessner et al. (1989), we found that in non-watered corms leaf growth was retarded and hysteranthy was enhanced (Figure 6). There seemed to be a relationship between the degree of hysteranthy and water availability (Figure 6).

CONCLUSIONS

The results presented herein fill a gap in the scientific knowledge on saffron. They show that temperature is the main factor which determines the rate of shoot growth, flower initiation and flower emergence. Also, we demonstrated that the optimal temperature is lower for flower emergence than for flower formation. This fact explains the differences in the timing of flower initiation in locations with contrasting climates.

Flower initiation occurs as the temperature rises during late spring above 20 ºC. It occurs earlier in warmer climates (Jerez and Valencia; figure 7). In these locations, too high autumn temperatures delay flowering as compared to traditional saffron growing areas (Albacete and Segovia). Flower emergence occurs as the air temperature fells below 16 ºC (Figure 7).

This knowledge allows to program saffron flowering at will. The protocols to force flowering from early September until the end of May have been described (Molina et al., 2004a).

ACKNOWLEDGEMENTS

This research was funded through a research contract with Compañía General del Azafrán de España. We thank Mr. Agustin Escandon, General Manager, for support and permission to publish these results.

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Figures

Fig. 1. Longitudinal section on a resting bud showing the shoot apex composed by a naked meristem without lateral organs protected by a sheath of cataphylls.

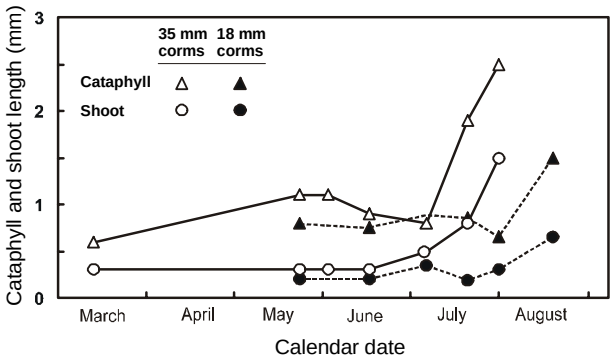

Fig. 2. Cataphyll (triangles) and shoot (circles) length in the apical buds of corms 35 mm in diameter (open symbols) and 18 mm in diameter (closed symbols). The measurements covered the period of time extending from the late stages of corm filling to the early stages of bud sprouting.

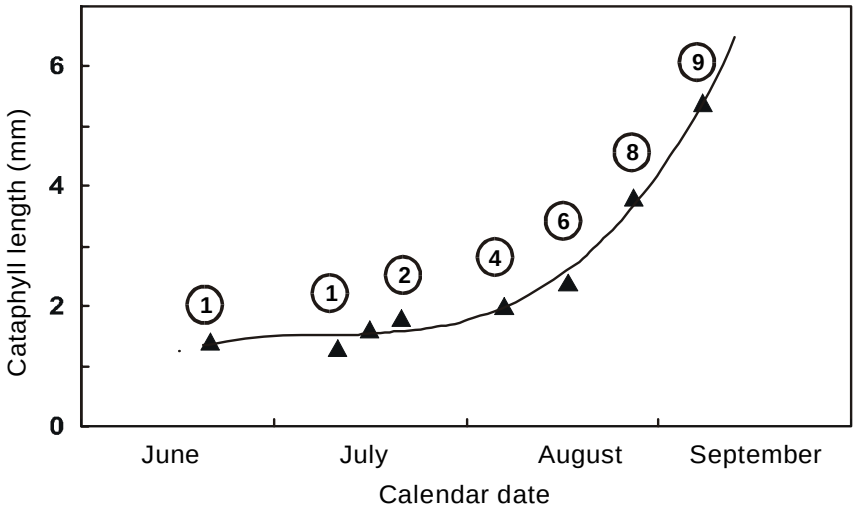

Fig. 3. Cataphyll length and flower morphogenesis in the apical bud of corms grown lifted by the end of June an incubated in the growth room at 25 ºC. The stage of flower development in the buds is shown in the circles, using the numerical score described in the text.

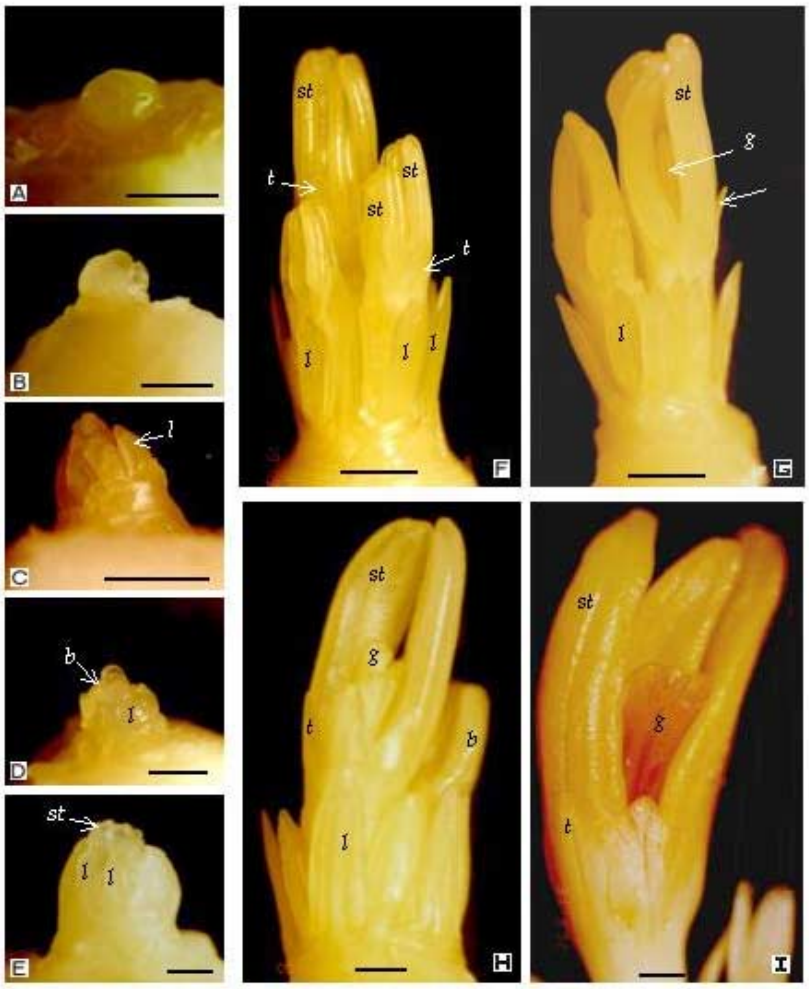

Fig. 4. Low magnification photographs of the naked shoot apex (having the sheath of cataphylls removed) at different developmental stages.
The flower score used in our work is also indicated. A, Resting bud photographed on 25 June. No lateral organs were initiated at this time (flower score, 1). B, Dome-shaped shoot apex photographed on 13 July. The leaf primordia were being initiated at the flanks of the meristem (flower score, 2). C, Shoot apex photographed on 20 July. The base of the dome-shaped meristem was covered by the developing leaf primordia (flower score, 3). D, Shoot apex photographed on 25 July, at the time the bracts were initiated at the edge of the meristem (flower score, 4). E, Stamen initiation.
Photograph taken on 6 August (flower score, 6). F, Bud with three developing flowers photographed on 14 August. The stamens were much longer than the leaves, a consequence of the hysteranthy of this species. At the dorsal side of the stamens, the first whorl of tepals was already initiated in two of the flowers (arrow; flower score, 8). G, Shoot apex with two developing flowers photographed on 20 August. The gynoecium was already initiated (arrow; flower score, 10). H, Further development of the flower, whose parts grew faster than the leaves. I, The gynoecium had reached one half of the length of the stamens. The developing style and stigmata showed a typical reddish colour. b, bract; g, gynoecium; l, leaf; st, stamen. Scale bars, 0.5 mm. Source; Molina et al. (2004d).

Fig. 5. Left. Longitudinal section of a shoot tip showing two flower primordia protected by bracts. Right. SEM view of a shoot apex with three developing flowers. The bracts wrapping the two flowers in the forefront are clearly discernable.

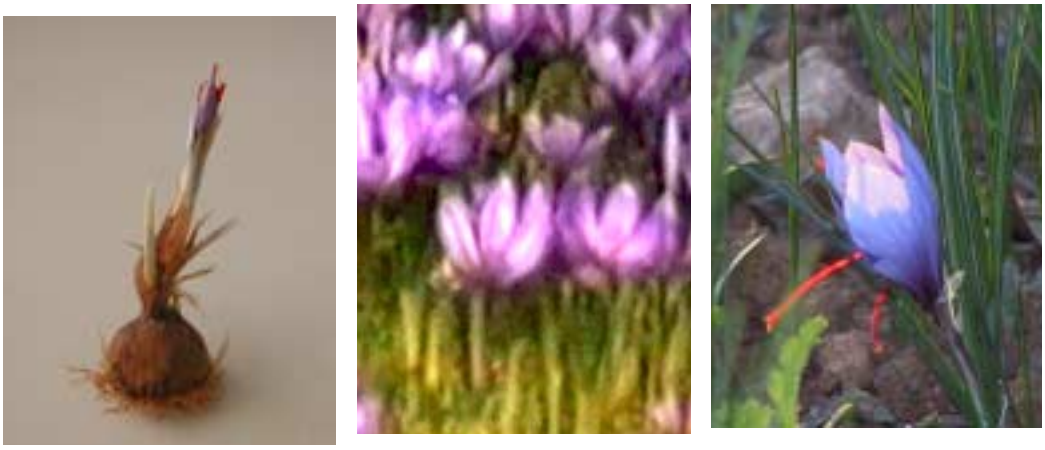

Fig. 6. Left. Hysteranthous behaviour of corms kept on the bench in the laboratory. These corms flowered at the same time than those grown in the open, and the flowers emerged long before leaf emergence. In corms watered before flower forcing, the corms showed a subhysteranthous behaviour; at the time of flower opening the leaves were visible, but smaller in size than the sheath of cataphylls. This circumstance occurred in the corms forced to flower under our experimental conditions (Centre). In well-watered corms grown in the soil, the leaves were already fully developed at the time of flowering (Right). Photograph at the right by courtesy of D.O. Azafrán de La Mancha).

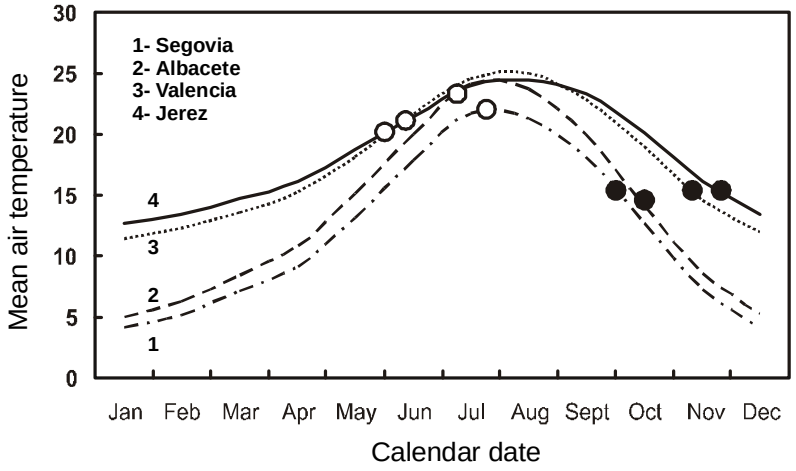

Fig. 7. Mean monthly air temperature and time of flower initiation (open circles) and flower emergence (closed circles) in four locations of Spain with contrasting climates. Albacete and Segovia are traditional saffron producing areas.