Temperature effects on flower formation in saffron (*Crocus sativus* L.)

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Abstract

The temperature conditions for shoot growth and flower formation were characterised for saffron (*Crocus sativus* L.). Leaf withering occurred during late winter or spring depending on location, and coincided with a rise in temperature. No growth was detectable in the buds during the first 30 days after leaf withering, neither in underground corms nor in lifted corms incubated in the laboratory under controlled conditions. Flower initiation occurred during the first growth stages of the buds. The optimal temperature for flower formation was in the range from 23 to 27 °C, 23 °C temperature being marginally better. To ensure the formation of a maximum number of flowers, the incubation at these temperatures should exceed 50 days, although incubation longer than 150 days resulted in flower abortion. Flower emergence required the transfer of the corms from the conditions of flower formation to a markedly lower temperature (17 °C). Incubation of the corms after lifting at a higher temperature (30 °C), reduced flower initiation and caused the abortion of some of the initiated flowers. No flowers formed in corms incubated at 9 °C. A variable proportion (20–100%) of the corms forced directly at 17 °C without a previous incubation at 23–27 °C formed a single flower. The wide differences in the timing of the phenological stages in different locations we found in this study seemed related to the ambient temperature. Leaf withering was followed shortly by flower initiation, which occurred during late spring or early summer as the rising temperature reached 20 °C. A long hot summer delayed flower emergence which occurred in late autumn as the temperature fell to the range of 15–17 °C.
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Keywords: *Crocus sativus*; Dormancy; Flower forcing; Flowering; Saffron; Temperature

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1. Introduction

Saffron (*Crocus sativus* L.) is an autumn-flowering geophyte extensively grown in the Near East and the Mediterranean basin since the Late Bronze Age (Zohary and Hopf, 1994; Negbi, 1999). Its long scarlet stigmas were highly valued for flavouring foods and for colouring them golden-yellow. They were also used for dying textiles. The appreciation for saffron as a food additive continues today and it has become the world's highest priced spice (Winterhalter and Straubinger, 2000). There is also a long tradition of saffron use in the traditional medicine of many cultures (Abdullaev, 1993; Ma et al., 2001). More recently, there has been increasing interest in the biological effects of the components of saffron and their potential medical applications, particularly those based on their cytotoxic, anticarcinogenic and antitumour properties (Abdullaev and Frenkel, 1999; Fernández Pérez and Escribano Martínez, 2000).

Despite these apparently bright prospects, there was a marked reduction in saffron production during the last two decades in some of the traditional producing countries, as Spain, Italy and Greece (Negbi, 1999). This reduction in production has been most marked in Spain, a country rightly considered in the near past as the leading producing country in the world, where the land area devoted to saffron cultivation fell from 2800 ha in 1990 to under 600 ha in 1999 (Ministerio de Agricultura, Pesca y Alimentación, 2000). These figures are well below the 47,000 ha devoted to saffron cultivation in Spain in the early 1970s and were accompanied by a parallel reduction in production from over 44 metric tons in 1976 to under 6 metric tons in 1999. The main reason for this reduction in production is that the technology of saffron production has not changed from the ancient times. Increasing labour costs have turned saffron production unprofitable despite its high market price. The picking of crocus flowers (0.25–2.5 million flowers ha^{-1}, depending on the productivity of the plantation) and the separation of the stigmata require an intensive hand labour. Saffron flowering lasts only 2–3 weeks, and picking of the flowers is required daily. A technological breakthrough to increase the profitability of saffron cultivation would be the cultivation under controlled conditions. In this way, flowering period could be extended, thus reducing the need of intensive labour. Further, it would be much easier to mechanise blossom collection in containerised plants than in those grown in the soil.

Despite its importance, the flowering process in saffron has not been characterised precisely. The life cycle of saffron is similar in all producing countries, but there are wide differences in the timing of events (Alarcón Molina and Sánchez Requena, 1968; Wilkins, 1985; Botella et al., 2002). Flowering occurs during autumn (October–November), and is followed by a vegetative stage throughout winter and the formation of replacement corms at the base of the shoots. At the beginning of the dry season (April–May), the leaves senesce and wither, and the bulbs go into dormancy. The transition from the vegetative to the reproductive stage occurs shortly afterwards in the apex of the buds of underground corms. This transition has been reported to start during March in Azerbaijan (Milyaeva and Azizbekova, 1978; Azizbekova and Milyaeva, 1999), from March to April in Israel (Greenberg-Kaslasi, 1991) and during July in Kashmir (Koul and Farooq, 1984). Differences in corm size or seasonal variations have been considered the cause of these differences in transition dates (Negbi, 1999). Flower formation is directly related to corm size (Negbi et al., 1989; De Mastro and Ruta, 1993) and a quantitative relationship

between these two parameters was found (Negbi et al., 1989). However, the role of ambient temperatures on flower bud differentiation and subsequent flowering is largely unknown. Plessner et al. (1989) reported the formation of a similar number of flowers in corms forced either under uncontrolled conditions (at around 15 °C) or in a phytotron at a 17/12 °C (day/night) cycle. However, neither the temperature nor the duration of corm storage before corm forcing was stated, apart from the rather imprecise statement ''under ambient room conditions (not controlled)''. Benschop (1993) quoted Le Nard to state that *C. sativus* corms can be stored at 30 °C and 80% relative humidity for up to 8 months, thus retarding flowering. However, no quantitative data were presented. Muñoz Gómez et al. (2002) stated that the storage of the corms at 30 °C for 45 days increased the number of flowers as compared to corms forced to sprout directly at 17/10 °C after leaf withering. However, the number of flowers formed was very low (under 0.3 flowers per corm).

In this study, we characterised the life cycle of saffron in several locations with different climates. Also, we determined the influence of temperature on the transition from the vegetative to the generative state, and on the dormancy of the corms.

2. Materials and methods

The corms used in this study were grown at Quero, Toledo, a traditional saffron producing area of Spain. Shortly after leaf senescence (late May to early June) the plants were lifted, the mother corm parts removed, and the new replacement corms separated and graded. The clean replacement corms were then dipped in a prochloraz solution (0.1%) to prevent *Fusarium* and *Penicillium* infestations and dried under forced ventilation for 5–7 h to remove the surface water. The dry corms were kept under shelter during 3–5 days at ambient temperature (17–20 °C) before the start of the experiments. Graded corms of the desired size were placed on a mat of dry rock wool in plastic trays (0.35 m × 0.5 m, and 9 cm height) and incubated in the dark at the desired temperature (±1 °C). Temperature and duration of incubation are given in Section 3 for every experiment. A humidity sensor-controlled water atomizer kept the relative humidity during this storage at 85 ± 2%. Ventilation was provided twice a day to keep the CO_2 concentration below 400 ppm. For each treatment (a set of temperature and duration of incubation) three trays (80 bulbs each) were prepared. Two trays were used for the visual inspection of time of flowering and flower count and to harvest of saffron. The third tray was used for the measurement of growth, the destructive characterisation of the flowering stage of the apex, to monitor weight loss by the bulbs and to take samples for histology. Samples for these observations consisted of ten corms selected at random.

After this incubation, the corms were covered with a 4–5 cm deep expanded clay (arlite) layer, watered with 1/4 strength Hoagland solution and transferred to a dark room at 17 °C for flower emergence. At the start of anthesis, light was provided by Pope FTD 58W/82 fluorescent lamps, with a photoperiod of 8/16 h (light/dark) and a photon flux density of 20 μmol m^{-2} s^{-1}. In some experiments, prior to the forcing at 17 °C, the watered corms were kept in the dark at an intermediate temperature (21 °C) for a variable length of time (6–18 days).
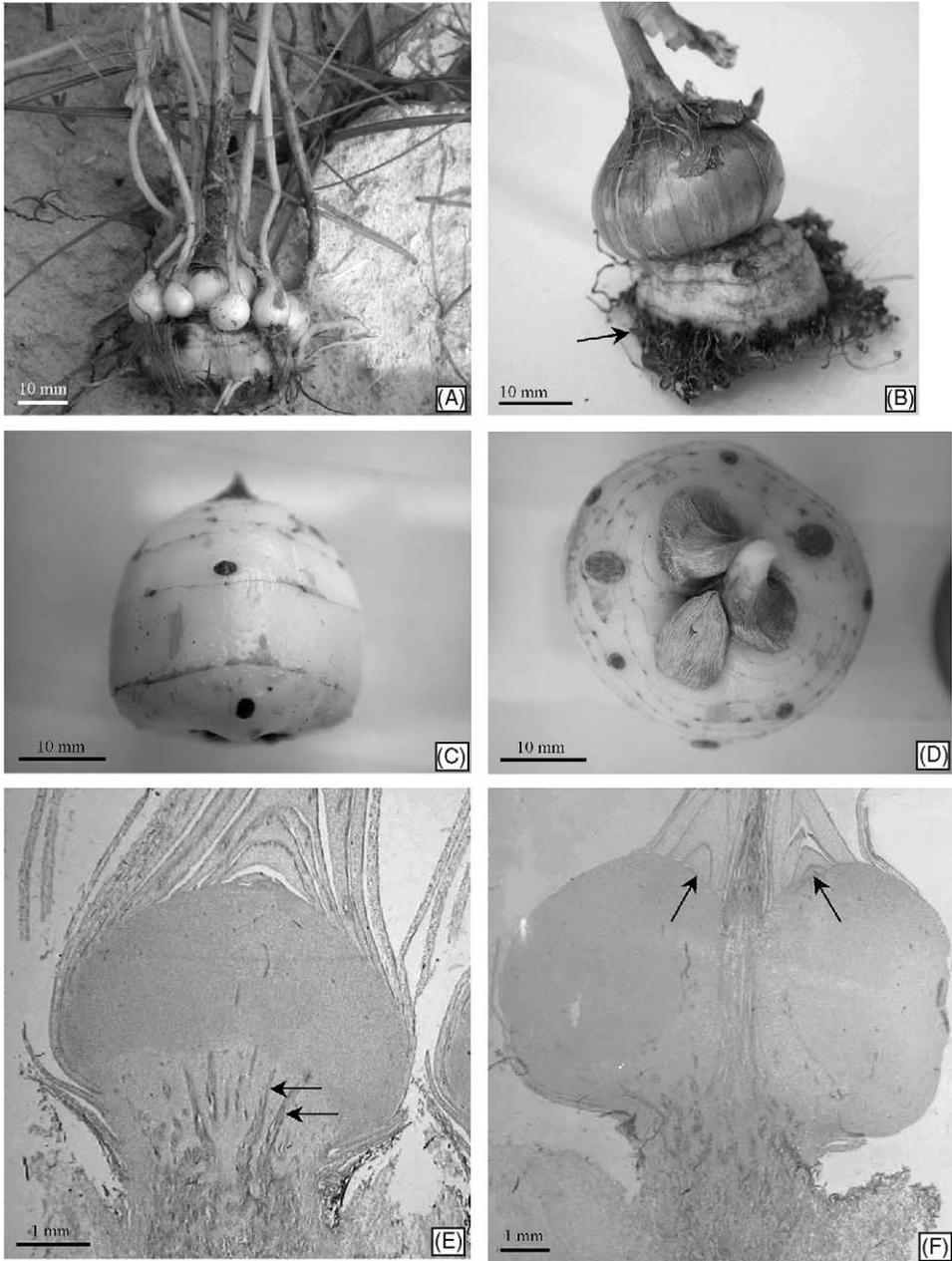
Fig. 1. (A) Multiple shoot development from a single corm. The growing replacement corms formed at the base of these shoots shall grow little and shall not flower the next season. (B) Replacement corm formed at the base of the single flowering shoot present in the mother corm. This corm shall grow to reach 30–40 mm of diameter and shall flower next season. Fibrous caulogenic roots (arrow) formed a root plate at the base of the mother corm. (C) Mature replacement corm formed at the base of a non-flowering shoot with the tunic (the expanded basis of the true and the sheathing leaves) removed to show the presence of a single apical dominant bud. (D) A mature

The stage of flower initiation and development was characterised using a numerical scale (see Section 3) based on the stages of development described by Beyer (1942). For histological observations, the growing point was removed with a minimal amount of corm tissue, fixed and embedded in paraffin. Longitudinal median sections were cut at a thickness of 10 μm, and stained with either the Schiff reagent or safranin and fast green (Johansen, 1940). The significance of the environmental effects on flowering was tested by means of an analysis of variance (ANOVA). Mean separation was done using a multiple range test (Snedecor and Cochran, 1967).

3. Results

3.1. The structure of the corms

A replacement corm formed at the base of every shoot developed from the maternal corm. The size and structure of these replacement corms depended on their number and on flower formation. A high number of shoots per corm resulted in the formation of several small-sized replacement corms which did not flower the following season (Fig. 1 A). The number of shoots in the mother corms was usually much lower, ranging from one (Fig. 1B) to three. The replacement corms formed at the base of non-flowering shoots kept the apical meristem of the shoot, which became the dominant, and usually the only one, sprouting bud the following season (Fig. 1C and E). The apical meristem of the flowering shoots was lost (Fig. 1F), and the replacement corms formed at the base of these shoots had two to three well developed axillary buds (Fig. 1D). All these buds sprouted the following season and formed a new replacement corm. This resulted in the increase in the number of corms. A sheath of cataphylls protected all the main buds. These cataphylls grew ahead of the shoot apex, and pierced the soil, thus protecting the shoot apex during emergence.

Replacement corms, either from flowering or non-flowering shoots, with a diameter ranging from 25 to over 40 mm, were used in our experiments.

3.2. The influence of location on ontogenesis

The ontogenesis of *C. sativus* plants grown in the open in four locations of different climates is shown in Fig. 2. The period without aboveground organs was much longer in Jerez (circa 9 months; from March to the end of November) than in Albacete (4.5 months; from early June to mid-October) or Segovia (near 4 months). At Valencia, this

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replacement corm formed at the base of a flowering shoot with the tunic removed to show three main big-sized axillary buds, which shall sprout in the following season. The smaller axillary buds at more basal positions in the corm (seen as small grey dots in the photograph) usually remain quiescent. (E) Longitudinal section of a corm formed at the base of a non-flowering shoot. The apical meristem has formed a protective sheath with several cataphylls. It shall become the apex of the replacement corm. Numerous vascular connections connect the mother corm and the replacement corm (arrows). (F) Longitudinal section of a replacement corm formed at the base of a flowering shoot. Flower formation resulted in the loss of the apical meristem of the shoot. Two axillary buds protected by a sheath of several cataphylls (arrows) have done a significant growth.
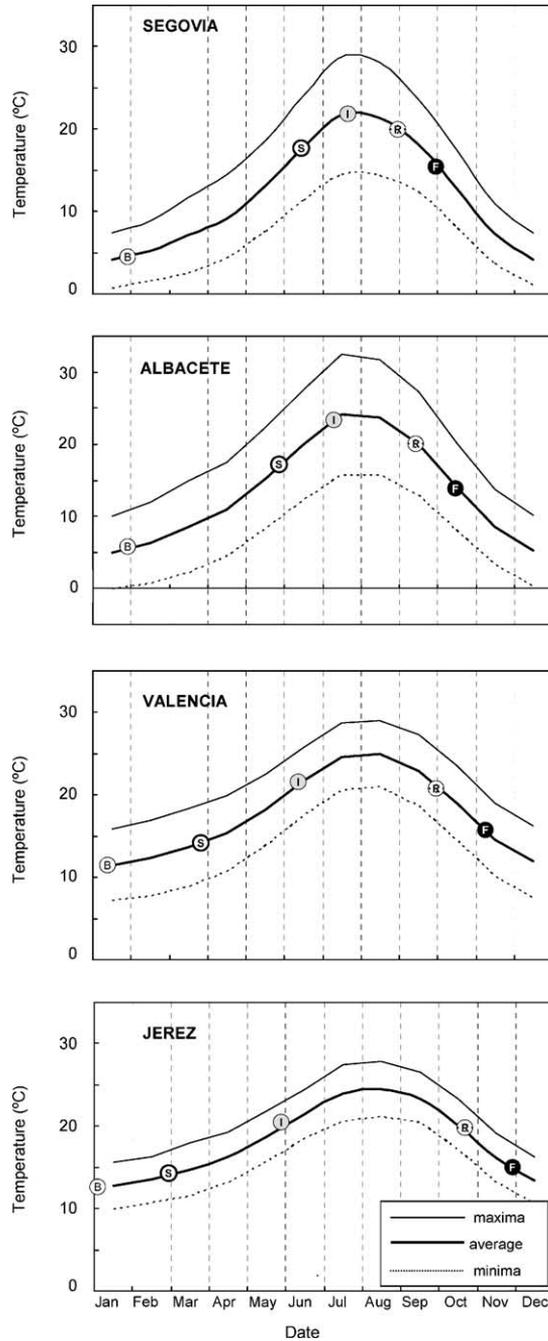
Fig. 2. Ontogenesis of *C. sativus* plants in four locations in Spain. Phenological data are mean from observations carried-out during three consecutive years on plantings started with a uniform batch of corms. Monthly maximum, average and minimum air temperatures (means for the period 1961–1990) were obtained from the Instituto
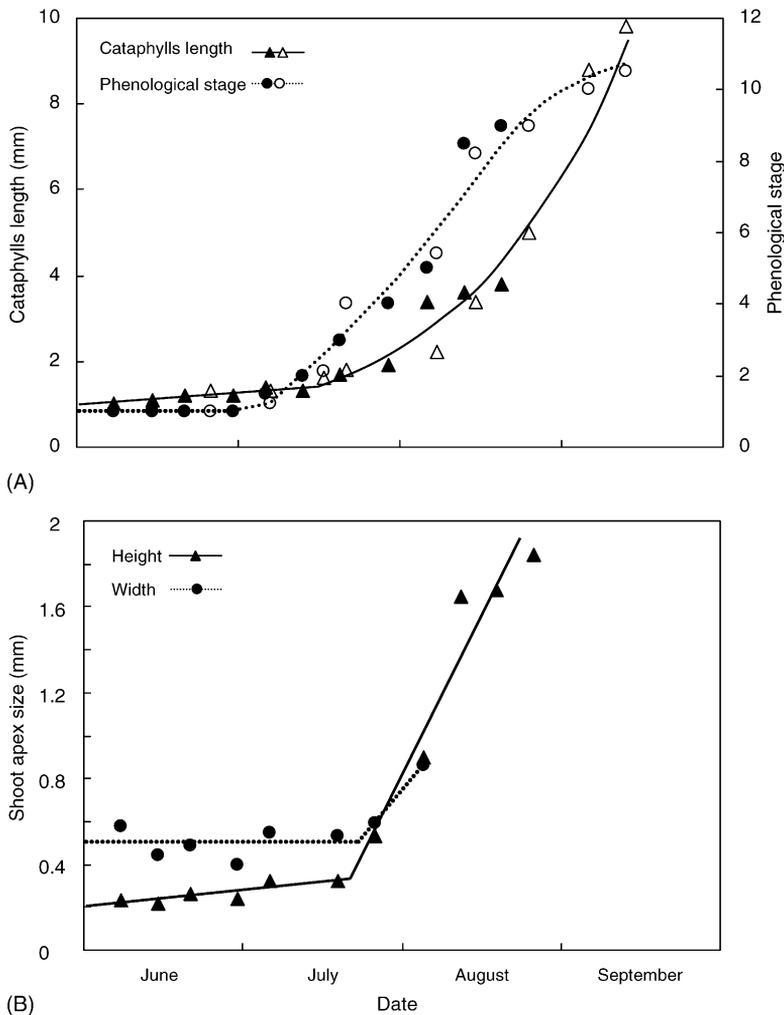
Fig. 3. (A) Apical bud growth and organogenesis in the apex during the summer in corms grown at Albacete, lifted on 23 June (23 days after leaf withering) and incubated under controlled conditions at 25 °C with 85% relative humidity (open symbols), and in corms grown at Quero (Toledo) and sampled periodically during summer (closed symbols). (B) Size of the shoot apex in the corms from Quero. For the measurements performed before August, the standard errors were smaller than symbols size; in later dates, smaller than 4% of the value of the parameter.

period had an intermediate duration (around 7 months, from early April to early November).

There seemed to be a close relationship between the air temperature and the timing of the phenological events (Fig. 2). The warmer winter climates of Jerez and Valencia resulted

Nacional de Metereología. The timing of flower differentiation was determined from the observation of the dominant bud from corms 30–40 mm diameter. *Symbols*: B, beginning of the formation of the replacement corm; S, senescence of the leaves; I, flower initiation in the dominant shoot apex; R, emergence of the caulogenic roots; F, anthesis.
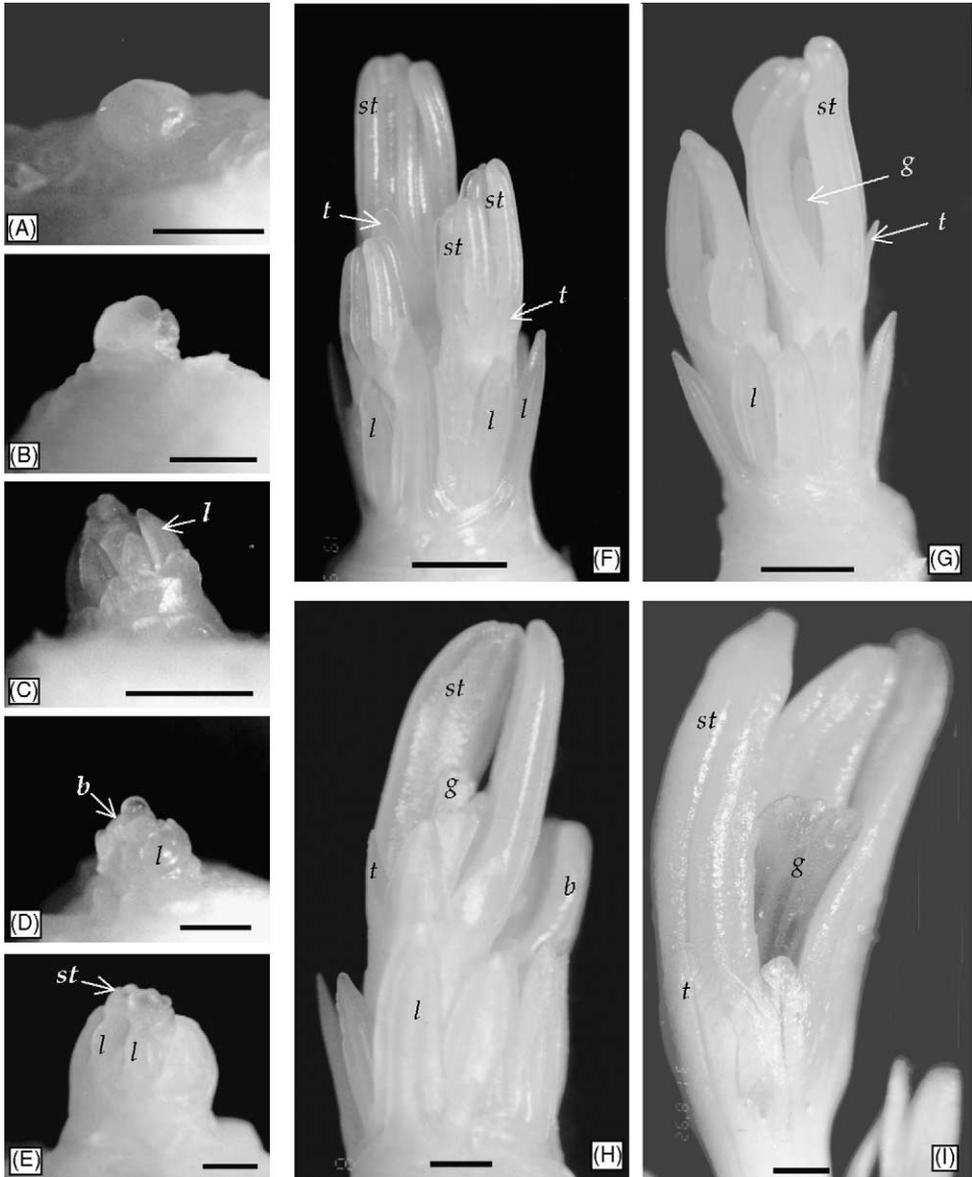
Fig. 4. Low magnification photographs of the naked shoot apex (having the sheath of cataphylls removed) at different developmental stages, and flower scores used in the present work. (A) Resting bud on 25 June. No lateral organs were initiated at this time (flower score, 1). (B) Dome-shaped shoot apex (13 July) with leaf primordia initiated at the flanks of the meristem (flower score, 2). (C) Shoot apex on 20 July. The base of the dome-shaped meristem was covered by the developing leaf primordia (flower score, 3). (D) Shoot apex on 25 July, at the time the bracts were initiated at the edge of the meristem (flower score, 4). (E) Stamen initiation (6 August; flower score, 6). (F) Bud with three developing flowers (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 on 20 August. The

in an early withering of the leaves and the shortening of the vegetative growth period. The earlier rise in temperature during early spring at these locations, accelerated flower initiation, but the high autumn temperatures delayed root formation and flowering. In all locations, flower differentiation occurred during late spring or early summer (early June to late July), when the mean air temperature rose to about 20 °C. Anthesis occurred in autumn, when the mean air temperature fell to 15–17 °C (Fig. 2). Flowering was always preceded by root formation.

3.3. Shoot growth and flower initiation

The time-course of shoot growth and flower differentiation in the corms during summer is presented in Fig. 3. The graphs show combined data from corms lifted after leaf senescence and incubated at 25 °C, and from corms kept in situ on the field at Quero, as no significant differences were observed in shoot development and the timing of morphogenesis. The numerical scale of flower differentiation we used was based on the stages of meristem development as defined by Beyer (1942). The main stages are illustrated in Figs. 4–6.

No change in the size of the bud (the length of the outermost cataphylls; Fig. 3A) or that of the naked shoot apex (without cataphylls; Fig. 3B) was observed from the time of corm lifting in early June to early July, some 40 days after leaf senescence. During this time, the buds made no growth and seemed to be dormant. No flower initials were present in the resting buds (flower score, 1; Figs. 4A, 5A and 6A). By early July, the apex become dome-shaped (flower score, 2; Figs. 4B and 6B). The increase in size of the apex was shortly followed by the formation of leaf primordia (flower score, 3; Figs. 4C, 5B and 6C). Shortly afterwards (16 July) the sheathing leaves started to grow at a faster rate than the shoot apex (Fig. 3A), piercing through the soil and protecting the growth of the young shoot and the scape. In succession followed the bract primordia stage (flower score, 4; Figs. 4D and 5C), the formation of stamen primordia (flower score, 6; Figs. 4E and 5D), the initiation of the perianth (flower score, 8; Figs. 4F, 5E and 6D) and the formation of gynoecium (flower score, 10 and higher; Figs. 4G and 6E). All the flower parts were already differentiated by the end of August (Fig. 4G and H). At this time, the sheathing cataphylls where 7–9 mm long (Fig. 3A).

The repeated observation of this sequence of flower morphogenesis in the same location during two consecutive years showed a difference in 12 days to attain the formation of the gynoecium stage.

3.4. Temperature requirements for flower formation

The influence of a constant temperature regime on flower formation was determined using corms lifted immediately after leaf withering (early June). These corms were

gynoecium was already initiated (arrow; flower score, 10). (H) Further growth of the flower parts, ahead of 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 (darker in the photograph). *Symbols*: b, bract; g, gynoecium; l, leaf; st, stamen; t, tepal. Scale bars, 0.5 mm.
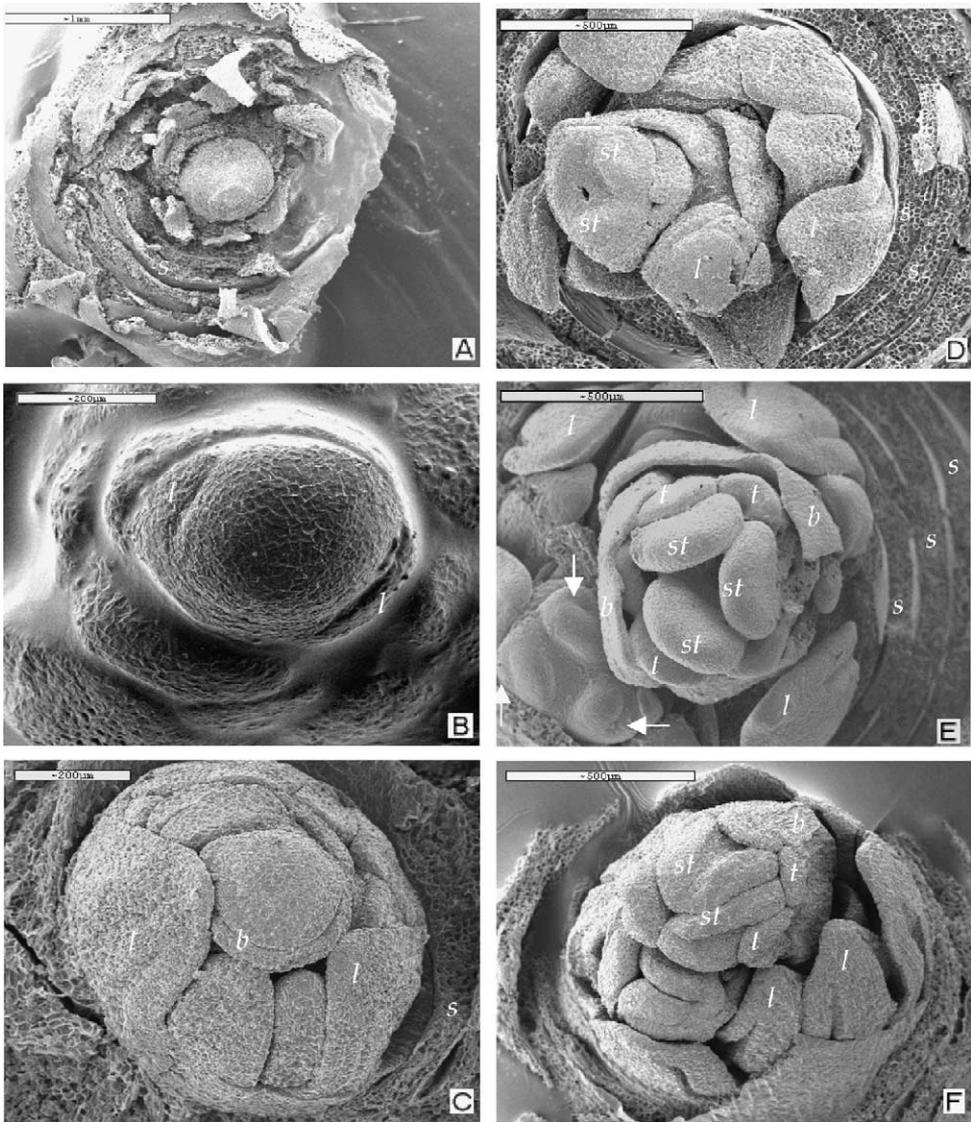
Fig. 5. Scanning electron microscope view of the naked shoot apex (with the sheath of cataphylls removed) showing the main stages of organ formation. (A) Resting bud (flower score, 1). (B) Leaf differentiation. The base of the leaf primordia almost encircled the shoot apex (flower score, 3). (C) Bract formation. The basal part of the shoot was protected by the tightly packed leaf primordia (flower score, 4). (D) Bud with the stamens being initiated (flower score, 6). (E) Bud with two developing flowers. In the oldest flower the six tepals were already differentiated (only five of them are shown in the picture as the sixth was removed during the preparation of the sample). In the younger flower, the first whorl of tepals was being initiated at the dorsal side of the stamen (arrows; flower score, 8). (F) Bud with two developing flowers. The bilobed stamens are easily distinguished from the arrowhead pointed leaves. At this stage, the gynoecium was being initiated (not seen in the picture; flower score, higher than 8). *Symbols*: s, cataphyll scar; other symbols as in Fig. 4.
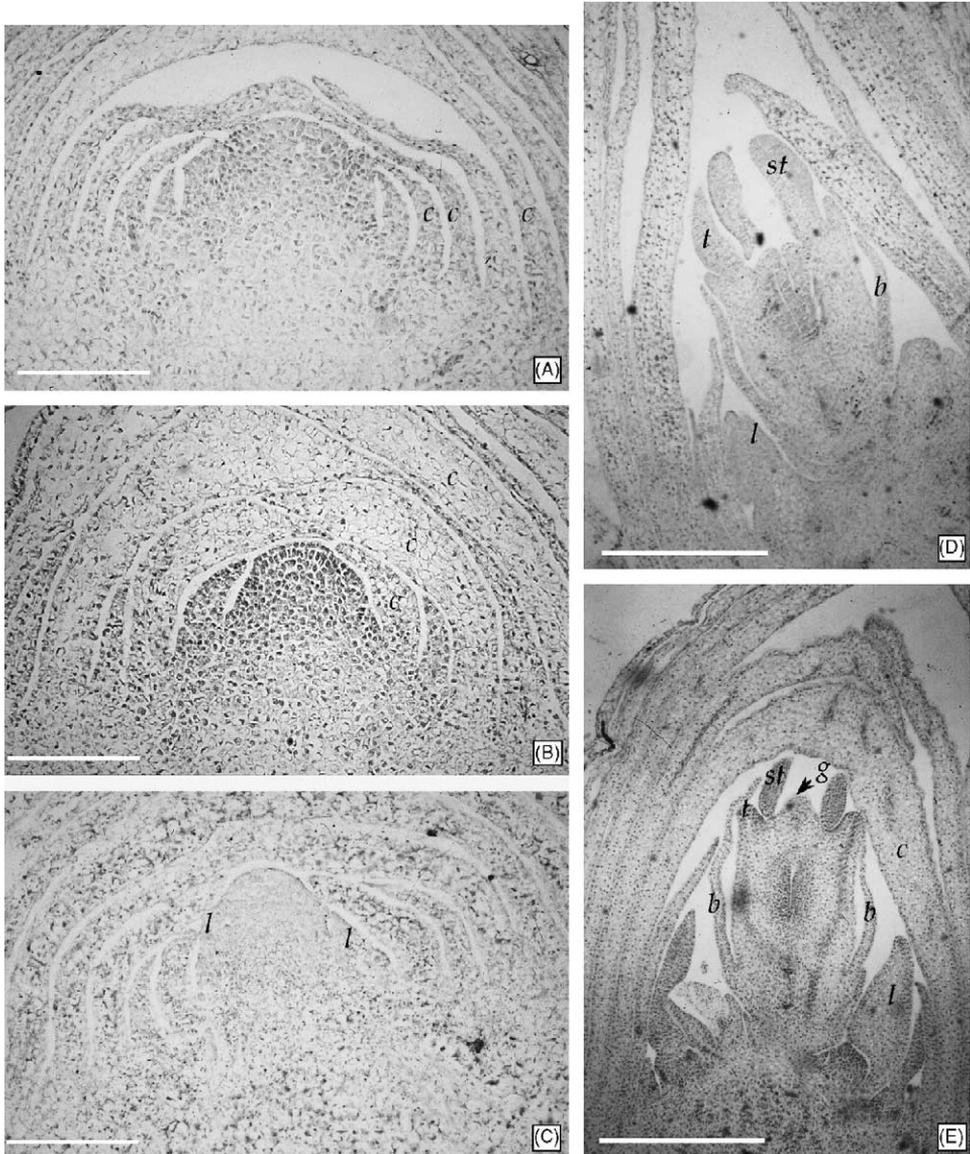
Fig. 6. Longitudinal sections of the shoot apex during the main stages of organ formation. (A) Sampled on 7 June. No lateral organs were differentiated. The apex was protected by several cataphylls (flower score, 1). (B) Sampled on 5 July. The shoot apex was dome-shaped, but no lateral organs were initiated (flower score, 2). (C) Sampled on 12 July. The shoot apex had increased in size and the leaves differentiated at the flank of it (flower score, 3). (D) Sampled on 11 August, with the tepals differentiated (flower score, 8). (E) Sampled on 20 August, showing the initial stages of gynoecium formation (flower score, 10). *Symbols*: as in Figs. 4 and 5. Scale bar: 0.5 mm.
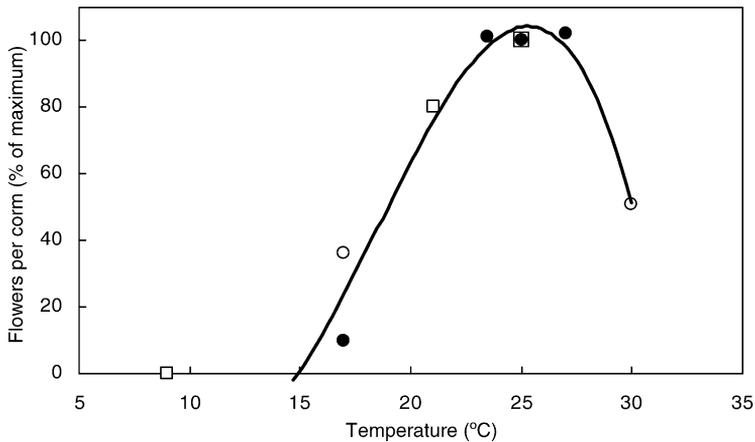
Fig. 7. The influence of incubation temperature on flower formation. The corms were incubated for 100–130 days at the temperature indicated before being transferred to 17 °C for flower emergence. Number of flowers expressed as a percentage of the flowers formed at 25 °C. Data from three separate experiments (indicated with different symbols). The incubation at 25 °C was performed in the three experiments. The number of flowers formed per corm at this temperature ranged from 2.4 to 2.8. The standard error was less than 0.12 flowers per corm (5% in the units of the graph).

incubated at the desired temperature (in the range 9–30 °C) for 100–130 days, and then forced to sprout at 17 °C. This temperature is close to the optimum for flower emergence as determined in prior experiments (evidence not presented). The results from three separate experiments carried-out in different years are presented in Fig. 7. The number of flowers formed was maximal for corms incubated at 23–27 °C. With the experimental material we used, the corms incubated at any temperature within this range for 90–150 days, formed between two and three flowers depending on the experiment. The growth of the shoot apex and floral morphogenesis were slightly faster, and anthesis occurred earlier, when the incubation was performed at the lowest of these temperatures (23 °C; Table 1). The corms held at this temperature also flowered slightly but significantly earlier. Within this range of temperature, there was no significant effect of the temperature of incubation either in flower number or in saffron production (Table 1).

Incubation at a temperature outside the range of 23–27 °C resulted in a reduction in flower number per corm (Fig. 7). This reduction was more marked at a higher (30 °C) than at a lower temperature (21 °C). At 21 °C, the number of flowers formed was close to that recorded at the optimal temperatures. A further reduction in the temperature of incubation resulted in a marked reduction in flower formation. No flowers formed in the corms incubated at 9 °C. A variable proportion (20–100%, depending of the experiment) of the corms incubated directly at 17 °C after lifting formed one single flower. This might indicate a marginal (and variable) effect of this temperature on flower formation. All the batches of corms used in our experiments were inspected under the dissecting microscope before incubation and we found no evidence of flower initiation at the time the incubation was started (the initial flowering score was invariably 1).

Table 1
The influence of incubation temperature on shoot growth and flowering[a]

Parameter	Temperature of incubation		
	23 °C	25 °C	27 °C
Shoot apex length (mm)[b]	16.9 a	8.8 b	6.3 c
Flower stage[b]	8.9 a	8.0 b	7.0 c
Days to flower[c]	123 a	128 b	141 c
Flowers per corm	2.2	2.2	2.3
Saffron per flower (mg)	10.3	10.2	10.3

[a] Values in the same row with different letters differ significantly at $P \leq 0.05$.
[b] Measured after 77 days incubation.
[c] For corms incubated for 102 days at the temperature indicated prior to forcing at 17 °C.

The effect of incubation duration on flowering was investigated for corms incubated either at 25 or 30 °C (Fig. 8). Flower formation was higher ($P \leq 0.01$) at 25 °C than at 30 °C (Fig. 8). The maximum number of flowers was obtained al 25 °C with an incubation lasting from 90 to 150 days (Fig. 8). A 180-day-long incubation resulted in flower abortion. Incubation at 30 °C only resulted in flower formation when it lasted from 90 to 120 days (Fig. 8). A longer incubation at 30 °C suppressed flower formation.

The visual inspection of the growing shoots after incubation and prior to flower forcing at 17 °C demonstrated that the smaller number of flowers formed in the corms incubated at 30 °C resulted from the combination of two factors; the smaller number of flowers initiated at 30 °C (as compared to corms incubated at 25 °C) and the abortion of some of these
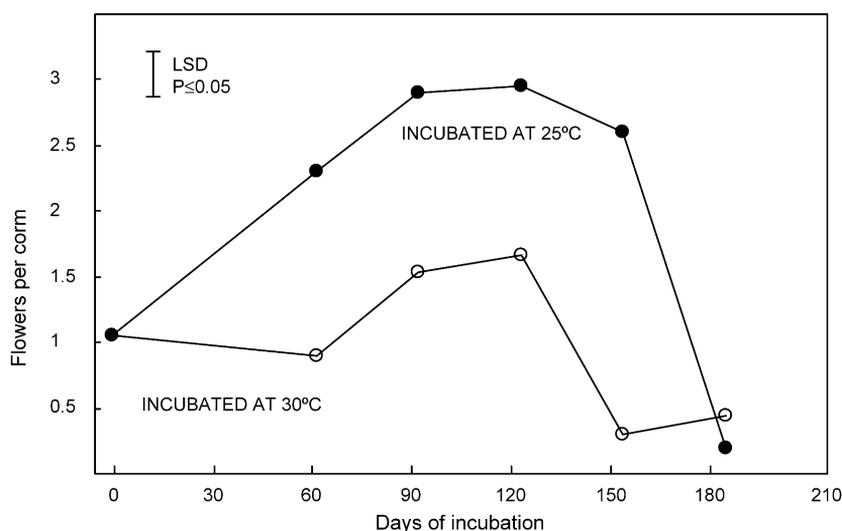

Fig. 8. The influence of the duration of incubation at 25 °C (closed signs) and 30 °C (open signs) on flower formation in corms lifted on 9 June (flowering score, 1) immediately after leaf withering. The controlled experimental conditions were set-up on 15 June. After the time of incubation indicated, the corms were forced to flower at 17 °C.

Table 2
Number of flowers formed per corm as determined from the visual inspection of the shoots at the end of the incubation at high temperature (25 or 30 °C)

Temperatures of incubation and parameter	Duration of incubation				
	59	91	115	150	178
Incubated at 25 °C					
Flowers formed	2.2	2.7	2.6	2.3	2.4
Flowers aborted	−0.1 ns	−0.2 ns	−0.3 ns	−0.3 ns	2.2[**]
Incubated at 30 °C					
Flowers formed	–	2.0	2.0	1.7	1.8
Flowers aborted	–	0.5[*]	0.4[*]	1.4[**]	1.4[**]

The number of flowers aborted is the difference to the number of flowers reaching anthesis after forcing the corms at 17 °C.
ns: the difference to zero was not significant.
[*] Significant at $P \leq 0.05$.
[**] Significant at $P \leq 0.01$.

flowers (Table 2). No abortion of flowers occurred in corms incubated at 25 °C unless the period of incubation extended beyond 150 days (Table 2). In these corms, the number of flowers counted at the end of incubation did not differ significantly from the number of flowers reaching anthesis (the difference between these two counts, which is the number of flowers aborted, did not differ from zero).

3.5. The effect of a post-incubation at 21 °C

Vigorous root growth occurred when the corms were transferred to the low temperature conditions (17 °C) for flower emergence. This root growth was caused by the combination of the lowering in temperature and of the watering of the corms. Root formation also occurred in non-watered corms after being covered with the arlite layer, but root growth was much weaker. The aims of this experiment, in which a short incubation at an intermediate temperature (21 °C) was performed before forcing the corms at 17 °C, were to determine the influence of this intermediate temperature on root growth and its effect on flower formation. The first of the goals could not be accomplished. Root growth was apparently more vigorous at 21 °C than at 17 °C. However, at both temperatures the vigorously growing roots penetrated the rock wool and made the quantitative measurements impracticable.

The effect of this intermediate temperature (21 °C) incubation on flower formation and characteristics in corms incubated for a different length of time at 25 °C is presented in Table 3. As expected from the results presented above, the duration of the incubation at 25 °C in the range of 77–104 days used in this experiment had only a marginal effect on flower number. Even so, the number of flowers formed per corm was slightly higher ($P \leq 0.01$) with 104 days incubation (2.4 flowers per corm for the mean of the three durations of incubation at 21 °C) than with 77 days (2.1 flowers per corm). The incubation at 21 °C had no effect either in flower number or in the time of flowering, but significantly increased flower size (saffron yield). This effect was already

Table 3
The influence of a post-incubation at 21 °C on flower formation and saffron yield in corms incubated at 25 °C for 77 and 104 days

Days of incubation		Flowers per corm	Saffron per flower (mg)	Days to flower (from 22 June)
25 °C	21 °C			
77	0	2.3	9.9	120
77	12	2.0	10.6	120
77	18	2.0	10.3	120
		ns	$P \leq 0.10$	ns
104	0	2.5	10.2 a	129
104	6	2.2	11.6 b	129
104	16	2.5	11.5 b	130
		ns	$P \leq 0.05$	ns

Corms lifted on 5 June (phenological stage of the apex, 1). Controlled conditions of incubation started on 22 June.

maximal after 6 days of incubation. It was more marked in the corms incubated at 25 °C for a longer time (Table 3).

3.6. The influence of the time of corm lifting

The corms lifted before 20 July formed few flowers when forced directly at 17 °C. The number of flowers formed with a direct forcing at 17 °C increased slightly but significantly at later dates, as the corms were lifted at a more advanced stage of flower differentiation (Fig. 9). The number of flowers per corm was close to one in the corms lifted on 15 August,
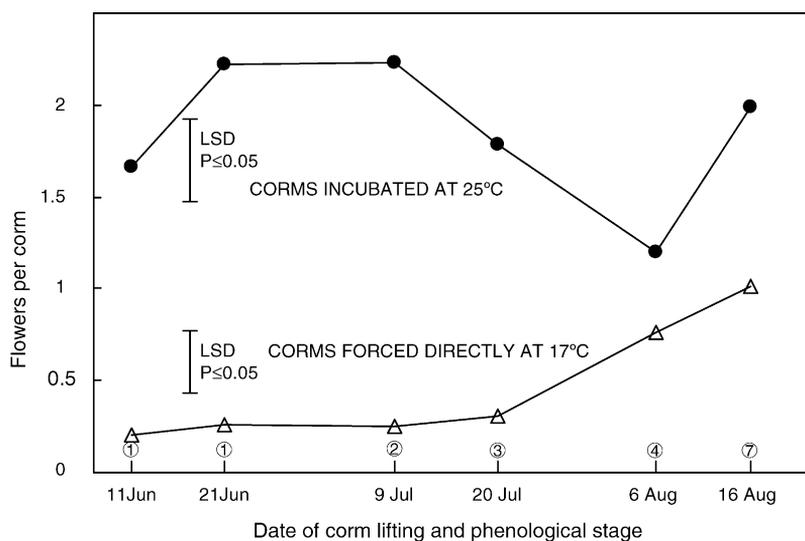

Fig. 9. The influence of incubation at 25 °C on flower formation in corms lifted at different stages of morphogenesis. The corms were lifted at the dates indicated in the figure (flower score at the time of corm lifting shown encircled), and forced to flower either directly at 17 °C (open triangles) or after being kept at 25 °C until 10 September (filled circles).

around 46 days after leaf senescence. At this date, the apical bud of the corms had reached the stage of stamen formation (flower score, 7; Fig. 9). The number of flowers formed increased markedly when the lifted corms were incubated at 25 °C until 10 September. All the corms incubated in this way were therefore kept at a high temperature for 103 days after the withering of the leaves and before flower formation. This figure results from adding to the time of incubation at 25 °C in the laboratory the time the corms were kept in the field under the uncontrolled but hot June and July conditions. Flower formation was significantly less in the corms lifted from 20 July to 6 August than in those lifted at earlier and later dates. During this period of time (20 July to 6 August) the bracts and the stamens were being initiated in the corms (Fig. 4).

4. Discussion

At the time of leaf withering, which occurred during late spring or early summer after the winter vegetative growth period, the meristems of the replacement corms were vegetative as ascertained by the morphological observations (Figs. 4–6). Flower initiation occurred during summer, as reported to occur in several spring-flowering bulbous plants, for example, *Crocus vernus* and *Crocus flavus* (Wilkins, 1985), *Hyacinthus*, *Iris* and *Tulipa* (Hartsema, 1961; Le Nard and De Hertog, 1993). The optimum temperature for flower initiation and development was in the range of 23–27 °C (Fig. 7). At variance with the above-mentioned spring-flowering species, which require a warm–cold–warm temperature sequence to flower (Wilkins, 1985), *C. sativus* flowered best in a warm–intermediate temperature sequence. The transfer of the corms after flower initiation at a temperature lower than 15–17 °C resulted in a reduction in flower formation (Molina et al., in press).

At the optimal temperature for shoot growth and flower development (23–27 °C; Fig. 7), apical bud growth started about 40 days after the withering of the leaves (Fig. 3). Flower formation followed shortly afterwards, and the first stages of flower initiation were already discernible by early August (Figs. 4–6). The release of bud dormancy, and hence the time needed for flower formation, could be accelerated by curing the *Crocus* corms at 30 °C for a short time (Molina et al., in press). A similar response was reported for the bulbs of *Tulipa* (De Hertog et al., 1983), *Freesia* (Imanishi, 1993), *Hyacinthus*, *Iris* and *Muscari* (Le Nard and De Hertog, 1993). While 20 days incubation at 30 °C was effective to release bud dormancy, a longer incubation at this temperature (60 days) resulted in a reduction both in flower initiation and in flower development (Figs. 7 and 8). Also, an incubation longer than 150 days at the optimal temperature range (23–27 °C) for flower initiation resulted in the abortion of the flowers (Fig. 8 and Table 2). Optimal flower formation required incubation for more than 60 days at the temperature range mentioned earlier (Fig. 8), followed by the transfer of the initiated meristems to a lower temperature. After 60 days of incubation, the meristems had formed the bract and in some of them the stamen could be discernible (Figs. 4 and 5). This stage is somewhat earlier than the perianth formation stage recommended for the low temperature forcing of the spring-flowering *Crocus* (Benschop, 1993). The optimal temperature for flower emergence was in the range 15–17 °C (evidence not presented; Plessner et al., 1989).

While the experiments discussed earlier demonstrated that a sufficiently long incubation at high temperature (23–27 °C) was needed to ensure the formation of a maximum number of flowers, the flowering behaviour of corms lifted at different times showed that additional factors were also affecting flower formation. The behaviour of the corms lifted during the bract and the stamen differentiation stages, which formed markedly less flowers ($P \leq 0.01$) than corms lifted either during earlier or later stages (Fig. 9) supports the saffron growers believe that disturbing the corms during flower initiation interferes with flower formation. The most obvious explanation for this behaviour, a putative stress resulting from the bright summer sunlight heating the lifted corms, may not be the reason. We lifted the corms before sunrise, and kept them protected under shelter during manipulation.

Incubation at an intermediate temperature prior to cool storage has been demonstrated to increase flower quality in *Tulipa* and other forced bulbous plants (Hartsema, 1961). In *C. sativus*, the incubation of the watered bulbs at 21 °C prior to forcing at 17 °C resulted in a significant increase in flower size (Table 3). Since this incubation enhanced root growth (quantitative data not available), we may speculate that root-produced cytokinins may have increased flower growth. Azizbekova et al. (1978) reported an effect of exogenously applied kinetin on shoot and flower growth.

Bulb and corm size is a major factor to determine the capacity of bulbous plants to flower (Le Nard and De Hertog, 1993). A tight relationship has been demonstrated between corm size and flowering in *C. sativus* (Negbi et al., 1989; De Mastro and Ruta, 1993). Negbi (1999) speculated that differences in corm size could be responsible for the wide differences reported in the time of flower initiation and flowering in different places (Milyaeva and Azizbekova, 1978; Koul and Farooq, 1984; Greenberg-Kaslasi, 1991). The wide differences we found at different locations both in the timing of flower initiation and in the time of flowering in plantings arising from a uniform batch of corms (Fig. 2), pointed-out to climatic factors as the main cause of these differences. The flowers were initiated as the mean air temperature increased during late spring above 20 °C (Fig. 2). The shoots remained underground during the hot summer, to pierce the soil and flower when the temperature fell below 15 °C. Hence, the warmer the climate during the winter months, the earlier was flower initiation and the later the time of flowering. The time-course for flower initiation was similar for corms kept in the orchard at Albacete and Quero and those incubated at the laboratory at the optimum temperature as determined in this study (Fig. 3). This demonstrated that the natural weather conditions during early summer in the main saffron producing area of Spain (Castilla-La Mancha) were close to optimal for flower initiation. Further, the vegetative growth of the plant lasted from mid-October to the end of May. This long period of photosynthetic activity (over 7 months as compared to 3–4 months in warmer climates) allowed the complete development of the replacement corms.

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