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TEMPERATURE EFFECTS ON CORM GERMINATION AND FLOWERING OF *CROCUS SATIVUS* L. (SAFFRON)

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ABSTRACT

Climate change has hindered *Crocus sativus* production since its early phases of development are vulnerable to temperature stress. Indoor cultivation of *C. sativus* in Malaysia is strongly encouraged as it provides optimum plant growth without being impacted by unfavourable weather or geographical limitation. The present study aims to investigate the effects of temperatures on corm germination and flowering of *Crocus sativus* in a controlled environment. Saffron, a spice derived from the flower of *C. sativus* is the world's most expensive spice and is native to the Mediterranean region. Three different temperatures (10°C, 23°C and 30°C) were tested for corm germination while the flowering process was tested in the temperature of 16°C, 23°C or 30°C using a randomized complete block design (RCBD). The results showed that *C. sativus* required a specific temperature setting and developed best in a sequence of high temperature during corm germination to a lower temperature during flower initiation. The optimum temperature for flower formation was 16°C provided that the corms were germinated at a higher temperature in the range of 23°C to 30°C. The results provide valuable information for the cultivation of *C. sativus* as a new prospect for Malaysia's economy, considering its high commercial and medicinal value.

Keywords: Corm, *Crocus sativus*, Malaysia, Saffron, Temperature

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INTRODUCTION

Crocus sativus L., commonly known as saffron crocus is among the most popular autumn-flowering *Crocus* that belongs to the Iridaceae family (Mathew, 1999). The plant is native to the Mediterranean region where it evolved in Attica, Greece (Nemati *et al*., 2019). It has been well adapted in areas characterized by cool to cold winters with autumn–winter–spring precipitation and warm summers with very little rainfall (Saxena, 2010). As geophyte, this species survives the summer drought below ground by its compact corm. Saffron, a spice produced from the dried stigmas of the *C. sativus* flower, is the world's most expensive spice by weight for decades. In 2022, the export value of saffron in the world was USD 188.592 million, led by Iran as the top-ranking exporter (ITC, 2022).

Saffron has been used worldwide for many purposes, not only as spice or flavour in food preparation but also as a food additive in the food industry and as a natural dye in the textile industry. It is also used as a food supplement as a source of natural polyphenols and incorporated in plant fertilizers from organic matter in the soil (Ahmed *et al*., 2020). Saffron is rich in carotenoid as its active compound, wherein crocin is the major component besides picrocrocin and zeaxanthin (Anuar, 2017). In modern medicine, saffron has been reported to have neuroprotective effects against cerebral ischemia stroke, besides having potent anti-cancer, anti-oxidant, anti-inflammatory, and anti-apoptotic properties (Lambrianidou *et al*., 2020; Azami *et al*., 2021). Recent research showed that crocin in saffron can potentially treat Alzheimer's disease (Bharate, 2018). Hence, it is worth developing saffron into a botanical drug as it showed promising medicinal benefits.

Despite its high commercial and medicinal value, the production of this spice is not steadily stable due to several issues. One of the constraints facing saffron cultivation is the global climatic change that has led to insufficient and irregular rainfall particularly in Kashmir, resulting in adverse effects on saffron cultivation. Farmers rely on rainfall for a good flush of blooms in September, which is the critical stage of normal flowering. However, due to the erratic weather, the delayed rainfall in late October caused flower abortion (Dass *et al.*, 2017). Plant disease such as corm rot caused by soil-borne microorganism also derails saffron production (Gupta *et al*., 2021).

In the temperate environment, seasonal behaviour or annual changes in temperature is important to control plant phenology such as flowering in numerous species, even when day length is controlled (Chen *et al*., 2014). In a review by Khodorova and Boitel-Conti (2013), they stated that seasonal thermoperiodicity which is the response of plants to alternation of a warm and cool period is more important compared to the effects of light on flower induction in most geophytes. A too-low temperature also could be detrimental to the plants. Previous trials to cultivate saffron in New Zealand reported that the flower did not open on frosty or wet days which resulted in quality deterioration of the stigmas (McGimpsey *et al*., 1997). Cultivation of this species has not only been enhanced in their native countries but has also been expanded out of its native climate to countries such as Australia, New Zealand, Saudi Arabia, and even the Southern Hemisphere of Netherlands (Sharaf-Eldin *et al*., 2013; Rotteveel, 2017).

Due to the commercial value of saffron and increasing interest in this species worldwide, there is a great potential for the introduction of *C. sativus* cultivation in Malaysia. As Malaysia experiences a tropical climate, the objective of the present study was to determine the effects of temperatures as a vital factor for corm germination and optimal flowering of this plant.

MATERIALS AND METHODS

Sample Preparation: Corms of *C. sativus* were purchased from Echilleuses, France, and received in their dormant state. The corms were germinated and grown at the Institute of Biological Sciences Laboratory, Faculty of Science, University of Malaya. The corms were graded into the desired size of about 3 to 4 cm in diameter to maintain uniformity as well as to avoid the influence of corm sizes. This experiment was carried out in two steps; the first was to study the effects of temperatures on corm germination, and the second was to study the effects of temperatures on flowering. Every step was repeated twice.

Effects of Temperatures on Corm Germination: For the first experiment, three different temperature treatments; 10°C, 23°C and 30°C ± 1°C (ambient temperature) as control were applied to determine the temperature requirement for dormancy break and germination. The dormant corms were subjected to 10°C to find the lower limit for corm germination. Moreover, a lower temperature than 10°C was unfavourable as *C. sativus* did not require cold storage (Molina *et al*., 2005). A temperature of 23°C was tested as it resembled the temperature in late summer where the dormant phase usually ended (Husaini *et al*., 2010) while 30°C was tested to investigate its adaption to the tropical ambient temperature.

Three sets of corms (135 corms in total) were placed separately in the laboratory growth chamber at 10°C, in a temperature-controlled room at 23°C, and in the laboratory room at the ambient temperature of 30°C ± 1°C. All corms were put in soilless, dark condition, with relative humidity in the range of 60 to 75% ± 5% RH (Digital Thermo-Hygrometer HTC-1). The dormant corms were observed weekly for eight weeks for any external morphological changes, with the dormancy considered 'released' when the sprout emerged. The corms were sprayed with a little distilled water every week to prevent them from drying.

Effects of Temperatures on Flowering: The following experiment was to study the effects of germination temperatures on the subsequent flowering response. The sprouted corms from the first experiment were maintained in or transferred to a temperature of 16°C, 23°C and 30°C ± 1°C (ambient temperature) as the control. They were sown at approximately 4 cm depth in transparent plastic containers filled with commercial garden soil.

Specifically, corms previously placed at 10°C (very low temperature) for germination were selected randomly to be transferred to 16°C, 23°C, and 30°C. Corms previously placed at 23°C (moderate temperature) for germination were transferred to 16°C and 30°C while some were maintained at 23°C for another two months. On the other hand, corms previously placed at 30°C (high temperature) for germination were transferred to 16°C and 23°C while some were maintained at 30°C as the control for another two months. The condition of the temperature-controlled rooms was 16 hours photoperiod with 1000 lux of light intensity and relative humidity in the range of 60 to 75% ± 5% RH. Since *C. sativus* is a Mediterranean plant where temperature differs from the tropical climate, therefore these temperatures almost resembled the temperature in autumn (16°C) and summer (23°C – 30°C). The plants were watered every week and their growth was observed every day for two months.

Experimental Design: As this experiment was repeated twice on different months and the corms were from different batches as well, therefore, a randomized complete block design (RCBD) was applied. Blocking is used to minimize the nuisance factors that might influence the response of the independent variables. In this case, anticipating that planting date and corm batches may affect the results was not an interest in this scope of study, thus, the data had two blocks.

For the first experiment (corm germination), each block consisted of three treatments (10°C, 23°C, and 30°C) with forty-five corms for each treatment. For the second experiment (flowering), each block consisted of nine treatments (3 levels of germination temperatures x 3 levels of flowering temperatures) with fifteen germinated corms for each treatment. Data were analysed using a

two-way analysis of variance for RCBD using IBM SPSS (version 22) statistical software. Comparison of the specific means was calculated using Duncan's Multiple Range Test at *p* value less than 0.05.

Data Collection: For the first experiment (corm germination), data were collected as average per corm for the height of apical shoot, the height of lateral shoot, and the number of lateral shoots. For the second experiment (flowering), data were collected as average per plant for days to flower initiation, days to flower blooming, and the number of flowers. Days to flower initiation were counted from the day of sowing the germinated corms in the soil to the emergence of the floral buds, whereas, days to flower blooming were counted from the day of sowing the germinated corms until the floral buds started to open. The number of flowers was counted for each plant regard less of whether the flower was fully bloomed or not.

RESULTS AND DISCUSSION

Effects of Temperatures on Corm Germination: The study of the effects of temperatures on corm germination started with the corms in the dormancy state, apparently showing no visible external growth. The responses of the dormant corms to different germination temperatures after eight weeks showed that the maximum height of apical shoots was at 23°C (3.30 ± 0.35cm) while the height of lateral shoots ranged from 0.17 ± 0.02 cm at 30°C to 2.04 ± 0.21 cm at 10°C. The results also showed that the number of lateral shoots was maximum at 10°C which was 9.22 ± 0.97 cm and the lowest number of lateral shoots was at 30°C which was 1.96 ± 0.21 cm (Table 1). Figure 1 depicted a marked difference in the growth of apical and lateral shoots of the corms stored at different germination temperatures.

Table 1. The effects of temperatures on *C. sativus* corms after eight weeks during corm germination.

Temperature (°C)	Apical shoot height (cm)	Lateral shoot height (cm)	Lateral shoot numbers
10	$2.28 \pm 0.05_b$	$2.04 \pm 0.21_a$	$9.22 \pm 0.97_a$
23	$3.30 \pm 0.35_a$	$1.30 \pm 0.14_b$	$5.43 \pm 0.57_b$
30	$0.44 \pm 0.05_c$	$0.17 \pm 0.02_c$	$1.96 \pm 0.21_c$

Means within the same column followed by different letters are significantly different at $p<0.05$ (Duncan's Multiple Range Test). Data are presented as mean ± SE, n = 45.

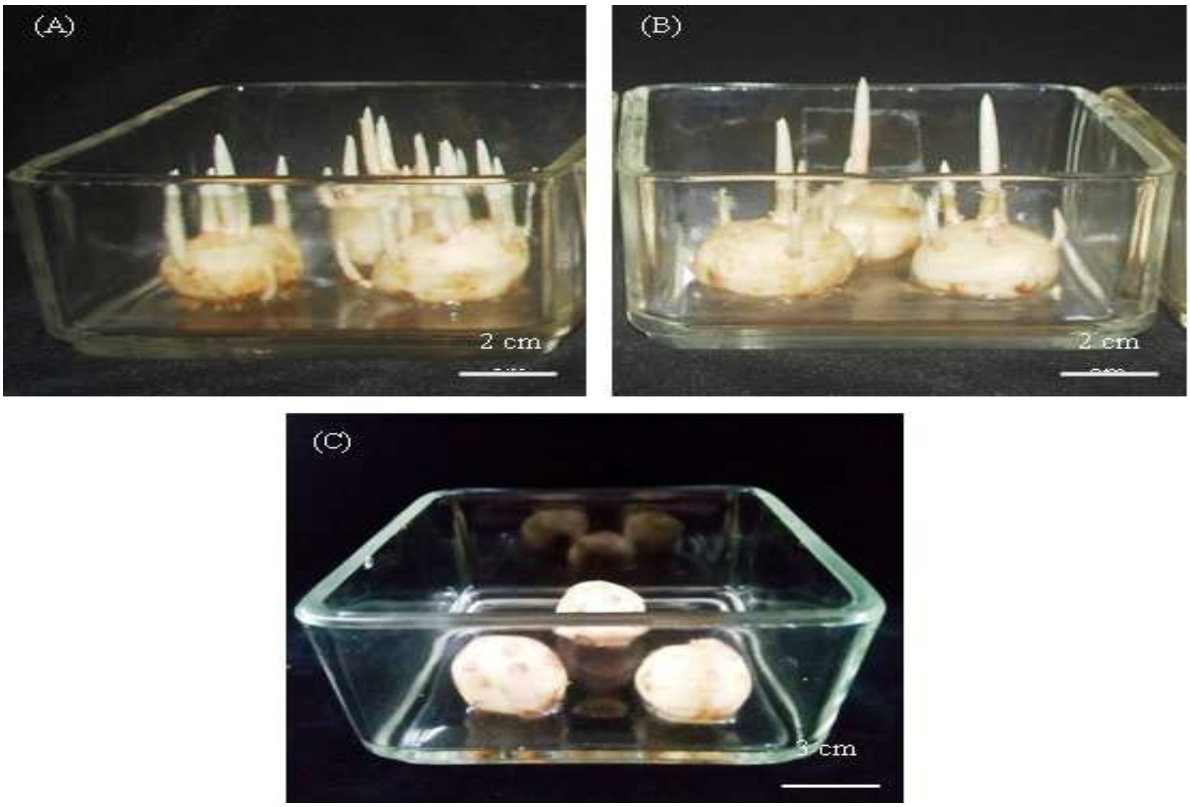

Figure1. Different morphological responses of *C. sativus* corms after eight weeks of storage at different temperatures of (a) 10°C, (b) 23°C, and (c) 30°C during the phase of corm germination.

Effect of Temperatures on Flowering: The germination temperatures used to germinate *C. sativus* corms were seen to significantly affect the flowering responses later on, as shown in Table 2. The results showed that there was no flower bud formation by corms initially germinated at10°C following transfer to a slightly higher temperature of 16°C (Figure 2A) and even higher temperature of 23°C and 30°C. Even though the lowest temperature (10°C) used in this study increased the chances of dormancy breaking as previously indicated in Table 1, the finding revealed that exposure to very low temperature was destructive to the flower formation later on. This finding was in line with Molina *et al*. (2005) who also found that too low temperature such as 9°C at the step of dormancy breaking resulted in no flower formation in *C. sativus*.

To a certain extent, frost during the period of flowering could be detrimental as the flowers were unable to bloom and the stigmas shrivelled, which in turn reduced saffron productivity (McGimpsey *et al*., 1997). According to Mzabri *et al*. (2021), cold storage of the *C. sativus* corms at 4°C for 7 and 14 days which were even shorter than the storage duration (8 weeks) applied in the present study, had harmed the flower and stigmas production. The cold period before growth at 10°C or 17°C also delayed the flowering in other crocuses such as *C. nevadensis* and *C. vernus* (Pastor-Ferriz *et al*., 2021). Morever, Zhao *et al.* (2022) stated that *C. sativus* corms did not require cold storage as mostly found in geophytes. Therefore, the present study suggested that incubation at a temperature of 10°C and below should be avoided in breaking the corm dormancy of *C. sativus* as it will inhibit flower development.

On the other hand, corms previously germinated at 23°C had faster flower initiation when transferred to a lower temperature of 16°C, which the plants only took 17.03 ± 0.13 days for the flower bud to be discernible from the moment they were sown (Table 2). The flowers also bloomed rapidly (18.20 ± 0.13 days), taking roughly a day after the flower bud appeared and the plants produced the highest number of flowers (2.87 ± 0.20 flowers) (Table 2 and Figure 2B). In this condition, the stigmas can be harvested in just less than three weeks after sowing. This temperature sequence is relevant as it almost simulates the summer-autumn temperatures in its natural conditions.

In contrast, the plants took a long time (37.63 ± 0.19 days) to initiate flowers when the temperatures for germination and flowering were retained the same at 23°C. In other words, prolonged temperature treatment at 23°C had delayed the flower initiation for almost three weeks compared to just 17.03 ± 0.13 days at 16°C (Table 2). The flowers were also retarded and did not fully bloom as depicted in Figure 3. This was probably because corm germination and flowering were two distinct growth phases which required different temperature sensitivity to activate the cell metabolism and triggered the specific gene activity in the plant organ (Ingram and Abrol, 1996).

Corms that were initially germinated at an ambient temperature of 30°C, also managed to initiate flowers after transferring to lower temperatures even though the earlier germination responses in terms of apical and lateral shoot formation were not so apparent. At a lower flowering temperature of 16°C, flower initiation was faster (41.93 ± 0.24 days) and more flowers were produced (2.50 ± 0.12 flowers) compared to the flowering temperature of 23°C (Table 2 and Figure 2C). Even though the plants showed asynchronous flower blooming but no flower abortion was observed (Figure 2C). Besides, no flower abortion was observed at both flowering temperatures. However, this finding contradicts the results obtained by Molina *et al.* (2005) who found that flower abortion occurred in corms initially incubated at 30°C upon being forced to flower at 17°C (flowering temperature used by the authors). A possible explanation of these differences is most likely due to a long incubation period applied by the authors in which the corms had been incubated for 91 to 178 days before being forced to flower, compared to only 56 days (8 weeks) applied in the present study.

Therefore, this study suggested that a shorter incubation period of 56 days at 30°C during the dormancy breaking is sufficient to promote flowering in *C. sativus* provided that the flowering temperature is within the optimal range. According to Paradiso and Pascale (2014), plant sensitivity to the inductive temperature during flower induction does not only depend on the plant's age and size but is also influenced by the duration and level of temperature in the vegetative period.

The results also showed at a flowering temperature of 30°C, no flower was formed although leaves had appeared, indicating that high temperature during the flowering period is damaging for flower morphogenesis, regardless of any initial germination temperature subjected (Table 2). The finding was comparable to that of Shahnawaz *et al.* (2017) who found that at a higher temperature of 45°C, the corms failed to achieve their flowering stage even though they exhibited normal vegetative growth.

Nevertheless, the results in the present study did not inclusively mean that the high temperature of 30°C was unsuitable. From another point of view, a higher temperature is necessary to hold the dormancy state of the corms for a longer period as it can overcome the seasonal limitation of this species. This also benefited the farmers especially when a large volume of corms, need to be handled at one time. Thus, efficient post-harvest storage of the planting materials can be achieved. In another experiment done by Saeidirad and Zarifneshat (2019), among three temperatures of 1°C, 10°C, and

25°C, the highest temperature of 25°C was the most appropriate for storing the *C. sativus* corms.

The findings also demonstrated that the variation in the flowering performance was affected by the germination temperature, indicating that there is a threshold of temperature tolerance and it changes throughout the plant developmental stages. The optimal temperature requirement for germination varies between plant species. In the rhizome of *Polygonatum kingianum*, a lower temperature of 2.97°C was considered to be the optimum temperature for bud dormancy release and 11.54°C was the upper limit for that event (Wang *et al*., 2020). In grapevine, the effective temperature for the dormancy release and bud break was even varied between cultivars (Camargo-Alvarez *et al.*, 2019).

Based on the findings in this study, it is proven that cultivation of *C. sativus* in Malaysia is feasible especially for indoor cultivation where the environmental factors such as temperature can be controlled, thus allows optimum growth of this species without the influence of adverse weather, pest infestation and geographical constraints. This study also lays a foundation in which the methods could be refined to the point where the plant can be cultivated for large-scale in cooler highland areas.

Table 2. The effects of temperatures on the flowering of *C. sativus*.

Temperatures (°C)		Days to flower initiation[X]	Days to flower blooming[Y]	Number of flowers
Germination	Flowering			
10		$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{d}$
23	16	$17.03 \pm 0.13_{d}$	$18.20 \pm 0.13_{d}$	$2.87 \pm 0.20_{a}$
30		$41.93 \pm 0.24_{b}$	$43.90 \pm 0.40_{b}$	$2.50 \pm 0.12_{a}$
10		$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{d}$
23	23	$37.63 \pm 0.19_{c}$	$41.47 \pm 0.18_{c}$	$1.30 \pm 0.09_{c}$
30		$53.90 \pm 0.39_{a}$	$56.40 \pm 0.35_{a}$	$2.10 \pm 0.11_{b}$
10		$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{d}$
23	30	$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{d}$
30		$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{e}$	$0.00 \pm 0.00_{d}$

Data are presented as mean ± SE, n = 15.
Means within the same column followed by different letters are significantly different at $p< 0.05$ (Duncan's Multiple Range Test).
[X]Days from sowing the germinated corms to the emergence of the floral buds.
[Y]Days from sowing the germinated corms until the floral buds started to open.

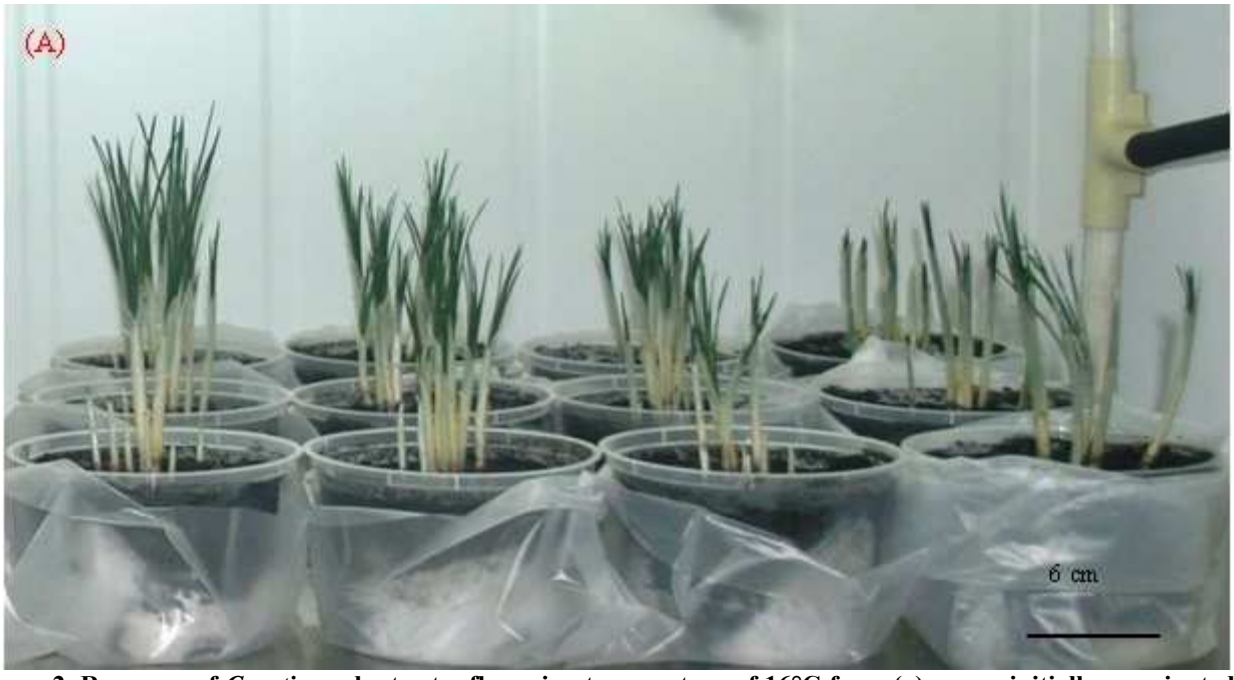

Figure 2. Response of *C. sativus* plants at a flowering temperature of 16°C from (a) corms initially germinated at 10°C, no flower formation, (b) corms initially germinated at 23°C, the flower bloomed synchronously, (c) corms initially germinated at 30°C, asynchronous flower blooming.

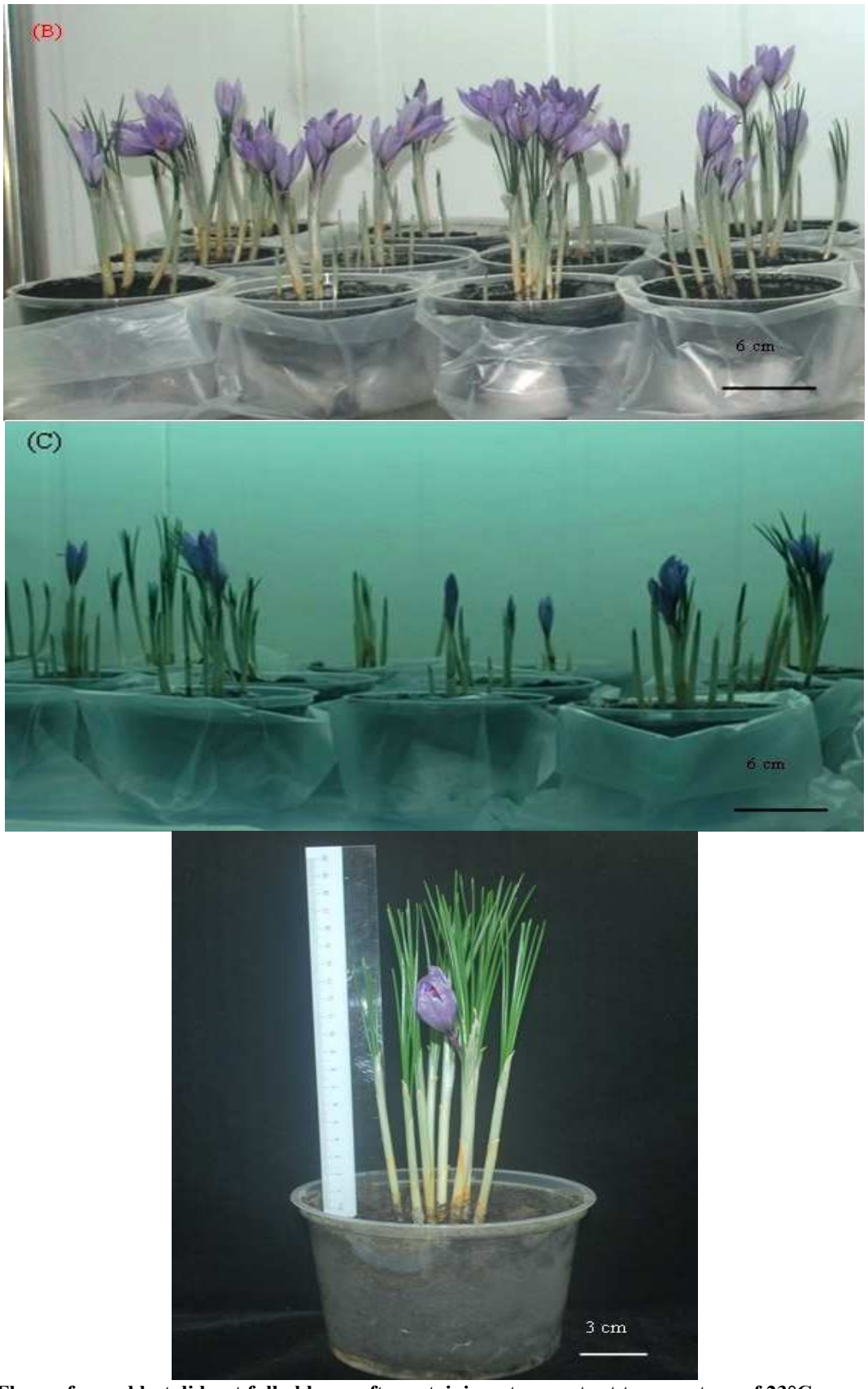

Figure 3. Flower formed but did not fully bloom after retaining at a constant temperature of 23°C.

Conclusion: It can be concluded that the flowering phase of *C. sativus* required a sequence of high temperature during germination to a lower temperature during flower initiation where the maximum number of flowers (2.87 ± 0.20 flowers) was successfully achieved when the corms were germinated at 23°C followed by a transfer to 16°C. A low temperature of 10°C is not recommended for corm germination as it hampered flower formation later on. Meanwhile, a high temperature of 30°C during the flowering inhibited flower formation even though the corms were able to break dormancy during the initial stage. This is the first successful attempt of *C. sativus* (saffron) cultivation in Malaysia since the flowering of this crop in Malaysia has never been reported before.

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