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Propagation of Saffron (*Crocus sativus* L.) Using Cross-Cuttings under a Controlled Environment

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Abstract: Saffron (*Crocus sativus* L.) is a valuable geophyte plant and one of the most expensive spices in the world. Recently, the demand for saffron spice has increased in worldwide markets owing to its enormous application and value. However, the production of saffron is limited by the vegetative propagation technique and the limited number of high-quality corms planted. Furthermore, climatic changes, notably increasing temperatures, negatively influence saffron multiplication and growth. Thus, it is important to develop alternative cultivation and propagation techniques for saffron under a controlled environment, which could ensure an increase in saffron yield and avoid the negative impact of climatic changes. The present study aimed to develop an alternative method for vegetative propagation of *Crocus sativus* under controlled conditions. The effect of different cross-cuttings, including basal cuttings (BCs) and top-to-bottom cuttings (CTBs), was evaluated on shoot, leaf, flower, and daughter corm production. All the growth parameters examined were influenced by the cutting treatment applied. The results showed that the highest number of shoots formed was obtained by BCs and CTBs, with an average of 6.68 and 5.47 shoots per corm, respectively, compared to the control with an average of 2.70 shoots per corm. The cutting treatment positively affected the formation of daughter corms in which, the high mean number of corms recorded was obtained by the BC treatment. Meanwhile, the lower size of the daughter corms was obtained after the cross-cutting treatment. This is the first report that provides an alternative propagation for saffron using a controlled environment, which could help to improve the production of saffron.

Keywords: basal cutting; corm propagation; cormlet; top-to-bottom cutting; saffron spice; vegetative propagation

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1. Introduction

Saffron (*Crocus sativus* L.) is a sterile triploid (2n = 3x = 24) perennial plant belonging to the Iridaceae family. Saffron cultivation dates back over 3500 years, and though its exact origin is not definitively known, evidence suggests two potential origin locations: one in Greece in the Mediterranean region, and the other in the East in Turkey-Iran-India [1]. Nowadays, saffron is cultivated in various countries, including Iran, India, Greece, Morocco, Italy, Spain, and the USA. Saffron is cultivated mostly for its spice extracted from the dried red stigmas, which is considered the most expensive spice in the international market. In fact, saffron spice is very well known for its special color, taste, and aroma related to its primary metabolites, including crocin, picrocrocin, and safranal [2,3]. Saffron has diverse uses and applications, mainly used in food coloring, perfumes, and cosmetics [4]. Recently, saffron has attracted increased attention from the pharmaceutical industries due to its high pharmacological activities reported, including anti-inflammatory activity, antioxidant activity, cardiovascular protection, cancer inhibition, and mitigation of anxiety and depression [5–7]. Moreover, a recent study highlighted saffron anti-inflammatory and antiviral potential against severe symptoms of COVID-19 [8,9]. All those valuable properties and applications resulted in a notable increase in saffron demand worldwide. Nevertheless, due to its sterility, saffron can only be propagated vegetatively through the formation of daughter corms from the mother corm.

Saffron is still grown traditionally by a perennial growing cycle in open field conditions. The yield production has a strong correlation with the corm size, corm density, physical and chemical properties of soil, and climatic conditions such as temperature, which could disrupt the vegetative reproduction of the saffron plant [10,11]. Nevertheless, due to the recent abiotic changes, including the increase in temperature and precipitation resulting from climatic change, field cultivation faces serious restrictions and limitations.

Morocco is considered the fourth largest producer of saffron spice in the world [12], with an average of 5–6 tons per year. However, climatic changes and stress linked to the soil of Taliouine, which is the main cultivation region in Morocco, and the limited healthy saffron plants are the primary challenging factors for large-scale production [4,13]. Because Moroccan saffron is only produced in open fields, there is a need to improve the rates of propagation with alternative methods.

In the last few decades, there has been growing attention toward enhancing saffron production and yield in open-field cultivation. Several studies have suggested the use of biological fertilizers or growth-promoting rhizobacteria (PGPR) to increase production [4,14,15]. However, negative climatic change outcomes and soil depletion affect saffron growth and flowering stages. Thus, protected cultivation of saffron in a greenhouse or under a controlled environment could represent a possible alternative solution to improve production [16–18].

Recently, some studies reported saffron production under protected propagation systems [18–21], including saffron cultivation under greenhouse conditions [20,21], aeroponics, and hydroponics culture [19,22], and a controlled environment under blue light [18]. However, propagation rates reported are still low, thus warranting further studies to enhance propagation rates of saffron.

Currently, in vitro saffron cormlet production is the only vegetative propagation method that has been extensively reviewed, wherein the significant effect of plant growth regulators PGRs, sucrose, and medium on the in vitro saffron buds, shoot and corm production were reported [13,23]. However, in vitro propagation could be very costly and could not radically improve the propagation; thus, it may not be the best solution to improve the propagation of most plant genera. Therefore, it is crucial to investigate other propagation techniques [24].

Previously, several studies reported the use of cross-cutting as an alternative vegetative propagation. Different cutting methods had a significant effect on the number of *Fritillaria persica* L. bulbs formed [25], with similar results reported for *Hypoxis hemerocallidea* cormlet propagation [24,26]. To the best of our knowledge, there are no studies reported on the use of cross-cutting on *Crocus sativus* propagation. Therefore, the current study aimed to improve saffron production by vegetative propagation, and evaluated the effects of two different cutting methods on shoot, leaf, and corm formation.

2. Materials and Methods

2.1. Plant Material

Crocus sativus plants were collected from the Taliouine-Taznakht Region in Morocco. Saffron corms of similar size, 2.5–2.9 cm, with an average weight of 7.60 g, were used as explants for this study, which were collected during their dormancy phase (July–August 2022). For each treatment and the control, 20 corms were used.

2.2. *In Vivo* Cross-Cuttings

Prior to the cross-cutting treatment, saffron corms were surface sterilized with 3% (*v*/*v*) sodium hypochlorite for 15 min, then rinsed with sterilized distilled water (SDW), and then dried under a laminar flow hood for another 30 min. The corm-cutting treatments used were as follows: (i) Basal cutting treatment (BC): Saffron corms were cut from the bottom to the top of the corm without complete separation of the 4 parts. The cut passed the basal part to encourage daughter corm formation. (ii) Top-to-bottom cutting treatment (CTB): Saffron corms were vertically cut through the center from top to bottom. All saffron corms were planted in nursery pots filled with perlite and peat moss mixture (1:1) under a controlled environment growth chamber.

The treatment and control groups were maintained under controlled environmental conditions, and light intensity of 500 $\mu mol/m^2/s$ with relative humidity (RH) of 70% at 15 °C for the flowering period (for 3 months) and 28 °C for corm formation.

2.3. *Statistical Analysis*

The experiments were performed using a completely randomized design. Each treatment contained 20 corms and was replicated a minimum of three times. Growth parameters data were collected from different growth stages, starting from two months after planting. The following variables were recorded: number of shoots, leaves, and flowers. Plant height was also measured in centimeters (cm). In addition, the number of daughter corms and corm weight and size were also measured. All data collected were subjected to analysis of variance (ANOVA) using SPSS (IBM SPSS Statistics version 27, SPSS, Inc., Armonk, NY:IBM Company) software. Differences between the mean values were compared using the post hoc test by Duncan's multiple range test (DMRT), at a significance level of $p < 0.05$. The results were expressed as the mean ± standard deviation (SD).

3. Results

The cutting method applied significantly affected saffron growth parameters, including flower, shoot, and cormlets regeneration and multiplication. As shown in Table 1, the type of cross-cutting treatment had a significant effect on the saffron flowering ratio and number of flowers produced. The highest flowering ratio was obtained from the control group (90%), followed by the BC group (60%), and CTB group (30%) (Table 1). Although the data on the effect of cross-cutting on flowering time emergence are not shown, it is worth mentioning that the flowering stage had started for the control group a week before the cross-cutting group flowering started. Further, the BC group flowered first, then the CTB group.

Table 1. Effect of cross-cutting treatments on saffron (*Crocus sativus* L.) flowering.

Treatment	Flowering Ratio %	Mean Number of Flowers per Mother Corm
Control	90^{a}	1.2 ± 0.61^{a}
BC	60^{b}	0.7 ± 0.65^{b}
CTB	30^{c}	0.3 ± 0.47^{c}

Data were means ± SE. The different letters within the same column indicated significant differences between the treatments according to Duncan's ($p < 0.05$). Experiments consisted of 20 saffron corms per treatment. BC: basal cutting; CTB: top-to-bottom cutting. Flowering ratio %: number of flowering corms/total number of saffron corms planted.

Regarding the shoot multiplication, the high number of shoots formed was recorded after the corm cutting treatment was applied; the average of shoots produced being 6.68 and 5.47 by the BC and the CTB groups, respectively, and the lowest shoot number (2.70) being recorded in the control treatment (Figure 1).

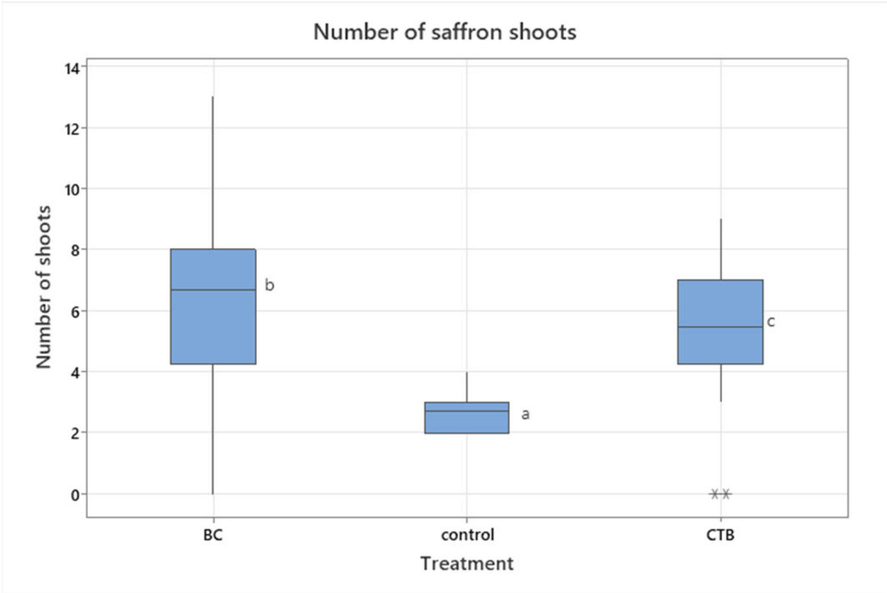

Figure 1. Number of saffron shoots formed under the control and the different cutting treatments: basal cuttings (BCs) and top-to-bottom cuttings (CTBs). Each box of the boxplots includes the median. The means with different letters are significantly different according to Duncan's test ($p < 0.05$).

The mean plant height was significantly affected by the cutting treatment. The highest average shoot height was 23.71 cm recorded for the control group, followed by 19.38 cm and 16.62 cm from the BC and CTB treatments, respectively (Figure 2). Furthermore, the cutting treatment also affected the number of leaves produced from the corm. The maximum number of leaves (12 per shoot) was obtained from the control group. However, no significant difference was found between the control and BC treatments, with averages of 6.15 and 5.85 leaves per shoot, respectively (Figure 3). However, a significant difference was found between the previous groups and the CTB treatment; where lower leaves were produced in the CTB treatment, with an average of 3.9 leaves per shoot (Figure 3).

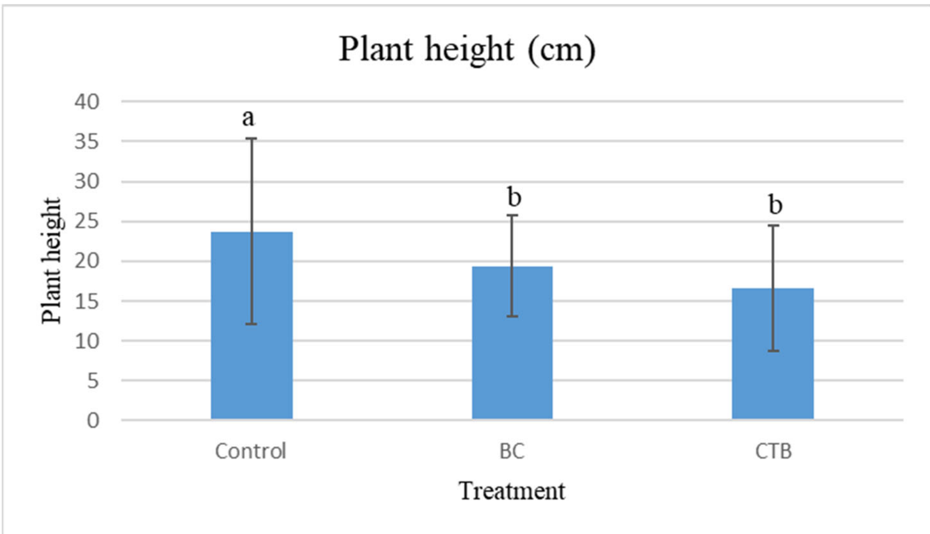

Figure 2. Height of saffron plants formed under the control and the different cutting treatments; basal cuttings (BCs) and top-to-bottom cuttings (CTBs). Values followed by different letters are significantly different according to Duncan's at $p < 0.05$.

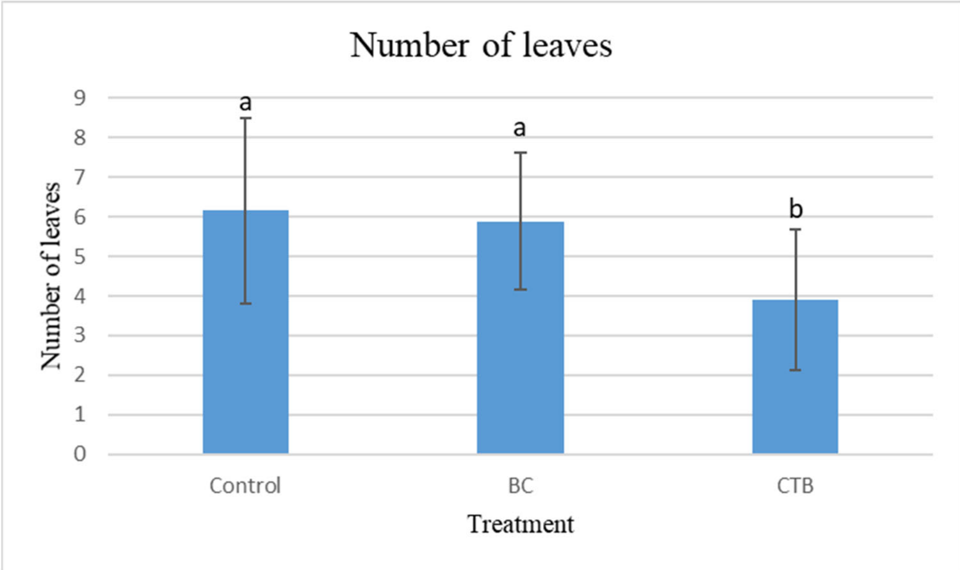

Figure 3. Number of leaves in saffron plants formed under the control and the different cutting treatments; basal cuttings (BCs) and top-to-bottom cuttings (CTBs). Values followed by different letters are significantly different according to Duncan's at $p < 0.05$.

The results collected after harvesting corms at the end of the vegetative phase, including the cormlet formation rate and total and mean number of daughter corms, are shown in Table 2.

Table 2. Effect of cross-cutting treatments on saffron (*Crocus sativus* L.) corm formation. BC: basal cutting; CTB: Top-to-bottom cutting treatment.

	Corm Formation Rate (%)	**Initial Number of Mother Corms**	**Total Number of Daughter Corms**	**Mean Number of Daughter Bulbs per Mother Corm**
Control	100 ^{a}	20	35	2.33 ± 0.90 ^{b}
BC	86.66 ^{a}	20	97	6.47 ± 1.96 ^{a}
CTB	80 ^{a}	20	76	5.08 ± 1.15 ^{a}

Means followed by the same letter indicate non-significant differences by Duncan's multiple range test (DMRT), at a significance level of $p < 0.05$.

Comparing the rate of corm formation, there was no significant difference between the control and the cutting treatments. The highest value in term of the ratio of corm formation was 100% obtained by the control group, followed by the BC and CTB groups with 86.66% and 80%, respectively. Regarding the number of daughter corms formed, we observed a significant difference between the cutting treatment and the control group (Figure 4). The number of corms produced ranged from 1 to 10 corms per mother corm. From the initial 20 mother corms for the control, and each treatment, a total of 35 corms were formed in the control group, 97 corms for the BC treatment and 76 corms for the CTB treatment (Table 1). The mean number of daughters corms formed per corm at the end of the experiment ranged from 2.33 to 6.47 (Table 1). The type of cross-cutting treatment had a significant effect on the corm formed; the highest mean of daughter corm was recorded for the BC treatment, followed by the CTB treatment, and the control group (Table 1). Additionally, the size of the daughter corms formed by the various treatments examined was significantly different for all the treatments (Figure 5). The higher mean diameter of the corm formed was recorded in the control group at 2.071 cm, followed by the BC and CTB treatments with an average of 1.43 cm and 1.14 cm, respectively. Regarding the weight of the corm formed, the highest mean weight of the daughter corm was recorded in the control group (4.9 g), which was found to be significantly higher than the corm formed in the cutting treatments with less than 2 g in both the BC and CTB treatments examined (Figure 6). The cross-cutting treatments are illustrated in Figure 7.

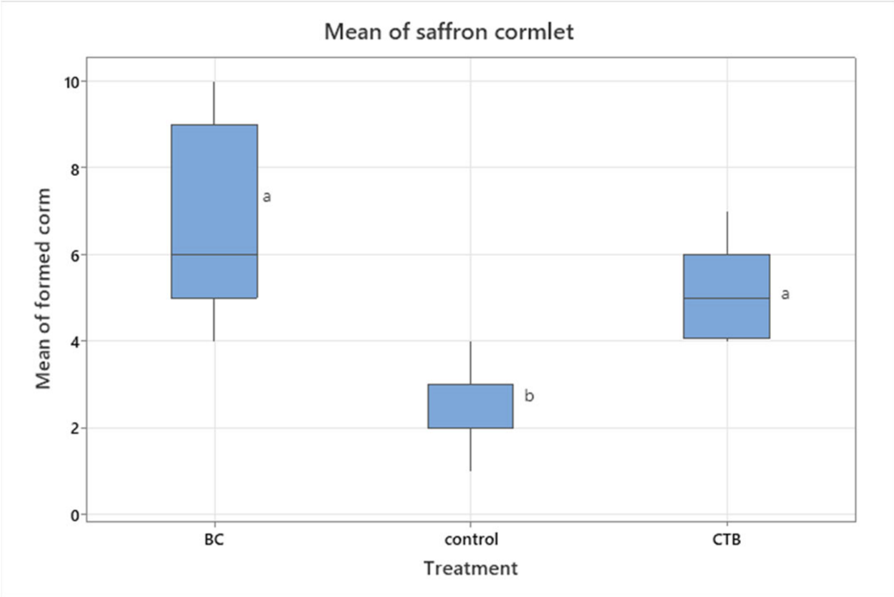

Figure 4. Box plot of number of daughter corms per mother corm. Each box of the boxplots includes the median. The means with different letters are significantly different according to Duncan's test ($p < 0.05$).

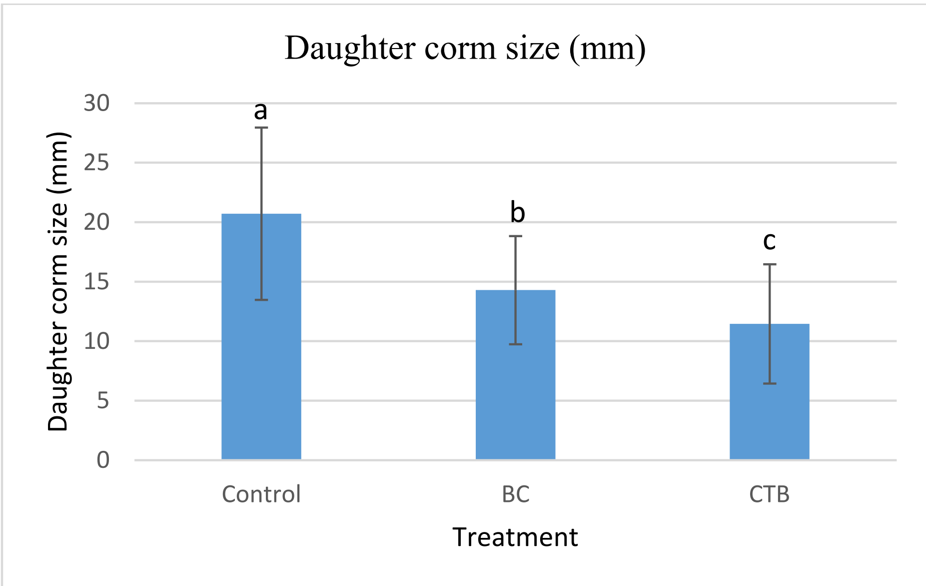

Figure 5. Size of daughter corms (mm) formed under the control and the different cutting treatments; basal cuttings (BCs) and top-to-bottom cuttings (CTBs). Means followed by the same letter indicate non-significant differences by Duncan's multiple range test (DMRT), at a significance level of $p < 0.05$.

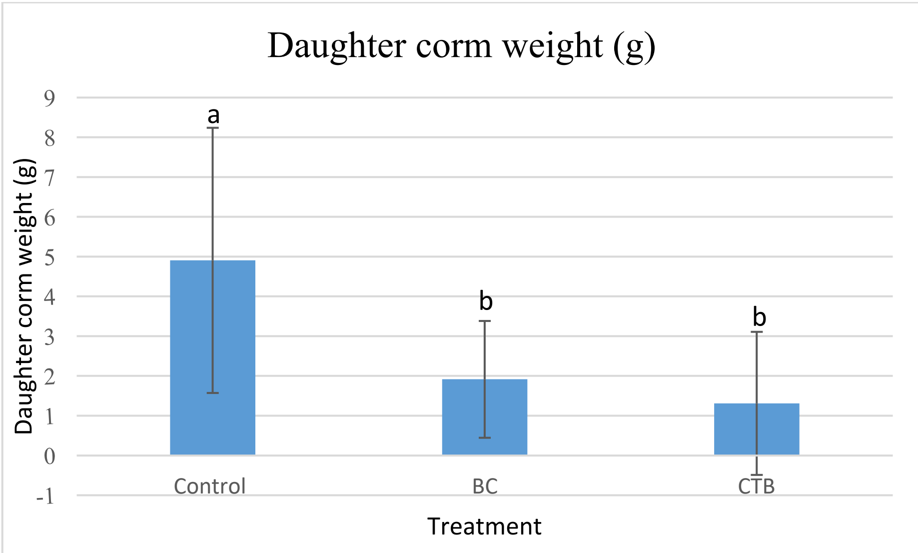

Figure 6. Daughter corms weight (g) formed under the control and the different cutting treatments; basal cuttings (BCs) and top-to-bottom cuttings (CTBs). Means followed by the same letter indicate non-significant differences by Duncan's multiple range test (DMRT), at a significance level of $p < 0.05$.

(**A**) (**B**) (**C**)

Figure 7. Saffron corms produced under a controlled environment with different cross-cutting treatments. (**A**): control group, (**B**): C cross-cutting, and (**C**): CTB cross-cutting.

4. Discussion

The main objective of the present study was to improve saffron production by vegetative propagation. Two different cutting methods were evaluated. According to this study, vegetative propagation by cross-cutting methods is applicable to saffron production. Overall, cross-cutting treatment of saffron corm without complete separation had a significant effect on the growth parameters measured. A clear morphological response was observed for all the saffron growth parameters with different cross-cutting treatments.

According to our results, saffron can grow successfully under controlled conditions. The saffron flowers emerged at 15 ± 2 °C. This result is similar to previous studies on saffron growth under controlled conditions [18,20]. Regarding the saffron flowering ratio, the highest flowering ratio was obtained with the control group followed by the BC and CTB groups, respectively. This was similar to previous results reported on *Crocus olivieri* ssp. Balansae with 26.7%, 20% [27], and no flowering for the control group, BC group, and CTB group, respectively. In contrast with the same study for *C. baytopiorum*, the highest flowering ratio reported was 6.7% with the BC treatment, while no flowering was recorded in the control and CTB groups [27].

The results of the current study clearly show the positive effects of cross-cutting treatment on the number of shoots and leaves per corm produced. The BC and CTB treatments regenerated a higher number of shoots compared to the control group. This result indicates that the cross-cutting treatment successfully overcame the apical dominance of apical meristems and led to the stimulation of the growth of axillary meristems [28,29]. Our current results were consistent with the findings reported on *Allium cepa* L., whereby removing a portion of the corm promoted shoot growth [30]. Furthermore, the cross-cutting treatment also had an effect on leaf growth and length, similar to a previous study on the Amaryllis bulb (Hippeastrum × johnsonii), where the maximum number of leaves was produced when the mother bulb was cut into 8 sections, whereas the lowest number was produced when the bulb was cut into 96 sections [31]. Mofokeng et al. (2020) [24] demonstrated that the cross-cutting of Hypoxis hemerocallidea bulb, followed by a 60 min treatment of 100 mg L^{-1} BA, resulted in a markedly higher number of leaves. However, there was no significant difference in leaf length observed when the bulb was cut and soaked in water or a solution of plant growth regulators (PGRs).

Our study revealed that the highest corm ratio was found in the control group (uncut), which is consistent with the results reported for both *C. baytopiorum* and *C. olivieri* ssp. balansae [27]. Meanwhile, for the cutting treatment, the highest ratio was obtained with the BCs followed by the CTBs, contrasting with the results reported for C. olivieri, where CTBs gave best results followed by BCs with a total of 86.7% and 80.0%, respectively, and an equal ratio for both cuttings at 60% for *C. baytopiorum* [27].

Concerning saffron corm multiplication, BC treatment showed the highest number of daughter corm formations, as similarly reported for *Fritillaria imperialis* [32]; furthermore, ref. [31] demonstrated that the type of cutting method and the number of cut sections evaluated had significantly influenced the bulb of Amaryllis, where the higher bulb formation was recorded after sectioning the bulb into eight segments. In another study, chipping a full Hypoxis hemerocallidea corm into four or eight segments significantly increased the number of corms formed; the cutting into eight pieces followed by soaking in distilled water showed a higher number of cormlets formed with an average of 5.29 daughter corms per corm [24].

The different cutting methods had a significant effect on the daughter cormlet size and weight; the lowest diameter and weight were observed when cutting CTBs and BCs, respectively, compared to the control group. These results are consistent with the previous study reported on *Fritillaria imperialis* L. bulbs, in which the cross-cutting and shipping of the bulb resulted in the lowest weights, 25.90 g and 11.74 g, respectively, compared to the control group with 84.60 g [32]. The same results were reported for the diameter of the bulb. The diameter of daughter Amaryllis (Hippeastrum × johnsonii) bulbs was affected by the cutting method [31]. The circumference and weight of the bulb depended on the size section cut performed of the bulb.

5. Conclusions

This is the first study reporting the use of cross-cutting under a controlled environment as an alternative propagation method for saffron. The effects of BC and CTB treatments on saffron growth parameters, including flower emergence, shoot multiplication, and cormlet formation, were evaluated. The BC treatment positively affected shoot and corm production, with the highest number of daughter corms formed. Based on the findings of the present study, saffron can effectively grow under controlled conditions, and the application of cross-cutting can lead to enhanced saffron daughter formation, thereby increasing the number of materials available for planting and avoiding the negative effects of climate change, which positively affects saffron production. Therefore, this proposed vegetative propagation by cross-cutting offers promise as a highly economical and efficient approach for the production of *Crocus sativus* planting stock. However, there is still a need to further improve saffron daughter cormlet size in indoor production by the application of biostimulants, PGRs, PGPRs, or the use of the blue light conditions in culture [4,18].

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