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Research Article

Study of the Effect of Pretreatment of Corms by Different Concentrations of Gibberellic Acid and at Different Periods on the Growth, Flowering, and Quality of Saffron in Eastern Morocco

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The present study was conducted to evaluate the influence of gibberellic acid (25, 50, 100, and 200 ppm) applied on saffron corms previously harvested during different periods (March, June, and before planting) on the growth and the quantitative and qualitative yield of saffron (*Crocus sativus* L.). The study was carried out in the field during the 2016-2017 and 2017-2018 seasons in the experimental station of the Faculty of Sciences of Oujda (Morocco). The measured parameters correspond, on the one hand, to morphometric measurements and to the determination of the saffron stigma yield and, on the other hand, to the evaluation of the quality of the spice. The results showed that almost all the parameters studied were significantly affected by the factors considered. The treatment of corms just before planting with concentrations of 100 and 200 ppm GA_3 showed the highest flower and leaf appearance rate and the highest leaf length and surface area. Moreover, the application of GA_3 during the month of March gave the best results in terms of stigmata yield, percentage of large-diameter daughter corms, and the ratio of the number of flowers produced to the total weight of corms. The results of the coefficient of corm propagation revealed that the application of gibberellin during any period improved this coefficient compared to the control. The results indicated that the application of GA_3 with concentrations above 25 ppm can improve the growth of saffron and increase its yield under the semiarid climatic conditions of eastern Morocco.

1. Introduction

The name saffron applies indiscriminately to *Crocus sativus* L., a sterile triploid plant that multiplies vegetatively by corms, and to the precious spice obtained from the dried stigmas of this plant [1]. The phenology of saffron includes dormancy, flowering, formation, and growth of the daughter corms. Ephemeral flowering, average yield, and high production cost have made saffron the most expensive spice in the world. For this reason, a lot of research has been launched in the world to increase saffron yield on the one hand and to reduce production costs on the other hand [2]. The application of plant growth regulators is one method to improve production and control plant flowering [3]. They are used at almost all stages of plant growth, from germination to postharvest stages. However, the dose and timing of application of these substances must be chosen so that the desired result is achieved. There are different methods of applying growth regulators, including preplanting soaking, foliar application, and direct watering. Direct drenching of corms is an effective method to achieve good results [4]. Many plant growth regulators have been widely used for the production of flowering, leafy, and many other ornamental plants [5, 6]. Among growth regulators, gibberellin was first recognized in 1926 by a Japanese scientist, Eiichi Kurosawa [7]. Numerous studies have focused attention on the use of

this phytohormone to improve the productivity and quality of several crops [8]. The most characteristic effects of GA_3 are elongation of internodes, increased leaf growth, increased flower number, and improved apical dominance [9]. Thus, many researchers have applied gibberellins to lift seed and bulb dormancy and increase germination rate [10, 11]. Gibberellins also initiate early flowering in many ornamentals and increase the number of flowers [12], which is the case in chrysanthemums (*Chrysanthemum parthenium* L.) [12]. Foliar sprays with low concentrations of GA3 have been tested with promising results on the performance, quality, and salt tolerance of various fruit and vegetable species in soil and hydroponic systems [13–15]. In saffron, corm dormancy is strongly associated with abscisic acid (ABA) [16], and the recovery of apical buds requires gibberellin (GA_3) [17]. There are a few reports on the effect of plant growth regulators on the recovery of saffron corms [1, 18–20]. As an extension of previous studies and considering the important properties of gibberellic acid, this experiment was conducted to study the influence of various doses of GA3 applied at different stages on the reproductive and vegetative characteristics of saffron.

2. Materials and Methods

2.1. Site Features. In order to study the effects of soaking saffron corms in GA_3 for 3 different periods on the quantitative and qualitative characteristics, an experiment was conducted in the open field at the experimental research station of the Faculty of Science in Oujda, located at an altitude of 661 m and at 34° 39′06–71″ north and 01° 53′58–80″ west (GPS Back Track Bushnell). Precipitation was modest for most of the trial period especially during the first year of the trial (131 mm) (Figure 1). Saffron water requirements were supplemented by regular irrigation. Average area temperatures above 15°C were recorded for the months of March to November, while minimum temperatures were recorded during the months of January and February (Figure 1).

2.2. Plant Material. In order to have a representative sample of the saffron corms, the corms were first visually examined and unsuitable, rotten, diseased corms were discarded and the rest were graded in such a way that only saffron corms larger than 2.5 cm in diameter were used in this trial. The corms used came from a saffron boat at the experimental research station of the Faculty of Science in Oujda.

2.3. Treatments and Trial Conditions. In order to determine the optimal stage and concentration of GA_3 to improve saffron yield and growth parameters, the mother corms were treated with 4 concentrations of GA_3 for 3 different stages. The corms were collected during 3 different periods, namely, March, during the transition from the vegetative to the reproductive stage; June, when the floral organs were present and finally just before planting. After removing the outer tunics covering the corms, they were immersed in different concentrations of gibberellin (25, 50, 100, and 200 ppm) for 2 hours. Then, microdrops of 10 μl of the hormonal solution were applied to the apical part of the corms. The control was treated only with distilled water (Figure 2). The corms were then kept at room temperature (20–22°C) for 24 hours to allow the hormone to penetrate the tissues, before being transferred to a storage chamber under 65% relative humidity and 20°C in dark conditions.

After the preparation of the soil and the layout of the land, the saffron corms with a diameter of 2.5 cm were planted on 01/09/2016 in plots of 1.5 ∗ 1.5 m at a depth of 7 cm, 10 cm between corms and 20 cm between lines. All maintenance techniques such as irrigation, weeding, and hoeing were carried out regularly, with no chemical inputs (pesticides or fertilizers). Some chemical and physical characteristics of the soil are presented in Table 1.

2.4. Measured Parameters

2.4.1. Performance Parameter

(1) Flowering Rate (TF). The flowering rate was calculated with reference to the method of [21], which is based on counting the number of flowers open each day. The number of open flowers per day (flowering rate) was calculated using the following equation:

$$\text{Flowering rate} = \sum_{i=1}^{n} \frac{\text{NF}}{\text{NJPF}}, \quad (1)$$

where NF is the number of flowers at each harvest date, NJPF is the number of days after the first bloom, and n is the harvest date.

(2) Stigma Yield (RS). The stigmas were separated from the flowers and weighed using a precision balance and stored at room temperature of 19°C for drying. Then, reweighed to an accuracy of 0.0001, the dry weight data were expressed in milligrams (mg).

(3) Saffron Quality. The amount of crocin, safranal, and picrocrocin in the 1% (E1%) aqueous solution of the dried stigmas was measured at wavelengths of 440, 330, and 257 nm, respectively, by UV-Vis spectrophotometer (PG instruments T80+).

2.4.2. Vegetative Parameters

(1) Leaf Appearance Rate (LAR). The leaf appearance rate is the number of days required for a leaf to appear [22]. This parameter is an excellent measure of plant development [23]. It has been calculated according to the equation developed by [21]:

$$\text{leaf appearance rate} = \sum_{i=1}^{n} \frac{\text{NF}}{\text{DPDI}}, \quad (2)$$

where NF is the number of leaves appearing on day n, DPDI is the distance of day n from the first irrigation date, and n is the day.

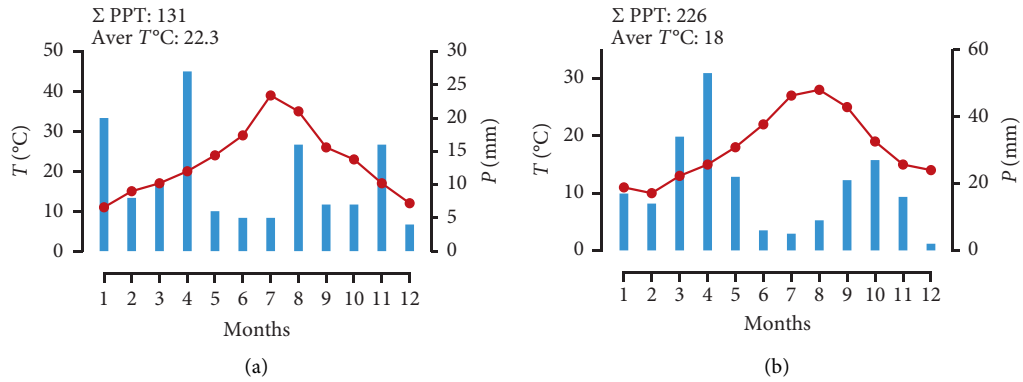

Figure 1: Average monthly meteorological data from the experimental station of the Faculty of Science in Oujda, for the experimental period January–December 2017 (a) and January–December 2018 (b).

Figure 2: Treatment of saffron corms by different concentrations of GA_3.

Table 1: Physicochemical characteristics of the soil (0–30 cm depth).

	Physical characteristics			Chemical characteristics	
Texture	Sand (%)	Limon (%)	Clay (%)	EC (ms/cm)	pH
Clayey	12.5	24.1	63.4	0.58	7.1

The interest of studying the rate of leaf appearance is to detect the effect of hormonal treatment on the early appearance of leaves in saffron. A low rate of appearance during the flowering period is desirable in order not to hinder the harvesting process.

(2) Length (LF) and Surface Area of Leaves (SF). Five (5) plants/treatment were randomly selected and used to count the number and measure the length of the leaves.

Given the morphology of the saffron leaves, the leaf area is estimated directly using AUTOCAD 2010 software after scanning the leaves with a flatbed scanner as an image (JPG).

(3) Chlorophyll Content a + b (CHLT). Fully developed leaves from each treatment were sampled. Chlorophyll (Chl a + b) was extracted by crushing the leaves in 80% acetone in the dark. The supernatant was then separated and analyzed by spectrophotometer (Ray Leigh, Vis 7220G) at wavelengths of 663 and 645 nm, as described by [24].

(4) Chlorophyll Fluorescence (FV/F0). Chlorophyll fluorescence was measured with a modulation fluorometer (Hansatech, England). The leaves were then darkened for 30 min and maximum fluorescence (Fm), variable fluorescence (Fv), and initial fluorescence (F0) were measured. Fv/F0 reflects the efficiency of electron donation to PSII controllers and the photosynthetic quantum conversion rate in PSII controllers. Fv/F0 was calculated using the following formula: $Fv/F0 = (Fm - F0)/F0$ [25].

(5) Parameters of the Underground Part. The parameters of the underground part (number, weight, and diameter of corms) were calculated at the end of the cultivation cycle after digging up the plants, the aerial part was separated from the underground part, the corms were removed from the topsoil and cleaned, and the number and weight of the corms were determined; thus, the size was measured with a calliper. The distinguished calibers are as follows: large size, $Ø > 2.5$ cm; medium size, $1.5 \text{ cm} < Ø < 2.5$ cm; small size, $Ø < 1.5$ cm. Subsequently, the coefficient of propagation of corms (CPC) that represents the percentage of corms

produced in relation to the initial weight of corms planted was determined [26].

2.5. Experimental Setup and Data Analysis. The experimental design adopted is split-plot with three replicates and 45 UE (5 corms/experimental unit, 75 corms/block and 225 corms/3 blocks), with large plots indicating the corm sampling period (March, June, and before planting), while the GA_3 concentration factor is allocated to small plots. The data obtained were subjected to an analysis of variance (ANOVA), using the software "GraphPad Prism for Windows version 7" and the comparison of the means is made by Duncan's test at the 5% significance level. The natural logarithms of the dry weight of the stigmas were used to meet the statistical assumptions.

3. Results and Discussion

3.1. Performance Parameters. The effects of gibberellin dose and application period were significant on the different components of saffron yield (Table 2). The highest rate of flower appearance was observed at the highest concentrations of GA_3 (100 and 200 ppm). The same trend was noticed during the two years of the experiment. On the other hand, the rate of flower appearance was affected by the timing of the hormone application, with the treatment just before planting achieving the best results compared to other treatment periods. The analysis of variance of the effect of the tested factors on the number of flowers was highly significant ($p \leq 0.01$) (Table 2). The application of 50, 100, and 200 ppm GA_3 increased flower numbers regardless of the application period. The most remarkable increase was recorded in 2017 in the 200 ppm GA_3 treatment during the March period, with a 114% increase over the control. This parameter also influenced the stigmata yield, the most important economic parameter in saffron (Table 2). The results showed that GA_3 significantly increased the dry weight of the stigmas, with the highest values observed in 2018 for the 100 ppm (1.89 mg/m^2) and 200 ppm (2.20 mg/m^2) concentrations, especially when the treatment was carried out in March.

Based on the results of this study, the application of gibberellin (GA_3), particularly at a concentration of 100 and 200 ppm, caused a break in the dormancy and stimulation of the recovery of saffron corms. These results are consistent with those found in saffron [27], chrysanthemum (*Chrysanthemum parthenium*) [28], carnation (*Dianthus caryophyllus* L.) [26], *Lilium* (*Lilium* L.) [29], and gladioli (*Gladiolus* spp. L.) [30]. The observed effect could be attributed to the effect of gibberellin on the decrease of abscisic acid concentration in buds, which induces floral initiation and thus early flowering [31]. Similarly, since saffron is a subhesive species, the production of flower buds depends strongly on the metabolism of the reserves stored in the corms. Hence, the important role of GA_3 in this physiological process by increasing starch catabolism and producing simple sugars [27]. The substantial increase in the number of flowers when corms were treated with GA_3 has a direct relationship with the same physiological process. These results have been confirmed by those reported by Rudnicki et al. [32] on tulips (*Tulipa gesneriana* L.), Kumar et al. [26] on carnations ((*Dianthus caryophyllus)*, and Ameri et al. [20] on saffron *(Crocus sativus)*. The same finding was observed in the second year of the experiment. This high flower production and thus yield in the second year could be attributed to the increase in the number and surface area of leaves, which improved the production and accumulation of photosynthates in daughter corms. The latter is considered to be the most influential factor in the yield of this species [33].

3.2. Saffron Quality. The study of the quality of the various samples was carried out with reference to the ISO 3632–1: 2011 standard, so the classification was made on the basis of the Moroccan standard NM 08.1.038. The concentration of the different qualitative components of saffron, namely, crocin, picrocrocin, and safranal, respectively, responsible for color, flavor, and odor was determined according to the absorbance of the solutions at different wavelengths 440, 330, and 257 nm. The results of this study showed that GA_3 had a slight effect on the concentration of the three components of saffron. The application of gibberellin with a high concentration (200 ppm) just before planting induced a slight increase in the picrocrocin concentration (127, +3.25%) compared to the control, while the treatment of the corms during the month of June with a concentration of 25 ppm GA_3 increased the picrocrocin level (140, +23%) in a highly significant way. On the other hand, the use of GA_3 during the month of March could not produce significant results for this compound (Table 3). On the other hand, the treatment of corms with high concentrations of GA_3 caused a decrease in the concentration of safranal in the saffron stigmas. The lowest amounts of this compound were obtained by applying a concentration of 200 ppm GA_3 regardless of the treatment period. For the qualitative evaluation of the stigmas of each treatment, only the 50 ppm GA_3 treatment (before planting) was placed in the first quality category on the basis of all indices (Table 3). Other treatments such as 50 ppm GA_3 applied in March and 200 ppm GA_3 applied just before planting were placed in category III. In the case of picrocrocin, all treatments were placed in quality category I. Limited research was conducted on the relationship between growth-regulating components and the quality of the active ingredients in saffron. In general, the results obtained in this section and the tendency to increase the quality of the active ingredients by applying GA3 are consistent with the results presented by Isfahani et al. [34] and Ameri et al. [20].

3.3. Vegetative Parameters. The results of the present study showed that the treated corms had a higher rate of leaf appearance than the control, whose increase in GA_3 concentration significantly increased this variable. According to the analysis of variance data, the effects of different GA_3 doses and different application periods on the rate of leaf appearance were statistically significant at 0.01 over the two

Table 2: Effect of different GA_3 concentrations and duration of application on flowering rate, number of flowers, and stigmata yield. Values with the same letter in each application period in the column are not significantly different ($p = 0.05$); ns: not significant; *significant at 5%; **significant at 1%; ***significant at 1‰.

Duration of application	Treatment GA_3	Flowering rate (day 1)		Number of flowers (size)		Yield in stigmas ($g/1\,m^2$)	
		2017	2018	2017	2018	2017	2018
Before planting	Control	1.75**d**	2.15**d**	1.49**d**	1.88**c**	0.28**c**	1.24**c**
	25 ppm	1.98**c**	2.67**b**	1.73**d**	1.82**c**	0.23**c**	0.57**d**
	50 ppm	1.36**e**	2.46 **cd**	1.87**b**	2.33**b**	0.34**b**	1.47**c**
	100 ppm	2.70**a**	3.21**bc**	2.30**a**	2.35**b**	1.31**a**	1.87**b**
	200 ppm	2.36**b**	3.85**a**	1.93**b**	2.71**a**	1.22**a**	2.03**a**
June	Control	1.48 **cd**	2.00**d**	1.56**b**	1.61**c**	0.30**b**	0.33**bc**
	25 ppm	1.34**d**	2.18**c**	1.44**bc**	1.56**c**	0.25**b**	0.27**c**
	50 ppm	2.08**a**	2.52**b**	1.23**c**	2.11**b**	0.18**c**	0.57**b**
	100 ppm	1.89**b**	2.44**b**	2.04**a**	2.32**a**	0.84**a**	1.00**a**
	200 ppm	2.17**a**	3.20**a**	1.51**b**	2.87**ab**	0.27**b**	0.35**bc**
March	Control	1.82**a**	2.35**d**	1.39**e**	2.30**c**	0.41**d**	0.79**d**
	25 ppm	1.65**c**	1.26**e**	1.87**d**	2.21**c**	0.36**d**	0.64**d**
	50 ppm	1.83**a**	1.55**c**	2.14**c**	2.65**bc**	1.10**c**	1.23**c**
	100 ppm	1.79**ab**	2.65**a**	2.54**b**	2.88**b**	1.87**b**	1.89**b**
	200 ppm	1.72**b**	2.11**b**	2.98**a**	3.12**a**	2.14**a**	2.20**a**
Source of variation	Treatment	0.604***	5.067***	2.623***	2.195**	4.307***	8.022***
	Period	1.840***	5.969***	3.801**	6.447***	9.314***	7.817*
	T ∗ P	3.149***	4.183**	2.664**	0.265ns	3.871***	2.607***

Table 3: Effect of different GA_3 concentrations and duration of application on phytochemical indices and quality grading of saffron stigmas.

Duration of application	Treatment GA_3	$E^{1\%}_{257nm}$		NM 08.1.038 picrocrocin		$E^{1\%}_{440nm}$		NM 08.1.038 crocin		$E^{1\%}_{330nm}$		NM 08.1.038 safranal	
		2017	2018	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
Before planting	Control	142	123	**I**	**I**	110	90	**II**	**HN**	50	54	**I**	**HN**
	25 ppm	119	134	**I**	**I**	87	79	**HN**	**HN**	71	63	**HN**	**HN**
	50 ppm	139	128	**I**	**I**	92	154	**HN**	**I**	42	48	**I**	**I**
	100 ppm	112	100	**I**	**I**	120	100	**III**	**III**	46	39	**I**	**I**
	200 ppm	103	127	**I**	**I**	102	116	**III**	**III**	62	40	**HN**	**I**
June	Control	125	109	**I**	**I**	82	94	**HN**	**HN**	77	69	**HN**	**HN**
	25 ppm	140	134	**I**	**I**	116	135	**III**	**III**	58	62	**HN**	**HN**
	50 ppm	115	127	**I**	**I**	128	92	**III**	**HN**	49	63	**I**	**HN**
	100 ppm	100	96	**I**	**I**	96	102	**HN**	**III**	40	51	**I**	**HN**
	200 ppm	132	120	**I**	**I**	122	108	**III**	**III**	65	55	**HN**	**HN**
March	Control	126	130	**I**	**I**	127	142	**III**	**III**	74	60	**HN**	**HN**
	25 ppm	104	97	**I**	**I**	95	113	**HN**	**III**	96	68	**HN**	**HN**
	50 ppm	123	100	**I**	**I**	88	133	**HN**	**III**	42	57	**I**	**HN**
	100 ppm	128	139	**I**	**I**	147	161	**III**	**II**	55	69	**HN**	**HN**
	200 ppm	111	125	**I**	**I**	137	105	**III**	**III**	61	58	**HN**	**HN**

years of the trial. The highest rate of leaf emergence (4.26 days) was observed in plants treated with 200 ppm GA_3 just prior to planting, while the lowest values were observed in the control for all treatment periods. Similarly, analysis of variance data showed a significant effect of GA_3 dose and application periods on leaf length. However, interactions between GA_3 dose and application period were not statistically significant. Overall, the largest leaf length values were obtained from 100 ppm GA_3 applied just before planting. However, the application of GA_3 in March did not show a significant difference between the different concentrations except for that of 200 ppm. The corms treated with GA_3 had the largest leaf area, while the control recorded the lowest values. The highest leaf area ($164\,cm^2$) was recorded following the application of 200 ppm GA_3 just before planting the corms. Based on the analysis of variance data, the effects of GA_3 dose, treatment period, and interaction ($GA_3 *$ period) were statistically significant at a level of 0.001. The effects of GA_3 dose, treatment period, and interaction ($GA_3 *$ period) were statistically significant at a level of 0.001 (Table 4).

Many authors have shown that phytohormones can influence plant growth and development by modifying the hormone content, which subsequently regulates crop yield

Table 4: Effect of different GA_3 concentrations and duration of application on leaf emergence rate, leaf length, and leaf area. Values with the same letter in each application period in the column are not significantly different ($p = 0.05$); ns: not significant; *significant at 5%; **significant at 1%, ***significant at 1‰.

Duration of application	Treatment GA_3	Leaf appearance rate (day 1)		Sheet length (cm)		Sheet area (cm^2)	
		2017	2018	2017	2018	2017	2018
Before planting	Control	1.33**e**	1.37**d**	25.6**c**	23.2**d**	91.2**b**	83.1**d**
	25 ppm	1.67**d**	1.89**d**	29.7**bc**	25.6**c**	87.6**b**	90.8**d**
	50 ppm	2.43**c**	2.64**c**	25.2**c**	25.9**c**	108.4**b**	130**c**
	100 ppm	3.07**b**	3.71**b**	32.5**b**	30.3**b**	139**a**	149.6**b**
	200 ppm	3.83**a**	4.26**a**	36.2**a**	38.7**a**	122.4**a**	164.5**a**
June	Control	1.12**c**	1.44**c**	26.8**a**	25.4**b**	71.5**c**	86.9**d**
	25 ppm	1.27**c**	1.57**c**	20.9**b**	20.5**c**	111.8**b**	130.2**c**
	50 ppm	2.88**b**	2.62**b**	21.1**b**	22.3**c**	128.4**ab**	140.6**b**
	100 ppm	2.71**b**	3.10**ab**	22.7**ab**	25.7**b**	139.1**a**	178.3**a**
	200 ppm	3.31**a**	3.76**a**	26.5**a**	30.2**a**	127.3**ab**	158.8**b**
March	Control	1.20**b**	1.37**c**	22.4**c**	23.1**b**	106.3**bc**	120**bc**
	25 ppm	1.12**b**	1.23**c**	23.9**b**	23.5**b**	89.1**c**	100.7**c**
	50 ppm	1.55**ab**	1.81**b**	23.4**b**	22.2**b**	118.4**b**	126.2**b**
	100 ppm	1.67**a**	1.73**b**	23.2**b**	23.9**b**	130.9**a**	122**b**
	200 ppm	1.82**a**	2.04**a**	23**a**	27.4**a**	115.3**b**	157.8**a**
Source of variation	Treatment	19.83**	24.14***	175.9*	534.7***	179.2***	519***
	Period	8.77***	10.14***	428.7***	171**	519***	179.8***
	T * P	4.05***	5.36***	150ns	135.2ns	976***	140.2***

[35]. The results of the present study showed that GA_3-treated corms exhibited a remarkable increase in vegetative growth parameters (number, length, and surface area of leaves) compared to controls. These results are supported by those reported by several authors on several crops [36–39]. These results could be attributed to the fact that GA_3 promotes cytogenesis, cell elongation, and tissue differentiation, which ultimately leads to an increase in leaf number and leaf area [40]. In addition, the observed action of GA_3 on the studied parameters could also be due to the improvement of enzymatic activity [41] and the increase in membrane permeability [42], which could facilitate the absorption and assimilation of mineral nutrients [43]. In the present study, dry conditions were dominant in the first year despite the water supply made to compensate for the plant's needs, which could explain the thin leaves observed during this year.

3.3.1. Total Chlorophyll Content and Chlorophyll Fluorescence. Chlorophyll levels and the associated variable Fv/F0 were slightly influenced by the factors studied (Table 5). With the exception of 100 and 200 ppm GA_3 concentrations, application of the low rates (25 and 50 ppm) just before planting did not show significant differences in total chlorophyll content compared to the control. However, plants whose corms were treated with low doses of GA3 (25 and 50 ppm) in June showed lower chlorophyll levels than the control (1.34 and 1.37). Total chlorophyll content decreased in the first year of the test compared to the control when GA_3 was applied in March at concentrations of 25–100 ppm, while at 200 ppm, the Chl a + b value was significantly higher than the control.

A similar trend was observed for the fluorescence of chlorophyll (Fv/F0), which showed higher values in the first year in plants treated with GA_3 compared to controls and the effect is all the more important when the concentration of GA_3 increases. However, during the second year of the experiment, the analysis of variance did not show an effect of hormone, application period, and interaction (period-$*$ GA_3) on chlorophyll fluorescence.

The results also showed that during the first year, total chlorophyll content was significantly higher in the treatments (GA_3) compared to the control. However, in the second year, no effect of the studied factors was detected on the photosynthetic parameters of the plant. The physiological response of plants to gibberellin application is still controversial. Decreases [44] and increases [45] in chlorophyll content have been reported after GA_3 application. Bruinsma et al. [46] have suggested that the increase in leaf area following gibberellin application may cause dilution of chlorophyll and thus a decrease in the content of this parameter compared to the control. Thus, the difference observed in the first year could be explained by the fact that the control plants were probably under stress conditions (dry year) and that the application of GA_3 was effective. Gibberellin has been shown to play a major role in the growth, development, and strengthening of the plant defense system. Application of GA_3 can neutralize the adverse effects of salinity on electrolyte leakage and chlorophyll content [47] by increasing the activity of the nonphosphate ribolose carboxilase oxigenase enzyme (Rabisco), which is a major enzyme of photosynthesis in plants [48]. GA_3 did not affect the Fv/F0 ratio during the two years of the trial. The fluorescence yield of chlorophyll can provide information on the state of the plant under stress [49]. Björkman and Demmig

Table 5: Effect of different GA_3 concentrations and duration of application on total chlorophyll content (a + b) and Fv/F0 chlorophyll fluorescence. Values with the same letter in each application period in the column are not significantly different ($p = 0.05$); ns: not significant; *significant at 5%; **significant at 1%, ***significant at 1‰.

Duration of application	Treatment GA_3	Chlorophyll content a + b (mg/g FM)		Fv/F0	
		2017	2018	2017	2018
Before planting	Control	1.30**b**	1.22**b**	0.73**c**	0.81**a**
	25 ppm	1.26**b**	1.20**b**	0.77**bc**	0.80**b**
	50 ppm	1.32b	1.24**ab**	0.80**b**	0.80**b**
	100 ppm	1.66**a**	1.26**a**	0.83**a**	0.82**a**
	200 ppm	1.41**ab**	1.21**b**	0.81**ab**	0.84**a**
June	Control	1.51**a**	1.41**a**	0.78**ab**	0.82**a**
	25 ppm	1.32**c**	1.34**b**	0.77**b**	0.81**a**
	50 ppm	1.36**b**	1.37**b**	0.79**a**	0.81**a**
	100 ppm	1.52**a**	1.48**a**	0.81**a**	0.82**a**
	200 ppm	1.36**b**	1.32**b**	0.79**a**	0.81**a**
March	Control	1.40**a**	1.32**b**	0.73**d**	0.82**a**
	25 ppm	1.34**b**	1.46**a**	0.76**c**	0.84**b**
	50 ppm	1.22**c**	1.41**a**	0.73**d**	0.82**a**
	100 ppm	1.31**b**	1.36**b**	0.84**a**	0.83**a**
	200 ppm	1.49**a**	1.44**a**	0.80**b**	0.84**a**
Source of variation	Treatment	0.241***	0.006ns	0.049ns	0.009ns
	Period	0.033*	0.208ns	0.001ns	0.004ns
	T * P	0.264***	0.092ns	0.022ns	0.018ns

[50] have reported that the Fv/Fm ratio is almost constant for many plant species under optimal conditions and is between 0.80 and 0.86.

3.3.2. Weight, Number, and Diameter of Corms Produced. The results showed that the treatment of saffron corms with GA_3 induced an increase in the total weight of daughter corms compared to the control, the analyses of variance confirmed that the effect of gibberellin and its period of application on the total weight of corms produced was significant ($p \leq 0.001$). The highest total weight (42.6 and 42.2 g/plot) was obtained for the application of 100 and 50 ppm GA_3, respectively, in March, and the lowest total weight (25.1 g/plot) was observed in the control. Likewise, the results showed a negative correlation ($R^2 = -0.98$) between the number and weight of daughter corms produced.

With respect to the diameter of daughter corms, treatment with GA_3 at low and high concentrations had the greatest effect on the percentage of large-diameter corms. These corms represent the most interesting category from an agronomic point of view, as it is responsible for the production of flowers and daughter corms in the coming years. According to analyses of variance, the effect of GA_3, application period, and interaction (GA_3 * period) was significant at levels of 0.001, 0.01, and 0.001, respectively (Table 6). The highest percentage of corms with large diameter was observed in the 25 ppm GA_3 treatment applied in March (29%), whereas the lowest percentages were obtained in the control with values of 0%, 5%, and 9%, respectively, for the preplanting, June, and March periods. Overall, large-diameter corms had the highest proportion in the 25 and 200 ppm GA_3 treatments.

The results of the coefficient of propagation of corms (CPC) showed that the application of gibberellin in any period improved this coefficient compared to the control. Concentrations of 25.50, 100, and 200 ppm GA_3 in June were able to produce heavier corms with increases of 46, 59.38, and 48%, respectively, over the control (Table 6). In order to get an idea of the relationship between the number of flowers and the weight of the corms, the ratio of the number of flowers produced to the total weight of corms (RFRPC) was calculated. This ratio is of great agronomic importance because a large proportion of the annually produced corms (small corms) does not have the capacity to produce flowers the following year. The most important values were obtained at the highest concentration of GA_3 (200 ppm) with values of 0.67, 0.73, and 0.90, respectively, for the periods before planting, June, and March, representing increases of 57, 48, and 24% compared to controls. These results confirm that GA_3-treated lots were able to produce large corms, which improved flower production.

Treatment with gibberellin has considerably increased the weight and diameter of the daughter corms. In addition, the diameter of corms per tuft was significantly ($p \leq 0.01$) and negatively correlated ($r = -0.88$) with the number of corms. The increase in the number of lateral buds and daughter corms in the control, for example, induced a decrease in the weight and diameter of corms. This last observation is in perfect agreement with the work of [51] on saffron. Thus, the results of previous research have shown that exogenous spraying of GA_3 in some bulbous plants inhibited the growth of lateral buds of mother bulbs, which gave rise to large replacement corms and, therefore, a better flowering yield [52, 53]. However, [54, 55] found that gibberellic acid had no effect on the number and weight of

Table 6: Effect of different GA_3 concentrations and duration of application on the total weight of corms, the percentage of large-diameter wire corms, the coefficient of corm propagation and the ratio of harvested flowers to total corm weight. Values with the same letter in each application period in the column are not significantly different ($p = 0.05$); ns: not significant; *significant at 5%; **significant at 1%, ***significant at 1%.

Duration of application	Treatment GA_3	Total weight of corms (g)	Large wire corms (%) CCF	The ratio of the weight of bulbs produced and bulbs planted (%) (CPC)	The ratio between the harvested flowers and the total weight of corms (RFRPC)
Before planting	Control	27.6**c**	0**e**	108.6**d**	0.68**b**
	25 ppm	26.3**c**	21**a**	114.3 **cd**	0.69**b**
	50 ppm	34.6**b**	10**c**	157.2**b**	0.67**b**
	100 ppm	30.4**b**	15**b**	126.6**c**	0.77**a**
	200 ppm	40.7**a**	15**b**	171.3**a**	0.67**b**
June	Control	25.1**c**	5**d**	114**d**	0.64**b**
	25 ppm	38.5**a**	22**a**	167.2**b**	0.40**d**
	50 ppm	38.2**a**	19**b**	182.7**b**	0.55**c**
	100 ppm	36.5**b**	12**c**	158.5**c**	0.63**b**
	200 ppm	38.9**a**	24**ab**	169.3**a**	0.73**a**
March	Control	30.8**c**	09**c**	130.2**c**	0.74**b**
	25 ppm	33.2**b**	29**a**	151.3**a**	0.66 **cd**
	50 ppm	42.2**a**	18**b**	136.9**b**	0.62**d**
	100 ppm	42.6**a**	15**b**	150.5**b**	0.67 **cd**
	200 ppm	34.3**b**	20**a**	162.7ab	0.90**a**
Source of variation	Treatment	676.9***	788.4***	894***	0.187***
	Period	176.2***	280**	166***	0.144***
	T ∗ P	444.5**	233.7***	563***	0.171**

suckers in black iris (*Iris nigricans* Dinsm.) and tuberose (*Polianthes tuberosa L.*). The increase in corm size and weight following the application of GA_3 was probably due to cell division and cell enlargement [56]. Similarly, translocation and accumulation of reserves, resulting from photosynthetic metabolism, from saffron leaves could be another possible reason for the increase in corm size and weight of the daughter corms in our case. In saffron, the weight and diameter of the corms are considered among the key indicators of yield, with daughter corms being able to flower only when they reach a base weight of 8 g [57–60]. Similarly, when the diameter of the corms reaches 3 cm, it can produce up to 6 flowers, while when the diameter reaches more than 4 cm, the number increases to more than 10 flowers/corms [61].

In terms of stigmata production, the highest concentrations of GA_3 had the highest values. This is certainly related to the weight of the corms produced in these treatments. The results of this study were consistent with those of [62–64], which showed that in saffron, there is a significant and direct correlation between the weight of mother corms planted and the number of flowers produced.

3.4. Analysis of Variance and Correlations. Analysis of variance showed a highly significant effect ($p > 0.001$) of hormone treatment (GA_3) and hormone application period (Period) for all traits studied, on total chlorophyll exception and photosynthetic activity Fv/F0. GA_3 ∗ Period interaction was also significant for all traits except for leaf log, total chlorophyll and Fv/F0 photosynthetic activity. These results confirm the existence of a remarkable effect on plant growth parameters and are in agreement with those found in other work on other species [53, 65].

The components of variance, which represent the contribution of each factor to the total variability, revealed that GA_3 treatment was the dominant factor for the majority of characteristics. In 2018, this represented between 5.4% of photosynthetic activity and 73.1% of the coefficient of corm propagation (Figure 3). A second factor of variability for stigmata-yielding traits and the majority of vegetative growth traits was the period of application. The GA_3 ∗ Period interaction had a more dominant influence on the flowering rate and on the total weight of daughter corms.

The differences between years, observed especially for parameters related to chlorophyll and flowering, would certainly be due to climatic factors and the biology of the saffron plant. Indeed, the chlorophyll level was significantly affected in the first year (2017) than in the second year (2018), when average temperatures during the vegetative phase were higher than in 2018. However, the climatic conditions do not seem to influence the qualitative treatment of saffron.

The increase in the number of flowers and thus the yield in stigmas as a function of time would be due to the increase in the number of corm threads capable of ensuring a flowering production in the years to come.

Apart from the relationships identified between characteristics of the same parameter, commented above, Pearson's linear correlation matrix showed several correlations between different measured parameters (Table 7). Indeed, a significant positive correlation ($p < 0.01$) was found between the rate of leaf appearance (TAF) and

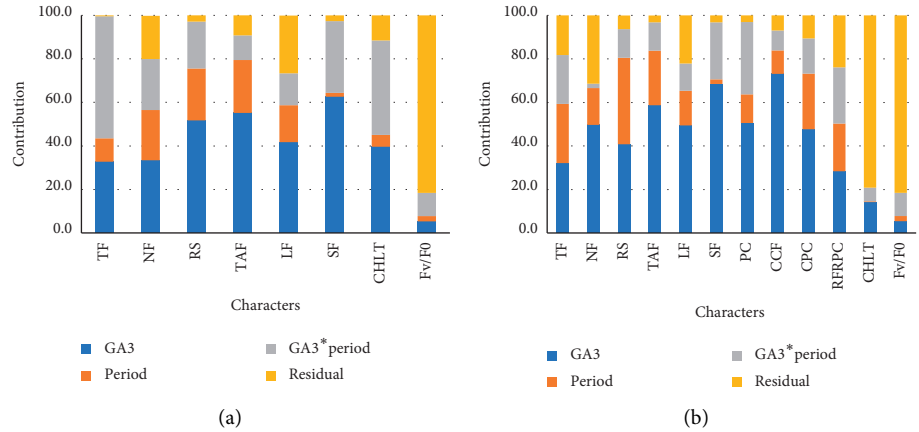

Figure 3: Contribution of factors to trait variances in the years 2016-2017 (a) and 2017-2018 (b). TF: flowering rate; NF: number of flowers; RS: stigma yield; TAF: leaf emergence rate; LF: leaf length; SF: leaf area; PC: corm weight; CCF: large daughter corms.

Table 7: Matrix of correlations between the different quantitative traits studied.

	TF	NF	RS	TAF	LF	SF	PC	CCF	CPC	RFRPC	CHLT	FVF0
TF												
NF	0.896*											
RS	0.901*	0.885*										
TAF	0.904*	0.992**	0.894*									
LF	0.944*	0.862	0.728	0.854								
SF	0.914*	0.987**	0.874	0.997**	0.881*							
PC	0.543	0.828	0.667	0.847	0.486	0.828						
CCF	0.699	0.925*	0.704	0.931*	0.706	0.928*	0.954*					
CPC	0.425	0.707	0.397	0.733	0.493	0.74	0.907*	0.919*				
RFRPC	0.87	0.644	0.644	0.618	0.899*	0.644	0.112	0.356	0.064			
CHLT	0.407	0.236	0.603	0.323	0.111	0.303	0.232	0.115	−0.033	0.134		
FVF0	0.407	0.236	0.603	0.323	0.111	0.303	0.232	0.115	−0.033	0.134	1.000**	

The colors red and green, respectively, indicate significant values at $p < 0.05$, $p < 0.01$. TF: flowering rate, NF: number of flowers, RS: stigmata yield, TAF: leaf appearance rate, LF: leaf length, SF: leaf area, PC: corm weight, CCF: large wire corms, CPC: ratio of weight of corms produced to weight of corms planted, RFRPC: ratio of harvested flowers to total corm weight, CHLT: total chlorophyll, and Fv/F0: chlorophyll fluorescence.

number of flowers (NF) ($r = 0.99$), leaf area (SF) and number of flowers (NF) ($r = 0.98$), leaf area (SF) and rate of leaf appearance (TAF) ($r = 0.99$), and total chlorophyll (CHLT) and photosynthetic activity (FV/F0) ($r = 1$). Similarly, positive correlations ($p < 0.05$) were noted between flowering rate (TF) and number of flowers (NF) ($r = 0.89$) and stigmata yield (RS) ($r = 0.90$) and leaf appearance rate (TAF) ($r = 0.90$). A positive correlation was also recorded between leaf area (SF) and percentage of large daughter corms (CWC) ($r = 0.92$) and coefficient of corm propagation (CPC) and total corm weight (PC) ($r = 0.90$) and percentage of large daughter corms (CWC) ($r = 0.91$).

These correlations between flowering parameters such as flowering rate and vegetative growth parameters such as leaf area and underground parameters underline the important role that photosynthetic organs and storage organs play in the precocity and productivity of saffron [20, 50, 57, 61].

4. Conclusion

Based on the results of the present study, it seems that soaking the saffron corms just before planting in a solution containing gibberellin reduced flower emergence time and significantly increased vegetative growth parameters such as number of leaves, leaf area, and total corm weight. The effect was all the more effective when the concentration of phytohormone increased. Moreover, the treatment of the corms with GA_3 during the month of March resulted in a significant increase in stigma yield, which is considered the most important parameter from an economic point of view, with a slight improvement in stigma quality.

In this respect, it is strongly recommended to adopt the treatment of mother corms with gibberellin, with concentrations higher than 25 ppm, as an agronomic technique to ensure a satisfactory production of stigmas and to increase the weight of daughter corms in the field.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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