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# Optimizing Saffron Flowering and Yield as Affected by Vernalization and Corm Size of *Crocus Sativus* L.

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**Declaration**

**Author's Contributions:** MA designed the experiment, SQAS performed the experiment and collected the data, HA and SJ, analyzed the data and prepared the manuscript, AN, MR, AH, SH and SA provided technical assistance during the experiment, MU, AU and IQ proofread the manuscript.
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## ABSTRACT

The absence of low temperature treatment leads to slow shoot growth in spring and results in flowering and various physiological disorders. Among several naturally occurring environmental factors, temperature is considered to play a predominant role in controlling proper growth and flowering in geophytes. Most of them require a "cold-warm" sequence to complete their annual cycle. Exposure of corms to vernalization is the most important physiological phenomenon and ecological factor governing variation of the vegetative apex into the reproductive one in saffron. Results findings showed that among different vernalization treatments, minimum days to emergence, highest emergence percentage, number of leaves plant-1, maximum leaf length (cm), number of cormels plant-1 were observed in saffron plants when corms were treated for 16 days vernalization while maximum number of sprouts corm-1, plant height (cm) and cormels weight (g) were recorded in saffron corms treated with 12 days vernalization. Among different corm sizes, minimum days to emergence, maximum emergence percentage, number of sprouts corm-1, average plant height (cm), number of leaves plant-1, leaf length (cm), number of cormels plant-1 and highest cormels weight plant-1 (g) were noticed in larger corm size (11g). It is concluded from the research findings that saffron corms of larger size when treated for 16 days vernalization could give better production.

## INTRODUCTION

Saffron botanically called (*crocus sativus* L) is commonly perennial plant belongs to family Iridaceae. The word saffron is derived from Arabic word Zafran which mean 'be yellow' (Winter and Straubinger, 2000) while the word crocus is a Greek word which is derived from Krokus mean

something like a string, fiber or horns like. The genus Crocus then has 85 species (Fernandez, 2004) but saffron (*Crocus sativus*) is most fascinating and intriguing species. It is propagated by vegetative corms (Kiran *et al.,* 2011) that serve as storage organa for winter survival or other adverse conditions (Babaei *et al.,* 2014). The species *Crocus staivus* L. is almost very expensive plant due its valuable part is stigma which has a huge range of usage as anti-carcinogenic agent, reduce levels of cholesterol in blood, combat against depression as well as alleviate arteriosclerosis (Melnyk *et al*., 2010). Saffron plant normally bears 5 to 11 true leaves that expand just after the flowers have opened. It has fragrant flowers, each flower has 3 corollas, 3 similar purple petals and 3 stamens in which red stigma is the edible portion of saffron which is known as commercial portion of saffron (Ghorbani, 2008). The flowers of saffron are sterile in nature which cannot produce viable seeds to re-germinate. Basically crocus corms can survives only for one season, and can able to produce ten or more than ten "cormlets" per mother corms which ultimately germinate when get appropriate cold temperature (Kaffi, 2002). Flowers normally appear just after sprouting but not necessary all the time. For better production, research have been done by focusing on different cold treatments, cultivation methods, irrigation, growing media and fertilization etc. (Unal and Cavusoglu, 2005). The production of daughter corms and the yield of saffron flowers were dependent on the size of the mother corms planted Kaushal *et al*. (2002). Depending on weather conditions, saffron can produce continually for eight to ten years (Kumar *et al.,* 2009).

Major producers of *Crocus sativus* are Greece, Iran, Morocco, Spain and India. Besides these, Italy, Turkey, Azerbaijan, Switzerland, Argentina, and Australia also produce outstanding amounts to the total world production of saffron (Fernandez, 2004). It has been estimated that saffron world production is 245 tons in which up to 93.7% of that is produced by Iran at an average of 4.6 kg/ha (Ghorbani, 2007). Iran area wise located in Middle East, and believed the largest saffron producer in the world, as producing up to three hundred and thirty six tons of saffron per year (Agricultural statistics, 2017). Based on arid and semi-arid climate of Iran, there are 6 Phonological stages in saffron plant; the first one is dormancy period starting from late of May until late of Oct. month which is divided into 2 phases, primary dormancy and secondary dormancy. During vegetative stage of saffron formation of daughter corms starts and early growth of plant starts from late of November until late of December. In forth stage daughter corm middle growth from late of December until late of first month of new year (January). Moreover, in fifth stage the daughter corms final growth starts from late of January until late of March and finally root growth cessation and initiation of dormancy phase begins again from late of March until late of May (Renau *et al.,* 2012). However, these stages in saffron plant can be affected by agronomic or cultural practices and sometime by environmental conditions (Koocheki, *et al.,* 2016).

In temperate regions, most of the annuals, biennial and perennial plants require naturally certain cold duration as an environmental signal for initiation of flowering known as vernalization because these species need to experience a period of cold winter to overcome a block to flowering. Vernalization treatment show positive response only when temperature is around or above freezing while below freezing point have negative effect on plant growth and reproductive stages because vernalization is an active process which requires changes in gene expression when plant are expose to cold treatment (Sung and Amasino, 2005; Amasino, 2004). The current project was designed by keeping in view the commercial importance of saffron, the role of vernalization and corm sizes on its production.

## MATERIALS AND METHODS

The experiment was conducted on effect of vernalization and corm size on flowering and production of saffron (*Crocus sativus* L.) at Ornamental Nursery, Department of Horticulture, The University of Agriculture Peshawar during 2017-18. The experiment was laid out in randomized complete block design (RCBD) with two factors factorial. Treatments were replicated three times. Three different sizes ($S_1$= Large (11g), $S_2$= Medium (7g) and $S_3$= Small (>3-5g)) of corms were subjected to low temperature (7°C) for

different times ($V_0$= Control (untreated), $V_1$=4, $V_2$= 8, $V_3$= 12 and $V_4$= 16 days (Figure 1).

**Media Preparation**

Media was comprised of leaf mould, well decomposed farm yard manure (FYM) and silt in 1:1:2 such that leaf mould and FYM was used of equal amount while fresh silt amount was used double of the amount of leaf mould and FYM. Corms were treated in such a way that all the corms were separately soaked for 10-15 minutes with fungicide to kill the entire microorganism and avoid corms rotting. All the cultural practices during the course of experiment were done uniformly. The saffron corms were sown on 23, October-2017. After sowing of corms sprinkler irrigation method without disturbing any corms. Weeding was done where needed. Common weeds flora observed were dandelion, malvaparviflora, bermuda grass and lambs quarter. Soil upper layer was softened time to time while hoeing to make it soft and easier for corms sprouting. The soil samples were tested in "Soil and water testing laboratory model farm service Centre Mansehra" and the results are tabulated as follows:

**Table 1**

*Soil analysis*

| pH | EC(dSm$^{-1}$) | OM% | Sand% | Silt% | Clay% | Texture class | K (ppm) |
|---|---|---|---|---|---|---|---|
| 8.02 | 3.17 | 1.20 | 6.9 | 56 | 5% | Silty loam | 148 |

**Parameters Studied**

During the experiment, the following parameters were studied while selecting five plants from each treatment in each replication.

**Days to Plant Emergence**

Data regarding plant emergence was recorded on randomly selected 5 plants from date of sowing till emergence of plants from each treatment in every replication. Then their average was recorded.

**Emergence Percentage (%)**

Data on emergence of corms were recorded from all the treatments in each replication and there percentage was calculated.

**Number of Sprouts Corm$^{-1}$**

Five plants from each treatment in every replication were randomly selected and numbers of sprouts corm$^{-1}$ were counted then their average was calculated.

**Plant Height (cm)**

Plant height of saffron was calculated from bottom of plant to tip by using measuring tap when the plants reach its maximum growth stage and average was taken.

**Number of Leaves Plant$^{-1}$**

Number of leaves was counted from each treatment in all replication and their average was calculated.

**Leaf Length (cm)**

Leaf length was calculated with the help measuring tap, the leaf length was taken from its base to its tip from five plants in each treatment and each replication and their mean was worked out.

**Leaf Fresh Weight (g)**

Data were recorded from fresh leaves taken from all treatments in each replication through digital weight balance in Horticulture laboratory and its average was determined.

**Leaf Dry Weight (g)**

Leaves of five plants were randomly collected from all the treatment and were oven dried for 24 hours at 70ºC and then weighed through digital weight balance in Horticulture lab.

**Number of Cormels Plant$^{-1}$**

When the plants leaves were completely dried then corms were uprooted and kept in dry, shady place. Soil was removed from corms, numbers of cormels plant$^{-1}$ were counted and there average was calculated.

**Cormels Weight Plant$^{-1}$ (g)**

All the cormels were weighed through digital weight balance from all treatment in each replication and their average was calculated.

## RESULTS AND DISCUSSION

**Days to plant emergence**

Statistical analysis of data shows that vernalization period (Figure 2) and corms size (Figure 3) significantly affected days to plant emergence in saffron while their interaction (Table 2) was found non-significant. Minimum days to emergence (19.57) were noted in corms treated with 16 days vernalization followed by days to emergence (21.58) recorded in corms treated with 12 days vernalization which were statistically at par with (22.80) recorded at 8 days vernalization, while

maximum days to emergence (27.0) were recorded in untreated corms. Data regarding corm size shows that minimum days to emergence (20.39) were observed in plants have large size corms followed by days to emergence (23.29) recorded in plants of medium size corms, while maximum days to emergence (25.96) was recorded in plants have small size corms.

Early emergence is desirable leading to early growth and development. Vernalization treatment resulted in early emergence of corms as compared to untreated corms. From the above results it was concluded that higher the vernalization temperature earlier will be the plant emergence. The results are also in line with the findings of Amir *et al.* (2013) who also reported early emergence of vernalized onion bulbs as compared to untreated onion bulbs. Kabir *et al.* (2008) reported more number of days to emergence in non-vernalized bulbs as compared to vernalized bulbs. Corm size also affected days to emergence. Bigger corm size resulted in early emergence of corms that might be due to more reserved food material present in large sized corms as compared to medium or small size corms. The results are in conformity with the findings of Samira *et al.* (2015) they also found that bigger size bulbs took least days to emergence as compared to smaller bulbs.

**Emergence Percentage (%)**

Statistical analysis showed that emergence percentage was significantly affected by vernalization (Figure 2) and corm sizes (Figure 3), whereas the interaction (Table 2) between vernalization and corm size was non-significant. Maximum emergence percentage (90.00%) was recorded in plants treated with 16 days vernalization treatment that was statistically at par with emergence percentage recorded in plants treated with 12 days of vernalization. Minimum emergence percentage (81.78%) was observed in control. Regarding corm size revealed that highest emergence percentage (95.79%) was noted in plants have larger size corms, followed by emergence percentage (85.52%) observed in plant have medium corms size, while lowest emergence percentage (77.70%) was observed in plants have small size corms.

Findings regarding vernalization treatment showed that almost 90% emergence percentage was observed when vernalization was increased up to 16 days to saffron corms. The results of current experiment are in conformity with the findings of Ami *et al.* (2013) who observed that increase in vernalization treatment resulted in an increase in emergence percentage. Emergence percentage was directly affected by corm size. Larger corms showed higher emergence percentage. It is one of the most important trait that determining the density of plants in a field as highest emergence percentage results in maximum production (Kochaki *et al.*, 2012).Current research work are in line with the findings of Andabjadid *et al.* (2015) who reported that bigger corm size have more emergence percentage as compare to smaller ones.

**Number of Sprouts Corm$^{-1}$**

Statistical analysis shows that different corms size (Figure 3) significantly affected saffron sprouts corm$^{-1}$ while different vernalization treatment and their interaction (Table 2) were found non-significant. Data pertaining to corm sizes showed that larger corm size resulted in maximum number of sprouts corm$^{-1}$ (5.56) followed by number of sprouts corm$^{-1}$ (4.84) observed in medium size corm, while minimum number of sprouts corm$^{-1}$ (3.75) was noted in smaller size corms.

The results showed that vernalization had no significant effect on sprouts corms$^{-1}$. According to the findings, corm sizes had significant effect on number of sprouts per corm in saffron plants as compared to small size corms. It might be due to the fact that larger corms have more food material for developing more sprouts corms$^{-1}$. The current findings of our experiment are in line with the findings of Eldin *et al.* (2013) who reported that corms with larger size had the ability to produce maximum sprouts.

**Plant Height (cm)**

The statistical analysis of the data showed that different treatment of vernalization (Figure 2) and corms size (Figure 3) significantly affected plant height of saffron while their interaction (Table 2) was found non-significant. Maximum average plant height (26.74 cm) was recorded in corms treated with 12 days vernalization, followed by plant height (25.20 cm) recorded in corms treated with 8 days vernalization treatment, while minimum plant height (21.26 cm) was observed in untreated corms. Regarding corms sizes, highest plant height (26.69 cm) was noted plants have larger size, which is statistically different from average plant height (23.83 cm), was observed in

plants with medium size corms, while minimum average plant height (21.77 cm) was recorded in smaller size corms.

The results showed that vernalization of corms for 12 days resulted in tallest plants. These results are supported by findings of Abreu (2000), who found that increasing the vernalization time to certain extend resulted in increase in plant height while further increase in vernalization timing up to three weeks reduced plant height in lilies. Rodrigues *et al*. (2010) found that when bulbs of Asian lilies were exposed to cold treatment for longer period of vernalization eventually reduced plant height. Bigger corms size has more reserved food, required for proper growth and development of plant. Hassnain *et al.* (2020) also stated that more availability of resources contributes to improved growth parameters like plant height. The results of experiment showed that in larger corms size, maximum plant height was observed as compared to medium and small corms. The findings of current research work are in line with findings of Takayama (1990) who reported the tallest plants from larger size corms in *Lilium auratum* L.

**Number of Leaves Plant$^{-1}$**

Different treatment of vernalization (Figure 2) and corms size (Figure 3) as well as their interaction (Figure 4) significantly affected number of leaves plant$^{-1}$in saffron. Maximum number of leaves plant$^{-1}$ (25.50) were noted in plants treated with 16 days vernalization, followed by number of leaves plant$^{-1}$ (23.13) observed in plants received 12 days vernalization treatment, while minimum number of leaves plant$^{-1}$ (17.17) were observed in control. Regarding corms size, highest number of leaves plant$^{-1}$ (23.69) were recorded in plants having larger corms size followed by number of leaves plant$^{-1}$ (21.55) noted in plants with medium size corms, while lowest number of leaves plant$^{-1}$ (18.64) were observed in plants having smaller corms. In case of interaction maximum number of leaves plant$^{-1}$ (28.21) were noted in large size corm when treated with 16 days vernalization, while minimum number of leaves plant$^{-1}$ (15.10) were observed in untreated small size corms.

Vernalization treatments resulted in increased number of leaves plant$^{-1}$. Saffron plants treated with vernalization up to 16 days resulted highest number of leaves plant$^{-1}$. The results are in agreement with the findings of Mahfoozi *et al*. (2001) who reported that vernalization treatment increased number of leaves. Paulo *et al*. (1979) also found that in gladiolus corms treated with vernalization at 4 $^{0}$C for two weeks increased the number of leaves. Among different corms sizes bigger corms had more number of leaves. It might be because of more availability of food reserves present in mother corms. Kaushal *et al*. (2002) found more production of leaves in larger corms in relation to corms weight.

**Leaf Fresh and Dry Weight (g)**

The statistical analysis showed that different vernalization treatments and corm sizes as well as their interaction (Table 2) had non-significant effect on leaf fresh weight that might be because of low temperature can inhibit internal leaf structure with ultimately decrease it fresh weight. Analysis of the data showed that leaf dry weight (g) of saffron was not affected by different treatment of vernalization, corm sizes and their interaction it may be due to low temperature can decrease leaf dry weight of saffron plants.

**Table 2**

*Number of sprouts, fresh leaf weight, dry leaf weight and all interactions of saffron as affected by vernalization and corm sizes*

| Vernalization periods (V) (Days) | Attributes | | |
|---|---|---|---|
| | Number of sprouts | Fresh leaf weight (g) | Dry leaf weight (g) |
| 0 | 4.81 | 0.131 | 0.031 |
| 4 | 4.80 | 0.135 | 0.033 |
| 8 | 4.62 | 0.130 | 0.031 |
| 12 | 5.00 | 0.132 | 0.032 |
| 16 | 4.37 | 0.130 | 0.033 |
| LSD (P≤0.05) | NS | NS | NS |
| **Corm sizes (C)** | | | |
| Large | 5.56a | 0.133 | 0.033 |
| Medium | 4.84b | 0.130 | 0.032 |
| Small | 3.75c | 0.132 | 0.032 |
| LSD (P≤0.05) | 0.435 | NS | NS |
| **VxC Interactions (P≤0.05)** | | | |
| Days to plant emergence | NS | Leaf length | NS |
| Emergence percentage | NS | Fresh leaf weight | NS |
| Number of sprouts | NS | Dry leaf weight | NS |
| Plant height | NS | Number of cormels | NS |
| Number of leaves | ** (Fig 3) | Cormel weight | NS |

*Means followed by similar letters are significantly at par with each other at 5% level of significance*
*NS: Non-significant*

***: Significant at 5% level of significance*

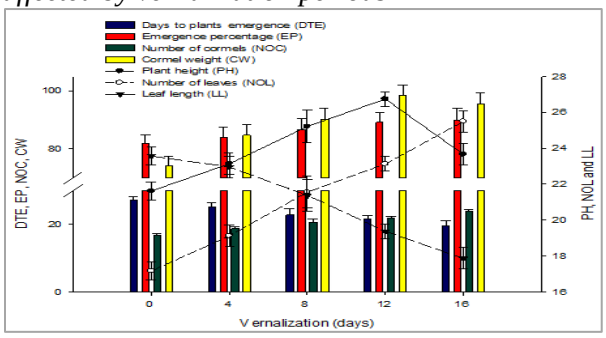
**Leaf length (cm)**

The statistical analysis showed that different vernalization treatments (Figure 2) and corm sizes (Figure 3) significantly affected leaf length (cm) of Saffron, while their interaction (Table 2) was found non-significant. Maximum leaf length (23.57 cm) was observed in control that was statistically similar with leaf length (22.96 cm) noted in plants that received 4 days vernalization treatment, while minimum leaf length (17.87 cm) was recorded in plants treated with 16 days vernalization. In case of corms size highest leaf length (23.59 cm) was noticed in plants have smaller corms size statistically different from leaf length (20.58 cm) observed in plants have medium size corms, while minimum leaf length (18.91 cm) was noted in larger size corms.

Result showed that vernalization had reduced the length of leaves in saffron plant. Current findings are in line with that of Aysun *et al*. (2010); they studied that cold treatment for extended period resulted in reduced leaf length. Mzabri *et al*. (2017) also found that low temperature treatment for longer time decreased the length of leaves in saffron. Among different corms sizes, smaller corms sizes have maximum leaf length but the leaves were thin and weak as compared to larger one. Mahmoud *et al*. (2013) also observed relatively smaller and thick leaves in plants produced from larger corms as compared to small size corms.

**Figure 2**

*Days to emergence, emergence percentage, plant height, number of leaves, leaf length, number of cormels plant$^{-1}$ and cormels weight of saffron as affected by vernalization periods*



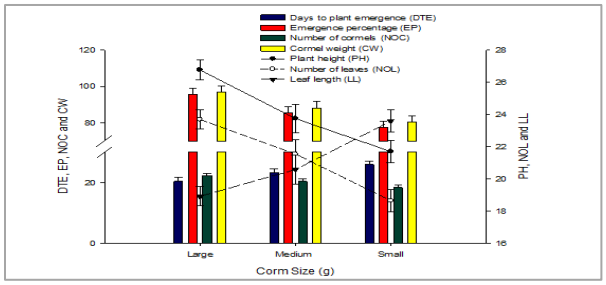

**Number of Cormels Plant$^{-1}$**

The analysis of variance showed that there were significant differences among the means of different treatment of vernalization (Figure 2) and corm sizes (Figure 3), while their interaction between vernalization and corm size (Table 2) was found non-significant. The mean data shows that among different treatment of vernalization, maximum number of cormels (23.73) plant$^{-1}$ were observed in plants treated with 16 days vernalization statistically followed by number of cormels (21.84) plant$^{-1}$ were recorded in 12 days vernalization treatment, while minimum number of cormels (16.75) plant$^{-1}$ were noted in control. Regarding corms sizes, maximum number of cormels (22.28) plant$^{-1}$ were recorded in plants having larger corms , followed by number of cormels (20.37) plant$^{-1}$ observed in medium size corms, while minimum number of cormels (18.37) plant$^{-1}$ were noticed in plants with smaller size corms.

Vernalization had positive influence on number of cormels in saffron. An increase in cold treatment resulted in increased number of cormels. The highest cormels in saffron were found in plants treated with 16 days vernalization. The current findings are in agreement with the findings of Hopkins, (1999), who reported that gladiolus corms exposed to low temperature consequently resulted in increased number of daughter corms and cormels. Corm size had positive effects on production of daughter corms hence bigger size corms had more number of cormels in saffron plant. Omidbaigi *et al*. (2002) reported that in saffron plants maximum numbers of cormels plant$^{-1}$ were obtained when plants were grown from bigger corms as compared to smaller ones. The current results are also in line with findings of Sadeghi (1994) who observed that saffron corms with bigger size had the ability to increase yield and produce more number of cormels. Our findings are also in agreement with the findings of Pandy and Srivastava (1979) who found that corms having larger size produced maximum number of cormels as compare to smaller size corms.

**Figure 3**

*Days to emergence, emergence percentage, plant height, number of leaves, leaf length, number of cormels and cormel weight of saffron as affected by different corm sizes*



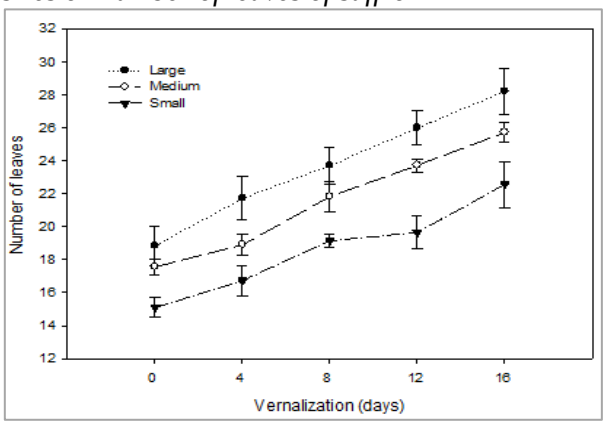

**Cormels Weight (g) Plant$^{-1}$**

The statistical results showed that different treatment of vernalization (Figure 2) and corm sizes (Figure 3) significantly affected cormels weight while their interaction (Table 2) was found non-significant. Maximum cormels weight plant$^{-1}$ (98.43 g) was observed in corms treated with 12 days vernalization, that was statistically similar with cormels weight (95.45 g) plant$^{-1}$ recorded in corms treated with 16 days vernalization, while minimum cormels weight (74.27 g) plant$^{-1}$ was recorded in control (untreated corms). Data regarding corms size showed that maximum cormels weight (97.24 g) plant$^{-1}$ was observed in plants have larger corms size followed by cormels weight (88.23 g) plant$^{-1}$ noted in plants have medium size corms. Whereas minimum cormels weight (80.61 g) plant$^{-1}$ was recorded in plants have smaller size corms.

Vernalization had positive effect on weight of cormels. Among various vernalization treatments maximum cormels weight plant$^{-1}$ was found in 12 days vernalization treatment. The results are in line with the findings of Alysun, (2010) who found that increasing time of vernalization resulted in an increase in weight of cormels while further increase in vernalization beyond level caused decline in weight of cormels. Bigger size corms increase maximum cormels weight (g) as compare to small size corms it may be due to small size corms are not able to produce flower and with maximum weight of daughter corms. The current research results are matched with the findings of Mashayekhi, (1998) they reported that big size had more food and are able to produce bigger (weight g) daughter corms because small corms are not able to produce optimum flower and daughter corms with maximum weight.

**Figure 4**

*Interactive effect of vernalization period and corm sizes on number of leaves of saffron*




## CONCLUSIONS

Saffron of large corms size, and vernalization treatment of 12 and 16 days significantly influenced most the studied attributes i.e. days to emergence, emergence percentage (%), number of leaves plant$^{-1}$, cormels plant$^{-1}$ were observed while, maximum number of spouts corm$^{-1}$, plant height (cm), cormels weight (g) plant$^{-1}$ except leaf fresh weight, leaf dry weight, leaf length (cm). Corms obtained from current research work were not able to produce flowers in saffron plant under various vernalization treatments. Saffron with large corm size treated for 16 days vernalization could be recommended to retain maximum growth and cormels production in saffron.

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