African Journal of Biotechnology Vol. 8 (4), pp. 601-604, 18 February, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

Full Length Research Paper

Stigma variability in saffron (*Crocus sativus* L.)

Seyed Mahmood Ghaffari[1]* and Abdolreza Bagheri[2]

[1]Institue of Biochemistry and Biophysics, Uninersity of Tehran, P. O. Box 13145-1384, Tehran, Iran.
[2]Ferdowsi University of Mashhad, College of Agriculture, Mashhad, Iran.

Accepted 11 July, 2008

The obstacle to improving *Crocus sativus* is its sterility caused by being triploid. Thus, the discovery of the new variant of saffron with increased number of stigmas was welcomed as a reason for improving its yield. The study of development and the process of budding of the corm of saffron showed that these flowers occur by fusion of two or more flowering buds on one corm. Cytological and morphological studies showed that this characteristic is unstable and is not genetically controlled.

Key words: Chromosome count, ***Crocus sativus***, saffron, stigma, triploid.

INTRODUCTION

Archeological and historical sources indicate that saffron (***Crocus sativus*** L., ***Iridaceae***) cultivation is a very old dating back to 2500 – 1500 BC, probably originated in Iran, Asia Minor or Greece and later became widespread in India, China, the Mediterranean basin and Eastern Europe (Tammaro 1987; Negbi 1999; Grilli Caiola et al., 2004). One of the world's most expensive spice, is the dry style and red-orange-colored stigmas of the saffron. In Iran, saffron is grown in the eastern part of the country, primarily in Khorasan province. Saffron is used mainly as a dye in industry, as a spice in cooking, as a food colorant, and as a component of drugs and perfumes (Mathew, 1982; Basker and Negib, 1983; Bowles, 1985; Francis, 1987; Behnia et al., 1999). Recently saffron extract has been successfully tested as an anticancer agent (Nair et al., 1991; Escribano et al., 1996; Abdullaev, 2004, 2007).

According to Karasawa (1933), saffron is a triploid species (2n = 24) whose chromosomes at metaphase form eight trivalents, indicating a probable origin from a diploid ***Crocus***. Ghaffari (1986) also considers ***C. sativus*** to have been derived from a sterile autoploid, whose sterility is due to its triploidy, combined with laggard chromosomes, nondisjunctions and rare inversion. According to Mathew (1982), ***C. cartwrightianus*** is the species most similar to ***C. sativus*** from morphological and cytological point of view. He considers it more probable that ***C. sativus*** originated from ***C. cartwrigh-tianus*** by autotriploidy and then it was selected by man for its stigmas which are used to produce saffron. However, recent quantitative and qualitative DNA analy-sis (Brandizzi and Grilli Caiola, 1996, 1998) indicated that DNA composition of ***C. sativus*** is more similar to that of ***C. cartwrightianus***. Grilli Caiola et al. (2004) also indicated that ***C. sativus*** is very closely related to ***C. carrtwrightianus*** by RAPD analysis.

Today saffron is an exceedingly expensive product, mainly because the crop does not lend itself to harvesting by mechanical means. The normal flowers have three stigmas, three anthers and six perianth parts. The diversity of variants of saffron with an increased number of stigmas has been reported by Aghamohammadi (1977) and Estilai (1978). Estilai assumed that this variability could be due to develop-mental abnormalities, to chromosomal variation and/or to a rare gene mutation. He did not investigate these possibilities, although he did determine the chromosome number of four of the rare plants by examining root tips, and found that the chromosome number was 2n = 24, as for normal saffron (Brighton, 1977; Chichiricco, 1984; Ghaffari, 1986; Chen and Kondo, 1990). In this paper the reasons for the variability are investigated.

MATERIALS AND METHODS

Eighteen farms with total size of about 18490 m^2 were selected randomly for collection and recognition of abnormal flowers in Bajestan and Gonabad region in Khorassan Province from 2002 to 2003 (Table 1). Collection of abnormal flower was carried out for

*Corresponding author. E-mail: ghaffari@ibb.ut.ac.ir. Tel: +98-21-66498819. Fax: +98-21-66404680.

Table 1. Investigated saffron farms.

No. of Farms	Size (m^2)	No. of stigmas						
		2	4	5	6	7	8	9
1	1200	0	16	8	5	0	0	0
2	1200	0	62	36	12	0	1	0
3	600	0	23	9	3	1	0	0
4	1320	5	42	35	20	2	0	2
5	870	1	5	9	6	2	0	0
6	1200	0	11	5	5	0	0	0
7	1500	10	50	60	30	10	5	2
8	600	0	12	2	1	0	0	0
9	1200	2	5	2	1	0	0	0
10	1000	0	19	18	6	0	0	0
11	1300	0	35	15	9	0	0	0
12	1700	0	44	16	7	0	0	0
13	750	0	10	2	0	0	1	0
14	1400	0	11	14	3	0	0	0
15	1100	0	9	6	2	0	0	0
16	800	0	11	0	0	0	0	0
17	1700	0	43	38	12	0	0	0
18	750	1	19	24	15	1	1	1
Total 18	18490 (m^2)	19	395	299	137	16	8	5

the determination of their frequency by the beginning of autumn for up to 35 days.

Abnormal flowers were separated according to number of stigmas and stored in different boxes for statistical analysis. In addition, the place of abnormal flowers with number of floral parts in the field was marked to recognise next year abnormal situation of flowers.

Some plants were transferred to pots and taken to the laboratory so that development of flower buds could be followed. For the study of mitotic chromosomes, the basal parts of young leaves of some of the abnormal plant were pretreated with saturated solution of *α*-bromonaphthalene for 7 h at 4^{o}C. They were then fixed in piennar's solution (6 parts absolute alcohol, 3 parts chloroform and 2 parts propionic acid) for 24 h and stored in 70% ethanol.

Staining was carried out by the Feulgen reaction enhanced by squashing in two percent acetocarmine. Photographs of abnormal plants were taken in the field and drawings were made from them.

RESULTS AND DISCUSSION

In all farms, normal flowers were mostly found with three branched stigma and the abnormal flowers with 2, 4, 5, 6, 7, 8 and 9 branches stigma were rarely found. According to the investigation of Estilai (1978), from an estimated 36 million flowers screened, 43 plants had abnormal number of stigmas. The frequency of abnormal flowers which were treated with different management varied. For example, the quantity of flowers with 4 stigmas in farms No. 9 and 2 were minimum of 5 and maximum of 62, respectively. Also, the quantity of flowers with 6 branched stigma in farms No. 9 and No 7, were minimum of 1 and maximum of 30, respectively (Table 1).

The yield rate of normal flowers showed that the weight of 1000 stigmas including styles in normal flowers was 7.50 g. But for the abnormal flowers with 4, 5, 6 and 7 branches in stigmas was 10.88, 14.28, 17.11 and 17.50 g respectively. This comparison shows that with increasing number of branches in stigma, the yield increases up to 133% (Table 2). If there was a correlation between the above trend and genetic variability, the stigma yield of saffron would also increase.

The study of development and process of budding of the corm in a few plant showed that frequently one or more large and some small buds grow on and around the tip of the corm, but only the large buds flower. When the large buds are close enough together, two or more flowers grow from one spathe (Figure 1). In the case of the abnormal flowers, the flowering buds merge completely so that the pedicels and floral parts combine with each other. In some cases the point of fusion of the pedicels was seen as an apparent groove. Also the study of ovary in the abnormal flowers showed that the ovaries of the abnormal flowers, despite their unity, had retained their own independent outer layer (Figure 2). When two or more flowering buds combine, parts of the flowers unite, so that in the resulting flowers the floral parts are not always present in multiples of three (Table 3).

Table 1 shows the frequency of flowers with 4 stigmas (395), 5 stigmas (299) and 6 stigmas (137), that their occurrence by fusion of two flowering buds. This phenomenon is observed more than occurrence of fusing in other stages (fusion of 3 or more bud flowers).

When the abnormal plants were observed the following

Table 2. Comparison of yield in normal and abnormal saffron flowers.

No. of stigmas	Weight of 1000 stigmas + styles (g)	Percent increase in yield as compared with normal (3 stigmas) flower
3	7.50	-
4	10.88	45
5	14.28	90
6	17.11	128
7	17.50	133

Figure 1. Two normal flowers in one spathe.

Figure 2. Normal (A) and rare (B) flower with six branches in stigma (ovaries are shown by arrows).

year, it was found that the abnormality had not been maintained and all flowers were normal. Moreover, both abnormal and normal flowers were seen on the same corm in rare cases (Figure 2). The above observations

Table 3. Variation in number of floral parts when combined from two flowers.

Flower	Number of floral parts		
	Stigmas	Anthers	Perianth
Normal flower	3	3	6
Two flowers	4	5	10
combination	4	4	8
	4	5	8
	4	4	9
	4	5	9
	4	5	11
	5	5	11
	5	6	10
	5	6	11
	5	4	8
	5	5	12
	5	5	10
	5	5	9
	5	5	8
	5	4	9
	6	5	12
	6	4	10
	6	6	11
	6	5	10
	6	4	9
	6	5	8
	6	6	10

show that the observed variability in saffron is not of genetic origin, and only occur by combination of two or more flower buds.

Chromosome counts were made by Estilai (1978) on the roots of the corm bearing abnormal flowers. Since the chromosome number found could not be regarded as the reason for chromosomal variation in the abnormal bud, we used meristem cells of young leaves of abnormal flowers.

The result of cytological experiments showed that no change in the chromosome number of the abnormal plants had occurred, and that all the cells had 2n = 24 chromosomes (Figure 3). Therefore it is clear that this variability has no genetic basis and it is not a stable and

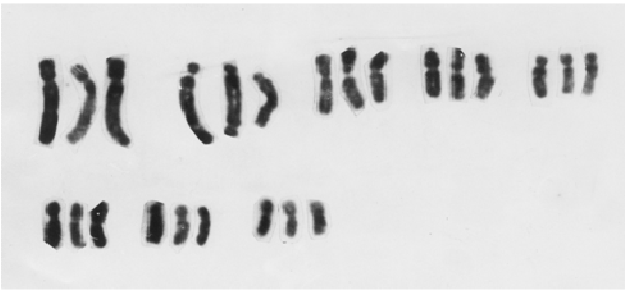

Figure 3. Karyotype of autotriploid saffron (2n = 3x =24) with six branched stigma. Initial magnification 1000 X.

repeatable event. Usually, such abnormal plants are found in the fields which have received plenty of compost. Since sterility has precluded traditional plant breeding effects to increase saffron yield, one expect considerable research on cultural practices and the application of fertilizer to improve productivity of saffron. Unfortunately, such studies are scarce (Behzad et al., 1992a, b; Behnia et al., 1999; Jahan and Jahani, 2007; Rezaian and Paseban, 2007; Rezvani et al., 2007).

ACKNOWLEDGEMENTS

This work was supported by grant (6401011/1/03) from research council of University of Tehran, and Iranian Research Organization for Science and Technology (Center of Khorasan).

REFERENCES

Abdullaev F (2004). Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention. Cancer Det. Preven. 28(6): 426-432.

Abdullaev F (2007). Biological properties and medical use of saffron (Crocus sativus). Acta Hort. 739: 339-345.

Aghamohammadi Z (1977). An investigation of asexual reproduction, induction of variability of mutagens and sexual sterility of saffron (***Crocus sativus***) as measured by pollen stainability and pollen germination in relation to chromosome paring. M.Sc. thesis, University of Tehran. Tehran, Iran.

Basker D, Negib M (1983). Uses of saffron ***Crocus sativus***. Econ. Bot. 37: 228-236.

Behzad S, Razavi M, Mahajeri M (1992a). The effect of various amount of ammonium phosphate and urea on saffron production. Acta Hort. Wager. 306: 337-339.

Behzad S, Razavi M, Mahajeri M (1992b). The effect of mineral nutrients (N.P.K.) on saffron production. Acta Hort. Wagen. 306: 426-430.

Behnia MR, Estilai A, Ehdaie B (1999). Aplication of fertilizers for increased saffron yield. Agron. Crop Sci. 182: 9-15.

Bowles EA (1985). Crocus and Colchicum. Waterstone, London, UK.

Brandizzi F, Grilli Caiola M (1996). Quantitative DNA analysis in different Crocus species (Iridaceae) by mean of flow cytometry. Giornale Bot. Italiano 130: 643-645.

Brandizzi F, Grilli Caiola M (1998). Flow cytometric analysis of nuclear DNA in ***Crocus sativus*** and allies (Iridaceae). Plant Syst. Evol. 211: 149-154.

Brighton CA (1977). Cytology of Crocus sativus and its allies (Iridaceae). Plant Syst. Evol. 128: 137-157.

Chichiricco G (1984). Karyotype and meiotic behaviour of the triploid ***Crocus sativus*** L. Caryologia 37: 233-239.

Chen KY, Kondo K (1990). Chromosome identification in triploid saffron by C-banding. Chromosome Info. Serv. 48: 25-27.

Escribano J, Alonso GL, Coca-Prados M, Fernandez JA (1996). Crocin, safranal and picrocrocin from saffron (***Crocus sativus*** L.) inhibit the growth of human cancer cells ***In vivo***. Cancer Lett. 100: 23-30.

Estilai A (1978). Variability in saffron (***Crocus sativus***). Experientia 34: 725.

Ghaffari SM (1986). Cytogenetic studies of cultivated ***Crocus sativus***. (Iridaceae). Plant. Syst. Evol. 153: 199-204.

Grilli Caiola M, Caputo P, Zaier R (2004). RAPD analysis in ***Crocus sativus*** L. accession and related Crocus species. Biol. Plant. 48(3): 375-380.

Jahan M, Jahani M (2007). The effect of chemical and organic fertilizers on saffron flowering. Acta Hort. 739: 81-86.

Karasawa K (1933). The triploidy of ***Crocus sativus*** L. and its high sterility. Jpn. J. Genet. 9: 6-8.

Mathew B (1982). The Crocus. A revision of the genus Crocus (Iridaceae). Batsford, B.T. Ltd., London.

Negbi M (1999). Saffron cultivation: past, present and future prospects. In: Negbi M (ed.): Saffron ***Crocus sativus*** L. Harwood Academy Publ. Amsterdam. pp. 19-30.

Nair SC, Pannikar B, Pannikar KR (1991). Antitumour activity of saffron (***Crocus sativus***). Cancer Lett. 57: 109-114.

Rezaian S, Paseban M (2007). The effect of micronutrients and manure fertilizers on the quantity and quality of ***Khorasan saffron***. Acta Hort. 739: 155-158.

Rezvani-Moghaddam P, Mohammad-Abadi AA, Sabori A (2007). Effect of different animal manure on flower yield and qualitative and quantitative characteristic of forage production of saffron (***Crocus sativus***) in Mashhadconditions. Acta Hort. 739: 159-163.

Tammaro F (1987). Notizie storico-colturali sullo zafferano (***Crocus sativus*** L., Iridaceae) nell, area mediterranea. Micol. Veget. Medit. 2: 44-59.