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Saffron (*Crocus sativus* L.) Strategies for Enhancing Productivity

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ABSTRACT

It is the member of Iridaceae family (Liliales, monocots) whose genomes are relatively large and are poorly characterized. Among the 85 species belonging to genus *Crocus*, saffron is the most fascinating and intriguing species. Saffron, *Crocus sativus* L., is a sterile triploid plant that is naturally propagated by daughter corms developed on mother corms. The intense orange color of saffron hints of its medical nature. Saffron is known for its possible therapeutic effect on cancer, recently received scientific recognition as a potential source of new medicines. The principal pigment of saffron is crocin, safranal, glycoside picrocrocin, crocetin, besides these, a new class of defense chitinase namely *Safchia* A has recently been isolated from saffron. There has been little success in enhancing the levels of these bioactive molecules of commercial importance. It has been observed that this plant represents variegated blend of genetically heterogeneous forms-clones and it is possible to create a new high yielding cultivars of this plant on the basis of clonal selection, mutation and polyploidy. The decrease of land surface dedicated to saffron crop in many areas has possibly resulted in corresponding genetic erosion that adds up to the limited genetic variation suspected for *C. sativus* due to its sterile habit. Thus, the situation seems dramatic at present time and compromises any attempt of genetic improvement regarding this highly-valued crop. In recent years, there is increasing interest to explants tissue culture and genetic engineering techniques for propagation and genetic improvement of saffron. Tissue culture is useful method for large scale production of pathogen free corms. Induction of callus and subsequent regeneration of plants is suggested as possible means of introducing new variation. *In vitro* tissue culture for product formation their utility in increasing the amount of crocin, picrocrocin and safranal. Selection at cellular level is likely to help in isolating cell lines rich in these three chemical compounds which account for popularity of saffron stigma.

Key words: *Crocus sativus* L., polypolidy, mutation, *in vitro* micropropagation, clonal selection, genetic engineering, production of pathogen free corms

INTRODUCTION

Saffron, *Crocus sativus* L., is an important crop cultivated as the source of its spice for at least 3,500 years. Dried stigmas of saffron flowers compose the most expensive spice which has been valuable since ancient times for its odoriferous, coloring and medicinal properties (Plessner *et al*., 1989). Saffron introduction into new areas should be encouraged as it is a unique crop in terms of its potential and is recognized as red gold. It is the highest priced spice in the world at around \$500 kg^{-1} of saffron (Fernandez, 2007). The name saffron is commonly used to refer both to the

spice and the plant itself. Some archaeological and historical studies indicate that domestication of saffron dates back to 2,000-1,500 years BC (Grilli Caiola, 2004). The origin of saffron is obscure, but the plant is believed to have originated in the eastern Mediterranean, probably in Asia Minor and Persia. The name 'saffron' is derived from Arabic Zafran which means 'be yellow' (Winterhalter and Straubinger, 2000). Owing to extremely high demand from the dye, perfumery and flavoring industries, it is one of the most expensive spices on earth. The components of the spice "saffron" are localized in the red stigmatic lobes of *C. sativus* flower and these are responsible for its distinct color, flavor and smell.

For color the principal pigment is crocin, for smell the main component is safranal and for the special bitter flavor the main compound is the glycoside picrocrocin (Basker and Negbi, 1999). These compounds are derived from oxidative cleavage of the carotenoid zeaxanthin (Bouvier *et al*., 2003; Moraga *et al*., 2004). Besides these, a new class of defense chitinase namely *Safchi* A has recently been isolated from saffron (Castillo *et al*., 2007). There has been little success however in enhancing the levels of these bioactive molecules of commercial importance. The information regarding flower initiation can be exploited by plant breeders to apply conventional breeding procedures suited to vegetatively propagated crops viz, mutation breeding or polyploidy induction using physical/chemical mutagens can be tried at this stage to hit the target site for generating variability with regard to floral traits or polyploidizing agents like colchicines can be applied for inducing hexaploidy in an otherwise sterile triploid (Zaffar *et al*., 2008).

A revolution in molecular biology, statistics and information technology has stimulated the merger of some advanced technologies for understanding the complex web of interactions linking individual components of a living cell to the integrated behavior of an entire organism (Bruskiewich *et al*., 2006). The marriage between these advanced technologies has given birth to the discipline of bioinformatics. As large-scale expression profiling experiments with saffron can generate huge amounts of data about the saffron transcriptome, the discipline of bioinformatics can be used to extract information from the data. Characterization of the transcriptome of saffron stigmas is vital for throwing light on the molecular basis of flavor, color biogenesis, genomic organization and the biology of the gynoecium of spices in general and saffron particularly. The information derived can then be utilized to construct biological pathways involved in the biosynthesis of the principal components of saffron i.e., crocin, crocetin, safranal, picrocrocin and safchiA.

Saffron production in the world: Saffron is currently being cultivated more or less intensely in Iran, India, Greece, Morocco, Spain, Italy, Turkey, France, Switzerland, Israel, Pakistan, Azerbaijan, China, Egypt, United Arab Emirates, Japan and recently in Australia (Tasmania). The world's total annual saffron production is estimated as 205 tons per year. Iran is said to produce 80 percent of this total; i.e., 160 tons and Khorasan province alone 137 tons of the totals. Behdani *et al*. (2008) reported that the age of saffron farms was the most important factor influencing yield and five years aged farms had the longest flowering period, also there is a positive linear relation between continuance of flowering and yield. The Kashmir region in India produces between 8 to 10 tons mostly dedicated to India's self-consumption. Greek production is 4 to 6 tons per year. Morocco produces between 0.8 and 1 ton. Saffron production has decreased rapidly in many traditionally producing countries and is abandoned in others such as England and Germany. Spain was used to be the traditional world leader and most reputed saffron producer for centuries in areas of La Mancha and Teruel. Nowadays, the production is only about 0.3-0.5 tons.

Productions of Italy (Sardinia, Aquila, Cascia) 100 kg; Turkey (Safranbolu) 10 kg; France (Gâtinais, Quercy) 4-5 kg; and Switzerland (Mund) 1 kg are nearly insignificant. All saffron producers in the European Union, as well as in Turkey, suffer from increasing labour costs (Fernandez *et al*., 2011). However, the demands of world industry for saffron product are very high. Among countries cultivating saffron, Iran is at the first place. More than 90% of the world production falls onto Iranian saffron which has a great importance in the economy of the country. The area under saffron cultivation in Iran reaches about 80,000 hectares with an annual production of about 250 tons of the dry stigmas. In recent years this amount has been remarkably increased which was achieved mainly by extension of the planting areas but not due to the increased yield capacity in the area unit. Beside other factors new high yielding cultivars of saffron are required to solve the problem.

Export and import of saffron: Export of saffron from India in small quantity has been a regular feature of international trade. However, among the saffron growing countries, Iran exports are the highest, with over 90% of its total produce being exported every year. Indian saffron is exported mainly to Spain, followed by France, USA, UK, UAE, Israel, Japan etc. However, the exports have been declining year after year, 9.77 MT in 1998-99 to 1.3 MT 2008-09, accounting for reduction of about 87%. This is primarily due to the decline in area and net productivity, lack of high yielding varieties. It is possible that the disillusionment of the farmers of the state with this crop could be also due to the declining trend in the domestic and international prices during the above period, thus contributing to the reduction in the net returns to the producers. Added to this is the major challenge thrown by the aggressive exports being made by Iran every year and the rampant adulteration that has plagued the saffron trade.

Saffron uses: The use of saffron goes back to the ancient times. It is most commonly used in medicine, as well as dye and spice in food industry (Basker and Negbi, 1999). Saffron has been also used as a drug to treat various human health conditions such as coughs, stomach disorders, colic, insomnia, chronic uterine haemorrhage, femine disorder, scarlet fever, smallpox, colds, asthma and cardiovascular disorders (Giaccio, 1990; Winterhalter and Straubinger, 2000; Abdullaev, 2003). It has been shown that saffron is a protective agent against chromosomal damage (Premkumar *et al*., 2001). Antinociceptive and anti-inflammatory (Hosseinzadeh and Younesi, 2002), antiseizure (Hosseinzadeh and Khosravan, 2002) and blood-pressure reducing (Fatehi *et al*., 2003) effects in animals were also reported. Saffron extract or its active constituents, crocetin and crocin, could be useful as a treatment for neurodegenerative disorders accompanying memory impairment (Zhang *et al*., 1994). Crocin extracts have been used for the treatment of nervous, cardiovascular and respiratory systems (Abe and Saito, 2000; Abdullaev, 2002) Crocin is also unique antioxidant that struggle with oxidative stress in neurons (Ochiai *et al*., 2004). The antidepressant effect of saffron petals and stigma in mice was also reported (Karimi *et al*., 2001). The positive effect of saffron extracts has also been described in patients suffering from allergic asthma (Haggag *et al*., 2003). Hartwell (1982) reported that saffron extracts were used against different kinds of tumors and cancers in ancient times. Therefore, liver, spleen, kidney, stomach and uterus tumors have been treated with pharmaceutical preparations of saffron. A number of studies in animal model systems have showed an antitumor effect of saffron on different malignant cells (Abdullaev, 2004). Khori *et al*. (2006) for the first time has explained the role of saffron on the protective mechanism of artrioventricular node against supraventricular arrhythmia. The results also showed the

non-specific effect of saffron on the transitional cells of fast nodal pathway which was manifested as a rate-independent increase of basic and functional (facilitation and fatigue) parameters of artrioventricular node.

Adulteration: Adulteration in Kashmir saffron is rampant and a most serious malpractice, making it a major constraint in reviving saffron cultivation in the state. It is a common sight to find imported Iranian saffron being mixed with Kashmir saffron and sold as Kashmir saffron to extract a higher price from the consumers/buyers. The adulterants detected were tetrazine-dyed starchy fibrous material (probably wheat flour and coconut fruit shell), dyed saffron stamens and dyed tender adventitious roots of Salix. Other adulterants reported are ray florets of marigold, safflower dyed with coal tar dyes viz, sunset yellow or matanil yellow; corn silk (stigma of maize), fibers of shredded meat coloured with saffron water, fibrous roots of various grasses, slender roots of willow and coloured nylon fiber. Fats, oils and glycerin are sometimes used to increase the weight of saffron.

Pests and disease: The major biotic stress being faced by saffron since last several years is the corm rot fungal infection, a soil borne disease caused by *Fusarium monliform* var *intermedium* and an unidentified mycelium of Basidiomycetous fungus. Out of six fungicides Blitox (copper-oxychloride), Difolatoan (captafol), Folpat (captafol), Bavistin (carbendazim) and Tecto (Thiobendazole) evaluated by Sud *et al.* (1999), only Bavistin and Tecto @ 0.2% as adip or drench gave a complete disease control. They further concluded that use of healthy corms followed by application of Bavistin or Tecto as a drench in the subsequent year appeared to be the best management strategy. Saffron belts of Kashmir are also infested with plant parasitic nematodes, namely *Helicotylenchus* sp. *Tylenchorynchus* sp. *Pratylenchus* sp. *Xiphinema* sp. etc as well as free living mites and thrips. Rodents and field rats also pose a serious problem.

WHY SAFFRON EXISTS JUST AS ONE CULTIVAR

In all over the world saffron is known as one cultivar, as descent of certain triploid sterile plant arisen once spontaneously in nature which was caught by sight of man and involved into cultivation (Mathew, 1977). It has been propagated and still continues to be propagated vegetatively. There is a supposition that saffron as a clone can be scarcely changed genetically and its improvement is hardly possible through clonal selection (Dhar *et al.*, 1988; Piqueras *et al.*, 1999). Meanwhile, other suppositions exist as well. For example, Rzakuliyev (1959) investigating in Apsheron (Baku) specimens of saffron obtained from 6 regions (2 regions in Italy, France, Istanbul, Yalta and Mashtaða) during 3 vegetations in 1934-1937 concluded that it is possible to create a new high yielding cultivars of this plant on the basis of clonal selection. Kapinos (1965) studied the morphogenesis and cytoembryology of *Crocus sativus* also under climatic conditions of Apsheron and came to the conclusion that this plant represents variegated blend of genetically heterogeneous forms – clones and clonal selection on it would be very promising. Apparently, the lack of new cultivars of saffron at present may not be explained by the impossibilities of the improvement of this plant through clonal selection. To solve this problem, it is necessary for a researcher to be properly acquainted with the biology and genetics of saffron and work up subtle puzzled methods of the clonal selection especially for saffron, different from those of the other plants. Two main difficulties of saffron breeding through clonal selection, in our opinion, are as follow: (1) Difficulty in the recognition of the clones. In any plantation saffron is represented by plants existing in highly

different "ages" of individuals connected with different sizes of corms underground. Above the ground, these plants differ in the size and number of their flowers (at the stage of flowering), also in their number and size of leaves. If some plants are sharply different from the others in certain characters, interesting for the aim of breeding, a researcher can not practically identify them. Naturally he does not see corms underground and can not elucidate the cause of the mentioned differences: whether these differences are due to the age (size) of corms, or because of the genotypes of plants. So, genetically different (if exist) and similar plants will continue to grow together and not be used as a subject for breeding. (2) Difficulty in the multiplication of clones and bringing them to cultivars. Let us suppose that farmer recognizes certain plant (s) which could be used as a clone with good economic characters. Multiplication of such clone (s) would be turned as insoluble problem. One saffron corm at planting with proper care produces on an average 4 corms of middle sizes during vegetation (one year). At such intensity of propagation it could be brought to about 100,0000 corms only after 17 years. This amount could be enough for planting on the area of 2 ha. It is clear that a farmer will accomplish never such an exhausting work of many years. Therefore the farmers would not pursue the aim to make new cultivar of saffron even if they have been lucky to find some clones with very highly expressed economically valuable characters. Concerning to the researcher, in our opinion, he could be able to do it in the favorable conditions, namely in the case of worthy appreciation of his long term hard work on this way by existing law on breeding. That is why in all over the world saffron has been remained as one cultivar despite of existence in culture during hundreds and thousands of years (Agayev *et al*., 2010). Alternatively, genetically changed superior plants of saffron could be propagated rapidly via *in vitro* technique. Investigations in this direction are very promising. Unfortunately, the experiments pursuing rapid corm propagation of saffron have not been successful so far and a few *in vitro* developed corms had been produced. Matured corms of *in vitro* origin in mass production had not been produced.

Origin: Seven species comprising this aggregate are: *C. sativus* Linn, *C. nivevs*, *C. cartwrightianus* Herb., *C. hadriaticus* Herb., *C. thomassi* Ten., *C. dispathacea* Bowles and *C. pallassii* Ancestry of *C. sativus* and its phylogenetic kinship have to be sought within this aggregate by using conventional and molecular techniques of genome analysis. The DNA polymorphism based AFLP method has confirmed the close relationship between these species, *C. sativus* to be derived from *C. cartwrightianus*.

Breeding strategies: *Crocus sativus* L., is a sterile triploid and propagated only vegetatively so conventional breeding methodologies applicable only to vegetatively propagated crops viz clonal selection, mutation, polyploidy and tissue culture have been tried and are being discussed briefly.

CLONAL SELECTION OF SAFFRON

With the object of breeding new cultivars of saffron with economically valuable traits, two principles can be applied: a) searching, identification and separation of superior clones in existing plantations, b) creating new valuable forms experimentally. Suggesting that in the existing plantations clonal selection of saffron is possible and promising, we are guided by following considerations. Having an ancient history of cultivation, saffron apparently should contain a lot of genetically changed forms (clones) as the result of mutations in somatic cells. The task is to find and study them individually, to separate the economically valuable forms and to bring them to the new industrial cultivars. But the matter is how to do it? In order to recognize the changed forms,

all of the corms were separated into groups according to their weights. Difference between adjacent groups was 1.0 g, or 0.5 g, or even less than 0.5 g. For the first time, we contented the difference of 1.0 g. The groups were as 3.0-3.9 g, 4.0-4.9 g, 5.0-5.9 g, etc. up to 16.0-16.9 g and more. Ignoring slight differences between weights of corms within each group (not more than 1.0 g), we conditionally accepted that they were of the same weight. The corms of each group were separately planted in lines in soil. In each hole (cluster) only one corm of a given weight was planted. So we were able to compare plants within each group separately and to investigate the existence and kind of changed forms. In this way, we could recognize within each group changed forms with valuable breeding characters such as multiflowering, larger flowers, flowers with stronger color and more aroma stigmas, tall flowers, early or late flowering, simultaneously flowering, lack of leaves at flowering time, number of leaves, their vigor, etc. These investigations were reiterated a few years for elucidation of the inheritance of established changes. At the end of such work, new clones with economically valuable characters were finally established. Next work will involve operations to propagate and use them in the breeding programs (Agayev *et al*., 2010).

Mutation and polyploidy: Increasing variability in Saffron through mutagenesis or changing ploidy level with chemical treatment has not received much attention. In vegetatively propagated crops, mutagenesis is considered a useful method for increasing the genetic variability to be exploited for improvement of different traits through selection. Akhund-Zade and Muzaferova (1975) irradiated Saffron corms with gamma rays which results in increase in corm production, flower number and stigma weight for 3 consecutive years in the population treated with 0.5 Kr. Mutagenesis in saffron should be done when the floral shoot has come out from the corm because meristem differentiation and maximum mitotic activity takes place during this stage. Formation of chimera will be low and recovery of solid mutants likely to increase Slower growth rate could be because of reduced rate of cell division, lower rate of growth hormones or lower metabolic activities. Increase in size of stomata reduction in its number may be due to higher gene doses as a result of chromosome number increase. Role of colchicines in bringing about chromosome doubling is well known. The plants showing variability for vegetatively floral traits have been tagged and will be observed during ensuing season. Evaluation data of C_3 generation along with chromosomal (root tip/PMC) studies will further confirm role of mutation/polyploidy in inducing variability in Saffron (Zaffar *et al*., 2004a). Preliminary results of induced genetic variability through gamma irradiation and induction of polyploidy through colchinization are not completely hopeful and probably require further work.

Molecular and biotechnological approach: Clonal selection offers the best chances of improvement in saffron provided a lot of genetic variability is present in its natural population. However measures of molecular and morphological genetic variations or often used to set conservation priorities and design management strategies for plant texa (Zaffar *et al*., 2009a). It seems that the genetic improvement of saffron and creation of new high yielding cultivars in the past was impossible owing to the complexity of the problem. Only just the traditional methods of breeding are not promising here. The literature on saffron breeding is very limited. There are known old works from Azerbaijan Republic more or less concerning with the breeding problems (Rzakuliyev, 1948, 1959; Kapinos, 1965; Muzaferova, 1970; Agayev *et al*., 1975). A lot of work has been carried out using tissue culture (Homes *et al*., 1987; Dhar *et al*., 1988; Munshi and Zargar, 1991; Munshi, 1992; Piqueras *et al*., 1999; Homes *et al*., 1987). Ascertaining the specified activities

at the same time it should be admitted that for today on arena there is only one cultivar of saffron. Our attitude to the given problem differs. The urgency of saffron breeding problems and the necessity to solve them with the application of new extraordinary approaches was stated before (Agayev, 1994a,b). It is believed that saffron plant being cultivated in different countries under different and constantly varying land and climatic conditions, has faced with countless different stress situations during a rather long period of time (thousands years) and undergone the various mutations. Although these mutations were not redistributed between plants because of their sterility, but are accumulated in populations and kept till now. With mutations, small and large, on our view, should be encompassed all attributes, morphological and physiological, peculiar to saffron plant. Clonal selection independently and in combination with the experimental polyploidy and hybridization involving wild close relatives of *C. sativus* is mostly promising. Methods of in vitro technique and molecular genetics should be also applied if necessary. RAPD marker and SSR markers have been used in saffron studies. Inter-SSR and inter-retroelement methods and gene based markers (PCR and hybridization based) may also be used to add the precision of molecular characterization studies.

TRANSCRIPTOMICS, TRANSGENOMICS AND GENE MINING

Gene profiles from DNA microarray technology provide a snapshot of life that maps to a cross section of genetic activities controlled by thousands of genes simultaneously. Transcriptome analysis of saffron plants, subjected to different photoperiod and temperature regimes can throw light on to genes that get up or down-regulated. Variable temperature during corm dormancy and subsequent low temperatures appear to be effective factors in saffron flower initiation (Koocheki *et al*., 2007). Comparison of environmental and management practices for saffron in Iran (Khorasan) and India (Kashmir) throw light on some basic climatic and topographic differences between the two regions viz., humidity, altitude, rainfall, soil-type and irrigation. The main similarities being in time of planting, harvesting and low temperatures (Kafi and Showket, 2007). How these differences and similarities, translating into gene expression, can be known using DNA microarray technology and bioinformatics tools. This kind of huge data bases generated by physiological, agronomic and gene expression studies can be analyzed under in silico to find agronomically important candidate genes in saffron and to identify chemical agent (s) that simulate the effect of these variable factors, so saffron can be grown under controlled conditions. Furthermore, molecular biologists and biotechnologists can utilize the knowledge generated to specifically tailor saffron plants for new geographical areas such as the East Midlands of England (Yadollahi *et al*., 2007). This will involve development of novel traits and agriculturally relevant characteristics through changes in gene regulation. Software tools can be employed for in silico analysis of the impact of such molecular intervention i.e., introduction of a regulatory sequence or a transgene, for enhanced adaptation to new geographical areas.

Bioinformatics tools and DNA microarray technology can be useful in locating sources of resistance and agronomically interesting traits for transfer to saffron by appropriate biotechnological tools. The removal of stamens and the hand separation of stigmas from saffron flowers is labour intensive and leads to the high cost of saffron stigmas (Tsaftaris *et al*., 2004). It is desirable to have saffron flowers which do not form stamens, or even have carpels in place of stamens, thus doubling saffron production in a single flower while lowering the production cost. As C-class MADS-box gene function is essential for both stamen and carpel formation (Tsaftaris *et al*., 2005) recently characterized the expression of MADS-box genes in crocus flowers using several

molecular biology techniques, bioinformatics tools and database resources. Such studies help in understanding and exploiting the molecular mechanisms that control flower development in crocus and in realization of the objective of producing flowers with carpels in place of stamens. Further, this knowledge can even be used in molecular medicine. Recently T and B-cell epitopes of Iranian *Crocus sativus* were mapped using bioinformatics tools and the predicted peptides were found useful for vaccine development.

FUNCTIONAL GENOMICS OF SAFFRON

The primary motivation of functional genomics research in saffron is to narrow the list of candidate genes implicated in the biological processes involved in the production of flavoring compounds and stigma pigments, so that their expression can be enhanced using a transgenic approach and hence improve quality of the saffron stigma. Bioinformatics can play an enormous technical role in sequence-level structural characterization of saffron genomic DNA. Earlier, the only major information resource for modern genotyping and sequence characterization available to saffron biologists was the *Arabidopsis thaliana* (L.) Heynh. genome, published in 2000 (The Arabidopsis Genome Initiative, 2000). This information resource was further strengthened with the completion of the rice (*Oryza sativa* L.) genome project and the availability of the rice genome sequence. Even though several crop genome-sequencing projects are rapidly constructing a rich and diverse repository of information about plant DNA sequences, an important database for saffron has been designed recently to manage and explore the Expressed Sequence Tags (ESTs) from saffron stigmas (D'Agostino *et al*., 2007). The database is the first reference collection for the genomics of Iridaceae, for the molecular biology of stigma biogenesis and for the metabolic pathways underlying saffron secondary metabolism.

GENES EXPRESSED IN CROCUS STIGMAS

D'Agostino *et al*. (2007) produced 6,603 high quality ESTs from a saffron stigma cDNA library and grouped these into 1,893 clusters, each corresponding to a different expressed gene. The complete set of raw EST sequences and their electopherograms are maintained in a database. This allows users to investigate sequence qualities and EST structural features. Putative transcripts determined to be associated with enzymes are organized into classes and can be viewed in terms of enzyme assignments to metabolic pathways. This represents a straight forward way to investigate the properties of the stigma transcriptome which contains a series of interesting sequences (putative sex determination genes, lipid and carotenoid metabolism enzymes, transcription factors), whose function can now be tested using *in vivo* or *in vitro* approaches. A contig (from contiguous) is a set of overlapping DNA segments derived from a single genetic source and is used to deduce the original DNA sequence of that source. Several such contigs have been characterized in saffron genome and based on the presence of tentative consensus sequences categorized into groups of putative function. The important ones include:

- **Cl000944:** 1 encoding non-heme-β-carotene-hydroxylase, highly expressed in saffron stigmas (Castillo *et al*., 2005)
- **Cl000627:** 1 encoding a putative glucosyltransferase, very similar to UGTCs2 which is able to glycosylate crocetin *in vitro* (Moraga *et al*., 2004)
- **Cl001532:** 1 and Cl001032:1 encoding putative isoprenoid GTases, one of which could represent the still missing enzyme responsible for the glycosylation of picrocrocin

- **Cl000348:** 1 encoding a Myb-like protein with high similarity to LhMyb (from Lilium, GenBank accession BAB40790), Myb8 (from Gerbera) (Elomaa *et al*., 2003) and Myb305 (from Antirrhinium) (Jackson *et al*., 1991) and probably acting as a putative transcription factor

Further, a large number of Cytochrome P450 sequences are expressed in saffron stigmas, some at very high levels (D'Agostino *et al*., 2007).

Tissue culture studies of monocots: Saffron is a monocotyledon member of the large family Iridaceae. Comparatively, bulbous and cormous monocotyledons are regarded as difficult *in vitro* material. Contamination is a serious problem during micropropagation of monocots especially if below ground organs, such as corms, bulbs, rhizomes and tubers, are used as an explants source. The size of geophyte, physical damage and dormancy are the other problems which make tissue culture studies difficult. Tissue culture is presently mainly used as a tool to facilitate a better understanding of the bio-chemical synthesis of Saffron secondary products. Regeneration/proliferation ability of the corms was dependent on genotype, type of explants, culture initiation time and composition of the culture medium. The plants were able to form shoots or corms within 5-30 weeks of the start culture (Zaffar *et al*., 2004b).

Schenk and Hildebrandt (1972) reported the importance of medium composition and techniques for induction and growth of monocotyledonous and dicotyledonous plants in cell culture. They found that a high level of auxin-type growth regulating substances generally favored cell cultures of monocotyledonous plants, while low levels of cytokinin were essential for most dicotyledonous cell cultures. Within the last few decades, an increasing number of bulbous and cormous monocotyledons have been successfully cultured. Tissue culture technology was greatly influenced by the demand of rapid multiplication and clonal propagation of slow-growing monocots. Several economically important monocot species constituting nutritional, medicinal or ornamental groups of plants were used for *in vitro* clonal propagation (Sutter, 1986) and production of secondary metabolites (Aslanyants *et al*., 1988). Organogenesis and somatic embryogenesis from differentiated tissues of bulbous and cormous monocots, such as *Crocus sativus* seeds. A corm survives for only one season, producing up to ten "cormlets" that eventually give rise to new plants. Therefore, reproduction is human dependent; the corms must be manually dug up, broken apart and replanted. The natural propagation rate of most geophytes including saffron is relatively low. Besides conventional methods of propagation, in vitro cultural methods contribute importantly for the propagation of many important and economic plants. Conventional propagation methods are very slow and propagation by tissue culture represents an important potential to effectively propagate it (Fernandez, 2004). Darvishi *et al*. (2006) reported that treatment containing 2 mg L^{-1} NAA and BAP with highest Mean Rank had the best effect on induction of nonembryogenic callus and treatment containing 1 mg L^{-1} 2,4-D and BAP had the best effect on induction of embryogenic callus.

Organogenesis of saffron: Organogenesis means the development of adventitious organs or primordia from an explant source. Direct and indirect are the two types of this method. In direct organogenesis, a cell or a group of cells differentiate to form organs. Indirect organogenesis is the development of adventitious organs originating from an intervening callus phase. Callus is an unorganized or undifferentiated mass of proliferative cells produced in culture and also in nature. It is made up of a mass of loosely arranged thin walled parenchyma cells arising from the proliferating cells of parent tissue. Many plant regeneration studies concerning saffron via direct and indirect organogenesis have been reported.

Organogenesis studies for *in vitro* propagation purposes: Plessner *et al.* (1990) reported *in vitro* corm production in saffron. Corms smaller than 1cm in diameter and isolated apical buds from larger corms were used as explants. The nutrient medium consisted of MS (Murashige and Skoog, 1962) minerals, supplemented with sucrose (3%), nicotinic acid (5 mg L^{-1}), pyrodoxine-HCL (1 mg L^{-1}), thiamine-HCL (0.5 mg L^{-1}), myo inositol (100 mg L^{-1}), adenine sulphate (160 mg L^{-1}) and casein hydrolysate (500 mg L^{-1}). The medium was solidified with 0.9% agar. 1 mg L^{-1} 2,4-D (Dichlorophenoxyacetic acid), 3-12 mg L^{-1} kinetin and 3 mg L^{-1} zeatin were used as growth regulators. They reported that cytokinins, particularly zeatin and the auxin 2,4-D were essential for regular bud development *in vitro*. They also examined the effects of ethylene and ethapon on organogenesis. Ethylene and ethaphon pretreatments inhibited leaf development but on the other hand, induced corm production as well as dormancy. However, rooting could not be achieved. Direct adventitious shoot regeneration from ovary explants of *Crocus sativus* L. was revealed by Bhagyalakshmi (1999). Media components, incubation conditions and age of the explants were the factors influencing the regeneration. Full strength MS medium supplemented with NAA (naphthelene acetic acid) and BA (benzyladenine) produced the best response towards caulogenesis (28%) with highest shoot numbers per ovary. On the whole, the best response toward shoot growth, both in terms of leaf length and number, was on the medium with 0.54 μM NAA and 2.22 μM BA. Ovaries of different growth stages having stigmas of pale yellow, pale orange and bright orange regenerated a maximum mean number of shoots per ovary. Further development of ovary-derived shoots was influenced by the composition of basal salts in the culture medium where full strength MS salts gave the best response of those tested. Regenerated shoots produced normal photosynthetic leaves and corms. Plant regeneration studies of saffron via indirect organogenesis from callus cultures have also been reported. Ilahi *et al.* (1987) described the morphogenesis in saffron tissue culture. Corms of saffron were cultured on half strength MS medium supplemented with different combinations of growth regulators; i.e., auxin and cytokinins and coconut milk. Callus was induced in a medium containing 0.5 mg L^{-1} each of 2,4-D and BAP and 2% coconut milk. The same culture was used for differentiation of callus into buds. They reported that an increase in 2,4-D also enhanced callus formation but suppressed shoot-bud formation. These shoots were induced to root when inoculated on a medium containing 2 mg L^{-1} NAA for 24 h. However, further growth of these roots was slow when re-inoculated on half strength MS containing 0.1 mg L^{-1} each of 2,4-D and BAP. In another set of experiments when a piece of callus, growing in similar conditions, was transferred to MS medium containing 0.5 mg L^{-1} NAA, 0.1 of either BAP or kinetin and 2% coconut milk, the nodules gave rise to roots after 4 weeks of culture with subsequent suppression of the shoot development. The micro-corms obtained with the help of tissue culture using Murashige and Skoogs medium supplemented with 6-12% sucrose, 0.1-1.0 mg L^{-1} it-benzylamino-purine (BA) and Paclobutrozol (PAC) 1-10 mg L^{-1} it have a high survival rate upon transferring directly in soil (Zaffar *et al.*, 2009b). Chaloushi *et al.* (2007) reported that the treatment of NAA and BAP (each 1 mg L^{-1}) as the best hormonal treatment for the plantlet regeneration from the domestic saffron calli.

Somatic embryogenesis of saffron: Somatic embryogenesis is the process of a single cell or a group of cells initiating the developmental pathways that lead to reproductive regeneration of non-zygotic embryos capable of germinating to form complete plants. There are few studies in the literature about the regeneration of saffron via somatic embryogenesis. Ahuja *et al.* (1994) indicated the somatic embryogenesis and regeneration of plantlets in saffron. Somatic

embryogenesis was initiated in *Crocus sativus* from shoot meristems on LS medium containing 2×10-5M BA and 2×10-5M NAA. They observed the various stages of somatic embryogenesis in the same medium and the development was asynchronous. Maturated embryos could be germinated on half strength MS medium containing 20 mg L^{-1} gibberellic acid (GA_3). Complete plantlets with well developed root system and corm formation were obtained on transferring germinated embryos to half strength MS supplemented with 5x10-6M BA, 5×10-6M NAA and 2% activated charcoal. Somatic embryogenesis in saffron was described by also Blazquez *et al.* (2004). They used MS culture medium supplemented with 0.5 mg L^{-1} BAP and 0.1 mg L^{-1} 2,4-D for induction of somatic embryogenesis. Embryogenic calli were subcultured in MS medium containing 1 mg L^{-1} BAP and 0.05 mg L^{-1} NAA for multiplication in solid medium. Temporary immersion systems (TIS) were used for this purpose. A four-fold increase in the production of embryogenic calli (fresh weight increase) was observed in tissue culture when compared to solid medium. They obtained the best result when 1 mg L^{-1} of paclobutrazol was added. They also improved the development of somatic embryos on solid medium supplemented with 0.5 mg L^{-1} jasmonic acid (JA) and obtained plant regeneration via somatic embryogenesis after eight weeks of treatment of JA in combination with sucrose According to Raja *et al.* (2007). Organogenisis and somatic embryogenesis have shown that auxin such as 2,4-D along with cytokinin BA is essential for somatic embryogenesis from leaf explants of saffron (*Crocus sativus* L.); the auxin or auxin in combination with cytokinins used in the medium can greatly influence the frequency of induction and also on maturation of somatic embroys.

Karamian (2004) indicated the plantlet regeneration via somatic embryogenesis in four species of *Crocus*. Shoot meristem culture on LS medium containing 4 mg L^{-1} NAA and 4 mg L^{-1} BA or 1 mg L^{-1} 2,4-D and 4 mg L^{-1} kinetin was used for somatic embryogenesis in *C. sativus, C. cancellatus, C. michelsonii* and *C. caspius*. Asynchronous somatic embryogenesis in all of the four species was investigated and various stages of somatic embryo development were observed when embryogenic calli with globular somatic embryos were transferred into half strength MS medium containing 1 mg L^{-1} abscisic acid. According to Karamian (2004), maturated embryos could be germinated on half strength MS medium supplemented with 25 mg L^{-1} GA_3. Finally, complete plantlets were obtained by transferring germinated embryos into half strength MS medium supplemented with 1 mg L^{-1} NAA and 1 mg L^{-1} BA at 20°C under 16/8 h (light/dark) cycle.

Organogenesis studies for secondary metabolite production purposes: *In vitro* production of stigma-like structures of *Crocus sativus* for the purpose of crocin, picrocrocin and safranal induction was also reported. Stigma-like structures were reported to be induced from almost every part of floral organs, including half ovaries (Himeno and Sano, 1987; Loskutov *et al.*, 1999), stigmas (Koyama *et al.*, 1988; Sarma *et al.*, 1990), petals (Lu *et al.*, 1992), anthers (Fakhrai and Evans, 1990) and stamens (Zhao *et al.*, 2001). Himeno and Sano (1987) described the synthesis of crocin, picrocrocin and safranal by saffron stigma-like structures proliferated *in vitro*. One young halfovary (0.3 mg, fresh weight) was placed on each medium and incubated at 20°C, in the dark. Some of the stigma-like structures were formed on Linsmaier-Skoog medium (LS) supplemented with NAA (10 ppm) and kinetin (1 ppm) and the others were formed on Nitsch medium supplemented with NAA (1 ppm) and BA (1 ppm). The stigma-like structures were formed directly on the explants after 10weeks. They found that the average of concentration of crocin and picrocrocin in the stigma-like structures grown on Nitsch medium were about 3-fold higher than those grown on LS medium, while the total contents in each structure were about the same. Loskutov *et al.* (1999) studied the optimization of *in vitro* conditions for stigma like structure

production from half-ovary explants of *Crocus sativus*. The optimum proliferation of stigma-like structure was observed on B5 basal medium (Gamborg B5 medium) containing NAA (5.4 μM), BA (44.4 μM), MS organics, casein hydrolysate (0.05%) and L-alanine (11.2 μM). They reported that the amounts of crocin, crocetin, picrocrocin and safranal in stigma-like structure, as determined by high performance liquid chromatography analysis, were similar to those found in natural saffron. Sarma *et al.* (1990) also reported *in vitro* production of stigma-like structures from stigma explants of *Crocus sativus* L. MS medium supplemented with NAA (10 mg dm-3) and BA (1 mg dm-3) induced the optimum response. NAA was found to be an important additive to achieve a good response. Zeng *et al.* (2003) recorded the increased crocin production and induction frequency of stigma-like structures from floral organs of *Crocus sativus* by precursor feeding. MS medium supplemented with 5 mg L^{-1} kinetin and 4 mg L^{-1} NAA was used as the basal medium. Almost all of the stigma-like structures formed directly from explants, instead of from callus. Induction of crocin, crocetin, picrocrocin and safranal synthesis in callus cultures of saffron was reported by Visvanath *et al.* (1990). Callus cultures were obtained from floral buds on MS medium supplemented with 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} kinetin. The cultures could be induced to produce red globular callus and red filamentous structures which produced crocin, crocetin, picrocrocin and safranal. Crocin production using *Crocus sativus* callus by two stage culture system was reported by Chen *et al.* (2003). Saffron callus was grown in a two-stage culture on B5 medium supplemented with casein hydrolysate (300 mg L^{-1}) at 22°C in dark with 2 mg L^{-1} NAA and 1 mg L^{-1} BA to give maximum biomass (16g dry wt/L) and with 2 mg L^{-1} IAA (indole-3-acetic acid) and 0.5 mg L^{-1} BA for crocin formation. The maximum crocin production (0.43 g L^{-1}) was achieved by this two-stage culture method. Chen *et al.* (2004) also examined the promotion of growth of *Crocus sativus* cells and the production of crocin by rare earth elements. They reported that La3+and Ce3+, either individually or as a mixture, promoted crocin production of *Crocus sativus* callus but Nd3+(40 μM) had little effect and all metal ions were toxic above 100 μM. La3+(60 μM) promoted growth of callus significantly but increased crocin only slightly. Ce3+(40 μM) significantly promoted crocin production but had little effect on cell growth. They showed that La3+(60 μM) and Ce3+(20 μM) together gave the highest dry weight biomass (20.4 g L^{-1}), crocin content (4.4 mg g^{-1}) and crocin production (90 mg L^{-1}). Although most of the studies about *in vitro* production of saffron secondary metabolites via direct and indirect organogenesis were reported in the literature, there were very few studies related to the achievement of a whole plant. All these studies make clear that there were no problems with the callus and shoot formation of saffron. However, corm and especially root formation were very rare events in organogenesis of saffron. Sheibani *et al.* (2007) reported that Thidiazuron (TDZ) concentrations affected the induction of somatic embryogenesis significantly while different types of corm explants showed no significant effect on this process. Among TDZ concentrations used, 0.5 mg L^{-1} was the most effective treatment for embryogenesis induction. Embryogenic calli (globular stage) proliferated well when subcultured into MS medium supplemented with 0.25 mg L^{-1} TDZ before transferring to hormone-free MS medium containing 6% sucrose for maturation (scutellar or horn-shape stage).

***Ex vitro* studies of saffron:** The effect of gibberellin on functional activity of dormant saffron corms were reported by Azizbekova *et al.* (1982). At the time of transplantation, saffron corms were immersed in a solution of gibberellin (100 mg L^{-1}) for 4 h. Corms immersed in water for 4 h served as the control. After these treatments, cormswere planted in soil. They demonstrated that treating saffron corms with gibberellin leads to subsequent acceleration of growth and development

processes, increase of leaf and root length and increase in the number of flowers. Unfortunately, there were not many *ex vitro* studies about saffron in the literature. However, *ex vitro* studies of *Gladiolus* were reported more than saffron as explained in the following section. This species is also monocot with corms and is very similar to saffron. Therefore, it was thought that these studies could also be applicable for saffron.

The effectiveness of chemicals such as gibberellins (Arora *et al*., 1992), BA (Goo *et al*., 1998), ethephon (Suh, 1989) and methyl disulfide (Hosoki and Kubara, 1989) in breaking the dormancy of *Gladiolus* corms and cormels has been studied extensively. Ram *et al*. (2002) indicated that plant growth regulators affect the development of both corms and cormels in gladiolus. Three plant growth regulators, BA (25, 50 and 100 mg L^{-1}), ethephon (100, 200 and 400 mg L^{-1}) and GA_3 (25, 50 and 100 mg L^{-1}) were tested on fresh cormels. The cormels were soaked in the solutions for 24 h and they planted in the field. In conclusion, ethephon at 400 mg L^{-1} was most effective treatment for many of the growth parameters including days to sprouting, percentage sprouting, corm diameter and cormel production and weight.

Genetic resources

***Ex situ* conservation and multiplication:** Genetic materials are preserved in the form of corms (saffron and wild relatives) and seeds (only wild species). General conservation and multiplication strategies for the collections are the standards for international genebanks outlined by Engels and Visser (2003). In addition, specific strategies and bank design for the ex situ conservation were established mainly based on information regarding the source of the species, reproductive biology, mode of multiplication, sample type and objectives of the collection. The design comprises three main collections.

- **Reserve vegetative collection (saffron and allies):** Ten corms of each accession were sown in special flower-pots with substrate of the collecting zone and specific mixture (soil enriched with organic matter mixed with sand) and placed in a greenhouse with semi-controlled conditions. Irrigation and weeding were done by hand when necessary (Khoury *et al*., 2010)
- **Exchange vegetative collection (saffron and allies):** The accessions (40 corms each) were sown in the experimental farm (field conditions) in a 10-15 cm furrow, with 15 cm among plants and 50 cm among furrows. Conventional laboring was used to prepare soil for seeding and previous fertilization applying N (80 UF), P (100 UF) and K (100 UF) was carried out. Irrigation schedule was applied depending on the climatic conditions
- **Seed collection (wild crocus):** The acquired seeds both from the wild and from seed harvesting in BGV-CU were placed in hermetic jars including silica gel inside and stored in a refrigerated chamber at 4°C and 30% relative humidity (Agrawal *et al*., 2007)

Germplasm distribution: A basic criterion for supplying plant materials including dates of request and sending has been established based on phenology of the materials and facilities at the BGV-CU. At the short time the provision of genetic materials (corms, leaves, styles, anthers and seeds) is addressed to carry out the complete characterization/evaluation of the collection. From the next 2 years users can consult the availability of materials (through the CIS or by contacting the curator) in order to request accessions. Before the user receives the materials a Material Transfer Agreement between donor and recipient must be signed. Germplasm characterization descriptor definition A descriptor list for whole characterization and evaluation of the genus has been defined

and improved during the last 3 years (unpublished data). It includes characterization descriptors (95 traits), evaluation descriptors (47 traits) and descriptors based on genetic markers technologies and cytological characters (14 traits) and embraces a diverse set of data (morphological, phenological, agronomical, phytochemical, molecular, etc.), with the aim of being a useful tool for the description of the genetic variation in the genus Crocus. Characterization/evaluation Germplasm characterization is an important operation for a genebank since the value of the germplasm collection depends on the availability and quality of the information relative to the preserved accessions. Therefore, one of the main goals of CROCUSBANK action is to strategically characterize and evaluate germplasm of saffron and allies at different levels. A partial characterization/evaluation of the collection have been developed during last years taking into account morphological, phenological, agronomical, resistance to salt stress, phytochemical and molecular characters. Sixty-six saffron accessions have been characterised/evaluated for morphological, phonological and agronomical traits and the existence of variability has been observed, suggesting the existence of genetic differences among the accessions related to the geographic origin of the materials. These preliminary results are being confirmed by the data obtained in other approaches. A different sensitiveness to saline stress has been recorded among some of the above mentioned saffron accessions in relation to genotypes origin (unpublished data). The phytochemical analysis using gas chromatography (GC-MS) and/or spectroscopic methods (FT-IR, Raman) also indicates that potential variability occurs among saffron accessions (unpublished data). In the same way genomic AFLP and SNPs markers have been identified in a subset of accessions, showing clearly the existence of genetic variation in saffron crop (unpublished data). In addition, these genetic markers provide a specific genetic fingerprint that could be very useful for the rationalisation of the bank. The significant genetic variability found in saffron, evidenced with the on-going characterization/evaluation studies, opens the door to unravel the peculiarities of "land varieties" of this minor but highly appreciated Mediterranean crop. Accordingly, these results scientifically support the importance of conserving the local and precious cultivated germplasm worldwide. Similar studies have been programmed or are being developed indeed for other Crocus species integrated in the WSCC, however, the shortage of materials in most accessions is by the moment a limiting factor to develop more extensive studies. Anyway, preliminary trials considering different kind of traits (mainly morphological, phenological and molecular, but also salt stress resistance and phytochemical in a lesser extent), have revealed both, interspecific and intraspecific variability, in 34 accessions belonging to 21 species (unpublished data). That information may be of interest for different purposes (commercial gardening, bank rationalization, taxonomic or evolution studies, etc.), although much work remains to be done in the future with these materials. Future actions and prospects on the WSCC (World saffron *crocus* collection). The WSCC has already a wide representation of the Crocus germplasm of plausible utility in saffron breeding which has never been achieved before. Additionally, for the first time worldwide it has been created a unique collection which contains a large part of the variability of the saffron crop and wild relatives at global scale for common use. Therefore, priority actions to make useful the genetic resources to potential users are needed (Pastor *et al*., 2010).

Evaluation and genetic enhancement: A coordinated and integrated effort to evaluate the WSCC(World saffron crocus collection) is needed to identify useful genotypes (e.g.) sources of biotic and abiotic tolerance, genotypes with optimal production of secondary genotypes interesting for

improving saffron yield, etc.) Rationalization of the collection (Fernandez, 2007). The reduction of the collection size, without loss in the genetic variability is a very desirable approach in order to preserve the collection and to promote its utilization (e.g. creation of a core collection) Utilization and dissemination. A new more dynamic portal exclusively for the WSCC (World saffron crocus collection) utilization and disseminations is being designed. It's based on Joomla CMS (www.joomla.org) and MySQL database with allows large numbers of people to contribute and share stored data and to improve communication between users through blogs, forums, etc.

Future strategies: Conventional breeding methodologies have not led to any improvement in saffron, necessitating exploring alternative procedures like biotechnological, molecular biological interventions. In order to achive short, medium and long term goals for bringing about enhancement in productivity per unit area which can lead to increase in net returns to farmers and encourage them to continue growing saffron, the following strategies are recommended.

- Exploring possibilities of induction of site specific mutations through *in vitro* culture for enhancing stigma length, size and its chemical composition
- Induction of polyploidy (hexaploidy) through *in vitro* culture in cell cultures for breaking sterility barriers and open vistas for over coming sterility barrier and application of breeding procedures in enhancing yield, tolerance to biotic and abiotic stresses
- Identification of putative parents (through molecular biological interventions) and their collection/conservation for future allele mining
- High priority should be given on collection/Characterization of Crocus taxa, wild relatives and local varieties
- Detailed genetic maps to be developed would provide valuable tool for the identification of important genes
- The establishment of markers for important genes should enable the selection of superior genotypes and the pyramiding of genes from several genetic background

REFERENCES

Abdullaev, F.I., 2002. Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus*). Exp. Biol. Med., 227: 20-25.

Abdullaev, F., 2003. *Crocus sativus* against cancer. Arch. Med. Res., 34: 354-354.

Abdullaev, F.I., 2004. Antitumor effect of saffron (*Crocus sativus* L.). Overview and perspectives. Acta Hort., 650: 491-499.

Abe, K. and H. Saito, 2000. Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation. Phytother. Res., 14: 149-152.

Agayev, Y.M., R.S. Muzaferova and S.P. Savchenko, 1975. Results of the experiments of saffron corm treatments with colchicine solutions. Vestnik Selskokhozyaystvennoi Nauki, 10: 121-123.

Agayev, Y.M., 1994a. Origin of saffron and its karyotype analyses. Proceedings of the 2nd Symposium on Saffron and Farming of Medicine Plants (SSFMP'94), Gonabad, Iran, pp: 13-13.

Agayev, Y.M., 1994b. Some urgent problems of genetics, cytogenetics and breeding of saffron. Proceedings of the 2nd Symposium on Saffron and Farming of Medicine Plants (SSFMP'94), Gonabad, Iran, pp:12-12.

Agayev, Y.M., A.M. Shakib, S. Soheilivand and M. Fathi, 2010. Breeding of Saffron (*Crocus sativus*): Possibilities and Problems. Agricultural Biotechnology Research Institute of Iran (ABRII), Iran.

Agrawal, R.C., D. Behera and S. Saxena, 2007. Genebank information management system (GBIMS). Comput. Elect. Agric., 59: 90-96.

Ahuja, A., S. Koul, G. Ram and B.L. Kaul, 1994. Somaticembryogenesis and regeneration of plantlets in saffron, *Crocus sativus* L. Indian J. Exp. Biol., 32: 135-140.

Akhund-Zade, I.M. and R.S. Muzaferova, 1975. Study of the effectiveness of gamma irradiations on the saffron. Radiobiologiya, 15: 319-322.

Arora, J.S., K. Singh, N.S. Grewal and K. Singh, 1992. Effects of GA3 on cormel growth in gladiolus. Indian J. Plant Physiol., 35: 202-206.

Aslanyants, L.K., Z.V. Marshavina and A.G. Kazaryan, 1988. Productivity of a cell culture of *Iris sibirica* L. grown on a simplified culture medium. Rastitel'Nye Resursy, 24: 107-110.

Azizbekova, N.S., E.L. Milyaeva and M.K. Chailakhyan, 1982. Effect of gibberellin on functional activity of dormant saffron buds. Fiziologiya Rastenii, 29: 1164-1169.

Basker, D. and M. Negbi, 1999. The uses of saffron. Econ. Bot., 3: 228-236.

Behdani, M.A., A. Koocheki, P. Rezvani and M.J. Al-Ahmadi, 2008. Agro-ecological zoning and potential yield of saffron in Khorasan-Iran. J. Boil. Sci., 8: 298-305.

Bhagyalakshmi, N., 1999. Factors influencing direct shoot regeneration from ovary explants of saffron. Plant Cell Tissue Org. Cult., 53: 205-211.

Blazquez, S., A. Piqueras, M.D. Serna, J.L. Casas and J.A. Fernandez, 2004. Somatic embryogenesis in saffron: Optimization through temporary immersion and polyamine metabolism. Acta Hortic., 650: 269-276.

Bouvier, F., C. Suire, J. Mutterer and B. Camara, 2003. Oxidative remodeling of chromoplast carotenoids: Identification of the carotenoid dioxygenase CsCCD and CsZCD genes involved in Crocus secondary metabolite biogenesis. Plant Cell., 15: 47-62.

Bruskiewich, R., T. Metz and G. McLaren, 2006. Bioinformatics and crop information systems in rice research. Int. Rice Res. Notes, 31: 5-12.

Castillo, R., J.A. Fernandez, L. Gomez-Gomez, 2005. Implications of carotenoid biosynthetic genes in apocarotenoid formation during the stigma development of *Crocus sativus* and its closer relatives. Plant Physiol., 139: 674-689.

Castillo, R., L. Gomez-Gomez and J.A. Fernandez, 2007. SafchiA is a new class of defence chitinase from saffron (*Crocus sativus* L.). Acta Hortic., 739: 195-202.

Chaloushi, B., R. Zarghami, C. Abd-Mishani, M. Omidi, Y.M. Agayev and B.P. Sardood, 2007. Effects of different hormonal treatments on the callus production and plantlet regeneration in saffron (*Crocus sativus* L.). Pak. J. Biol. Sci., 10: 1625-1631.

Chen, S.A., X. Wang, B. Zhao, X. Yuan and Y. Wang, 2003. Production of crocin using Crocus sativus callus by two-stage culture system. Biotechnol. Lett., 25: 1235-1238.

Chen, S., B. Zhao, X. Wang, X. Yuan and Y. Wang, 2004. Promotion of the growth of *Crocus sativus* cells and the production of crocin by rare earth elements. Biotechnol. Lett., 26: 27-30.

Darvishi, E., R. Zarghami, C.A. Mishani, M. Omidi and A. Sarkhosh, 2006. *In vitro* production of pathogen-free plantlets via meristems culture in saffron (*Crocus sativus* L.). Biotechnology, 5: 292-295.

Dhar, A.K., R. Sapru and K. Rekha, 1988. Studies on saffron in Kashmir. 1. Variation in natural population and its cytological behavior. Crop Improvement, 15: 48-52.

D'Agostino, N., D. Pizzichini, M.L. Chiusano and G. Giuliano, 2007. An EST database from saffron stigmas. BMC Plant Biol., 7: 53-53.

Elomaa, P., A. Uimari, M. Mehto, V.A. Albert, R.A.E. Laitinen and T.H. Teeri, 2003. Activation of anthocyanin biosynthesis in *Gerbera hybrida* (Asteraceae) suggests conserved protein-protein and protein-promoter interactions between the anciently diverged monocots and eudicots. Plant Physiol., 133: 1831-1842.

Engels, J.M.M. and L. Visser, 2003. A Guide to Effective Management of Germplasm Collections. IPGRI, Rome, Italy.

Fakhrai, F. and P.K. Evans, 1990. Mophogenic potential of cultured floral explants of *Crocus sativus* L. for the *in vitro* production of saffron. J. Exp. Bot., 41: 47-52.

Fatehi, M., T. Rashidabady and Z. Fatehi-Hassanabad, 2003. Effects of *C. sativus* petals extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and guinea pig ileum. J. Ethnopharmacol., 84: 199-203.

Fernandez, J.A., 2004. Biology, biotechnology and biomedicine of saffron. Re. Res. Devel. Plant Sci., 2: 127-159.

Fernandez, J.A., 2007. Genetic resources of saffron and allies (*Crocus* spp.). Acta Hortic., 739: 167-185.

Fernandez, J.A., O. Santana, J.L. Guardiola, R.V. Molina and P. Heslop-Harrison *et al*., 2011. The world Saffron and *crocus* collection, Strategies for establishment, management, characterization and utilization. Genet. Resour. Evol., 58: 125-137.

Giaccio, M., 1990. Components and features of saffron. Proceedings of the International Conference on Saffron (ICS'90), L'fAquila, Italy, pp: 135-148.

Goo, D.H., I.S. Park and K.W. Kim, 1998. Effects of low temperature and growth regulators on dormancy breaking of *Gladiolus cormlets* produced *in vitro*. J. Korean Soc. Hort. Sci., 39: 479-482.

Grilli Caiola, M., 2004. Saffron reproductive biology. Acta Hort., 650: 25-37.

Haggag, E.G., M.A. Abou-Moustafa, W. Boucher and T.C. Theoharides, 2003. The effect of a herbal water extract on histamine release from mast cells and on allergic asthma. J. Herb. Pharmcother., 3: 41-54.

Hartwell, J.L., 1982. Plants Used Against Cancer: A Survey. Quaterman Publications, Lawrence, CA.

Himeno, H. and K. Sano, 1987. Synthesis of crocin, Picrocrocin and safranal by saffron stigma-like structures proliferated *in vitro*. Agric. Biol. Chem., 51: 2395-2400.

Homes, J., M. Legros and M. Jaziri, 1987. *In vitro* multiplication of *Crocus sativus* L. Acta Hortic., 11: 675-676.

Hosoki, T. and T. Kubara, 1989. Breaking corm dormancy and accelerated flowering of gladiolus with methyl disulfide. Hort. Sci., 24: 968-971.

Hosseinzadeh, H. and V. Khosravan, 2002. Anticonvulsant effects of aqueous and ethanolic extracts of *Crocus sativus* L. stigmas in mice. Arch. Iran Med., 5: 44-47.

Hosseinzadeh, H. and H.M. Younesi, 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts. BMC. Pharmacol., 2: 7-7.

Ilahi, M., M. Jabeen and N. Firdous, 1987. Morphogenesis with saffron tissue culture. J. Plant Physiol., 128: 227-232.

Jackson, D., F. Culianez-Macia, A.G. Prescott, K. Roberts and C. Martin, 1991. Expression patterns of myb genes from antirrhinum flowers. Plant Cell., 3: 115-125.

Kafi, M. and T. Showket, 2007. A comparative study of saffron agronomy and production systems of Khorasan (Iran) and Kashmir (India). Acta Hortic., 739: 123-132.

Kapinos, G.E., 1965. Biology of Development Bulbous and Cormous Plants in Apsheron. Azerbaijan National Academy of Sciences, Baku.

Karamian, R., 2004. Plantlet regeneration via somatic embryogenesis in four *Crocus* specie. Acta Hortic., 650: 253-259.

Karimi, G.R., H. Hosseinzadeh and P. Khaleghpanah, 2001. Study of antidepressant effect of aqueous and ethanolic of *Crocus sativus* in mice. Iran. J. Basic. Med. Sci., 4: 11-15.

Khori, V., M. Nayebpour and A.R. Mansourian, 2006. The effect of aqueous extract of *Crocus sativus* on the electrophysiological properties of isolated perfused rabbit AV-node. Pak. J. Biol. Sci., 9: 2647-2651.

Khoury, C., B. Laliberte and L. Guariano, 2010. Trends in *ex situ* conservation of plant genetic resources: A review of global crop and regional conservation strategies. Genet. Resour. Crop Evol., 57: 625-639.

Koocheki, A., A. Ganjeali and F. Abbassi, 2007. The effect of duration of incubation and photoperiod on corm and shoot characteristics of saffron plant (*Crocus sativus* L.). Acta Hortic., 739: 61-70.

Koyama, A., Y. Ohmori, N. Fujioka, H. Miyagawa, K. Yamasaki and H. Kohda, 1988. Formation of stigma-like structure and pigment in cultured tissues of *Crocus sativus*. Planta Med., 54: 375-376.

Loskutov, A.V., C.W. Beninger, T.M. Ball, G.L. Hosfield, M. Nair and K.C. Sink, 1999. Optimization of *in vitro* conditions for stigma-like structure production from half-ovary explants of *Crocus sativus* L. *in vitro*. Cell. Dev. Biol. Plant, 35: 200-205.

Lu, W.L., X.R. Tong, Q. Zhang and W.W. Gao, 1992. Study on *in vitro* regeneration of style-stigma-like structure in *Crocus sativus* L. Acta Bot. Sin., 34: 251-256.

Mathew, B., 1977. *Crocus sativus* and its allies (Iridaceae). Plant Syst. Evol., 128: 89-103.

Moraga, A.R., P.F. Nohales, J.A. Perez and L. Gomez-Gomez, 2004. Glucosylation of the saffron apocarotenoid crocetin by a glucosyltransferase isolated from *Crocus sativus* stigmas. Planta, 219: 955-966.

Munshi, A.M. and G.H. Zargar, 1991. Variation in natural population of saffron (*Crocus sativus* L.) crop in Kashmir and performance of some selected subpopulation. Phytobreedon, 7: 62-67.

Munshi, A.M., 1992. Genetic variability for important traits in saffron (*Crocus sativus*). Crop Res., 5: 326-332.

Murashige, T. and F. Skoog, 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant, 15: 473-479.

Muzaferova, R.S., 1970. Primary results of a study of influences of the mutagenic factors on changing of saffron. Proceedings of the Institute of Genetics and Selection of the Academy of Sciences of Azerbaijan SSR (IGSASA'70), Baku, Elm, pp: 194-203.

Ochiai, T., S. Ohno, S. Soeda, H. Tanaka, Y. Shoyama and H. Shimeno, 2004. Crocin prevents the death of rat pheochromyctoma (PC-12) cells by its antioxidant effects stronger than those of α-tocopherol. Neurosci. Lett., 362: 61-64.

Pastor, R.T., E. Sanch?s, A. Garcýa J.L. Guardiola, R.V. Molina, J. Medina and J.A. Fernandez, 2010. Current state of the panish germplasm collection of saffron and wild relatives. Acta Hort., 850: 303-311.

Piqueras, A., B. Hee Han, J. Escribano, C. Rubio, E. Hellin and J.A. Fernandez, 1999. Development of cormogenic nodules and microcorms by tissue culture, a new tool for the multiplication and genetic improvement of saffron. Agronomic, 19: 603-610.

Plessner, O., M. Negbi, M. Ziv and D. Basker, 1989. Effects of temperature on the flowering of the saffron crocus (*Crocus sativus* L.): Induction of hysteranthy. Israel J. Bot., 38: 1-7.

Plessner, O., M. Ziv and M. Negbi, 1990. *In vitro* corm production in the saffron *Crocus (Crocus sativus* L.). Plant Cell Tissue Org. Cult., 20: 89-94.

Premkumar, K., S.K. Abraham, S.T. Santhiya, P.M. Gopinath and A. Ramesh, 2001. Inhibition of genotoxicity by saffron (*Crocus sativus* L.) in mice. Drug Chem. Toxicol., 24: 421-428.

Raja, W., G. Zaffar and S.A. Wani, 2007. *In Vitro* microcorm formation in saffron (*Crocus sativus* L.). Acta Hort., 739: 291-293.

Ram, R., D. Mukherjee and S. Manuja, 2002. Plant growth regulators affect the development of both corms and cormels in gladiolus. Hort. Sci., 37: 343-344.

Rzakuliyev, I.M., 1948. Flowering biology of saffron (*Crocus sativus* L.). Izvestiya Azerb SSR (Baku), 2: 105-114.

Rzakuliyev, I.M., 1959. A study of different specimens of saffron in condition of Apsheron. AGU Uchyonyye Zapiski Biol. Ser., 5: 3-8.

Sarma, K.S., K. Maesato, T. Hara and Y. Sonoda, 1990. *In vitro* production of stigma-like structures from stigma explants of *Crocus sativus* L. J. Exp. Bot., 41: 745-748.

Schenk, R.U. and A.C. Hildebrandt, 1972. Medium and techniques for induction and growth of monocotyledons and dicotyledonous plant cell cultures. Can. J. Bot., 50: 199-204.

Sheibani, M., A.V. Azghandi and S.H. Nemati, 2007. Induction of somatic embryogenesis in saffron using thidiazuron (TDZ). Pak. J. Biol. Sci., 10: 3564-3570.

Sud, A.K., Y.S. Paul and B.R. Thakur, 1999. Corm rot of saffron and its management. J. Mycol. Plant Pathol., 29: 380-382.

Suh, J.K., 1989. Corm formation in gladiolus as influenced by dormancy breaking methods and cormel planting and corm harvesting dates. Proc. Korean Soc. Hort. Sci., 7: 158-159.

Sutter, E.G., 1986. Micropropagation of *Ixia viridifolia* and a Gladiolus X Homoglossum hybrid. Sci. Hortic., 29: 181-189.

The Arabidopsis Genome Initiative, 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature, 408: 796-815.

Tsaftaris, A.S., K. Pasentsis, I. Iliopoulos and A.N. Polidoros, 2004. Isolation of three homologous API-like MADS-box genes in *Crocus sativus* L. and characterization of their expression. Plant Sci., 166: 1235-1243.

Tsaftaris, A.S., K. Pasentsis and A.N. Polidoros, 2005. Isolation of a differentially spliced C-type flower specific AG-like MADS-box gene from *Crocus sativus* and characterization of its expression. Biol. Plantarum, 49: 499-504.

Visvanath, S., G.A. Ravishankar and L.V. Venkataraman, 1990. Induction of crocin, crocetin, picrocrocin and safranal synthesis in calluscalture of saffron-*Crocus sativus* L. Biotechnol. Applied Biochem., 12: 336-340.

Winterhalter, P. and M. Straubinger, 2000. Saffron-renewed interest in an ancient spice. Food Rev. Int., 16: 39-59.

Yadollahi, A., S. Azam-Ali, E. Cocking and Z.A. Shojaei, 2007. Possibility of growth and development of saffron in the UK. Acta Hortic., 739: 139-149.

Zaffar, G., G.H. Zargar, A.W. Shafiq and M.Y. Ghani, 2004a. Strategies for improvement of productivity in saffron (*Crocus sativus* L.). SKUAST J. Res., 6: 151-159.

Zaffar, G., S.A. Wani, T. Anjum and N.A. Zeerak, 2004b. Colchinicine induced variability in saffron. Acta Hort., 650: 277-280.

Zaffar, G., S.A. Wani and A.B. Shikari, 2008. Corm development and flower formation in saffron. Book on (Saffron Production in Jammu and Kashmir). Directorate of Extension Education SKUAST-K, Srinagar, J&K, pp: 87-98.

Zaffar, G., S. Imran and F.A. Nehvi, 2009a. Studies in relation to molecular variability in saffron. Proceedings of the 3rd International Symposium on Saffron Forthcoming Challenges in Cultivation Research and Economics, May 20-23, Krokos, Kozani, Greece, pp: 3-3.

Zaffar, G., S.A. Wani and N.A. Zeerak, 2009b. Effect of media composition and growth conditions on *in vitro* micro corm formation in saffron. Proceedings of the 3rd International Symposium on Saffron Forth Coming Challenges in Cultivation Research and Economics, May 20-23, Krokos, Kozani, Greece, pp: 9-9.

Zeng, Y., F. Yan, L. Tang and F. Chen, 2003. Increased crocin production and induction frequency of stigma-like-structure from floral organs of *Crocus sativus* by precursor feeding. Plant Cell Tissue Organ Cult., 72: 185-191.

Zhang, Y., Y. Shoyama, M. Sugiura and H. Saito, 1994. Effects of *Crocus sativus* L. on the ethanol-induced impairment of passive avoidance performances in mice. Biol. Pharm. Bull., 17: 217-221.

Zhao, J., F. Chen, F. Yan, L. Tang and Y. Xu, 2001. *In vitro* regeneration of style-stigma-like structure from stamens of *Crocus sativus*. Acta Bot. Sin., 43: 475-479.