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Review

Breeding and biotechnological opportunities in saffron crop improvement

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Saffron (*Crocus sativus* L.) being triploid in nature is propagated by vegetative means through corms. The natural propagation rate of such plant species is relatively low, therefore some breeding and biotechnological technique like introduction, clonal selection, mutagenesis, micro-propagation and molecular markers have been used earlier as an alternative method of propagation for saffron. The creation of a germplasm bank consisting of superior elite clonal selections can be considered as a great achievement and in addition, the identification of selections as sources of variation can play an important role in improvement of this crop. The use of mutagenesis could increase the natural variability for important characters and may help in overcoming sterility barrier in autotriploid saffron by colchinization. Genetic improvement through mutation is one more important research area in saffron crop improvement and efforts made by different scientists have already given a base line idea for the improvement of this crop. Molecular markers have very good potential for study of species/clone level variation within saffron species. Use of molecular markers in saffron crop improvement will help in identifying the accessions and species which will be used for commercial exploitation and making of hybrids. At present we are having very few SSR markers in saffron, therefore both genomic and genic SSR markers need to be developed so that variation at genomic and expression level can be exploited.

Key words: Saffron, hybridization, clonal selection, introduction, mutagenesis, in-vitro regeneration, molecular markers.

INTRODUCTION

Saffron (*Crocus sativus* L.) is an autumn flowering cormose plant, cultivated for numerous properties ascribed to the stigmatic lobes and used as spice, condiment and for medicinal purposes (Figure 1). The corms reproduce annually, only vegetatively as the plant is sterile (Chichiricco, 1984) autotriploid (2n=3x=24) and seeds are unknown. Studies have revealed that the sterility is related to meiotic abnormalities producing both pollen grains, which display low/defective germination, and partially nonfunctional macrospores (Chichiricco, 1989). Sterility in saffron limits the application of conventional breeding approaches for its further improvement. All over the world saffron is known as one cultivar, a 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

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Figure 1. Saffron in full bloom.

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). It is supposed that saffron, a sterile clone of triploid origin that has been cultivated from times immemorial (the period about 3.5 to 4.5 thousand years) has passed an original evolution. Growing in the various countries under various soil-climatic conditions, during many centuries, saffron has been influenced by various stressful factors and has undergone different sorts of mutations. Despite of sterility, genetic changes could partly happen as a result of somatic recombination, deletions, inversions, translocations, polyploidy, incomplete segregation, segregation distortion, mutations, trans-versions, transitions etc.

Each plant that has undergone a genetic change has become a unique, new clone but clones in populations grow together, in a mixture. Thus they never combine genetically because of their sterility (Agayev et al., 2010). Studies in relation to genetic variability and divergence in saffron to generate information on the nature and magnitude of component of phenotypic variability including, heritability, genetic gain, nature of interrelationship among components of economic worth, contribution of different morphological and yield component traits and extent of divergence saffron populations collected from natural saffron growing areas have been studied (Anonymous, 2006). Genetic variation and heritability of agro-morphological and phytochemical traits in saffron populations have been studied and populations were found significantly different for most evaluated traits like leaf number per plant, leaf length, flower number per plot, dry stigma weight per plot, spathe number and the content of crocins, picrocrocin and safranal (Baghalian et al., 2010). Moraga et al. (2009) found that saffron is a monomorphic species as revealed by RAPD, ISSR and microsatellite analyses. Pardo et al. (2004) investigated the distinction and variability of *Crocus sativus* from several geographic areas (Italy, Iran, Greece and Spain) using molecular markers and dry stigmas as plant material. Zubor et al. (2004) used AFLP markers for study of genetic diversity among different saffron species and found close relationship between these species. Retero-transposons have also been used for studying the genetic diversity among different saffron species, genetic variation was observed within and between species and in some cases variation was found among ecotypes of the same species from different geographical regions (Alavi-Kia et al., 2008). Use of molecular markers as a tool for identification of variability among different saffron clones is an important area for improvement of this crop through breeding. Sequencing of corm cDNAs at different developmental stages would increase our knowledge about the physiological processes occurring in this organ. However, little work has so for been done in these areas. Development of gene expression in saffron corm at different time intervals has been studied (Orti et al., 2004). Different ESTs with respect to corm development, signal perception and transduction, defence against pathogen and stress, metabolism, development and gene regulation, cell organization, protein metabolism, transport etc have been identified. EST data base from saffron stigmas has been produced (Agostino et al., 2007) which will be very useful for detecting the level of expression of different components in saffron genotypes. Differential expression of apocarotenoid biosynthetic genes has been observed during different stages of stigma development in saffron (Mir et al., 2012). Beside other factors new high yielding cultivars of saffron are required to solve the problem. 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 because the traditional methods of breeding were not promising (Agayev et al., 1975). A lot of work has been carried out using tissue culture (Dhar et al., 1988; Chichiricco, 1999; Munshi and Zargar, 1991; Munshi, 1992; Piqueras et al., 1999). Ascertaining the specified

activities at the same time it should be admitted that for today on arena there is only one cultivar of saffron. The urgency of saffron breeding problems and the necessity to solve them with the application of new extraordinary approaches was stated earlier (Agayev, 1994a, b). Clonal selection independently and in combination with the experimental polyploidy and hybridization involving wild close relatives of *C. sativus* is most promising along with application of the methods of *in vitro* technique and molecular genetics. Thus, we need to exploit different research areas which can decipher the path for improvement of this crop. Following are some opportunistic research areas which need to be studied in detail for qualitative and quantitative improvement of saffron crop.

CLONAL SELECTION OF ELITE GERMPLASM

Clonal selection plays an important role with reference to improving the traits of cultivated saffron. There is a specific belief among some researchers that clonal selection of saffron will not result in large scale success with respect to improving the productivity of saffron because saffron, as a cloned species, does not have sufficient genetic variability for use in plant selection programs. In addition, mutations that have been identified as resulting from experimental or natural mutagenesis are not maintained as they are not heritable; consequently, they disappear in subsequently vegetative generations. There is also the problem of sterility, caused by the triploid nature of saffron, which prohibits its use in hybridization programs. Therefore, methods of conventional breeding are not relevant in terms of saffron breeding programs, and others believe that experimental mutagenesis and *in vitro* techniques must be focused upon. However, to date, both experimental mutagenesis and procedures aimed at doubling a chromosome set of saffron have not lead to encouraging results (Agayev et al., 1975; Bagheri and Vessal 2003; Khan 2004, 2007; Zaffar et al., 2004; Nehvi et al., 2007a, b). Moreover, 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 (Fernandez, 2004). Consequently, the creation of a germplasm bank consisting of superior elite clonal selections can be considered as a great achievement in the first place. In addition, the identification of selections as sources of variation with respect to some valuable traits like apocarotenoid biosynthetic potential, stigma length variation, variation in stigma number and yield etc can play an important role in improvement of this crop. Therefore superior clones showing better stigma characteristics need to be selected and mass multiplied. Furthermore such genotypes need to be analyzed in detail to find out the active principal behind their superiority, which can be exploited for saffron crop improvement. Therefore, utilization of heterogenity in the natural population which is due to genetic and environmental factors offers a tremendous scope for saffron improvement. Natural variability can occur due to deletions, translocations, inversions at chromosomal level. Changes at DNA level due to transitions and transversions, mutations, SNPs etc will lead to development of stable variants in saffron. Stress and other natural factors can also induce changes which can be useful for breeding programmes.

HYBRIDIZATION WITH CLOSE RELATIVES

C. sativus was generally assumed to be of autotriploid or hybrid origin. Now we have several data that support the alloploidy of *C. sativus* being the parents *C. cartwrightianus* and *C. hadriaticus*, both with 2n=16 and present currently in Greece but not in overlapping areas. Other possible parents, e.g., *C. thomasi*, from Italy and Croatia, *C. mathewii* from Turkey, and *C. pallasii* ssp. *haussknechtii* from Iran-Iraq-Jordan, cannot be excluded. The complexity of the evolutionary history of the genus crocus suggests an intensive species hybridisation and explosive speciation in crocus evolution that could be on the basis of the origin of saffron. We now are sure that saffron is an allopolyploid but the localization of the hybridisation event has not been ascertained so far. If the event took part several times could have generated different amphiploids and, in consequence, different saffron lines. In saffron the potential of the spore mother cells is limited by their triploid genome which causes meitic abnormalities, followed by variations in sporogenesis and gametogenesis, as a result, abnormal gametophytes are generated. However, the reproductive system of the saffron, like that of fertile crocus species, supports inter-specific crosses with related species. This potential cross-compatibility opens the door to breeding programmes for genetic improvement of the saffron. It is therefore possible to transfer the traits from other species into the saffron through hybridization or change the ploidy level of this species through hybridization with close diploid relatives. History suggested that such events led to the development of this crop therefore those events can now further be used to intensify the improvement of this crop.

INTRODUCTION OF ELITE GERMPLASM

Sustained efforts are required towards genetic improvement of saffron to develop high yielding cultivars through mutual exchange of germplasm base across

saffron growing countries of the world, followed by their molecular characterization and further evaluation. Germplasm having tolerance to biotic and a-biotic stresses and possessing high quality flower traits can improve the saffron production and quality. Germplasm susceptible to local biological agents particularly *Fusarium* should not be introduced as such germplasm can have detrimental effects on growth and development of the crop.

MUTAGENESIS FOR INDUCING VARIABILITY AND POLYPLOIDY

Induction of genetic variability through mutagenesis is another very important area of research in this crop. The use of mutagenesis could increase the natural variability for important characters such as high content of active principles per stigma, variability in morphological and flower component traits, change in flowering pattern, increase in stigma number etc. Also mutageneisis may help in overcoming sterility barrier in autotriploid (2n=3x=24) saffron (*Crocus sativus* L.) by colchinization, or any other means. Genetic improvement through mutation is one more important research area in saffron crop improvement. Different efforts made by different scientists (Khan, 2004, Khan 2007; Nehvi et al., 2005) have already given a base line idea for the improvement of this crop. Due to triploid sterile nature of this crop mutation breeding is very helpful in development and isolation of even-ploidy level selections. Saffron being triploid sterile plant and often propagated vegetatively through corms thus allowing the detection, selection and conservation of mutants within the M1-generation. Thus mutation breeding combined with *in vitro* culture techniques may lead to rapid success in generation of stable clones possessing even level of ploidy, which can lead to fertility of this crop. Polyploidy induction has already been studied for generation of stable hexaploids using colchicines (Zaffar et al., 2004).

IN-VITRO REGENERATION SYSTEM

Since the saffron reproduce only vegetative by the corms any attempt to modernize saffron cultivation will therefore require efficient mass production of pathogen free corms. Micro propagation of saffron has therefore been advocated to be the best alternative for its propagation. Thus through *in-vitro* multiplication of somaclonal variants can be obtained which will act as source of variation with respect to different traits in saffron and for induction and isolation of mutant cells, *in-vitro* regeneration system needs to be exploited as a tool. Rapid rates of multiplication and assured health status of propagules that can be attained in culture. Embryo culture is of most interest to the breeder as a means of producing novel inter-specific and inter-generic hybrids. Cultured embryos can be used as experimental systems for studying the biochemistry and molecular biology of storage product synthesis and accumulation as well. The techniques of cell culture and somoclonal variation to select variants mutants for various biotic and abiotic stresses would be fruitful. It is expressed that biotechnology will find higher application in saffron improvement.

OTHER BIOTECHNOLOGICAL INTERVENTIONS

Genetic modification using biotechnological interventions can also lead to variation. Transgenic saffron can be produced having additional traits as a source of variation. But such research areas can only be taken up after standardization of efficient micropropagation protocols and identification of genes and their regulatory behaviour fulfilling the requirements for release. There is very little knowledge on the inheritance of traits of agronomic relevance. Several approaches have been taken to overcome the constraints in the current methodologies for the genetic improvement of saffron. Evaluations at early stages of selection allow for estimates of general combining ability effect or breeding values of parental lines. Molecular markers can play an important role for early stage selection for genetic estimates and for selection of superior saffron clones having potential for utilization in clonal selection programmes. Extent of variability can be searched through exhaustive selection and identification of elite clones will be very useful for improvement of this crop through further breeding programmes. Molecular markers have very good potential for study of species/clone level variation within saffron species. Use of molecular markers in saffron crop improvement is an open area of research. These studies will help in identifying the accessions and species which will be used for commercial exploitation and making of hybrids. At present we are having very few SSR markers in saffron, therefore for scanning large saffron genome which is about 30,000 Mbp development of large number of SSR markers is needed. Both genomic and genic SSR markers need to be developed so that variation at genomic and expression level can be exploited. ESTs can be used for studying the expression level among different species of saffron. Species variation with respect to different metabolites using ESTs data can be studied, which will be very useful in identifying the variants useful for further breeding programmes. Since literature suggests that there is very low level of variability at genomic level but cultivation of saffron is done under different environmental conditions which can lead to induction of variation at transcription level hence utilization of EST-SSR can have more potential for revealing the variability between different saffron clones. Gene expression studies during the development of saffron and between the saffron clones is also an

important area of research. These studies will be very useful for quantitative measurements of genetic diversity of saffron on one hand and identification of different developmental stages of saffron on other hand. Researchers successfully develop *in-vitro* stigma likes structures in saffron (Mir et al., 2010, 2012) having high potential for apocarotenoid biosynthesis. Gene expression studies will be very useful for identifying the actual stage of stigma development under *in-vitro* conditions. Also gene expression during flower development in saffron can be correlated with apocarotenoid biosynthetic gene expression which can help in identifying the clones of high quality stigma with better flowering traits. Saffron genome sequencing partially or as a whole will also help in deciphering different genetic mechanisms which are hidden so for. The origin of saffron from wild Crocus species is not fully proven. So there is need to investigate the structure of genes which are conserved across all plant species thereby to define how the saffron genome is related to other species. Gene annotation will play an important role in identifying the level of synteny between saffron and other species.

DEVELOPMENT OF DESCRIPTOR FOR SAFFRON

There is an immediate need to construct a list of morphological, molecular, phonological, agronomical and biochemical descriptors valid for the genus crocus besides descriptors of susceptibility to stress factors, resistance to diseases and abiotic factors. The creation of collection will contribute not only to slow down the intense genetic erosion but also will make available a wide variety of crocus genotypes of potential carrier of interesting genes for plant breeders, e.g., resistance to biotic and abiotic stress, reserve accumulation, biosynthesis of secondary metabolites, identification of accessions of saffron within the collection those are genetically identical using different levels of indicators etc.

Conflict of Interest

The authors have not declared any conflict of interest.

REFERENCES

Agayev YM, Muzaferova RS, Savchenko SP (1975). Results of experiments of treatment of saffron corms in colchicines solution. Vestnik Selskokhozyaystvennoi Nauki (Moscow) 10:121–123.

Agayev YM (1994a). 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 P. 12.

Agayev YM (1994b). Origin of saffron and its karyotype analyses. Proceedings of the 2nd Symposium on Saffron and Farming of Medicine Plants, (SSFMP'94), Gonabad, Iran P. 13.

Agayev YMO, Fernandez JA, Zarifi E (2010). Clonal selection of saffron (*Crocus sativus* L.), the first optimistic experimental results. Euphytica 169:81–99.

Alavi-Kia SS, Mohammadi SA, Aharizad S, Moghadam M (2008). Analysis of genetic diversity and phylogenetic relationships in crocus genus of Iran using inter-retrotransposon amplified polymorphism. Biotechnol. Eq. 22:795-800.

Anonymous (2006). Studies in relation to genetic variability and divergence in saffron (*Crocus sativus* L. SKUAST(K), Srinagar, J & K, India.

Baghalian K, Shabani SM, Jamshidi AH (2010). Genetic variation and heritability of agro-morphological and phytochemical traits in Iranian saffron (*Crocus sativus* L.) populations. Ind. Crops Products 31:401-406.

Bagheri A, Vessal S (2003). Saffron improvement in Iran, breakthroughs and barriers. In, Proc 3rd Natl Symp Saffron, Mashhad pp. 1–10.

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

Chichiricco G (1999). Sterility and perspectives for genetic improvement of *Crocus sativus* L. In, Saffron, *Crocus sativus*. Medicinal and Aromatic Plants – Industrial Profiles. (Ed. By Negbi, M.). Hardwood Academic Publishers P. 127-135.

Agostino DN, Pizzichini D, Chiusano ML, Giuliano G (2007). An EST database from saffron stigmas. BMC Plant Biol. 7:53-53.

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

Fernandez JA (2004). Biology, biotechnology and biomedicine of saffron. Recent Res. Dev. Plant Sci. 2:127–159.

Khan IA (2004). Induced mutagenic variability in saffron (*Crocus sativus* L.). Acta Hortic. 650:281–283.

Khan IA (2007). Development of high yielding saffron mutant. Acta Hortic. 739:255-257.

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

Mir JI, Ahmed N, Wani SH, Rashid R, Mir H, Sheikh MA (2010). In-vitro development of microcorms and stigma like structures in saffron (*Crocus sativus* L.). Physiol. Mol. Biol. Plants 16(4):369.

Mir JI, Ahmed N, Wafai AH, Qadri RA (2012). Relative expression of CsZCD gene and apocarotenoid biosynthesis during stigma development in *Crocus sativus* L. Physiol. Mol. Biol. Plants 18(4):371-375

Moraga AR, Rambla JL, Ahrazem O, Granell A, Gomez-Gomez L (2009). Metabolite and target transcript analyses during *Crocus sativus* stigma development. Phytochemistry 70:1009–1016.

Munshi AM (1992). Genetic variability for important traits in saffron (*Crocus sativus*.). Crop Res. Hisar. 5:326–332.

Munshi AM, Zargar GH (1991). Variation in natural population of saffron (*Crocus sativus* L.) crop in Kashmir and performance of some selected sub-population. Phytobreedon 7:62–67.

Nehvi FA, Agarwal SG, Mir MA, Dar SA, Mir ZA, Nusrat N (2005). Quality drying of Saffron. SKUAST J. Res. 7(2):343-346.

Nehvi FA, Wani SA, Dar SA, Makhdoomi MI, Allie BA, Mir ZA (2007b). New emerging trends on production technology of saffron. Acta Hortic. 739:375–381.

Nehvi FA, Wani SA, Dar SA, Makhdoomi MI, Allie BA, Mir ZA (2007a). Biological interventions for enhancing saffron productivity in Kashmir. Acta Hortic. 739:25–31

Orti MA, Gómez LG, Rubio Á, Escribano J (2004). Development and Gene Expression in Saffron Corms. Prokisch Proc. Ist IS on Saffron Eds, J.-A. Fernández & F. Abdullaev. Acta Hortic. 650:35-38

Pardo J, Fernández JA, Gómez LG (2004). Development of molecular markers for origin determination in saffron. Acta Hortic. (ISHS) 650:95-98

Piqueras A, Han BH, Escribano J, Rubio C, Hellin E, Fernandez JA (1999). Development of cormogenic nodules and microcorms by tissue culture, a new tool for the multiplication and genetic improvement of saffron. Agronomy 19:603–610.

Zaffar G, Wani SA, Anjum T, Zeerak NA (2004). Colchicine induced variability in saffron. Acta Hort. 650,277–280

Zubor AA, Suranyi G, Gyori Z, Borbely G, Prokisch J (2004). Molecular biological approach of the systematics of *Crocus sativus* L. and its allies. In, Abdullaev F (ed). First International Symposium on Saffron Biology and Biotechnology pp. 85–93.