Genetic Mapping and Molecular Markers in Saffron

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Abstract

Saffron (*Crocus sativus* L.) is a sterile triploid ($2n = 3x = 24$) plant with a big complex genome and rare and limited breeding history. Breeding and genetic improvement of saffron is not easy due to its male sterility caused by triploidy and lack of enough diversity, whereas several hybrids are introduced by crossing the wild *Crocus* species. Poor breeding background in saffron led to genetic erosion and lack of superior cultivar (s) adapted to diverse geographical conditions, and different biotic and abiotic stresses. Success in saffron breeding depends on the selection of the best elite genotypes/and clones. As a result, collecting, screening, acquiring reliable information about genetic diversity and population structure, selection, and protection of genetic resources of saffron are essential. To achieve these goals different morphological, molecular, biochemical, and cytological markers are considered as the major tools to study the genetic diversity of clones, similarities and differences within and among populations, assess the genetic structure and phylogeny of saffron and *Crocus* species. New advances based on OMICS approaches have enabled genetic improvements in saffron through the molecular breeding programs which has encouraged breeders to adopt precision breeding approaches in *C. sativus*. Followed by identification of diversity among saffron populations, it is possible to preserve such a valuable saffron gene pool for initiating a comprehensive breeding program.

5.1 Introduction

Saffron (*Crocus sativus* L.) is a sterile triploid ($2n = 3x = 24$) monocot that belongs to Iridaceae. It is the most precious spice in the world known as red gold, and Iran is known as the world's largest producer of saffron (Vahedi et al. 2014, 2018; Taheri-Dehkordi et al. 2020, 2021; Moradi et al. 2021, 2022). Sterile saffron plant has a big complex genome and rare breeding history (Vahedi et al. 2019). Nevertheless, its wild allies are diploid and produce fertile seeds. Breeding and genetic improvement of saffron is not easy, because of its male sterility caused by triploidy (Schmidt et al. 2019), whereas several hybrids are introduced by crossing wild *Crocus* species. Regardless of how saffron emerged as an

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J. Vakhlu et al. (eds.), *The Saffron Genome*, Compendium of Plant Genomes,
https://doi.org/10.1007/978-3-031-10000-0_5

auto triploid or allotriploid species, limited success has been achieved using conventional breeding methods in saffron and hence new approaches such as protoplast fusion, mutagenesis using different mutagen agents, chromosome doubling and polyploidy may facilitate its breeding programs (Ahooran et al. 2009). Probable wild allies could be considered as potential candidates to alter saffron traits by cross-pollination. Breeding mainly requires diversity which has been neglected in saffron so far. Such a poor breeding background in saffron led to genetic erosion and lack of superior cultivar (s) adapted to diverse geographical conditions, and biotic and abiotic stresses mainly viral, fungal and bacterial diseases, salinity and moisture stress. Restoration of fertility will enable seed propagation, introduction of genetic variability by recombination, and selection for saffron breeding (Schmidt et al. 2019).

New advances in genomic technologies, transcriptomics, proteomics, and metabolomics, have enabled genetic improvements in saffron through the molecular breeding programs. The application of genomic tools and techniques has encouraged *C. sativus* breeders to adopt precision breeding approaches. Using OMICS approaches and data integration help us to better characterize the genome, transcriptome, proteome, and metabolome of saffron and other *Crocus* species towards a deeper insight into molecular basis of flavor and color biogenesis in saffron which definitely pave its breeding programs (Husaini and Asharaf 2010; Haq et al. 2022).

Success in saffron breeding depends on the selection of the best elite genotypes/and clones, but without genetic diversity, all efforts to introduce new cultivars will be failed. As a result, collecting, screening, selection, and protection of genetic resources of saffron are essential. Plant genetic resources are potentially used for future breeding programs. Acquiring reliable information about genetic diversity and population structure is a prerequisite for plant selection and consequently breeding a superior variety (Karp et al. 1997). To achieve these goals different morphological, molecular, biochemical, and cytological markers are considered as the major tools to study the genetic diversity of clones, similarities and differences within and among populations, assess the genetic structure and phylogeny of *Crocus* species. Therefore, to discover authentic genetic markers, distinguish single nucleotide polymorphisms (SNPs), functional genes related to secondary metabolites, unlock the secret of saffron origin, and improvement of saffron breeding, we will present an overview of molecular approaches towards saffron breeding in this chapter. Breeding and genetic improvement of saffron, possibilities and challenges, resources and tools are considered in the present report. Identification of saffron germplasms and wild species, screening and estimating their potential will lead to preserve genetic resources and select superior clones/ecotypes to breed new saffron varieties adapted for specific geographical regions.

5.2 An Overview of Breeding in Saffron and Wild Allies

One of the inevitable consequences of modern agriculture and using modified varieties with maximum yield and acceptable quality is the reduction of genetic diversity which leads to loss of many useful genes and genetic resources. This issue is more complicated in a plant such as saffron which naturally is sterile triploid. Poor genetic and breeding background in saffron led to genetic erosion which is a great threat to this plant against various biotic and abiotic stresses. In this regard, it is necessary to first estimate the level of genetic diversity in germplasm assemblages and plant populations that are used to improve plants and genetic studies. Genetic diversity is the process of expressing differences or similarities between species, populations, or individuals using specific statistical methods or models. Towards this goal, various approaches can be used, one of which is the application of molecular tools (Mohammadi and Prasanna 2003). Genetic relationships can be used as a guide for selection of useful genes and traits (Etminan et al. 2013).

Although, the most important plant in the Iridaceae and in the genus *Crocus* is *C. sativus*, however, wild *Crocus* species are of considerable importance because of their relevance to *C. sativus* (Fernandez 2004), theories of saffron origin and being considered as the saffron parental species (Schmidt et al. 2019), high potential alternative sources for extraction of apocarotenoids (Ordoudi et al. 2019), use as a ornamental bulb (Taheri-Dehkordi et al. 2020), and their potential for pharmaceutical uses (Zengin et al. 2020). The evolutionary history of wild *Crocus* species defiantly is longer than the cultivated saffron variety. *C. sativus* L. has been cultivated for its spice for at least 3500 years. The history of saffron appearance, its origin and domestication are not clear but perhaps the natural breeding was happened long time ago in this plant. Saffron wild allies are diploid and produce fertile seeds which are easy to breed. One of the ancestors of saffron is very likely the diploid *Crocus cartwrightianus* ($2n = 2x = 16$) native to Greece (Aegean islands), while its triploid mutant "*C. sativus*" was selected and domesticated (Fernández 2004; Nemati et al. 2019). This species has floral morphology that is quite close to that of *C. sativus*. In many studies, *C. sativus* L. has been considered as a result of triploid mutation from wild species of autotriploid (Mathew 1983; Chichiriccò 1984; Negbi 1999; Negbi and Negbi 2002; Nemati et al. 2019). In some studies, saffron is a hybrid result of allotriploidy between two wild diploid species (Agayev 2002; Maggi et al. 2011). Molecular data support the involvement of *C. cartwrightianus* and *Crocus thomasii* as parental species (Brandizzi and Caiola 1998; Tsaftaris et al. 2011) or *C. cartwrightianus* and probably *Crocus pallasi* (Harpke et al. 2013). Moreover, *Crocus mathewii* from Turkey, *Crocus serotinus*, *Crocus hausknechtii*, *Crocus michelsonii*, and *Crocus almehensis* from Iran were mentioned as possible parents (Frello and Heslop-Harrison 2000; Alavi-Kia et al. 2008; Petersen et al. 2008; Tsaftaris et al. 2011; Gismondi et al. 2013; Erol et al. 2014). The allotriploidy hypothesis is also supported by heterosis, expressed in more vigorous perianth parts and style branches compared to those of *C. cartwrightianus*, and by amphiplasty, where in a hybrid the formation of a satellite in one of the satellite chromosomes is inhibited by the other(s) (Agayev 2002). By comparative FISH study of different *Crocus* species including *C. sativus*, *C. cartwrightianus*, *C. thomasii*, *Crocus oreocreticus*, *Crocus asumaniae*, *Crocus hadriaticus*, *Crocus pallasii* ssp. *pallasii*, and *Crocus cancellatus*, this hypothesis took hold that saffron (*C. sativus*) is an autotriploid hybrid derived from heterogeneous *C. cartwrightianus* cytotypes. It seems that heterogeneous *C. cartwrightianus* cytotypes encompass most of the chromosomal diversity required for the saffron formation. According to these findings, an evolutionary scenario for the formation of the autotriploid saffron was proposed (Fig. 5.1).

Cross-pollination of *C. sativus* with *C. thomasii* has been reported to produce some viable seeds (Chichiriccò 1989; Caiola 1999), which is promising to start breeding programs based on hybridization. However, being triploid, saffron never bears seed naturally and it is propagated extensively by daughter corms annually. As a result, saffron has a rare breeding history and limited success has been achieved using traditional breeding methods. Although, saffron as a clone naturally can be scarcely and slowly changed genetically and its improvement is hardly possible through clonal selection (Dhar et al. 1988; Piqueras et al. 1999), however, a long history of cultivation in different geographical regions with various climatic conditions and selection by humans defiantly had evolutionary impact on the genetic background of saffron clones through different sorts of mutations, deletions, inversions, translocations, transversions, transitions, polyploidy, incomplete segregation, somatic recombination, and segregation distortion. Several documents have reported that local saffron specimens/ecotypes were selected based on clonal selection in different countries, i.e., "Ghayenat" in Iran, "Lacha" in India, Abruzzo and Sardinia in Italy, Castilla-La Mancha in Spain, and Kozani in Greece. These ecotypes were completely differing in their yield and quality.

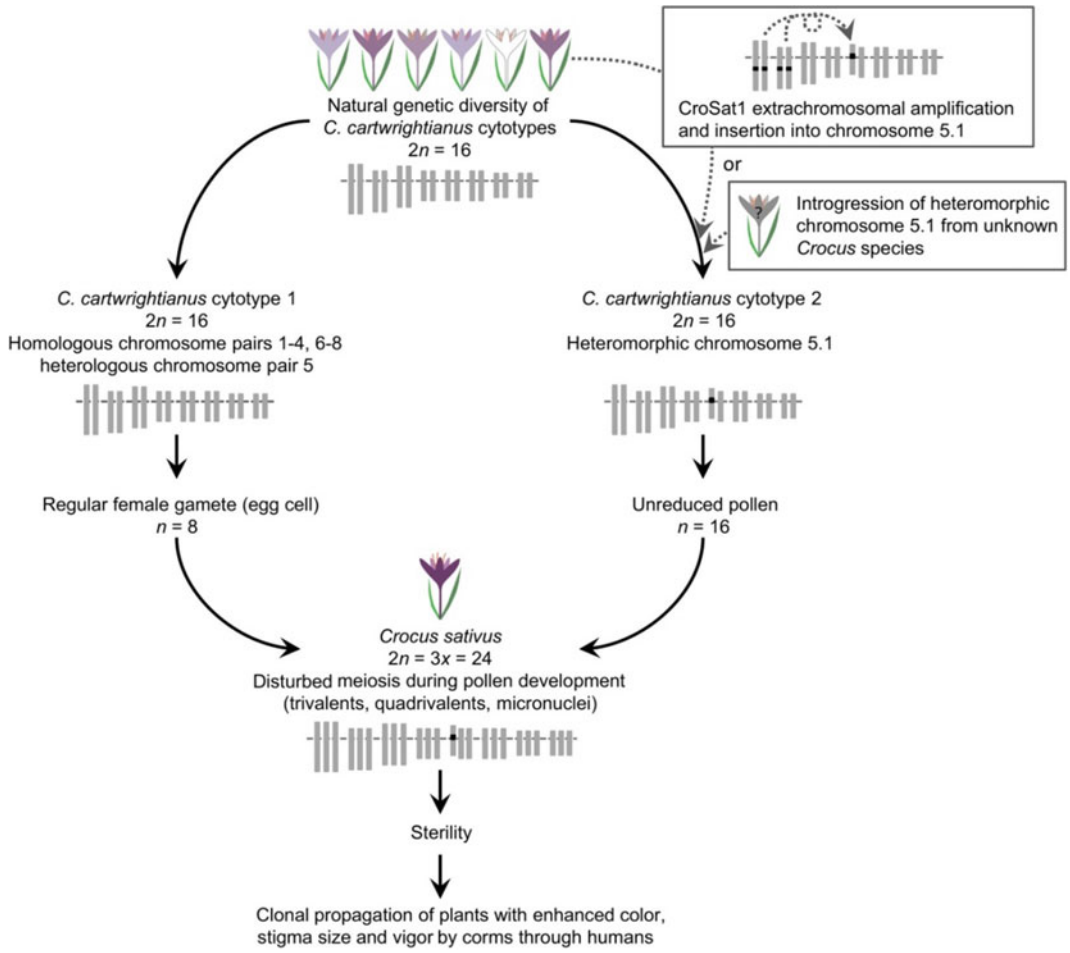

Fig. 5.1 Evolutionary scenario revealed by FISH illustrates the emergence of saffron (*C. sativus* L.) from a cross between heterogeneous *Crocus cartwrightianus* cytotypes with phenotypically different ranges of flower colors from white to purple and chromosomal variation. Cytotype 1 contained homologous chromosome pairs 1–4 and 6–8, all traceable to at least one of the five *C. cartwrightianus* cytotypes. This cytotype 1 plant was assumed that fertilized by unreduced pollen (n = 16) of cytotype 2, as unreduced gametes. Cytotype 2 provided two chromosomes to each triplet. The heteromorphic chromosome 5.1 might result from rearrangement by extra chromosomal amplification of CroSat1, or has been introgressed from an as yet unknown *Crocus* species. The offspring are today's triploid saffron with severe disturbances of pollen meiosis, including trivalents, univalents, and laggard and chromatin bridges. Consequently, these saffron plants are sterile and can only be propagated vegetatively by corms. From Schmidt et al. (2019)

Success in saffron breeding depends on the selection of the best elite genotypes/clones, but without genetic diversity, all efforts to introduce new cultivars will be failed. Acquiring reliable information about genetic diversity in an existing germplasm is a prerequisite for selection, breeding, and conservation programs (Karp et al. 1997). Several characteristics could be considered as breeding traits to introduce new "superior" clones of saffron such as large numbers of flowers and large corms, daughters corm bearing attributes, length and thickness of the stigmas,

dry stigma weight, higher appocarotenoids content particularly crocin content, and tolerant to biotic and abiotic stresses.

Clonal selection independently and in combination with polyploidy and hybridization with wild close relatives of *C. sativus* is mostly promising (Mir et al. 2015). Methods of in vitro technique and molecular approaches should be also applied if necessary. Many attempts have been conducted using tissue culture approaches which have been reviewed comprehensively in Chap. 12. Other approaches such as molecular, morphological, biochemical, and cytological markers have also been used to assess diversity in saffron.

5.3 Molecular Markers in Saffron and Its Wild Allies

Before designing a breeding program for saffron, one should have enough information about the genetic diversity of the relevant germplasm and quantitative and qualitative improvement in saffron requires accurate identification of the genetic structure as a prerequisite of breeding programs. In this regard, information about morphological, biochemical, and molecular diversity is crucial (Baghalian et al. 2010; Gresta et al. 2008).

Compared to other types of markers, genetic molecular markers have very few limitations. These markers cover much of the genome, affected neither by environmental conditions nor developmental stages, require less analysis time (Mammadov et al. 2012). DNA markers have been shown to be valuable in plant breeding, study of genetic diversity and gene mapping. Molecular markers have been widely used to reveal the level of polymorphism in different crops. These markers have potential to identify the variation at DNA level which is not observable in plant morphology. Molecular markers are divided into two categories: protein markers and DNA-based markers. DNA-based markers are used to determine genetic diversity and phenotypic association in different species. In these markers, a specific sequence of DNA molecules is easily detected and their inheritance can be seen. These markers are divided into two general categories of DNA markers based on hybridization and DNA markers based on polymerase chain reaction (PCR) depending on how they show polymorphisms. PCR-based approaches are in demand because of their simplicity and also because they can be carried out with only small quantities of sample DNA. Genetic diversity and relationships among species or populations is an important topic in genetics and plant breeding. Some of molecular markers such as RAPDs and ISSRs are simple, cheap and fast and require little amount of DNA with no need for prior genomic information. Some other markers such as SSRs and AFLPs are more complicated but more informative (Sarikamiş et al. 2010). Nevertheless, all of them consider as useful tool for assessing the genetic diversity, population structure and for map-based cloning approaches towards breeding.

Different types of molecular markers have been used in saffron to assess the genetic diversity and polymorphism, heritability of agro-morphological and phytochemical traits, distinction and variability of *C. sativus* from several geographic areas, molecular phylogeny and taxonomic analysis of different species of genus *Crocus*, genetic variations within and between species, variability among different saffron clones, saffron origin, and authenticity (Pardo et al. 2004; Zubor et al. 2004; Caiola et al. 2004; Alavi-Kia et al. 2008; Moraga et al. 2009; Baghalian et al. 2010). These included RAPDs (Moraga et al. 2009; Ali et al. 2013; Beiki et al. 2010; Caiola and Canini 2010; Imran et al. 2010; Keify and Beiki 2012; Qadri et al. 2012; Zheng et al. 2013), ISSRs (Moraga et al. 2009; Zheng et al. 2013), SSRs (Moraga et al. 2009; Namayandeh et al. 2013; Nemati et al. 2012; Zheng et al. 2013), AFLPs (Caiola and Canini 2010; Fernández et al. 2011; Nazzal et al. 2011; Siracusa et al. 2013; Zubor et al. 2004), Retrotransposons (Alavi-Kia et al. 2008), SNPs (D'Agostino et al. 2007; Fernández et al. 2011), and ESTs (D'Agostino et al. 2007).

Saffron breeders are suspicious about the existence of genetic variability in this plant, although, deep efforts need to assert or reject this.

Assay with RAPDs has revealed limited genetic differences among saffron samples from Italy, Iran, India, Greece, and Spain. Nevertheless, analysis of phenotypes revealed differences in flower size, tepal shape, and color intensity in plants from Israel and Italy. Some researchers revealed that saffron is a monomorphic species in nature due to its triploidy and vegetative reproduction, and therefore there is no significant variation among saffron ecotypes (Alavi-Kia et al. 2008; Moraga et al. 2009; Fluch et al. 2010). As a result, phenotypic differences in saffron have been created only depending on the environmental conditions (Siracusa et al. 2010; Maggi et al. 2011), hence, only one saffron cultivar is cultivated worldwide. Other researchers have shown limited genetic differences in DNA level and found some variations among different saffron clones (Álvarez-Ortí et al. 2004; Sik et al. 2008; Nemati et al. 2012; Keify and Beiki 2012; Siracusa et al. 2013; Nemati et al. 2014). As a result, by selecting the best clones in saffron, high-yielding corms with higher flowering rate and other commercial attributes can be achieved.

In saffron, RAPD markers have been used for investigating the variability and distinction of *C. sativus* from several geographical areas (Pardo et al. 2004). Caiola et al. (2004) evaluated the genetic diversity of 24 wild *Crocus* species alongside six saffron ecotypes using RAPD markers. A total of 233 bands were observed in saffron ecotypes, 14 of which showed polymorphism. In wild species, out of 227 amplified bands, 53 showed polymorphisms. RAPD and SRAP markers have also been used for identifying molecular variation among different saffron genotypes from Iran (Beiki et al. 2010). RAPD markers were found to show promise in identifying variation among different saffron genotypes from Kashmir region (Imran et al. 2010; Qadri et al. 2012). Keify and Beiki (2012) using 26 RAPD markers showed that clones collected from Iran are genetically diverse. RAPD and ISSR markers have been used in many studies to study the molecular diversity of saffron and the result has been significant (Shokrpour et al. 2017). Izadpanah et al. (2015) showed morphological and molecular differences among 36 samples of saffron were collected from Khorasan Razavi and South Khorasan. Moraga et al. (2009) studied domestic saffron ecotypes from different countries along with *Crocus kotschyanus* by ISSR, RAPD, SSR markers, however, no polymorphic band was observed among plants. They reported that domesticated saffron is cloned and that the specimens are not only morphologically identical but also molecularly similar. While in *C. kotschyanus* bands with different sizes and sequences were observed.

Molecular markers like sequence-related amplified polymorphisms (SRAPs) have also been promising to access the genetic diversity of saffron (Keify and Beiki 2012). The SRAP markers are simple, reliable, and highly functional (Li and Quiros 2001). Due to its obviousness and production of high-resolution bands, it is suitable for gel extraction and sequencing subsequently (Sun et al. 2006).

Diversity and relationships within and between species of *C. sativus* and its relatives analyzed by inter-retroelement amplified polymorphism (IRAP). High polymorphisms were identified between accessions of wild relatives with further variation between the species. In contrast, no polymorphisms were seen among 17 *C. sativus* accessions obtained from different geographical regions from Kashmir through Iran to Spain. IRAPs did not generate a tree position suggesting origination from one diploid species, and autopolyploidy was not supported (Alsayied et al. 2015).

Micro-satellite markers are also robust discriminative tools to study genetic diversity of different species. SSR markers were successfully used in saffron, and DNA polymorphisms were reported using this set of molecular markers (Nemati et al. 2012, 2014). SSRs can be isolated from different sources. The fiasco technique is one of the fastest and most effective methods of separating microsatellite markers that have been used in various plant species (Aibin et al. 2008). Twenty-seven SSR markers were evaluated on eight saffron ecotypes in Iran and 29 wild allies to evaluate the molecular diversity and capacity of these markers according to their effectiveness in establishing genetic relationships in *Crocus*

ecotypes (Nemati et al. 2014). Genetic diversity of twenty-two saffron ecotypes was studied using 25 SSR and 5 SNP markers. Filthy alleles were amplified using SSR primers, among them 33 alleles were polymorphic. Five polymorphic SNP markers were classified into transitions (C/T and A/G) and transversions (A/T and C/G) according to their substitution types, and the transitions were more common than the transversions (Javan and Gharari 2018).

AFLP markers have also been used for the study of genetic diversity among different saffron species (Zubor et al. 2004) and for identifying genetic variability in saffron from different origins (Siracusa et al. 2013). Recent reports have analyzed 112 cases using methyl-sensitive-AFLP to look for changes at the genetic and epigenetic levels. These studies show the presence of high epigenetic diversity (35.57% of polymorphic peaks and 28 types of effective epigenotypes (Busconi et al. 2015).

Retero-transposons have also been used for studying the genetic diversity among different saffron species (Alavi-Kia et al. 2008). Expression Sequence Tags-ESTs and EST-SSRs also have the potential to identify the molecular variations at functional/transcriptional levels (D'Agostino et al. 2007).

Molecular markers can also be used for taxonomic studies. The taxonomy of *Crocus* is extremely complicated due to the lack of clear distinctive characters, wide range of habitats and heterogeneity of the morphological traits, and cytological data (Moraga et al. 2009). Whether saffron has undergone modifications along its millenarian cultivation and whether it has one or more ancestors is still uncertain (Caiola et al. 2004). Genetic relatedness has been studied using molecular markers and it was found that *C. sativus* is very closely related to *C. cartwrightianus* and is also similar to *C. thomasii* (Caiola et al. 2004). Wild *Crocuses* are valuable considering their ornamental and pharmaceutical properties (Taheri-Dehkordi et al. 2020). Wild species are also highly valued for their resistance to pests, diseases, and non-living stresses such as salinity, cold, and heat. Beiki et al. (2013) investigated the genetic diversity of *C. sativus*, *Crocus speciosus*, *C. cancellatus*, *Crocus caspius*, and *Crocus haussknechii* by ISSR markers. The results showed that *C. sativus* with a similarity coefficient of 0.48 is very similar to *C. cancellatus* from Fars province. SSR markers have been successfully adopted for the analysis of genetic diversity in different types of plants, especially in saffron (Sarikamiş et al. 2010). Study of genetic diversity and phylogenetic relationships using inter-retrotransposon markers among *C. almehensis*, *C. michelsonii*, *C. cancellatus*, *C. speciosus*, *C. caspius*, *Crocus gilanicus*, and *Crocus haussknechti* in Iran showed that *C. almehensis* and *C. michelsonii* are most similar to *C. sativus* and might be possible ancestors of saffron. While Alavi-Kia et al. (2008) previously reported that *C. cartwrightianus*, which originated in Greece, is very similar to the saffron species and therefore can be considered as the possible ancestor of saffron (Caiola et al. 2004; Moraga et al. 2009; Nemati et al. 2019).

5.4 Approaches Towards Saffron Breeding

Genetic diversity is crucial in all breeding programs. Crop improvement relies on new gene combinations and their consequent expression in a new genetic context. Ancestral and wild species are a major source of genetic diversity (Vaughan et al. 2007; Heslop-Harrison and Schwarzacher 2012). With a basic chromosome number of $x = 8$, the saffron is a sterile species propagated exclusively by vegetative corms (Petersen et al. 2008; Agayev et al. 2009). Low but existing variations in saffron clones can be due to environmental effects and clonal variations (Siracusa et al. 2013; Babaei et al. 2014). Therefore, its breeding is applicable through clonal selection, mutagenesis, polyploidy, tissue culture, and gene/genome editing approaches such as CRISPR-Cas9. During long history of saffron cultivation worldwide, saffron lines have been selected clonally for better quality and higher yield, Multi-flowering, larger flowers and corms, flowers with stronger color and more aroma

stigmas, early or late flowering, simultaneously flowering, lack of leaves at flowering time, number of leaves, their vigor, length, thickness and weight of the stigmas, higher appocarotenoids content, better adaptation and tolerant to biotic and abiotic stresses. However, there has been little success due to saffron triploid sterile nature. Two major difficulties of saffron breeding through clonal selection include proper clone recognition and difficulty in bringing clones to cultivars. Clonal selection in saffron involves screening and identification and of superior clones and consequently creating new valuable forms experimentally. Corms and flowers mainly stigmas are the main targets for saffron breeding. Breeding through mutations or polyploidy induction can be exploited by breeders for generating variability in corms and flowers (Zaffar et al. 2008).

Although mutation induction using physical and or chemical mutagens (irradiation, colchicines, etc.) is considered a useful method for increasing the genetic variability and crop improvement in vegetative propagated crops, it has not been effective enough in saffron yet. There are a few reports concerning successful mutation/polyploidy induction in saffron but they never ended up with a new saffron cultivar. Irradiation of saffron corms with 0.5 Kr Gamma rays resulted in plants which produced higher number of corms and flowers and heavier stigmas. On the other hand, Gamma irradiation may result in chimera plants, plants with slower growth rate, increase in size of stomata reduction in its number (Akhund-Zade and Muzaferova 1975). Evaluation data of C3 generation along with chromosomal (root tip/PMC) studies will further confirm the role of mutation/polyploidy in inducing variability in saffron (Zaffar et al. 2004). Clonal selection independently and in combination with the polyploidy and hybridization involving wild saffron allies is mostly promising. To detect variability molecular markers are the best offer.

The main purposes to coordinate OMICS studies in saffron are crop improvement, adulteration and origin of saffron, traceability of the product and determination of authenticity. New advances in OMICS technologies may enable genetic improvements in saffron through the molecular breeding programs. Molecular markers lead to answer a plenty of questions that exist in saffron. Today, rely on the advance in PCR, sequencing, and OMICS technologies; a new path has been taken in determining plant species relationships (Nazzal et al. 2011). Conventional and novel molecular markers such as Genotyping-by-Sequencing (GBS) have been developed which are able to deeply survey the differences at DNA level. Using the GBS, it is possible to find and introduce molecular markers and determine the simultaneous genotype in a wide range of species including saffron and its wild allies (Elshire et al. 2011; Poland and Rife 2012; Narum et al. 2013; Nemati et al. 2019). GBS has been used as a promising molecular marker for analyzing plant genetic diversity and population structure analysis (Soorni et al. 2017). Moreover, GBS is used for studying genetic structure and molecular phylogeny of *Crocus* species and populations (Nemati et al. 2019). GBS is a fast, high-throuput and robust approach based on the sequencing of fragments produced by special restriction enzyme(s). By selecting the appropriate restriction enzyme(s), duplicate areas of the genome are not considered, and areas with low copy number can be targeted with two or three times more efficiency (Gore et al. 2007). The GBS data provide information about millions of potential single nucleotide polymorphisms (SNPs), and small INDELs across the genomes as the basis for high-performance genotyping (Soorni et al. 2017). GBS was used to unravel the saffron origin and its relationships to examine parents. According to these data, 99.3% of saffron alleles are similar to *C. cartwrightianus* (Nemati et al. 2019). GBS has been also used to determine the genetic diversity and population structure of different saffron ecotypes were collected from different regions of Iran alongside discovering authentic genetic markers and single nucleotide polymorphisms (SNPs), mining functional genes related to secondary metabolites and improvement of saffron breeding. GBS data confirmed the existence of genetic variability among Iranian

saffron populations collected from different geographical regions (Unpublished data). Altogether, these results provide useful information on genetic diversity of saffron that can be used for further genetic studies and to preserve and utilize such valuable genetic resources. These findings will help breeders to improve saffron production. Also, GBS would be considered to generate genetic linkage maps, performing genome-wide association studies, and genomic selection for traits of interest towards saffron improvement. Due to the identification of diversity among saffron populations, it is possible to preserve this valuable saffron gene pool for initiating a comprehensive breeding program.

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