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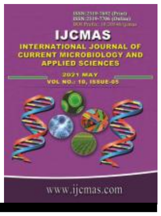

Review Article

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Breeding Aspect for Improvement in Chrysanthemum: A Review

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ABSTRACT

Chrysanthemum (*Dendranthema grandiflora* Tzvelve syn. *Chrysanthemum morifolium* Ramat.) is one of the most important flowering crop in the world. Flowers are highly prized for its varied color, form, size, shape and use. Developing Chrysanthemum cultivars with novel characteristic for its different flower color, shape, size, flowering time, postharvest quality tolerance for biotic and abiotic stress. In recent years, researchers have used various conventional and non-conventional breeding techniques to understand the classification studies, correlation and association both at morphological and molecular level, including transgenic technology, genome editing, and marker-assisted selection (MAS) with the wild relatives for introducing various ornamental traits from wild types to cultivated varieties. Moreover, the recent extensive advances in high-throughput technologies, especially genomics, transcriptomics, proteomics, metabolomics, and microbiomics, which are collectively referred to as omics platforms, have led to the collection of substantial amounts of data. Major traits which are targeted through biotechnological approaches includes development of novel flower colours, altered flower and plant morphology, insect-pest and disease resistance and enhanced post-harvest attributes. This review summarizes the recent achievements in conventional and modern molecular breeding methods and emerging technologies for improvement in chrysanthemum for floriculture point of view.

Keywords

Breeding, Chrysanthemum, mutation, floriculture, x-ray

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Introduction

Chrysanthemum (*Chrysanthemum morifolium* Ramat.) belongs to the Asteraceae family. It is one of the most versatile, economically important and internationally recognized floriculture crop. Chrysanthemum has a long history of cultivation; it was first cultivated in China as a herb in approximately the 15th century BC and was then successively introduced to Japan, Europe, and the United States (Andorson, 2006). Maximum diversity of chrysanthemum is scattered in eastern parts of the world hence also recognized as 'Queen of East', 'Glory of East' or 'Autumn Queen'. Fukai *et al.*, (1995) mention that

Florists Chrysanthemums (2n=54) originated by crossing and doubling between *Chrysanthemum zawadskii* var *latilobum* (Maxim.) kitamura (2n= 18) *Chrysanthemum indicum* var. *Procumbense* (Lour.) Kitamura (2n=36). Cultivated Chrysanthemum is a complex hexaploid that also exhibits aneuploidy, in which chromosome numbers vary from 47 to 67 (Dowrick, 1953). However, a chromosome number of 54 is the most frequent and stable conformation (2n = 6x = 54) (Roxas *et al.*, 1995). The genus chrysanthemum comprises of 100 to 200 species which varies in their morphological attributes like growing habit, form and colour. The cluster analysis of ISSR-PCR for 86 plants of chrysanthemum revealed that *Chrysanthemum vestitum* is closest to medicinal and large flowered chrysanthemum in genetic distance and the evolution of chrysanthemum may be mainly in one way that is from wild chrysanthemum to medicinal chrysanthemum to ornamental Chrysanthemum (Zhou, 2009).

The market demands for chrysanthemum increase annually, forcing scientists and breeders to create new cultivars with novel appearances and improved stress tolerance and quality attributes. Conventional breeding approaches, including crossbreeding and mutation breeding, are the most common approaches used to develop new Chrysanthemum cultivars. Although, in classical approaches, breeders mainly use phenotypes to select superior progeny or mutants, a process that is often laborious and ineffective, especially for traits with significant genotype × environment (G × E) interactions (Ibitoye and Akin, 2011). In recent years, transgenic molecular breeding has been extensively employed by introducing foreign genes into Chrysanthemum using *Agrobacterium*-mediated transformation and biolistic transformation and has led to considerable progress in horticultural character improvement. Due to the rapid development of DNA-based molecular marker techniques offers plant breeders a new opportunity to employ molecular marker-assisted selection (MAS) in breeding, which allows the indirect selection of target traits without regard to environmental factors or plant growth phases and shows great potential to increase the effectiveness of plant breeding (Das *et al.*, 2017). With the advent and improvement of next generation sequencing (NGS) technology has accelerated the generation of multiomic data at the DNA, RNA, protein, and metabolite levels at an unprecedented rate, leading to a new era of big biological data (Choi, 2019).

In this review summarizes and focus on recent advances and technologies in chrysanthemum for further breeding programs.

Conventional breeding Crossbreeding

Chrysanthemum is a highly heterozygous plant and the variability in habit, height, vigour, period and quality of bloom, colour, size and shape of flowers, that shows inbreeding depression and self-incompatibility; as a result, conventional crossbreeding is a powerful method for developing modern chrysanthemum cultivars. Crossbreeding is simple and effective, and the F1 progeny derived from two parents with contrasting target traits generally exhibit a wide phenotypic variation in Chrysanthemum. Still, there are several factors that must be taken into consideration when crossbreeding Chrysanthemum, such as the fertility of a certain cross combination, qualitative analysis of the target traits, and superior hybrid progeny selection. New varieties are developed through conscious/selective artificial cross-pollination. This classical breeding uses deliberate interbreeding (crossing) of closely or distantly related individuals to produce new varieties with desirable properties. Plants are crossbred to introduce traits/genes from one variety or line into a new genetic background.

Interspecific and intergeneric hybridization have efficiently been used with the aid of embryo rescue techniques such as embryo and ovule culture to breed novel agronomic traits and to overcome incompatibility (Watanabe, 1977). Intergeneric hybrids between *Chrysanthemum morifolium* Nannongxiaoli and *Artemisia vulgaris* Variegata showed enhanced resistance against both aphids and alternaria leaf spot using ovule rescue technique as the hybrids inherited the flower quality of Nannongxiaoli' and favorable resistances to aphids and alternaria leaf spot from Variegata (Zhu *et al.,* 2014). The inheritance of branching traits identified in different cultivars of Chrysanthemum revealed that two cross combinations both had cultivar Fukashi as a parent exhibited branching traits as highly heritable (Yang *et al.,* 2015). Sun *et al.,* (2010) reported that, crossed between the *Chrysanthemum grandiflorum* Yuhuaxingchen (excellent ornamental cultivar with low drought tolerance) with *C. indicum* having (drought tolerant) and six true hybrids, with that the improved drought tolerance were obtained.

Mutation breeding

Mutation breeding is a useful strategy in vegetatively propagated ornamental plants that has been widely exploited to modify one or a few traits in outstanding cultivars. Normally, mutations occur spontaneously, although they can be induced by physical and chemical methods. The mutation technique has successfully produced a large number of new chrysanthemum varieties that have been commercialized. Chrysanthemum when exposed to the effect of mutagen, most often the colour of the inflorescence changes which determines the decorative value of cultivars. Various chrysanthemum cultivars with novel traits, especially new flower colors and shapes, have been produced by X-ray radiation, gamma radiation, and ion-beam irradiation. In present, microwave radiation, which is a form of electromagnetic radiation, has been used to induce genetic and phenotypic variations (novel flower shape and color, increased inflorescence diameter, and prolongation of bud coloration) in chrysanthemum (Miler and Kulus, 2018). The advantage of mutation breeding is that the high heterozygosity of chrysanthemum can increase the apparent mutation rate and produce many excellent mutation types in a short period of time. Even so, the primary limitations of this type of breeding are that mutations occur unpredictably throughout the genome, and often, only a single change is produced.

Induction of mutation not only used for the improvement of flower quality parameters but also agronomic traits e.g. salt tolerance (Hossain *et al.,* 2006). Lema-Rumińska and Zalewska, (2005) obtained, from violet pink original cultivar Richmond, containing anthocyanins, mutants in which there was identified the presence of carotenoids or no anthocyanins at all in ligulate florets. The ionization irradiation can result in a partial or complete inactivation of the genes encoding the enzymes of pathway for the biosynthesis of anthocyanins. The mutations of that type which concern single genes result in the accumulation of intermediate compounds which leads to change in colour. Quantitative and qualitative changes in the content of pigments in inflorescences of the cultivars were obtained by the induction of mutagenic gamma radiation.

Molecular breeding Transgenic breeding

Transgenic technology can be used to transfer genes to a host plant from any source and to repress or enhance gene expression in a programmable manner. Compared with conventional breeding strategies, the transgenic method possesses greater prospective potential for producing innovative phenotypes. Cheng *et al.,* (2013) reviewed that transgenic technology has become an important for breeding in new chrysanthemum cultivars and has led to great achievements related to floral attributes, plant architecture, postharvest, flower longevity, biotic and abiotic stress tolerance. Transgenic ornamental plants are becoming beneficial for both growers and consumers due to their novel appearances and enhanced stress tolerance without presenting food safety concerns, in contrast to fruits and vegetables. Ledger *et al.,* (1991) first tried to generate transgenic chrysanthemum (*Dendranthema indicum* Korean) using Agrobacterium strain LBA4404, but reported to have very low transformation frequency (1.7%). Hence, this review describes only the most recent reports of several useful traits that have been introduced into chrysanthemum.

Abiotic and biotic stress tolerance

The industrialization of Chrysanthemum is limited by several abiotic and biotic stresses, including drought, salt, extreme temperature, and insect pests. With this, the development of new cultivars with increased tolerance has always been a goal of breeders. Ling (2018) suggests that DgWRKY2 could be used as a reserve gene for salt-tolerant plant breeding. Lower accumulation of reactive oxygen species (ROS) and higher enzymatic activity of peroxidase, superoxide dismutase and catalase in CmWRKY10 over expressed lines than that of WT demonstrates its role in drought tolerance. The CmWRKY10 works as a positive regulator in drought stress by regulating stress-related genes (M. Abuzar Jaffar *et al.,* 2016). Over expression of the *C. lavandulifolium* gene ClCBF1 in the chrysanthemum cv. ‘White Snow’ improved its salt and drought tolerance. The transgenic Cm- BBX24 plants also responded to salt and drought stresses, possibly due to changes in gibberellic acid (GA) biosynthesis.

Flowering time

Flowering time is the main determinant of successful commercial plants, and the development of early flowering cultivars helps meet consumers’ needs by allowing plants to bear more flowers or be produced in sufficient numbers for the celebration of particular festivals. Yang *et al.,* (2014) created transgenic RNA interference (RNAi)-suppressed chrysanthemum plants that flowered ~20 d earlier than Cm-BBX24-overexpressing and wild plants under long-day conditions. Wei *et al.,* (2017) showed that age-dependent regulation of SPL TFs by miR156 influences flowering by controlling CmNF-YB8 expression in chrysanthemum. More recently, overexpression of CmERF110 in transgenic Arabidopsis accelerated flowering by ~7 d compared with non-transgenic plants (Xing *et al.,* 2019).

Flower color

Flower color is a major objective in Chrysantemum breeding due to its strong influence on consumer choice. By the several internal and external factors, such as vacuolar pH, metal ions, and coexisting colorless compounds, also affect the formation of different colour pattern in flowers. Seita *et al.,* 2006) reported that diverse flower colors have been observed in chrysanthemum during its long history of cultivation, violet and blue colors are lacking due to a deficiency in flavonoid 3′5′-hydroxylase (F3′5′H) activity. Ohmiya *et al.,* (2006) reported that suppression of CmCCD4a expression by RNAi in white-flowered chrysanthemums produced yellow-flowered transformants. In other experiments (Yoshioka *et al.*, 2010) involving crosses between white- and yellow-flowered chrysanthemums indicated the presence of CmCCD4a led to the development of white ray petals. F3′5′H is a key enzyme for delphinidin biosynthesis in most blue-hued flowers. In the past, transgenic chrysanthemums with a violet ray-petal color were produced by expressing a heterologous F3′5′H gene under the control of ray-petal-specific promoters (Noda *et al.*, 2013).

Genome editing

Recently, genome editing has been used as a vital tool for functional genomics and biotechnology research and has become available as a precision-breeding approach for modifying traits in plant species. Genome-editing techniques theoretically allow researchers to introduce mutations into any targeted genomic sequence, and mutations in genome-edited plants are heritable. Now a days, few reports have demonstrated successfully on genome editing for flower color in ornamental plants by using the CRISPR/Cas9 method. Kishi- Kaboshi *et al.*, (2017) first attempted to use multicopy transgenes instead of endogenous genes as targets for genome editing in chrysanthemum.

The resultant transgenic chrysanthemum possessed over five copies of the yellowish-green fluorescent protein gene (CpYGFP) from Chiridius poppei, allowing the visualization of gene editing progress. Two single-guide RNAs (sgRNAs) were used to target different positions in the CpYGFP gene, and transgenic lines with mutated CpYGFP genes were acquired, showing that axillary buds and callus regeneration contribute to mutation.

Linkage maps and QTL mapping

A linkage map provides a basis for the identification of genomic regions related to traits of interest and the necessary infrastructure for MAS breeding.

Quantitative trait locus (QTL) mapping is a conventional tool for identifying the genes that control a trait, and it is useful for genome-wide scanning for QTL detection based on a linkage map in plants. Generally, linkage maps in chrysanthemum are most efficiently constructed according to the ‘double pseudotestcross’ mapping strategy in an F1 population. Zhang *et al.*, (2019), produce first preliminary linkage map of chrysanthemum from a bi-parental cross using a combination of RAPD, AFLP, and inter simple sequence repeat (ISSR) markers.

In some studies it is identified that molecular markers associated with the selected characters, preliminarily screened excellent progeny, and provided important basic genetic resources for chrysanthemum breeding programs.

Omics technologies Genomics

Genomic research has a vast capacity to accelerate the breeding process and presents applications for genetic improvement such as MAS and gene pyramiding. The genomic information of cultivated chrysanthemum has not been reported, mainly due to its unknown origin, heterozygosity, extremely large genome size (9.4 Gb, ttp://www.etnobiofic.cat/gsad_v2/), and high repeat content. Early on, bacterial artificial chromosome (BAC) libraries were widely used for whole-genome sequencing (WGS). However, this approach consisted of a laborious process of constructing a physical map composed of many BAC clones (Das *et al.*, 2017).

Table.1 List of chrysanthemum cultivars released by different institutes in India

Institutes	Hybridization	Selection	Mutation
NBRI, Lucknow	Ajay, Appu, Apsara, Apurva, Apurva Singar, Arun Kumar, Arun Singar, Bindiya, Birbal Sahani, Dhawal, Diana, Gauri, Gulal, Guldasta, Haldighati, Hemant Singar, Himanshu, Jaya, Jayanti, Jubilee, Jwala, Jyoti, Jyotsna, Kargil 99, Kaumudi, Kiran, Kirti, Kundan, Lal Kila, Lalima, Lalpari, Lilith, Maghi, May-Day, Mayur, Meghdoot, Mini-Queen, Mohini, Mother-Teresa, NBRI Pushpangadan, NBRI Khoshoo, NBRI Kaul, NBRI Himanshu, NBRI Little Orange, NBRI Little Hemant, NBRI Little Kusum, NBRI Little Pink, NBRI Yellow Bud Sport, Neelima, Niharika, Nirmal, Pancho, Peet Singar, Phuhar, Priya, Prof. Harris, Puja, Ragini, Rangoli, Sadbhavna, Shanti, Ratna, Sharda, Sharad Kanti,‘ Sharad		**Sharad Mukta, Sharad Sandhya, Sharad Shobha, Sharad Singar, Shizuka, Shyamal, Suhag Singar, Sujata, Suneel, Sunayana, Suparna, Surekha Yellow, Surya, Swarn Singar, Sweta Singar, Tushar, Vandana, Vasantika, Vijay, Vijay Kiran, Vinaya, White Charm, White Profile, Y2K, Yellow Charm,**
IARI, New Delhi		Pusa Aditya, Pusa Sona, Pusa Anmol, Pusa Chitraksha, Pusa Guldasta, Pusa	**Pusa Arunodya, Pusa Kesari**
IIHR, Bengalore	Arka Ganga, Arka Pink Star, Arka Ravi Arka Swarna, Chandrakant, Chandrika, Indira, Kirti, Nilima, Pankaj, Rakhee, Ravikiran, Red Gold, Yellow Star, Yellow Gold, Usha Kiran		
PAU, Ludhiana	Anmol, Baggi, Gul-E-Sahir, Royal Purple, Yellow Delight, Autumn Joy, Garden		
	Beauty, Winter Queen, Punjab Gold, Punjab Shyamli		
YSP, Nauni		Solan Shringar	
TNAU, Coimbatore	**CO1, CO2, MDU**		

(Sangeeta *et al.*, 2019)

Table.2 List of mutant variety released by different countries.

Countries	Varieties released	
Belgium	7	**Marconi, Copper Marconi, Red Marconi, Dark Red Marconi, Torino, Dark Torino, Yellow Torino**
Brazil	3	**Repin Rosa, Ingrid, Cristiane**
China	19	**Xishihanxiao, Chuntao, Yingsidai, Mantianxin, Zixia**
India	49	**Agnisikha, Navneet, Subama, Sonali, Surekha Yellow, Sharad Har, Navneet Yellow, Jugnu, Batik, Raktima, Kesar, Lalima Tubular**
Japan	56	**Amazon, Araddin, Baiogiku Rainbow orange, Baiogiku Rainbow Peach, Baiogiku Rainbow Pink, Baiogiku Rainbow Red, Baiogiku Rainbow White, Baiogiku Rainbow Yellow, Yellow Prism etc**
The Netherlands	80	**Amber Boston, Apricot Impala, Blue Star, Blue Winner, Bronze Star, Dark Milos, Yellow Winner, Yellow Westland, Yellow Clingo**
Germany	34	**Izetka Filmstar Bronze, Izetka Herbstgold, IIzetka Kopenicker Barbarossa Rotstern, Izetka Marienhain Cremeweiss etc**
Poland	6	**Lady Amber, Lady Bronze, Lady Salmon etc**
Russian	17	**Radii, Saputnik, Selena, Sointse, Saturn etc**
Vietnam	3	**VCM 1, VCM 2, VCM 3**
Korean Republic	2	**ARTI Purple, ARTI Queen**
Thailand	**1**	**Golden Cremon**

(Sangeeta *et al.*, 2019)

Table.3 Some of the genetic transformation studies in chrysanthemum

Species/Cultivars	Foreign genes	References
Dendranthema grandiflora	NPT II, GUS	(Van Wordragen *et al.*, 1991)
***Dendranthema grandiflora* cv. 'Yellow Spider'**	GUS, NPT II	(Pavingerova *et al.*, 1994)
***Dendranthema grandiflora* cv. 'Kitamura'**	NPT II, GUS	(Seiichi *et al.*, 1995)
***Dendranthema grandiflora* cvs. 'Polaris', 'Hekla', 'Iridon'**	GUS, NPT II	(Sherman *et al.*, 1998)
***Dendranthema grandiflora* cv. 'Peach Margaret'**	NPT II	(Boase *et al.*, 1998)
Dendranthema grandiflora	**GUS**	(Seo *et al.*, 2003)

(Sangeeta *et al.*, 2019)

The expansion of gene families by duplication events may lead directly to variation in the ornamental and medicinal characteristics of chrysanthemum. The genome assembly of *C. seticuspe* using the Illumina sequencing platform produced 2.72 Gb of sequences consisting of 354,212 scaffolds with an N50 length of 44.7 kb, covering ~89.0% of the estimated genome size. SNP identification and annotation performed by mapping the transcriptome data of six chrysanthemum varieties onto the *C. seticuspe* genome demonstrated that the *C. seticuspe* genome is applicable to genetic analysis in cultivated chrysanthemums (Jiangshuo *et al.*, 2019).

Plant growth and development

Plant growth and development is a complicated hierarchical system that is dynamically controlled by a network composed of various genes. Flowering is a complex developmental process in plants during which morphological and physiological changes affecting several external and internal factors occur (Amasino, 2010). Further studies showed that *TCP4* and *TCP9* may function as positive regulators of chrysanthemum petal growth. Novel flower shape is of great importance for marketing in ornamental plants. A comparative transcriptome analysis between the florets and leaves revealed several key DEGs involved in flower development, flower organ differentiation, and anthocyanin biosynthesis (Liu *et al.,* 2015).

Stress resistance

Mining crucial genetic resources controlling resistance characters will greatly contribute to breeding programs. Transcriptome-sequencing technology has been widely used for the identification of major regulatory genes and gene networks controlling responses to several abiotic stresses (i.e., drought, salt, cold, heat, and waterlogging).

Chrysanthemums are easily attacked by aphids throughout the growing season, seriously affecting their growth and ornamental value. MicroRNAs (miRNAs), which are ~22 nt in size, are the most abundant class of endogenous noncoding small RNAs (smRNAs) in plants and play important roles in plant biological processes (Sun, 2012). Designing a rational set of experiments to investigate common genes or pathway responses to multiple stresses would provide breeders with an opportunity to improve the synthetic resistance of chrysanthemum by developing gene-specific molecular markers or pursuing transgenic breeding. The responses of plants to stresses and the acquisition of stress resistance are regulated by a complex system of interacting signals and are susceptible to some environmental factors.

Proteomics

Ornamental chrysanthemums are traditionally cultivated by vegetative stem cutting, after which regeneration occurs via adventitious roots; therefore, the investigation of the molecular mechanisms underlying adventitious root formation is of particular significance in chrysanthemum breeding. Proteomic approaches have been successfully used in several sequenced plants, such as rice and Arabidopsis, to study diverse bioprocesses and environmental adaptations (Vanderschuren, *et al.,* 2013). A comparative proteomic analysis of postharvest medicinal chrysanthemum flowers under normal or UV-B radiation conditions allowed the detection of 43 differentially accumulated protein spots, some of which were identified as participating in photosynthesis, respiration, and defense (Yao, *et al.*, 2015).

Prospects of chrysanthemum breeding

With the rising demand for chrysanthemum, the development of new attractive cultivars with comprehensive resistance to various environmental stresses is urgently needed**.** Molecular and omics methods are expected to expedite the breeding process compared with conventional methods, which remain the mainstay of practical breeding programs for hexaploid chrysanthemum. Selecting suitable parent or populations with desired traits from the abundant germplasm resources of Chrysanthemum, which were materials used for genetic studies, it is the fundamental basis of breeding programs. The exploration of feasible methods for employing these new technologies is still in the initial stage for chrysanthemum. Despite the restrictions discussed in this article, the combination of modern breeding strategies with conventional methods would clearly be a superior approach for improving major horticultural characteristics, incorporating multiple characters into a single cultivar and expanding the usage of chrysanthemum in the floriculture industry.

It is concluded that, presently commercial floriculture being the most profitable business is expanding rapidly all over the world. Use of advanced science based techniques has given an impetus to the growth of this industry. An important driving force for the floriculture industry is the development of novel features in ornamentals. Chrysanthemum is one of the most important floricultural crops in the cut flower, flowering pot plants and herbaceous perennial markets of the world. Conventional breeding relies primarily on selection, using natural processes of sexual and asexual reproduction. This method has long become the base of development of a lot of cultivars. While some the advanced methods like mutation breeding and genetic engineering has played key role in the development of novel and desirable traits in plants. Mutation breeding has played tremendous role in the development of large number of cultivars. Breeding for novel color, such as rare blue colour, would be valuable for the flower industry and its consumers. Genetically modified crop plants are now grown over a very large area in several countries which is true for agriculture crops. In the Chrysanthemum the new varieties being produce for the commercialized among the farmers.

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