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Strategies for the development of unique flower forms in ornamental crops: A review

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Abstract

Ornamental plants play a crucial role in human's day to day life because of its aesthetic value. Any uniqueness in its forms is always welcome. Phenotype with unique forms of flower or, the double flower has higher ornamental value than the single one. Key transcriptional factors for the identification of floral organs have been identified and new ABCDE model has been proposed based on original ABC model. Here A+E specifying sepals, A+B + E petals, B+C+E stamens, C+E carpels, and D+E ovules. Different strategies are being applied like hybridization, mutation, polyploidy, genetic engineering etc to bred cultivars with new flower forms. Single, semi-double & double types of flower are genetically controlled either by a single gene or multiple genes hence by selecting suitable hybridization method double flower can be bred. Similarly, by induced mutagenesis, the mutant can be easily selected with modified visible characters, as flower color, shape, and size, or leaf form and growth habit. In polyploidy breeding, doubling the chromosome number whose main consequences are, increase in size and shape of plants/leaves/ branches, flower parts etc. With the advancement in biotechnological tools like RNAi, CRES-T, miRNA, various traits viz, flower color, fragrance, abiotic stress resistance, disease resistance, pest resistance, manipulation of the form and architecture of plants and/or flowers, flowering time, and post-harvest life etc are being manipulation by genetic engineering. These techniques have been successfully utilized to modify the shape of torenia, chrysanthemum, morning glory, petunia, orchids, gentian, cyclamen, rose plant etc. Even though there are many techniques available but, very few varieties have been developed for the commercial purpose.

Keywords: Flower crops, ABCDE model, double flower, flower form, mutation, polyploidy, genetic engineering

Introduction

Ornamental plants play a crucial role in human's day to day life and modifying surrounding environmental conditions for better living. These plants have ornamental value, of due to their morphology, flower colour or shape. Flower shape is one of the most important characteristics of ornamental plants. Creation of new flower shapes in these plants is always a major breeding target of any breeder. Any modification in flower shape either in a complete flower like semi-double or double or in its parts like petal or sepal or serration is of high commercial value because of its uniqueness. Key transcriptional factors for the identification of floral organs have been clarified by analyzing model plants such as *Arabidopsis Antirrhinum majus* etc (Bowman *et al*, 1989; Coen and Meyerowitz, 1991) [6, 13]. Generally, Phenotype with unique flower forms or, the double flower has higher ornamental value than the single one. The double flower is characterized by multiple whorls of the petal either by excessive development or conversion of another floral organ into petals, making it one of the most important traits of ornamental flowering species. Human selection since civilization over aesthetic traits has played pivotal roles in the development of double flowers and currently existing varieties of cultivated double flowers (Abbo *et al*, 2014; Ross-Ibarra *et al*, 2007) [1, 47]. In general, most of the double-flower varieties of ornamental crops were derived from single-flowering wild ancestors (Liu *et al*, 2013) [32].

ABCDE Flower Development Model

The ABCDE model for flower development proposes that floral organ identity is defined by five classes of homeotic genes, named A, B, C, D and E (Coen and Meyerowitz, 1991; Rijpkema *et al.*, 2010) [13]. According to the floral quartet models of floral organ specification (Coen and Meyerowitz, 1991; Smaczniak *et al.*, 2012) [13], the A and E-class protein complex develop sepals as the ground-state floral organs in the first floral whorl, the A, B and E-class protein complex specify petals in the second whorl, the B, C and E-class protein complex specify stamens in the third whorl, and the C and E-class protein complex specify carpels in the fourth whorl. Cloning of ABCDE homeotic genes has been done in *Arabidopsis*,

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which revealed that they encode MADS-box transcription factors. The only exception was class A gene, APETALA2 (AP2) (Jofuku *et al.*, 1994). In Arabidopsis, the class A MADS-box gene is AP1, the class B genes are AP3 and PISTILLATA (PI), the class C gene is AGAMOUS (AG), the class D genes are SEEDSTICK (STK), SHATTERPROOF1 (SHP1) and SHP2 and class E genes are SEPALLATA1 (SEP1), SEP2, SEP3, and SEP4. Report suggests that D-class proteins in interaction with the E-class proteins specify ovule identity, while class E genes show partially redundant functions in identity determination of sepals, petals, stamens and carpels (Mandel *et al.*, 1992; Jack *et al.*, 1992; Goto and Meyerowitz, 1994; Yanofsky *et al.*, 1990; Favaro *et al.*, 2003; Pinyopich *et al.*, 2003; Pelaz *et al.*, 2000; Ditta *et al.*, 2004) [35, 92, 21, 59, 18, 45, 44, 15]. The diversification of MADS-box genes during evolution has contributed to the wide variation of flower forms in angiosperms (Litt and Kramer, 2010) [31].

Genetic analysis of floral development using homeotic mutants

Mutations that occur in homeotic genes are called homeotic mutations. These genes encode transcription factors that control development by regulating the body parts identity and thus give rise to the body plan. Homeotic mutants are displayed as the transformation of one body part into another. These mutations were used in the model plant *Arabidopsis thaliana* to determine the model for floral development. Plants with mutated ABC genes produce homeotic mutant flowers. Here, type A and C genes are reciprocally antagonistic (Bowman *et al.*, 1991) [7] i.e. loss in function of A gene results in expression of function of C gene. Mutations in A genes affect calyx and corolla, and instead develop carpels in place of sepals and stamen in place of petals (APETALA2 mutant in *A. thaliana*) while, Mutation in B genes affects the corolla and the stamen, resulting in development of sepals instead of petals and carpels in the place of stamen (APETALA3 and PISTILLATA mutant in *A. thaliana*). Any mutation in C genes, directly affects reproductive parts i.e. stamens and the carpels, here instead of the stamen, petals develop while sepals develop in place of carpels (AGAMOUS mutant in *A. thaliana*) (Bowman *et al.*, 1989; Bowman *et al.*, 1991) [6, 7]. Hence, loss of C function or modifications in its

expression plays an important role in the production of excessive numbers of petals and thus double flower. Yan *et al.*, (2016) [58] reported that the phyllody phenotype in the *Rosa chinensis* cv. Viridiflora is associated with an up-regulation and ectopic expression of RcSOC1 and A-class genes along with the down-regulation of the B, C, and E class of floral organ identity genes.

Strategies for improving flower shape include

1. Hybridization

The best way to achieve unique shape is by crossing two different forms. The result may give various shapes viz, single, double, semi-double depending upon genetic constitutions'. These single, semi-double and double types of flower in ornamental crops are genetically controlled either by a single gene or multiple genes. By selecting suitable genotype as a parent one can develop new cultivars having required flower type. The inheritance of the double flower form in rose is controlled by a dominant allele (Debener 1999) [14], and hence to develop new double type cultivar, cultivars having double type flower need to cross with suitable single flower form cultivar. Chen *et al.* (2012) [10] proposed that double flowering phenotype in Periwinkle (*Catharanthus roseus*) is controlled by a recessive gene. He also proposed that single flowers are controlled by dominant allele either in the homozygous or heterozygous state. When he crossed TYV1, a double-flowered mutant of *Catharanthus roseus* with single-flowered 'Little Pinkie' cultivar, he only got the single flower in F1 but in the F2 generation and Test cross he recovers the double flower types in 3:1 and 1:1 ratio respectively. In Hydrangea double-flowered cultivars have only a few stamens, so seed production is very poor. Therefore it is necessary to cross double-flowered cultivars with single flowered ones to breed new double-flowered cultivars (Suyama *et al.*, 2015) [55]. He reported that double-flowered phenotype of decorative hydrangea flowers is controlled by a single recessive gene and showed that double-flowered progeny can be obtained when a pair of recessive genes are identical. In *Mathiola incana* also Double-flowered plants can only be obtained from the seed of singles that are heterozygous (Ss) for doubleness (recessive to singleness).

Table 1: Inheritance of double flower character in flower crops

Character	No. of Gene	
	Single	Multiple
Doubleness is dominant	Rose, Marigold, Cyclamen	<i>Impatiens wallerana</i> , Pelargonium
Doubleness is incompletely dominant	Carnation	-
Singleness is partially dominant	Chrysanthemum	-
Singleness is dominant	Stock, Periwinkle, Petunia, Antirrhinum, <i>Eschscholtzia californica</i> , Hydrangea, <i>Nicotiana glauca</i>	Zinnia

2. Mutation

Ornamental plants are most ideal systems for mutagenic treatments because their various characters of economical interest can easily be monitored after treatment and characterized. Furthermore, many ornamental species are heterozygous and often propagated vegetatively thus allowing the detection, selection, and conservation of mutants within the M1-generation (Schum and Preil 1998) [48]. Induced mutations observed in ornamentals comprise features such as flower characters (colour, size, morphology, and fragrance), leaf characters, growth habit, and physiological traits such as changes in photoperiodic reaction, early flowering, free

flowering, keeping quality as well as tolerance against biotic and abiotic stress factors (Schum and Preil 1998) [48]. The morphology of flowers and inflorescences can be affected by mutation induction as well. An increase and, more frequently, an unfavorable reduction of flower size was reported. For many species, mutation induction led to changes in petal shape, which sometimes were of ornamental value. Furthermore, an increase or decrease in petal numbers was recorded as result of mutagenic treatment. In Compositae, an increase in whorls of ligulate florets as well as a conversion from ligulate into tubular florets was described.

Table 2: Induced mutation for change in flower morphology in flower crops

Sl. No	Crop	Mutagen	Character	References
1	Dendrobium Orchid	Gamma rays	Narrow, elongated or broad or curled petals, Veinous sepals. Short and broad lip. Small flower etc.	Ariffin and Basiran, 2000 [3].
2	Chrysanthemum	Gamma rays	Tubular ray florets Tubular and Flat shaped florets Spoon-shaped, tubular and irregular ray florets Reduced flower head size	Banerji, and Datta, 1992 [5]. Misra <i>et al.</i> , 2003 [37], Soliman <i>et al.</i> , 2014 [51] Singh and Bala, 2015 [49] Kumari <i>et al.</i> , 2013 [30]
3	<i>Catharanthus roseus</i>	Gamma rays	Development of four petals instead of five	El-Mokadem, 2014 [7].
4	<i>Torenia hybrida</i>	Gamma rays Forchlorfenuron	Erode petal margins, extra petals, extra stamens or missing petals Serrate petal margins	Suwanseree <i>et al.</i> , 2011 [54] Nishijima and Shima, 2006 [40]
5	Carnation	Heavy ion beams	Change from serrate to rounded petals	Okamura <i>et al.</i> , 2003 [42]
6	Cyclamen	Ion-beams	Typical petal mutant	Sugiyama <i>et al.</i> , 2008 [53]
7	Rose	Ion-beams X-rays	Change in no of petal, shape, and size Increase and decrease in petal number	Murugesan <i>et al.</i> , 1993 [38] Yamaguchi <i>et al.</i> , 2003 [57] Van & Broertjes. 1989 [56]
8	Tulip	X-rays	Parrots, fringed and double	Van & Broertjes. 1989 [56]
9	Begonia rex	Gamma rays	Shape mutants	Buiatti (1990) [9]
10	Hibiscus	Gamma rays	Single flower type	Banerji, & Datta, 1986 [4]
11	Petunia	MMS, MNNG	Dissected and Dentate corolla	Mahna & Garg, 1989 [34]
12	Tuberose	Gamma rays	Large flower size	Patil <i>et al.</i> (1975) [43]
13	Bougainvillea	Gamma rays	Variegated flower	Srivastava, 2002 [52]
14	Gerbera	Gamma rays	Change in flower morphology	Jain <i>et al.</i> (1998) [29]
15	Dahlia	X-rays	Development of white tip	Broertjes, & Ballego, 1967 [8].

3. Polyploidy

Polyploidy breeding is an effective method for doubling the chromosome number of a species. It is also used to create variations in the species where the natural variations are limited. Genetic variations thus created can be further used in breeding program. Chromosome doubling has been extensively useful in several crops for breeding purpose. These new forms with improved plant architecture provide good material for the breeding programme and for further development of cultivars (Mata, 2009) [36]. The major consequences of induced polyploidy reported are increased in size and shape of plants; their leaves, branches, flower parts, fruits, and seeds (Chopra, 2008) [12]. Tetraploids are more vigorous and larger in size. To induce polyploidy mainly Colchicine and oryzalin are used, where its concentration and duration of treatment plays very important role. Several works had been done in this aspect, Gantait *et al.*, (2011) [20] had reported having vigorous plant growth with longer stem length and higher flower diameter in tetraploid *Gerbera jamesonii* Bolus cv. Sciella. Similarly Hanzelka and Kobza, 2001 [23] also reported increased flower diameter (4.20 cm) after polyploidy induction using colchicine.

4. Genetic Modification

Genetic Modification offers new opportunities for breeders of ornamental plants. Development of new ornamental varieties through gene transfer is possible by this technique. In some ornamentals, development of new varieties through hybridization or mutagenesis is very difficult or lengthy or is not an option if varieties are completely sterile, as in orchids. In these cases, GM provides an avenue for variety improvement. Genetic engineering can introduce traits which can't be generated by conventional breeding. Major traits which can be manipulated by genetic modification includes flower color, fragrance, abiotic stress resistance, disease resistance, pest resistance, manipulation of the form and architecture of plants and/or flowers, modification of flowering time, and post-harvest life etc. Ex: Chrysanthemum, *Torenia*, Cyclamen, Petunia etc.

Different techniques for Genetic Modification are:

- RNAi or Gene silencing
- Chimeric REpressor gene-Silencing Technology (CRES-T)
- Micro RNA

A. RNAi or Gene silencing

RNA-mediated interference (RNAi) is a simple method of silencing gene expression. It is a novel gene regulatory mechanism which limits the transcript level by either suppressing transcription (transcriptional gene silencing [TGS]) or by activating a sequence-specific RNA degradation process (posttranscriptional gene silencing [PTGS]/RNA interference [RNAi]). Gene silencing is a consequence of degradation of mRNA into short RNAs that activate ribonucleases to target homologous mRNA of a target gene. The resulting phenotypes either are identical to those of genetic null mutants or resemble an allelic series of mutants. These techniques are used widely for loss-of-function studies where a particular gene is specifically silenced and hence, that character is not expressed. Noor *et al.*, (2014) [41] induced double flower formation in four cultivars of *Petunia hybrida* by virus-induced gene silencing (VIGS) of two C-class MADS-box genes, pMADS3 and FBP6. He reported complete conversion of stamens into petaloid tissues in flowers induced by pMADS3/FBP6-VIGS, besides significant enlargement of upper limb-like tissues giving it a decorative appearance. Heijmans *et al.* (2012a) [24] had also reported that flowers in fbp6/fbp6 pMADS3-RNAi plants showed complete conversion of carpels into secondary flowers, giving it voluminous appearance. Similar works for double flower development was also reported in Japanese gentian (Nakatsuka *et al.*, 2015) [39], *Thalictrum thalictroides* (Galimba *et al.*, 2012) [19], Phalaenopsis orchids (Hsieh *et al.*, 2013) [26], Aquilegia (Gould and Kramer, 2007) [22] etc.

B. Chimeric REpressor gene-Silencing Technology (CRES-T)

Chimeric repressor gene-silencing technology (CRES-T) is a

powerful tool that has recently been developed for the functional analysis of plant transcription factors and for the genetic manipulation of plant traits. For CRES-T, a transcription factor is converted to a strong repressor by fusion with an SRDX repression domain, which is then expressed in plants to induce a loss-of-function phenotype. The chimeric repressor dominantly represses the expression of target genes, even in the presence of redundant endogenous transcription factors, resulting in a loss-of-function phenotype of the transcription factor (Ishida *et al.*, 2007) [27]. CRES-T has been successfully utilized to modify the shape of torenia (Shikata *et al.*, 2011), chrysanthemum (Narumi *et al.*, 2011), morning glory (Sage-Ono *et al.*, 2011), cyclamen (Tanaka *et al.*, 2011) and rose plants (Gion *et al.*, 2011).

C. Micro RNA

MicroRNA (miRNA) is a small non-coding RNA molecule (about 22nucleotides) found in Eukaryotes, which functions in RNA silencing and post-transcriptional regulation of gene expression. miRNAs are involved in almost all biological and metabolic processes. Different miRNA discovered which are related to plant architecture: miR156 (Jiao *et al.*, 2010), Leaf & Petal morphogenesis in Snapdragon: miR319 (Carle *et al.*, 2007).

Conclusion

Creation of new flower form in ornamentals plants is a major breeding target as it increases its commercial value by the virtue of its uniqueness. It has been discovered that floral organ identity is defined by five classes of homeotic genes, named A, B, C, D and E and to develop a new form, modification of these genes are required. By using various strategies like hybridization, mutation, polyploidy, genetic engineering etc it is possible to develop unique flower form including double flower. Nowadays new techniques like RNAi, CRES-T, miRNA and other gene silencing techniques are available which can be applied to silent a particular gene in order to develop plants with altered flower form. Even though there are many techniques available but, still very few variety has been developed for commercial purpose by Genetic transformation.

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