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Biotechnological interventions in floriculture

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Abstract
Biotechnology offers new opportunities for breeders to the development of new ornamental varieties through gene transfer. Genetic engineering can introduce traits not be generated by conventional breeding. Now a day's gene silencing techniques like RNAi, CRES-T, miRNA are being used to manipulate the floricultural traits but, very small number of varieties has been developed for commercial use by genetic transformation. Moon series of transgenic carnations and transgenic blue roses were recently developed by genetic engineering and these are commercialized in North America, Australia and Japan. In petunia senescence is delayed by incorporation of $etr1^{-1}$ gene and ethylene regulation. The biotechnological manipulation of floral scent can be achieved by isolation of scent biosynthetic genes. Our review summarized biotechnological efforts related to genetic manipulation of floricultural traits like flower color, disease and pest resistance, fragrance, plant stature, change in flowering time, and post-harvest life of flowers.

Keywords: Flower modifications, biotechnology, transgenic plants, cut flowers, genetic engineering

Introduction

Biotechnology in floriculture

Floriculture is a branch of horticulture which deals with commercial growing, marketing and arranging flowers and ornamental plants. Commercial floriculture has higher potential per unit area of land than most of the other field crops, and important in export angle. Globally, 118 countries are involved in the export of flowers with an export value of 13 billion US dollar in 2015. The Compound Annual Growth Rate (CAGR) of World's flower export during 2006–2015 is 5 percent [1]. Floriculture as a sunrise industry because of hundred percent export oriented value identified by government of India. Indian floriculture industry has been changed from loose flower production to cut flower production for the export purposes [2]. Ornamental plants are being widely used in professional landscaping, home gardening and also as a cut flowers so it requires new varieties with modified traits such as improved floral color, stress tolerance, and disease and pest resistance [3, 4, 5]. The global floriculture industry prospers on novelty for that genetic engineering is expanding the floricultural gene pool to promoting the production of novel varieties of cut flowers. Flower traits are modified by gene silencing techniques like RNAi, CRES-T, and miRNA. In the traditional breeding methods has limitations for developing new varieties *i.e.*, degree of heterozygosity [6, 7]. For that technique like genetic engineering (GE), genome editing has feasible methods to deal with limitations of traditional techniques [8]. Genetically modified crop area (181.5 mha in 2014) has increased in global level during the last few years [9, 4]. Recently private and government sector were interested toward the biotechnology and genetic engineering for the improvements in herbicide and pesticide tolerance and also focused on enhancement of quality traits [9, 10, 11]. The main benefit in adopting genetic engineering is very possible to introduce genes for disease resistance & stress tolerance in ornamental plant species [12, 13, 14, 15]. Similarly, floricultural traits like flower color, fragrance, resistance to abiotic and biotic stress and post-harvest life can be addressed through genetic engineering. So far mostly the color variant cut flower varieties are released *e. g*., rose [16].Transgenic cut flower crops may become more benefit to growers and buyers due to their modified floral traits [3] even though very small number of transgenic plant varieties has been field tested and released. Therefore, in this review we have highlighted recent progress in application of genetic engineering and biotechnology upon flower crops, and also we have tried to point out awareness for far reaching useful benefits for sustainability of technology & society.

Genetic engineering and gene transformation techniques

Approaches for improvement of traits through conventional methods are hybridization and mutation and non-conventional methods are biotechnological approaches *i.e.* genetic engineering and tissue culture. Genetic engineering (GE) is used to alter the plant characteristics through recombinant DNA technology. It can be used to transfer new specific traits into the plant without specific to plant species. Genetic incapability was present in conventional breeding method. Agrobacterium mediated gene transformation is an indirect method in this method *Agrobacterium tumefaciens* and *A. rhizogenes* were provide vector systems for the generation of genetically modified plants. After that incorporation of the transfer-DNA to host plant cell but protocols have been followed for using this biological vector. Particle bombardment is a direct gene transformation method. It is used to acceleration of DNA coated micro particles which carries the genes into cell [17, 18]. The genes coated on the particle have biologically active and 1 shot can transfer the genes into more cells. In another direct gene transformation micro capillaries and microscopic devices are used to incorporate DNA into target cells then those cells survive and proliferate [19], this technique is called as microinjection. It has generates transgenic clones from protoplasts [20, 21]. It requires more skills and instrumentation and one injection per cell for DNA delivery. Temporary openings of plasma lemma by the discharge of a capacitor across cell groups this technique is called as electroporation; it is also direct gene transformation method. If the DNA is direct contact with the membrane it facilitates entry of DNA molecules into cells. It is one of several standard techniques for efficient transformation [22, 23].

Genetics for flower colour improvement

Flower colour is the most important trait, dictating consumer attraction. The role of flower colour and colour pattern was attraction of pollinators, function in photosynthesis, protecting tissue against photo-oxidative damage, helps symbiotic interaction, act as intermediary compound for other compounds and the differential accumulation of pigments cause the colour pattern (Table 1). The flower colour can alter and improved by changing the plant's genetic makeup with deliberate crosses between two parents in the conventional hybridization like inter-specific hybridization, mutation and polyploidy. In non-conventional method genetic engineering technique is used. Two types of genes involved in the pigment synthesis one is structural gene which was codes for any RNA/protein product other than a regulatory protein and second type gene is regulatory gene which was influence the type, intensity and accumulation pattern of flavonoid but don't encode flavonoid enzyme (Table 2). The flower colour modification done by structural genes over expression, utilization of sense (or) antisense enzyme construction, and inhibition/addition of key biosynthetic enzymes in biosynthetic pathways.

Table 1: Major pigments in flower crops

Pigments	Type of Compounds	Examples	Colors
Porphyrins	Chlorophyll	Chlorophyll a and b	Green
Flavonoids	Anthocyanins, Anthoxanthins, Flavonols, and Flavans	Apigenin, Biochanin, Catechin, Cyanidin, Delphinidin, Kaempferol, Naringin, Pelargonidin, and Quercetin	Red, Blue, Violet Yellow and Colorless
Carotenoids	Xanthophylls/Xanthophylls	Canthaxanthin, Cryptoxanthin, Lutein, Violaxanthin, and Zeaxanthin	Yellow, Orange, Red
Betalains	Betacyanins/Betaxanthins	Miraxanthin & Portulaxanthin	Reddish To Violet Yellow To Orange

Adopted from [24]

Genetic engineering of blue colour carnation and rose

Naturally rose plants could not have the true blue colour flowers because lack of 'Flavonoid 3', 5'- hydroxylase' enzyme and delphinidin which are involved in flavonoid biosynthesis pathways which were responsible for blue colour pigment production. The scientists of Florigene Company were isolated the blue gene from the petunia in the year 1991 and they were got patented in the year 1992. Incorporation of the petunia gene into a carnation which was responsible for blue colour by that the carnation plant produces the blue colour flowers but this technique is not giving the good results in case of rose. Mauve coloured carnation variety that is 'moondust' developed by Florigene Company in the year of 1996. It was the 1st genetically modified variety of carnation which is in commercial market. Another variety of carnation was 'moonshadow' which is purple in colour developed by Florigene Company. Also they used to incorporation of both F3'5'h and Dfr genes into Dfr deficient white carnation plants for the successful development of transgenic violet colour carnation flowers [25]. The genetically modified rose 'Applause' was commercially released and marketed in year of 2009 in Japan [26]. The demand of blue rose was very high *i.e* $22 to $33 per a single stem (The Japan Today).

Today many people want to hunt black roses and for some people, when they hear black roses, surely what comes to mind is fiction, but in fact this flower has been found. This beautiful rose has been found in the village of Halfeti in Turkey. Nutrition in the soil from the Euphrates River makes this area the only place where these flowers can grow. The content that is in the ground where this flower grows that affects the colour of this flower Although they look black, blooms start out as red in spring and dark in summer. The rose is different from other roses, because it only blooms twice a year when spring or spring comes. Black rose is only able to survive within 15 days and the price of black rose is 320 rupees.

Table 2: Enzymes and genes assimilated in pigment synthesis

Enzymes and Genes	Plant species
Chalcone synthase (Chs)	Antirrhinum, Chrysanthemum, Dianthus, and Rosa
Flavonoid 3' hydroxlase (F3'h)	Antirrhinum, Aster, Chrysanthemum, and Orchid
Flavonol synthase (Fls)	Petunia and Rosa
Flavone synthase (Fns)	Antirrhinum and Gerbera
Dihydroflavonol-4-Reductase (Dfr)	Antirrhinum, Aster, Dianthus, Orchid, and Petunia

Adopted from [27].

Transgenic red/orange colour flowers: Cyanidin and delphinidin derivatives are synthesized in petunia but the pigment pelargonidin derivatives are not produced because of substrate specificity of Dfr gene in petunia. Flavonoids are responsible flower colour in petunia. Production of purple to white (or) from purple to red colours flower in the commercial varieties of *Petunia hybrid* was successfully achieve by suppression of endogenous flavonoid biosynthetic genes and expression of heterologous gene (or) combination of both genes [28]. The down-regulation of the F3'h gene & the expression of the rose Dfr gene cause the production of red to orange colour flowers. These results show that functionality of metabolic engineering of the flavonoid biosynthetic pathway to change flower color but very few of the genetically modified petunia show phenotypic stability [29]. For the commercialization purpose need to produce many self-sufficient transgenic lines, select stable phenotypes and keep them in tissue culture further multiplication.

Genetic engineering for yellow colour flowers

Flavonoids and anthocyanins and their biosynthetic pathways are well established and they are important for the determination of colour in most of the flowers [30, 31]. The modifications of flavonoid biosynthetic pathway by the genetic engineering would provide novel flower colour in floriculture, which was not possible in conventional breeding technique [30]. In *Antirrhinum majus* and *Dahlia variabilis* has the bright yellow colour flowers due to aurone flavonoids pigments. Aureusidin synthase (AmAS1) was the key enzyme that catalyzes biosynthesis of aurone from chalcones in *Antirrhinum majus* but the overexpressing AmAS1 gene can failed to generate aurones which are responsible for yellow colouration in transgenic plants. Ono *et al*. (2006) [32] report that co-expression of chalcone 4'-O-glucosyltransferase (4' CGT) and AmAS1 genes and down-regulation of anthocyanin biosynthetic pathway by RNA interference can produce yellow flowers in transgenic plants. Glucosylation and acylation modifications occurred before output is transported to the vacuole in the biosynthesis process. It is the way to production of yellow flowers in ornamental species which are lacking yellow colour flowers (Table.3).

Table 3: Flower colour modifications by regulating biosynthesis pathways

Plant species and genes	Methods	Flower colour original to modified	References
Cyclamen (F3'5'h)	Antisense	Purple to Red/ pink	Boase *et al*. (2010) [33]
Cup flower (F3'5'h)	Antisense	Violet to Pale blue	Uyema *et al*. (20006) [34]
African daisy (F3'5'h)	RNAi	Magenta to Reddish	Seitz *et al*. (2006) [35]
Gentiana (F3'5'h)	RNAi	Blue to Lilac	Nakatsuka *et al*. (2010) [36]
Bush rose (F3'5'h)	Over expression	Red to Bluish	Katsumoto *et al*. (2007) [37]

Genetic engineering for longer vase life

Flowers normally show senescence at particular period of time after harvest. Senescence is a physiological process in which fading of flowers will take place. Due to premature petal senescence the lifespan of flowers were reduced. The increased ethylene production were triggers senescence process which leading to fading of flowers. A major goal in cut flower industry is to increase vase life of cut flower. Antisense RNA has played a major role in cut flower industries particularly increase in vase life. The long postharvest longevity increases the value of cut flower. Senescence is highly controlled process requires active gene expression & protein synthesis for programmed cell death. Generally, different types of chemicals are required for increasing the cut flowers vase life [38]. Different biotechnological techniques have been used for vase life increase [39]. Ethylene is essential for senescence in fruits and flowers. In plants the biosynthesis of ethylene process starts with, the conversion of *S*-adenosyl methionine (SAM) to 1-aminocyclopropane-1-carboxylic acid (ACC) in the presence of ACC synthase (ACS) then after the conversion of ACC to ethylene are catalyzed by ACC oxidase (ACO) [40, 41]. By using a complementary DNA clone which was representing carnation ACO from *Dianthus caryophyllus* L. cv. Scania. They have produced transgenic carnation plants containing an antisense ACO gene.

Silencing the ACO gene that down regulate ethylene synthesis in transgenic carnation then the plants exhibiting delayed flower senescence. Scania and White Sim transformant cultivars containing the antisense gene which were exhibiting decreased ethylene production and delayed flower senescence. The antisense ACO flowers produce very less or not detectable ACO mRNA (or) ACS mRNA. However, by applying exogenous ethylene in these transgenic flowers the ACS and ACO genes can be induced [42]. Vase life of genetically modified carnation flowers was increased three times compared with control by the harboring Arabidopsis etr-1 gene [43]. The antisense ACO gene has suppressing the level of ACO mRNA, eliminated ethylene production and extended carnation flower vase life. The expression of the ACS & ACO genes in petals must be dependent on the presence of ethylene. It is useful approach for further analyzing the different molecular and physiological processes influenced by the ethylene in carnation plants. Savin *et al.* 1995 [42] also investigated the stability of antisense gene suppression during multiplication of carnation. Similar findings in Oncidium and Odontoglossum can be achieved by mutating ethylene receptor gene for the inhibition of ethylene biosynthesis genes [44]. In rose cultivar 'Linda' Chlorophyll amount was increased by Agrobacterium mediated transformation of PSAG12-ipt. Moreover, the ipt expression level in transgenic plants upon exposure to darkness and ethylene application [45]. These are strengthening the concept of improving postharvest qualities by genetic engineering rather than by conventional breeding (or) chemicals application.

Genetic engineering for biotic and abiotic stress resistance

The wide variety of flower crops and ornamental plants grown as pot plant, cut flower, or garden plant is challenged by various microorganisms during production and postharvest period. Traditionally, flowers crops have been grown in India in the open fields, where they have been exposed to both biotic and abiotic stresses during the cultivation. Hence, the quality is not up to the standards for export of such flower produce. However, in the era of globalization as there is lot of demand for various floricultural products in the export market and the modern floriculture will meet demand of the present day's consumers. Disease resistance breeding in floriculture is still underdeveloped compared to other crops because of host specificity for example *Phytophthora quercina* on oak are host specific [46] while *P. ramorum* has more than a hundred host species [47].

The wide diversity in floricultural species and diseases with their specific host ranges makes breeding for disease resistance complex [48]. For growers and consumers of flower crops, temperature, light intensity, humidity and salinity have an impact on the ability to produce a marketable product on the season. Genetically modification research for improving drought tolerance is being explored by Ornamental Biosciences at Stuttgart region, Germany [49]. Potentially increase the range of environments in which petunia grown as bedding plant by the transfer of the CBF3 gene from Arabidopsis to petunia for increasing the frost tolerance [50].

Plants challenged by pathogens are able to induce expression of pathogenesis-related genes, such as b-1, 3-glucanase, chitinase, and cysteine-rich antimicrobial protein genes [51, 52, 53]. Build-up of pathogenesis-related proteins enhanced resistance to subsequent challenge by pathogens; it is known as systemic acquired resistance [54, 55]. Recently a lot of studies have reported on development of genetically modified plants harboring pathogenesis-related protein genes, and expression of these genes has enhanced disease resistance [56, 57, 58]. Therefore, using the genetic engineering to develop powdery mildew resistance roses is a highly useful approach. Lepidopteran insects resistance chrysanthemum cultivar 'shuho-no-chikara' developed by modified of delta-endotoxin gene cry1ab (mcbt) from *Bacillus thuringiensis*. The transgenic chrysanthemum resistance against chrysanthemum stunt viroid (csvd) and *Fusarium oxysporum f. Sp. Dianthi* [59].

Table 4: Specificity of various genes in flower crops

Gene source and gene	Achievement	Reference
Rockcress (Asl38)	Increased column patterns in Celosia cristata flower	Meng *et al*. 2009 [60]
Chrysanthemum morifolium (Ls)	Reduced branch number	Jiang *et al*. 2010 [61]
Agrobacterium rhizogenes (ipT)	Increased branches in chrysanthemum	Boase *et al*. 2004 [62]
Dianthus caryophyllus (ACO/ACS)	Vase life increase	Zuker *et al*. 2001b [63]
Orchid species (MADS-Box)	Converted 2nd row petals into calyx	Thiruvengadam, Yang. 2009 [64]

Genetic engineering for morphological traits of plant

Reports on homeotic mutants have shown many more important aspects of genetic control on flower shape and size. The combination A, B, and C genes involved in development of flower parts. A is responsible for development of sepals, the both A and B produce the petals, B and C responsible for the stamens and the only C gene is produced the carpels [65]. The ABC model and its modified types [66] are known to be applicable for flower part development in a wide range of flower crops [67]. An interest increased for developing novel flower shape and size through molecular manipulation because of AGAMOUS gene isolation from *Antirrhinum majus* and the consequences of A, B, and C genes deficiencies in flowers. The modification of sepals to petals in transgenic torenia plants because of constitutive expression of *Antirrhinum majus* genes such as DEF and GLO in torenia. A carpeloid structure developed in the place of sepals because of constitutive expression of 'C' gene which was isolated from *Rosa rugosa* [68].

In floriculture controlled plant is very important and it can achieve by silencing of both DmCPD and DmGA20ox genes to generate a short height and delayed-flowering in chrysanthemum using the RNAi vector. The resulted binary vector was inserted into wild-type plants *i.e* Pink chanetii through Agrobacterium mediated T-DNA transformation. 6 individual transgenic plants were confirmed to be positive by PCR analysis. From those 3 transgenic lines RNAi-1, RNAi-3, and RNAi-10 resulted good inhibitory effect. The period plant development the transgenic lines shown a reduced growth rate, small and dark leaves [69].

Genetic engineering for floral scent enhance

Flower fragrance plays very important to increase the demand of flowers because a consumer choice depends on scent associations while flower purchase. Flower produce a specific metabolites to attracts pollinators, increases aesthetic traits, stimulate (or) repress signaling pathways and protect against pathogens and it have vital function in reproductive process of plants [70, 71]. 700 floral scents have been identified in 60 families of different flowering plants such as categorized of terpenoid (Monoterpenes and Sesquiterpenes), benzenoid, and aromatic amino acid [72, 73]. The specific metabolites are produced in low quantities. Thus, their detection and characterization is difficult. Therefore, increased production of specific metabolites may involve in detection, isolation, and identification scent and pigmentation in flowers. Although the fragrance biochemistry identified number of scent controlling genes. A fragrant biosynthetic gene in a transgenic flower can lead to a modification of flower fragrance for example in carnation flower the synthesis of volatiles regulated, membrane associated, and also cytosol partitioning occurs [74]. In snapdragon petal cuticles produce volatiles compounds [75]. Roses produce 400 and above volatile compounds and appear likely to be source of several floral scent genes such as those encoding S-adenosylmethionine: orcinol O-methyltransferase [76, 77, 78, 79] and terpenoid biosynthetic enzymes.

Conclusion

New transgenic flower crops may provide potential profits to growers and consumers by producing variety of flower appearances. The conventional breeding limitations were overcome by the genetic engineering approaches. Recent researches come up with improvement of flower colour, vase life, fragrant and biotic and abiotic stress resistance in flower crops. Difference in flower colour is determined by the various classes of pigments, and knowledge at biochemical and molecular level has made to evolve new flower color. We promote that modification of flower crops through genetic engineering is useful in commercial perspective and we should not be bothered about sale of transgenic plant and flowers in the market. The very recent genetic engineering in flower crops has revealed examples such as transgenic carnation and transgenic blue rose and those were shown the satisfaction over the transgenic flower. Vegetative propagation was approached for propagation of transgenic crops and has not shown harmful effect on environment and consumer and grower. New genes discovery, development of markers, and genome sequences of flower crops will leads to production of novel transgenic lines in floriculture. Additionally, techniques such as Zinc finger nuclease, Transcription activator-like effector nuclease, and clustered regularly interspaced short palindromic repeats are facilitating to modify the targeted genome and enhance in commercialization of genetically modified flower crop crops. Floral traits governed by several genes that have not been identified and isolated, high technical knowledge, expensive equipment's, and government rules and regulation were limited in genetic engineering in floriculture. However in future floriculture industry will gain stable profits by production of transgenic flower crops.

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