Available online at www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.5491

ISSN: 2320 – 7051

Int. J. Pure App. Biosci. **5 (6):** 633-638 (2017)

Review Article

International Journal of Pure & Applied Bioscience

OPEN ACCESS

Transgenic Flowers for Novel Colour

Subiya, R., Kengond, Priyanka, T., Kurabet. Humajahan, S. Vadrali and Shafeeq, Bawoor

Department of Floriculture and Landscape Architecture,

Kittur Rani Channamma College of Horticulture Arabhavi, 591 218

*Corresponding Author E-mail: subiyark@gmail.com

Received: 21.08.2017 | Revised: 25.09.2017 | Accepted: 30.09.2017

ABSTRACT

Transgenic flowers for novel colour are the one in which genes are modified or introduced through genetic engineering and has integrated in its genome for pleasing look. Flower colour is a key element in consumer selection between ornamental varieties available in the market place. Thus introduction of novel colour, forms, through genetic engineering by the way of producing transgenic flowers is likely to have large influence on the floriculture industries. In this review we have describe the experimental data of manipulation of flower for novelty by the different methods of genetic engineering and have compared the results in terms of colour change and stability for long term. Metabolic engineering involved the manipulation of anthocyanin biosynthesis pathway, down and up regulation of flavonoid anthocyanin pathway. Molecular breeding included gene silencing technology for colour modification in transgenic flowers. Among different types genetic engineering tools RNAi technique found to be superior and powerfull method to obtain transgenic flowers with aimed phenotype. This modification of flower colour has applied both for ornamental as well as commercial flower crop species.

Key words: *Transgenic flowers, RNAi technique, Metabolic engineering*

INTRODUCTION

Ornamental plants paint the world around us with a plethora of flower and leaf colour. Man has pursued his quest to improve flowering plants from the domestication or import of wild species to the increasingly sophisticated breeding strategies.Some ornamental flowers only have a narrow colour spectrum, while in others species, specific colours are lacking. Genetic engineering played a vital role in floriculture industry to broaden the colour spectrum of flowers. The extensive information available on the genetics and biochemistry of pigment biosynthesis gives a strong base in the production of transgenic flowers.

Transgenic flowers for novel colours are the one in which gene are modified or introduced from different species & artificially inserted in its genome through metabolic engineering. The second generation of transgenic flowers use metabolic engineering to insert new pathways into plants or improve expression of enzymes in existing ones. Understanding the biosynthetic pathways of flavonoid provide background to produce novel colours.

Cite this article: Subiya, R., Kengond, Priyanka, T., Humajahan, K., Vadrali, S. and Bawoor, S., Transgenic flowers for novel colour, *Int. J. Pure App. Biosci.* **5(6):** 633-638 (2017). doi: http://dx.doi.org/10.18782/2320-7051.5491

History of transgenic flowers

GM of flower color was first demonstrated more than 20 years ago. (White petunia for creation of novel orange colour by maize DFR gene).Petunia, tobacco and torenia have been model species. Delphinidin production presents a major breakthrough in the achievement of blue roses. In 1993 the gene encoding flavonoid 3', 5' hydroxylase (F 3',5' H) was isolated, providing a tool for the development of the color modifications in *D. caryophyllus* and *R. × hybrida*. Florigene has already successfully created blue carnations using gene technology and these are available in markets of Japan, USA and Australia since 1996. Carnations are the first genetically modified commercial flowers.

Role of novel colour in flowers : Attraction of pollinators and protecting tissue against photo oxidative damage, Symbiotic plant-microbe interactions, The flavonoids include protectants against herbivores and many phytoalexins, Anthocyanins help in protecting plants against excessive light, Many flavonoids act as antioxidants.

Major Pigments Responsible for Colour: Chlorophylls, Carotenoids, Flavonoids, Betalains.

Flavonoid biosynthetic pathway

The pathway starts with the condensation of coumaroyl-CoA and malonyl-CoA via a polyketide folding mechanism by chalcone synthase (CHS) to form the intermediate, chalcone, the primary precursor for all classes of flavonoids. After the action of chalcone isomerase (CHI), chalcone is modified to its isomer naringenin, which is further hydroxylated by a group of cytochrome P450-dependent monooxygenases to produce flavanones. Cytochrome P450-dependent monooxygenases include flavonoid 3-hydroxylase (F3H), flavonoid 3'-hydroxylase (F3'H) or flavonoid 3'5'-hydroxylase (F3'5'H) for dihydrokaempferol, eriodictyol or pentahydroxyflavanone, respectively. These three flavanones can be modified again by the catalysis of F3H, F3'H and F3'5'H to produce dihydroflavanols, dihydrokaempferol, dihydroquercetin and dihydromyricetin. Dihydroflavonol 4-reductase (DFR) further reduces these dihydroflavonols to colorless leucoanthocyanidins, which are catalyzed by anthocyanidin synthase (ANS) to their corresponding anthocyanidins, pelargonidin, cyanidin and delphindin.

Transformation is carried through gene transfer which is either vector mediated or direct gene transfer. *Agrobacterium mediated* & T-DNA are vector mediated gene transfer techniques and Electroporation, Microinjection, Particle bombardment are direct gene transfer techniques.

After Transformation: Plant tissue culture also has very important role in the production of genetically modified plants because after the transferred cell or tissue are need to regenerate into new plant and production of more plants from that genetically modified plant. Plant tissue culture techniques can be direct (Meristem culture & Nodal culture) as well as indirect (Leaf, ovule, anther, petal and cell culture).

Flower color modification

- The modification of flower colour via genetic engineering has generally focused on metabolic engineering of the flavonoid pathway.
- In many ornamentals color range is limited by the genetics of the plant species.
- GM is one of the powerful tool to overcome this limitation.

- **Different factors that contribute to the spectrum and intensity of color**

1. The type and level of anthocyanins
2. The complexing of anthocyanins with metal ions
3. The presence of co pigments (flavones or flavonols)
4. The modification of basic anthocyanins .
5. The variation in vacuolar pH

Modifying the flavonoid biosynthesis pathway

- Down regulation of flavonoid genes.
- Over expression of flavonoid genes.
- Over expression of foreign gene to introduce novel functions in target plants.

1. **Down regulation of flavonoid genes:** Reppression of the flavonoid pathway at a

single enzyme step resulted in the impairment of pigment synthesis. Gene silencing is a suitable genetic tool for down regulation of flavonoid genes. Sense RNA (Co-suppression), Antisense RNA, RNAi (RNA interference).

2. **Over expression of flavonoid genes:** This strategy aims at increasing the synthesis of existing compounds or enabling the synthesis of new flavonoids by boosting or rerouting the subtrate flow towards end products of pathway. To accumulate higher levels of anthocyanins.
3. **Over expression of foreign gene in to target plants:** Over expression of foreign genes mainly aimed to introduce novel traits in transgenic flowers.Typical example in this is to introduce F3’5’H activity in species lacking blue colors and Transgenic violet carnations.

Amir *et al.*[1] studied the Modification of flower color by antisense suppression of the flavanone 3-hydroxylase gene. He considered Flowers of carnation cv Eilat which had dark orange colour with reddish edges. Here F3H activity was inhibited using antisense suppression of the corresponding gene. For this F3H was cloned into binary vector in an antisense orientation under the regulation of CaMV 35S promoter and used to transform in cv Eilat.Following transformation carnation plants regenerated and grown in the green house. All the plants developed & flowered normally but few of them exhibited colour modification from attenuated to complete loss of orange colour. In attenuated one, Fig1 (b) 70% pelagonodin was detected and in other two Fig 1(c,d) there was complete loss of pelargonidin content was found.

To evaluate the expression of F3H in the transgenic plants, Northern blot analysis was performed, this clearly revealed the dramatic suppression of F3H transcript level in F3H-11 & F3H-14 in contrast to control and F3H-33 lines. Thus this study concluded that the use of antisense suppression of F3H gene was successful in reducing the level of anthocyanin concentration in coloured cultivar of carnation.

Murray *et al*[3] studied the Isolation and antisense suppression of *flavonoid 3', 5'-hydroxylase* modifies flower pigments and colour in cyclamen. In this study two cultivars of minicyclamen were considered ie purple and wine red. Antisense CpF3'5'H transformants were produced from purple cultivar using pPN48 or pPN51 constructs. Antisense CpF3'5'H transformants were produced from wine red cultivar using pLN96 or pPN50 constructs. These binary vectors harboured in their T-DNAs the cyclamen antisense F3'5'H gene under a CaMV35S promoter and either nptII or hpt selectable marker genes under a NOS promoter.

The modification of cyclamen transgenic flowers with respect to colours indicated the ositive correlation with the loss of endogenous F3’5’H transcripts. In purple cultivar, there was aloss of purple colour and became pink. In wine red cultivar colour remained with pinkish hue only but with reduced intensity chroma. Anthocyanin concentration in transgenic lines showed drastic reduction in comparision to control lines. In conclusion that antisense suppression of F3’5’H was successfully used in changing anthocyanin profiles and flower colour in cyclamen.

Noriko *et al*.[4] studied about RNAi suppression of the anthocyanidin synthase gene in *Torenia hybrida* yields white flowers with higher frequency and better stability than antisense and sense suppression. *Torenia hybrida* cv summerwave (blue) was considered in this study using binary vectors pSPB805, pSPB806, pSPB807 for RNAi, sense and antisense suppression respectively.

The suppression of anthocyanin synthase gene was done using three method of post transcriptional gene silencing, which involved antisense suppression, sense suppression and RNAi suppression. Antisense suppression gave only few white flowers, sense suppression gave no white flowers and RNAi suppression yielded flowers which were maximum in white colour. This study showed the usefulness of RNAi technique to suppress the target gene. Among all the transgenic lines

interestingly one transgenic line 805-36 exhibited novel colour phenotype which was very different from wavy colouration of petals of host plant

To confirm the ANS gene down regulation, RT-PCR analysis was performed, here it was clearly shown about the suppression of ANS transcript in transgenic lines in copmarision to control line.In this study they concluded that RNAi is a powerfull tool to obtain transgenic plants with aimed phenotype and ANS gene can be used successfully in blocking the anthocyanin biosynthetic pathway.

Shinzo *et al.*[5] studied the Flower color modification of *Petunia hybrida* commercial varieties by metabolic engineering. The objective was to produce flowers of white colour by down regulation of endogenous gene towards acyanic petunias in *P.hybrida* cv surfinia purple. The phenotypes generated by *CHS* gene suppression resulted into transgenic flowers which did not remain as vigorous as those of host one. As there was complete loss of flavonoids resulted in the less protection to various kinds of stress. And became unstable after a year of cultivation in green house. As an alternative DFR & F3H genes became suitable targets of gene suppression to obtain white or paler shades of flowers.

Binary vectors pBPFT1 (sense suppression) of F3H gene and pBPDF1 (antisense suppression) of DFR gene were constructed and subjected to transformation of surfinia purple mini.resultant flowers exhibited modified colour like by F3H gene suppression yielded star shaped flowers, Suppression of only DFR gene yielded star shaped flowers with spots and F3H & DFR gene suppression yielded acyanic /pale flowers

For obtaining orange coloured petunias there was a need to suppress an endogenous *F3'H* gene and overexpress the rose *DFR* gene, pSPB520 (antisense suppression of *F3'H* gene) and pSPB538 (RNAi suppression of *F3'H* gene) were constructed and used to transform Baccarat Red. The RNAi construct (pSPB538) yielded higher frequency of flower color changes, which indicates that RNAi is superior to antisense to achieve the suppression of the target gene.

This study concluded with the usefulness of RNAi technique in yielding higher frequency of flower colour modifications in petunia.

Yukihisa *et al.*[2] studied about the Engineering of the Rose Flavonoid Biosynthetic Pathway Successfully Generated Blue-Hued Flowers Accumulating Delphinidin. Usually flowers of rose lack violet to blue colours due to the absence of delphinidin based anthocyanin as they do not possess F3'5'H activity a key enzyme for delphinidin biosynthesis.

The binary vectors used in this study (pSPB130, pSFL207, pSFL236 and pSPB919) fig 11. The vector pSPB130 is designed for the constitutive overexpression of the viola F3050H BP40 gene and the torenia 5AT gene in rose. The binary vector pSFL207 is used for the constitutive overexpression of the viola F3050H gene alone, and pSFL236 is for the co-expression of the viola F3050H and the iris DFR genes. The vector pSPB919 is to down-regulate the endogenous rose DFR gene using RNA interference (RNAi) and to overexpress the iris DFR and the viola F3050H genes. The transgenic roses exhibited various concentrations of delphinidin content and flower colour variation, here fig 12 BCDF achieved novel color which was not achieved by hybridization programme.

The flower colour alterations of transgenic roses were subjected to flavonoid analysis. Here the presence of delphinidin and myricetin indicates the introduction of F3'5'H gene function in transgenics roses.They observed that incorporation of only viola F3'5'H gene alone would not generate roses containing exclusive accumulation of delphinidin. Hence this prompted them to design another vector pSPB919 in order to down regulate an endogenous pathway that potentially competes against the introduced F3'5'H. they considered cv Lavande rose to evaluate the efficacy of binary vector pSFL207, pSFL236 & pSPB919. Transgenic

roses harbouring pSPB919 produced highest concentration of delphinidin%.

Higher the delphinidin % bluer the flower colour was achieved in transgenic roses. Additional expression of iris DFR gene pSFL236 increased delphinidin concentration indicating that iris DFR is useful to change the metabolic flux towards delphinidin%. But where as in transgenic roses harboring pSPB919 exhibited exclusive accumulation of delphinidin % than the other two vectors.

Develpoment of the Moon Series of Carnation (Florigene Ltd. and Suntory Ltd)

This was the first strategy experimented prior to the generation of moon series carnations by Over-expression of a petunia F3'5'H gene under the control of a constitutive promoter in a pelargonidin producing carnation variety produces petals in which delphinidin derivatives contribute to about 70% of total anthocyanins . However, there was only a slight colour change toward blue.In order to increase the content of delphinidin derived anthocyanins it was necessary to avoid competition for substrates between two key endogenous enzymes of the anthocyanin pathway (DFR and F3'H) and the introduced enzyme (F3'5'H). To achieve this white carnation cultivars were selected that were specifically deficient in the DFR gene.

Expression of petunia F3'5'H (under the control of a promoter region from the snapdragon CHS gene) and petunia DFR (under the control of a constitutive promoter) genes in one such DFR mutant resulted in exclusive accumulation of delphinidin derivatives and significant colour change toward blue (FLORIGENE®Moondust, this was the first genetically modified floricultural crop to be sold in the world. Expression of a pansy F3'5'H gene (under the control of a promoter region from the snapdragon CHS gene) and a petunia *DFR-A* gene (under the control of its own promoter and terminator regions) resulted in transgenic plants which also exclusively accumulated delphinidin but at a higher concentration. These flowers had a dark violet colour LORIGENE®Moonshadow. The same gene combinations were subsequently introduced into a white standard-type carnation (also a mutant in DFR). In these transgenic plants different amounts of delphinidin based pigments accumulated in the petals, depending on the transgenic events. Four events that exhibited stable colour were selected and released in following names.

FLORIGENE®Moonvista
FLORIGENE®Moonshade
FLORIGENE®Moonlite and
FLORIGENE®Moonaqua.

Production of world's first blue roses (*Florigene* company)

Florigene company followed three crucial steps for the production of blue roses in that first one was to stop rose DFR gene by using gene silencing technology.gene silencing uses natural mechanism that degrades RNA the courier that delivers the gene instructions to make proteins like the enzyme DFR .second step was to open the door for the production of blue pigment this became possible by inserting pansy gene for blue pigment production.once the red pigment production ceases using gene silencing . Next step was to find DFR gene good at producing blue pigment and placing it in rose. Hence this florigene decided to replace DFR rose gene with DFR gene from iris which is excellent at producing blue pigment. This DFR gene from iris inserted into rose and subsequently a rose with blue pigment produced.

This study concluded by highlighting the major role of gene silencing technology in producing blue roses successfully.

Barriers to transgenic flowers for commercialization

1. Product development cost
2. The cost of Regulation
3. Identity preservation
4. Acceptance in the marketplace

CONCLUSION

Genetic modification of an ornamental plant can be a successful venture, from both a scientific and a commercial perspective. The transgenic varieties have proven to be genetically very stable during mass scale vegetative propagation and there have been no

unexpected effects on either the environment or on the health of those handling the flowers. Major obstacle to dozens of genetically modified ornamental products entering the marketplace is that the high costs and expertise required for commercial development. To ease this burden the regulatory requirements for ornamentals, should be reduced.

REFERENCES

1. Amir, Z., Tzvi, T., Ben-Meir, H, Marianna O., Elena S., Hanan I., Forkmann G., Stefan M., Neta-Sharir I., David Weiss1. and Alexander V Modification of flower color and fragrance by antisense suppression of the flavanone 3-hydroxylase gene. *Mol Breed,* **9:** 33–41 (2002).
2. Yukihisa, K,, Fukuchi-Mizutani, M., Yuko, F., Filippa, B., Timothy, A. H, Mirko, K., Noriko, N., Yonekura-Sakakibara, K., Junichi, T., Alix, P., Guo-Qing, T., Narender, S. N., Chin-Yi, L., Barry, K.D, Shinzo, T, Toshihiko, A., Takaaki, K., John, G. M. and Yoshikazu, T., Engineering of the Rose Flavonoid Biosynthetic Pathway Successfully Generated Blue-Hued Flowers Accumulating Delphinidin. *Plant Cell Physiol,* **48(11):** 1589–1600 (2007).
3. Murray, R. B., David, H. L., Kevin, M. D., Gayle, B. M., Deepa, P., Kathy, E. S. and Simon, C. D., Isolat ionland antisense suppression of *flavonoid 3',5'-hydroxylase* modifies flower pigments and colour in cyclamen. *BMC Plant Biol,* **10:** 107 (2010).
4. Noriko, N., Fukuchi-Mizutani, M., Kiyoshi, M., Kenichi, S,, and Yoshikazu, T., RNAi suppression of the anthocyanidin synthase gene in *Torenia hybrida* yields white flowers with higher frequency and better stability than antisense and sense suppression. *Plant Biotechnol,* **23:** 13–17 (2006).
5. Shinzo, T., Yuko, F., Noriko, N., Yukihisa, K., Yonekura-Sakakibara, K., Fukuchi-Mizutani, M, Kazuyuki, O, Yukiko, U, Hideo, O, Timothy, A. H, Takaaki, K. and Yoshikazu, T. Flower color modification of *Petunia hybrida* commercial varieties by metabolic engineering. , Flower color modification of *Petunia hybrida* commercial varieties by metabolic engineering. *Plant Biotechnol,* **21:** 377–386 (2004).
6. Yoshikazu, T., Filippa, B. and Steve, C., Recent Progress of Flower Colour Modification by Biotechnology. *Int. J. Mol. Sci.* **10:** 5350-5369 (2009).