

Breeding, physiological and management approaches for modification of flower colour

¹Harshitha M., ²M. S. Biradar, ³R. M. Hosamani

¹M. Sc. (Agri.) student, ²Professor of Horticulture, ³Professor of Horticulture

Department of Horticulture

College of Agriculture, University of Agricultural Sciences, Dharwad, India.

Abstract - Every flower is a symbol of nature's vitality, contributing to the aesthetic appeal of our surroundings through its captivating hues. The market value of a flower is primarily by its colour, a critical factor from the consumer's perspective. Despite the abundant variety of colours naturally occurring in flowers, there persists a desire for fresh and unique shades. Various internal and external factors influence the development and regulation of flower coloration. Flower colours are primarily determined by three classes of pigments: flavonoids, carotenoids, and betalains. While flavonoids and carotenoids are widely distributed, betalains are specific to plants within certain genera of the Caryophyllales order. Among these pigments, flavonoids, particularly anthocyanins, are the most prevalent in flowers, offering a spectrum of colours ranging from yellow to orange, red, and purple. Through interventions such as modifying the pigment biosynthetic pathway, manipulating metal ions, and shaping surface cell structures, novel colours can be cultivated in flowers. This review delves into various methodologies, including hybridization, mutation, polyploidization, genetic engineering, regulation of vacuolar pH, use of plant growth regulators, and tinting techniques, each explored in detail with relevant studies. These approaches offer promising avenues for modifying and enhancing flower colour, catering to evolving consumer preferences and market demands.

Key - Pigments, Genetic engineering, Mutation, Co-pigments, Vacuolar pH, Tinting.

I. INTRODUCTION

Flowers, often hailed as nature's laughter, hold a special place as divine creations. Botanically, they are the reproductive structures of seed-bearing plants, comprising the calyx, corolla, and reproductive organs. Their vibrant colours add beauty to surroundings, with each hue playing a vital role in attracting pollinators and safeguarding floral organs. Despite the diverse array of colours, some plant species lack specific hues, prompting breeders to pursue flower colour enhancements (Zhang *et al.*, 2023). The ornamental plant industry, valued at over \$70 billion annually, continues to evolve, with a focus on generating new colours (Zhao and Tao, 2015).

This review article is based on Master's Seminar- I presented during semester-II of year 2022-2023.

Chemically distinct pigments—betalains, carotenoids, and anthocyanins—contribute to this vibrant spectrum. Research has revealed that flower colour development is influenced by factors such as petal tissue structure, pigment distribution, and environmental conditions, offering avenues for regulation through genetic engineering (Tanaka *et al.*, 2005). The modification of flower colour remains a key area of botanical research and horticulture, driven by the desire to enhance visual appeal and meet market demands. Innovative approaches, including genetic engineering, have been employed to overcome limitations in natural colour variation. As the industry flourishes, understanding and manipulating flower colour continue to offer both visual diversity and insights into genetic principles.

II. ROLE OF FLOWER COLOUR:

1. **Pollinator Attraction:** Flower colours serve as visual cues to attract pollinators like bees, butterflies, and birds, facilitating the transfer of pollen for fertilization.
2. **Photosynthesis Aid:** The pigments responsible for flower colour, particularly chlorophyll, assist in photosynthesis, the process by which plants convert light energy into chemical energy.
3. **Protection Against Photo-Oxidative Damage:** Flower pigments help absorb excess light energy, shielding delicate tissues from harmful photo-oxidative damage caused by reactive oxygen species.
4. **Intermediary Compounds:** Flower pigments can serve as intermediaries in biochemical pathways,

contributing to various physiological processes within the plant.

5. **Horticultural Therapy:** The vibrant colors of flowers are utilized in horticultural therapy to alleviate mental ailments, promoting emotional well-being and relaxation.
6. **Dye Extraction:** Flowers with pigmented petals are a source of natural dyes used in textiles, cosmetics, and food colouring.
7. **Health Benefits:** Some flower pigments, such as anthocyanins and carotenoids, possess antioxidant properties and can act as precursors of essential nutrients like vitamin A, contributing to human health.
8. **Stress Resistance:** Flower coloration may confer resistance to both biotic (e.g., pests and pathogens) and abiotic (e.g., UV radiation, drought, and temperature extremes) stresses, enhancing plant resilience.
9. **Symbiotic Interactions:** Flower colours can influence symbiotic relationships between plants and beneficial microbes, such as mycorrhizal fungi, aiding in nutrient uptake and overall plant health.

(Bordoloi *et al.*, 2019)

III. FLOWER PIGMENTS:

Flower pigments play a pivotal role in determining the vibrant colours seen in landscapes and floral displays. These pigments accumulate on petals, creating captivating patterns and hues. They primarily belong to three major groups:

- **Flavonoids:** This group includes subclasses such as anthocyanins, metalloxanthins, chalcones, and aurones. Anthocyanins give rise to red, purple, and blue colours, while metalloxanthins produce yellow to orange hues.
- **Carotenoids:** Carotenoids are divided into carotenes and xanthophylls. Carotenes contribute to yellow to orange colors, while xanthophylls produce yellow to red hues.
- **Betalains:** Betalains are a taxonomically restricted group comprising betacyanins (red) and betaxanthins (yellow). They add distinct coloration to flowers, contributing to their visual appeal.

IV. WHY MODIFICATION IN FLOWER COLOUR MAY BE DESIRED:

Agronomic or Consumer Preferences: Modification allows for the development of flower colours that align with desired traits, such as a preference for white carnations over red-flowering varieties due to aesthetic or symbolic reasons.

Introducing New Colours: Modification enables the introduction of flower colours that do not naturally occur in a particular crop, meeting consumer demand or expanding the aesthetic range of flowers, such as achieving blue hues in roses, carnations, or orchids.

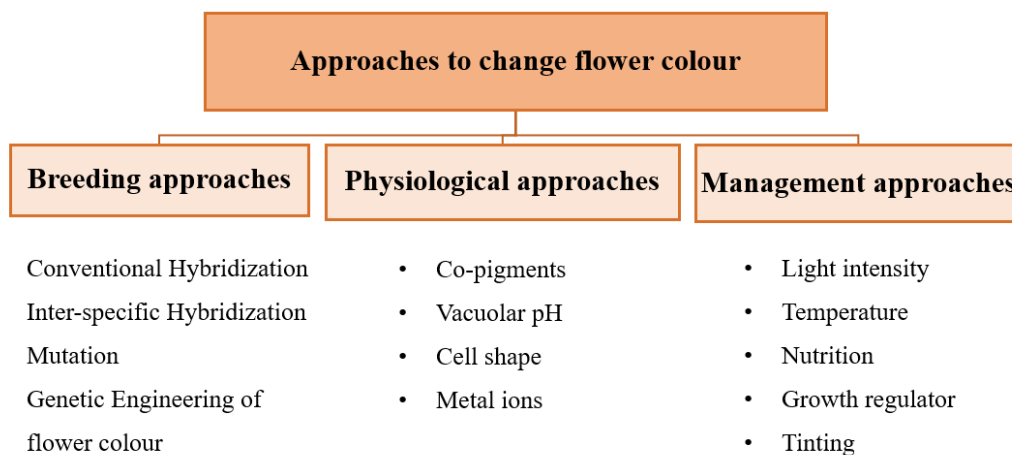
Temporal Changes in Colour: Some flowers change colour during different stages of growth, such as morning glories that exhibit varying colours from bud to flowering.

Trends and Fashion: Colour preferences can change over time, with trends shifting from season to season or year to year. Modification allows breeders to adapt to evolving consumer preferences and market demands by offering novel and trendy colours.

Market Value: Flowers with novel or rare colours often command higher prices due to their exclusivity or novelty. For example, the rarity of blue roses contributes to their high market value, making modification for such colours financially appealing.

V. APPROACHES TO CHANGE FLOWER COLOUR

Understanding the pigment biosynthetic pathway would be helpful to know the function of the various enzymes responsible for the end product. Identifying and isolating the genes responsible for particular enzymes could aid in further colour modifications. Various approaches like hybridization, mutation, polyploidization, genetic engineering, regulation of vacuolar pH, use of plant growth regulators and tinting can be adopted to modify the flower colour by targeting the particular step or particular enzyme in the biosynthetic process and are discussed with suitable literatures.



VI. BREEDING APPROACHES

i. Conventional Hybridization

It is defined as the crossing of two or more plants which are genetically different from each other to produce a new crop. Hybridization is effective to combine all the good characters in a single variety to create genetical variation and to exploit the hybrid vigour.

A study was conducted aiming to produce novel red-purple *Delphinium* flowers by introducing the *F3'H* gene from *Delphinium zalil* into *Delphinium cardinale*. They found that flavonoid 3'-hydroxylase was transcribed in the hybrid plants, leading to the biosynthesis of cyanidin-based anthocyanins. This resulted in the generation of novel purple-red flowers and concluded that in delphiniums, delphinidin is dominant over pelargonidin, and cyanidin is also dominant over pelargonidin (Sakaguchi *et al.*, 2019).

A study investigated chrysanthemum hybridization using transgenic lines 2081-33 and 2013-03 from 'Sei Arabella' and T37, transformed with pB423 containing a *F3'5'H* gene. Wild-type 'Sei Arabella' and T37 were controls. All pigmented progeny from the pink wild-type 'Sei Arabella' and T37 plants exhibited pink petals. When blue transgenic plants were used, progeny displayed white, pink, and blue petals. Progeny generally exhibited hues similar to parents but with paler colours. T37 transgenic progeny showed a slightly purplish hue compared to the bluer T37 parent, potentially due to higher accumulation of cyanidin-based anthocyanin (Aida *et al.*, 2020).

ii. Interspecific Hybridization

Interspecific hybridization is the crossing of two species of the same genus. This allows the exploitation of useful genes from wild, unimproved species for the benefit of the cultivated species.

A study conducted on interspecific hybridization aiming to transfer yellow flower colour from yellow-flowered carnations ($2n=2x=30$) and *Dianthus knappii* ($2n=2x=30$) to a white-flowered cultivar of the garden pink, *Dianthus plumarius* ($2n=6x=90$). Progeny from carnation crosses were pink, while those from *D. knappii* crosses were pale cream to yellow, with varying intensities. *D. knappii* displayed high levels of flavone and flavonol glycosides, absence of chalcones, and very low levels of carotenoids. Conversely, yellow pigments in carnation cultivars were confirmed as chalcones. F1 hybrids with *D. knappii* were yellow because they contained the same pigments, while hybrids with carnations were pink due to inability to convert chalcones to dihydroflavones and anthocyanins (Gatt *et al.*, 1998).

A study was conducted by Aida *et al.* (2018) on Interspecific hybridization of chrysanthemum. The purple flower line 1434-18 and blue flower line 1575-14 are transgenic plants of *Chrysanthemum morifolium* 'Taihei'. The progeny of the pink wild-type 'Taihei' only had pink petals, not purple or blue of the transformants. When a purple transformant (1434-18) was used as the seed parent, nine white, one pink, and two purple petaled plants were produced. PCR analysis confirmed the presence of the transgene in all hybrids, indicating its role in blue petal formation. Notably, flower pigmentation in all offspring was lighter than in parental 'Taihei'. This phenomenon was attributed to hybridization with *C. japonense* var. *japonense*, which naturally produces white flowers, resulting in reduced anthocyanin precursor levels and diminished anthocyanin accumulation.

iii. Mutation:

Mutation is the sudden heritable change in the phenotype of an individual. It may be spontaneous or induced, macro or microlevel (Sangeetapriya *et al.*, 2020). The elements responsible for mutation are called mutagens. There are two types of mutagens namely physical mutagens and chemical mutagens. Ornamental plants are ideal for mutation. First officially released commercial mutant cultivar is Tulip (cv. 'Faraday' from cv. 'Fantasy by irradiation) expressing an altered flower colour in 1936 (Broertjes and van Harten, 2013). Approximately 55% of the mutant cultivar changes in flower colour due to mutation. Some of the successfully achieved crops are Chrysanthemum, Bougainvillea, Rose etc., (Datta *et al.*, 2001).

Prasad *et al.* (2008) identified novel coloured radio mutants within the irradiated population of chrysanthemum cultivar Ajay, a pink-coloured spray cultivar. These mutants, observed in the form of chimeras, were isolated, purified, and proliferated. Micro-plantlets derived from these mutants were successfully established in the field, resulting in the development of a new mutant variety named Pusa Anmol. The yellowish-pink mutant, named Pusa Anmol, was successfully established in the field, evaluated for three consecutive years, and is set for release. It is also photo/thermo intensive and blooms two to three times a year alongside its parent cultivar.

A study on in vitro mutagenesis in rose (*Rosa hybrida* L) cv. Raktima to induce novel traits was conducted by Bala Madhu and Kanwar Pal Singh (2015). Maximum flower color variation (8.51 %) was observed in treated nodal explants exposed to 55 Gy treatments, with frequencies of 3.92 % and 7.46 % in 25 Gy and 40 Gy treatments, respectively. Two distinct flower colour variants were isolated and propagated through tissue culture, RK-1 variant features red-coloured flower buds with stripes, in contrast to the solid red colour of the original cv. Raktima (control). RK-2 exhibited fully opened large flowers with dark-coloured petals and streaks, unlike the solid red flowers of the original cv. Raktima.

In the study, Gerbera "Opal" in vitro shoots were utilized and irradiated using three types of ion beams with varying line energy transfers (LETs). The total number of trait/morphological mutants observed were seven following Carbon (C) irradiation, 23 following Argon (Ar) irradiation, and 14 following Iron (Fe) irradiation. The mutation rate was highest at 10 Gy for C irradiation (2.2%), 5 Gy for Ar irradiation (14.1%), and 5 Gy for Fe irradiation (4.6%). A total of 34 plants (1.4% of the total irradiated plants) exhibited single mutations, with flower colour mutants observed across all ion beam irradiations, resulting in 21 plants (0.9% of the total irradiated plants). Petal shape changes manifested in four patterns: slender shape, sword shape, receptive shape, and semidouble. Complex mutations often coincided with flower color changes, yielding seven plants (0.3% of the total irradiated plants). These included mutations such as flower color/petal slender shape, flower color/semidouble, and petal slender/male sterility, obtained from various ion beam irradiations (Hosoguchi *et al.*, 2021).

iv. Genetic Engineering of flower colour

Genetic engineering techniques focus on manipulating the flavonoid biosynthesis pathway, crucial for determining flower colour. Various approaches include:

1. Over-expressing or silencing structural genes in the flavonoid biosynthetic pathway:

Chalcone synthase (CHS): Over-expression or silencing of CHS alters flower colour in Petunia, Torenia, Chrysanthemum, lisianthus, etc., generating white flowers.

Chalcone isomerase (CHI): Mutant plants with altered CHI expression accumulate yellow-coloured chalcones, seen in aster and carnation.

Flavanone hydroxylase/Flavonoid-3'-hydroxylase/ Flavonoid-3',5'-hydroxylase: Mutation in the f3h locus leads to white flowers in Petunia and Antirrhinum.

Dihydroflavonol-4-reductase (DFR): Transgenic carnations carrying sense dfr and sense f3'5'h from Petunia produce violet flowers.

Anthocyanidin synthase (ANS): Application of transgenic ANS for pigment modification is less common.

Flavonoid 3-O-glucosyltransferase (3GT): Overexpression of snapdragon 3GT cDNA in lisianthus results in novel anthocyanins.

2. Other enzymes:

Aureusidin synthase (AS): In species like snapdragon, cosmos, and dahlia, AS produces chalcone-aurones, imparting a yellow color.

Chalcone reductase (CHR): CHR, along with CHS, catalyzes the production of iso-liquiritigenin, a precursor of yellow pigments in certain species.

3. Transformation with multiple genes:

Petunia and torenia transformed with F3'5'H and DFR genes exhibit altered flower colors.

Table 1: Enzyme and genes with different methods used to change flower colour

Plant species and genes	Methods	original colour	Modified colour	References
Cyclamen(F3'5'h)	Antisense	Purple	Red/pink	Boase <i>et al.</i> (2010)
Cup flower (F3'5'h)	Antisense	Violet	Pale blue	Uyema <i>et al.</i> (2006)
African daisy(F3'5'h)	RNAi	Magenta	Reddish	Seitz <i>et al.</i> (2006)
Gentiana (F3'5'h)	RNAi	Blue	Lilac	Nakatsuka <i>et al.</i> (2010)
Bush rose (F3'5'h)	Over expression	Red	Bluish	Katsumoto <i>et al.</i> (2007)

v. Generation of variegated flowers by using transposons

Insertion or excision of transposons in flavonoid biosynthetic or regulatory genes produces a mosaic or variegated phenotype. Insertion of a transposon results in white sectors of a colored background, and excision of such a transposon results in coloured sectors on a white background. The sizes of sectors: Depend on the timing of insertion and excision Morning glory and Petunia etc.,

vi. Co expression

A study was conducted by Shin *et al.* (2023) on Flower colour modification through co-overexpression of the *VtF3'5'H* and *RhNHX* genes in *Rosa hybrida*. They introduced the *Rosa hybrida NHX (RhNHX)* and *Viola tricolor F3'5'H (VtF3'5'H)* genes into the *Rosa hybrida* line "KR056002" by using Agrobacterium mediated transformation to determine whether the increase in pH and the overexpression of anthocyanin synthesis genes in petals and young leaves can improve anthocyanin biosynthesis. Petal colour shifted notably from white to red-purple in transgenic lines compared to NT plants. The introduction of the *VtF3'5'H* gene notably stimulated delphinidin anthocyanin synthesis, while the upregulation of the *RhNHX* gene contributed to raising the pH levels in petals and young leaves of transgenic lines. Ultimately, this genetic manipulation resulted in a significant colour transformation, shifting the petals of transgenic plants from white to red-purple. This study presents a promising approach for achieving colour modification in rose plants

vii. PHYSIOLOGICAL APPROACHES

Despite the breeding approaches, the final colour outcome results from a combination of factors:

1. Pigment Structure: The molecular arrangement dictates light absorption and reflection.
2. Pigment Type and Concentration: Different pigments and their concentrations influence colour intensity.
3. Co-pigments: Interactions between pigments or co-pigments create unique shades.
4. Metal Ion Type and Concentration: Certain metal ions, such as aluminium, interact with pigments, affecting colour.
5. Vacuolar pH: The acidity or alkalinity of the cellular vacuole influences pigment stability.
6. Shape of Surface Cells: The physical structure of flower surface cells impacts light interaction and perceived colour.

Understanding these factors enhances our appreciation of flower colours and provides insights for horticultural practices, allowing for the manipulation and enhancement of floral pigments for aesthetic and commercial purposes.

i. Co pigments

Copigments, such as flavonols and flavones, are frequently linked with anthocyanins, serving to stabilize the coloured pigments. While most flavones and flavonols lack color themselves, they provide substance to white, cream, and ivory-hued flowers. Through the action of flavonol synthase (FLS), dihydroflavonols like dihydrokaempferol, dihydroquercetin, and dihydromyricetin undergo a conversion to flavonols such as kaempferol, quercetin, and myricetin, respectively, by introducing a double bond between positions 2 and 3 of

the C-ring in the flavonoid structure. The genes responsible for encoding FLS have been isolated from various plant species. Additionally, in certain plants like *Petroselinum*, *Chrysanthemum*, *Dahlia*, and *Gerbera*, flavones can also be produced from flavonols through the action of flavone synthase (FNS) (Tanaka, 2005).

ii. Vacuolar pH

Anthocyanin colour shifts depending on pH: red in strong acidity, purple in neutrality, and blue in alkalinity. However, plant cell vacuoles typically maintain a weakly acidic pH, leading to unstable purple anthocyanins. To stabilize flower color, three mechanisms are proposed: metal chelation, altering vacuolar pH, and forming molecular associations

The vacuole's pH, typically around 5.5, plays a crucial role in stabilizing anthocyanins, with even slight pH shifts affecting flower colour visibly. Generally, pH decreases lead to a redder hue, while increases result in a bluer tone. In *Petunia*, specific loci (ph1 to ph7) have been identified, mutations in which cause the flowers to turn blue (Bordoloi *et al.*, 2019).

Morning glory (*Ipomoea tricolor*) undergoes a striking transformation: its initially strong reddish-purple buds' transition to light blue open flowers within a span of four hours. This colour change is facilitated by the PURPLE (PR) protein, which transports sodium ions (Na⁺) into the vacuole while expelling hydrogen ions (H⁺), leading to an increase in vacuolar pH from 6.5 to 7.5.

Yoshida *et al.* (2021) investigated the relationship between sepal color variation and vacuolar pH in *Hydrangea macrophyllum* using micro-spectrophotometry and proton-selective microelectrodes. They observed that blue cells (with a peak wavelength at 589 nm) exhibited a significantly higher vacuolar pH of 4.1 compared to red cells (peak wavelength at 537 nm) with a pH of 3.3. The blue cells also contained higher levels of Al³⁺, suggesting its contribution to the development of blue coloration. Differences in Al³⁺ content between blue and red cells may stem from variations in cultivars or soil pH.

iii. Cell shape

The accumulation of anthocyanin pigments is influenced by the shape of cells. For instance, in wild-type snapdragon petals, the conical shape of epidermal cells enhances light absorption, resulting in a velvet sheen. Conversely, a mutant displaying a fainter coloration was observed to have flattened epidermal cells. While the mechanisms governing cell shape remain unclear compared to other factors, there have been no reports of molecular approaches to manipulate cell shape yet (Bordoloi *et al.*, 2019).

VIII. MANAGEMENT APPROACHES

i. Light Influence on flower colour

Anthocyanin biosynthesis is dependent on light, and researchers have often used artificial light during the post-harvest period to enhance the colour intensity of certain fruits. Flowers such as roses, petunias, and carnations exhibit faded colours when they open under low light intensities. This fading is attributed to a reaction mediated by UV photoreceptors, cryptochromes, phytochromes, and sugar production by leaves or stems.

Researchers demonstrated that localized photomorphogenic responses within flowers play a crucial role in mediating anthocyanin production. They found that shading flowers led to a reduction in anthocyanin biosynthesis (Herrero, 2015). Conversely, in *Antirrhinum majus*, increasing light intensity was associated with higher anthocyanin content. Additionally, Dai (2013) showed that different types of light affect anthocyanin content differently, with fluorescent and blue light increasing anthocyanin levels, while red light and incandescent lamps decreased them. Gao *et al.* (2020) further revealed that fluorescent lamps, particularly in the 470-600 nm wavelength range, provided optimal efficiency for achieving vibrant flower coloration

ii. Temperature

Research has extensively explored the impact of temperature on anthocyanin synthesis and flower colour development during post-harvest storage. Studies indicate that low temperatures can enhance the transcription of genes involved in phenylpropanoid biosynthesis, such as phenylalanine ammonia-lyase (PAL), and genes responsible for enzymes in flavonoid and anthocyanin production (Boulton, 2001). Conversely, high temperatures often lead to reduced anthocyanin concentration in plants due to decreased biosynthesis and increased degradation (Berardi *et al.*, 2021).

Moreover, elevated temperatures, especially when combined with higher metal concentrations, have been observed to diminish anthocyanin accumulation in flowers, leading to petal discoloration. This effect is attributed

to a decrease in the activity of enzymes like PAL and CHI involved in anthocyanin synthesis. Conversely, lower temperature regimes have been linked to more vibrant flower coloration compared to higher temperatures (Araceli *et al.*, 2009).

iii. Effect of nutrition and growth regulators on flower colour

Adjusting NPK administration through fertilization can significantly enhance fruit and flower coloration. Positive effects have been observed in various flowering plants, such as gladiolus and geranium, where nitrogen and potassium fertilization influenced the colour of flower spikes and leaf pigmentation, respectively. Phosphorus deficiency stress in geranium resulted in darker purple rings on leaves due to increased anthocyanin production.

Controlled-release fertilizers have shown superior effects on flower colour intensity and quality compared to traditional water-soluble fertilizers. For instance, in *Anthirrinum* flowers, benzyl-adenine (BA) and abscisic acid (ABA) led to increased anthocyanin content, while gibberellic acid (GA) and indoleacetic acid (IAA) decreased it at certain concentrations (Prisa, 2023).

In Tulipa, experiments conducted by Hojjati *et al.* (2019) investigated the effects of three phytohormones (ABA, GA3, JA) and sucrose on flower colour composition and secondary metabolites. Results indicated that GA3 at 500 mg/L significantly increased anthocyanin accumulation and postharvest performance. ABA and JA, despite enhancing anthocyanin accumulation, were associated with reduced vegetative growth parameters and vase life.

Additionally, a study by Adeoye *et al.* (2020) found that increasing NPK concentration in sunflower varieties led to brighter yellow ray florets. Higher levels of NPK resulted in increased floral colour intensity, with the Jos local variety displaying 97% colour saturation at 90 kg/ha.

iv. Tinting

Tinting is a crucial method for enhancing flower colour when natural pigments are weak. Ranchana *et al.* (2017) noted its effectiveness in achieving desired colours post-harvest. For aesthetic purposes, tinting white flowers may be the only option to attain specific hues, often fetching a premium in the market. This process allows for adding one or more colours to cut flowers, improving their appearance and appeal, whether fresh or dried.

Tinting, an artificial colouring process, is typically applied to white or light-coloured flowers like Carnations, Orchids, Roses, and Chrysanthemums, using dyes such as Bromocresol red/green, Eosin yellow, ammonium purpurate, phenol red, and various food colorants (Sowmeya *et al.*, 2017).

A study was conducted by Ranchana *et al.* (2017) on the standardization of tinting techniques in China aster cv. Local White. The study revealed that food dyes such as Apple Green, Lemon Yellow, and Orange Red exhibited rapid uptake, requiring only 2.1, 2.4, and 2.3 hours respectively, resulting in excellent final coloration. Using the RHS color chart, it was noted that higher dye concentrations corresponded to increased color intensity in the flower petals. However, higher dye concentrations were associated with reduced vase life in tinted spikes. Despite this, the highest colour intensity was achieved with a 4% dye concentration compared to other treatments. While tinted flowers exhibited attractive coloration suitable for flower arrangements, they also displayed shorter vase life. Therefore, tinted flowers are recommended for display for only one day, despite their visual appeal.

In the study conducted by Kashyap *et al.* (2022) on tuberose flower spikes. Overall, treatment T15 (pH 5 + 4 percent sucrose + 40,000 ppm of royal blue colour) excelled in several parameters including physiological weight loss, water loss, color intensity, floret opening, and vase life prolongation. Thus, it is deemed beneficial and economical for tinting tuberose flowers. Not only does it enhance colour variation, but it also improves various post-harvest parameters and proves economically viable. Therefore, treatment T15 is recommended for tinting tuberose flowers to achieve desirable colour variation, prolonged vase life, and enhanced durability.

IX. CONCLUSION

The floriculture industry aims to innovate by creating flowers with novel colours, recognizing the important role of colour in consumer preference. Flower colour is primarily determined by various pigments, alongside factors like vacuolar pH, co-pigments, and metal ions. Leveraging advancements in biochemistry and molecular biology, researchers can manipulate flower colour, offering a wide array of hues. Methods such as heavy ion beam radiation induce mutations, leading to colour variations, while genetic engineering enables the modulation of target genes for colour modification. Additionally, tinting presents a cost-effective approach to alter flower colour, providing avenues for enhancing colour vibrancy. Moving forward, the emphasis should be on enhancing

the stability of colour modifications in plants. This involves exploring techniques to preserve colour consistency throughout the plant's lifecycle, ensuring that desired hues remain vibrant over time. By continually innovating in these areas, the floriculture industry can meet consumer demands for an array of captivating flower colours.

X. REFERENCES

- Adeoye O, Pitan O, Akinkunmi O and Akinyemi O, 2020, Synergistic interactions between honeybee *Apis mellifera* L. and flower colour of sunflower in response to NPK fertilizer application. *Ethiopian Journal of Environmental Studies & Management*, 13(4): 495- 508.
- Aida R, Douzono M, Yoshioka S, Noda N, Tsuda M and Ohsawa R, 2018, Inheritance of bluish flower colour of transgenic chrysanthemum by interspecific hybrids. *Japan Agricultural Research Quarterly*, 52(4): 339-345.
- Aida R, Noda N, Yoshioka S, Douzono M, Tsuda M and Ohsawa R, 2020, Crossability between transgenic blue chrysanthemums and the wild *Chrysanthemum* species *Chrysanthemum japonense* var. *japonense*. *Japan Agricultural Research Quarterly*, 54(4): 335-340.
- Araceli C O, Lourdes P H M, Elena P H M, Andrés G V C, 2009, Chemical studies of anthocyanins: a review. *Food Chemistry*, 113 (4): 859–871.
- Bala M and Singh K P, 2015, In vitro mutagenesis in rose (*Rosa hybrida* L.) cv. Raktima for novel traits. *Indian Journal of Biotechnology*, 14(1): 525- 531.
- Berardi A E, Korinna E Lea J, Therese M, Cannarozzi G M, Cris K, 2021, Complex evolution of novel red floral colour in *Petunia*. *Plant Cell*, 33(2): 2273–2295.
- Boase M, Lewis D, Davies K, Marshall G, Patel D and Schwinn K, 2010, Isolation and antisense suppression of flavonoid 3', 5' -hydroxylase modifies flower pigments and colour in cyclamen. *BMC Plant Biology*, 10(1):107-110.
- Bordoloi D, Sarma A and Sarma D, 2019, A Review of Genetic Improvement of flower Colour. *Bulletin of Environment, Pharmacology and Life Sciences*, 8(2): 9-16.
- Boulton R, 2001, The pigmentation of anthocyanins and Its role in the colour of red wine: a critical review. *American journal of Enology and Viticulture*, 52 (2): 67-87.
- Broertjes C and van Harten A M, 2013, Applied mutation breeding for vegetatively propagated crops. Elsevier, 42(2): 110-115.
- Dai L, 2013, Analysis on components and stability of anthocyanidin from rhododendron with different colours. *Journal of Anhui Agricultural Sciences*, 41 (14): 6455-6458.
- Datta S K, Chakrabarty D and Mandal A K A, 2001, Gamma ray - induced genetic manipulations in flower colour and shape in *Dendranthema grandiflorum* and their management through tissue culture. *Plant Breeding*, 120(1): 91-92.
- Gao B, Jin L, Li Y, Zhang P, Zhang X, Zhu Y, Si J, 2020, Changes of pigment components in *Dendrobium catenatum* flower under different storage conditions. *China Journal of China Materia Medica*, 45 (4): 829–837.
- Gatt M K, Hammett K R, Markham K R and Murray B G, 1998, Yellow pinks: interspecific hybridization between *Dianthus plumarius* and related species with yellow flowers. *Scientia Horticulturae*, 77(4): 207-218.
- Herrero J A and Frutos M J, 2015, Influence of rutin and ascorbic acid in colour, plum anthocyanins and antioxidant capacity stability in model juices. *Food Chemistry*, 173(1): 495–500.
- Hojjati Y, Shoor M, Tehranifar A and Abedi B, 2019, Modification of flower color pigments and color composition with hormonal treatments and sucrose in *Tulipa gesneriana*'Kingsblood'. *Journal of Ornamental plants*, 9(2), 73-91.
- Hosoguchi T, Uchiyama Y, Komazawa H, Yahata M, Shimokawa T and Tominaga A, 2021, Effect of three types of ion beam irradiation on gerbera (*Gerbera hybrida*) in vitro shoots with mutagenesis efficiency. *Plants*, 10(7): 1480-1489.
- Kashyap M, Tamrakar S K and Khilari M, 2022, Evaluation of varying levels of pH, sucrose and colour concentrations on tinting and vase life of tuberose (*Polianthes tuberosa* L.) cut spike. *The Pharma Innovation Journal*, 11(4): 1318-1326.

- Katsumoto Y, Mizutani M F, Fukui Y, Brugliera F, Holton T A and Karan M, 2007, Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. *Plant Cell Physiology*, 48(11): 1589-1600.
- Nakatsuka T, Mishiba K I, Kubota A, Abe Y, Yamamura S and Nakamura N, 2010, Genetic engineering of novel flower colour by suppression of anthocyanin modification genes in gentian. *Journal of Plant Physiology*, 167(4): 231-237.
- Prasad K V, Kumar S, Raju D V, Swarup K, Singh O and Patil M R, 2008, In vitro isolation, purification, rapid bulking and field establishment of a promising radio-mutant Pusa Anmol from spray Chrysanthemum cv. Ajay.
- Prisa D, 2023, Biology, abiotic factors and biotechnology influencing flower colour.
- Ranchana P M, Ganga M, Jawaharlal and Kannan M, 2017, Standardization of Tinting Techniques in China aster cv. Local White. *International Journal Current Microbiology and Applied Sciences*, 6(9): 27-31.
- Sakaguchi K, Isobe C, Fujita K, Ozeki Y and Miyahara T, 2019, Production of novel red-purple delphinium flowers containing cyanidin-based anthocyanin using hybridization breeding. *The Horticulture Journal*, 88(4): 514-520.
- Sangeetha Priya S, Bhatt S, Bhatt T and Chawla L, 2020, Approaches for modification of flower colour—a review. *Journal of Emerging Technologies and Innovative Research*, 7(4): 1337-1343.
- Seitz C, Vitten M, Steinbach P, Hartl S, Hirsche J and Rthje W, 2007, Redirection of anthocyanin synthesis in *Osteospermum hybrida* by a two-enzyme manipulation strategy. *Phytochemistry*, 68(2): 824-833.
- Shin J Y, Xu J, Park P M, An H R, Kim Y J, Kim S J and Lee S Y, 2023, Flower colour modification through co-overexpression of the *VtF3' 5' H* and *RhNHX* genes in *Rosa hybrida*. *Plant Cell, Tissue and Organ Culture*, 153(2): 403-416.
- Sowmeya S, Kumaresan S and Priya L S, 2017, Effect of multi colors in tinting techniques in cut flowers (Rose and Carnation). *Chemical Science Review letter*, 6(24): 2250-2253.
- Tanaka Y, Katsumoto Y, Brugliera F and Mason J, 2005, Genetic engineering in floriculture. *Plant Cell, Tissue and Organ Culture*, 80(1): 1-24
- Ueyama Y, Katsumoto Y, Fukui Y, Mizutani FM, Ohkawa H and Kusumi T, 2006, Molecular characterization of the flavonoid biosynthetic pathway and flower color modification of *Nierem bergia* sp. *Plant Biotechnology*, 23(1): 19-24.
- Yoshida K, Toyama-Kato Y, Kameda K and Kondo T, 2021, Sepal color variation of *Hydrangea macrophylla* and vacuolar pH measured with a proton-selective microelectrode. *Plant Cell Physiology*, 44(3): 262-268.
- Zhang D, Xie A, Yang X, Yang L, Shi Y, Dong L, Lei F, Sun L, Bao M and Sun X, 2023, Analysis of physiological and biochemical factors affecting flower colour of herbaceous peony in different flowering periods. *Horticulturae*, 9(4): 502-524.
- Zhao D and Tao J, 2015, Recent advances on the development and regulation of flower color in ornamental plants. *Frontiers in Plant Science*, 6(1): 1-13.