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Floriculture and Transgenic

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Abstract

“Genetic engineering has revolutionized floriculture by enabling the modification of plant species and the creation of new flower color varieties (Chandler, Stephen et al, 2010). Despite the global cultivation of transgenic crops, floriculture has been slow to adopt transgenic breeding. "Floriculture, with its cut flower market as a significant component, is on track to achieve a market size of $43.8 billion by 2027, according to Markets and Markets. This industry plays a crucial role in uplifting rural economies through international trade. For instance, floriculture products from Africa find their way to markets in the U.S. and Europe. Similarly, Asian nations such as Japan, China, and India are strengthening their financial positions through the import and export of floriculture commodities (Tanaka, Yoshizu et al., 2009). One of the notable advancements in floriculture has been the creation of new flower color varieties by manipulating pigment metabolic pathways in plants. However, there are challenges. Some flowering plants, despite their high market value, do not realize their full economic potential due to issues like prolonged flowering seasons and diseases." These challenges can be addressed through the production of transgenic plants using genetic engineering and plant tissue culture techniques. The study aims to explore the potential of genetic engineering associated with plant tissue culture techniques in enhancing floriculture, particularly in developing new flower color varieties and improving the production of high-value flowering plants, thereby highlighting the economic impact of floriculture globally.

Introduction

The aspect of floriculture can be seen throughout the world, but Europe, North America, and Japan can be prominently said to be the main markets for floriculture products. (Chandler and Tanaka, 2007). In the cut flowering industry, the byproduct of floriculture is only known as the flower, where the sole purpose of the flower is for sale for decoration and gifting purposes. (Chandler and Tanaka, 2007; Tanaka and Ohmiya, 2008).

Building on this, the industry is now exploring the potential of genetic engineering to enhance the aesthetic and commercial value of flowers. Techniques such as somatic embryogenesis and shoot regeneration are being employed to create transgenic varieties of ornamental flowering plants. And while using those processes foreign genes can also be introduced to the article plant for transgenesis. The most commonly used gene transfer methods are the Agrobacterium mediated gene transfer method (indirect) and the particle gun method (direct). These are highly used because those techniques are capable of successful gene transfer in both monocotyledonous and dicotyledonous flowering plants. For dicotyledonous species Rose, Carnation geranium and for monocotyledonous Lily and tulip, those 2 above mentioned processes are extremely helpful. (Shibata, 2008). There are several kinds of transgenic modification can be done in ornamental flower plants, but most of the transgenic modifications are associated with the flower colour. As an example, flavonoid’s pigment is responsible for the colours yellow, orange, red, and purple, whereas carotenoids pigment is responsible for the colours red, orange,

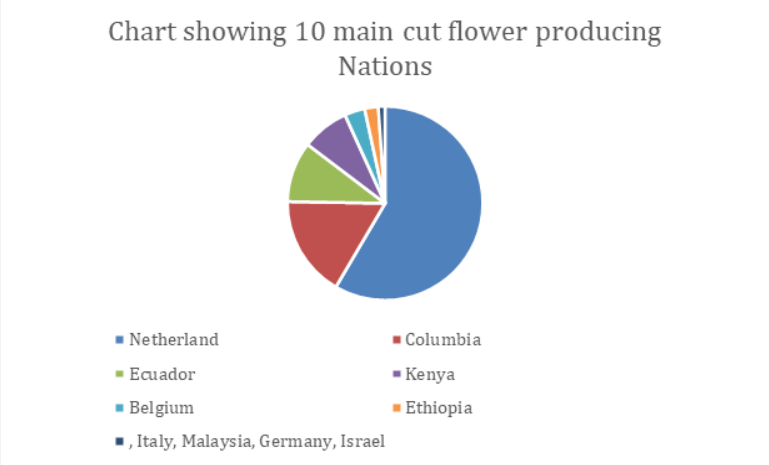
and yellow. Those pigments can be found in an embedded form inside the membranes of chloroplasts and chromoplasts. Those pigments are produced by following several biochemical pathways, and 2 or more than 2 genes are responsible for the production of such colour pigments. THC, for example, is responsible for the yellow color of flowering plants like snapdragon. The first common flavonoid compound is THC. The UDP-glucose enzyme chalcone 4'-O-glucosyltransferase (4'CGT) first converts THC into THC 4'-O-glucoside, which is then transformed into aureusidin 6-O-glucoside (yellow color) by the aureusidin synthase route.

The determination of the in vitro activity of the aureusidin synthase mechanism, catalyzing THC into aureusidin (a yellow pigment), has been reported in other plants by the induction of 2 genes isolated from snapdragon. The genes are AmAS1 and AM4'CGT. Invitro cloning of those 2 genes gave the concept that both genes must be expressed together for the biosynthesis of the product aurone and yellow colour pigment. This process can be obtained using PCR (for gene amplification) and the Agrobacterium mediated gene transfer method (Chen-Kuen et al, 2015). Several more techniques of gene transfer and micropropagation are available for efficient and time effective floriculture. This review will be enlighten of those techniques associated with floriculture and transgenesis of plants.

Floriculture

"Floriculture" is a term that simply means that anything is related to flowers. Floriculture is also commonly referred to as flower farming. And It is also a branch of horticulture. According to the report, the "Netherlands" is the core of the global floriculture sector. The prosperity of that country shows that it has good breeders, infrastructure, a good marketing and exporting plan, and strong networking. After analyzing the Dutch market, it is apparent that the floriculture sectors play a significant role in strengthening the country's economy. Several advancements occurred in Europe throughout the twenty-first century, including new markets, virtualization, and the easing of trade barriers. There are several developments that happened in the Europe in 21st century, like new markets, virtualization, liberalization of trade barriers. These factors assisted countries such as the Netherlands in significantly improving their trading systems (U. Rickert and G. Schiefer 2012).

The important hubs for the cut flower business can be recognized using the pie chart below, as well as the contribution of those countries to the worldwide cut flower market.



Graph-1 showing main cut flower producing nations.
(Amita Abrol and HS Baweja, 2019)

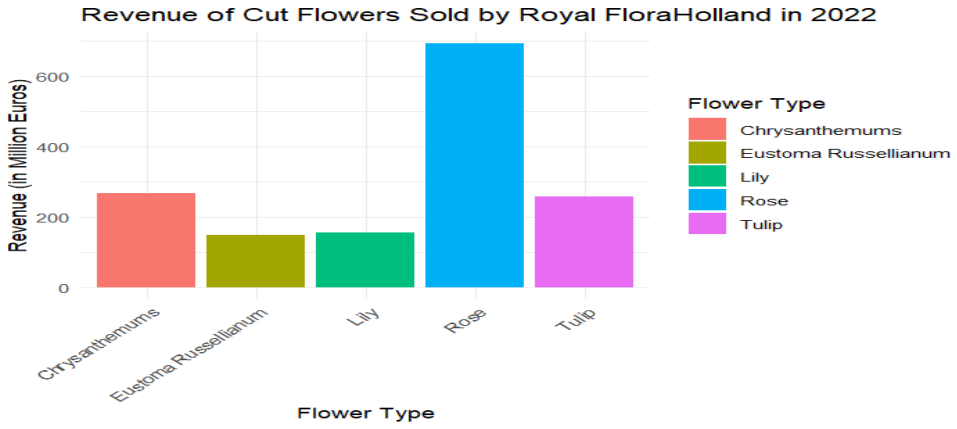
The economic value of cut flowers is significant. According to data from American and Dutch papers, between the late 1990s and the early 20s, around 11 billion dollars was invested in the cut flower industry for their applications, with 44 billion dollars created in the early twenties. Analytically, the average annual growth rate was 6-9% (Van Uffelen and Groot 2010). The revenue amount was the reason why several European nations received significant investment in the cut flower market. According to the industrial viewpoint, the cut flower business is separated into two broad subcategories: cut flowers and loose flowers. Examples of these two types are shown below.

Cut_Flower	Loose_Flower
Rose	Marigold
Gerbera	Jasmine
Tuberosa	Crossandra

Table-1, contains the examples of cut flower and loose flower.

These are the categories created based on the industrial relevance of cut flower products. There are also other categories, such as flower seed and bulb production, essential oil perfumes, and protected cultivation. However, the cut flower is most commonly used for ornamentation. Cut flowers may include exotic flower kinds that are not available locally, whereas loose flowers are traditional flowers that can only be obtained in that specific place (ICAR). According to the current state of floriculture in India, the top states contributing to India's cut flower business are Paschimbanga, Andhra Pradesh, Tamandu, Orissa, Gujrat, Karnataka, and Rajasthan Narendra K. Dadlani, (2020). Floriculture is an extremely effective way to contribute to the economy, as European and some Asian countries have proved. However, floriculture in India is not at its optimum performance, but it is extending its strong roots throughout the country. The government has launched various missions through the Ministry of Agriculture to educate flower producers about the benefits of floriculture (Dr. Thankham Ghule, 2012).

A new statistical data collection from market research giant "STATISTA" reveals the most popular flowers on the market. The information is about a corporation called "Royal Flora Holland" that transports floriculture-based products all over the world. According to the firm, over 2.8 billion roses were sold in 2020, while lilies were fifth, with over 273 million lilium (lily) flowers sold on that particular year. This facts can be explained clearly by certain sales data published recently.



Graph-2, showing the sales volume of cut flowers by Royal Flora Holland (C. Simionato, 2022). (STATISTA)

According to another report published by DUA_Enterprises, the top cut flower producing countries are selling a lot of flower bouquets worldwide. And it is becoming a prominent way of earning foreign currency.

Country	Year	Revenue_in_Billions
Netherlands	2022	6.80000
Colombia	2023	1.91000
Ecuador	2022	1.04000
Kenya	2023	0.73000
Malaysia	2024	1.12000
Italy	2022	0.13490
Germany	2023	0.35000
Israel	2022	0.02982
Belgium	2021	0.02600
Ethiopia	2022	0.60000

Table-2, illustrates the recent data of cut flower bouquet sales value around the world by top producing countries.

The success of the floriculture industry is indeed largely dependent on the cultivation of high-value flowers, many of which are monocots. In the next section, certain genetic engineering-based approaches will be described in relation to Tulips and Lilies, which are economically significant members of the monocot family.

Floriculture and Trans genesis in Monocots (Tulips, Lilies)

Monocot is a term widely used in botany. This term is usually associated with the differentiation of plants according to their embryonic leaf number. In monocots, only one embryonic leaf, or cotyledon, is present. There are several other characteristics that are present in a monocot plant, like the number of petals, stamens, and confirmation of vascular bundles. By looking at those physical characteristics, it can be determined whether the plant is monocot or not. Tulips and lilies have a higher economic value in floriculture than other flowers of the monocot variety. Some monocot flowering plants have some economic significance, especially iris and daffodils. These flowers are largely grown in India. However, according to global data on cut flower industries, tulips and lilies are more economically important than others. This is because of their diverse colouration and beauty, along with their specific geographical availability. So, in this section, the discussion will be related to some of the economically important monocot flowering plants along their floriculture technique and transgenic modifications.

Tulip

The word "tulip" is associated with the country of the Netherlands. The primary gene center of the genus Tulipa L is located in the Pamir-Alai and Tien-Shan Mountain ranges in Central Asia (Hoog MH, 1973). The flower tulip first came into existence in the Netherlands in 1594, and in 1637, when the highest price for a tulip bulb was listed in the market. After investigating the popularity of tulips in the Netherlands, the rich capitalists developed interest in tulip breeding. Because of that capitalistic approach, along with investments abundance after 1600, the Netherland became the main hub for tulip breeding. (Mike Dash, Tulipomania Review, 1999). There are about 45–100 species of tulip flower all over the world

(Botschantzeva et al 1962). Tulips are basically grown by a vegetative propagation technique. Seeds are also used for the tulip harvest process. Seeds of tulip low temperate for germination along with bulb formation. Niimi's (1978) research highlights the influence of temperature on the initiation and development of a bulb primordium in isolated tulip embryos. This suggests that careful temperature control is essential in tulip cultivation. In the conventional method of tulip growing, four main factors are considerd. temperature, light, humidity, and air composition. By means of temperature, the tulip needs hot summers followed by cold winters. The propagation of the tulip done with the bulb needs a cold climate to sustain initially. Eventually the temperature of 17-20°C in summer in in winter 2-9°C is required for the proper growth (M Nayeem1 and Adnan Qayoom, 2015). Tulip bulbs are mainly used in tulip harvesting. Several physiological conditions are determined before the selection of bulbs. The circumference of 6-8 cms are generally preferred for the bulb selection. The tulips required 4-5 years of growth period to reach into flowering state. The size of the pants varies from 10-70 centimeters. The plants generally have 2-6 leaves, and some of the species have 12 leaves. Typically, the plant will have 1 flower per stem during the flowering period (Gardenology). There are multiple times in the year occurs, when the initiation of tulip harvesting can be done. The plantation of bulbs starts in autumn. The early spring period is helpful for the rapid hormonal activity and growth purposes. At the end of spring, the newly formed bulbs pass through differentiation. So, at the end of the year a complete plant is achieved along with all functional organs present on it.

Tulips can reproduce sexually, with a central bundle of pollen tubes developing after adequate pollination. Pollen tubes bend sideways, growing towards and into the ovules. At 15˚C, the first ovules appear 1-3 days after pollination. A pollen tube penetrates approximately 68%-83% of the ovules. This penetration procedure can occur after 3-9 days of ovule development. The optimal temperature for pollination is 15°C. Suspensors form on the chalazal side of the prembronal cell mass in 3 to 6 weeks after pollination. At this stage, endosperm covers both the suspensor and the embryo. At the advanced globular embryo stage, the suspensor degenerates. The spherical embryo will expand longer and develop into a spindle-shaped embryo. The majority of spindle-shaped embryos are discovered nine weeks following pollination. Around 12 weeks following pollination, mature seeds can be harvested (Jaap M. Van Tuyl et al, 2007).

The previous aspects were all about the mechanism of conventionak method of tulip harvesting, but in recent years, somatic embryogenesis, a concept of plant tissue culture, has been raised because of its accuracy and efficiency. And researchers have found promising results in tulips by using this technique. In the somatic embryogenesis-based plant tissue culture method, the stems from tulips with a length of 50–60 mm are collected. Then freezing of these explant sections is done for 24 weeks. Before using the explant, 1-2 mm-thick slices of the stems are cut. After that, the surface sterilization of the explant is done with ethanol and with a commercial plant tissue culture-grade detergent. Induction of callus is the best way to grow tulip in invitro conditions because controlling growth will be much easier after callus induction. M.s agar, which is the basic media in plant tissue culture, is used during the process. Some hormonal supplements like 2,4- dichlorophenoxyacetic acid (2,4-D), 4-amino-3,5,6-trichloropicolinic acid (Picloram), α-naphthaleneacetic acid (NAA), and cytokinins: (0.5–50 μM) benzyladenine (BA) and zeatin (ZEA) are used during the callus formation. After 4-6 weeks of culture, the embryogenic callus develops. The colour of the callus and structure varied depending upon the used growth regulators. Explants cultured on media containing 2,4-D (10–50 μM) or Picloram (10– 100 μM) with addition of BA (0.5–50 μM) gave rise to nodular embryogenic callus. In somatic embryogenesis culture of tulip, it has been observed that an increase in auxin concentrations, particularly Picloram, resulted in a significant enlargement of explants

(diameter of explants). Embryogenic callus formed after 4–6 weeks of cultivation. In this process another important thing has been observed that the chilling process of explants and usage of endogenous growth regulators really helps in differential of callus and plant let growth After the formation of premature leaf segments, the plantlets are transferred in to multiplication media containing 5 µM BA and0.5 µM NAA. The medium had a pH value of 5.8 (Agata Ptak & Anna Bach, 2007).

Successful genetic alteration of tulips is good news for tulip breeders. In recent days, various foreign transgenic traits have been induced in tulip. Mutation breeding is displacing hybrid breeding due to its generation-wise genetic stability. Mutation breeding is a strategy used to genetically modify tulips. In the early stages, the ray-based mutagen can be utilized to cause mutation in both mother and daughter bulbs. Radiation exposure varies between 350 and 550 rad. Radiation can be sprayed before planting bulbs in the field. This mechanism can result in several types of mutations. Mutations like as flower color, flower shape, and various colors around the edges of flowers are simple to achieve. According to Yirui Li et al. (2022), under a treatment of 5 Gy (Gy unit of ionizing radiation dose) by gamma radiation, the height of the tulip plants slightly increased. Comparably, under the 5 Gy treatment, the flowering rate and petal count increased initially, but as the irradiation dose grew from 5 to 100 Gy, they declined. So, it can be said that radiation has a significant impact on plant's growth and morphology.

In other gene transfer-based genetic alteration processes, agrobacterium-mediated transformation is not a viable method for genetically engineering the monocot tulip. The particle gun method is the most effective way to produce genetically engineered tulip plants. This transformation strategy produced GUS positive and ppt-resistant tulip plants (Wilmink, A, et al 1992).

Lily

Lilies are recognized for their exotic beauty and elegant fragrance. Lilium is one of around 220 genera in the Liliaceae family (ANUSHRI VARSHNEY et al 2000). Native Lilium species are distributed throughout the Northern Hemisphere, particularly in Asia, North America, and Europe. Lilies play an important role in the horticulture sector; they are now frequently utilized as cut flowers, potted plants, and garden plants. In 2000, around 1500 million bulbs were manufactured over the world. In 2002, the Netherlands was the world leader in bulb cultivation, with 4523 hectares. And in recent days, production rate of lilies are simultaneously increasing in the United States, Japan, and more recently, in the Southern Hemisphere, including Chile, Australia, and South Africa. Lily is now the Netherlands' fourth most important cut flower in the horticulture sector.

The "bulb" is the most vital portion of the lily plant. In the traditional lily cultivation approach, the bulb is the most commonly used plant component. For optimal plant growth, bulbs should be chosen based on their size. For example, selecting smaller bulbs produces weaker stalks and fewer flowers. Blossoms are rarely produced by much smaller bulbs. After selecting the bulbs, these must be stored in an appropriate environment. And Precooling is a critical step before freezing. In the precooling process, the bulbs are typically held at 1-2°C for 6 to 8 weeks. The bulbs are unusable if the shoots of unplanted lilies have grown over 2 inches. And after that freezing of the bulbs are done at -2.2 to-1.6 °C at peat moss. Freezing has its own significance. Freezing helps bulbs stop sprouting, lowers the loss of energy in the bulbs, and reduces the risk of disease.

And thawing is also a very necessary step before the plantation of bulbs. For the thawing process, bulbs are stored in a room overnight at a temperature not higher than 15 °C.

There are several methods are available for growing lilies. Lilies can be grown in green houses, in high tunnels and in raised beds in the field.

The use of a raised bed for growing lilies has a good influence on growth. A raised bed can be built by using pressure-treated wood to create a 6-inch-high side. Those types of raised beds can help with proper water drainage and prevent diseases like root rot. Soil selection is an important aspect of each and every harvesting method. In the case of lily, the typically used soils have an organic matter percentage of 2–5%, and the soil should have a pH of 6.3–6.8 and be well-drained.

For the green house-based harvesting process, the soil selection parameters will be the same, but plastic-made shipping crates can be used for the harvesting process. Along with soil, leaf compost, sand, and coarse peat in a 1:1:1 ratio can be beneficial for lily growth.

Keeping the crates in a cool environment (10–24 °C) is necessary before putting them in the green house. This process must be continued at 12°C for 7 to 20 days. This step will help prevent the bulbs from developing shoots before the rooting process begins. When the sprouts have grown to a height of 2 to 3 inches, it is critical to move the trays out of the green house region and into the light. Some calcium-based supplements, along with fertilizers, are good for the lily's growth. A ratio of 2:1 for calcium nitrate and potassium nitrate can be used with the fertilizers for better growth (Dole, J. H., Wilkins, 2005, & De Hertogh, 1996).

The plant tissue culture-based method has its own advantages. It can help in the rapid regeneration of plants, along with virus-free plant characteristics. Lily has also shown promising growth improvement in the invitro condition. The requirements of nutrition in in vitro conditions are very basic. Morishige and Skoog supplemented with 3% sucrose are mainly used at the initiation of the culture. Hormonal supplements like a-naphthaleneacetic acid, indole-3-acetic acid (IAA), ndole-3-butyric acid (IBA), ndole-3-butyric acid (IBA), N-6-benzyladenine (BA), and kinetin are used. At the initial stage of the culture, bulb scale portions are used (basal, middle, and apical), and the NAA concentration of 0-1.25 Mm in the in the basal segment shows significant growth results. .

During the bulblet multiplication period, the effects of several energy sources (sucrose, glucose, and fructose) at varying concentrations (87.6, 175.28, and 262.9 mM) were checked. Bullet multiplication and subsequent growth were investigated in the first set. In this case, 0.5 µM NAA was added to MS semi-solid medium, and each energy source was supplied separately at various doses. The second experiment was conducted to optimize the sucrose concentration (which proved to be the best energy source) on bulblet multiplication and growth: up to 438 mM sucrose was added to the medium, and the cultures were kept under standard culture-room conditions (16/8 h light/dark), 24 h light, or complete darkness. After 6 weeks of protocol, some of the bulblet cultures showed proper root formation. The bulblets' leaves and roots were removed, and the bulblets were sliced into little pieces and placed in aluminum foil packets on petri plates in a hot-air oven at 600 °C for 10±15 minutes. After executing all standardization protocols, it has been observed that none of the bulblets showed dormancy at 3% concentration, and because of that, in the multiplication stage, a high concentration of sucrose was used. So, sucrose is the most important nutritional element in the in vitro multiplication of lilies (ANUSHRI VARSHNEY et al., 2000).

In the transgenic modification aspect of lilies, diseases are taken firstly in to the consideration. Diseases like basal rot are common in lilies. This disease is basically caused by a soil-borne pathogen named Fusarium oxysporum. But in the case of lilies, the identification of disease-resistant genes is a hard procedure. For eradicating the problem, an AFLP molecular marker-based approach is used (Vos et al., 1995; van Heusden et al., 2000). After the identification of disease-resistant genes from different species,

the amplification of the target gene is done using the PCR method. In Asian hybrids of lily, Fusarium oxysporum resistance genes are found. So, after the successful isolation of the gene from Asian hybrids, an agrobacterium-based genetic transformation process is performed to deliver the gene to the non-resistant lily species (Bino et al., 1992). Through this marker-assisted breeding process, many disease-resistant transgenic varieties of lilies are produced.

A research conducted by Yue chen et.al, (2023), mentioned about the successful transfer of 2 cold resistant genes in lily. The study began with the establishment of a lily regeneration system. This entailed screening various explants,

After establishing the regeneration mechanism, researchers refined the lily's genetic transformation system. This procedure included determining the critical concentration of antibiotics, which are employed in plant tissue culture to eradicate or minimize bacterial contamination. They also identified the appropriate concentration of the bacterial solution and infection time, both of which are critical parameters in Agrobacterium-mediated transformation.

The later part of the study was most important and successful as two specific genes were introduced into the lilies. The first gene, LlNAC2, is a comparatively lily gene for freezing tolerance, which shows that this gene is involved in lily's ability to cope with the cold. LaKNOX1, is another gene related to bulblet induction, quite possibly the development of new bulblets is controlled by this gene.

After thorough tests for stability and toxicity, the genes were successfully transferred to the varieties of Siberia and Sorbonne. The transgenic lily lines were thus each created

The conducted research is indeed a pioneering progress in gene manipulation of lilies, potentially leading to the production of improved varieties with cold resistance and bulblets.

Floriculture and Transgenesis in Dicots

Dicot plants have many more varieties than monocot plants. Because of the woody support system in their roots and stems, which allows them to grow in all shapes and sizes, from trees to daisies, Dicots are mechanically strong and stable compared to monocots. There are many varieties of dicotyledonous ornamental flower varieties on the market. However, according to floriculture market figures, roses, gerberas, and chrysanthemums are at the top of market demand. And, in most situations, standard breeding techniques for such flowers are simple and successful if carried out appropriately. So, in this section of the review, some economically important flowers that belong to the dicotyledonous family will be discussed, along with their floriculture, transgenic, and plant tissue culture aspects.

Rose

The rose (Rosa hybrida L.) is one of the most beautiful creations of nature. And it is known to be the most popular flower in the world. It has long been referred to as the "Queen of Flowers," and it is one of the most popular decorative plants. Rose is a commercial crop farmed all over the world, and it is dominating the international cut-flower markets (Ladha and Gunjal, 2004). The flower rose is widely used for ceremonial purposes, but it has many more uses in the cosmetic industry, perfumery, and medicine industry (Krussmann, 1981).

The conventional breeding method for roses is easy. Several types of grafting processes are used for the production of rose plants. But the conventional breeding method is slow and prone to many diseases (Gamborg and Phillips, 1995). The breeding of the roses can also be done using seeds, but the produced

seedling plants are not true to type (Horn, 1992). By looking at problems in conventional breeding, it is better to approach the plant tissue culture-based method for rose breeding.

In the plant tissue culture of roses, several explants are used. Explants like axillary buds (4-5 mm), shoot tips (8–10 mm), and nodal segments (2–3 cm long) are mainly used for the culture. The explants are collected from a mature, disease-free plant with an age of around 5 years. After the collection of those explants, the explants are surface sterilized using chemicals like detergent, HgCl2, and NaOCl. After the surface sterilization process, all three types of selected explants were inoculated aseptically in MS (1962) medium containing different concentrations (0.0, 1.0, 1.5, 2.0, 2.5, and 3.0 mg L-1) of cytokinins (BAP and Kinetin) alone and in combination with 0.1 mg L-1 NAA, in addition to 3.0 percent sucrose and 0.8 percent agar (w/v). 5.8 pH was maintained before the addition of agar in the liquid MS medium. After that, it is necessary to keep cultures at 25±1°C. A photoperiod of 16/8 h (light/dark) is required with a light intensity of 2000 lux using a fluorescent lamp. After one week of culture, visible, swollen, and expanded green buds can be observed. Then the cultured bulbs are transferred to the media containing different concentrations of BAP and kinetin, along with 0.1 mg L-1 NAA. Proper shooting requires 3–4 weeks. After that, the proliferated shoots are taken out of the jar, and the initiation of the rooting process starts. In the rooting process, half-strength MS media is basically used along with the addition of plant growth hormones like NAA and IBA. The cultured bottles should be kept at a temperature of 25±1 with a 16/8 h (light/dark) photoperiod provided by a 2000 lux fluorescent light source. Plants with good shoots and roots are transferred to the clay pots containing an autoclaved potting mixture of sand, soil, and FYM (1:1:1, v/v). After that, acclimatization and hardening processes are subsequently performed (Ram C. Yadav et al., 2013).

Nowadays, getting exotic, unusual-colored roses is not a matter of astonishment. By altering pigmentation pathways, it is now easily reproducible. Other than the pigment metabolic pathway alteration, more advancements are being made in the field of flower color alteration. In research, it has been discovered that flowers in the pH range of 4 to 5 can produce blue pigmentation. Sepal color variation of Hydrangea macrophylla and vacuolar pH measured with a proton-selective microelectrode (Yoshida K et al., 2003). In other research, it has been discovered that the PH1–PH7 gene loci in petunia are thought to be involved in controlling the pH of petunia petals (de Vlaming et al., 1983; van Houwelingen et al., 1998). The proton pump H+ P3A-ATPase encoded by PH5 is found in the vacuolar membrane. When the PH5 gene is mutated, the pH in the vacuole rises, causing the petals to change color from purplish red to bluish violet (Verweij et al. 2008). Furthermore, the PH5-encoded H+ P3A-ATPase physically binds to the PH1-encoded P3B-ATPase, resulting in increased proton transport activity and a more acidic vacuolar pH. (Faraco et al., 2014). Furthermore, the transcription factors PH3 and PH4 influence PH5 expression directly. PH6, also known as ANTHOCYANIN1 (AN1), is a bHLH protein that interacts with PH3, PH4, and the WD40 protein AN11. Anthocyanin is not generated when a transposon is placed into AN1, and the pH of the petals rises.

This kind of pH-based influence on petal color can also be observed in roses. The development of the red color in the rose happened because the petal maturation occurred in an acidic pH. So, the insertion of a gene that can introduce the alkaline pH-triggering mechanism during petal development can be an easy and sustainable solution for blue rose production.

Gerbera

Gerbera is a significant cut flower in the market, and it is a member of the Asteraceae family. It is indigen-

nous to South Africa's Natal and Transvaal regions. Gerbera is a genus of about forty species, of which only Gerbera jemesonil is commercially farmed.

Gerbera is typically grown in the open using seeds and rhizomes. To achieve quality standards for exports and the home market, the majority of cultivars are commercially cultivated under protection in greenhouses or poly/shade houses. To manage light and intensity, as well as solar radiation, a shade net of 50 to 70% is required. The majority of the cultivars thrive at temperatures ranging from 10 to 21°C on average. The optimal temperature for blooming commencement is 23°C, while for leaf unfolding, it is 25 °C to 27°C. The plants, on the other hand, would blossom at temperatures ranging from 13 to 32°C. Flowering begins when the temperature falls below 12°C and rises above 35°C. The ideal humidity level within the polyhouse should be between 10 and 75°C. The humidity level must be kept below 70 percent during the day and below 85% at night. It is crucial to have good internal air circulation and ventilation in the greenhouse at night, as well as ventilation during the day.

Gerbera can be propagated using seeds, suckers, and tissue culture methods. The tissue culture-based propagation method is very useful for producing virus-free plants. Gerbera is a popular cut flower and potted plant worldwide. Some of the variety shows excellent agronomic characteristics such as color, floral diameter, stem length, and vigor, which make this plant of commercial importance. Rapid multiplication of gerbera can be achieved by both direct and indirect tissue culture methods. Direct shoot regeneration of gerbera was accomplished from stem apices on MS medium supplemented with 1 mg/L 6-benzyladenine (BA) and 1 mg/L kinetin. Indirect shoot generation is generally achieved by the callus induction method. For callus induction and differentiation, MS medium containing 2 mg/L 2,4-dichlorophenoxyacetic acid, 0.5 mg/L indole-3-acetic acid, and 2 mg/L BA is required. After achieving a shoot length range of 4-5 cm, the shoot bases are dipped for 3-5 s in a 2,000 mg/L indole-3-butyric acid solution, followed by transfer to the pots containing farmyard manure, soil, and sand (1:1:1 by volume). Watering, maintenance of humidity (85–90%), and maintenance of temperature (25 +/- °C) are very necessary in this period. Within 10–15 days, shoots get fully developed. After 7 days, the removal of the jars for a few hours per day allowed plants to acclimate in ex vitro conditions. After one month of complete removal, it is important to transfer the plants (4–5 cm long with 5–6 leaves) to the glasshouse to progressively expose them to the ex-vitro conditions (Minareva Ghani and Surinder Kumar, 2013).

Gerberas are commonly grown for their bright and cheerful daisy-like flowers. The main feature of this flower that attracts the most is its color variety. So, in Gerbera, various color-related modifications have been achieved by using multiple gene transfer techniques.

A lot of transgenic modified gerbera varieties can be seen on the market. In Gerbera, genetically engineered bower color was obtained using antisense transformation with several flavonoid pathway gene constructs. From the original red-colored variety, Terra Regina (co), a creme and differently shaded pink lines were produced using gene transfer. Terra Regina gene insertions such as Terra Regina; 1, 2 anti-gchsl transformants; 3 antigchs2 transformants; and 4 anti-gdfr transformants Transformation of del and the insertion of an anthocyanin pathway regulatory gene from Antirrhinum majur pf 355 promoters into Gerbera resulted in strongly increased anthocyanin pigmentation in leaves and flower scapes.

The modification of the structure of the Gerbera flower has been done in inflorescence by transforming several cDNAs belonging to the MADS-box gene family into Terra Regina (Kotilainen et al., 1991; Yu et al., 1999). For example, transformation of the Arabidopsis agamous gene (Yanofsky et al. 1990) under the CaM V 35S promoter resulted in transgenic lines that displayed smaller capitulum and ray flowers with reduced pink corollas. This was due to the partial homeotic change of petals into structures with stamen-

like characteristics presented by Coen and Meyerowitz (1991) (M. Kotilainen, D. Yu, and V. Albert, unpubl.). Similar phenotypes have been obtained by overexpressing the gerbera C-class MADS-box genes, gagal and gaga2 (Yu et al. 1999).

Most of the time, in those transformation processes, the target expressible genes are transferred to the subject through a plasmid vector. Several selective markers are added to the vectors. which basically helps to detect the expression of a gene in vivo. Before transformation of gerbera, the E. coli intermediate vectors are conjugated with the Agrobacterium tumefaciens strain, where the plasmid cointegrates with the disarmed Ti plasmid.

Chrysanthemum

Because of its diverse range of plant and flower colors, shapes, and forms, chrysanthemum will continue to be a major global floricultural crop leader. Along with conventional harvesting processes, modern approaches like in vitro regeneration from thin cell layers and biotechnological breeding protocols involving somatic embryogenesis, intergeneric somatic hybridization, and mutation are used for chrysanthemum breeding.

Tropical and subtropical climatic conditions are good for chrysanthemum development in conventional breeding. Chrysanthemum species shows better growth in properly drained red loamy soil with a temperature of 20–28 °C and at night, 15-20°C. Commercial propagation of chrysanthemum can be done from terminal cuttings (5-7 cm long), suckers, or seeds. The June-July period is the timing for the plantation. The flowers are harvested at four-day intervals, beginning in the third month. The standard breeding strategy can provide an average yield of 20 t/ha from plant crops and 10 t/ha from rat crops (Agritech).

The major explant employed in the plant tissue culture-based strategy for chrysanthemum breeding is nodal segments (1.0–1.5 cm) with one node cut from five- to six-month-old plants. The selected explants are then cultured in Murashige and Skoog medium with a sucrose concentration of 3%, and the media can be supplemented with different concentrations of BAP, Kn (0.5–5.0 mg/l), and IAA (0.1–0.5 mg/l). IAA (0.1–0.3 mg/l) can also be used along with BAP and Kn. After the inoculation of explants into the media, the cultures are incubated at 26 ± 2 ºC under a 16-hour photoperiod. 3000 lux of light intensity is required for that stage. The subculture can be done in a 15-day interval. For root induction, well-developed shoots were removed from the culture jars and then cultivated separately on half-strength MS medium reinforced with auxins (IBA, IAA, and NAA). Plantlets were removed from the culture vessels after sufficient root development and rinsed with sterilized distilled water to remove all trace of medium attached to the roots. After being rinsed, plantlets were transferred into small plastic pots containing autoclaved garden soil and compost (1:1). The pots were covered with transparent polythene bags and left at room temperature for 10 days to maintain high humidity. After 10 days, the pots were opened and subjected to partial and then full direct sunlight. This sunlight-exposing strategy eventually helps in the hardening of plants (Sabina Yasmin et al., 2017).

The chrysanthemum (Chrysanthemum morifolium) is one of the world's most popular ornamental plants. Cut flowers or potted plants are the most common uses of chrysanthemum. Chrysanthemum flowers can be seen in variety of colours like purple, yellow, red, white and, pink. However, the deep red and blue chrysanthemum flowers are not found naturally. So, for the expression of these 2 desired colours, researchers had selected two chrysanthemum cultivars, C morifolium 'LPi' and C morifolium that 'LPi', these varieties only accumulate flavonoids in their ligulate flowers. Researchers have also isolated seven

anthocyanin biosynthesis genes, named CmCHS, CmF3H, CmF3'H, CmDFR, CmANS, CmCHI and Cm3GT from the above-mentioned cultivars (Huang He etal, 2013). Anthocyanins are phenolic pigments that are water soluble, and it is responsible for the colour expressions like colours, red, purple, and blue in flowers, fruits and vegetables (Hock Eng Khoo et al 2017). After the analysis of the 7 isolated genes by RT-PCR and qRT-PCR techniques it has been found that CmF3′H was the most important enzyme required for cyanidin biosynthesis. Researchers performed the downregulation of CmF3'H gene using Rna interference technology to rebuild the delphinidin pathway and for the over expression of F3′5′H (PCFH) gene in chrysanthemum. To create vector 35S-PCFH, full-length PCFH cDNA was subcloned into the binary vector pBI121 in substitution for the GUS structural gene. CmF3′H was chosen as the RNAi target (Nishihara et al 2005). The vector was transferred to the transverse thin cell layers (tTCLs) of the LPi were used as explants. After the conduction of the experiment chrysanthemum plants has shown higher accumulation of cyanidin content with brighter red petals. But the accumulation of delphinidin pigment was not observed. These findings suggested that CmF3′H is required for anthocyanin accumulation in chrysanthemum, while Senecio cruentus F3′5′H only demonstrated F3′H activity in chrysanthemum but did not reconstruct the delphinidin pathway to generate blue chrysanthemum (HH H. Ke H and Keting XQ, 2013). Occurrence of flower color is completely natural but now with the help of genetic engineering process it is possible to create such transgenic plants with desired flower color.

Future Perspective

By looking at the current scenario, predictions can be made that the cut flower market will flourish rapidly in the running decade. In the floriculture sector the cut flower market is valued at$36.7 Billion in 2022 and now it is expected to reach US$45.5 Billion by 2027 (Markets and Markets). Expectations are quite legitimate as the requirement for ornamental flowers will always be there. In ceremonies and functions, decorations without ornamental flowers are worthless. And the rising demand for the original fresh flower will drive the market. Previously barriers like climate, geographical locations, prolonged flowering period, and disease-related problems negatively influenced the cut flower industry, but now with the help of plant tissue culture and transgenic plant production approach, the mentioned problems can be overcome. And with the rapidly evolving technology in floriculture, will encourage farmers to take a growing interest in cut flower harvesting. Because of the better technological and business-based strategies several European nations are experiencing their peak performances in the cut flower industry. In the previous decade, India had promising growth in cut flower production and exports. Understanding the importance of floriculture, the Government of India has implemented numerous developmental programmes through the Ministries of Agriculture (National Horticulture Board), Commerce and Industry (APEDA), and Technology Mission for North Eastern States. These projects will bring new technology and give farmers with the necessary assets for better and faster production of cut flowers, allowing them to gather cut flowers more readily and contributing to India's foreign currency gains.

Conclusion

Due to a variety of agroclimatic zones, unique production systems, and stabilized genetic modification of plants, floriculture has become much more commercialized than subsistence farming. Looking at the demand for floriculture-based products in the market, production has increased in all geographical locations. Entrepreneurs recognize all possibilities to enter markets in countries with favorable growth circumstances. The cut flower business around the world is in a state of change. By looking at the market

opportunity, new exporting countries are emerging (Ecuador, Kenya), while India, China, and the Republic of Korea are vying to be the next generation of successful emerging exporters (Amita Abrol and HS Baweja 2019). In the previous decade, India has also influenced the cut flower market with a promising performance. In 2018, the Indian horticulture market was worth INR 157 billion. The market is expected to reach INR 472 billion by 2024 (APEDA). And due to the rise in government initiatives in regards to the agriculture sector, it also helps Indian harvesters produce and export more cut flowers. International commerce in ornamental products has expanded in pace with the rise in consumer demand. Consumption has increased globally in places where ornamental use is expected to develop, as has the level of prosperity. Overall, the global flower market appears to be becoming more competitive. For high-cost farmers looking to stay competitive in the global floriculture market, rapid innovation is a potential option.

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