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# Physical and Genetic Modulation of Strawberries in Relation with Qualitative and Quantitative Traits – A Comprehensive Review

***Rupesh Kaushik, Harjinder Kaur*, Rahul R. Rodge, Lakhwinder Singh, Sanjeev Kumar and Khan Jabroot J.***
*Department of Horticulture, School of Agriculture,*
*Lovely Professional University, Phagwara-144411 (Punjab), India.*

*(Corresponding author: Harjinder Kaur*)*
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**ABSTRACT: Strawberry breeding began in the 15th century with the selection and cultivation of European strawberry species in Western Europe, followed by a similar discovery and cultivation in Chile. The most popular variety of strawberries today is the garden strawberry, a hybrid of two different species with the scientific name *Fragaria ananassa*. However, there are numerous strawberry varieties, some of which are to some extent farmed. Strawberry species are divided into numerous genetic subcategories based on the number of chromosomes they possess. Over the years, strawberry farmers have employed a variety of breeding techniques, starting with conventional plant breeding and moving on to molecular breeding and genetic engineering in the twentieth century. In this review essay, about various breeding techniques used in strawberry breeding were discussed. However, there have been numerous obstacles to strawberry production, which has put pressure on scientists around the globe to create fresh adaptation strategies to meet the rising demand for high-quality strawberry production. The pressure from pests and disease, as well as extreme weather, is the biggest threats to strawberry production. To address some of these issues and meet the demands of consumers for fruit quality, cultivars have been created. The overall acceptability of fruit quality is a key factor in determining the success of a breeding programme because most developed varieties with desirable traits like resistance to biotic and abiotic stresses could not be commercialised and are not grown in commercial settings due to their poor quality traits. Numerous factors, including long juvenility, tall stature, environmental stress, and high heterozygosity, impede the improvement of fruit crop quality. It is challenging to improve specific desirable traits because quality traits in fruit crops are polygenic and controlled by numerous genes. Although perennial fruit crops neatly neglect this issue, numerous attempts have been made to improve the qualitative traits of annual crops. Therefore, using a combination of traditional and contemporary breeding techniques could help to solve these issues. When dealing with laborious fruit crops, biotechnological and molecular methods like marker-assisted selection, transgenics, genomic editing, genomic cis-genics, and candidate genes offer accuracy and dependability to shorten the breeding cycle. This review's main topics will be the difficulties in fruit breeding and the current state of various breeding methods for improving fruit quality in fruit trees.**

**Keywords: S**trawberry, Molecular breeding, Tissue culture, Hybridization.

## INTRODUCTION

The garden strawberry (*Fragaria* × *ananassa* Duch.) is one amongst the foremost delicious, refreshing and soft fruit of the planet. Due to its genotypic diversity, extreme heterozygosity, and wide range of environmental variations, it is the fruit crop that is most widely distributed throughout the biosphere. Due to its extraordinarily high returns per square foot within the shortest timeframe, strawberry cultivation has recently gained popularity in Asian countries. In India, the states of Himachal Pradesh, Western Uttar Pradesh, Uttarakhand, Haryana, Punjab are where strawberries are cultivated commercially. Almost 15600 area units are used for strawberry farming in total (Cosme *et al.*, 2022). Strawberries include a wide range of nutritive and non-nutritive bioactive substances that are considered to have positive effects on both health and illness. Several studies have looked at the benefits of strawberries, including the decrease of obesity-related diseases and heart disease risk, the prevention of oxidative stress and inflammatory disorders, and the protection against various cancers (Afrin *et al.*, 2016). A lot of items, including confectionery, soap, lip gloss, perfume, and many more, contain artificial strawberry flavours and fragrances. Size, colour, flavour, shape, fertility, ripening season, disease susceptibility, and plant constitution vary across strawberry varieties. On average, a strawberry's exterior membrane contains 200 seeds. Some have distinct vegetation, and some have diverse sexual organ material development. Most of the time, the blooms look hermaphrodite, yet they might be either male or female. Because the strawberry is an octoploid (2n=8x=56), some nuclear and live material germplasm was used to collect the strawberry's genetic history (Pincot *et al.*, 2021). The allo-octoploid (2n = 8x = 56) domesticated garden strawberry (*Fragaria ananassa*) has a unique natural and domestication history. Around 300 years ago, it was originally produced as an interspecific hybrid between two wild octoploid progenitor species (Edger *et al.*, 2019). The progenitor species *Fragaria virginiana* and *Fragaria chiloensis* genomes, which were produced by the fusion and interactions of the subgenomes of four diploid progenitor species roughly a million years ago, are the products of polyploid evolution (Njuguna *et al*., 2013). Furthermore, ellagic acid, which is known to be an anti-carcinogenic substance, is present in high amounts (Ansary *et al*., 2021). Strawberry, however, was first consumed in Bangladesh twenty years ago and has recently grown in popularity. A strawberry cultivar called BARI Strawberry-1 was just made public by the Bangladesh Agricultural Research Institute (BARI). There are different methods of strawberry breeding like hybridization, introduction of cultivars, tissue culture, molecular breeding and mutations, all of them we are going to discuss one by one (Saha *et al*, 2022). The world's strawberry production is steadily rising as a result of the numerous breeding and biotechnology projects that have produced novel varietals, as well as the numerous studies that have been conducted to learn more about plant physiology and create new cultivation methods. Access to genetic resources is still necessary. The creation and management of these technologies are dominated by a few research for breeding procedures that can now incorporate cutting-edge genomic and molecular technological advance, but there are also a lot of new businesses on the scene that can create innovations with significant local and even global implications. Important public research projects that are also of global significance support these activities, but it is still crucial to maintain a connection with the businesses that need to access the findings to increase their capacity for innovation (Menzel *et al.,* 2023). The ultimate goal is to continue growing the strawberry crop by creating new cultivars that are highly adaptable to various environments and above-ground greenhouse growing systems (field) with less negative environmental effects and higher consumer safety and quality standards (Mezzetti *et al*., 2018).

## INTRODUCTION OF CULTIVARS

The strawberry is the most significant type of berry and a perennial herbaceous plant. There were some problems which were faced in strawberry then some new varieties or cultivars are introduced which have some quality which was adaptable in different environment conditions.

The two cultivars ‘ROMANIA’ and ‘CRISTINA’ which have high sensorial and nutritional value (Aaby *et al*., 2007). Recently they are released from Marche Polytechnic University (Ancona, Italy) which show high adaptability to non-fumigated soil, Romina is of interest for its early ripening time. It is a cross between 95.617.1 and 'Darselect (Azzini *et al.,* 2010). The 'Romina' plant exhibits strong vigour, high uniformity, and a medium number of crowns. The medium-sized, conic or biconical yellow achens are medium in size and easily detached from the calyx. Romina's early production duration is comparable to that of other widely used varieties in Europe like Clery and Alba, but it differ significantly from those cultivars in that its red fruit has a greater firmness (494 g/N), a longer shelf life, and a higher yield and similar fruit weight and commercial production characteristics (Diamanti *et al*., 2015). Cristina is a cultivar that was created through a cross between two late Italian selections. It is a short-day in nature and its late (IP 154—comparable to "Sveva") ripening time makes it effective. The "Cristina" plant is medium-strong vigour and has a significant crown number. The "Cristina" plant is of medium strength vigour and has a large number of crowns. The ripening and blooming periods follow "Adria" and "Sveva" very late (IP 154) respectively (IP 151 and 153). High fruit productivity (1094 g/plant), lower than 'Adria' (1451 g/plant), but higher than 'Romina' (831 and 767 g/plant, respectively). In contrast to all other cultivars, the fruit of the cultivar "Cristina" weighs 33.4 g and is bright red, flavorful, and enormous (Capocasa *et al*., 2016).

In Bosnia and Herzegovina, strawberry production is rising because of its high profitability so newly introduced varieties must be included before their mass introduction into production. Alba, Asia and Joly are three newly introduced cultivars in which examines the fruit's morphological characteristics, such as its weight, width, and thickness, for the three cultivars and height of the fruit (Weissinger *et al*., 2010). Joly cultivar has best morphometric properties and chemical properties among three i.e. in first picking cycle and this have highest yield in that area. While Asia and Alba have recorded the most notable decline in morphometric characteristics from the first to the third picking cycle (Kiprijanovski *et al.*, 2010). Therefore, the cultivar Joly has the best outcomes in fruit morphology and chemical properties allow it to be recommended to nearby strawberry growers (Skender *et al*., 2016). As the high costs of implementation and the high price of the finished product, cultivar selection is critical in the strawberry production chain. Cultivar selection, in combination with producer management and cultivar adaptability, becomes the key to success (Ruan *et al*., 2013). The main cultivars used in Brazil come from breeding programmes in Spain and the United States. 'Benicia', 'Cristal', 'Florida Elyana', 'Florida Fortuna', 'Jonica', 'Mojave', 'Pircinque', ‘Sweet Ann 'Sabrina', and 'Sabrina' were the cultivars introduced and registered in the last five years (MAPA, 2016). The program's main goal is to develop cultivars that are adaptable to a variety of growing environments, pathogen-resistant, highly productive, and bear fruits with high physicochemical characteristics, particularly sweetness, flavour, and firmness, as well as off-season production. Some disease resistant cultivar was introduced to resist from *Verticillium dahlia* which is a pathogen that is extremely harmful to soil-cultivated strawberries (*Fragaria* × *ananassa*). It is a soil-borne plant pathogen that negatively affects soil cultivation yields significantly (Sowik *et al.*, 2003).

The soil-grown strawberry, *Fragaria × ananassa,* is severely harmed by the pathogen *Verticillium dahliae*. It is possible to lessen the damage caused by the disease by breeding Verticillium wilt resistance into cultivars of commercially available strawberries. In this experiment, we found brand-new resistance sources that were discovered across several strawberry populations, providing breeders with a tremendous amount of knowledge. Through studying *V. dahliae* subclade II-1 specific resistance in the cultivar "Redgauntlet" and subclade II-2 specific resistance in the cultivars "Fenella" and "Chandler," experiments informed by pathogens have made it possible to distinguish between subclade-specific resistance responses (Cockerton *et al*., 2019).

**Table 1: Different introduced varieties of strawberry and their traits.**

| Sr. No. | Introduced variety of strawberry | Introduced from | Traits | References |
|---|---|---|---|---|
| 1. | Romania and Cristina | Ancona, Italy | High sensorial and nutritional value | (Capocasa *et al*., 2016). |
| 2. | Fenella, Flamenco and Chandler | United Kingdom | Disease tolerant from *Verticillium dahlia* | (Cockerton *et al*., 2018) |
| 3. | Pircinque, Jonica | Brazil | High production | (Fagherazz *et al*., 2016) |
| 4. | Joly | Bosnia and Herzegovina | Morphological fruit weight affects the yield and chemical properties | (Skender *et al*., 2016 ) |
| 5. | Senga Sengana | Poland | highly tolerant to *Verticillium dahlia* | (Sowik *et al.*, 2016). |
| 6. | K40 ( somaclone of Elsanta ) | Dutch | resistant to *Verticillium dahlia* | (Sowik *et al.*, 2003). |

## HYBRIDIZATION IN STRAWBERRY

Hybridization's main goal is to create variation. When two plants with different genotypes are bred the genes from both parents are combined in Fl. Many new gene combinations are formed in F2 and subsequent generations as a result of segregation and recombination. Natural variety in self-pollinated populations is exhausted during selection; therefore, fresh genetic variability must be created by crossing two separate pure lines for further advancements. The crossing of two plants or lines of distinct genotypes are referred to as hybridization., as well as succeeding generations, are referred to as segregating generations. Today, hybridization is the most frequent form of crop development, and the majority of crop varieties are the result of hybridization. One goal of hybridization is to create genetic variety (Rho *et al*., 2012).

**Strawberry Whole Genome Sequencing Advances Using NGS Technologies.** One of the most important technological developments of the twenty-first century, next generation sequencing (NGS), Agriculture is currently experiencing a significant improvement, particularly in breeding. De novo whole genome assembly is required since the advancement of genomic studies depends on the reference genome. It has been tested on a range of plants, including the all-octoploid flowering plant strawberry (*Fragaria* × *ananassa*) species with four different species (2n = 8x = 56) Strawberry's whole genome assembly has been regarded as a challenging task due to its complex genome de novo structure (Isobe *et al*., 2020).

**Genomic Selection in Strawberry F1 Hybrid Breeding (*Fragaria* × *ananassa*).** The cultivated strawberry is the crop among about all fruit that is consumed most in the world, hence several breeding programmes are being carried out to enhance its agronomic characteristics, such as fruit quality. There was a significant danger of viral and insect infection while propagating strawberry cultivars vegetatively using runners. F1 hybrid seed development has been proposed as an alternative strategy for breeding strawberry to address this issue. In this investigation, we assessed the potential for Breeding strawberry F1 hybrids using genomic selection (GS) (Bassil*et al*., 2015). To serve as possible parents for strawberry F1 hybrids, we created 105 inbred lines. To create test F1 hybrids for GS model training, 275 randomly selected parental pairings from the 105 inbred lines were crossed. 20,811 single nucleotide polymorphism sites were found in the 105 inbred lines using whole-genome shotgun sequencing. These sites were subsequently used in GS analysis. The presence of dominant effects in a GS model build indicated a small increase in GS accuracy. With the exception of Brix, the GS models using the inbred lines showed predictability for the test F1 hybrids and vice versa in the across population prediction analysis. In comparison to F1 hybrids predicted to have high fruit firmness or high pericarp colour, as expected, the F1 hybrids chosen for different purposes expressed greater identified phenotypic values. The research in this work shows that GS can be used in strawberry F1 hybrid breeding (Yamamoto *et al*., 2021).

**Based on maturity indices, experimental strawberry (*Fragaria* × *ananassa*) hybrids were selected.** The only fruit or vegetable that belongs to the rosacea family is the strawberry (*Fragaria* × *ananassa* Dutch). Strawberry species today have descended from wild species and are members of this genus. Due to the strawberry's octoploid structure and variability following hybridization, Selecting a character or characters could result in unwanted genotypes and even the marginalisation of desirable alternative because they have demonstrated negative genetic correlations that result in ineffective selection, which can lead to undesirable genotypes. The populations resulting from their crossings yielded 103 hybrids and seven commercial cultivars, which were both used. Seven physical-chemical traits and four agronomical traits were evaluated during the experiment's augmented blocks. The selection of strawberry clones is advised using the Mulamba and Mock, Smith, and Hazel indices, those with the highest proportion of selection gains (Vieira *et al.*, 2017).

## RECURRENT SELECTION

One of the breeding strategies is recurrent selection in which reselection is done generation after generation with the selecting interbreeding parents. This selection is used for improving the characters of desirable alleles in a breeding population. This is the modified form of progeny selection and important method for crop improvement. Many of breeding programs are done for improving the agronomic traits of strawberry. Strawberry cultivated by the vegetative means of propagation via runners and by this Insects and viruses carry a high risk of infection. For resolving this problem, the development of F1 hybrids seeds has done through alternative breeding strategy. By using the breeding method crossing has been done in between the selected parents for getting a superior resulting progeny **(**Yamamoto *et al*., 2021). In forcing culture, strawberry (*Fragaria annassa Duchesne*) lines that grew well with short days and cool temperatures were developed. In **Vigorous strawberry lines.** (Nagano *et al*., 2017) observed that the Strawberry (*Fragaria × annassa*) cultivated 36 non-everbearing cultivar. The strawberry cultivar differentiates into three groups in the basis of their genetic differences. Recurrent selection was performed in set self and mixed pollination. In these two or three cycles of recurrent selection and testcross lines are crossed with the selected lines and eleven are existing cultivars. The vigorous lines are observed by the recurrent selection in all three groups. Recurrent selection used for the most vigorous cultivar 'Kaorino' for slower the leaf area reduction.

**Recurrent Reciprocal Selection for Genetic Improvement.** The breeding method is one of the techniques used for the genetic improvement. The only breeding technique that has been used in any significant way to improve this crop has essentially involved sets of one cross-pollination and one self-pollination, recurrent selection was used. Breeding was done primarily to produce cultivars with consistent plant vigour during the harvest time. Recurrent selection over two or three generations led to the production of very robust lines. 'Kaorino' is one of Japan's most robust strawberry cultivars. had a leaf area that was larger on average than that of the largest leaf, which is a gauge of plant vigour. Two times as much leaf area as "Kaorino" was produced in one line. Although strawberry is typically prone to inbreeding depression, some recurrently selected self-pollinated lines had leaves that were greater than those of 'Kaorino' (Kataoka & Noguchi 2016).

**Genomic selection for F1 hybrids breeding.** Strawberry (*Fragaria × ananassa*) were cultivated through the runners by the vegetative propagation method. By the runners it can adapt the high risk of infection in strawberry cultivar. For resolving that problem, the seed propagated strawberry was developed by the use of several breeding methods. For best strawberry cultivar F1 hybrid seeds are produced through breeding. Genomic selection was used for qualitative traits improvement. Genomic selection used for efficient F1 hybrids breeding. 'Reikou' strawberry cultivar's first allo-octoploid genome sequence (Hirakawa *et al*., 2014).

crossing two parents of choice and choosing a desired superior type from the offspring. The original material consisted of five choices from each of the two variety crosses. The five choices were numbered A-1 to A-5, with one being a cross between Valentine and Sparkle. The selections for the other cross, *Valentine × Fairfax*, ranged from B-1 to B-5. Every A selection was crossed with every B selection, and 15 duplicate plant plots of each offspring were grown. The fruit yield, plant stand, and foliage ratings for the F1 self-fruiting strawberry varieties were comparable to those of grown standard strawberry varieties (Cavalier, Redcoat, Catskill and Sparkle). Berry appearance was comparable to that of these varieties, but fruit uniformity scores were lower than those of the majority of varieties (Hancock *et al*., 2008).

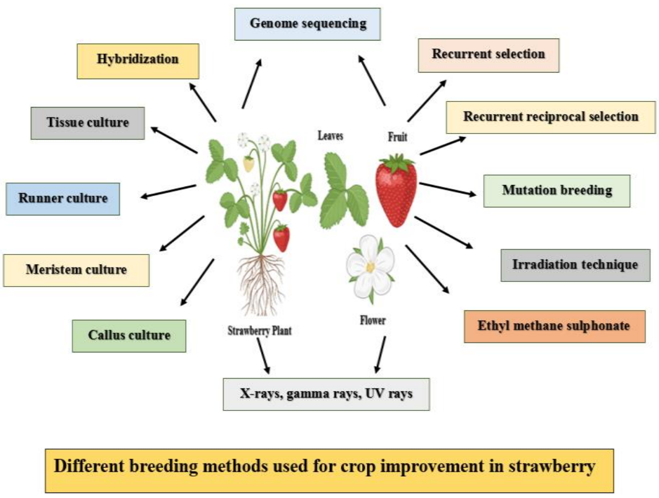


**Fig. 1.**

## TISSUE CULTURE

Tiny segments of plant tissue are removed and then placed in an artificial environment where they can live and grow as part of the tissue culture method for biological research. For the purpose of creating plant clones, in tissue culture, a single cell, a group of cells, an entire organ, or a section of an organ may all be present. In tissue, a single cell, a group of cells, an entire organ, or a section of an organ may all be present. The resulting clones are kind of true to a genotype selection (Thorpe, 2007). The plant grows more easily in the controlled environment provided by tissue culture. The environment that is offered ought to be fully nutrient-dense, at an ideal temperature and pH level, with full gaseous and liquid conditions. Recent years have seen a significant industrial shift towards the use of plant tissue culture as a method of plant propagation. The production of many plants using plant tissue culture is possible. In order to create hundreds of thousands of plants, only a small piece of tissue is needed (Akin-Idowu *et al*, 2009). An alternative effective method to implement strawberry improvement is in vitro methods. Utilizing meristem culture, axillary bud growth, somatic embryogenesis and adventitious shoot regeneration, and, strawberry species are reproduced in vitro. It is an efficient method of propagation that is used for viral sanitation, clonal multiplication, and germplasm improvement. New cultivars with characteristics like high productivity and resistance to both abiotic and biotic stresses are generated through germplasm development, as well as helps to promote already existing cultivars. Micropropagation may be a very practical method for commercial plant production, and it has a number of advantages over traditional methods such as seed, cutting, air layering, grafting, etc. Plants free of viruses can be produced using tissue culture, which is a quick propagation method. Standardized strawberry in vitro regeneration methods exist. The production of millions of plants involves a number of techniques, including the use of anthers, immature embryos, meristem tips, and first axillary buds of stolons (Debnath & Teixeira 2007). Growing somaclonal and gametoclonal variations in plants through plant tissue culture has been found to be the most efficient way to improve crops. In a suitable manner, micropropagation has grown and preserved species that are threatened, endangered, and rare types (Adhikary *et al*., 2021).

**Tissue culture of strawberry.** While using biotechnological methods to develop strawberries is thought to be more effective, importing strawberry mother plants is not an economically sensible option. By using traditional methods, it is difficult to find healthy stalks for propagation. In vitro techniques in plants are crucial for introducing novel traits into specific plants, reproducing desirable cultivars, and growing them with the least amount of effort (Taji *et al.,* 2002). However, since it is quite expensive to import the mother plant, the propagation of a specific variety of strawberry trees is of particular importance. Since some strawberry varieties can disappear due to genetic unpredictability, seed propagation is not very common in this plant. Strawberry plants were traditionally multiplied vegetatively using root runners. However, this method is useless due to low rooting rates, ecological risks, disease susceptibility, labor-intensiveness, etc (Sakila *et al*., 2007). Better substitutes include other propagation methods like micropropagation. Several studies that were done in this area showed that micropropagation is a much more effective method for strawberry propagation than any conventional method (Karhu and Hakala 2000; Mahajan *et al*., 2001; Mohan *et al*., 2005). According to this field's research, micro propagated strawberries outperformed other conventionally propagated varieties in terms of traits like flowering procedure, and number of runners, crown dimension. Gomes and Canhoto (2003) stated that micropropagation plays a crucial role, when rejuvenation problems continue and more growth is required to satisfy demand, it is challenging to achieve cover using conventional methods.. Axillary shoot proliferation is thought to be the most popular micropropagation method for Ericaceae clonal propagation, out of a number of methods that are in use (Jain and Häggman 2007). In the past, runners were used to grow strawberries (Sakila *et al*., 2007), which was labour intensive, expensive; time taking and it led to spread of viral diseases (Gautam *et al*., 2011). A shoot renewal scheme is helpful for identifying or inducing somaclonal variations as well as for creating transgenic plants after plant cells have undergone genetic conversion. The nodal cutting in vitro culture has been successful in the micro propagation of strawberry plantlets (Ghaderi & Siosemardeh 2011; Karhu and Hakala 2000). Naing *et al*. (2019) stated that using the tissue culture method, different strawberry plants could also be produced over the course of a year from various mother plants. It has been determined that this approach is suitable for introducing contemporary cultivars. Runners and in vitro micropropagation are two common methods used for strawberry propagation. According to reports, strawberry micropropagation, which differs from the standard root runner technique of propagation, is effective in growing a significant number of disease-free plants (Moradi *et al*., 2011). To avoid the majority of plant and soil transmissible diseases, strawberry plants that were micropropagated were introduced. For success in those aseptic conditions, micro propagation differs from all related conventional propagation approaches.

**Runner culture.** Strawberry in vitro propagation was done at the BARI Biotechnology Lab in Joydebpur, Gazipur. Five BAP concentrations—0.0 (Control), 0.5, 1.0, 1.5, and 2.0 mg/l—were used to initiate a shoot and four IBA concentrations—0.0 (Control), 0.5, 1.0, and 1.5 mg/l—were used to initiate root. At a concentration of 0.5 mg/l BAP, the highest average number of shoots (7), and the longest average shoot length (3.34 cm), were noted. At the same concentration, the highest average number of leaves (5) was also noted. The IBA @ 0.5 mg/l rooting concentration outperformed the other four rooting concentrations in every parameter tested. This concentration also yielded the roots with the greatest number (6 per culture) and the longest roots (3.05 cm). IBA concentration was not present in half-strength MS media, so root induction was not effected (Ashrafuzzaman *et al*., 2013).

**Meristem culture.** A reliable clonal propagation method that guarantees stable transfer of desired traits into commercial genotypes and regenerates plants true to type without associated with sex recombination is in vitro production through meristem culture. It is also an effective tool for widespread plant propagation (Kumar and Reddy 2011). The meristem that was removed from the runner tip was the explant used for strawberry micropropagation (Sowik *et al.*, 2001). A meristem that has no leaf primordia or, at most, just a single or two is removed and cultured in the proper medium (Kumar and Reddy 2011). The keystone of the micropropagation method is an apical shoot meristem's capacity to regenerate an entire plant (Debnath and Silva 2007). The shoot tips of young runners are chosen, and the meristems are cut off and cultured on a medium high in nutrients but low in cytokinins (Sowik *et al.,* 2001). After that, the explant is cultured in a medium with more cytokinins to promote axillary budding without callus development. With the help of cytokinins, apical dominance can be reduced and it is possible to enhance the side branches of buds from the leaf axis. More axillary bud branching cycles result in the production of more shoots (Debnath 2003). Cultures can be established and kept up on MS Medium with BAP, IBA, and GA3 or (Boxus, 1974) medium with Knop's macronutrients, Murashige and Skoog's (1962) micronutrients, and organic substances (Mozafari & Gerdakaneh 2012).

**In vitro regeneration via. Callus culture.** The propagation and improvement of crop plants can benefit significantly from plant regeneration from in vitro callus tissue culture (Yeoman and Macleod 1997). For the first time, plant regeneration from strawberry callus has been evidenced (Egbuna *et al.*, 2021). Efferth (2019) found that after 24 weeks, the *Fragaria ananassa* cv. Redcoat callus culture completely lost its ability to regenerate. Several cultivars have successfully achieved adventitious shoot regeneration using a variety of explants, including leaf explants, petioles, runners, and immature embryos (Mercado *et al*., 2007). It was found that strawberry adventitious buds, which appear between the two points of the leaf stipule and proliferate quickly with axillary branching, are of particular interest. They came to the conclusion that the regenerated plants' performances were comparable to those of the control plants. The control plantlets were more or less anthocyanin-colored, whereas they noticed that the roots of stipular plantlets were exclusively white (Badjakov *et al*., 2021). The study of adventitious shoot regeneration using various explants revealed significant variations in the ability of calluses derived from the leaf, petiole, and root tissues, resulting in shoot regeneration. However, these variations vanished when complete leaves, including the stipules, were utilised as explants (Chung & Ouyang 2020). Passey *et al.* (2003), used leaf discs, roots, petioles, and stipules as explants to study seven commercial strawberry cultivars. All cultivars' highest rates of regeneration were found in the leaf discs, where more than 90% of explants produced shoots.Samir & Debnath (2005) reported that in vitro regeneration of shoots on strawberry calyxes that had been removed was described as an effective method. They discovered that within 4 to 5 weeks of culture initiation, sepal cultures on 2.4 mM 1-phenyl-3-(1,2,3thiadiazol-5yl) urea (thidiazuron, TDZ)-containing without a callus phase in between, the shoot induction medium generated numerous buds and shoots. Another finding was that the high rate of differentiation was produced by young, expanding sepals that were kept in the dark for 14 days, adaxial side in contact with the culture medium. It was also noted that explants, such as sepals, outperformed leaf discs and petiole segments to encourage the development of calluses and reducing shoot elongation in media containing TDZ (0.5–4.0 mg/l). After one more subculture, after being moved into a medium containing 2.4 mM zeatin, TDZ-initiated cultures produced usable shoots before rooting on a medium devoid of growth regulators. Qin *et al*. (2005) reported that cultivating leaf explants for 10 days in a medium for shoot regeneration was more effective when $AgNO_3$ was present, and adventitious bud development was accelerated. According to Samir and Debnath (2006), adding 2, 4 or 8μ M thidiazuron (TDZ) to the growth medium accelerated the rate of shoot regeneration from young, expanding sepals, leaf discs, and petioles without a callus-forming phase in between. When it came to regenerating shoots, sepals outperformed leaf discs and petiole segments.

**Mutation breeding in strawberry.** Mutation, it is fast transmissible changes within the desoxyribonucleic acid (DNA) sequence that don't seem to be derived from genetic segregation or recombination and gaining are popularity in breeding of different produces. It has different types out of which spontaneous is one. Spontaneous mutation happens at an awfully low frequency one in ten lacs or 10-6. The highest degree of spontaneous bud mutation is known as a "bud sport" in fruit crops. Fruit breeders have become interested in breeding through iatrogenic mutation because of the prevalence of an excessive variety of natural bud sports in citrus, mango, grapes, and other fruits. Due to the fact that mutations produce variation, they serve as the ultimate foundation for the evolution of new forms, varieties, or species. Mutations can lead to chromosome deletion, inversion, translocation, and base substitutions in ester compounds. Mutations are frequently caused artificially, or iatrogenicly, by a variety of physical and chemical agents known as mutagens. Most widely employed Gamma rays and EMS (Ethyl Methane Sulphonate) are two examples of physical and chemical mutagens, respectively (Pereira *et al*., 2023). The best rates of mutation can result from doses that cause 25–5 LD50) in mutated plants. Fruit crop improvement through conventional breeding is constrained by their perennial nature, prolonged immature phase, state, sexual incompatibilities, etc. To improve fruit crops, the development needs to be created while not modification within the style and colour of fruits and it are often best achieved through mutation breeding as a result of, it causes modification within the genetic background of parental material and improve varied husbandry characters in count to yield. Mutation breeding helps positive upgrading of characteristics of many crops and therefore the technology is comprehensively exploitation by scientists with success (Wang *et al*., 2023). Traditional crossbreeding techniques have been primarily used to develop strawberry cultivars. However, because strawberry cultivars have a high degree of heterozygosity, many of the traits of the offspring produced by cross-breeding are dissimilar to those of the parent cultivars (Kim *et al*., 2019). In contrast, Techniques for mutation breeding could greatly increase genetic diversity and enable the introduction of novel traits without compromising the desirable qualities of the best cultivars. (FAO/IAEA 2018). Many plants have been genetically modified using chemical and physical mutagens. Because of the high frequency of nucleotide substitutions it causes to be found in different genomes, ethyl methane sulphonate (EMS), one type of chemical mutagen, is frequently used in plants (Adak & Kaynak 2018)

**Improvement of strawberry germplasm through irradiation.** Strawberry is wholesome and delicious foreign fruit custom-made in Bangladesh, already acquire attention from each Government and farmers and general individual of Bangladesh. Various cultivars are available there but they don't have satisfactory performance. Jamal *et al.* (2012) conducted and experiment for the improvement of strawberry germplasm with the help of irradiation. They use four different germplasm say viz. V1 (Germplasm-01), V2 (Germplasm-02), V3 (Germplasm-03) and V4 (Germplasm-04) and irradiations, X-ray and UV-ray. Out of which they found that the most superior germplasm is V1 (Germplasm-01). The quality and yield of strawberry fruit are positively mutated by X-ray irradiation among the various irradiation techniques.

**Induced Mutations and *in vitro* Culture in Strawberry *(Fragaria* × *ananassa)* Improvement.** *Ethyl Methane Sulphonate* (EMS) is the most frequently used chemical potent mutagen under *in vitro* and *in vivo* conditions. Various structural variations were formed via EMS treatments. Furthermore, EMS has extensively been applied to many plant species, such as soybean, banana, loquat. Tissue culture is not only a method of clonal propagation but also used in plant breeding studies. It increases the efficiently of mutagenic treatment, because of that reason tissue culture-induced-mutations have been widely considered as a foundation of plant upgrading programmes. Adak and Lamikaynak (2018) conducted an experiment by application of EMS improvement of strawberry through inducted mutation in in-vitro culture and found the increased rate of survival, number of shoots, number of roots, root length and number of leaves. Similarly Bhat *at al*. (2017) conducted on experiment on camarosa variety of strawberry. To ascertain the impact of induced mutation on the growth parameters in vivo. Ex-plant (Runner tips, shoot tips, and leaf disc) was exposed to various concentrations for various lengths of time. Excellent runner tip was discovered among them. With the increase in *EMS* concentration and time duration rate of survival of ex-plant decrease.

**Efficiency of gamma ray irradiation and *ethyl methane sulphonate* on *in vitro* mutagenesis of strawberry.** The use of mutagens was proposed as a method to improve fruit quality while increasing genetic diversity and changing only one or a few selected cultivar-specific features (Predieri, 2001). The introduction of several advantageous features that impact plant growth, flowering time and fruit maturity, fruit color, greater quality, self compatibility, selfthinning, and disease resistance have all been achieved in the past through the application of mutagenesis in fruit crops (Kaushal *et al.,* 2004). The effects of gamma radiation and ethyl methane sulphonate with gamma rays were studied in an experiment by (Murti *et al.,* 2013). 'DNKW001 accession' ('DNKW001') received treatments with EMS 7M (GRE) and doses of 0, 30, 80, 130, 180, 230, 280, 300, and 325 Gy in addition to comparable gamma radiation doses. Irradiated with the doses of 0, 30, 80, 130, 180, and 230 Gy. With increasing doses, the survival rate and plantlet performance of DNKW001 significantly decreased, and the lethal dose (LD50) was lower (104 Gy) than the lethal dose from gamma irradiation alone (177 Gy. Several plantlets were seen before and after acclimation, including those with misshapen leaves and bigger petioles. While monosomic octoploid plants functioned as dwarf plants, hexadecaploids of Akihime, pentadecaploids with 13x+4 chormosomes, and diplodecaploids of DNKW001 were shown to be strong plants with thicker leaves and bigger pollen than octoploid plants. Exceptional variations were selected from the PCA plot based on weight of fruit, color and total soluble solid content, and they were later shown to be DNA mutants. Gamma radiation and EMS together made it simpler to induce variations of more sorts and magnitudes. An irradiation dosage of less than 130 Gy was sufficient to create strawberry variants.

**Influence of gamma rays and NaCl on the induced somaclonal variation in the strawberry cultivar Arnavutköy.** Strawberry is affected by salinity in temperate and tropical regions. Kapenek (2016) conducted an experiment on induced somaclonal variant of strawberry which is produced by the process of organogenesis. All this is done to find of the best plant which is resistant to salinity. They concluded that if strawberry is treated with 40Gy along with 500ppm of NaCl plant show most resistance to salinity and if used properly can be used for the development of varieties which are resistant for salinity.

**$^{60}$Co-gamma ray effect on runner strawberry.** With the rapid increase of strawberry production in China, new strawberry types have been introduced and bred. It is critical to develop novel strawberry germplasm using a variety of breeding techniques. In plant breeding, induced mutation is an excellent way for obtaining novel genetic resources and variations. Weimin *et al.* (2009) conducted an experiment in which the effects of three different dosages of $^{60}$Co-gamma radiation on runner plants of five strawberry types were tested. They used five different strawberry cultivars for this study and found that as the dose of $^{60}$Cogamma radiation was increased, the death rate of the cultivars varied. The sensitivity of the different kinds to the irradiation of 60Co-gamma ray varied, with 'Akihime' being the most sensitive, followed by 'Hokowase,' 'Chun Xu,' and 'Sheng Xing,' with 'Shuo Feng' being the least susceptible.

**Improvement of Salt Stress Tolerance in Strawberry by Ethyl Methane Sulfonate Treatment.** EMS was applied to the "Fortuna cv" runner tips for 30, 60, and 90 minutes at three different concentrations (0.0, 0.1, and 0.3%). In order to evaluate vegetative growth and yield traits, strawberry seedlings were planted in an open field. After choosing the best strains, the strains were tested under saline stress conditions (500 and 1000 ppm of NaCl). To find the potential variability effects of EMS, RAPD-PCR was used. The findings demonstrated that, in comparison to the control, EMS treatment enhanced the characteristics of vegetative growth and yield under salt stress. The results also showed that EMS-treated plants had higher fruit quality parameters than control plants. Four RAPD primers were used to detect a total of 15 polymorphic bands, of which 12 were the results of the EMS treatment. Eleven distinct bands were seen, eight of which came from EMS treatment facilities, and the remaining three bands were found among the rival plants. This study demonstrated the potential of EMS, a chemical mutagen, in improving salt tolerance (Moneim *et al.*, 2021).

**Table 2: Different mutagen and their effect on strawberry cultivars.**

| Sr. No. | Mutagen | Concentration | Duration | Effect | References |
|---|---|---|---|---|---|
| 1 | X-rays and UV-rays with different germplasm | - | - | X- rays with germplasm VI gives best result | Islam (2012) |
| 2 | EMS *ethylmethane sulphonate*) | 0.4% | 60mins | Maximum survival rate | Adak and Lamikaynak (2018) |
| 3 | EMS *ethylmethane sulphonate*) | 0.2% | 60-90mins | For multiplication and rooting | Adak and Lamikaynak (2016) |
| 4 | EMS (*ethyl Methane sulphonate*) | 0.1% | 1.5 hr | Minimum days to bear first flower (25.5) and maximum number of flowers per plant (25.5). | Bhat *et al.* (2017) |
| 5 | EMS (*ethyl Methane sulphonate*) | 0.4% | 1.5, 2.5, 3.5 hrs respectively | Lethal to plant | Bhat *et al.* (2017) |
| 6 | Gamma rays + NaCl | 40Gy + 500ppm | - | Show high level of resistance to salinity | Kapenek (2016) |
| 7 | EMS | (0.0, 0.1 and 0.3 %) | for (30, 60 and 90 min) | Improved plant height, leaf count, chlorophyll content, fruit count, and fruit weight; these are all aspects of vegetative growth and yield. | Moneim *et al*. (2021) |

**Biotechnology in strawberry**

**Biotechnology for berry nutritional quality.** Mezzetti (2013), founded that although there is currently considerable concern regarding transgenic techniques in modern agriculture, particularly when genes from organisms other than plants are employed, they can provide an alternative. Transgenic techniques have so far proved successful in increasing the nutritional value of a number of important crops. Rice, for example, is grown all over the world. The demonstration that D-galacturonic acid can be used to biosynthesise L-ascorbic acid in ripe strawberry fruit was the first exciting result in the field of berries. In addition, over expression of the strawberry gene *Gai*UR was shown to boost vitamin C levels in *Arabidopsis thaliana* plants. Dgalacturonic acid reductase is also is encoded by *Gai*UR gene.

**The diploid strawberry (*Fragaria nilgerrensis*) has a high-quality genome that offers new information about anthocyanin synthesis.** A wild variety of diploid strawberries termed *Fragaria nilgerrensis* from Asia's east and southeast, offers a richness of genetic variation for strawberry improvement. Pacific Biosciences sequencing uses single-molecule real-time (SMRT), chromosomal conformation capture (Hi-C), and genome scaffolding. (Zhang *et al*., 2020) He displayed *Fragaria nilgerrensis'* chromosome-scale assembly, and researchers found that sequential variability in the upstream regulatory region of FnMYB10—a crucial transcriptional inducer of anthocyanin biosynthesis—led to reduced expression of the gene. This is likely why *Fragaria nilgerrensis* only produces white fruit.

**The fruit colour of both wild and cultivated strawberries is changed by genetic RAP modulation.** Gao *et al*. (2020), found that they tested the viability of RAP in changing fruit colours by overexpressing RAP in *Fragaria vesca* and knocking down RAP in the cultivated strawberry *Fragaria ananassa*. They noticed that red fruit developed when RAP was overexpressed in the Yellow Wonder background. In addition, the red hue first appears in the loral stage 10 and stays throughout the early stages of fruit development in the receptacle. According to their findings, increasing anthocyanin production in the early stages of fruit transport could switch the metabolic flow from proanthocyanidin to anthocyanin.

**Table 3: Different molecular tools and their result in strawberry.**

| Sr. No | Fruit Crop | Tool Used | Results | References |
|---|---|---|---|---|
| 1. | Strawberry | *Gai*UR | Boost Vitamin C levels | (Agius *et al*., 2003) |
| 2. | Strawberry | Chromosome conformation capture (Hi-C) genome scaffolding (SMRT) and Pacific Biosciences' sequencing. | The FnMYB10 gene's decreased expression level, which is most likely what causes the white fruit phenotype in *F. nilgerrensis*, was caused by Sequence changes in the gene's upstream regulatory region. An important transcriptional regulator of anthocyanin biosynthesis is FnMYB10. | (Zhang *et al*., 2020) |
| 3. | Strawberry | RAP | The formation of red fruit was caused by the over expression of RAP in the Yellow Wonder background. | (Gao *et al*., 2020) |
| 4. | Strawberry | FaRVd2B and FaRVd6D are resistant markers from the "Redgauntlet" and "Chandler," respectively. | Disease resistant from *Verticillium dahlia* | (Cockerton *et al*., 2019) |
| 5. | Strawberry | When the two resistance alleles were combined, the co-dominant resistant QTL FaRVd3D, which is present in the cultivars "Redgauntlet" and "Hapil," displayed 48 a major effect of 18.3%. | Disease resistant from *Verticillium dahlia* | (Cockerton *et al*., 2018) |

## CONCLUSIONS

At the end of the paper, we concluded that Cristina, a new cultivar with improved fruit quality that integrates delayed production with greater productivity has snuffed out their enthusiasm. In Europe, Romina is competing for early production with other well-established cultivars like Clery, Alba, Flair, and others. Recurrent selection is valuable for producing improved plant as a new cultivar by selecting the superior breeding material. By the breeding genetic variability sustain and produce diseases resistance cultivars. Recurrent selection producing vigorous lines in two or three cycles and in these lines the leaf area was larger by this plant remain dwarf. In November these lines leaf area was larger than the existing and smaller from November to March. In winter the selection lines where more vigorous due which yield became higher in winter. One of the most efficient ways to lower the cost of micropropagation is to use an appropriate protocol for automation in a bioreactor. The high scale of propagation can be effectively controlled by growth regulators like BA, IBA, or NAA. In comparison to conventional propagated plants, in vitro raised plants produced the most runners. In most of the cases mutation is done is strawberry to increase the germplasm so that new best cultivars can be produced. EMS (*ethyl methane sulphonate*) mutant is most commonly used in strawberry mutation programme. The methodology of gamma rays+ NaCl is used to develop a new cultivar which show resistance to salinity. Breeding programmes are taken from molecular techniques used in biotechnological processes. The discovery of genetic links between plants, the production of genetic maps, the determination of candidate markers, and their use in early selection are all successful in strawberry breeding. ISSR and RAPD are powerful molecular markers for detecting somaclonal variations.

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