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Review Article

Advancements in Mutation Breeding in Phalsa (*Grewia asiatica* L.) Crop Improvement: A Comprehensive Review of Radiation and Chemical Induced Mutagenesis Studies

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Abstract

Phalsa (*Grewia asiatica* L.), a member of the Tiliaceae family, is a remarkable fruit-bearing shrub with significant medicinal and nutritional value. Originating in South Asia, it has gained popularity worldwide, particularly in subtropical and tropical regions. This review comprehensively examines the botanical characteristics, medicinal properties, nutritional composition, cultivation practices, and challenges faced by Phalsa growers, with a focus on its potential for crop improvement through mutagenesis and polyploidization techniques. With around 150 species, Grewia is the sole genus in the Tiliaceae family that produces edible fruit. Phalsa bushes, known for their rapid growth, yield orbicular fruits with moderately acidic yet nutritious pulp. The fruit is esteemed for its medicinal benefits, ranging from anti-inflammatory and anti-diabetic properties to its use in treating various respiratory and cardiovascular ailments. Despite its nutritional and medicinal significance, Phalsa cultivation faces challenges such as poor post-harvest management, limited germplasm diversity, and abiotic stress susceptibility. To address these challenges and enhance Phalsa's agricultural potential, mutagenesis and polyploidization techniques have been explored. Induced mutagenesis offers a promising avenue for creating genetic diversity and improving traits such as stress tolerance and disease resistance. However, culture contamination remains a significant obstacle in achieving optimal shoot initiation and propagation efficiency. Overall, this review underscores the importance of Phalsa as a valuable crop with immense medicinal and nutritional benefits. By leveraging mutagenesis, polyploidization, and tissue culture techniques, Phalsa growers can overcome existing challenges and unlock its full agricultural potential, contributing to food security and public health.

Keywords: Phalsa, Mutation breeding, crop improvement, mutagens, electron beams, gamma radiation, colchicine, ascorbic acid, fruit quality, shelf-life extension, disease resistance, mutagenesis methods, fruit crops, physiological changes, biochemical changes, agricultural innovation.

INTRODUCTION

Phalsa (*Grewia asiatica* L.), commonly known as 'Star Apple,' is a Tiliaceae family member (Ghosh *et al*., 2017). The Tiliaceae family has around 50 genera and 450 species (Paithane and Bhuktar, 2012). Grewia has 150 species, including tiny shrubs and trees. Grewia is the only genus in the *Tiliaceae* family that produces edible fruit (Zia-ul-Haq *et al*., 2013). It originated in South Asia and is now extensively distributed throughout the world's subtropical and tropical areas. Major Phalsa-producing countries are India, Pakistan, Sri Lanka, the Philippines, and Bangladesh. It is planted on 1241 acres in Pakistan and produces 4209 tonnes per year. It may be cultivated in Pakistan in warm locations like Punjab and Sindh which have high temperatures during the ripening period. It is planted across 661 acres in Sindh, with an annual yield of 1738 tonnes. It is cultivated on 580 acres in Punjab, with an annual yield of 2471 tonnes (Fruit, Vegetables & Condiments Statistics of Pakistan, 2019). Phalsa is a hardy shrub that grows quickly to a height of 4 to 5 meters. The plant's leaves range in length from 5 to 18 cm, is wider at the tip and pointed, and have an oval shape (Hiwale *et al*., 2015). Flowers with 5 sepals and petals are tiny, about 1-2 cm in diameter, and vivid orange-yellow (Dey and Das, 1995; Paul, 2015). Just below the pedicles, bracts can be found. The Phalsa fruits

are orbicular in shape. Flowers usually start in January and February and harvesting of fruits starts at the end of May and continues till June due to abrupt flowering and inadequate ripening of fruit (Morton *et al*., 1987). Phalsa bush develops from self-fertilization (self-pollinated) (Malik *et al*., 2010). Phalsa is highly valued in the indigenous medicinal system. Almost every species and plant component in the Grewia genus is used as a medicinal treatment for a range of illnesses and infirmities. The fruit of *G. asiatica* has astringent and cooling effects. The unripe fruit of the Phalsa bush soothes inflammation and may be used in respiratory, blood, cardiac, and fever conditions. The fruits are also beneficial for liver and heart diseases, indigestion, anorexia, toxemia, thirst, asthma, hiccough, and diarrhea are used to treat (Morton *et al*., 1987). Antioxidant characteristics of leaf and fruit extracts, i.e., anticancer, liver associated illnesses, and breast cancer. Therefore, fruits and extracts of Phalsa leaves can be used to help prevent human cancer. The root bark has been recommended for rheumatism, as well as an infusion that may be used as a medication. Phalsa leaves can be applied to the skin to help with skin blemishes. Its juice, along with its low glycemic index, may be utilized to treat diabetes. Carbohydrates with a low glycemic index are slowly converted into their basic form in the human stomach. This type of food is also recommended to reduce the likelihood of heart disease and obesity (Tiwari *et al*., 2014). Phalsa requires a hot summer to fruit. In the winter, Phalsa drops its leaves and goes dormant. It shoots anew in the spring when the temperature is warm. When the temperature is high, the fruit ripens and matures faster. It can survive in a variety of soil types but prefers clay loam soils with a high nitrogen concentration. A fertilizer that is applied correctly enhances its shelf life and productivity.

Exploring the Potential of Phalsa: From Nutritional Powerhouse to Commercial Challenges

The ripe Phalsa fruits are edible, although they have a moderately acidic flavor, and Phalsa leaves are a superior method to gain protein and tannin-free. The Phalsa fruit is low in calories and fat, but abundant in vitamins, minerals, fiber, and essential minerals. Phalsa fruits are well-known for their anti-inflammatory properties and are frequently used to treat respiratory, aging, diabetes, cardiovascular, rheumatoid arthritis, and atherosclerotic illnesses (Sinha *et al*., 2016). Various plants can also be utilized in herbal therapy to treat cancer, fever, aging, and diabetes, among other disorders (Wani *et al*., 2015). Some of the phytochemicals found in Phalsa variants include glycosides, alkaloids, steroids, saponins, functional acids, and flavonoids (Khan *et al*., 2019). The Phalsa business in Pakistan is hampered by a variety of abiotic conditions, including stress tolerance to salt and drought. Poor post-harvest management, several harvesting, and inconsistent fruit ripening with short shelf life are additional significant constraints (Jayswal *et al*., 2017). Furthermore, due to a limitation of broad germplasm introduction, selection, and characterization, just two commercial variants are available. Furthermore, it is not widely produced in Pakistan and is characterized as a small fruit due to lack of awareness and expertise among producers and consumers. In India, seeds are commonly used to commercially grow Phalsa. Furthermore, when kept under ambient conditions, the seed tends to lose viability significantly after three months, while only fresh seeds from fully ripe fruits should be cultivated. The Phalsa plant may be easily reproduced by rooting hardwood cuttings or layering (Samson *et al*., 1986). The regeneration potential of cuttings is influenced by a variety of aspects such as cutting pretreatment, developmental stage, external factors, and so on (Jadhav *et al*., 2007).

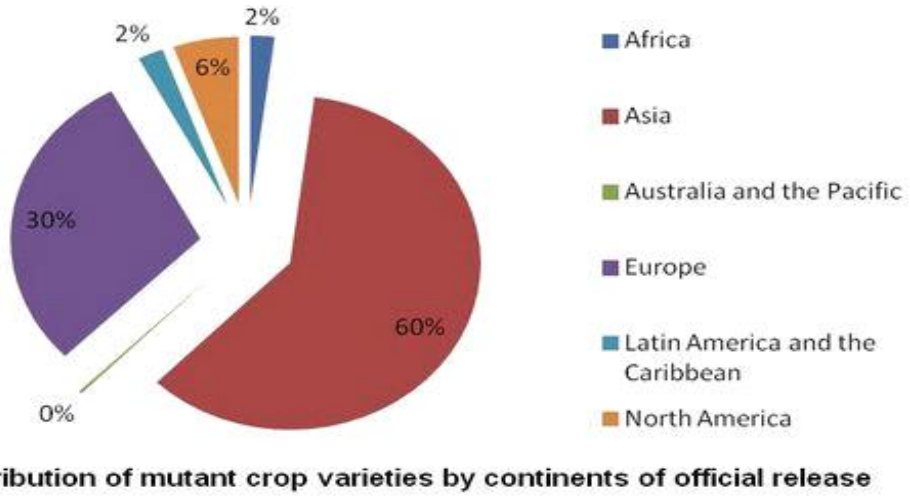

Figure 1: Distribution of mutant crop varieties by continents of official release (Mba, 2013)

Unleashing Genetic Diversity: The Role of Plant Mutagens in Fruit Crop Enhancement

Plant mutagens have a significant role in enhancing the variety of desired attributes in fruit crops. The most successful approach for creating genetic diversity and gene regulation is induced mutagenesis (Kozgar *et al*., 2012). Furthermore, it is a potential strategy for developing novel varieties with higher

quality traits, such as stress tolerance against a variety of biotic and abiotic challenges and genetic improvement in crops. Mutagens can also aid in genetic mapping and linkage studies (Chaudhary *et al*., 2019).

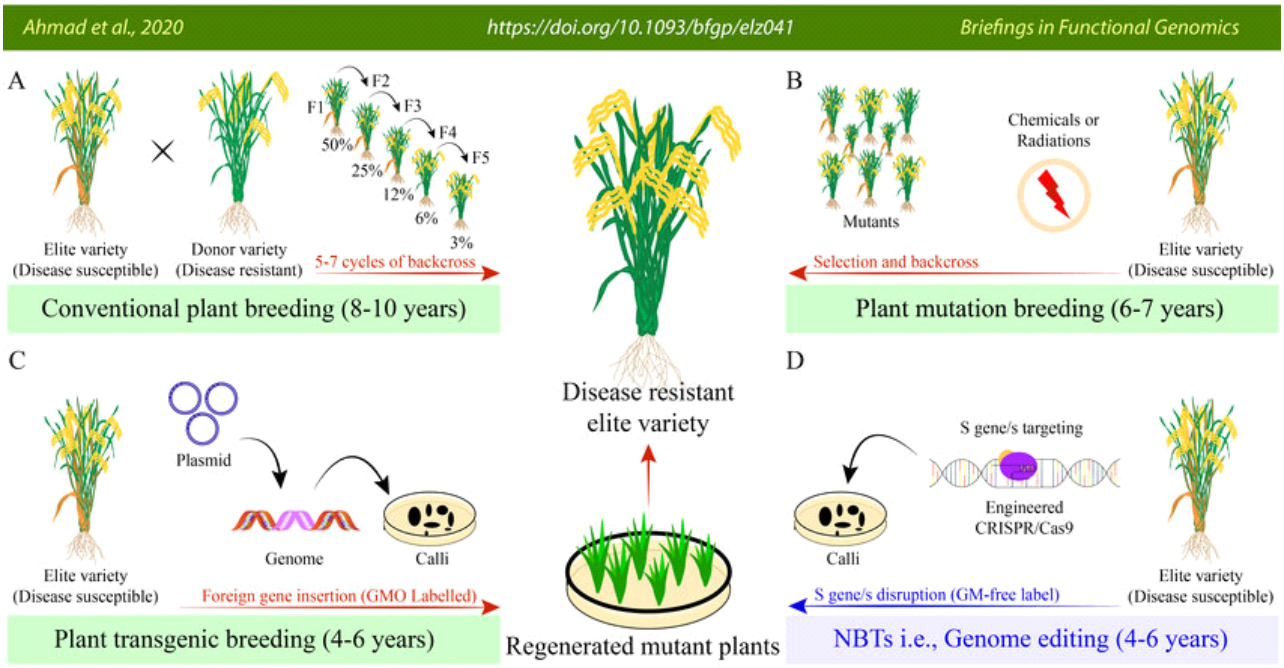

Figure 2: Comparison of plant breeding and mutagenesis methods for crop improvement, adapted from Chen *et al*. (2019). (A) Conventional plant breeding, known as crossbreeding, involves crossing an elite recipient parent line with a donor line carrying desirable traits, followed by successive backcrossing and rigorous selection to obtain progeny with desired traits, such as disease resistance. This method is time-consuming, labor-intensive, and has limitations. (B) Mutation breeding involves treating seeds with chemical or physical mutagens to induce mutations in the plant genome. Mutants are selected based on desirable phenotypes, but the process is time-consuming, tedious, and prone to random mutations that are sometimes difficult to detect and predict. (C) Transgenic breeding involves inserting a gene of interest from one genome into another to improve traits. Despite precision and accuracy in gene insertion, genetically modified plants may face public acceptance issues and regulatory scrutiny. (D) New genetic modification techniques, such as genome editing with CRISPR/Cas9, offer hope for sustainable crop improvement by targeting and disrupting specific negative regulators or genes. This approach is efficient, cost-effective, and may bypass GMO legislation due to the absence of foreign DNA. (Adapted from: "CRISPR/Cas9 for development of disease resistance in plants: recent progress, limitations and future prospects" by Chen *et al*., 2019)

Mutagenesis has been widely employed in the development of several features in fruit crops, including plant height, shelf-life, fruit skin color, and disease resistance (Lamo *et al*., 2017; Arshad *et al*., 2024)). The screening of mutants is a technique that includes choosing individuals from a large population of mutants that meet selection criteria such as particular early flowering and disease resistance in comparison to the parent. However, the results of this selection are frequently regarded as presumptive mutants. The method of identifying mutants with high percentages in a repeated scenario is known as mutant identification. Following the outcomes of this technique, many putative mutants are discovered to be false mutants. In general, crop improvement mutations include single bases and may or may not affect protein synthesis (Mba *et al*., 2013).

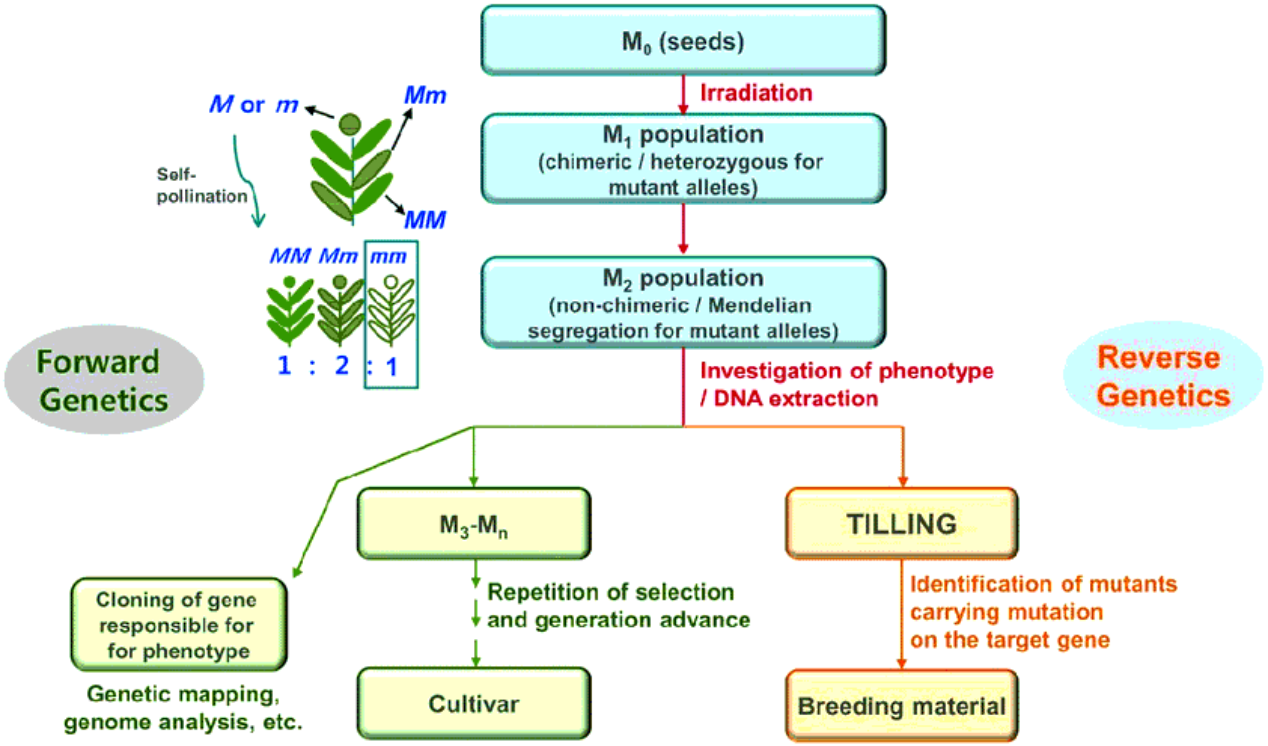

Figure 3: Illustrates the general procedure for mutation breeding of seed-propagated crops. Initially, the M1 plant is chimeric because it arises from irradiated seeds and consists of multiple cells with various mutation types. Selections based on either phenotype or DNA sequences typically occur with M2 populations, which do not contain any chimeric individuals. In a forward genetics approach, individuals exhibiting the desired phenotype are chosen from the population, followed by the study of the gene responsible for that phenotype. Conversely, in reverse genetics approaches, lines with mutated targeted genes are selected first, and then the resulting phenotype is investigated (Frequency and Spectrum of Radiation-Induced Mutations, 2024)

Unveiling the Power of Polyploidy: A Narrative Journey Through Crop Improvement

Polyploidization is the most helpful element in crop improvement and flowering plant evolutionary research (Martin *et al*., 2019). I t is considered a significant adaptation and speciation process, as well as a key agent in evolution and crop development (Mason *et al*., 2016). In some plant species, the involvement of unreduced gametes in the creation and development of polyploids has been extensively characterized (Sattler *et al*., 2016). Polyploids offer various advantages over diploid species, including larger fruit size, higher production, disease resistance, and a smaller number of seeds (Gu *et al*., 2005; Arshad *et al*., 2024a). Furthermore, polyploids have an important function in increasing adaptation, phenotypic variation, and genetic diversity (Zhou *et al*., 2020). The most common polyploidization strategies are in vivo, *In vitro*, and ex *vitro* systems. The most extensively used method of polyploidization is *In vitro*, which may lead to fast polyploid growth in confined spaces (Eng and Ho *et al*., 2019; Abbas *et al.*, 2021a). In an *In vitro* system, aqueous antimitotic agent solutions may also be utilized in culture media and simply delivered to plant tissues. Polyploidy induction *In vitro* focuses mostly on organ-genic and somatic embryogenic systems for regeneration (Touchell *et al*., 2020). A variety of explants, as well as apical meristems, have been exposed to antimitotic agents for the development of polyploidy *In vitro* in diverse plants (Zhang and Gao, 2020), nodal segments (Shmeit *et al*., 2020; Abbas *et al.*, 2021), leaf segments (Zhang *et al*., 2020), seeds (Carbajal *et al*., 2019; Arshad *et al*., 2024c), and embryogenic suspension cultures (Acanda *et al*., 2015; Arshad *et al*., 2024c). *In vitro* autopolyploid generation is more dependable than in vivo approaches due to higher mutation rates and a lower frequency of chimeras (Fu *et al*., 2019) it may be necessary for medicinal plants since identifying plant chimeras takes time. However, *In vitro* polyploidization, evaluation of acquired polyploids, and the use of molecular markers to differentiate the polyploidy impact *In vitro* are crucial (Iannicelli *et al*., 2020; Rafeeq *et al.*, 2020). Hu *et al*. (2021) assessed the phenotypic characteristics and consistency of the plant in diploid and polyploid carambola field trials. In general, artificially created ploidy plants were associated with improved agricultural qualities. Triploid and tetraploid crops produced harder roots and petioles, more seeds, wider and broader leaves, larger grains, and pollen-filled flowers. There was a considerable decrease in the number of blooms seen in polyploid plant species. In comparison, using a scanning electron microscope, the high number of triploid plant pollen grains was irregular, with a weakened structure and two different pollen grains.

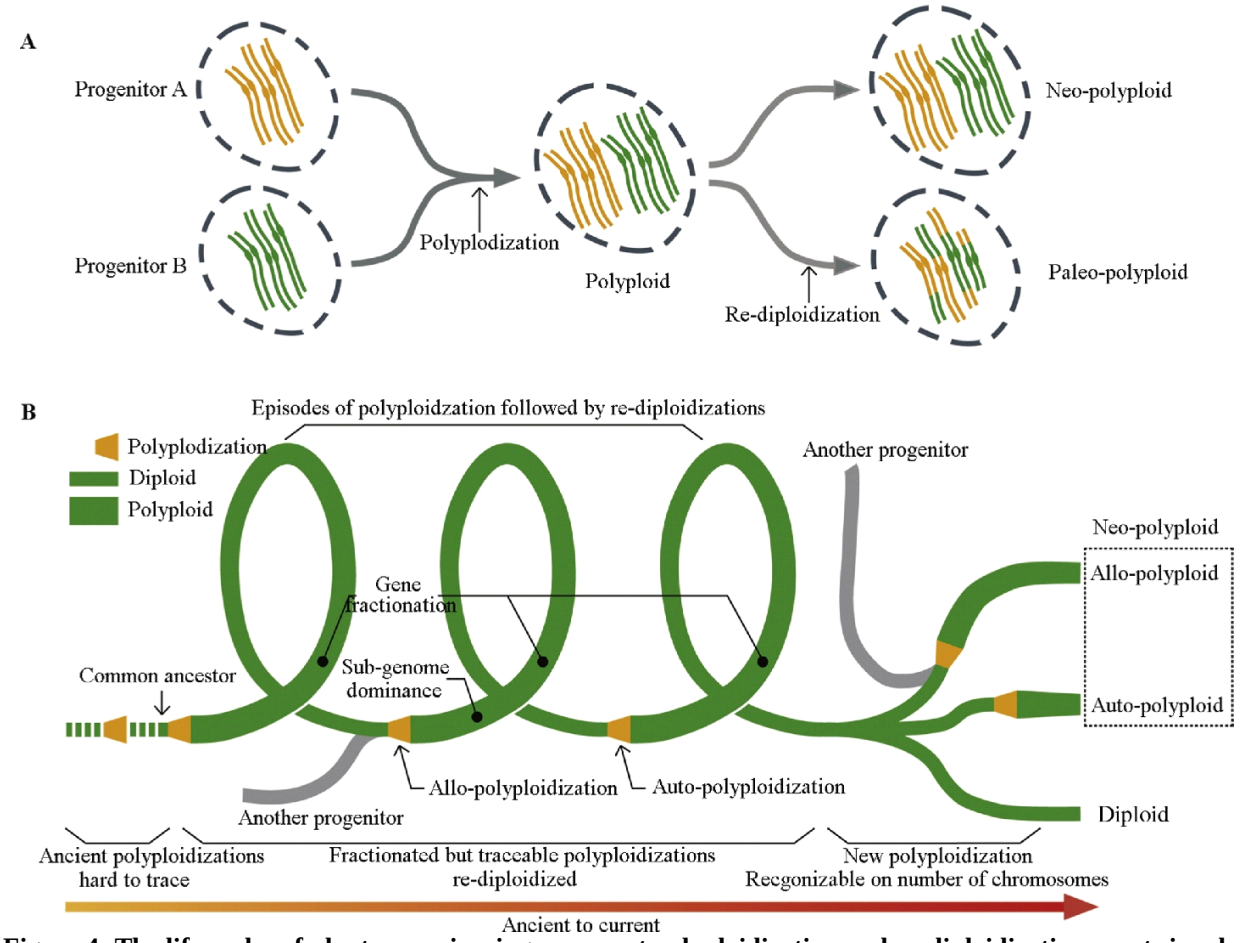

Figure 4: The life cycles of plants experiencing recurrent polyploidization and re-diploidization events involve complex genetic processes that impact their reproductive cycles and evolutionary trajectories (Zhang *et al.*, 2019)

This study is expected to result in the development of a method for breeding new polyploid carambola varieties with distinct agronomic traits, higher fruit quality, and a longer self-life. Cimen *et al*., (2020) used colchicine to create a tetraploid in C35 citrange. Explants were obtained from seeds obtained during a germination trial with a few dosages of GA_3. C35 seeds cultured without seed coat in WM enriched media with 4 mg GA_3 resulted in a phenomenal germination rate (84%). Explants were treated with the antimitotic drug colchicine at 0.1 percent for 48 hours. This treatment resulted in a maximum tetraploid proliferation of 15%. The seedling survival rate was lowered by the high dosage of colchicine and the duration of exposure. Flow cytometry revealed that the nuclear genome size of tetraploid seedlings was larger than that of diploid seedlings. In general, the leaf area and stomata size rose as the ploidy level increased. Mo *et al*., (2020) inducted polyploids was created in *Rhododendron fortune* Lindl by employing the antimitotic drug colchicine at a dose of 0.1 percent for 24 hours. It was discovered that the maximal ploidy induction rate was 36.67%. Flow cytometric analysis revealed 69 tetraploids and 29 octoploids in the regenerated plants tested. According to phenotypic analysis, tetraploid and octoploid plants have thicker and narrower leaves, as well as more frequent and longer epidermal hairs than diploids. Furthermore, polyploid stomata were broader and thinner than diploid stomata. Polyploids also contain more chlorophyll, which results in deeper green leaves. Hailu *et al*., (2020) used colchicine at varying concentrations and time exposure to induce polyploidy in the garlic "Tawangmangu Baru" variety for genetic diversity. This experiment consisted of two factors. One factor for colchicine dosages of 0.00, 0.02, 0.04, 0.06, 0.08, and 0.10 percent, and another for periods of 24 and 48 hours. According to the data, BDS + 0.4 mg resulted in 4.72 percent callus induction. Both BDS + 1.5 mg generated L-1 kinetin and 4.0 percent callus proliferation at a concentration of 2 mg L-1. 2,4-D in the presence of 1 mg L-1 MS +1.5 mg and L-1 kinetin 2,4-D in the presence of 1 mg L-1 L-1 kinetin. The control medication had a higher death rate than the colchicine treatment after 48 hours. The number of shoots was larger in 0.1 percent colchicine at 48 hours and lower at 24 hours, but 0.1 percent colchicine at 24 and 48 hours had the highest ploidy level of total nuclear DNA, as measured by flow cytometry. Before assessing the phenotype, the putative lines created following 0.1 percent colchicine treatment were inoculated to create new mutants. Hassanzadeh *et al*., (2020) & Arshad *et al*., (2021) experimented on salvia Officinalis L. to examine polyploidy induction with colchicine at concentrations of 0, 0.05, 0.1, 0.25, and 0.5 percent for 12 hours, 24 hours, and 48 hours. The

induction of polyploidy was first noticed visually and anatomically and was later validated by flow cytometry and leaf chromosomal analysis. The concentration of colchicine and the length of immersion time were shown to have a substantial influence on plant survival and the percentage of tetra-ploidy induction. By increasing the immersion period and colchicine concentration, the percentage of plants that survived was lowered. Moreover, the maximum rate of induced tetraploid plants was obtained at 0.25 and 0.5 percent colchicine, with immersion periods of 48 and 24 hours, respectively. Tetraploid plants vary from diploid plants in terms of leaf length, width, height, number of leaves, nodes, internode length, stomata size, catalase, total phenolic, and flavonoid content, and stomata count, whereas diploid plants differ in terms of stomata count. Singh *et al*., (2020) performed research on sweet orange cv. Mosambi mutants were created by treating them with different concentrations of colchicine (0.05, 0.10, 0.15, and 0.20 percent) and propagating them on Jatti Khatti rootstock. The mutants were investigated two years after the plants were transplanted in the field for their distinct growth characteristics and leaf nutrient absorption. Colchicine had both inhibitory and stimulatory effects on the mutants that were created at different doses. The use of colchicine lowered stem girth and TCSA by 0.05 and 0.20 percent, respectively. Plant growth characteristics were boosted by mutants developed at 0.10 percent and 0.15 percent colchicine concentrations. Less macronutrient absorption was seen in the leaves of mutants developed at 0.10 percent -0.15% dosages, but significant nutritional uptake was observed at 0.05 percent and 0.20 percent doses. The variation established in mutants by colchicine indicated that both low and large dosages of mutagenic treatments may result in economically important mutants, suggesting its potential use as a mutagen. Talei *et al*., (2020) investigated the use of Colchicine to produce polyploid induction in *Stevia reaudiana.* The stevia seedlings were treated with four different concentrations of the antimitotic drug colchicine 0.05, 0.1, 0.2 percent, control, and 48-hour period. Before blossoming, plant morphological and phytochemical properties were evaluated. According to the findings, colchicine concentrations impacted plant height, number of leaves, branches, leaf length, stomata size, and density. The higher the colchicine rate, the greater the rise in aforementioned morphological parameters, as well as stomata size, and the greater the decrease in stomata density ($P \leq 0.01$). In contrast, the combination of colchicine concentration and exposure period had a significant impact on leaf length. According to scientists, determining the number of physiological alterations and secondary metabolites is one of the most accurate techniques for screening polyploid plants in polyploidization breeding programs. Ren *et al*., (2019) used *Hibiscus* breeding. Hibiscus seeds were polyploidized, and mutations were discovered using morphological and cytological approaches. The scientists discovered that 2 days after seedling development, 0.2 percent colchicine dipping for 12 hours and a variability factor of 100 percent was the optimal quantity of Hibiscus seed polyploid formation. The mutant plants exhibited a variety of morphological frequency responses, including dwarfing, coarsening, and shortening of internodes; stomata also revealed a polyploid impact with bigger and smaller densities. Flow cytometry was used to easily and accurately identify the induction outcomes from the DNA content histogram. Kushwah *et al*., (2018) investigated the development of colchitetraploidy in a local Chrysanthemum. Two metrics are determined and compared to the control treatment morphological and cytological. The phenotypic characteristics of the tetraploid plant were a slower development rate, seeds, and bigger and thicker leaves. When compared to the control treatment, cytological metrics such as increased cell size, chiasma level, DNA contents, and chromosome doubling were seen in colchitetraploid plants. The growth pattern, morphology, pollen staining ability, and seed set were all observed, with the chromosomal behavior of control and isolated colchitetraploidy receiving the most attention. Chromosome counts, stomatal density and size, and other physical features were used to determine polyploidy (Akhter *et al*., 2017; Pervaiz *et al*., 2024). Mondin *et al*., (2018) used the antimitotic drug colchicine to induce polyploidy in two cassava cultivars *In vitro*. Explants were taken from *In vitro*-produced plants of two cassava cultivars. Explants were treated for 48 or 96 hours in MS medium control and with colchicine at 90 rpm in the dark (0.05, 0.10, or 0.15 percent). Both cultivars produced normal-like and presumptive polyploid phenotypes *In vitro.* Flow cytometry or chromosome counting revealed that all plants in the polyploid group were tetraploids, and there was no correlation between ploidy level and phenotype in plants. Mixoploids have also been found in both cultivars. Vassourinha reacted to colchicine therapy better than Porquinho. Tetraploids have fewer stomata guard cells but bigger stomata than diploids. 0.10 percent colchicine for 96 hours resulted in a high number of tetraploids in both cultivars. Nasirvand *et al*., (2018) used the antimitotic drug colchicine to promote polyploidy in parsley. This experiment included two parts first phase of *in vivo* seed treatment and second phase of *In vitro* node therapy. The survival rate of nodes and seeds decreased significantly as colchicine concentration and time of treatment increased. However, polyploidy induction in node explants was superior to that in seeds. At 0.05 percent colchicine, the maximum amount of tetraploidization of plants from seeds was attained after 24 and 48 hours. *In vivo* and *in vitro*, the greatest induction of tetraploid plants was seen 24 hours after treatment with 0.05 percent and 0.1 percent colchicine, respectively. In addition, morphological studies showed that tetraploid plants had larger stomata and leaves than diploid plants. Conversely, the density of stomata was lower in tetraploids than in diploids. Ghafari *et al*., (2017) investigated polyploidy in Troyer citrange produced by colchicine. In the first step, 96 seeds were soaked in various doses of colchicine for 30 hours before

being planted in pots. When the seeds reached the four-leaf stage in the second phase, the meristems were treated with colchicine's. The results showed that treated seeds or seedlings with 1 and 1.5 percent colchicine's had reduced height and stomata density, but were more robust than the control, which might be useful in rootstock breeding.

Harnessing Tissue Culture Techniques for Polyploidy Induction in Crop Improvement

Tissue culture techniques are now frequently employed for crop improvement and effective replication of particular clones. There is currently a growing interest in applying these technologies in breeding, propagation, disease eradication, rejuvenation of older cultivars, production of clones resistant to abiotic and biotic stresses, genetic resource conservation, and so on (Lal *et al*., 2015). Micropropagation is a vital tool for disease-free plant production on a huge scale, and it offers significant advantages over traditional vegetative propagation techniques (Kozai *et al*., 199; Abbas *et al*., 2021)). It is an efficient method for achieving a large number of plant populations in a limited area in a short time (Loberant *et al*., 2009). Cost savings, greater efficiency, the development of new technologies, and the integration of micropropagation with other platform methods such as micro-cuttings, hydroponics, and aeroponics are some of the issues facing micropropagation in the twenty-first century (Cardoso *et al*., 2018; Rehman *et al*., 2021). Culture contamination is a major issue that reduces shoot initiation frequency from source explants and total shoot number developed at different cycles owing to culture loss. Studies have endeavored, often unsuccessfully, to remove all microbes from plant tissue cultures. Even in an obviously aseptic environment, obtaining true axenic cultures may be difficult. Previous research, on the other hand, suggested that plant tissues might be cultured in the presence of bacterial or fungal contamination (Herman *et al*., 1997; Arshad *et al*., 2021). Aziz *et al*. (2021) determined how effective bio-catharanthine is as a polyploidy agent against the phenotype of Rutaceae fruits crop cultivated in South Sulawesi. Alauddin Makassar used a randomized complete block design with two conditions (bio-catharanthine concentration levels C1, C2, and C3=0.05, 0.075, and 0.1 cents, respectively, and time exposure T1 for 3 hours, T2 for 6 hours, and two immersion sessions) (T1 for 3 h & T2 for 6 h). The results indicate that bio-catharanthine at doses of 0.05 and 0.075 for 6 hours elicited a stronger response. Talebi *et al*. (2017) used various colchicine levels (0, 500, 1250, and 1750 M), trifluralin (0, 10, 50, and 100 M), and oryzalin (0, 10, 50, and 100 M) to create an autotetraploid population in *Agastache foeniculum* L. During the first part of the experiment, seedlings from the apical meristematic area were treated with colchicine at the time of leaf emergence. In the second and third stages, colchicine was administered to seeds and seedlings for 6, 12, and 24 h. After six weak, the survival rate was observed. Flow cytometric examination of stomatal characteristics was used to demonstrate polyploidy induction. Several colchicine levels had a substantial impact on the survival rate. As the quantity of colchicine was raised, the survival rate decreased drastically ($p \leq 0.05$).

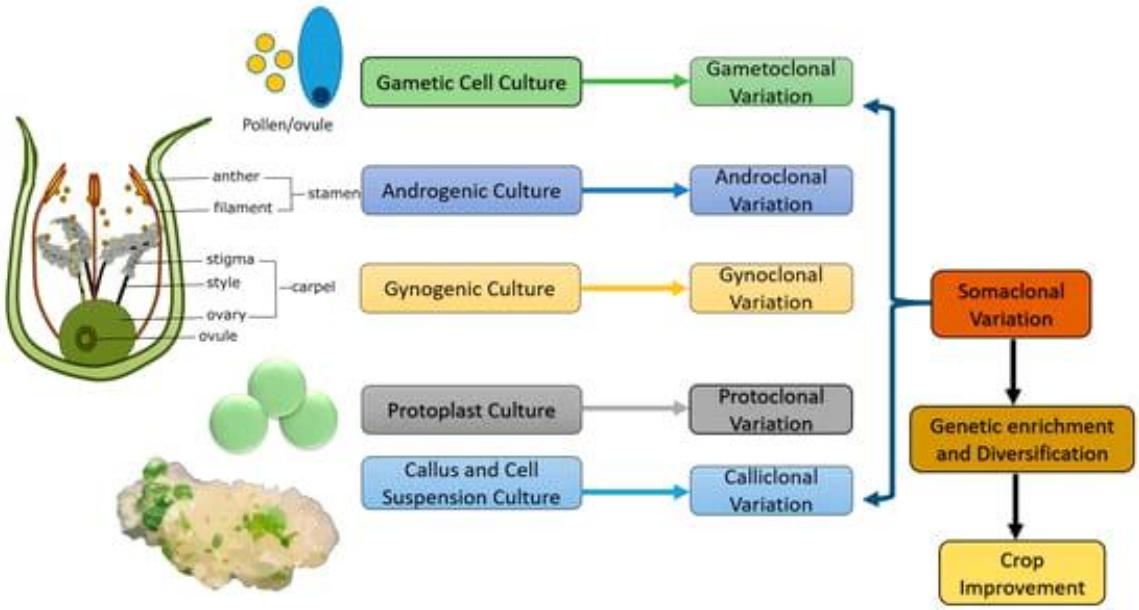

Figure 5: Different types of somaclonal variations arise depending on the tissue from which the variation originates. These variations include gametoclonal variation, which occurs among plants regenerated from gametic cultures, androclonal variation observed in plants regenerated from anther or pollen cultures, gynoclonal variation arising from ovule or ovary culture, protoclonal variation observed in plants regenerated from protoplast cultures, and callioclonal variation seen in plants regenerated from callus cultures (Wijerathna-Yapa *et al.*, 2022)

Although seeds treated with 100 M oryzalin for 24 hours produced the largest proportion of tetraploid plants, 17,500 M colchicine and 50 M trifluralin caused the highest rate of tetraploid proliferation in seedlings and apical meristems, respectively. Stomatal size and density, morphological traits, and physio-biochemical properties in polyploid plants ($p \leq 0.05$). Tavan *et al*., (2015) studied an *In vitro* approach for inducing polyploidy in *T. persicus* plants. Polyploidization was achieved *In vitro* by dipping shoot tips in colchicine (0, 0.05, 0.1, 0.3, and 0.5 percent) for 12 to 48 hours. The ploidy levels of regenerates in metaphase were measured using flow cytometry or chromosome counting. The mother diploid had a chromosomal number of 2C DNA = 1.20 g, whereas this induced auto tetraploid had a chromosomal number of 2C DNA = 2.39 g. (CV 4 percent). Tetraploid was found in 7.80 and 1.04 percent of 960 shoot tip sections treated with colchicine, respectively. For developing polyploidy, 0.3 percent colchicine for 12 and 24 hours produced the best results. Polyploidization's effect on growth, anatomical characteristics, and PTs production was also investigated. Tetraploids had considerably lower plant height, shorter roots, darker foliage, longer or wider leaves, and reduced stomatal density. Furthermore, triterpenoid production was shown to be more favorable in tetraploid and mixoploid plants. These findings imply that tetraploids might be used in future breeding efforts to develop a wide range of triterpenoids with improved medicinal properties. Blasco *et al*., (2015) developed tetraploid in loquat by employing the antimitotic drug colchicine according to market demand. Colchicine was administered to the stalk apex of *In vitro* produced plants, complete plants, and ungerminated seeds. There were no permanent polyploidy treatments identified on the shoot apex or the submerged entire plant. Colchicine is being utilized to suppress the growth of ungerminated seeds. In the 0.5 percent (w/v) configuration, two triploids and one tetraploid survived after 24 hours. The presence of triploids among treated seeds showed that these plants were initially accessible in halves and halves alone. Bagheri *et al*., (2015) investigated the efficiency of colchicine in producing polyploidization in (*Cannabis sativa L.*), as well as the effect of polyploidy on several primary and secondary metabolites. Shoots were dipped in various concentrations of colchicine (0, 0.1, and 0.2 percent w/v) for 24 or 48 hours. The 24-hour treatment in 0.20 and 0.10 percent w/v obtained the largest number of near tetraploids (43.333 percent) and mixoploids (13.333 percent). Colchicine at 0.2 percent concentration for 48 hours was more deadly than colchicine at 0.1 percent concentration for 24 hours. According to the biochemical findings, mixoploid plants had significantly higher levels of soluble sugars, reducing sugars, total protein, and total flavonoids than tetraploid and diploid plants. Tetraploid plants showed higher total protein, total flavonoid, and carbohydrate levels than control plants. The results show that polyploidy induction can only raise tetrahydrocannabinol levels in mixoploids, whereas tetraploids had lower concentrations than diploids. Elyazid *et al*., (2014) intended to conduct a study on citrus *Reticulata blanco* utilizing *In vitro* colchicine to explore polyploid induction. Seeds were dipped in a variety of colchicine concentrations (0.01, 0.05, 0.1, and 0.2 percent for time exposures of 12, 24, and 48 hours) before being grown *In vitro* on WM enriched medium at half strength. The rate of seed survival decreased with increasing colchicine concentration and treatment period, with the lowest proportion at 0.2 percent after 48 hours. The greatest DNA level was measured at 0.2 percent for one day. Colchicine treatments decreased the number of stomata per unit area; stomata length and width were also measured. According to the findings, 0.1 percent colchicine treatment for 48 hours exhibited the highest tetraploid induction effectiveness percentage. Atichart *et al*., (2013) tested *Dendrobium chrysostom* L. for colchicine concentration and duration to produce polyploidy and plant regeneration. Diploid *D. Chrysostom* was treated for 1, 2, 3, 4, and 5 days with colchicine dosages of 0.01, 0.02, 0.03, 0.04, and 0.05 percent. According to flow cytometry, the most effective therapy was 0.04 percent colchicine for 1 day, which resulted in approximately 84 percent surviving PLBs and 47 percent tetraploid orchids. The highest number of shoots (2.36 per explant and 2.44 per explant, respectively) were generated from 1 mg NAA and 0.5 mg BA treated Proton bodies with 0.01 percent and 0.02 percent colchicine levels. The culture media supplemented with 0.5 mg of BA and 1 mg of BA generated the most shoots (3.40 per explant and 4.35 per explant, respectively) when treated with 0.03 percent and 0.04 percent colchicine levels. Wu *et al*., (2012) designed in-depth research on the effects of colchicine-induced chromosomal doubling on fruit size, morphology, and crop loading in diploid Actinidia chinensis kiwi-fruit. The flow cytometric approach was utilized to assess the plant's leaf surface, and genetic analyses were performed on flower buds and root tips. For the third year and three years in a row, the weight, size, and crop load of fruits were calculated in since planting in the field. They discovered a significant increase in fruit size in induced autotetraploid of several *A. chinensis.* Nucleotide duplication in auto tetraploid A. Chinensis has been proven to significantly increase fruit quality. Rodrigues *et al*., (2011) investigated the effect of colchicine concentration and exposure time on diploid banana plant chromosomal doubling. Colchicine was administered to banana stem tips from 1304-04 (Malsaccensis x Madang) and 8694-15 (Calcutta x Galeo) x SH32-63 genotypes. Colchicine was utilized at doses of 1.25, 2.5, and 5.0 mM, including the control therapy. The results revealed that a 5.0 mM concentration for 48 hours generated 50% tetraploids.

Utilizing Gamma Radiation for Crop Improvement

Gamma radiation has emerged as a powerful tool in agricultural research, offering unique opportunities for crop improvement. Studies on citrus fruits, guava, and papaya have highlighted its potential to

induce beneficial mutations, enhance pest resistance, and extend shelf life. By irradiating seeds, shoot tips, or bud wood, researchers have successfully generated genetic variability, leading to the development of new cultivars with desirable traits. Furthermore, gamma radiation coupled with other treatments, such as waxing or modified atmosphere packaging, has been shown to preserve fruit quality and reduce postharvest losses. These findings underscore the significance of gamma radiation as a sustainable approach to address challenges in modern agriculture and ensure food security. Khan *et al*., (2019) investigated the effects of waxing and gamma radiation on insect reduction and physicochemical properties of Kinnow Citrus *Reticulata blanco*. Kinnow, waxed and unwaxed, were irradiated and then stored for one month at (15°C), RH60 5%. The researchers discovered no significant changes in the biochemical and organoleptic properties of the Kinnow fruit after exposing it to 0.5kGy of gamma radiation. The percentage of weight loss increased with storage duration for both waxed and unwaxed fruits. The least quantity of vitamin C loss was seen with increased storage period and radiation. TAA and TSS rose throughout a 30-day storage period in both non-irradiated and irradiated trials. The sensory quality of radiation apples remained unchanged. Overall, the data demonstrated that 0.5kGy radiation employed as a phytosanitary therapy is reliable. Fruit waxing, followed by radiation, is effective in keeping Kinnow's sensory quality for at least 30 days. Nam *et al*. (2019) explored how changes in microbiological and physicochemical parameters were measured after mandarins were treated with doses of 0.4 and 1 k Gy gamma radiation and kept at 4 °C for 15 days. Microbial growth was seen in non-irradiated fruits during storage, but irradiated fruits demonstrated a dosage proportion rate for more than 15 days. Radiation did not affect its moisture or total phenolics. However, after 5 days of storage, all organic acids, hesperidin, radical activity, reducing & sugars percent were significantly reduced in all samples. Even though radiation at 0.4 and 1.00 kGy did not prevent changes in stored mandarins, radiation at 0.4 kGy had minimal effect on the key important characteristics or physical appearance of mandarins while also allowing microbial eradication. Rime *et al*., (2019) used mango cultivars to create low vigorous or dwarf cultivars for 'HDP' to increase production. Mutants of the mango hybrid cv. Arka Puneet was created in an attempt to promote variability by employing different dosages of (EMS). The study's goal was to investigate the probable mutant germplasm of mango cv. (Arka Puneet) utilizing physiological, morphological, and biochemical characteristics. High mutagen dosages lowered biochemical features like total chlorophyll concentration while increasing enzyme activities like peroxidase and hormones like ABA. Morphological changes in dwarf mutant populations included lower plant height, thicker stems, and shorter internodal length. There was a decrease in stomatal number with a rise in total phenolics in the leaf in 0.8 percent EMS mutants, where the greatest dwarf plants were detected. Gamma radiation was utilized by Rattanpal *et al*., (2019) to promote variability in Kinnow. 400 buds were budded on the rough lemon rootstock after being irradiated at 30 Gy. The investigation revealed that 188 MV1 plants were grown in the field, and observations were made in the experimental setting. The number of big seeds per fruit ranged from 0.4 to 30.8 in the group addressed from irradiated buds.6.4 percent of all branches had seed numbers less than 10.3, 39.4 percent had bold seed numbers ranging from 10.33 to 20.33 per fruit, and 54.70 percent had seed numbers more than 20.3 per fruit. The mean fruit weight in the treated plants ranged from 94.3 to 253.2 g, with 46.8 percent yielding little fruits, 44.3 percent bearing medium fruits, and 8.9 percent bearing greater fruits. Among the 188 M1 plants, 11 Kinnow mutant plants with an average seed count of fewer than 8 were found, displaying distinctive needed traits and being extensively assessed in comparison to the parent variety Kinnow. Devi *et al*. (2018) used-acceptable levels of electron beams of 0.25, 0.5, 0.75, and 1.0 kGy to increase the nutritional content of citrus jambhiri fruits. Moisture, ash, crude fat, crude fiber, and energy levels were increased at low gamma-ray doses (0.25-0.5 kGy) and decreased at higher doses (0.75-1.0 kGy). However, when gamma-ray doses grew, so did available carbohydrates and energy. The ascorbate content of fruits rose at 0.25 kGy but decreased at higher doses. Higher doses of 0.75-1.0 kGy resulted in a greater reduction in anti-nutrient content, such as lipid peroxidation and tannin concentration. Sukhjinder *et al*., (2018) designed a study to produce variations in guava cv. Shweta by irradiating bud sticks with varying doses of gamma rays. Guava buds were irradiated with gamma rays at various dosages, namely 10, 20, 30, 40, 50, 60, and 70 Gy, before patching budding mutant buds on L-49 rootstock. After 40 days of budding, the control had the most sprouting and the 10 Gy treatments had the least. The same sprouting pattern was seen after 50, 60, 70, 80, and 90 days after budding. After 90 days of budding, the control had the most sprouting, followed by the 10 Gy treatments. However, even after 90 days, no sprouting occurred in buds treated at 40, 50, 60, and 70 Gy. Wani *et al*., (2018) in an experiment employed a combination of gamma light with modified air or MAP bundling to extend the period of usage and capacity nature of tasty cherries. Sweet cherries were harvested, modified environment packed, and then exposed to varying amounts of gamma radiation, including 0.3, 0.6, 0.9, 1.2, and 1.5 kGy. The gamma-irradiated cherries and the control cherries were maintained at 25 degrees Celsius and cooled at 3 degrees Celsius. According to the findings, a 1.2 kGy dosage of gamma radiation mixed with MAP extended the shelf life of cherries stored at both ambient and refrigerated temperatures. Furthermore, when cherries were stored in the refrigerator, the combination of MAP and gamma radiation decreased degradation. The dose of 1.2 kGy was discovered to be the most efficient in increasing the shelf life of fruits. Saini and Gill *et al*., (2016) designed

a study to assess the use of gamma rays in terms of vegetative features and LD50 dosage in rough lemon. Seed germination was reduced when gamma radiation dosages were increased. As the dose of gamma radiation rose, seedling height and leaf size dropped, however branching on apices, the total number of branches per seedling, number of variegated per albino seedling, and the number of leaves increased. Seeds treated with an 8 kr gamma radiation exhibit the finest changes in seedling height, number of leaves, leaf color, size, internodal length, and proportion of apical branching after two months of planting. Islam *et al*., (2015) determined the optimal response of gamma-ray dosage on biochemical and morphological characteristics of grape seedlings. A pot experiment was conducted with different levels of gamma radiation (5, 10, 15, and control) administered at different vegetative bud stages, and four different radiation doses (5, 10, and 15 Gy, and control) were utilized. At a radiation dosage of 5 Gy, the maximum growth characteristics and chlorophyll-a and b concentrations were shown. Mahadevamma *et al*., (2012) intended to conduct a study on the effects of gamma radiation on papaya seedlings. Variability was induced in two papaya cultivars, Coorg Honey Dew and Sunrise Solo, using gamma rays. After being treated with 100 ppm gibberellic acid, the seedlings were subjected to gamma radiation at dose rates of 10, 20, 30, 40, and 50 KR (GA). In both papaya cultivars, gamma rays had a substantial effect on germination and survival percentage, number of leaves, seedling height, total chlorophyll content, stomatal number, and length and width. Zamir *et al*., (2009) used gamma radiation to generate mutations in guava. Irradiated seeds, shoot tips, and bud wood. Shoot tips were subjected to gamma rays ranging from 15 to 90 Gy before being cultured in a glutamine and BAP-fortified medium. The LD50 for a 45 Gy irradiated shoot tip was observed. When the dosage exceeded 75 Gy, all explants were considered fatal. Although seed was treated with gamma rays of 50-300 Gy and bud wood with a dosage of 20-100 Gy. The maximal dosages were found to be fatal, with an LD50 of 190 Gy for seeds. Lower dosages resulted in the most germination. Bud wood was discovered to be more resistant to gamma radiation than seeds. Doses of 150 to 200 Gy were found to be the most effective, resulting in a significant increase in fruit size, quantity, and weight. Zeng *et al*., (2006) conducted research to double the chromosome number of kumquat (Fortunellacrassifolia) and frost embryogenic callus through colchicine. Colchicine treatment reduced the viability of protoplast, postponed the division of protoplast and repressed callus formation that is the indication of poison in cells. Cell lines originated from protoplast of Meiwa treated with 0.01% and 0.1% colchicine for 8, 16 and 24 hours each concentration showed a different response on culturing on embryo induction medium. Flow cytometric analysis revealed that tetraploids were found in cell lines and embryoid from all treatments, and the highest frequency was 19.23%. Zhang *et al*., (2007) carried out a study in which triploid seedless citrus were produced from tetraploid plants. An efficient protocol was used for the development of autotetraploid sweet oranges. Cell division activity was observed by flow cytometry analysis to determine the rate of cell division and the callus was treated with 1000mg/L colchicine. The No. of cells in callus were observed to be increased from 11.0% to 44.4% and to 59.0% for liquid and solid media respectively. About 20 tetraploid plantlets were recovered from 47 plantlets via embryogenesis. Autotetraploid could be used for the commercial generation of seedless citrus.

Usman *et al*., (2012) conducted research to explore various cultivars of grapefruit for the induction of polyploidy by colchicine using embryo culture on MS medium. Embryo germination was highly dependent on colchicine. The highest no. of embryos per seed was observed in shamber and frost fresh 2.89 and 2.27 respectively, compared to other cultivars. Colchicine halted the shoot growth while no. of leaves increased in foster and red max cane foster compared to control. Leaf lamina was also observed higher in treated ones in comparison with control. Number of stomata becomes lesser while length and width increased in treated leaves in shamber. Elyazid and Ali (2014) conducted an experiment to check the effect of different levels of colchicine in mandarins. Seeds were soaked in different concentrations of colchicine (0.01%, 0.05%, 0.1% and 0.2%) for different time spans (12, 24 and 48 hr), then seeds were cultured *in vitro* on MS media. The poor seed germination was observed on the higher level of colchicine that is 0.2% for 2 days. The number of stomata per cell decreased while the DNA recorded in high amount at 0.2%. But 0.1% colchicine for 48 hours showed best tetraploid induction. This research work was done to consecration of polyploidy in citrus via colchicine. Seeds were treated with different levels of colchicine for different varieties (0. 0.2%, 0.6%, 1.2%) citrumelo, citrange and sour orange respectively. These polyploids were detected by using flow cytometer, the results showed that higher concentration of colchicine lowers the durability. Guerra *et al*., (2014) an experiment was conducted to investigate the huge morphological differences among tetraploids and diploids. The purpose of this study was to compare physical characters and growth rate of tetraploids and diploids of acid lime and grapefruit during a period of 12 months. Color, tallness, petiole size, leaf length and central leaflet breadth of diploids were studied after every 45 days. Mark able differences were observed in these parameters among diploids and tetraploids. Petiole length was observed to be more in diploid while leaf length and central leaf width were more in tetraploids. Furthermore, polyploids have thicker leavers with darker color.it was resulted that diploids have more growth rates than tetraploids.

CONCLUSION

The review paper comprehensively explores the botanical characteristics, medicinal properties, nutritional composition, cultivation practices, and

challenges faced by Phalsa growers, highlighting its immense potential for crop improvement through mutagenesis and polyploidization techniques. Phalsa, with its significant medicinal benefits and nutritional value, faces challenges such as limited germplasm diversity, poor post-harvest management, and susceptibility to abiotic stresses. Mutagenesis techniques offer a promising avenue for creating genetic diversity and improving traits such as stress tolerance and disease resistance in Phalsa. Various studies have demonstrated the effectiveness of induced mutagenesis in enhancing fruit quality, shelf life, and disease resistance. Polyploidization techniques, including in vitro methods, have also shown potential for rapid crop improvement by generating larger fruit sizes, increased production, and disease resistance. Tissue culture techniques, particularly micropropagation, have emerged as vital tools for disease-free mass plant production and the conservation of genetic resources in Phalsa. However, culture contamination remains a significant obstacle in achieving optimal shoot initiation and propagation efficiency. Moreover, radiation-based approaches, such as electron beams and gamma radiation, have been explored to enhance fruit quality, extend shelf life, and induce beneficial mutations in Phalsa and other fruit crops. These techniques offer promising solutions to address post-harvest losses and ensure food security. In conclusion, leveraging mutagenesis, polyploidization, tissue culture, and radiation-based techniques can significantly contribute to overcoming existing challenges in Phalsa cultivation and unlock its full agricultural potential, ultimately benefiting food security and public health.

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