Environment and Ecology 40 (4B) : 2412—2422, October—December 2022
ISSN 0970-0420

Mutation and Polyploidy Breeding in Vegetable Crops : A Review

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Received 12 July 2022, Accepted 13 September 2022, Published on 10 November 2022

ABSTRACT

Physical and chemical mutations can cause such genetic changes naturally at low rates or spontaneously. Polyploidy has played a significant role in higher plant evolution and diversification. Chromosome doubling chemicals including colchicine, trifluralin, and oryzalin have been used to promote artificial polyploidization. Ploidy manipulation can cause morphological and physiological changes in plants by changing nuclear DNA content, gene expression, and developmental processes. Polyploid plants have more biomass, higher yield, better biotic and abiotic stress tolerance, and higher primary and secondary metabolites. Plant's ploidy can be determined in two ways : Directly and indirectly.

Keywords Polyploidization, Gene expression, Molecular mutation, Mutation, Polyploidy.

INTRODUCTION

Polyploidy is defined as the existence of more than two complete sets of chromosomes in an organism. It is responsible for expanding genetic variety and developing larger, more vigorous, and disease-resistant species (Dhar *et al.* 2017). Polyploidy is a major driving force in both wild and farmed plant evolution. Polyploid organisms often have more vigour than their diploid counterparts and in some situations, surpass them in multiple ways. Many plant breeders have sought to exploit polyploids' superiority during the last century, inducing polyploidy and/or using natural polyploids in a variety of methods to produce more enhanced plant cultivars (Sattler *et al.* 2016). Not only are polyploids widely spread in floral plants (Cui *et al.* 2016, Zhang *et al.* 2019) but are also prevalent in lower plants, including gymnosperms (Li *et al*. 2015, Zhang *et al.* 2019). A mutation is an abrupt genetic change that does not result from genetic segregation in the DNA of a living cell (Pathirana 2011). The primary cause of all genetic diversity in both plants and other animals is mutation, a natural process that generates new variants (alleles) of genes (Oladosu *et al.* 2016, Beyaz and Yildiz 2017).

Mutation breeding in vegetable crops

Mutation breeding, recombination breeding, and transgenic breeding are the three types of vegetative breeding. The creation of new mutant alleles is the most fundamental and distinctive feature of mutation breeding. Mutation breeding is the process of using induced mutations to improve crops. Induced mutagenesis was frequently used in the United States, Europe, Japan, and China during the 1950s. The investigation of differences in sensitivity of different genotypes and plant tissues, which is commonly quantified using the fatal dose, is one of the most important procedures (LD). By affecting existing loci as well as alleles at previously identified loci, mutations can cause both qualitative and quantitative change in a relatively short amount of time, in addition to changing linkage groups. Induced mutagenesis has been utilized to obtain direct mutants or to employ these

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Table 1. The total number of officially released mutant types on each continent. Source : FAO/IEAE Mutant variety database 2022.

Continents	Number of mutant varieties
Africa	82
Asia	2087
Australia and Pacific	9
Europe	960
North America	211
Latin America	53
Total	3402

Table 2. Official release of mutant varieties across the various countries in the world. Source : FAO/IEAE Mutant variety database 2022.

Continents	Number of mutant varieties
China	817
Japan	500
India	345
Russia	216
Netherlands	176

mutants in hybridization to overcome yield plateaus and produce desirable horticultural features (Gupta 2019). Currently, the development and formal distribution of 3402 mutant varieties around the continent and release of mutant varieties across the top countries in the world (Tables 1-2).

History of vegetable mutation

Wilhelm Roentgen's discovery of X-rays in 1895 led to Muller's (1927) use of X-rays to induce mutations in *Drosophila melanogaster* and Stadler's use of X-rays to induce mutations in barley (1928). For genome discovery, this technique became the most essential tool for finding genes on chromosomes, analyzing gene structure, gene expression and regulation. *Nicotiana tabacum* was the first plant to produce the "chlorine type," the first marketable mutant cultivar. Ionizing radiations such as X-rays and gamma rays are generally used due to their ease of use, great penetration, repeatability, high mutation frequency, and low settling problem. Phosphate groups, as well as purine and pyrimidine bases, are alkylated by all chemical mutagens when they react with DNA. In any mutagenesis program, the amount of mutagen used is a critical factor to consider. The lethal dose-50 (LD_{50}) gives an indication of the mutagens' optimal dose. The ideal dosage produces the greatest number of mutations while posing the fewest risks. High mutagen doses kill a large number of plants, while low levels create a limited mutation spectrum and frequency. The mutagenic dosage is mostly determined by the concentration, treatment period, and treatment temperature. The carcinogenic effect is modulated by pre-soaking, pH of the solution, metal ions, carrier agent, post-washing, post-drying and seed storage. Physical and chemical mutations result in three outcomes : Physical damage, gene mutation, and chromosomal abnormality. Although gene and chromosomal alterations can be passed down over generations, physiological effects are usually limited to the M_1 generation. Ionizing radiation was used to induce mutagenesis artificially in the early twentieth century (Gupta 2019).

Tilling : A best screening tool for mutant plant population.

The power of tiling was first demonstrated in model systems such as *Arabidopsis* and *Drosophila*, where it was shown that single mutations in specific genes could be identified. Subsequent tillage has been successfully applied in many plant systems, including barley, wheat, corn, rice, oats, peas and soybeans. In recent years, the availability of genomic sequences from many plant species and the development of a wide range of molecular-genetic technologies have enhanced our ability to detect or engineer such variation at specific genetic loci (reverse genetics), have expanded our capacity for both. Examining gene function and genetic engineering. Gupta (2019) found a new reverse genetic strategy that combines the high density of point mutations provided by conventional chemical mutagenesis with rapid mutational screening to search for induced lesions. Minoia *et al.* (2010) developed a new mutant genetic resource for improving tomato crop by tilling technique. The general applicability of tillage makes it suitable for genetic modification of vegetable crops. After mutagenic treatment with ethyl methane sulfonate (EMS), the resulting M_1 plants are self-fertilized to obtain M_2 individuals that are used to prepare DNA samples for mutagenic screening.

Table 3. Mutant varieties of vegetables crops released for cultivation in India. Source : Chakraborty and Paul 2013.

Crop	Number of varieties	Specific crop and number of varieties
Vegetables	24	Tomato (4), bitter gourd (1), brinjal (1), green pepper (1), okra (2), ridge gourd (1), snake gourd (1), cluster bean (1), Cowpea (10), pea (1), frenchbean (1)

Effect of physical mutagens in vegetable crops

Ane (2014) also studied the effect of gamma radiation on yield due to yield in two varieties of peas (*Pisum sativum* L.). Dose-dependent reduction in yield is observed in most of the two cultivars, but the genotype of var. *arvense* is known to be more sensitive to gamma radiation doses than var. *hortense*. Table 3 provides detailed information on the number of mutant varieties released for cultivation in India and the overall number of variations released for the vegetable crop. Table 4 provides the properties of physical mutagens.

Effect of chemical mutagens in vegetable crops

Elangovan and Pavadai (2015) carried out an experiment to ascertain the impact of various concentrations of diethyl sulfate (DES) and ethyl methane sulphonate (EMS) (DES). The EMS and DES treatments had the highest mean values for all parameters, 0.5% and 0.4%, respectively, compared to the other therapies. The 0.4% DES treatment saw the highest weight of 100 seeds recorded.

Combined effect of both physical and chemical mutagens in vegetable crops

Spontaneous mutation in crop plants occurs naturally during adaptation and developmental processes at a very low rate i.e. 10-5-10-8. This frequency is sufficient to cause variation in the genetic composition of the crop to improve desirable traits (Zhong-hua *et al.* 2014).

Mutations may occur spontaneously or may be induced artificially. Artificial mutagenesis can be induced by physical mutagens such as X-rays, Y-rays and neutrons and chemical mutagens such as ethyl methanesulfonate (EMS) (Pathirana 2011). Physical mutagenesis are used more often than chemical mutagens and in physical mutagens, γ-rays are more commonly used than X-rays (Beyaz and Yildiz 2017). Mutation screening is the selection of individuals from a large mutational population that meet specific selection criteria, and mutation confirmation is being re-evaluated (Oladosu *et al.* 2016). Table 5 shows number of mutant varieties developed by different mutagen treatments. Table 6 shows number of mutant varieties released in vegetables legumes crops.

Economic impact of a new mutant variety

The economic value of a new variety can be arrived at by several parameters. These include : The area planted for the variety and the percentage of the area under crop in the field; increased yield, increased quality, less use of insecticides and fungicides, water savings (short growth period and drought tolerance). Increase in land use through early maturity to facilitate crop rotation, Enhanced/rapid cropping system with altered maturity or response to photoperiod, Improved processing quality and value of products (eg., oils, starches, malts, beer and whiskey), quality preference by the consumer (new flower and leaf color in jewellery, skin and flesh color in root and tuber crops and fruit crops, aroma and sticky nature in rice, and kernel color in wheat). Increase nutritive value, high lysine and vitamin, increase oil-shelf life, reduce toxins; increase the yield of essential oils; new speciality and designer crops; ease of harvest, threshing; increase in export earnings; Reduction in imports (Zakir 2018, Chakraborty and Paul 2013).

Polyploidy breeding in vegetable

The “Gigass” effect, also known as gigantism, is brought on by polyploidy and results in larger plant organs and the “Gigass” effect. Triploids have three sets of chromosomes, are sterile and hence yield fruit without seeds. This watermelon trait of being seedless has been extensively used. In addition to being seedless, triploids have a larger yield than diploids. Greater vigour and improved performance are displayed by polyploid organisms compared to their

Table 4. Different types of mutagens and their characteristics. Source: Raina *et al.* 2018.

Mutagens	Characteristics
X-rays	Electromagnetic radiation; ionizing, penetrate tissues from a few millimeters to many centimeters
Gamma rays	Electromagnetic radiation, ionizing, very penetrating into tissues; sources are Co^{60} and Ce^{137}
Neutrons	Uncharged particles; penetrate tissues to many centimeters; source is U^{235}
Beta particles	Negatively charged electrons; ionize; shallowly penetrating; sources P^{32} and C^{14}
Alpha particles	Helium nucleus capable of heavy ionization; very shallowly penetrating
Proton	Positively charged particles; penetrate tissues up to several centimeters

diploid counterparts. Particularly in green vegetables and asexually propagated tuber crops, polyploidy has a strong future (Budhani *et al.* 2018). Bharathi *et al.* (2014) tetraploid *Momordica subangulata* subsp. Renigera (2n = 56) with *Momordica dioica* (2n = 4x = 56) produced tetraploidy in an effort to create a new synthetic species of *Momordica*. This hybrid creates adventitious root tubers via which it survives, reproduces as its female parent, and passes on to its progeny its morphological traits. The hybrid is a fantastic option for a new vegetable crop because it is naturally fertile and has better agronomic traits than both parents. *Momordica saboica* Bharati is the name of the new species. Liu *et al.* (2015) research on the characteristics of tetraploid pumpkins revealed that these plants had female flower nodes that were located lower on the plant. Tetraploid fruit weighed 2.9 kg, compared to 2.2 kg for diploid fruit. Comparatively to diploid fruit, tetraploid fruit had roughly 30 seeds per fruit (122 seeds). Tetraploids had a 50% increase in flavonoid content but a 90% increase in photosynthetic rate. It has been shown that polyploidy, though not always, leads to an increase in gene activity and enzyme diversity, photosynthetic capacity, yield, and biomass, as well as in the size of the tuber, rhizome, root, fruit, and flower as well as the length and thickness of the leaves as well as the intensity of their colors. In medicinal plants, nutritional deficits, illnesses, pests, drought, and cold stress can boost secondary metabolite production in addition to main metabolism, dismantle self-incompatible systems, promote fertilisation, and result in dwarfism (He *et al.* 2016).

Traditionally, polyploidy has been divided into two main categories : (i) Allopolyploidy, which occurs more frequently in nature as a result of the hybridization of two or more different species and the subsequent chromosome duplication in the offspring; and (ii) Autopolyploidy, which is the homologous doubling of a species' genome (Hegarty *et al.* 2013).

Applications of polyploidy in plant reproduction

Polyploidy induction has become a reliable method for plant improvement. Many vegetatively propagated flowers and fruits as well as crop plants are polyploid (Dhooghe *et al.* 2011, Corneillie *et al.* 2019). The impact of polyploidy in plants frequently seems to be linked to observable phenotypic changes, such as greater vigour and the ability of freshly generated polyploids to adapt to new circumstances (Sattler *et al.* 2016). These interesting characteristics displayed by polyploid individuals have led to increased interest in developing artificial polyploids (Dhooghe *et al.* 2011, Sattler *et al.* 2016). Polyploid plants' genotypes can alter as a result of heterozygosity, gene silence, gene dosage effects, or interactions between the genetic and epigenetic systems (Dewitte *et al.* 2011). Genomic alterations include aneuploidy, DNA sequence modifications, deletion of duplicated genes, and gene conversion. They also include structural chromosomal rearrangements. DNA methylation, histone acetylation, chromatin remodelling and RNA interference are other epigenetic alterations that influence gene expression (Sattler *et al.* 2016, Ding and Chen 2018). Affected traits are diverse, including flowering time, biomass, leaf morphology, which are subject to selection and can lead to domestication of crop plants (Ding and Chen 2018). Tetraploid cells are roughly twice as large as their diploid counterparts, which is typical of polyploids, but this does not necessarily mean that the entire plant or its organs would become larger as fewer cell divisions occur in polyploids (He-

Table 5. Number of mutant varieties developed by different mutagen treatments. Source : FAO/IEAE Mutant variety database 2020.

Mutagen treatment	Number of mutant varieties
Chemical	384
Physical	2610
Combined	37
Somaclonal variation	3
Total	3034

garty *et al*. 2013, Sattler *et al.* 2016). The volume of the nucleus rises by up to 1.6 times with a doubled cell genome, which may upset the equilibrium between chromosomal and nuclear components. The changing ratio of nuclear/cytoplasmic volume is hypothesised to cause disruptions in metabolism and general development in polyploids. As a result, plants with dwarf and stunted growth can result from high amounts of polyploids, such as octoploids, because of somatic instability and excessive gene redundancy (Manzoor *et al*. 2019). Self-fertilization is frequently enabled by self-incompatibility mechanisms being disrupted in polyploids (Hegarty *et al*. 2013). Decreased seed sterility is another common consequence of autopolyploidy and may result from meiosis (Sattler *et al.* 2016). As a result, species grown for their vegetative organs and those with vegetative propagation, like Triploid watermelon, benefit from autopolyploidy induction in breeding efforts (Acquaah 2015, Sattler *et al.* 2016). As a result of the lack of chromosomal pairing during meiosis, meiotic hybrids are typically sterile. By preparing each chromosome, acquiring its perfect copy, and establishing chromosome homology, chromosome doubling offers a technique to eliminate inter-specific hybridization and large chromosomal variances in intra-specific hybrids, or to create viable offspring following interploidy crossings (Hegarty *et al*. 2013, Manzoor *et al*. 2019). Along with morphological changes, polyploid plants exhibit larger, lower-density stomata, increased vessel diameter, larger vacuoles, thicker leaves, denser pubescence, lower transpiration rates, higher rates of photosynthetic activity, and lower specific hydraulic conductivity, which may increase their resistance to drought stress (Manzoor *et al.* 2019). Autopolyploids may also be more compatible with environmental conditions such as nutrient deficiency, temperature, pest and pathogen stress (Miri 2020). Table 7 detail of aneuploids where 2n diploid number of chromosome

Table 6. Number of mutant varieties released in vegetables legumes crops. Source : FAO/IEAE Mutant variety database 2020.

Legumes vegetable	Number of mutant varieties
Dolichos lablab L. (Hyacinth bean)	1
Phaseolus vulgaris L. (Common bean)	59
Pisum sativum L. (Pea)	34
Vicia faba L. (Faba bean)	20
Vigna unguiculata Walp. (Cowpea)	15
Total	129

and the number indicate extra number or missing number of chromosome.

Methods of polyploidy induction

It was generally believed that polyploids in plants are induced through two mechanisms : Sexual (meiotic) polyploidization or doubling in the meristem tissue of somatic (mitotic) sporophytes (Miri 2020).

Sexually polymorphic

Meiosis, pre-meiotic genome doubling, and post-meiotic genome doubling are the three development-specific classes that make up the mechanisms of 2n gamete production. Plants rarely exhibit pre- or post-meiotic genome duplication, whereas meiosis is the primary process that produces undifferentiated gametes. At the conclusion of meiosis II, meiosis transforms into a non-reducible process like mitosis in this process, producing dyads (and triads) instead of the typical tetrad (de Storme and Geelen 2013). Polyspermy-derived triploids are longer and produce larger organs than monospermic plants, however, it is lethal in many eukaryotes and generally regarded as an unusual mechanism of polyploid formation (Nakel *et al.* 2017). The key benefit of sexual polyploids over somatic polyploids is that they increase the genetic diversity of the progeny, enabling the maintenance of a higher level of heterozygosity and, consequently, a potentially higher level of trait expression (Sattler *et al.* 2016). The majority of uncontrolled pollen production is found to be genetically determined, but numerous studies have shown that genes involved in the regulation of 2n pollen production are strongly influenced by environmental factors like temperature, light, herbivory, wound, water stress, and nutrient

Table 7. Classification of aneuploids where 2n diploid number of chromosome and the number indicate extra number or missing number of chromosome.

Term	Chromosome number
Monosomy	2n-1
Nullisomy	2n-2
Trisomy	2n+1+1
Tetrasomy	2n+2
Pentasomy	2n+3
Sources : Meru 2012	

stress, with light and temperature, particularly changes in temperature during gametogenesis, having a disproportionately large impact on meiotic abnormalities (Martin *et al*. 2019). Additionally, various attempts have been attempted to increase the generation of 2n gametes by using gene silencing techniques such RNA interference, virus-induced gene silencing, anti-tubulin compounds, nitrous oxide (N_2O) and ethyl methane sulfonate (EMS) (Dewitte *et al.* 2011).

Somatic polyploidy

Several crop species have undergone somatic polyploidization, which is the stimulation of chromosomal doubling in somatic tissues (Sattler *et al.* 2016). The term "antimitotic agents" refers to a broad class of both natural and artificial substances that have been shown to impede the cell cycle, mainly at the late metaphase stage (Salma *et al*. 2017). Nevertheless, progress in artificial polyploidy induction was accomplished only after the introduction of colchicine. Colchicine is a metaphase inhibitor and its mechanism of action includes binding to α- and β-tubulin dimers, disruption of microtubule assembly during the cell cycle, and inhibition of polar chromosome migration during anaphase, resulting in ploidy levels (Sattler *et al.* 2016). Due to its strong binding to an animal cell's microtubules, it is extremely poisonous to humans. However, it requires the usage of quite large concentrations and has a weak affinity for plant tubulin. Due to these shortcomings, a cell cycle inhibitor replacement with a better affinity for plant tubules is needed (Dhooghe *et al.* 2011). About 25% of all herbicides likely to affect mitosis of plants can be used as antimycotic agents at low concentrations. They belong to various chemical classes, including vinblastin, acenaphthene, dinitroanilines (trifluralin, oryzalin, benfluralin, ethalfluralin, pendimethalin, butralin, dinitramin), pyridines (dithiopyr, thiazopyr), benzamides (pronamide, propyzamide), phosphoroamidates (amiprophos-methyl, butamiphos), benzoic acid (chlorthaldimethyl), carbamates (chlorpropham, isopropyl N-(3-chlorophenyl) carbamate) and others (Roughani and Miri 2018b). These antimycotic medications, including colchicine, are metaphase inhibitors with the exception of carbamates. The microtubule organising core is disrupted and fragmented by carbamates, but the microtubule is not depolarized. Instead, they change how spindle microtubules are organized, causing many micronuclei to develop (Dooghe *et al*. 2011). Pliankong *et al*. (2017) demonstrated that colchicine was more successful than orzalin at inducing polyploidy in *Capsicum frutescens.* Roughani *et al.* (2017) colchicine, trifluralin, and oryzalin were applied to the seeds of *Spinacea oleracea*, and it was discovered that all three antimycotic agents may be effective in increasing polyploidy induction. However, oryzalin was discovered to have low toxicity, be inexpensive, and have the ability to increase ploidy levels at lower doses.

Ex vitro polyploidy induction

Antimicrobial agents are applied by two methods *Ex-vitro* and *in vitro* (Roughani and Miri 2018b). Although antimicrobial chemicals are typically applied by foliar spray or cotton plug method, pre-germinating seeds with developing roots is the most efficient way to induce tetraploidy (Manzoor *et al.* 2019). However, Noh *et al.* (2012) by treating the seed, shoot apex, and inverted hypocotyl with 0.1% and 0.2% colchicine, it was possible to get an effective approach for inducing tetraploids in *Citrullus lanatus*. It was then discovered that the hypocotyl component of the plant had the greatest rate of tetraploids (29.5%).

In vitro polyploidy induction

In vitro polyploidy induction is recommended because supplements enhance the uptake of plant growth regulators in media and also reduce time and space (Hegde *et al.* 2015). The efficiency of *in vitro* chromosome doubling is influenced by a number of variables, including the target species' ability to multiply, the type and concentration of antimitotic

Table 8. Polyploid vegetable crop variants and their distinguishing characteristics. Sources : Pal and Bal (2020).

Variety	Crop	Salient Features
Sree Harsha	Cassava	Plants are triploid and non-branching, yielding 35-40 t/ha and containing 39.05 percent starch
Pusa Jyoti	Palak	Tetraploid with large, thick, soft, succulent dark green leaves, fast rejuvenation, and yields of 50 tonnes^{-ha}
Arka Madhura	Watermelon	TSS 13-14 percent, extended shelf life and transit quality, appropri ate for year-round production under protected conditions, yields 50-60 t^{-ha}
Pusa Bedana	Watermelon	Seedless triploid hybrid having aborted embryos and false, rudi mentary, least perceptible seeds

agent used, the length of exposure, the technique used to apply the antimitotic solution, the type of explant used, and the regrowth medium. Therefore, it takes several attempts to create an *in vitro* polyploidy induction technique and find the best concentration and exposure period for each species of antimycotic medication (Sattler *et al.* 2016).

Genotype

Chromosome doubling is genotype-dependent and plants with lower ploidy levels have a higher propensity for polyploidy induction (Roughani and Miri, 2018b). Some research has indicated that oryzalin was less efficient for chromosome duplication in cassava (Mondin *et al.* 2018) and chili peppers (Pliankong *et al.* 2017). Mondin *et al.* (2018) Studying the *in vitro* polyploidy development of two different strains of cassava revealed that Colombia 22 and Vasorinha genotypes responded differently to colchicine.

Explant source

The crucial stage in inducing polyploidy is making a true choice of explant. Several explants, including seedlings, shoot tips, calluses, somatic embryos, seeds, single nodes, tuber segments, cotyledons, hypocotyls, and root tips, have been used as starting materials for effective polyploidization (Dhooghe *et al.* 2011). Given that endosperm is a triploid tissue, it makes sense to think that endosperm culture is a helpful procedure for creating naturally triploid plants from diploid ones ; nonetheless, this methodology produces triploid plants faster than traditional procedures. But regeneration from cultured endosperms is frequently technically difficult, and explant stage, medium composition, and additions, particularly plant growth hormones, are significant variables (Wang *et al.* 2016).

The most popular antimitotic drug used to make plants polyploid is colchicine. Even after being autoclaved, it keeps its multiplicity. Colchicine does, however, have certain negative effects on different plant species, such as poor viability, sterility, chromosomal abnormalities, and gene changes brought on by aberrant growth, irregularly shaped nuclei, and micronuclei. Trifluraline and oryzalin are frequently preferred over colchicine because they promote explant survival and can be employed at lower dosages (Dhooghe *et al*. 2011) In an experiment on Petroselinum crispum, the induction of polyploidy was higher in node explants than in seeds (Nasirvand *et al*. 2018).

Antimitotic agent

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Concentration and exposure time of the antimitotic agent

Explants can be treated with low doses of antimycotic

agents in liquid or solid culture medium throughout a subculture or for a brief period of time in liquid medium with high concentrations of antimycotic agents before being cultured on fresh medium to promote polyploidization (Dhooghe *et al.* 2011). There are reports that survival rates of explants decreased with increasing concentration of the antimycotic agent and the duration of its treatment, whereas very low concentrations were unsuccessful (Nasirvand *et al.* 2018). He *et al*. (2016) indicated that shorter duration of treatment at higher concentrations of colchicine and longer duration at lower concentrations may achieve similar desirable effects on ploidy induction. Plants of tetraploid *Petroselinum crispum* in 0.05% colchicine increased the treatment duration from 12.5 - 75% by increasing the treatment duration from 8-48 h, respectively (Nasirvand *et al.* 2018).

Colchicine dissolved in 3-4% DMSO increased the incidence of tetraploidy but decreased exploratory survival as compared to water or liquid media. Other solvents can be chosen as an alternative to prevent over-toxicity, such as oryzalin, which can be combined with liquid MS media (for *in vitro* polyploid induction) or ethanol (96%) or NaOH (1 M in acetone, trifluralene 100%), and colchicine in water (Dhooghe *et al.* 2011).

Regrowth medium

The crucial aspect in this strategy is growth recovery following adequate antimicrobial therapy. For the maintenance and growth of polyploidy, several methods were followed (Gantait *et al.* 2018). Most researchers have suggested modified MS media for shoot multiplication of polyploids because it contains all the essential nutrients for in vitro growth (Miri and Roughani, 2018b, Parsons *et al*. 2019, Podwyszynska and Pluta 2019).

Assessment system

Following the induction of chromosome doubling, it is important to confirm the polyploidy status in plants because their tissues may contain multiple polyploid chains or chimeras (Sattler *et al.* 2016, Manzoor *et* al. 2019). Methods for the detection of polyploidy are classified as direct and indirect. Indirect methods include anatomical, morphological and physiological symptoms that are quick and simple but are often inaccurate. In contrast, confirmation through direct methods is accurate and sometimes necessary, for example, chromosome counting and measurement of the nuclear genome by flow cytometry (Sattler *et al.* 2016).

Indirect assay

Morphological and physiological characteristics are examples of indirect methods for identifying polyploidy. Plant height, seedling number and length, root number and length, leaf size, and pollen grain diameter are all considered in morphological evaluation. Stoma cells are physiologically evaluated in terms of their frequency, size, and chloroplast density (Zahedi *et al.* 2014). Stoma cell frequency, size, and chloroplast density are assessed physiologically (Sattler *et al.* 2016). Pliankong *et al.* (2017) indicated a decrease in the density of stomata per unit leaf area and an increase in the size of guard cells' stomata. Similarly, Nasirvand *et al.* (2018) found that tetraploid *Petroselinum crispum* plants had larger stomata and leaf sizes than diploids, but reduced stomata density. Tetraploid-induced plants showed reductions in plant height, internode length and root length compared to normal diploids (Tavan *et al.* 2015), but a higher width/length ratio of leaf and stem diameters was observed (Zhang *et al.* 2016). Pollen diameters in polyploid plants were larger than those in diploid plants, according to research on the subject, however these plants had poor viability and *in vitro* and *in vivo* germination (Martin *et al*. 2019).

Direct assay

However, the time-consuming nature of cytogenetic methods and the need for highly customised protocols for every species make them difficult to use (Sattler *et al.* 2016). Additionally, it is challenging to count the chromosomes since they appear so tiny under an optical microscope, like little dots (Tavan *et al*. 2015). Additionally, temperature, pH, osmotic balance, and fixation period all affect how well a stain takes. A small number of cells could only be checked because insufficient staining could happen if any of the factors were off. For this reason, tiny chromosome counts is

frequently inaccurate (Miri 2020). As an alternative, FCM, which has been widely used since the 1980s, is a quick, precise, and easy method to estimate ploidy level and genome size. It can be employed in the early phases of plant growth and is a quick and effective way to determine the nuclear DNA content of huge populations (Hannweg *et al.* 2016). The FCM technique follows an extraction of the cell nuclei using a razor blade chopping or bead beating method and subsequently, DNA is stained by a DNA fluorochrome such as DAPI (4′,6-diamidino-2-phenylindole) or PI (propidium iodide) that binds to the DNA (Roughani and Miri 2018a). With the use of a flow cytometer, the stained nucleus emits a fluorescence that can be detected, and the strength of the fluorescence is directly related to the ploidy level (Salma *et al.* 2017). The correct nuclear DNA content and ploidy level are associated in the FCM study (Salma *et al.* 2017). Therefore, it is hypothesised that a rise in the number of chromosomes causes an increase in the DNA content (Sattler *et al*. 2016). An internal reference standard (a plant with known nuclear DNA content, processed along with the sample) is required for flow cytometric genome size determinations. Many studies use species with consistent genome sizes, such as *Allium cepa*, *Pisum sativum* and *Petroselinum crispum*, as internal standards (Sattler *et al.* 2016, Sliwinska, 2018). FCM can efficiently discriminate the polyploids from diploids and mixoploids (Salma *et al.* 2017).

Opportunities for determining polyploidization have been made possible by developments in molecular technologies. Guo *et al.* (2016) created an analytical toolset using molecular markers and FCM to identify polyploid Salix. With this analytical toolset, polyploids may be quickly screened from a large number of natural stands, and they discovered that the results from marker-aided selection were similar with those from FCM measurements.

4. Impacts of polyploidy induction on crop improvement

Vegetables

Kihara created the tetraploidization process for growing seedless watermelons in 1951. By interbreeding a tetraploid (female parent) and a diploid (male parent) inbred line, seedless varieties (2n = 3x = 33) are created. The triploid hybrid fruit is seedless since the triploid hybrid female is sterile. Since triploids lack viable pollen, at least 20% of the diploid cultivar must be grown in the production area in order to provide pollen that promotes fruit set (Miri 2020). Due to its special qualities, including its small size, vigorous growth, high fruit number per plant, high sugar content, flesh firmness, thin rind, and potential extended shelf life, triploid seedless watermelon is well-liked commercially and commands a premium price on the global market (Noh *et al.* 2012). Pliankong *et al.* (2017) produced polyploidy in *Capsicum frutescens* and discovered that polyploid fruit was larger and contained more capsaicin than diploid fruit. Vitamin C concentration has also been reported to be high in polyploids caused by colchicine (Miri 2020). Tetraploid *Petroselinum crispum* plants treated with colchicine had larger leaves and stems than diploid plants (Nasirvand *et al.* 2018). Raphanus sativus induced tetraploid plants have huge leaves and a taproot in addition to having high levels of soluble sugar and protein, vitamin C, and antioxidant enzyme activity (peroxidase). Additionally, qRT-PCR results revealed that FLC1.1, a gene known to suppress flowering, was highly expressed in tetraploid plants at the flowering stage, whereas VRN2 and AGL24 Levels, positively regulating genes for flowering and bolting, were expressed at levels that were lower in tetraploid than in diploid radish at those same stages (Pei *et al.* 2019). Table 8 indicate some polyploid vegetable crop variants and their distinguishing characteristics.

CONCLUSION

One of the most typical manifestations of diversity, adaptation, and evolution in flowering plants is polyploidy. Numerous vegetable crops that may serve as a source of food, animal feed and raw materials for industry may domesticate more quickly as a result of the induced mutation. The enzymatic activity of pathways that make secondary metabolites is significantly impacted by polyploidy, which has a knock-on effect on the patterns of secondary compound production in plants. One of the most effective ways to produce variety and genetic variation is through induced mutation.

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