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Review Article

Ploidy manipulation for enhancement in fruit quality

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Abstract

Fruits are storehouses of phytochemicals and other bioactive substances promoting good health. Numerous environmental and genotypic variables may influence the quality of fruits. Ploidy manipulation is one of several methods used to improve the quality of fruits, including breeding and other biotechnological methods. Colchicine and oryzalin, antimitotic agents that alter ploidy levels, are being utilized widely. Increasing up and down the number of chromosomes in a species within a polyploid sequence constitutes ploidy manipulation. There are several ways to manipulate ploidy, including endosperm culture, chromosomal duplication, interploid hybridization, sexual polyploidization, and the creation of haploids. Ploidy modification may be a practical approach to improve the fruit quality attributes with these cutting-edge methods.

Keywords: Chromosome Doubling, Colchicine, Fruit Quality, Haploids, Ploidy Manipulation, Polyploidy

Abbreviations

MT: Metric Tonnes; 2x: Diploid; 4x: Tetraploid

Introduction

Fruits are a crucial component of our daily diet because they are abundant sources of many nutrients that are good for our health, including vitamins, minerals, and various polyphenolic compounds. The synergy or interactions of bioactive chemicals and other nutrients are ascribed to the health benefits derived from fruits [31]. To avoid the onset of malnutrition and non-communicable illnesses, the WHO [81] has advised consuming 400 g of fruits and vegetables per day. The total fruit production in the world touched a milestone of 883 million tonnes in 2019 [13]. India, with 107.1 million MT of fruit production [35], is the second largest producer of fruits in the world.

Fruit quality is a dynamic synthesis of all its physicochemical characteristics that affect how consumers perceive it [28]. Numerous environmental factors, as well as genotypic traits, have an impact on fruit quality. Ploidy manipulation is one of several methods used to improve the quality of fruits, including breeding and other biotechnological tools. Polyploidy is one of the elements driving plant species evolution [62], which is defined as "the development of a greater chromosomal number by the inclusion of additional complete chromosome sets existing in one or more ancestral organisms" [16].

Polyploidy occurs when an organism has two or more chromosomal sets [68]. Ploidy manipulations, commonly referred to as analytical breeding, involve shifting the proportion of a species' chromosomes to a higher or lower level within a polyploid sequence [7]. This technique may be used to improve fruit quality.

Ploidy manipulations are primarily influenced by the type of fruit crop concerning its ploidy behaviour, type of propagation, and access to a wide range of genetic resources belonging to different ploidy levels [48].

Historical background

The first instance of creating an artificial polyploid in a lab was the 4x form of the Solanaceae family plant *Solanum nigrum* [65], which was created by the regeneration of the callus tissue [80]. Additionally, the use of colchicine for chromosomal doubling in several species was shown in the second quartile of the 20th century [4]. Soon after this original discovery, many experiments examining colchicine for ploidy modification were conducted. In 1941, the popular journal 'American Naturalist' supported a symposium on different aspects associated with agrarian crop plants due to the heightened interest generated by this new element of polyploid studies [70]. The first detailed dialogue on the benefits of artificial ploidy manipulation to improve ornamental crops was provided in the same issue of 'The American Naturalist' [11]. Due to these early experiments, polyploidy has become a crucial component of crop improvement for many commercial crops, including fruit crops.

The rapidly developing plant tissue culture industry boosted polyploidy investigations in the 1960s. The *in vitro* cultivation method was successfully used to produce 4x plants which provided the early indications of using tissue culture techniques for ploidy manipulations [40]. Colchicine was also used to develop polyploid sugarcane cell cultures [21]. *In vitro* polyploid induction has been more popular during the past two decades. This growth may be partly explained by the creation and spread of tissue culture methods for various species [73].

Types of polyploids

Autopolyploids and allopolyploids are the two primary categories of polyploids that are often taken into account. An allopolyploid develops when many sets of chromosomes with distinct structural sets are combined, unlike an autopolyploid, which multiplies a single set of chromosomes [25,65]. The polyploids are divided again into five conventional categories [16,65,68].

- Strict autopolyploidy (AAAA): These polyploids are formed within a species, either from genome doubling in a single individual or fusion of unreduced gametes from genetically similar individuals. E.g., aonla, bael, litchi, jackfruit, etc.
- Interracial autopolyploidy (AAAA): These polyploids evolved within a genetically distinct species but have structurally similar chromosomes.
- Segmental allopolyploidy ($A_sA_sA_tA_t$): These are the polyploids formed within a species but from the parental genomes that differ in many genes or chromosomal segments. These are unstable polyploids that evolve toward auto- or true alloploidy. E.g., Pinoche Creek larkspur (*Delphinium gypsophilum*)
- True (genomic) allopolyploidy (AABB): These polyploids are derived from the hybridization between distantly related species. E.g., mango, European plum, strawberry, etc.
- Autoallopolyploidy (AAAABB): These polyploids develop from genome doubling followed by an allopolyploid formation. E.g., Jerusalem artichoke (*Helianthus tuberosus*)

Here, subscripts denote unique genomes from the same species, whereas A and B represent the different parental genomes.

Agents for ploidy manipulation

To inhibit chromosome pole movement during anaphase, ploidy modification requires that the cell cycle be disturbed [73]. Chemicals that have been demonstrated to produce polyploidy include antimicrotubule herbicides, coffee [71], and nitrous oxide [69]. These substances are collectively known as "antimitotic agents." Colchicine, oryzalin, and other antimicrotubule substances have been primarily employed for effective *in vitro* polyploid induction. The brief categorization of antimitotic agents is shown in figure 1.

Methods of ploidy manipulation

It was previously believed that either sexual (meiotic) polyploidization or somatic (mitotic) doubling in sporophyte meristem tissue would cause polyploidy in plants [55]. In addition, numerous alternative techniques for manipulating ploidy have been reported from diverse investigations and are discussed below.

Sexual polyploidization

Unreduced gametes with a complete complement of chromosomes are created throughout the process of gametogenesis, and abnormalities in typical cell cycle activities such as spindle formation and cytokinesis may be responsible [56].

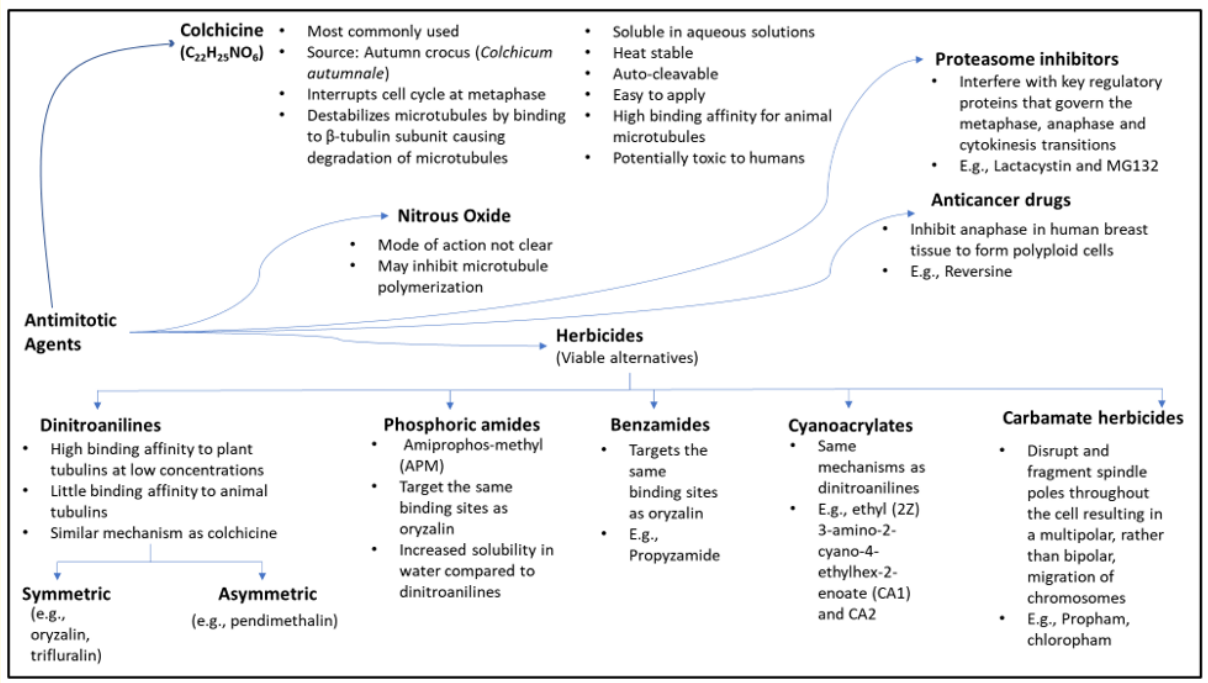

Figure 1: Brief classification of antimitotic agents [derived from 3,23,24,26,29,32,37,38,41,51,73,74,76]

Meiotic restitution occurs when meiotic cell division is changed into a mitosis-like non-reductional process and produces dyads (and triads) instead of the typical tetrads after meiosis II, it is the most common mechanism of unreduced gamete generation [9]. Triploid and tetraploid embryos can be created by unionizing reduced (n) and unreduced gametes or merging two unreduced gametes. It is believed that unreduced gametes (2n pollen or 2n eggs) are an effective mechanism of polyploid formation [56]. The sexual polyploids differ from somatic polyploids depending on the genetic variation present in the progeny resulting in greater heterozygosity and further expression of the associated traits [60].

Among fruit crops, the strawberry (*Fragaria* × *ananassa*) is a prime example of naturally occurring polyploidization. Various ploidy levels, including diploid, triploid, tetraploid, pentaploid, and octoploid, have been seen in the genus *Fragaria* [19]. Given that unreduced gametes are frequent in *Fragaria*, numerous researchers have hypothesized that 2n gametes' unification led to the development of polyploidy in this species [19]. Additionally, tetraploids and diploids can be crossed to generate triploids [56]. According to [61], tetraploid-diploid crossings produced 22.4 percent diploid, 54.0 percent triploid, and 23.4 percent tetraploid. In contrast, diploid-tetraploid crosses produced plants that were 31.8 percent diploid, 68.0 percent triploid, and 0.1 percent tetraploid. The most effective method of making polyploids for some crops, such as the triploid banana species, *Musa acuminata,* and *M. balbisiana*, may be sexual polyploidization. However, the limited frequency of creation of unreduced gametes limits the use of sexual polyploidization [60].

Production of haploid and doubled haploids

Microspore culture, pollen culture, and anther culture all result in the creation of haploid cells; the process that employs female gametes is known as gynogenesis, while the process that uses male gametes is known as androgenesis. The fundamental concept is to induce immature gametic cells to stop developing into gametes and instead drive them to grow into haploid plants [8]. Due to parthenocarpy, self-incompatibility, high heterozygosity, and long generation intervals in fruit trees, the breeding process of fruit crops is complex. The availability of haploids has significant consequences on the genetic development of fruit trees [54]. Using

embryo rescue methods, the haploid embryo must be preserved and grown into a haploid, and then chromosomal doubling must be carried out to create doubled haploids.

Haploids that undergo chromosome doubling become doubled haploids. The smaller and male-sterile haploid plants cannot produce pollen or eggs because their homologous pair is absent during meiotic division. Although doubled haploids are derived from a single breeding cycle with unquestionably complete homozygosity, homozygous lines take 6-7 breeding cycles to be created. Fruit breeders are interested in haploids and doubled haploids because they may be able to develop homozygous lines more quickly than using traditional breeding methods [14]. In citrus and related genera, advanced techniques such as anther culture and pollen irradiation have been used to produce doubled haploid plants [86].

Chromosome doubling

Another technique to change the ploidy level is to use various antimitotic agents to double the initial set of chromosomes. The use of unreduced pollens [34], temperature shocks [5], inducing injury to plant cells [56], radiation treatment of plant parts [59], and chemical treatments are just a few examples of methods that can cause chromosome doubling [82,84]. Since colchicine only affects dividing cells, it should only be taken while tissues actively divide. To double the number of chromosomes in a high number of shoot apex cells, multiple treatments spaced at close intervals should be employed because only a tiny portion of cells would be dividing at any given moment [27]. With concentrations of 0.2% colchicine more typical, seed treatment may be applied for 1 to 10 days. To allow for aeration, seeds are often soaked in a shallow container. The shoot buds of woody plants are frequently treated with 1 percent colchicine. Colchiploidy was used effectively in a variety of fruit crops, including banana [18], kiwifruit [84], pineapple [39], ber [17], pomegranate, passion fruit, guava, papaya, Annona, and grape [27]. Kiwifruit tetraploids treated with colchicine were 50–60% bigger than their diploid ancestors [82].

Interploid hybridization

Interploid hybridization is the process of breeding two distinct individuals with different levels of ploidy, and the offspring generated by this process is known as interploid hybrids [77]. Interploid hybridizations commonly cause smaller, abnormally developed seeds in angiosperms and seed abortion. Whether the crosses are diploid, tetraploid, or reciprocal, citrus breeders more frequently adopt interploidy crosses to produce seedless hybrids [77]. Triploid recovery is mainly prevented by polyembryony and early endosperm development. Tetraploid diploid hybrids with a more significant proportion of triploids were created using monoembryonic seed progenitors [6]. In citrus, adopting the embryo rescue approach employed in interploidy hybridization permitted the recovery of diverse triploids [77].

Endosperm culture

Due to double fertilization, which occurs only in higher plants, the endosperm of diploid plants is a triploid (i.e., possessing three sets of chromosomes) tissue. The process of double fertilization, in which one of the two male gametes fertilizes the egg to create a zygote. At the same time, the other unites with secondary nuclei to form a triploid endosperm and produces the endosperm [66]. Chromosome variety and significant polyploidy levels are typically seen in the endosperm tissue. Other essential characteristics of endosperm tissues include chromosomal bridges, erratic mitosis, and laggards. Endosperm cultivation offers a straightforward 1-step approach for the generation of triploid plants compared to conventional methods [72]. The lesser number of seeds or complete seedlessness is the trait that is attracting fruit breeders in recent times, and triploid induction using ploidy manipulation techniques can help produce good quality seedless fruit crops [72]. Triploid plants are helpful in plants where the vegetative components are valuable since they develop vegetatively more quickly than diploid ones. Triploids often grow faster than their respective diploid counterparts [72].

Factors affecting ploidy manipulation

Genotype

Plants with lower ploidy levels have a high tendency for polyploidy induction even though chromosome doubling is genotype-dependent. The effectiveness of antimitotic agents like oryzalin and colchicine has been the subject of conflicting reports [36], and it was discovered that responses to the induction of polyploidy were genotype-dependent [22,58,84].

Type of tissue

The technique of manipulating ploidy is substantially impacted by the choice of explant material or tissue. Several researchers have

used a variety of plant tissues and materials, including petioles in kiwifruit [84], hypocotyl segments and shoot apices in watermelon [42,57], hypocotyl segments in passion fruit [58], axillary buds in apple [22], and shoots [43]. Although there are few technological limitations [72,78], using endosperm as an explant material directly generates natural triploid plants in a concise amount of time [66].

Type of antimitotic agents, dosage, and exposure period

Various factors, such as the capacity to generate polyploidy, lethality, solubility, heat stability, etc., influence the choice of an antimitotic agent. Dinitroanilines, an antimitotic agent from the herbicides group (Figure 1), have been demonstrated to have a high affinity for plant tubulins and are used to induce polyploidy *in vitro*. Despite this, in most fruit plant species, colchicine was the preferred antimitotic agent since it is the most effective [22,43,58,84]. Antimitotic chemical concentration and exposure time are variables that are frequently examined. The interplay between exposure duration and concentration is not entirely understood, even though low exposure levels are ineffective and high exposure levels are fatal [73].

Regrowth media

A proper growth medium is necessary for polyploids to recover well after antimitotic treatments. *In vitro* growth media are frequently supplemented with antimitotic agents, and it's plausible that media elements might interact with antimitotic agents to affect chromosomal doubling [73]. For instance, pH and sucrose significantly impact how well dinitroanilines bind to -tubulin [23,37]. Vitamins, amino acids, plant growth regulators, and other regrowth medium elements significantly impact the survival of experimentally produced polyploids [1,12].

Assessment of ploidy level

Recent years have seen other approaches for determining the ploidy level in plant tissues, mainly flow cytometry, which has revolutionized this task [47]. The techniques, such as the determination of the size and density of leaf stomata [10,75], length of pollen grains [87], and cell size [20], have generally proved to be not sufficiently reliable [45,46]. Conversely, the nuclear DNA content was directly correlated with the ploidy level [63], and flow cytometry has arisen as a far more reliable methodology for the determination of the ploidy level [47]. The technique of flow cytometry is being effectively utilized to determine the ploidy level in fruit crops such as kiwi [50], plum [15], persimmon [53], and many other fruit crops.

Impact of ploidy manipulation on fruit quality

Polyploidy is a widespread phenomenon among various plant species, and among the cultivated species, approximately 40% are polyploids [64]. The development of artificial polyploids can be an efficient technique to facilitate fruit breeding and improve fruit quality. Some significant advantages of polyploids are the 'Gigas effect' (enhancement in plant vigor), buffering of deleterious mutations, increased heterozygosity, creation of a novel genetic resource, and restoring fertility and heterosis [60]. Polyploids often possess novel biochemical, physiological, morphological, and ecological traits. Presumably, the more significant environmental adaptability of the polyploids has allowed them to establish and sustain the severity of environmental change over the evolutionary time scale [30]. It has been demonstrated that polyploids show, although not consistently, increased yield and biomass, fruit and flower size, color intensity, flowering time, secondary metabolite production as well as primary metabolism [30,67,82,84]. These features of ploidy attracted several researchers to develop artificial polyploids and evaluate their effect on fruit quality, summarized in table 1.

Fruit Crops	Ploidy Level	Fruit Quality Traits Improved	Reference
Grape	Triploid	Seed lessness	[49]
	Tetraploid	Muscat flavour	[33]
Passion fruit	Tetraploid	Higher fruit weight, juice per fruit, soluble solids, juice/seed, and low seed count	[58]
Kiwi Fruit	Tetraploid	Fruit size (50-60% larger than diploid)	[83]
Apple	Tetraploid	Larger size of flowers, fruits, and seeds than the diploid cultivar	[85]
	Triploid	Regular fruit-bearing, more marketable fruit of the larger size	[61]
Citrus (*Fortunella* sp.)	Tetraploid	Thicker pericarp and high TSS	[44]

Ber	Tetraploid	Higher fruit weight, ascorbic acid content, titratable acidity, and soluble sugars	[79]
Bilberry	Tetraploid	Large flower and late blooming	[52]
Banana	Tetraploid	High lutein content	[2]

Table 1: Influence of ploidy manipulation on fruit quality traits.

Conclusion

Several tools and techniques are in practice to achieve the target of better fruit quality. Indeed, a particular method has a few advantages over another and vice versa. Nevertheless, ploidy manipulation opens up a new horizon regarding fruit quality enhancement. The techniques of ploidy manipulation are more efficient and provide economy in space and time since they can be performed *ex vitro* and *in vitro*. The experimental findings suggest that ploidy manipulations improved fruit quality parameters in a few fruit crops and enhanced their tolerance to various abiotic and biotic stresses. Further, ploidy manipulations may be utilized to develop varieties with improved fruit quality traits. Since no direct manipulation is involved, the varieties grown will be accepted and welcomed by the masses.

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