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PLANT BREEDING REVIEW

Synthetic polyploidy in spice crops: A review

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Abstract

Spice crops comprise diverse, economically valuable crops with numerous applications ranging from culinary to pharmaceutical industries. Spices are an integral part of cuisines worldwide, imparting characteristic flavor, aroma, and pungency to food. Breeding and crop improvement efforts in spices have focused on enhancing yield and quality parameters (essential oil, oleoresin, fiber, etc.) along with bioactive chemical constituents. The prominent breeding strategies followed are selection, hybridization, mutation, in vitro approaches, and transgenics. Polyploidy is one of the drivers of speciation and evolution, increasing the biological diversity in many crops, including spices. Polyploidy, either through natural means or artificial induction, broadens the scope of crop improvement. The artificial induction of polyploidy is usually done via antimitotic chemicals, duplicating the complete chromosomal set and allowing for genetic alterations and rearrangements that result in phenotypic changes across the board. As a result, increasing ploidy in crops often results in improved yield, biomass, vigor, biotic and abiotic stress tolerance, and secondary metabolite production, all of which can contribute to the economic success of these crops. This review provides an overview of research on artificial polyploidization in spice crops, including the polyploidy induction system, polyploidy generation, and screening methods to select the polyploids of interest. Thus, we have summarized the significant applications of artificial polyploidy in crop improvement that can serve as a potent reference for future research works in the same direction in the under-explored spice crops.

1 | INTRODUCTION

Spices are widely used as flavoring agents in cuisines across the world. They are dried portions of plants and mostly have an aromatic nature (Vázquez-Fresno et al., 2019). More precisely, the U.S. Food and Drug Administration defines spices as "aromatic vegetable compounds, whether whole, broken, or ground, that primarily serve as seasonings in food rather than as sources of nourishment" (Embuscado, 2015). These plant parts can range from dried buds/flowers (clove and saffron), rhizomes (ginger and turmeric), fruits/berries (pepper and chilies), bark (cinnamon), to seeds (coriander, cumin, fennel, and fenugreek) (Sung et al., 2012). Spices have been used since the dawn of human civilization. In addition to their use as flavoring agents and preservatives, they also find application in the pharmaceutical and cosmetic industries (Tajkarimi et al., 2010).

International Organization for Standardization has identified 109 spices worldwide. Although spices are cultivated worldwide, India is still regarded as the "Home of Spices" by the entire world (Gidwani et al., 2022). On a global scale, spice crops are grown in an area of 15.6 million ha,

Abbreviations: APM, amiprophos-methyl; DAPI, 4′,6-diamidino-2-phenylindole; DH, double haploid; DMSO, dimethyl sulfoxide; DNA, deoxyribonucleic acid; EMS, ethyl methanesulfonate; FCM, flow cytometry; MS, Murashige and Skoog.

contributing 45.5 million tonnes annually (FAOSTAT, 2019). The production of different spices in India has grown steadily over the last few years. Being the leading producer and exporter of spices, India produced 10.88 million tonnes of spices from 4.42 million ha area and exported 1.53 million tonnes worth US$4,102.29 million during 2021–2022 (Spices Board, 2022).

Spices are a storehouse of bioactive secondary metabolites. These comprise polyphenols, alkaloids, and terpenoids that are found to possess antioxidant, antimicrobial, and antifungal properties (Gottardi et al., 2016). The secondary metabolites are responsible for the medicinal properties exhibited by the spices. The broad utility spectrum of spices, with their known and potential medicinal properties, has enhanced the economic value of spice crops, leading to crop improvement programs. Increasing the overall quality and yield is the principal goals of crop improvement in spices (Letchamo & Craker, 1996). The quality parameters are divided into internal and external quality. The "external quality" of the spice includes the size, color, and shape that influence the consumer's preference. Internal quality refers to nutritional attributes such as starch, sugar, protein content, flavor, bioactive phytochemicals, culinary value, cooking characteristics, and processing features (Peter et al., 2006). Yield is governed by biomass production and the fresh or dry weight of the plant produced. The prominent breeding methods practiced are selection, hybridization, and mutation, along with in vitro-based approaches such as micropropagation, somaclonal variation, and the development of transgenics (Babu et al., 2013). Among the various crop improvement methods, polyploidy breeding has drawn attention as one of the most versatile plant breeding techniques. Polyploidy, the occurrence of more than two sets of chromosomes, is relatively common in flowering plants. Synthetic polyploidization helps to overcome sterility and incompatibility barriers and has played a pivotal role in angiosperm evolution and diversification (Sattler et al., 2016). To trigger novel phenotypes, whole genome duplication empowers organisms with the ability to respond and survive (Rutland et al., 2021). Polyploidy encourages the diversification of *Allium* species through shifts in morphology, ecology, or both (Han et al., 2020). Thus, polyploidy breeding that utilizes either natural or induced polyploids broadens the scope of crop improvement in spice crops through increased heterozygosity and genetic enhancement.

Artificial polyploidization, which has now become a potent tool for plant breeding, primarily utilizes antimitotic chemicals for ploidy alteration (J.-T. Chen et al., 2020). The polyploidy induction results in chromosomal duplication that causes additional changes at the genomic, epigenetic, and gene expression levels (Z. J. Chen & Ni, 2006). It can affect a wide range of phenotypes due to the increase in the number of copies of the gene (Adams & Wendel, 2005). Alterations in genome size can influence the developmental process, as there is a strong correlation between genome size and cell size. This effect of polyploidization is known as "gigas" effect (Balao et al., 2011). However, an increase in cell size does not necessarily guarantee an upsurge in the size or overall biomass of the entire organism, as the polyploids often have a lesser number of total cell divisions (Stebbins, 1971). In the case of plants, alterations in the ploidy levels have an impact on morphological, physiological, and biochemical traits. Regarding the general plant morphology; plant height; habit; number of shoots; roots; leaf characteristics (number of leaves, leaf shape, length/width ratio, etc.); number and size of the flowers, seeds, and pollen; and the composition of the cell wall are the frequently noticed changes brought on by ploidy alteration in plants. Polyploidization has resulted in improvement in morphological attributes, yielding polyploids with superior characteristics compared to diploids (Trojak-Goluch et al., 2021). In comparison with their diploid counterparts, polyploids have larger leaves, flowers, and seeds; improved photosynthetic abilities; reduced transpiration; and higher biomass production (Miri, 2020; Trojak-Goluch et al., 2021). Physiological changes such as size and density of stomata have been reported in polyploids, with stomatal density as a significant indicator of ploidy level (McGoey et al., 2014). Studies have reported a larger stomatal size but a lesser stomatal density in polyploids (Bomblies, 2020; Padoan et al., 2013; Wilson et al., 2021). Polyploidization has an effect on the developmental cycle of plants such as delayed flowering, sluggish growth, better tolerance to nutrient and mineral insufficiencies, improved resistance to biotic and abiotic stress factors, and hence better adaptation to habitat disturbance (Corneillie et al., 2019; Doyle & Coate, 2019; Tossi et al., 2022; Van de Peer et al., 2021; Vichiato et al., 2014). The effects of polyploidization can be harnessed for economic purposes to obtain desirable levels of metabolites in plants (Gantait & Mukherjee, 2021). Hence, artificial polyploidization offers advantages to agricultural crops by improving their biomass, yield, vigor, and stress tolerance by altering their anatomical, physiological, and morphological

Core Ideas

- Spices have been an indispensable part of human life since time immemorial as food and medicine
- The domestic and industrial usage of spices demands an increase in overall production
- Polyploidization could offer rapid phenotypic alterations and better agronomical traits
- Synthetic polyploidy can be instrumental in creating superior genotypes in spice crops.

characteristics, all of which could be advantageous for the economic success of the crops.

In spices, there is a significant scope for varietal improvement through polyploidization. This can result in genotypes with improved yield and quality parameters. It also has potential application in these crops for the enhancement of secondary metabolites, as many of these spices are valuable sources of phytochemicals and medicinally important bioactive molecules. So, in this review, we have discussed the most significant applications of polyploidy in the improvement of the major spice crops, which can serve as potent references for future works in the same direction in other less explored spice crops.

2 | COMMON SYSTEMS OF POLYPLOIDY GENERATION

Polyploids can occur naturally or created artificially. The emergence of new polyploid lineages in plants is enabled by several pathways, including the spontaneous doubling of chromosomal sets in somatic cells and the reunion of unreduced gametes (Otto & Whitton, 2000; Tayalé & Parisod, 2013). Somatic chromosome doubling can occur at any point in the life cycle of a plant, which can lead to a mixoploid organism or the origin of polyploidy meristematic cells that eventually give rise to new polyploid organisms (Grant, 1981). Different meiotic defects, such as abnormal spindle formation and orientation, absence of first or second meiotic division, or disrupted cytokinesis that result in the formation of unreduced gametes have been revealed in a range of plant species (Brownfield & Köhler, 2011). The union of diploid gametes facilitates the formation of both auto and allopolyploids under natural conditions. Noticeably, this sexual polyploidization has been used successfully in the breeding processes of several economically valuable crops such as alfalfa, potato, yam, and rose (Ramanna & Jacobsen, 2003). Nair et al. (1993) identified polyploidy in a cultivar of black pepper and aneuploids in its open-pollinated seedling progenies, giving insights into the natural incidence of autotriploidy and inter-specific hybridization. Multiple polyploidy events have occurred in the commercially significant genus *Brassica*. The allotetraploid *Brassica juncea* (mustard) has formed from the inter-specific hybridization of diploid species under this genus (Baker et al., 2017). However, the rarity of these occurrences hinders the breeding process.

Polyploidy can be artificially created primarily by antimitotic chemicals such as colchicine, oryzalin, and trifluralin and is a potent tool for crop improvement. To begin with this, efficient polyploidization systems are required. The two primary techniques for polyploidization are *in vitro* and in vivo systems (Nasirvand et al., 2018). The most popular method of polyploidization is the in vitro system, which can hasten polyploid development in a confined and controlled space. The establishment of effective in vitro culture protocols is a prerequisite for the success of in vitro polyploid induction (George et al., 2022; Touchell et al. et al., 2020). Higher mutation rates and a lower incidence of chimeras make in vitro autopolyploid induction more effective than in vivo approaches (Fu et al. et al., 2019). In vitro polyploidization can be accomplished by either treating the explant with antimitotic chemicals before inculcating it into the culture medium or by directly adding aqueous solution of antimitotic agents to either liquid or solid culture media and allowing it to interact with the explant. This technique has been used on a variety of spice crops, including *Zingiber officinale* (George & Prasath, 2023; Smith & Hamill, 1997), *Punica granatum* (Shao et al., 2003), *Aframomum corrorima* (Wannakrairoj & Tefera, 2013), *Trachyspermum ammi* (Noori et al., 2017), *Allium cepa* (Yun et al., 2021), *Allium sativum* (Wen et al., 2022), and *Thymus vulgaris* (Navrátilová et al., 2021) for the successful induction of polyploidy (Table 1). Conversely, in vitro systems require technical expertise and expensive laboratory equipment to conduct these procedures.

In vivo polyploidization is achieved through the application of an anti-mitotic agent to portions of an intact plant or plant parts such as seeds, nodal segments, rhizome buds, and corms, which are easy to get and manage and are frequently used as the initial material for polyploidization (Hassanzadeh et al., 2020; Prasath et al., 2022; Samadi et al., 2022). Chemicals can be applied directly through various methods, such as immersion of the seedling as in *Agastache foeniculum* (Talebi et al., 2017), injection by syringe in *Papaver somniferum* (Mishra et al., 2010), the cotton plug method in *Capsicum annuum* (Kulkarni & Borse, 2010), immersion of the root tip in *Ocimum basilicum* (Omidbaigi et al., 2010), and dropwise application to the apical meristem in *A. foeniculum* (Talebi et al., 2017). There is no need for skilled workers and fully equipped laboratories for the execution of in vivo protocols, but it has a reduced rate of polyploidy induction (Salma et al., 2017). The major drawback of this system is that it takes longer to establish and multiply a polyploid population.

3 | FACTORS AFFECTING ARTIFICIAL POLYPLOIDY INDUCTION

The induction of polyploidy is complicated by several elements, giving it a multi-variant, unexpected, and nondeterministic character.

3.1 | Plant parameters

The primary and most significant factor influencing the development of polyploidy in plants is genotype. The responsiveness of several genotypes and ecotypes of a single plant species to polyploidy induction is obviously variable (Niazian & Nalousi, 2020). This is particularly true for a technique that

TABLE 1 The reported synthetic polyploidy in major spice crops employing different explants, anti-mitotic agents, concentrations, and durations

Crop	Explant	Method of induction	Antimitotic agent	Concentration and duration	Optimum concentration and time for polyploidization	Induced ploidy and polyploidization efficiency	Reference
Black pepper (*Piper nigrum* L.)	Seeds	In vivo	Colchicine	0.05% and 0.1%; 4 h	0.05%; 4 h	Tetraploidy, NR	Nair and Ravindran (1992)
Ginger (*Zingiber officinale* Rosc.)	Rhizome buds	In vivo	Colchicine	0.25%; 2 days	0.25%; 2 days	Tetraploidy, NR	Ramachandran and Nair (1992)
	Shoots	In vitro	Colchicine	0.5%; 2 h	0.5%; 2 h	Tetraploidy, NR	Smith and Hamill (1997)
	Shoot tip	In vitro	Colchicine	0.2%; 4, 8, 12, and 14 days	0.2%; 8 days	Tetraploidy, 36.5%	Adaniya and Shirai (2001)
	Shoot tip	In vitro	Colchicine	0.1%, 0.2%, 0.3%, and 0.4%; 0, 6, 12, 18, 24, 30, 36, 42, or 48 h	0.2%; 30 h	Tetraploidy, 33.3%	Kun-Hua et al. (2011)
	Buds	In vitro	Colchicine	50, 100, 150, 200 mg L^{-1}; 3, 5, and 7 days	150 mg L^{-1}; 7 days	Tetraploidy, 18%	Zhou et al. (2020)
	Rhizome buds	In vivo	Colchicine	0.025%, 0.05%, 0.075%, and 0.1%; 24 and 48 h	0.1%; 48 h	Tetraploidy, NR	Prasath et al. (2022)
	Rhizome buds	In vivo	Colchicine	0.05%, 0.1%, 0.15%, and 0.2%; 24 and 48 h	0.15%; 24 h	Tetraploidy, NR	George and Prasath (2022)
	In vitro shoot-tips	In vitro	Colchicine	0.025%, 0.05%, 0.75%, and 0.10%; 24 and 48 h	0.10%; 48 h	Tetraploidy, NR	George and Prasath (2023)
Chilli (*Capsicum annuum/frutescens* L.)	Seeds	In vivo	Colchicine	0.05%, 0.1%, 0.2%, and 0.4%; 1, 2, 4, 6, and 8 days	0.2%; 4 days	Polyploidy, 80%	Pal and Ramanujam (1939)
	Seedlings	In vivo	Colchicine	0.2%; 6, 12, and 24 h	0.2%; 12 h	Polyploidy, 80%	Raghuvanshi and Joshi (1964)
	Seedlings	In vivo	Colchicine	0.3%; 12, 18, and 24 h	0.3%; 24 h	Octaploidy and tetraploidy, NR	Panda et al. (1984)

(Continues)

TABLE 1 (Continued)

Crop	Explant	Method of induction	Antimitotic agent	Concentration and duration	Optimum concentration and time for polyploidization	Induced ploidy and polyploidization efficiency	Reference
	Seeds and seedlings (two leaf stage)	In vivo	Colchicine	0.05%, 0.1%, 0.2%, and 0.4%; 24, 48, and 72 h	0.1%, 48 h; 0.2%, 24 h	Tetraploidy, NR	Kulkarni and Borse (2010)
	Seeds	In vivo	Colchicine	0, 100, 200, and 300 mg $L^{-1;}$ 6 h	300 mg L^{-1}; 6 h	Tetraploidy, 70%	Pliankong et al. (2017)
			Oryzalin	0, 10, 20, and 30 mg L^{-1}; 6 h	20 and 30 mg L^{-1}; 6 h	Tetraploidy, 40%	
	Seeds	In vivo	Colchicine	0.025%, 0.05%, 0.075%, and 0.1%; 24 h	NR	Mixoploidy, NR	Tammu et al. (2021)
Coriander (*Coriandrum Sativum* L.)	Seedling	In vivo	Colchicine	0.10%, 0.25%, and 0.50%; 0.5, 1, and 2 h	0.5%; 2 h	Tetraploidy, NR	Sharma and Datta (1957)
	Seedling	In vivo	Colchicine	0.1%, 0.2%, and 0.3%; 3 h for 3 days	0.2%; 3 h for 3 days	Polyploidy, 25%	Purbiya et al. (2021)
Garlic (*Allium sativum* L.)	Callus	In vitro	Trifluralin	0, 50, 100, and 200 μM; 5, 10, or 15 days	100 μM; 15 days	Tetraploidy, 3.3%	Cheng et al. (2012)
	Stem disc	In vitro	Colchicine	0.25%, 0.50%, and 0.75%; 36, 48, or 72 h	0.5%;36 h	Tetraploidy, NR	Dixit and Chaudhary (2014)
	Cloves	In vivo	Colchicine	0.2%, 0.4%, and 0.6%; 12, 24, 36, and 48 h	0.6%; 24 h	Tetraploidy, 26%	Yousef and Elsadek (2020)
	Stem disc	In vitro	Colchicine	0.02%, 0.04%, 0.06%, 0.08%, and 0.10%; 24 and 48 h	0.1%; 24 and 48 h	Tetarploidy, 16.6%	Hailu et al. (2020)
	Immature inflorescences	In vitro	Colchicine	0, 125, 250, 500, 1000, and 2000 mg L^{-1}; 5, 10, 15, 20, 25, or 30 days	2,000 mg L^{-1}; 20 days	Tetraploidy, 21.8%	Wen et al. (2022)

(Continues)

TABLE 1 (Continued)

Crop	Explant	Method of induction	Antimitotic agent	Concentration and duration	Optimum concentration and time for polyploidization	Induced ploidy and polyploidization efficiency	Reference
			Oryzalin	0, 15, 30, 60, 120, and 240 μM; 5, 10, 15, 20, 25, or 30 days	60 μM; 20 days	Tetraploidy, 4.3%	
Fenugreek (*Trigonella foenum-graecum* L.)	Seeds and seedlings	In vivo	Colchicine	0.5% and 0.15%; 4 h	0.15%; 4 h	Tetraploidy and hexaploidy, NR	Shambulingappa et al. (1965)
	Seeds	In vivo	Trifluralin	7.5, 15, and 22.5 μM; 12 and 24 h	22.5 μM; 24 h	Tetraploidy, NR	Afshari et al. (2009)
	Shoot meristem	In vivo	Colchicine	0.5%; 3 days	0.5%; 3 days	Mixoploidy, NR	Marzougui et al. (2011)
	Germinating seeds	In vivo	Colchicine	0.05%; 4 h	0.05%; 4 h	Mixoploidy, NR	Omezzine et al. (2012)
	Seed, root meristem and terminal meristem	In vitro	Colchicine	0.05%, 0.1%, 0.2%, and 0.5%; 12, 24, 48, and 72 h	0.5%; 72 h	Tetraploidy, NR	Keshtkar et al. (2019)
	Embryos	In vitro	Colchicine	0, 75, 150, 300, and 600 μM; 8, 12, 24, and 48 h	150 μM; 24 h	Tetraploidy, NR	Alavi et al. (2022)
			Trifluralin	0, 125, 250, and 500 μM; 24, 48, and 72 h	500 μM; 72 h	Tetraploidy, NR	
Onion (*Allium cepa* L.)	Gynogenic embryos (*n*)	In vitro	Trifluralin	5 and 50 μM; 24 and 72 h	50 μM; 72 h	Diploidy, 32.5%,	Grzebelus and Adamu (2004)
			Oryzalin	50 μM; 24 and 72 h	50 μM; 72 h	Diploidy, 34.9%	
			APM	50 μM; 24 and 72 h	50 μM; 72 h	Diploidy, 32.6%	
			Colchicine	25 and 125 μM; 24 and 72 h	25 μM; 72 h	Diploidy, 19%	

(Continues)

TABLE 1 (Continued)

Crop	Explant	Method of induction	Antimitotic agent	Concentration and duration	Optimum concentration and time for polyploidization	Induced ploidy and polyploidization efficiency	Reference
	Three-week-old plantlets	In vitro	Colchicine	0.625, 1.25, and 2.5 mM; 24 h	0.625 mM; 24 h	Diploidy, 62%	Geoffriau et al. (1997)
			Oryzalin	10, 50, 100, and 150 μM; 24 h	10 μM; 24 h	Dipoidy, 33.3%	
	Three-month-old plantlets	In vitro	Colchicine	2.5, 7.5, and 12.5 mM; 24 h	2.5 mM; 24 h	Diploidy, 65.7%	
			Oryzalin	50, 100, 150, and 200 μM; 24 h	50 μM; 24 h	Diploidy, 57.1%	
	Gynogenic embryos	In vitro	Oryzalin	2.5, 5, 7.5, 10, 25, 50, and 75 μM;	50 μM (APM); 3 days (solid medium)	Diploidy, 36.7%	Jakše et al. (2003)
			APM	30 and 60 min (liquid medium), 1–3 days (solid medium)			
	Whole basal explant	In vitro	Colchicine	750 and 1000 μM; 48 h	1000 μM; 48 h	Diploidy, NR	Alan et al. (2007)
			APM	50, 100, and 150 μM; 48 h	150 μM; 48 h		
			Oryzalin	50, 100, and 150 μM; 48 h	100 μM; 48 h		
	Haploid plantlets	In vitro	Colchicine	625 and 1250 μM; 24 and 48 h	625 μM; 48 h	Diploidy, 70.4%	Foschi et al. (2013)
			APM	50, 100, and 200 μM; 24 and 48 h	50 μM; 48 h	Diploidy, 57.7%	
	Embryos	In vitro	APM	25 and 50 μM; 24, 48, and 72 h (solid and liquid medium)	25 μM; 24 h	Diploidy (solid medium), 35%	Fayos et al. (2015)

(Continues)

TABLE 1 (Continued)

Crop	Explant	Method of induction	Antimitotic agent	Concentration and duration	Optimum concentration and time for polyploidization	Induced ploidy and polyploidization efficiency	Reference
	Bulb/shoot tips	In vitro	Colchicine	250, 750, and 1250 µM; 2, 4, and 6 days	250 µM; 4 days	Tetraploidy, octaploidy and mixoploidy, 42.22%	Ren et al. (2018)
			Pendimethalin	10, 30, and 50 µM; 2, 4, and 6 days	30 µM; 6 days	Tetraploidy, octaploidy and mixoploidy, 41.11%	
	Callus	In vitro	Oryzalin	0, 25, 50, 75, 90, and 120 µM; 2 drops	75 µM	Tetraploidy, NR	Yun et al. (2021)
Black cumin (*Nigella sativa* L.)	Seeds	In vivo	Colchicine	0%, 0.025%, 0.05 0.1%, and 0.2%; 8, 24, and 48 h	0.05%; 48 h	Tetraploidy, 9.9%	Zishan et al. (2016)
	Seeds	In vivo	Colchicine	0.00625%, 0.0125%, 0.025%, 0.05%, and 0.1%; 8 h	0.025%; 8 h	NR	Gupta et al. (2021)
Thyme (*Thymus vulgaris* L.)	Nodal segments	In vitro	Oryzalin	20, 40, 60, and 80 µM; 24 and 48 h	80 µM; 24 h	Tetraploidy, 7.5%	Shmeit et al. (2020)
	Nodal segments	In vitro	Oryzalin (solid MS)	0.346, 1.73, and 3.46 mg L^{-1}; 2 weeks	0.346 mg L^{-1}; 24 h	Tetraploidy, 40%	Navrátilová et al. (2021)
			oryzalin (liquid ms)	1.73, 3.46, 5.19, 6.92, and 8.65 mg L^{-1}; 24 h	3.46 mg L^{-1}; 24 h	Tetraploidy, 25%	
Mint (*Mentha piperita* L.)	Stem	In vitro	Colchicine	50, 100, 150, and 200 mg L^{-1}; 24, 48 and 72 h	150 mg L^{-1}; 48 h	Tetraploidy, NR	Zhao et al. (2017)
	Rhizome	In vivo	Colchicine	0%, 0.0125%, 0.025%, and 0.05%; 3, 6, and 9 h	0.05%; 9 h	Hexaploidy, 15.27%	Moetamedipoor et al. (2022)
	Nodal segments	In vitro	Oryzalin	20, 40, and 60 µM; 24 and 48 h	40 µM; 48 h	Hexaploidy, 8%	Bharati et al. (2023)

(Continues)

TABLE 1 (Continued)

Crop	Explant	Method of induction	Antimitotic agent	Concentration and duration	Optimum concentration and time for polyploidization	Induced ploidy and polyploidization efficiency	Reference
Caraway (*Carum carvi* L.)	Seedling	In vivo	Colchicine	0.2%; 2 days	0.2%; 2 days	Tetraploidy, 3%	Dijkstra and Speckmann (1980)
	Seedling	In vivo	Colchicine	0.2, 0.5, 0.75, and 1 g L^{-1}; 6, 12, and 18 h	0.5 g L^{-1}; 6 h	Tetraploidy, NR	Akbari et al. (2019)
Saffron (*Crocus sativus* L.)	Corms	In vivo	Colchicine	0.05% and 0.025%; 12 and 24 h	0.025%; 12 h	NR	Samadi et al. (2022)
Basil (*Ocimum basilicum* L.	Seeds	In vivo	Colchicine	0%, 0.05%, 0.10%, 0.20%, 0.50%, and 0.75%; 6, 12, and 35 h	No polyploidy	No polyploidy	Omidbaigi et al. (2010)
	Meristem tip of seedling	In vivo	Colchicine	0%, 0.1%, 0.2%, 0.5%, and 0.75%; 3 days	0.5%; 3 days	Tetraploidy, 8%	
	Root	In vivo	Colchicine	0%, 0.05%, 0.10%, 0.20%, 0.50%, and 0.75%; 6, 12, 24, 36, and 48 h	No tetraploidy	No tetraploidy	
Poppy (*Papaver somniferum* L.)	Seeds	In vivo	Colchicine	0.25% and 0.40%; overnight	NR	No polyploidy	Mishra et al. (2010)
	Shoot meristem	In vivo	Colchicine	0.25% and 0.40%; 2 days	NR	Tetraploidy, NR	
Parsely (*Petroselinum crispum* L.)	Seeds	In vivo	Colchicine	0.025%, 0.05%, 0.1%, and 0.2%; 8, 24, and 48 h	0.05%; 24 h	Tetraploidy, 75%	Nasirvand et al. (2018)
	Nodal segments	In vitro	Colchicine	0.025%, 0.05%, 0.1%, and 0.2%; 8, 24, and 48 h	0.1%; 24 h	Tetraploidy, 100%	
Pomegranate (*Punica granatum* L.)	Single node shoots	In vitro	Colchicine (solid MS)	10 mg L^{-1}; 30 days	10 mg L^{-1}, 30 days	Tetraploidy, 20%	Shao et al. (2003)
			Colchicine (liquid MS)	5000 mg L^{-1}; 96 and 114 h	5000 mg L^{-1}; 96 h	Mixoploidy, 25%	

(Continues)

TABLE 1 (Continued)

Crop	Explant	Method of induction	Antimitotic agent	Concentration and duration	Optimum concentration and time for polyploidization	Induced ploidy and polyploidization efficiency	Reference
Sage (*Salvia officinalis* L.)	Seeds	In vivo	Colchicine	0.05%, 0.1%, 0.25%, and 0.5%; 12, 24, and 48 h	0.25%; 48 h 0.5%; 24 h	Tetraploidy, 9.67% Tetraploidy, 9.25%	Hassanzadeh et al. (2020)
Ajowan (*Trachyspermum ammi* L.)	Seeds	In vitro	Colchicine	0.025%, 0.05%, 0.1%, 0.2%, and 0.5%; 6, 12, 24, 36, and 48 h	0.05%; 24 h	Tetraploidy, 11.53%	Noori et al. (2017)
Java cardamom (*Amomum compactum* Soland ex, Maton)	Seeds	In vivo	Colchicine	0%, 0.05%, 0.10%, and 0.15%; 6 h	NR	NR	Komala et al. (2022)

Abbreviations: APM, amiprophos-methyl; MS, Murashige and Skoog; NR, not reported.

induces polyploidy in vitro since genotypes can influence the rate of regeneration and, consequently, the level of polyploidy induction in relation to antimitotic agents and in vitro regeneration variables. Two ecotypes of the Iranian indigenous mint (*Mentha mozaffarianii*) were subjected to polyploidy induction, and the results revealed distinct effects on the two ecotypes (Ghani et al., 2014). Moreover, Adaniya and Shirai (2001) achieved in vitro induction of tetraploidy in *Z. officinale* Roscoe cv. 'Sanshu' by treating with 0.2% colchicine for 8 days. In China, Kun-Hua et al. (2011) reported the highest percentage of tetraploidy in 0.2% colchicine for 30 h in *Z. officinale*.

In addition to plant genotypes, plant parts such as explants play a crucial role in polyploidy induction. The best plant communicators are those with active cellular division, such as somatic embryos, calluses, nodal segments, apical buds, juvenile root tips, immature inflorescence, and germinated seeds. Fu et al. (2019) revealed that the capacity to induce chromosomal duplication varies among explants. Omidbaigi et al. (2010) investigated the impact of colchicine on various explants of *O. basilicum* and reported more polyploids from the meristem tip treatment of seedlings. In *A. foeniculum*, the highest percentage of tetraploidy (20%) was obtained from seeds compared to the apical meristem and seedlings (Talebi et al., 2017). The age of the explant is also crucial for the successful establishment of the polyploids. Additionally, the totipotency of the cells may vary with the age of the explant and thus have a considerable impact on regeneration after chemical treatment, particularly in in vitro pathways. The ability to regenerate a whole plant from a single or a small number of cells can enhance the formation of homogenous polyploids and reduce the likelihood of cytochimeras, having cells with different ploidy levels (Touchell et al., 2020). Pre-treatments have occasionally been used to synchronize the cell cycle to enhance the impact of antimitotic agents. Smith et al. (2004) cultured the shoot tips of *Z. officinale* on half MS media for 7 days before treating with colchicine. The culture conditions after antimitotic treatments have been found to affect chromosomal doubling in the context of in vitro induction.

3.2 | Anti-mitotic agents

Antimitotic compounds are crucial and responsible for the inhibition of spindle fiber formation at the metaphase stage, which leads to the nondisjunction of chromosomes and increases the ploidy level. A few contributing factors are considered, including type, dosage, exposure time, and mode of application. An experiment to induce polyploidy can use a variety of spindle blockers. Colchicine is the most frequently used antimitotic drug in chromosomal duplication investigations. It is an established mitotic arresting alkaloid that binds

to tubulin and hinders the development of tubulin dimers and cytoskeleton (Salma et al., 2017). Colchicine seems to have a low selectivity for tubulins in plant tissue and a strong affinity for tubulins in animal tissue; it is highly toxic to humans despite its widespread use in polyploidy induction experiments (Huy et al., 2019). High colchicine concentrations must be considered to produce polyploidy in plant cells. Besides, elevated doses of colchicine may cause mutation, malformation, and an increased rate of mortality. Thus, colchicine results in reduced formation of polyploidy in plants (Manzoor et al., 2019). Hence, modest doses with long exposure times are thought to be effective in reducing its toxic effects and increasing the rate at which polyploids are produced. Substitutes to the colchicine which are less harmful are oryzalin, trifluralin, flufenacet, amiprophos-methyl (APM), pronamide, and nitrous oxide gas (Afshari et al., 2009; Alavi et al., 2022). APM and oryzalin are reported to be better than colchicine due to their higher selectivity for plant tubulins and lack of affinity for animal cells (Jakše et al., 2003; Rauf et al., 2021). Additionally, the optimum concentration is 50–250 times lower than colchicine. Wannakrairoj and Tefera (2013) reported that the rate of in vitro polyploidy induction in *A. corrorima* by oryzalin (10 M) was comparable to that of colchicine (125 M). Oryzalin was more efficient than trifluralin and colchicine in the seed treatment of *A. foeniculum* (Talebi et al., 2017).

Dimethyl sulfoxide (DMSO) is frequently added to aqueous solutions at 1.0%–2.0% to increase the permeability and penetration of chemicals into cells. Two percent DMSO was used to dissolve (APM for doubled haploid production in *A. cepa* (Foschi et al., 2013). In addition to DMSO, detergents such as Tween 20 or Triton X-100 were also used to increase cell permeability of the mutagen in the chromosome doubling of mint (Moetamedipoor et al., 2022) and onion (Jakše et al., 2003). The mixture of explants and chemical solution is constantly agitated using a shaker at various rpm to improve contact, which increases the penetration of anti-mitotic chemicals into the cell. The shaking speed depends on the explant type, maturity, and size. The optimum shaking speed reported is 100 rpm for buds (Komala et al., 2022), 120 rpm for nodal segments (Nasirvand et al., 2018), and 90 rpm for rhizome buds (Lindayani et al., 2010). Shaking ensures the explants receive the same exposure to an antimitotic agent, maintaining uniformity.

The exposure time and dosage of the spindle inhibitors are variables that are frequently examined. It is obvious that the ideal concentration of an antimitotic drug is essential for a positive polyploidy induction, and treating explants over the threshold can be fatal, while modest exposure levels are ineffective. Consequently, determining the antimitotic agent's minimal optimal dose is crucial (Podwyszyńska et al., 2018). Wen et al. (2022) noticed a considerable decrease in the explant viability and shoot regeneration capability with an increment in colchicine concentration and treatment time in *A. sativum*. There is also a substantial interaction between the concentration and duration of the treatment on the viability and induction frequency of tetraploids. In *C. annuum*, 0.4% colchicine for 72 h was found to be lethal (Kulkarni & Borse, 2010). Different spice crops respond differently toward concentrations of antimitotic chemicals and are species specific. In vitro studies of *Z. officinale* indicated maximum polyploidization at 0.5% colchicine for 2 h (Smith & Hamill, 2002), whereas in *Petroselinum crispum*, the highest polyploidy induction was obtained in 0.1% colchicine for 24 h (Nasirvand et al., 2018).

4 | IDENTIFICATION OF PUTATIVE POLYPLOIDS AND ITS VERIFICATION

The identification of induced polyploids plays a crucial role following treatment with antimitotic agents. The polyploids can be identified either directly or indirectly. The majority of polyploidization experiments use indirect identification strategies for the initial selection of putative polyploids followed by direct identification methods for further confirmation of their ploidy.

4.1 | Indirect identification methods

Indirect strategies are simple and quick, but they may not be accurate because they rely on changes in morphological, physiological, and anatomical characteristics. This technique involves a preliminary screening to pick a small population from the initial large population. Leaf parameter, shoot diameter, plant height, and floral character are the commonly used morphological characters for the detection of putative plants (Salma et al., 2017) (Figure 1). The leaves of polyploids frequently exhibit several unusual traits, such as wider, thicker, greener, and alterations in the leaf margin (Cheng et al., 2012). This approach has been used as ploidy markers for early population screening in *Zingiber offcinale* (Zhou et al., 2020), *A. cepa* (Yun et al., 2021), and *A. sativum* (Yousef & Elsadek, 2020). Marzougui et al. (2011) reported the appearance of malformed leaves in *Trigonella foenum-graecum* after colchicine treatment, which revealed mixoploidy as well as tetraploidy. Pollen size was also influenced by ploidy number. The tetraploid plantlets have bigger pollen grains than diploid plantlets (Omidbaigi et al., 2010). Selection based on the size and shape of the pollen grain and seed size has proved to be an efficient way to identify mixoploid plants (Dijkstra & Speckmann, 1980; Omezzine et al., 2012). This measurement is, however, seldom used in the ploidy screening procedure. The use of antimitotic chemicals may also lead to alterations in the floral morphology, such as flower size, length, and color. Samadi et al. (2022) observed deformed flowers with

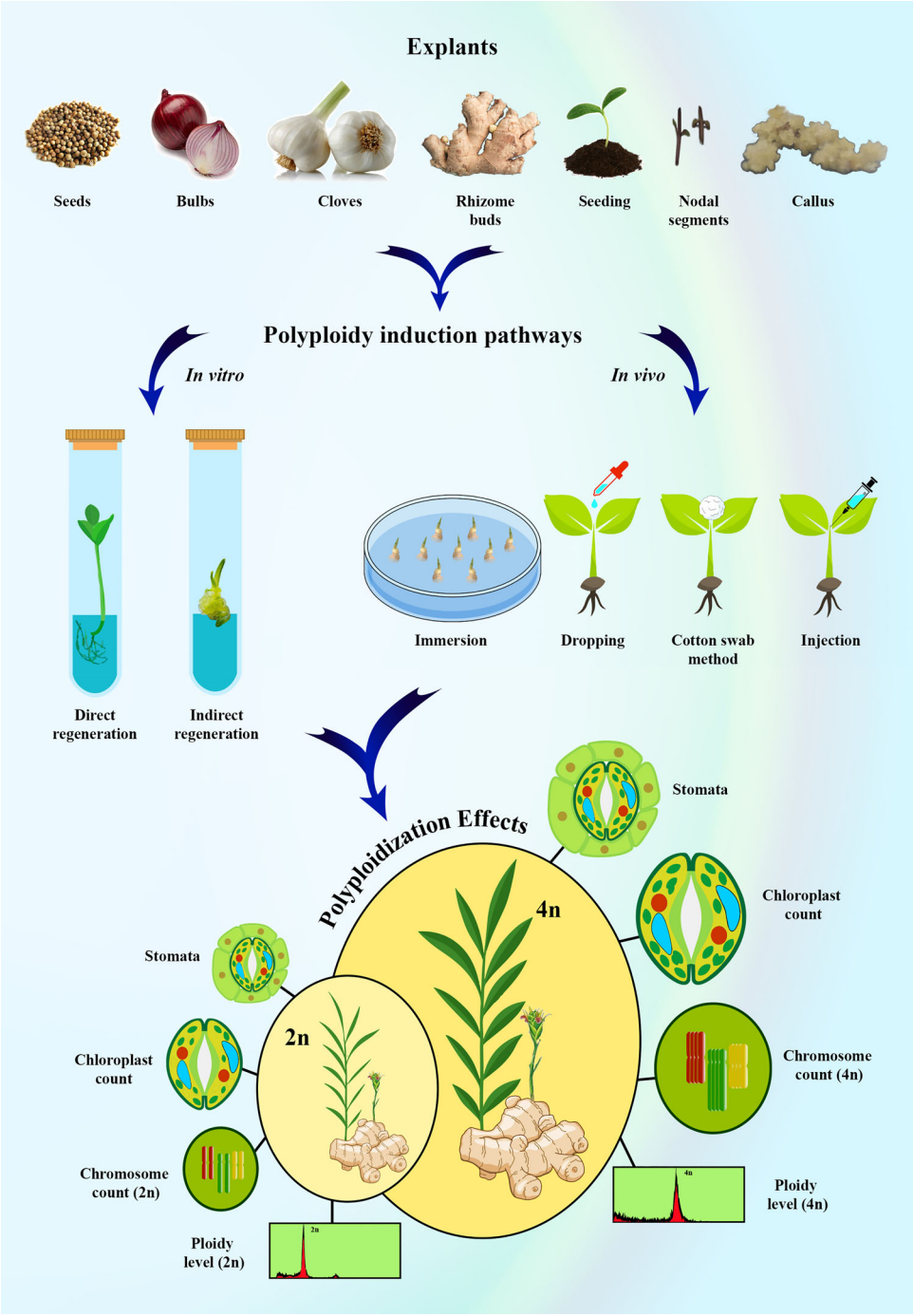

FIGURE 1 Schematic representation of polyploidy induction pathways and associated changes.

incomplete tepals and stigma in induced tetraploids of *Crocus sativus*. All these visual cues clearly demarcate the mutated ones from treated diploids, making it easier to recognize and characterize polyploids from a large population of treated plants.

Stomatal characteristics, including stomatal frequency, length, and width as well as chloroplast count in the stomatal guard cells, are helpful in identifying mutated plantlets (Beck et al., 2003; Hodgson et al., 2010) (Figure 1). Polyploids exhibit larger stomata with a lesser frequency compared to diploids. Several studies provide convincing evidence to corroborate this result (George & Prasath, 2022, 2023; Kun-Hua et al., 2011; Mishra et al., 2010; Nasirvand et al., 2018; Noori et al., 2017). Among the various indirect assays, those linked to stomata (density and length) may offer a rapid method for separating and pre-selecting suspected polyploid plants (Wannakrairoj & Tefera, 2013). Subsequently, a considerable increase in the number of chloroplasts in stomatal guard cells

was also exploited to detect this whole genome duplication in *A. foeniculum* (Talebi et al., 2017). Given that the external influences might affect morphological and physiological features, they are not entirely accurate and dependable for ploidy detection.

The identification of plant ploidy is now frequently performed via flow cytometric (FCM) analysis, which is significantly quicker and more precise than conventional techniques. FCM analysis involves the estimation of nuclear DNA content with high precision (Doležel et al., 2007). Following the extraction of cell nuclei using a razor blade chopping (Galbraith et al., 1983) or a bead beating method (Roberts, 2007), DNA is then labeled with fluorochrome and evaluated using a flow cytometer. The stained nuclei are exposed to laser rays to emit fluorescence. The intensity can be measured with a flow cytometer, which is directly correlated with the ploidy level (Doležel et al., 2007). Sample preparation often takes a few minutes, requires only a small amount of tissue for analysis, and is typically nondestructive (Sattler et al., 2016). The fact that tissues containing dividing cells are not necessary for ploidy calculation is a significant benefit. This analysis can be performed at any growth stage without compromising the entire plant. A sample of known ploidy can be used as a standard to determine the ploidy status of an unknown sample (Doležel & Bartoš, 2005). FCM is effective at separating polyploids from diploids and distinguishing mixoploids within the treated population. Sometimes, the typical approaches might not work with certain samples for several reasons. Procedure optimization should be conducted on various plants to produce trustworthy results (Suda & Trávnícek, 2006). The freshness of the sample, the presence of cytosolic chemicals, and the lack of globally accepted DNA reference standards can all have an impact on the precision and accuracy of FCM analysis (Doležel & Bartoš., 2005). This method of ploidy verification is extensively used in spice breeding programs by researchers over all other indirect methods as it gives more accurate results much more quickly (George & Prasath, 2022, 2023; Hassanzadeh et al., 2020; Keshtkar et al., 2019; Prasath et al., 2022).

4.2 | Direct identification methods

The most effective direct method for determining the proper ploidy level is chromosome counting (Maluszynska, 2003). Nevertheless, the classical chromosome counting technique allows the visual verification of the exact number of somatic chromosomes in a dividing cell at its metaphase stage. Although chromosome counting can be done on a variety of plant parts, root tips have been the most widely employed (Eng & Ho, 2019). It is a laborious process that involves the following three major steps: (1) material preparation and pre-treatment, (2) fixation, and (3) preparation and staining (Mirzaghaderi, 2010; Ochatt et al., 2011). Moreover, tissues with actively dividing cells are necessary for clear and countable imaging and necessitate unique protocols for each species. As a result, it is a challenging procedure to quickly assess ploidy levels, which also requires extremely skilled operators (Niazian & Nalousi, 2020). Polyploidy in a black pepper cultivar was identified with the help of the chromosome counting technique (Nair et al., 1993). Noori et al. (2017) used the chromosome counting method to verify the ploidy status of colchicine-treated ajowan plants. Purbiya et al. (2021) analyzed the pollen mother cell to visualize the meiotic chromosomes and the segregation process in both diploids and colchicine-induced tetraploids of coriander. This fundamental method of ploidy detection is still practiced by researchers for determining the ploidy of treated spice crops (Alavi et al., 2022; Bharati et al., 2023; Prasath et al., 2022).

5 | EFFECTS OF POLYPLOIDY

As it is widely known, polyploidization has inherent and obvious genomic consequences. The most obvious of them is the increased genome size of the organism. This increase in genome size is followed by dynamic changes in the genetic landscape, altering the genetic architecture and gene expression profiles (Wang et al., 2021). This includes alteration in chromatin topology which affects chromatin accessibility and can contribute to changes in the gene expression pattern. At the allelic or locus level, major changes are an increased number of alleles and an increased heterozygosity (in allopolyploids and heterozygous autopolyploids) which can result in heterosis (Birchler et al., 2010). These dramatic genomic changes can have repercussions in the cellular and biochemical interactions that drive morphogenesis, affecting the morphological attributes of the organism. Although the whole developmental processes are robust to change until these changes cross a threshold level, these can result in the alteration of morphogenesis and developmental events and thus produce novel phenotypes (Madlung, 2013).

5.1 | Polyploidization effects in spices

Even though spices encompass a diverse array of plants from different families, widely varying in ploidy levels and propagation means, polyploidization-based breeding strategies offer tremendous potential across spice crops. Regardless of the mode of propagation, polyploidy breeding continues to serve as a faster and more efficient method to meet the ends of crop improvement (Table 1). There have been studies altering the ploidy level in many spice crops, leading to morphological, biochemical, and physiological changes which is summarised below.

5.2 | Morphological effects of polyploidization in spices

The most obvious and direct consequences of polyploidy induction include an increase in ploidy level and cellular changes in many spice crops, followed by a gigas effect for most of the morphological traits. The most common morphological changes observed upon ploidy induction across spice crops are in the leaves, shoots, roots, flowers, and fruits. Here, polyploidization has resulted in larger and thicker green leaves in garlic (Dixit & Chaudhary, 2014; Hailu et al., 2020, 2021; Wen et al., 2022), onion (Ren et al., 2018; Yun et al., 2021), fenugreek (Marzougui et al., 2011; Shambulingappa et al., 1965), ginger (Adaniya & Shirai, 2001; Prasath et al., 2022; Ramachandran & Nair, 1992; Smith & Hamill, 1997; Zhou et al., 2020), hot pepper (Kulkarni & Borse, 2010; Tammu et al., 2021), thyme (Navrátilová et al., 2021), and ajowan (Noori et al., 2017). In addition to large green leaves, polyploids exhibit gigas characteristics for plant height (Navrátilová et al., 2021), floral characters (Noori et al., 2017; Shambulingappa et al., 1965), larger stem size (Noori et al., 2017; Shambulingappa et al., 1965; Smith & Hamill, 1997; Zhou et al., 2020), large rhizomes with higher yield (Smith & Hamill, 1997; Zhou et al., 2020), size and density of trichomes (Zhao et al., 2022), larger bulb size (Hailu et al., 2020), peduncle and seed length (Dijkstra & Speckmann, 1980; Gupta et al., 2021), and so on. Nair and Ravindran (1992) observed thick leaf and stem with vigorous growth in tetraploid black pepper. Even the root system embodies the gigas effect, as observed in the roots of tetraploid plants of hot pepper with increased root size and a higher number of lateral roots (Kulkarni & Borse, 2010). Regarding fruit characters, polyploids had an increased fruit size than diploids in hot chilies (Pliankong et al., 2017). In coriander, a considerable increase in the size of flowers, fruits, and umbel was noticed (Purbiya et al., 2021; Sharma & Datta, 1957). In addition, it is common observation that there is a characteristic reduction in the number of branches or shoots along with polyploidization, in addition to the gigas characteristics (Shambulingappa et al., 1965; Smith & Hamill, 1997). Some researchers reported reduced plant height or dwarfing (Komala et al., 2022; Wen et al., 2022) and shortened internodes after polyploidization.

5.3 | Physiological and biochemical effects

Polyploid induction alters gene expression or physiological processes. Enhanced genetic activity, along with increased cell size, improved water interactions, and hormonal conditions can lead to an increase in the rate of photosynthesis in each cell (Lavania, 2005). The complex interplay of these aspects is responsible for the generation of bio-active phytochemicals from secondary metabolism (Dhawan & Lavania, 1996). Like in other crops, studies in spices also show that polyploidization can result in an increase in the chlorophyll index (due to an increased chloroplast number in most cases), which can lead to an enhancement in the photosynthesis rate or sometimes slow down the photosynthesis rate (Talebi et al., 2017). Bharati et al. (2023) noticed high chlorophyll content in the oryzalin-treated hexaploid *Mentha spicata*. Takizawa et al. (2008) revealed the effect of polyploidy on the physiology of capsicum and indicated that tetraploids have increased water and nutrient uptake, resulting in an improved photosynthetic rate. A similar result was observed in fenugreek with increased mineral contents (Marzougui et al., 2009). There was a substantial variation in stomatal characteristics and the number of chloroplasts in guard cells between triploid and hexaploid mojito mint (Moetamedipoor et al., 2022). Changes in stomatal density upon polyploidization have also been reported in many plant species. Here, most studies report a lower stomatal density in polyploids compared to diploids due to the increased size of stomata per leaf area (Foschi et al., 2013; Hailu et al., 2021). This may be due to an increase in the mean length and width of stomatal guard cells and the mean leaf area with an increase in ploidy level (Nasirvand et al., 2018; Zhao et al., 2017). Various studies, such as those conducted by George and Prasath (2023), Talebi et al. (2017), Yun et al. (2021), and Zhou et al. (2020), have reported these findings in spices.

Physiological processes such as flowering and pollen germination are affected by polyploidization. Even though the entire mechanism underlying the delayed growth response is unclear, it is closely associated with whole genome duplication. The increased cell size that occurs in neopolyploids may induce substantial physiological shift, suggesting the reduced growth rate (Roddy et al., 2020). This gigas effect necessitates the synthesis and deposition of additional cell wall material during flowering, pollen germination, and pollen tube growth (Bomblies, 2020). For instance, polyploidization has resulted in delayed flower and leaf emergence in saffron (Zaffar et al., 2003). While some of the studies report low pollen fertility in polyploids that can have adverse effects on fruit yield (Kulkarni & Borse, 2010), other works report polyploids with restored and high pollen fertility of more than 80% (Ramachandran & Nair, 1992) and increased pollen viability (Adaniya & Shirai, 2001; Jakše et al., 2003). The morphological, anatomical, and physiological traits associated with ploidy change can favor abiotic stress tolerance in plants. Polyploids with bigger, darker green, and thicker leaves, as well as greater resistance to abiotic stresses, have been recorded in chili (Kulkarni et al., 2008). Marzougui et al. (2010) reported improved salt tolerance in tetraploid fenugreek.

The effects of polyploidization on biochemical characteristics are well studied, most of which often manifest as alterations in the production pattern of plant secondary metabolites (Bagheri & Mansouri, 2015; Cao et al., 2018; Caruso et al., 2013; Madani et al., 2021). These changes in the metabolic profile of plants are attributed to altered genetic activity that follows genomic rearrangements. Studies across spice crops report an increase in the protein content and enzyme activity per cell in response to the increased DNA content (Marzougui et al., 2010; Talebi et al., 2017). On the other hand, studies also reported stable or even decreased enzyme levels (Zhou et al., 2020). Several studies have found that polyploidization can affect the yield and composition of essential oils. For example, Dijkstra and Speckmann (1980) and Prasath et al. (2022) reported an increase in oil yield, while Bertea et al. (2005) found changes in composition. The amount of essential oil in tetraploid *Carum carvi* has increased by 60%–85% (Zderkiewicz, 1962, 1964, 1971) and 30% in *Mentha arvensis* (Janaki Amal & Sobti, 1962) compared to diploids. However, some studies have reported a decrease in oil content (Ramachandran & Nair, 1992). Polyploidy induction can result in an alteration in biochemical composition and the metabolic profile. Increased secondary metabolites have also been reported in spices, some of which are an increased allicin concentration in onion (Dixit & Chaudhary, 2014) and enhanced capsaicin in hot chili (Pliankong et al., 2017). Besides, even the mixoploids generated through colchicine treatment exhibited variations in the chemical composition and plant parameters (Omezzine et al., 2014; Tammu et al., 2021). Amplification in secondary metabolite production including alkaloids, phenolics, and flavonoids can accelerate medicinal and pharmacological properties associated with it (Marzougui et al., 2012).

In general, the effects of artificial polyploidy induction can be unpredictable as it depends on intrinsic and extrinsic factors. In saffron (Samadi et al., 2022), increased expression levels of the prominent biosynthetic genes were not reproduced at the level of the metabolite. Further, these effects appear more complex, as evident from another study wherein the levels of most phytohormones were significantly lower in tetraploid garlic compared to diploid controls. This resulted in dwarfism in tetraploids due to altered hormonal regulation, but the prominent secondary metabolites showed an increased production level (Wen et al., 2022). All these studies thus point to a complex crosstalk between hormonal and metabolite production following genetic changes in polyploidization that finally result in altered phenotypes. The knowledge of these intrinsic molecular and biochemical mechanisms is crucial to achieving desirable polyploidization effects in crops of our interest.

6 | POLYPLOIDY AS A BREEDING STRATEGY FOR SPICES

The search for compounds extracted from natural resources that are pharmacologically potent and have few to no side effects for application in preventive medicine, cosmetics, and the food business has recently gained more attention on a global scale. Spices are an incredible source of phytochemicals, which makes them money-grubbing in the worldwide market. Since ancient times, it has been an integral part of world cuisines and pharmaceutics (Jiang, 2019). The demand for spices is dependent on the quality and quantity of the essential oil, oleoresins, and other secondary metabolites, which can be immensely affected by several factors, such as biotic and abiotic stresses, agro-climatic conditions, nutrition, and manuring (Askary et al., 2018; Selmar et al., 2017). The utilization of biotechnological approaches to accomplish the above through commercial propagation and the creation of novel kinds has increased dramatically over the past few years (Babu et al., 2015). Polyploids of ornamental and horticultural crops have been successfully developed in recent decades (Manzoor et al., 2019). Synthetic polyploidy has emerged as a vital technique for plant breeding, and the use of antimitotic drugs results in whole genome doubling, leading to profound phenotypic alterations.

For crops that are vegetatively propagated and tree spices, traditional breeding is difficult, as it takes several years for hybridization and selection processes, and it is impossible in self-incompatible and pollen-infertile lines. Therefore, additional breeding techniques like polyploid breeding and mutation are needed to produce breeds with enhanced genetics. Synthetic polyploidy can produce novel genotypes with improved morphological, physiological, and biochemical characteristics by altering plant genetic material. In 1937, the discovery by Blakeslee and Avery (1937) that treatment with colchicine can induce chromosome doubling in plant cells was a breakthrough in plant breeding, leading to the emergence of synthetic polyploidy. Although in vitro and in vivo polyploidy induction methods have been used for many years in breeding techniques, attempts to induce polyploidy in spices date back to the late 1930s. An initial report on polyploidy induction in spices was published by Pal and Ramanujam (1939). They studied the influence of colchicine on chili by immersing the seeds in different concentrations for different time intervals. Few of the treated plants exhibited various abnormalities in growth, such as slow growth, thickening of leaves, and doubling of flowers, and they were identified as tetraploid and triploid using chromosome counting. There were other reports on the colchicine treatment of chili seeds to induce polyploidy (Pal et al., 1941; Panda et al., 1984;

Raghuvanshi & Joshi, 1964). It is unusual to see octoploids produced directly from diploids. Panda et al. (1984) reported octaploid chilies from the colchicine treatment, where the size and vigor of the plants significantly decreased compared to tetraploids, indicating that an increase in the ploidy level is not necessarily accompanied by gigas effects. Similarly, Kulkarni and Brose (2010) demonstrated the potential use of colchiploidy to generate novel alterations in root systems. Colchicine treatment influenced the thickness and number of fruits in capsicum (Tammu et al., 2021). Hybridization of these novel polyploids with other materials with commercially important traits may provide promising avenues in the breeding procedure.

Ramachandran published the first study on induced polyploidy in ginger in 1982 (Ramachandran, 1982). Later, Ramachandran and Nair (1992) developed autotetraploids of ginger by treating rhizome buds with 0.25% colchicine solution. They reported more vigorous growth, high yield, and bold rhizomes in tetraploids. The tetraploid induction in *Z. officinale* has been the subject of numerous research (Adaniya & Shirai, 2001; Kun-Hua et al., 2011; Prasath et al., 2022; Ratnambal & Nair, 1982; Zhou et al., 2020). Smith and Hamill (1997) initiated an intriguing systematic research to increase the knob size of the ginger rhizome in Queensland, Australia. This group developed autotetraploids with large rhizomes, and one of the autotetraploids was chosen for commercial release as "Buderim Gold" after several seasons of field testing (Smith & Hamill, 2002; Smith et al., 2004).

The creation of synthetic polyploids enables quick genetic advancement in plants, making polyploidization one of the most significant and common technologies employed in plant breeding. The chances of being chosen for agronomical uses are augmented due to morphological and physiological traits coupled with polyploidy induction (Osborn et al., 2003). Scientists experimented with inducing autoploidy in the *Allium* genus. The genetic potential of *A. sativum* has been enhanced in vitro by the use of colchicine treatment and gives a potential key advancement for the enhancement of *Allium* species in the future (Hailu et al., 2020, 2021; Wen et al., 2022). Dixit and Chaudhary (2014) demonstrated that their tetraploids displayed an increase in bulb size and allicin production and expected an improvement in garlic production and yield. The size of the vegetative parts in *A. cepa* was similarly increased by anti-mutagenic treatment (Ren et al., 2018; Yun et al., 2021). Synthetic polyploidy is also exploited to develop double haploids (DH) in *A. cepa* (Alan et al., 2004, 2007; Campion et al., 1995; Fayos et al., 2015; Foschi et al., 2013; Geoffriau et al., 1997; Grzebelus & Adamus, 2004; Jakše et al., 2003) using an in vitro regeneration system. DH lines exhibited unexpectedly vigorous growth, and the resulting bulbs were big and homogeneous in morphology. The formation of gynogenic doubled haploids would result in perfect homozygous lines, permitting the evaluation of genetically complicated traits through multiple rounds of screening (Khan et al., 2020).

Saffron (*C. sativus*) is one of the most expensive spices, valued for its red stigma. The vital compounds are produced and stored in the thread-like flower stigma (Yue et al., 2020). Samadi et al. (2022) documented the effects of chemical mutagens (colchicine and EMS) on the floral characters and revealed the variation in the expression of genes involved in the biosynthesis of apocarotenoids. Polyploidy breeding can bypass challenges faced during *C. sativus* breeding programs as a sterile triploid.

Numerous researchers have also discussed the impact and influence of polyploidization on the synthesis of primary and secondary metabolites (Gantait & Mukherjee, 2021; Hailu et al., 2020; Samadi et al., 2022). Polyploidy induction in ajowan (*T. ammi* L.) has led to an increase in the thymol content of the induced tetraploids compared to the diploids, so this ploidy induction protocol can be used to accelerate the thymol content of ajowan, the major bioactive compound of this medicinal spice (Noori et al., 2017). Authors reported an increase in the yield of essential oil in *T. vulgaris* (Shmeit et al., 2020) and *Mentha piperita* (Zhao et al., 2022). Conversely, Navrátilová et al. (2021) could not find any differences in the total terpene content of thyme between tetraploids and diploids; however, they did vary in the relative amounts of each of the different terpenes. Similarly, no significant difference was observed in the gingerol content between the tetraploid ginger and its diploid parent (Wohlmuth et al., 2005). A significant increase in the morphine content and opium yield was exhibited by the tetraploid *P. somniferum* (Mishra et al., 2010). Nevertheless, the essential oil of the tetraploid cytotype of *Acorus calamus* contained up to 96% β-asarone, whereas diploid was distinguished by the lack of β-asarone (Bertea et al., 2005). Higher ploidy resulted in increased enzyme activity as well as phenolic and flavonoid levels in *Salvia officinalis* (Hassanzadeh et al., 2020), *Nigella sativa* (Gupta et al., 2021), and *T. foenum-graecum* (Marzougui et al., 2009). The method for creating polyploidy is well established in most of the spice crops (Table 1). Although there are certain areas yet to be explored, they provide new opportunities for spice crop improvement and breeding. The health benefits, economic worth, and industrial uses of spice crops have led to an upsurge in their demand on a global scale in recent years. Consequently, to satisfy consumer demand and prevent the extinction of certain spices, sustainable and continuous production techniques are needed.

7 | CONCLUSION

Spices, which are primarily cultivated through vegetative propagules, often exhibit limited genetic diversity. Artificial polyploidization has emerged as an effective technique in

spices breeding and crop improvement to enhance the characteristics of desirable traits by altering their genomic content. Mainly done with the help of various antimitotic agents, artificial polyploidy induction leads to the generation of higher ploidy levels in spices with changes in general morphology, physiology, and biochemical nature. The enhanced gene expression after polyploidization has resulted in increased secondary metabolite production in many spice crops. The selection, concentration, and duration of application of antimitotic agents for polyploidization vary depending on the species. When it comes to antimitotic chemicals for in vivo and in vitro methods, colchicine has traditionally been the preferred choice. However, it is worth exploring other options that may offer better polyploidization abilities while being less harmful to both plants and humans. In addition, techniques such as embryo culture have shown promise in achieving whole genome doubling in a simple and convenient manner through in vitro methods.

Earlier studies in other plant species have shown that the relationship between polyploidization and plant characteristics may not be as linear as it seems but may involve complex molecular and epigenetic mechanisms resulting in either enhancement or repression of gene expression, resulting in an altered phenotype. Hence, a deeper understanding of these mechanisms, along with the experimental improvements in polyploidy induction systems and protocols, enables us to better harness the polyploidization effects. The rapid advance of molecular approaches, such as multi-omics, high-throughput biology, and CRISPR genome editing, will speed up the understanding of artificial polyploidy biology and, in turn, result in novel mechanistic insights and novel polyploids in spices for cultivation.

AUTHOR CONTRIBUTIONS

Maria George Neenu: Conceptualization; data curation; investigation; writing—original draft. **Akkaraparambil Aswathi**: Formal analysis; validation; writing—original draft. **Duraisamy Prasath**: Conceptualization; methodology; project administration; supervision; writing—review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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