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Polyploidy and its relevance in crop improvement

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Abstract

Many areas of research in crop production have been geared towards crop improvement and increased yield. Crop improvement include but not restricted to; plant introduction and acclimatization, domestication, ploidy manipulation (polyploidization), recombinant DNA technology, crossing for superior selection (cultivar development), molecular genetics, etc. Polyploidy is a condition where the genome of an organism has more than the usual number of complete sets of chromosomes and the product of this phenomenon is called a Polyploid. Polyploidy occurs naturally, and can be induced chemically using antimitotic agents or physically using protoplast fusion and temperature shock. It is mostly artificially induced through a process called polyploidization. Polyploids are more advantageous in important plant attributes than the regular diploid. Relative success has been reported in the application of polyploidization for crop improvement which resulted chiefly in increased amount of beneficial secondary metabolites (phytochemicals), larger stomata and leaves, improved adaptation to stress and unfavourable conditions, to mention but a few. Therefore, it is imperative to state that polyploidy is an area of research that has been and will continually be deployed in crop improvement.

Keywords: Chromosome; colchicine; crop improvement; diploid; polyploidy; secondary metabolite
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Introduction

Over the years, agriculture has changed a great deal due to natural occurrences, government policies, urbanization, need for space, preference and other factors directly and indirectly affecting output. Crop improvement is an important aspect of agricultural discipline that offers lasting solutions to the dire need for food and raw material in our world today. Discovery of polyploidy in 1907 was a major event in eukaryotic evolution that took place in many plants, animals, and fungi (Yang et al., 2011). Polyploidy is the multiples in the chromosome number that is higher than the usual diploid sets and believed to play the major role in speciation and diversification (Soltis et al., 2009). Polyploidy is the possession of three or more complete sets of chromosomes, and this phenomenon is estimated to occur in 47 % to 70 % of all living angiosperms (Ramsey and Schemske, 1998). Sattler et al. (2016) opined that in the last century, polyploidy is undisputedly amongst the major channels of plant adaptation and speciation that has been extensively considered.

Traditionally, polyploidization has been contemplated to be an occurrence that mediates ecological differentiation (Castro et al., 2018). Historically, in the evolution of all organisms, it has been postulated that polyploidization has happened at least once and this is the major driving force in speciation (Blanc and Wolfe, 2004; Chen and Gao, 2007; Soltis and Soltis, 2009; Jiao et al., 2011; Sattler et al., 2016). Diversity of plant species is significantly affected during the evolution of flowering plants by polyploidy which is a regular process (Manzoor et al., 2019). Polyploidization plays a key role in plant breeding and crop improvement (Hailu et al.,

2021). Polyploidization can be induced by several antimitotic agents (chemicals that inhibit chromosome segregation during cell division); the most frequently used chemicals are colchicine, trifluralin, and oryzalin (Salma et al., 2017), although, colchicine is the major antimitotic agent employed, it is a toxic alkaloid obtained from *Colchicum autumnale* (Nilanthi et al., 2009).

Blakeslee and Avery (1937) initiated the approach of artificial mitotic polyploidization technique and effectively applied it in agriculture in the 1930s. Nevertheless, the first *in vitro* polyploidization was reported in tobacco (Murashige and Nakano, 1966). This technique is simpler to conduct *in vitro* (tissue culture) and more efficacious in induction of polyploidy due to the controlled atmosphere than in the greenhouse. Advancement in plant tissue culture has made the process more popular and successful since the 1990s. The *in vitro* aseptic culture of cells, organs, tissues, or whole plant under controlled nutritional and environmental conditions is known as tissue culture (Thorpe, 2007). In the tissue culture process, a whole new plant that possesses the genetic makeup and information as the mother plant is regenerated.

Different explants have been explored for effective polyploidy induction and further proliferation, but apical bud or shoot tips have been accepted as the explant of choice (Chen and Gao, 2007; Kaensaksiri et al., 2011; Tavan et al., 2015; Yan et al., 2016). Low concentration of antimitotic agent but longer exposure period exhibit better conversion percentage and thrive more (Salma et al., 2017). Many authors recommend full strength Murashige and Skoog (1962) (MS) medium for regrowth and proliferation of the polyploids (Gao et al., 2002; Tavan et al., 2015; Widoretno, 2016).

Adaniya and Shirai (2001) used shoot tips for polyploidization in 0.2 % colchicine for 8 days, regenerated in MS + 2.0 mg/l Benzyladenine (BA) + 0.05 mg/l Naphthaleneacetic acid (NAA) for Ginger (*Zingiber officinale*) and got 36.5 % polyploids. Gomes et al. (2014) used nodal bud for polyploidization in 30 µM colchicine for 1 week, regenerated in MS for (*Pfaffia glomerata*) - Brazilian ginseng and got 66.2 % polyploids. Castro et al. (2018) suggests the use of the lowest colchicine concentration tested, that is, 0.1 % colchicine and postulated the use of older seedlings as it increases survival rates. Chemically induced polyploids were obtained by colchicine treatment of shoot tips of hops (*Humulus lupulus* L. 'Sybilla') (Trojak-Goluch and Skomra, 2013).

There are two methods (direct and indirect) for the ploidy determination of plants. Indirect methods are simpler but more inaccurate; which involves the relationship between ploidy level (number of chromosome sets), morphological attributes (e.g. plant height, leaf size and pollen diameter) and anatomical structures (e.g. stomatal frequency and size, and chloroplast number in guard cells). Direct methods include chromosome counting in mitotic cells of root-tips using microscope and flow cytometry; the direct methods are accurate techniques for the determination of ploidy level in plants (Miri, 2020). It is noteworthy to state that in order to confirm the induction of polyploidy, deoxyribonucleic acid (DNA) of a known ploidy level is employed as a standard for direct methods while a diploid plant is used as a standard for indirect method.

Merits Of Polyploidy

1. *Breeding:*

Interestingly, many important crops contain polyploid sets of genomes naturally that attract plant breeders to utilize it as a means to artificially induce the introduction of important and desirable traits. Conventional breeding programmes are normally environment dependent and are susceptible to various biotic and abiotic stresses as well as the usually too low content of secondary metabolites. In this context, developing polyploid genotypes artificially would be a remarkable approach to increasing vigour and attain to this objective. Polyploids often exhibit some morphological features that are different or greater in forms than their diploid progenitors (Salma et al., 2017). In polyploidy, there is a formation of heterosis due to allopolyploids and heterozygous autopolyploids (types of polyploids) that confers transgressive performance and hybrid vigour compared to its diploid relatives (Birchler et al., 2010). In addition, the double dose of a gene due to the increased number of alleles in a polyploid corresponds to the masking of recessive lethal mutations (Gu et al., 2003).

Polyploidization plays a key role in plant breeding and crop improvement.

Important features in relation to plant breeding such as "gigas" effect (improved plant attributes), addressing deleterious mutations, increased heterozygosity, and heterosis (hybrid vigour) are inherent in polyploids (Sattler et al., 2016), which results in production of cultivars with higher yield levels, and increased tolerance to both biotic and abiotic stressors. Colchicine at different concentrations and time durations of exposure have been used to induce polyploidy in many ornamental crops through different application methods like dipping, whole plant immersion, soaking or use of cotton wool and lanolin methods (Manzoor et al., 2019). In comparison to diploids, polyploid results in a higher expression of genes (Majdi et al., 2014). Genetically, a polyploid organism possesses multiple copies of alleles which may be important in increasing allelic diversity, providing several evolutionary and adaptability advantages (Rauf et al., 2021). Polyploidization is a significant platform for restoration of the fertility of sterile hybrids, and for genetic transfer between species in which direct cross is not possible. More so, the application of polyploidy as a plant breeding tool has afforded plant breeders the potential of developing adapted and productive cultivars (Sattler et al., 2016). Above all, polyploidy has long been used in yield improvement of many crop plants and can be considered as one of the most promising tools in plant breeding programmes (Miri, 2020).

2. Improved adaptation

Polyploids can thrive in extreme environments, including xeric climates, subarctic regions, and high altitudes. It is hypothesized that polyploid species can prosper much more effectively than diploids for their vigorous morphological, physiological, and developmental differences that might be the reason for their higher stress endurance (Moghbel et al., 2015). In contrast to diploid species, it has been demonstrated that polyploids show advanced morphology and superior resistance ability to environmental stresses (Kaensaksiri et al., 2011).

Rao et al. (2020) induced polyploidy in *Lycium ruthenicum* and compared the abscisic acid signaling with the diploid. It was found that the tetraploids produced proteins that improve stress tolerance of the plants at the translational level. Therefore, by monitoring the phenotypic, hormonal and molecular changes induced by chromosome doubling, it was concluded that tetraploids exhibit superior drought resistance than diploids and that the internal environmental adaptation of tetraploids differed dramatically from that of diploids under normal growth.

Moreover, the polyploids exhibited larger stomatal aperture, enlarged vessel diameter, and reduced specific hydraulic conductivity that significantly offers superior drought tolerance than the diploids (Maherali et al., 2009). According to Rauf et al. (2021), it has been communicated in research that polyploid species possess substantial biomass yield, stamina and regeneration after grazing for forages, and superior tolerance to abiotic stresses than diploid.

3. Production of bioactive compounds

The non-existence of natural polyploidy in most genera and in view of the robust effects of polyploidy, the technique has been selected and applied in many important medicinal plants. Furthermore, in regard to the medicinal plants, there is an augmentation in the rate of secondary as well as primary metabolites production. For example, tetraploids of *Atropa velladonna* have alkaloid content 1.5 times higher than the diploids (Huang et al., 2010) while the *Salvia miltiorrhiza* exhibited higher terpenoids and flavonoids in tetraploids than the diploid plant (Gao et al., 1996).

Artificial induction of polyploidy may be a prospering approach to improve pharmaceutically important secondary metabolite production (Salma et al., 2017). Genetic multiplication of chromosomes inadvertently increases the production of secondary metabolites quantitatively and qualitatively in pharmacologically important medicinal plants (Majdi et al., 2010; Zahedi et al., 2014). Salma et al. (2017) reported that Research Institutes around the World are actively involved in artificial polyploidization of medicinal plants in order to investigate its significance in relation to production of bioactive compounds. Kim et al. (2021) recorded a dramatic increase in strong antioxidants (gentisic acid, naringin and salicyclic acid) and phenol production upon

tetraploidization of *Cnidium officinale*, an important medicinal plant in Asia.

4. Others

Polyploidy is important in horticulture for the development of new ornamental varieties with desirable morphological traits in terms of plant size and vigour, leaf thickness, larger flowers with thicker petals, intense color of leaves and flowers, long lasting flowers, compactness, dwarfness and restored fertility (Manzoor et al., 2019). Chromosome doubling of alleles may result in evolving new or varied functions (neo-functionalization or sub-functionalization), providing ecological niche expansion and increased resistance to diseases and environmental changes (Lynch, 2007). Some plants with successful polyploidization are listed in Table 1.

Table 1: Some plants with successful polyploidization

Crops	Old ploidy; New ploidy	Colchicine treatment; explant	Success recorded	Reference
Zingiber officinale Roscoe	2x=22; 4x=44	0.2 % for 8 days; shoot tip 0.15 % for 7 days; stem segment	Higher pollen fertility and germination ability, increase in carotenoids concentration	Adaniya and Shirai, 2001; Zhou et al., 2020
Vicia faba L.	2x=12; 4x=24	0.005 % for 8 hours; seed	Gigantism, bigger leaves, flowers and pods	Joshi and Verma, 2004
Vitis vinifera	2x=38; 4x=76	0.02 % for 24 hours; somatic embryo	Enlarged leaf stomata	Yang et al., 2006
Citrus sinesis	2x=18; 4x=36	0.1 % for 23 days; callus suspension culture	Production of a breeding material for seedless fruits	Zhang et al., 2007
Pennisetum purpureum x *Pennisetum glaucum*	3x=21; 6x=42	0.1 % for 24 hours	Larger stomata size	Campos et al., 2009
Pyrus communis	2x=34; 4x=68	0.4 % for 48h; leaf	Higher specific leaf mass and larger stomata	Sun et al., 2009
Feverfew (*Tanacetum parthenium* Schulz-Bip.)	2x=18; 4x=36	0.05 % for 6 hours; shoot tip meristem	Increased secondary metabolite (parthenolide), flower and leaves	Majdi et al., 2010
Humulus lupulus L.	2x=20; 4x=40	0.05 % for 48 hours; nodal explant	Increased morphological characteristics and secondary metabolite (humulene)	Trojak-Goluch and Skomra, 2013

Table 1 (continued): Some plants with successful polyploidization

Crops	**Old ploidy; New ploidy**	**Colchicine treatment; explant**	**Success recorded**	**Reference**
Orchid (*Dendrobium nobile* Lindl.)	2x=38; 4x=76	0.1 % for 96 hours; leaf	Increased number of internodes and floral pieces, greater height of flower and width of thelp	Vichiato et al., 2014
Nigella sativa L.	2x=12; 4x=24	0.05 % for 4 hours; seed	Increase in secondary metabolite (thymoquinone)	Dixit et al., 2015
Marigold (*Tagetes erecta*)	2x=24; 4x=48	0.1 % for 3-6 hours and 0.2 % for 3 hours; seed	Improved quantitative traits, larger stomata size and larger flower morphology	He et al., 2016
Anoectochilus formosanas Hayata	2x=24; 4x=48	0.1 % for 72 hours; nodal stem	Increase in important phytochemicals (total flavonoids and gastrodin)	Chung et al., 2017
Cannabis sativa L.	2x=20; 4x=40	0.2 % for 24 hours; apical meristem	Increase in cannabinoid	Mansouri and Bagheri, 2017
Chrysanthemum carinatum	2x=18; 4x=36	0.2 % for 72 hours; apical meristem	Larger flowers and seeds	Kushwah et al., 2018
Eclipta alba L. Hassk	2x=22; 4x=44	0.1 % for 24 hours; shoot tip	Increase in wedelolactone	Salma et al., 2018

Table 1 (continued): Some plants with successful polyploidization

Crops	**Old ploidy; New ploidy**	**Colchicine treatment; explant**	**Success recorded**	**Reference**
Stevia rebaudiana Bertoni	2x=22; 4x=44	0.05 % for 48 hours and 0.1 % for 24 hours; seed	Larger stomata, higher chlorophyll content indices and increased secondary metabolite	Zhang et al., 2018
Citrus limon L. Osbeck	2x=18; 4x=36	0.025 % for 24 hours; seed with radicle	Increase in essential oil and limonene	Bhuvaneswari et al., 2019
Goldenberry (*Physalis peruviana* L.)	2x=24; 4x=48	0.6 % for 24 hours and 36 hours; seed	Increase in total anthocyanin, flavonoid and phenol	Çömlekçioğlu and Özden, 2019
Lemon balm (*Melissa officinalis* L.)	2x=32; 4x=64	0.05 %, 0.1 % for 24 hours and 48 hours; seedling	Increase in carotenoid, flavonoid and phenolic content	Talei and Fotokian, 2020
Lyceum ruthenicum	2x=24; 4x=48	0.1 % for 48 hours; leaf	Induced the expression of osmotic proteins to increase the stress tolerance of the plant	Rao et al., 2020
Garlic *(Allium sativum* L.)	2x = 16; 4x=32	0.25 – 0.5 % for 24, 36 and 42 hours; meristematic basal disc	Increased genetic potential, greater bulb size and larger tuber size	Hailu et al., 2021

Conclusion

Polyploid organisms have been reported to often display increased vigour and, in most cases, outperform their diploid relatives in several aspects. This innate capacity of polyploids has been the target of many plant breeders in recent times and time past. With the advent of polyploidy, more beneficial secondary metabolites useful in pharmaceutical and food industries can be isolated from plants, which will however give prospects to polyploidy research on more crops. It is noteworthy to state that chromosome doubling may not increase the production of bioactive compounds in all plant species.

Polyploidy may be much helpful and priceless due to the increased content of active ingredients, higher yield levels, and increased tolerance to both biotic and abiotic stresses. In the case of ornamental plants, the increased size of the flowers is of much aesthetic value and can adequately add more value economically.

Reports on the prospects of polyploidy in more common plants have not been so recorded; nevertheless, its (polyploid) usefulness cannot be over emphasized in plants where successful polyploidization has been reported. Polyploidy is also a good teaching tool for indicating plant transformation. More so, tissue culture seems to be a veritable tool for achieving, maintaining and massively regenerating transformed (polyploids) genotypes.

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