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Polyploidy: A Novel Technique for Improvement of Vegetable Crops

(*Twinkle, T.[1], Diksha, R.[1], Emmi, R.[1] and Sarkar, M.[2])
[1]Department of Vegetable Science, ASPEE College of Horticulture, NAU, Navsari
[2]College of Agriculture, Waghai (The Dangs), NAU, Navsari
*Corresponding Author's email: twinkletandel8110@gmail.com

Polyploidization refers to the multiplication of a complete chromosome set of a certain species to give birth to a new species. These multiple sets of chromosomes coexist in one nucleus and can be stably inherited to progenies. It was first discovered in 1907 and is one of the more prominent mechanisms of speciation and diversification in higher plants. The estimation of polyploidy frequency in angiosperms (flowering plants) is widely variable in the literature, ranging from 30-80% for with most estimates about 50-70%. It is responsible for increasing genetic diversity and producing species with increased size, vigour and resistance to diseases. Thus, the production and application of polyploidy breeding have brought remarkable economic and social benefits (Can, 2012). Paleo-polyploid species evolved from polyploid ancestors that underwent polyploidization event, but they existed as diploids themselves that re-diploidized through the reshuffling and rearrangement of multiple sets of parental chromosomes in their polyploid ancestors (Fig. 1, A). Neo polyploids possessed multiple sets of chromosomes at the time of polyploidization when the parental chromosomes merged, and they were independent of each other (Fig. 1, A). Polyploids are not only ubiquitously distributed in flowering plants but also are widely found in lower plants, such as gymnosperms, ferns, and diatoms, as well as in animals, including frog, fish and, insects, and mammal. Along with the rapid development of genome sequencing and analysis technologies, the number of sequenced genomes has significantly increased in recent years, which greatly facilitates comparative genomic studies on polyploidy evolution. To date, increasing polyploidization events have been identified in plants, rendering polyploidy one of the most topical research fields in plant science.

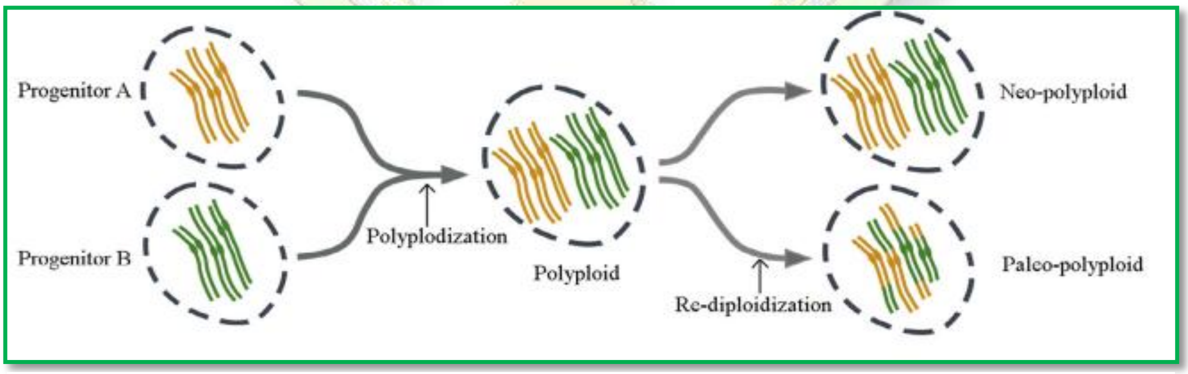

Fig. 1 Life cycles of plants experiencing recurrent polyploidization and re-diploidization events

Types of Polyploidy

According to the status of the parental chromosomes following polyploidization, polyploids can be divided into two groups: paleo and neo-polyploids

1. Types based on origin

A. Naturally occurring: Produced due to mutation in gene which codes for the spindle forming protein during cell division, or due to the production of unreduced gametes.

B. Induced: Induced by application of colchicine @ 0.01-0.5% (or other chemicals like acenaphthene chloralhydrate, sulphanilamide, ethyl mercury chloride, colchicine, oryzalin, trifluralin etc) which dissolves the spindle fibber during cell division and results in chromosome doubling. Blackslee and Avery (1937) first showed method of induction in Portulaca and Datura plants by application of alkaloid colchicine (isolated from autumn crocus plant, Colchicum autumnal).

II. Types based on genome content

A. Auto-polyploids: Multiple chromosome sets derived from a single species and can arise from a spontaneous genome doubling (e.g. potato) or fusion of 2n gametes.

B. Allo-polyploids: Multiple chromosomes derived from different species and they are result of multiplying the chromosome number in an F1 hybrid.

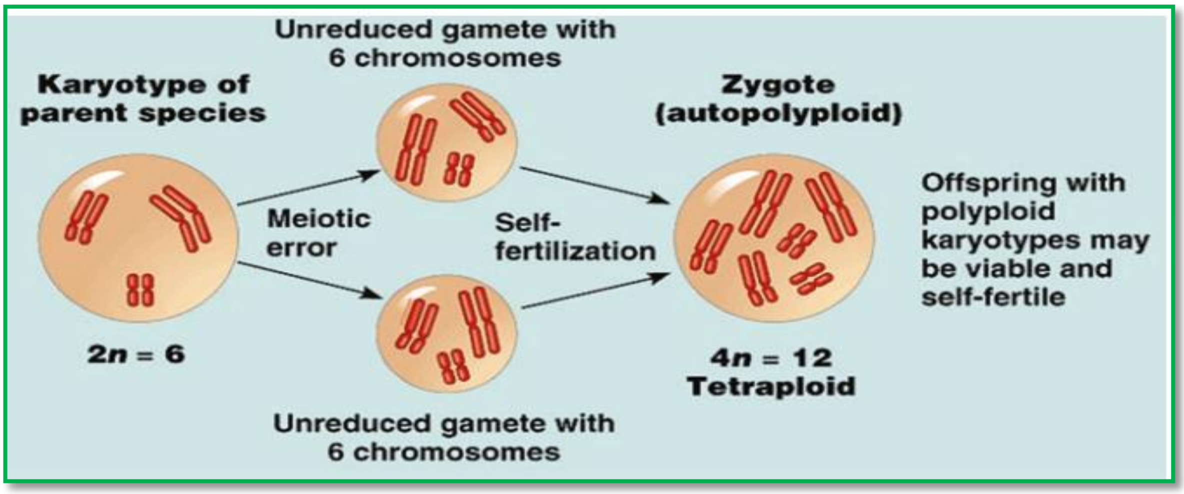

Fig.2 Showing the origin of autopolyploid from unreduced gametes

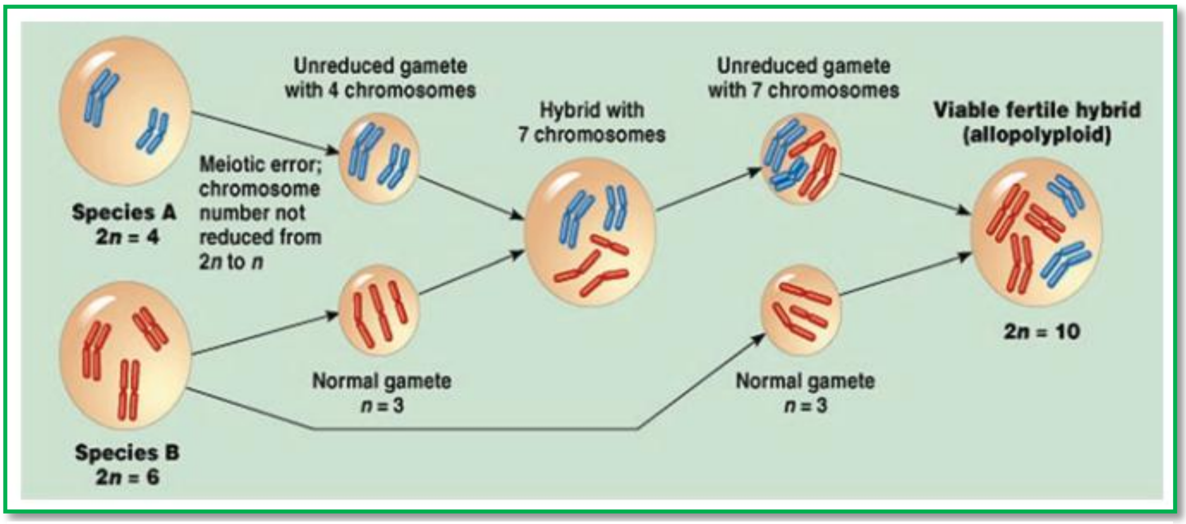

Fig.3: Showing the origin of allopolyploid from unreduced and reduced gametes

Advantages of Polyploid

The advantages of polyploids are heterosis and gene redundancy. Heterosis causes the polyploids to become more vigorous than their progenitors, whereas, gene redundancy shields polyploids from the deleterious effects of mutation in one or more vital genes. The most widespread consequence of polyploidy in plants is the increase in cell size, due to genome duplication, which is known as the gigas effect. Therefore, organs of polyploid individuals may exhibit an increase in size compared to their diploid progenitors, such as roots, leaves, tubercles, fruits, flowers and seeds.

Methods of Polyploidy Induction

It was generally assumed that polyploids in plants are induced through two mechanisms: sexual (meiotic) polyploidization or somatic (mitotic) doubling in meristem tissue of sporophytes (Ramanna and Jacobsen 2003).

1. **Sexual polyploidization**: Before the discovery of colchicine in the 1930s, meiotic polyploidization was commonly used for obtaining polyploids (Ramsey and Schmeske 1998; Ramanna and Jacobsen 2003). This process involves the generation of unreduced (2n) gametes during gametogenesis, which contain the full somatic chromosome number. Meiotic aberrations related to spindle formation, spindle function and cytokinesis have been implicated as the cause of unreduced gamete production (Ramsey and Schmeske 1998). The mechanisms of 2n gamete formation can be divided into three developmental-specific classes: pre- and post-meiotic genome doubling and meiotic restitution. Pre- and post-meiotic genome duplication is only rarely recorded in plants, whereas meiotic restitution is the predominant mechanism of unreduced gamete formation. In this process, meiotic cell division is converted into a mitosis-like nonreductional process, generating dyads (and triads) instead of the normal tetrads at the end of meiosis II (De Storme and Geelen 2013). The fusion of reduced (n) and unreduced gametes, or of two unreduced gametes, can produce triploid and tetraploid embryos, respectively. It is believed that unreduced gametes (2n pollen or 2n eggs) to be a major mechanism of polyploid formation (Ramsey and Schmeske 1998).
2. **Somatic polyploidization:** Somatic polyploidization implies the induction of chromosome doubling in somatic tissues, and has been performed in several crop species (Sattler *et al.* 2016). There is a wide range of natural and synthetic compounds that are reported to interrupt the cell cycle mainly in the late metaphase stage and known as the antimitotic agents (Ascough *et al.* 2008; Salma *et al.* 2017). Initial efforts to induce somatic polyploidy were made through other methods, such as exposure to high or low temperature (Blakeslee and Avery 1937). An increased frequency of tetraploid cells was observed in root-tips of *Pisum sativum* and *Zea mays* plants which had been exposed to hot water at 40 °C (Randolph 1932). Colchicine is a toxic alkaloid extracted from seeds and bulbs of *Colchicum autumnale* L. and the most widely employed antimitotic agent for polyploidy induction (Sattler *et al.* 2016). Pliankong *et al.* (2017) demonstrated that the induction of polyploidy in *Capsicum frutescens* by colchicine was more effective than oryzalin. Roughani *et al*. (2017) treated the seeds of *Spinacia oleracea* with colchicine, trifluralin and oryzalin and found that all three antimitotic agents could be effective in the increase of polyploidy induction, but oryzalin was preferred for its low toxicity, low cost and ability to increase ploidy levels at lower doses.

Application of Colchicine

A. Seed treatment: Imbibed and germinating seeds can be treated with 0.01 to 0.8 % colchicine in solution or in agar media for 3-4 hours. Followed in plants, which become large and woody within few days after germination due to faster growth rate.

B. Immersion method: Flexible twigs are kept immersed in 0.25 % colchicine solution for one day.

C. Mixtures with lanolin: Mixtures with lanolin have been successfully used in inducing 4n tissue but considerably higher concentration of solution is needed.

D. Covering the bud with cotton: The solution is soaked in a piece of cotton and the target bud is kept covered with that cotton. This is most commonly used method.

E. Capillary string method: A string is immersed in solution and the other end wrapped around the bud and the colchicine solution moves by capillary action.

F. Single drop application: A single drop of colchicine solution is applied on the target bud and repeated several times. It is more economical as very less solution is needed.

Detection of Higher Ploidy Level

Conventionally ploidy levels are determined by counting the chromosomes of meristematic tissue (i.e. root tips at metaphase phase of cell division) from individual plants. However, to screen large number of plants requires trained skills, labour intensive, time and resource consuming as well. Among the several alternative and indirect methods of ploidy determination, the following one are generally employed

a. Counting chloroplast number in the stomatal guard cell: positive correlation exists between the number of chloroplasts in the guard cell and the ploidy level in many plant species.

b. Measuring stomatal length: Length of the stomata is measured under ocular microscope. Longer the stomata, higher the ploidy within the same species.

c. **Measuring pollen grain diameter**: plants having higher ploidy levels produce larger pollen grains.

d**. Flow cytometry**: It is a method of measuring nuclear DNA content in plants. It more accurate than the above three methods. However, this method requires the use of expensive equipment's.

Polyploid varieties released in vegetable crops and their salient feature

Variety	Crop	Salient features
Sree Harsha	Cassava	Triploid, stout non branching plants, yields 35-40 t/ha and contains starch 39.05
Pusa Jyoti	Palak	Tetraploid, very big, thick, tender, succulent dark green leaves, quick rejuvenation, yields 50 t/h
Arka Madhura	Watermelon	Triploid seedless, TSS 13-14 %, longer shelf life and transport quality, suitable for year-round production under protected condition, yields 50-60 t/ ha
Pusa Bedana	Watermelon	Seedless triploid hybrid having aborted embryos and false, rudimentary, least perceptible seeds.

Ploidy Manipulation in Vegetable Crops

1. **Triploid Watermelon (3x = 33):** Triploid seedless watermelons were first produced by Dr. Kihara who started the experiment by treating a normal diploid (2x = 22) plant with colchicine to obtain a tetraploid from (4x = 44), and then used the pollen of the diploid to pollinate the stigma of the induced tetraploid, producing the triploid progeny (Crow, 1994). Since the triploids were infertile, they did not produce sufficient viable pollen for pollination and fruit development. Consequently, diploid plants were planted in the same area of the triploids to supply the required pollen for seedless fruit production. Seedless watermelons are popular in Israel, Japan and the Northern territory in Australia. In India, it is slowly gaining popularity among the consumers.

2. **Triploid Sugar Beet:** Sugar beet is a crop of major importance for sugar production in temperate areas, and exists in diploid (2x = 18), triploid (3x = 27) and tetraploid (4x = 36) forms (Smulders *et al.* 2010). Triploid sugar beets are produced by crossing male diploid sterile plants with tetraploid pollinators or by reciprocal crossing, i.e., between male tetraploid sterile plants and diploid pollinators (Kinoshita and Takahashi 1969). Triploid sugar beets have larger roots than diploid ones, but maintain the same sugar content of the diploids and thus yield more sugar per unit area. Moreover, triploid sugar beets are highly sterile and do not produce seeds, which is not a disadvantage, since only their roots are relevant for commercial purposes (Dabholkar 2006).
3. **Polyploid Onion**: McCallum (1988) treated F1 of Allium cepa × Allium fistulosum and reciprocal crosses with colchicines and recorded C2 population with good seedling vigour and winter hardiness at Beltsville during the winter as compared to normal diploids.
4. **Polyploid Asparagus**: Sheidai and Inamdar (1992) studied meiosis in distinct polyploid species of Asparagus. The tetraploid species (n =20) A. *racemosus var. javanica, A. densiflorus* cv. Myers, *A. racemosus* var. *subacerosa* and hexaploid species (n = 30). A. gonoclados recorded high pollen fertility and fruit set. This was due to regular disjunction of chromosomes at anaphase. Further, the tetraploid species had larger pollen grains than diploids.
5. **Polyploidy in the *Genus Brassica* L.**:Nagaheru (1925) proposed the evolution theory of natural-amphidiploid Brassica species from three diploid species.
6. **Fenugreek:**Marzougui *et al.* (2009) evaluated morphological and chemical comparison of diploids and induced autotetraploid of *Trigonella foenum-graecum* and found that the autotetraploid had higher leaf area and productivity concerning the number of seeds, pods and branches compared to the diploids. Its leaves also are richer in potassium, sodium, calcium and phosphorus.

Conclusions and future perspectives

- Polyploidy is associated with extensive structural, developmental, physiological and biochemical changes in plants that result in wide variation in these traits. Therefore, it can be said that polyploidization provides new options for plant breeders to induce ex vitro and in vitro synthetic polyploids and select suitable plants depending on the purpose such as medicinal, ornamental and resistance applications, and so on.
- Polyploids are also important as bridges for germplasm transfer between species where direct crossing is not possible, as well as to restore the fertility of sterile hybrids.
- Polyploidy generally results into gigantism in plants. More beneficial for those crops like potato, sweet potato, taro, yam and the leafy vegetables where vegetative parts are consumed.
- Natural polyploids in potato and sweet potato helped to increase the storage organs and became the major source of starch worldwide.
- Production of Doubled Haploid (DH) plants reduced the breeding cycle as well as helped the breeders to generate F2 mapping population in crops like carrot and onion which show very high rate of inbreeding depression.
- Diplotization of the chromosomes helped to overcome fertilization barriers with distantly related species.
- Seedlesness is achieved in watermelon through triploidy.

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