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Production of triploids and seedless genotypes in different horticultural crops

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Abstract

Seedlessness is a desirable character in fruit crops and fetches good prize in domestic and international markets owing to their high quality. There are certain causes of evolution of seedlessness in fruit crops ranging from evolutionary changes, genetic makeup, induced hybridization, mutations, and polyploidy etc. However seedless fruits have certain limitations like low recovery of seedless types and lack of proper markers for judging it at early age. Triploidy is one of the major ways to induce seedlessness in fruit crops which comes in with other advantages like prolonged flowering period, greater biomass, ease in genome evolution and genome plausibility. Natural Selection, artificial hybridization, endosperm culture *in vitro* and fusion of somatic diploid protoplasts with haploid microspore cells are possible traditional ways to induce triploidy in fruits along with some recent biotechnological advances. Barring some limitations production of seedless fruits is highly advantageous as in year-round production, processing, gustatory advantages etc. and thus need to expanded in times to come.

Keywords: Endosperm, hybridization, polyploidy, seedlessness, triploidy

Introduction

Seedlessness is a relative phenomenon where it may be due to occurrence of the following situations namely reduction in size of the seed, number of seeds per fruit, presence of soft/semi soft (Mellowness), rudimentary/papery seed. Seedlessness may be due to genetic/other factors like incompatibility, triploidy, gynoecey or due to cultural and environmental conditions. Genetically seedless fruits are produced as a result of Parthenocarpy. Seedlessness is appreciated by consumers both for fresh consumption (e.g., grape, citrus, and banana) as well as in conserved or processed form. This is of much more importance in types that have strong, hard seed and impart off flavour and taste. Further, seeds in some cases, can produce substances that accelerate the senescence (Sajana *et al.*, 2020)^[15]. The independence (whole or partial) of fruit development from pollination and subsequent fertilization is advantageous in horticultural crops in case where rate of fruit set is low. Pollen development, maturation and fertilization are affected by environmental factors such as light, temperature, relative humidity as unfavourable environmental conditions tend to drastically affect pollination and fruit development. These problems are occurring in crops/varieties where parthenocarpic fruit development is the norm. In horticulture, parthenocarpy can be exploited for timing the production regarding the ready availability of fruits for the fresh horticultural produce in all seasons.

Characteristics of seedless fruit

- Seedless fruits are generally smaller in size compared to their seeded counterparts.
- In seedless fruits, other forms like reduced seed number, soft seed, hollow seed (floater) (Ex. grapes) can also be noted.
- Seedless fruits may result in thickening of pedicels in some cases like pear.
- Fruit surface is normally very smooth in case of seedless fruits compared to seeded counterparts except in case of guava where the surface is warty.
- Seedless fruits in general mature later than their seeded counterparts.

Need for Seedless fruits

- Seedlessness is a desired trait by consumers & buyers owing to better eating quality.
- Seedless fruits generate less waste and therefore are preferred by processors.
- It improves the acceptability plus it creates the novelty in fruit crops.

Reasons/ Causes for formation of Seedlessness in fruit crops

1. Evolution caused majorly due to spontaneous mutation in nature. Although the biological function of fruiting is the production and dissemination of seeds, humans have developed seedless fruits in several plant species to facilitate consumption
2. **Genetic makeup:** Seedlessness in many fruits is a highly desirable trait and is due to natural causes, not genetic engineering techniques. All seedless fruit fall under a general category called parthenocarpy and can be vegetative, stimulative and in some cases stenospemocarpy is also observed.
3. Hybridization & complex nature (due to several processes/ existence of extra chromosome satellite chromosomes)
4. Induced mutations/ Isolation of natural bud/limb sports
5. Polyploidy (Accessions of triploids- Apple, pear, Tahiti: All aneuploids)
6. Parthenocarpy
7. Incompatibility owing to pollen deformity, sterility (Self-sterility in Litchi)
8. Climatic conditions (Fig)
9. Location (Latitudinal changes- Eg: Grapes)
10. Chromosomal imbalances due to polyploidal crosses
11. **Other factors:** Damage by insects, abnormal/unseasonal adverse weather condition growth tensions and plant growth conditions like excessive vegetative growth reduced crop density, root destruction, tree injury may also lead to seedlessness.

Difficulties in developing Seedless varieties in fruit crops

- a. In conventional breeding, only seeded genotypes can be used as seed parents, hence there is lot of limitations.
- b. When seedless genotypes are used as male parents, the pollen quality, viability etc. vary considerably and hence the results are not consistent.
- c. The average frequency of seedless progeny in a cross involving seedless parent can only give rise to 10-15% (on avg.) seedless progeny which is further reduced by poor germination.
- d. Lack of availability of morphological marker for adjudging seedlings at the early age
- e. In the present day, the traditional breeding involves use of genotypes with high seed index namely Hur, Anngurkala, Kattakurgan (Grapes) which are expected to give high proportion of seedless progeny.
- f. Introgression breeding to transfer berry quality and seedlessness and an alternative approach involving different crosses (A x B and A x C)
- g. In-ovulo embryo rescue and use of marker: In present day, several grape breeding programmes globally following in-ovulo embryo rescue followed by recovery of seedless progeny from crosses involving Seedless x Seedless genotypes. In such population the time for evolving seedless hybrid is reduced to 5-6 years compared to 12- 15 years under the conventional system.

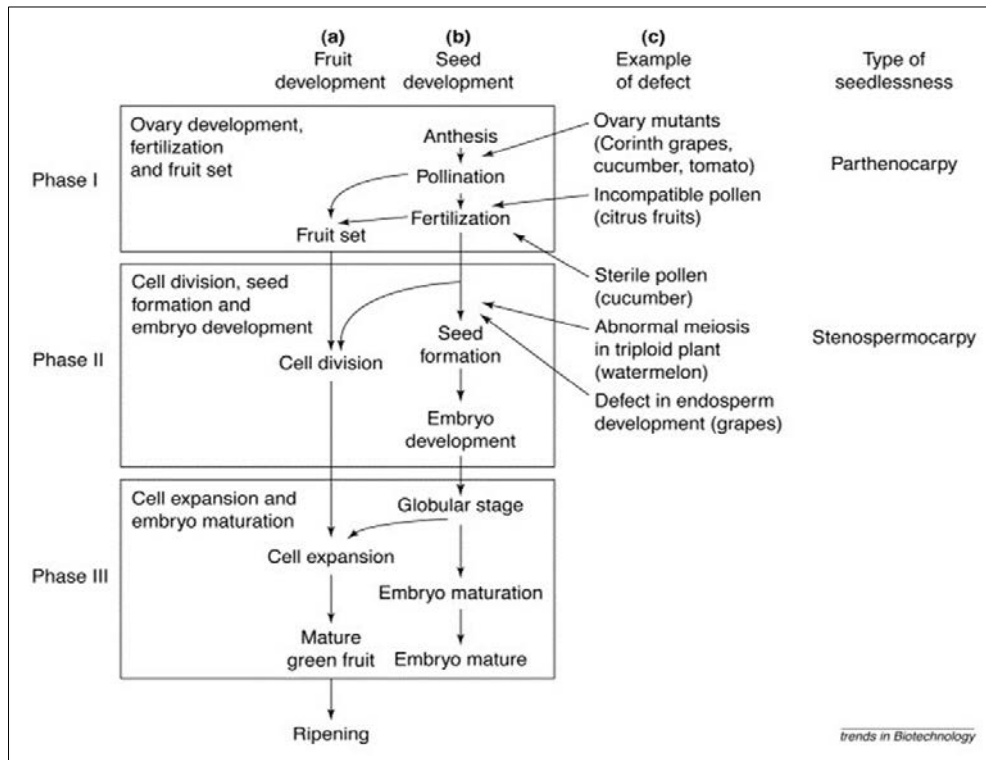
Mechanism of fruit development and seedless fruit formation

In most plants, early fruit development can be divided into three phases. Earliest phase involves the development of the ovary and the decision (signal, stimulus, and process of development) to abort or to proceed with further cell division, differentiation and fruit development which is generally referred to as fruit set. In second phase, fruit growth is primarily due to cell division. The third phase begins after cell division ceases. During this phase, fruit growth continues, mostly by cell expansion, until the fruit reaches its final size. This growth phase is the most visible and physiologically most significant because of the strong sink activity exerted by the expanding cells. All the stages and possible seedlessness stage and reason are shown through following figure (Varoquaux *et al.*, 2000) ^[17]. Cell expansion commonly increases fruit size by 100-fold, and this makes the greatest contribution to the final size of the fruit. At the end of early development, a green fruit is obtained, which has the size of a mature fruit, and the maturation phase occurs from this point onwards. During early fruit development, many pathways of communication between the sporophyte and the gametophyte are established. It is generally considered, for instance, that the decision of whether to set fruit is dependent on the successful completion of pollination and fertilization. During early fruit development, many pathways of communication between the sporophyte and the gametophyte are established. It is generally considered, for instance, that the decision of whether or not to set fruit is dependent on the successful completion of pollination and fertilization.

Triploids and its role in seedlessness

Triploidy is the term referring the presence of three sets of haploids (single) chromosomes in an organism or cell line and is termed $3n$, where $1n$ is the haploid chromosome number for the species concerned (Wang *et al.*, 2016). Triploids are both euploid and polyploid in a sense that they contain a completely balanced extra set of chromosomes to the normal diploid ($2n$) state. Triploid plants have three sets of chromosomes, and many desirable characteristics, including greater vigour; broad, thick, dark green leaves; and larger flowers or fruit, which result in higher yield or higher harvest index. For example, the Vertigo watermelon variety ($2n = 3x = 33$) has produced the highest watermelon yields (41,000 lb/acre). Triploid cassava also has a high yield with outstanding culinary and industrial qualities. Triploid plants produce seedless fruits in different species like citrus, banana, and watermelon. Only in citrus, international markets demand fruits without seeds and this characteristic is one of the most important for citrus and with special emphasis in mandarins. Sterile triploid crop and horticultural plants can reduce or eliminate the undesirable spread of non-native invasive crop plants that produce numerous seeds into natural areas. Thus, triploid plants will play an even more important role in agriculture, forestry, and ecology in the future.

One of the new trends in genomic research is to create synthetic polyploid plants to provide materials for studying initial genomic responses immediately after polyploid formation. Thus, triploid plants have attracted more attention and there has recently been great progress in understanding the details of their formation after decades of investigation.



Characteristics and application of triploid plants

- 1) Prolonging flowering period:** Although one of the chief characteristics of true triploids is partial or total sterility, this sterility can be horticulturally useful. Flowers of triploid plants are generally larger and more colorful than those of their diploid counterparts partly because the energy that is normally devoted to seed formation is used for flowers or other organs. Triploid flowers often have longer shelf life and the triploid plants require little or no 'dead-heading' (the removal of faded or dead flowers from plants to maintain both a plant's appearance and to improve its overall flowering performance).
- 2) Neutralizing invasive plants:** Gene flow mediated by pollen has also been demonstrated between commercial cultivars and weedy relatives. Thus, sterile triploid cultivars can be a vital strategy for reducing the invasiveness of crop plants. Many invasive plant species are considered noxious because they produce massive amounts of seeds, which can be dispersed by birds or other means and colonize surrounding areas of native flora, resulting in major transformation of ecosystems such as forests, roadsides, parks, preserves, wildlife refuges, and urban areas. However, if this seed production can be blocked, these plants may behave well as crops or high-quality ornamentals without this invasive tendency. One potential solution good for both the horticultural industry and for the environment is to create seedless versions of plants that have been shown to be, or that have potential to be invasive. Thus, seedless triploid varieties can play an important role in neutralizing the invasiveness of introduced plants.
- 3) Triploid plants with larger organs and greater biomass:** Triploid production increases the size of somatic cells and guard cells, and increases chloroplast number, which results in strengthening photosynthesis. Therefore, many triploid plants are relatively more vigorous; have short internodes; broad, thick, dark green leaves, resulting in greater biomass or crop yield per

plant. Hoshino *et al.* (2011) ^[7] found that triploids, including cassava (*Manihot esculenta* C.), watermelon, little gourd (*Coccinia grandis* (L.) J. Voigt), had higher yields and higher starch content. Today, over 80% of the watermelons produced in the US are seedless triploid. The triploid seedless watermelon commands premium prices because of its high-quality flesh that is virtually free of seeds. The protein content of triploid mulberry leaves is 4.14% higher than that of diploid mulberry. Therefore, the edibility and digestibility of triploid mulberry leaves is higher for silkworms. When fed triploid mulberry leaves, silkworms grow more rapidly, which reduces the length of their life cycle by about 2–3 days and increases whole cocoon weight, cocoon layer weight, and pupal weight over those fed with diploid leaves. Cocoon production is also increased by 14–16%, and fecundity improved by about 11%.

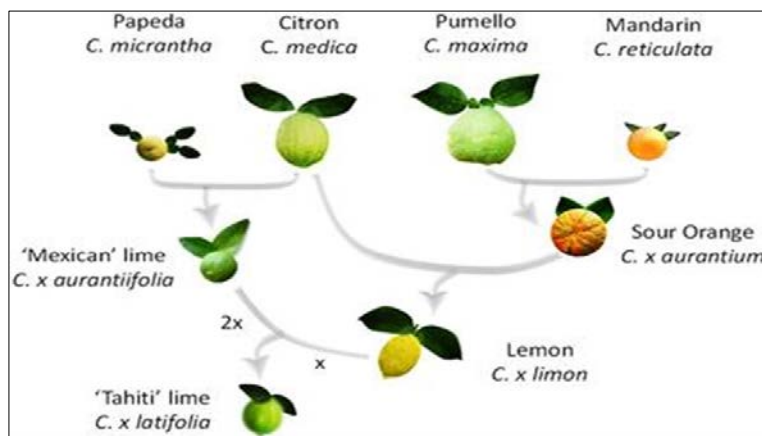
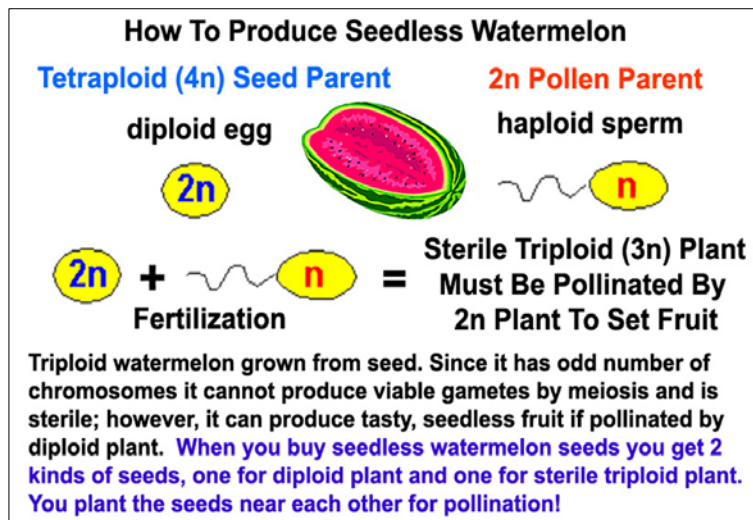
- 4) Use of triploid plants for studying genome evolution and genome plausibility:** Triploid plant species provide some unique characteristics for genome studies. After genome doubling, genomic characteristics at the individual or population level can be affected, including imbalances in gene dosage, genetic or epigenetic changes, genome size, genomic diversity, and genomic rearrangements. The DNA methylation status of newly formed species appears to be consistently affected following polyploidization. Triploids, which carry three complete sets of chromosomes, in particular produce offspring with different chromosome numbers, including diploid and tetraploid progeny, as well as a swarm of aneuploid progeny. Thus, there is strong selection against imbalance in gamete ploidy in crosses between triploids and diploids, even in the absence of aneuploidy.

Triploidy in different horticultural crops

- 1. Triploidy in Watermelon:** Seedless watermelons are gaining popularity as both their seeds and the mature melons become more available in the general market.

These watermelons have excellent flavor and good disease resistance. They ship well due to a good, thick rind. The seedless melons may also have a longer shelf life than standard watermelons. Standard watermelon cultivars are diploid, and contain two sets of chromosomes, designated 2X. Seedless watermelons are

triploid (3X) which causes them to be sterile, or seedless. The triploid seeds are created by crossing a normal diploid (2X) melon as the pollinator with a tetraploid (4X) parent. Each parent contributes half its respective chromosomes, 1X from one parent and 2X from the other (Wijesinghe *et al.*, 2020)^[19].



Triploidy breeding in citrus

The triploid 'Tahiti' lime (*C. x latifolia*) (Yu. Tanaka) Tanaka naturally originated from a merger between a haploid ovule of lemon (*C. x limon* (L.) Burm) and a diploid pollen from a 'Mexican' lime (*C. x aurantiifolia*) (Christm.) Swing). Most *Citrus* species and related genera are diploid with a basic chromosome number $x = 9$. However, some triploid and tetraploid plants were encountered in the citrus germplasm. The triploid Tahiti Lime has been bred as shown in fig of crosses between Citrus family (Ahmed *et al.*, 2020)^[2].

Ways to produce Triploid Plants

There are 4 major ways to produce triploid plants which are as follows:

1. Natural Selection
2. Artificial Hybridisation
3. Endosperm culture *in vitro*
4. Fusion of somatic diploid protoplasts with haploid microspore cells

Natural Selection: Natural triploid poplar, especially *Populus tremula* L., is widely present in nature. In 1936, Nilsson discovered a natural triploid of *Populus tremula* L. in Sweden. It is named gigas form of *P. tremula* due to its huge

leaves, rapid growth, and tall stature. Compared with other trees of the same age, it has obvious advantages, therefore, it aroused the interest of scholars in various countries who carried out research on this variant. These triploid poplars also had more desirable characteristics of volume growth, resistance, stem straightness, and fibre than did diploids of the same species.

Unreduced gametes seem to occur more frequently when plants experience environmental stresses, such as frost, wounding, herbivory attack, and water or nutrient shortage. Noticeably, the frequency of unreduced gamete production occurs up to 50-fold more often in hybrids between divergent genomes than in non-hybrid systems. Nishiwaki *et al.* (2011) found that new, naturally derived *Miscanthus* (*Poaceae*) triploid genotypes were identified more efficiently by flow-cytometry screening of seeds harvested from areas where tetraploid *M. sacchariflorus* F. plants grow sympatrically with diploid *M. sinensis* F. plants, than by random identification of triploids in the field.

Artificial Hybridization: Triploid plants can be recovered by $2x \times 2x$, $2x \times 4x$, $4x \times 2x$ or $2x \times 3x$ sexual hybridization. Most conventional methodology is from $2x \times 4x$ and $4x \times 2x$ hybridization. Since 1996, the Plant Protection and

Biotechnology Center of the Instituto Valenciano de Investigaciones Agrarias (IVIA, Moncada, Spain) has developed an extensive citrus triploid breeding program based on interploid sexual hybridizations. A lot of citrus triploid hybrids have been successfully recovered from different $4x \times 2x$ and $2x \times 4x$ sexual hybridizations. Most natural species and hybrids are diploid and spontaneous tetraploidy is extremely rare. So artificial induction of tetraploid lines is necessary. Doubling of the diploid chromosome number may be achieved using spindle inhibitors, mutation breeding, protoplast fusion mediated by electricity or PEG. Mechanical damage such as top pinching repeatedly can also achieve tetraploidy. Colchicine was one of the most used spindle inhibitors and has been used to good effect in numerous plants either *ex vitro* or *in vitro*. Oryzalin, and trifluralin were also used to disrupt spindle formation and preventing nuclear and cell division. The effectiveness of these compounds depends strongly on the concentration applied, the duration of treatment, the type of explant, and the penetration of the compound. Colchicine has been used effectively in concentrations ranging from 0.25 mM to 38 mM. Dimethyl sulfoxide (DMSO) can improve the permeability of drugs. But colchicine-induced tetraploids *ex vitro* was confronted with low mutation rate, high chimeric rate, and reverse mutation, were difficult to select and vulnerable to environmental disturbance. Fusion of protoplasts together with colchicine-induction created homogenous tetraploid of *Citrus reticulata* Blanco. But fusion of protoplasts requires too much technical expertise.

Triploid production efficiency is determined by pollen viability, parents compatibility and the frequency of unreduced gametes. The pollen germination rates were more dependent on genotypes than polyploid. Yang *et al.* (2000) found that both $4x \times 2x$ and $2x \times 4x$ crosses could generate a small number of triploid mulberry but the germination percentage of seeds from $4x \times 2x$ was small. In $4x \times 2x$ or $4x \times 2x$ hybridizations, three seed types are obtained: undeveloped seeds, developed seeds (normal seeds) and developed small seeds. Only the developed normal seed can germinate. Seeds tend to abort due to endosperm degeneration during early embryogenesis.

Aleza *et al.* (2012)^[3] confirmed those triploid plants could be originated because of unreduced megagametophyte with haploid pollen grain with a most suitable endosperm/embryo ploidy ratio (3/5) or maternal/paternal contribution. So, embryo rescue is an indispensable technique for triploid breeding programs that are based on interploid hybridizations. Embryo rescue was utilized by plant breeders to rescue inherently weak, immature and/ or abortive embryos, breeding seedless crosses and triploid plants, and distant hybridization between different species. Thus far, embryo rescue was extensively applied in rescuing many fruit crops, including apple, banana, citrus, grape etc. According to the results of citrus embryo rescue, the undeveloped seeds were 49–75% smaller than normal seeds and the undeveloped seeds had either one (monoembryonic) or multiple embryos (polyembryonic) which is difficult to individualize or isolate. All normal seeds contained only one well-formed embryo. But the efficiency of triploid plants recovered from small seeds was higher than from normal seeds. These results provided great help for breeding of triploid plants. In the future, maker-assisted selection technique together with embryo rescue technique will continuously play an important role in the efficient evaluation and selection of the triploid

hybrids.

Endosperm culture *in vitro*: As endosperm is a triploid tissue, it would be reasonable to assume that natural triploids could be successfully regenerated plants from endosperm tissues. The first attempts at endosperm culture *in vitro* took place in the 1930s. Endosperm culture has now been attempted for triploid plant regeneration in nearly 64 species, but successful initiation of buds or shoots from endosperm explants has been reported in only 32 species. Triploid plantlets have been regenerated only from 15 of these species. Thus, regeneration from endosperm tissues is often technically challenging.

Endosperm is a unique tissue in its origin, ploidy level and nature of growth. It is mostly formed by the fusion product of three haploid nuclei, one from the male gametophyte and two from the female gametophyte and is, therefore, triploid. The triploid nature of the endosperm suggests that the direct production of triploid plants from diploid plants is possible if plants could be regenerated from cultured endosperm. Among polyploid cultivars, triploidy, a genomic condition that is favorable for vigor and vegetative productivity, has proved the best for agricultural use. Triploid plants are usually seed sterile and is undesirable where seeds are of commercial value. But in cases where the seedlessness is employed to improve the quality of fruits, such as in banana, apple, citrus, grapes, papaya, etc., induction of triploid plants would be of immense use. Seedlessness continues to be a key commercial objective in papaya improvement programs, being driven by consumers' preference for seedless fruit. By using endosperm culture triploid plants have been developed in *Malus*, *Citrus*, *Prunus*, *Emblica*, *Annona*, *Pyrus*, *Diospyros*, *Passiflora* *Morus*, *Caricacapaya* and diploid *Actinidia kolomikta*, a cold-hardy kiwifruit relative. Genotype, sampling times, and culture media are important aspects of endosperm culture systems. First, the efficiency of endosperm response has been found to be genotype-dependent in many species. Second, because either immature and mature endosperms have been used for successful endosperm culture (18 out of 30 species from mature endosperm and 14 out of 30 species have successfully used immature endosperm), it is not clear how critical endosperm developmental stage is for regeneration. Third, culture media composition can be the decisive factor that determines the success of triploid plant development. Most experiments on endosperm culture attempt to determine the most efficient media for the given species. (1) MS is the most used basal medium. (2) Plant growth regulators are essential for regeneration from immature endosperm in most species. About 70% of the reports we have identified have found that 2,4-D is the most effective plant growth regulator to induce callus from endosperm. And BAP appears to be the most popular cytokinin in endosperm culture. Successful callus induction without cytokinin has been reported, but there have been no reports of successful callus induction without auxin. The concentrations and ratios of cytokinins (BA, KT) and auxins (2,4-D, NAA) are key factors for different genotypes or endosperm stage.

Protoplast fusion: Plant somatic hybridization via protoplast fusion has become an important tool for ploidy manipulation in plant improvement schemes, allowing researchers to combine somatic cells from different cultivars, species, or genera, resulting in novel allotetraploid and autotetraploid genetic combinations. Protoplast fusion technology has been

utilized in many crops to generate allotetraploid somatic hybrids, and sometimes autotetraploids as a by-product of the process. Protoplast fusion has become a significant tool in ploidy manipulation that can be applied in various cultivar improvement schemes. In rare cases, a new somatic hybrid may have direct utility as an improved cultivar; however, the most important application of somatic hybridization is the building of novel germplasm as a source of elite breeding parents for various types of conventional crosses for both scion and rootstock improvement. Somatic hybridization is generating superior allotetraploid breeding parents for use in interploid crosses to generate seedless triploids; several thousand triploid hybrids have been produced using somatic hybrids as the tetraploid parent. Protoplast fusion is also being utilized to produce somatic hybrids that combine complementary diploid rootstocks, which have shown good potential for tree size control. This technique can facilitate conventional breeding, gene transfer, and cultivar development by bypassing some problems associated with conventional sexual hybridization including sexual incompatibility, nucellar embryogenesis, and male or female sterility. Somatic hybridization is generating superior allotetraploid breeding parents for use in interploid crosses to generate seedless triploids. By utilizing somatic hybridization seedless fruits from triploid hybrids produced by interploid crosses using somatic hybrid pollen parents. 'Sugar Belle' x 'Nova' + 'Succari' somatic hybrid; 'Todo del Ano' lemon x 'Mexican' lime + 'Valencia' somatic hybrid; 'Todo del Ano' lemon x 'Hamlin' + 'Femminello' has been developed. Somatic hybrids such as sour orange + rangpur lime or sour orange + Palestine sweet lime, can yield over 22 tons (20,000 kg) fruit per acre, have better soil adaptation than Flying Dragon, which does not perform well on high pH, calcareous soils. UFR-6' (identified as "Changsa + 50-7") is a new and distinct allotetraploid citrus rootstock was developed for tree size control and improved disease resistance (Huanglongbing disease). Calamondin (*C. madurensis* Lour.) + 'Keen' sour orange (*C. aurantium* L.) somatic hybrid showed tolerance against Citrus tristeza virus (CTV) (Abbate *et al.*, 2019)^[1].

Some Biotechnological and transgenic approaches

Parthenocarpy is an important agricultural trait and therefore, a target for biotechnological research. Auxin and GAs play important roles in parthenocarpic fruit development. Increased levels of these hormones in the ovary or ovule can substitute for pollination and can trigger fruit development, and this has been used for the induction of Parthenocarpy in genetic engineering studies. The *barnasesuicide* gene, a cytotoxic ribonuclease of *Bacillus amyloliquefaciens* Fukumoto, was transformed into tobacco plants, producing transgenic plants with female sterility under the control of a stigma-specific promoter and male sterility under the control of the tapetum-specific promoter. These approaches involve targeting the seedcoat or embryo could result in embryo abortion, and thus stenospermocarpy. Reported on a transgenic 'Ponkan' mandarin transformed with the *barnasesuicide* gene under the control of a tapetum-specific promoter (pTA29). These efforts may yield male-sterile mandarins, and therefore seedlessness. Another important technique is the somatic hybridization via protoplast fusion and development of cybrids. The first somatic hybrid of citrus was produced in 1985 and by now numerous inter and intrageneric somatic hybrids have been produced. This

approach is now becoming the most important in the creation of triploids and novel germplasm for improving rootstock and scion varieties. Transgenic lime (*C. aurantifolia*) plants containing gene for decreased seed set were obtained from seedling hypocotyl and epicotyl segments using *Agrobacterium* mediated transformation. Putative transformants were identified by polymerase chain reaction, (PCR). If haploid lines are obtained and then crossed with diploid lines via protoplast fusion, triploids can also be produced. This allows the insertion of a haploid genome to the whole diploid genome of high organoleptic quality cultivars. It can be done via electro fusion of protoplasts (Premachandran *et al.*, 2019).

Advantages of Seedlessness

- 1) Year-round production:** Production of fruits is greatly influenced by the pollen and studies have shown that effective pollination is a prerequisite for the quality fruit production. An alarming situation is prevailing in the world that effective pollination is becoming a hindrance and due to many factors like pollinators environmental factors that affecting the fruit production. Malformed fruits are produced due to this reason. So, it will be a effective way to produce fruit without effective pollination. By breeding seedless varieties foremost production can be made year-round without worrying about the availability of pollen and pollinators.
- 2) Gustatory advantage:** Seedless fruits have many gustatory advantages. Seeds are often hard, can have a bad taste and can be harmful; for example, grape seeds can bring about digestive problems. Furthermore, seedlessness in citrus is gaining popularity among the consumers it could be mainly due to the tiresomeness to remove the seed from it. Studies have also shown that seedless tomato fruits are tastier than the seeded variety. Indeed, seedless tomato fruits exceed seeded fruits in dry-matter content by up to 1%, contain more sugars, less acidity, less cellulose and have considerably more soluble solids than seeded cultivars.
- 3) Processing:** Seedlessness will be a boon to the processing industry which remove many of the procedure in the fruit processing related to the seed and it is known that 80 per cent of the grapes cultivated in the world are seedless and there are mostly used for wine making and resin making.
- 4) Seed cavity can be filled with fruit tissue:** The seeds and their cavities are replaced with edible fruit tissue; this is more attractive to the consumer. An illustration of this is the seedless pickled gherkin, which is more crunchy, firmer and fleshier than its seeded variety. It is possible to speculate that this advantage might be even greater for species with a large seed, such as peaches and mangos, or for those with a large cavity that is filled with numerous seeds, such as melons and papayas.
- 5) Improve shelf life:** The shelf life of seedless fruit is expected to be longer than seeded fruit because seeds produce hormones that trigger senescence. This effect has been observed in watermelons, in which seeds are the origin of fruit deterioration. Seedless watermelons develop a meaty texture and become overripe significantly later than seeded varieties.
- 6) Fruit quality:** Fruit weight, size, acidity, maturity index and harvest time, as well as chemical and nutritional composition are all important quality traits, consequently

different fruit quality features have been evaluated in a study conducted by with mandarin varieties to produce seedless clones by way of irradiation showed that there are some characters of fruit which are improved when it is being irradiated like fruit peel colour, fruit diameter, fruit length, easiness in peeling. It is very evident that due to irradiation seed in the fruit are greatly reduced than the in irradiated ones Seed numbers and pollen germination (%) are less in several 'Moncada' clones, obtained in forced pollination.

- 7) **Organic acids and Flavonoids:** The content of vitamin C which is an important antioxidant and other organic acids in fruits and vegetables can be influenced by various factors such as genotypic differences, climatic conditions and cultural practices. Furthermore, their nature and concentration largely affect taste characteristics and organoleptic quality. A study was conducted by on mandarin varieties to produce seedless clones by way of irradiation. It was observed that certain clones produced high number of organic acids than control (seeded) which are ascorbic acid, malic acid and citric acid while succinic acid concentration did not significantly change in case of flavonoids and hesperidin concentration was higher than control in most of the clones.
- 8) **Carbohydrates, carotenoids and essential oils:** The main portions of carbohydrates in citrus fruits are three simple sugars: fructose, glucose and sucrose, they represent the largest percentage of total soluble solids of citrus juice, and the ratios of fructose: glucose: sucrose are generally about 1:1:2. A study conducted by with mandarin varieties to produce seedless clones by way of irradiation showed that the ratio was similar for the irradiated "Moncada" clones under study, and sucrose was present in the largest amounts for all clones. In case of carotenoids β -carotene increased significantly in clone's *limonene* was identified as profound essential oil present in the citrus and shown significant increase in irradiated clone than control.

Limitation of seedless fruits

1. **Lack of stability and uniformity in the expression of Parthenocarpy:** The expression of Parthenocarpy in the fruit is highly variable and unstable. still in many fruit crops Parthenocarpy is reported but it is not stable in crops like mango, jamun, mangosteen.
2. **Small sized fruits:** Parthenocarpic fruits are generally small. This could of various reasons such as hormonal changes inside the fruits due to the absence of seeds and the space of the seed will also be considered.
3. **Malformed fruits:** Parthenocarpic fruit sometimes will be resulted in a malformed fruit the reason pointing to the hormonal imbalance in the fruit development.
4. **Hampers the production of commercial seeds:** Seedlessness results in hampered seed production and there is no room for the commercial seed production.

Conclusion

Due to inviable seeds and resulting lack of progeny triploid plants are rare in nature. Also, it is very difficult to detect naturally occurring triploids however due to desirable attributes like faster growth and seedlessness they are proved to be useful in improving biomass, fruit and flower traits, and other qualities of economically important food, medicinal,

bioenergy, and ornamental plants, reducing or eliminating the invasiveness of many crop and horticultural plants. As endosperm is a triploid tissue, it is thought that endosperm culture is the most direct and efficient method for production of triploid plants and many protocols have been developed in last 15 years. As reviewed in this paper in order to advance in this field further there need to be a proper mix of traditional methods with modern methods to promote development of breeding triploid. With the rapid development of genomics research and advanced biology technologies, perhaps new methods to induce formation of triploids and new avenues of research into and using triploid plants will become possible in years to come.

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