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Somatic hybridization on different horticultural crops: A review

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Abstract
A promising method for producing asymmetrical and unbalanced polyploidy somatic hybrids in multiple kinds of plant species is somatic hybridization through protoplast fusion. By introducing new lines which are practical as superior breeding material for scion and rootstock enhancements, this strategy possesses the capacity to get better traditional breeding programmes. Somatic hybridization is noteworthy because it tackles issues related to sexual hybridization, including male/female sterility, nucellar embryogenesis, and sexual incompatibility. The successful application of somatic hybridization in horticulture is illustrated by the diffusion of genes that resist into multiple kinds of crops from similar plants, such as citrus, potato, brinjal, tomato, mango, avocado, banana, strawberry, pear, and cherry, for both biotic and abiotic stresses. Somatic hybridization permits for the exchange of many uncloned genes without being restricted by legal formalities, unlike transgenic technology. As opposed to sexual hybridization, somatic hybridization has various restrictions and constraints that may prevent it from being widely used. However, new genomic technologies provide hope in this regard. With an improved comprehension of plant genomes provided by these technologies, somatic hybridization may be used more broadly and more effectively to benefit agriculture.

Keywords: Somatic, hybridization, tomato, mango, polyploidy

Introduction
Protoplast fusion-based somatic hybridization (SH) is a key mechanism for producing interspecific Interfamilial hybrids. The steps involved in SH are combining Protoplasts from distinct sources genomes, choosing the Wanted hybrid somatic cells, and finally growing the hybrid plant again. By providing an efficient Mechanisms for genetic conveyance between species, the technique helps parents' the mitochondrial and atomic genomes for integrate and overcome obstacles to crossing over. The process of SH involves several stages, including the separation and merging of protoplasts, growing and regenerating the post-fusion blend, and detecting Somatic fusions within the sprouts that have recovered.SH has been extensively used to generate innovative hybrids with enhanced productivity and improved disease resistance in various horticultural crops. In addition, SH has been employed for the generation of seedless triploids, improve rootstock, transfer cytoplasmic male sterility (CMS), increase quality, and improve salt tolerance (Wang *et al.,* 2013) [57]. In members of "model horticultural families," including Rutaceae, Brassicaceae, and Solanaceae, SH has produced a number of positive results. In SH, protoplast fusion has produced hybrids that are both symmetric and asymmetric. A "symmetric hybrid" is a combination of two maternal cytoplasmic genomes and diploid nuclear genomes. Conversely, "asymmetric hybrids"-also called cybrids-are produced when the genomes of two different parents combine or when one parent's genome is combined with the cytoplasm of another parent. Somatic hybrids may have phenotypes or performances that are unpredictable owing to the manifestation of both good and bad features inherited from the fusion parents. Because they have both parents' genomes, these hybrids might not possess any instant use (Xu *et al.,* 2007) [60]. According to (Grosser *et al.,* 2000) [30], there's one chance that both advantageous and disadvantageous features will be passed down simultaneously even in cases when only a partial or combination manifestation of genomes takes place.

Genetic Unbalance could also occur from the target genes being incorporated with significant amounts of outside genetic material. As noted by (Liu and Deng, 2000) [31], this imbalance may cause the fruit to develop unfavourable traits like thick and uneven skin, which would restrict how widely it may be used. A more successful tactic would be asymmetric fusion in cases when the vast bulk of them genes granting resistance to specific biotic or abiotic stimuli are found on one or a few chromosomes. According to (Xia., 2009) [61], this strategy leads to the integration of a section of a foreign genome into the receptor genome. In other cases, a reduced contribution from one parent makes it easier to produce children without the unwanted genetic consequences, they are usually connected toallopolyploidy (Xu *et al.*, 2007) [60]. Compared to a thorough whole-genome transfer, this partial transfer might be more tolerated. Protoplast fusion opens doors for overcoming central and intracellular genome-based obstacles to genes transfer and sexual reproduction, which expands the genetic bank for domesticated species. Furthermore, protoplast fusion works well for producing new seed for high-level breeding via traditional crosses, which improves Growth in crops in already-existing cultivars. Due to reproductive barriers like conflict, differing bloom phases, male and/or female sterility, nucellar development, as well as polyembryony, traditional techniques in breeding, such as vaginal recombination face obstacles in transferring certain features of superior mating (e.g., qualities of perfection, Immunity against illness, and CMS) as explained by (Aleza *et al.* 2010) [1]. Protoplast fusion, often known as SH, offers a way across sexual boundaries. According to research by (Mwangangi *et al.*, 2019) [44] this procedure fuses using protoplasm from two distinct creatures create a novel species cross that has traits from both parent species. Because somatic hybridization makes it easier to create hybrids between distinct species of plants and animals, it is an essential tool for improving crops and plant breeding. According to (Imandi, and Bahadur, 2023) [32], this process entails mixing two distinct protoplasm cultures genomes, choosing desired somatic hybrid cells, and growing hybrid plants. It functions as a useful technique for creating hybrids by combining protoplasts from different kinds, species, or plants. This unconventional sources genetic process, which involves the *in vitro* union of isolated the protoplasts and the subsequent growth of their descendants into hybrid plants, results in the ensuing hybrids, known as somatic hybrids. According to protoplast fusion is an effective method of moving genes with desired features from a single species to another, which has a major impact on agricultural advancements. Intrinsic fusions are produced by fusing pure proximal elements made up of two separate sources-different tissues, plants, species, or genera, for example-together. Somatic hybridization is the term for this novel form of genetic recombination that involves protoplast fusion also *in vivo* the subsequent production of a hybrid plant. Somatic hybridization is mostly used to improve different kinds of plants, such as agricultural and medicinal plants. Somatic hybridization can improve a trait by modifying its quality, quantity, resistance to illness, or other characteristics. For example, reproductive hybridization utilized for give potato plants (*Solanum tuberosum*) opposition to the potato leaf rolling disease. The three main stages of somatic hybridization are hybrid plant identification, hybrid cellular selection, and protoplast fusion. Numerous good genetic features have been able to spread among plants because to this mechanism. The progression of intergeneric hybrid crops within the family Brassicaceae is an example of the promptness with which somatic breeding in important crop plants (Panesar *et al.*, 2013) [47].

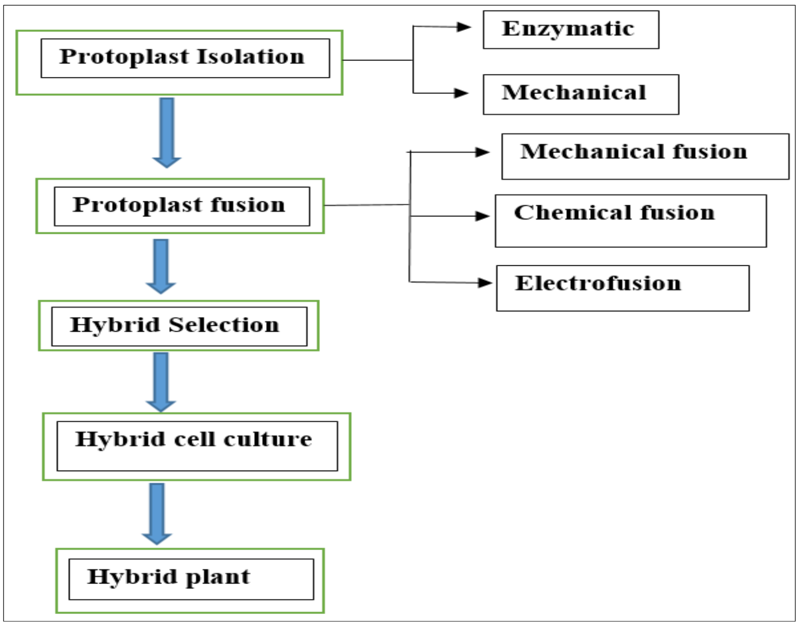

Fig 1: Flowchart on steps of Somatic Hybridization

Principles and facets of somatic hybridization

Sexual hybridization has been the conventional method for improving the attributes of cultivated plants lengthy duration. Sexual hybridization's restriction to inside species of plants or closely associated species, however, limits the assortment of possible advancements and is a crucial disadvantage. Somatic cell Synergy has the potential for create a viable hybrid that overcomes species constraints to plant improvement that arise from amalgamation of gender. To process of confluence of embryonic protoplasm within two distinct plant genera or varieties results in hybrid plants, a phenomenon known as somatic hybridization. In general, this procedure entails the *in vitro* fusing of separated protoplasts to produce a hybrid cell, which then develops into a hybrids plant. Protoplasts are joining together is a relatively new and flexible method for stimulating or aiding biological repurposing in a variety of prokaryotic and eukaryotic cells. Hanstein first used the name "Protoplast" in 1880. A protoplast is a neuron that's experienced its cell wall destroyed, either by enzymes or mechanical means. Klercker achieved the first isolation of protoplasts using a mechanical method in 1892. The mechanism of random fusing in dynamically isolated protoplasts was. In 1960, Cocking made a major contribution to the field of protoplast research by removing the cell wall by an enzymatic approach. Plant protoplasts are extremely useful for developing plant cells, genetic engineering, and agricultural improvement. Therefore, protoplasts offer a special way to create cells with a different genetic composition. This technique provides an amazing way to overcome barriers resulting from sexual incompatibility between different species of plants. Notably, a whole tobacco plant was successfully regenerated from protoplasts by Somatic hybridization-the fusing of protoplasts-is a relatively new and adaptable method of initiating or promoting genetic recombination in a variety of cells, both prokaryotic and eukaryotic.

Approach to somatic hybridization

Plants can have their genetic makeup significantly altered through the procedure for protoplast fusion. Plant tissues contain protoplasts that might have arisen freed by soaking them in digestive enzymes may demolish cellular barriers (George. 2007) [26]. There are a few actions to stimulate protoplasts to fuse together. These techniques include exposure to electrical pulses previously stated by (Compton *et al.*, 2018) [19] or implantation in PEG (polyethylene glycol) (Compton *et al.*, 2018) [19]. Cultures can be utilized to regenerate autologous hybrid plants after these fusion procedures.

Plant Protoplast

Plant tissues contain large populations of protoplasts that might be isolated. Certain formulations that contain pectinases, hemicellulases, and cellulases effectively disintegrate walls of cells and release protoplasts in a matter of hours. To preserve their integrity and viability, the isolated protoplasts are subsequently carefully processed and cultivated in a nutritional solution enhanced with osmotic stabilisers. Protoplasts can be isolated and manipulated using standard protocols from cells grown in culture, leaves, and other organs of many important crops (Reed *et al.*, 2021) [50]. Protoplasts can undergo division and rebuild a cell wall in the right culture circumstances (Compton *et al.*, 2018) [19]. Individual plant cells are stored in the protoplasts. Tiny droplets, neatly distributed in glass petri dishes and ranging in size from 50 to 200 µl, are best for cultivation. Freshly isolated protoplasts are devoid of a cell wall and is suitable for protein absorption combining (Compton *et al.*, 2018) [19], or organelle translocation.

Fusion of Protoplast

The spontaneous protoplast merger that takes place throughout the isolation phase has been suggested to be explained in part by plasmodesmata. The right conditions must be created to encourage broad transmembrane contact and activation when the deliberate fusing of the protoplasts via two sources is desired. It was discovered recently that polyethylene glycol, or PEG, is an incredibly powerful agent for protoplast union (Compton *et al.*, 2018) [19]. Protoplasts clump together when exposed to 2,8% of a PEG, or polyethylene glycol concentration in a molecular mass range of 1500–6000. PEG acts to bring the opposing membranes into close contact, and as the PEG concentration is gradually diluted, the fusion process proceeds to completion). PEG utilisation is quite successful; heterokaryon production rates range from 20 to 30 percent. Protoplasts show resistance to the treatment, and the fusion products regenerate and divide as cells in the following culture (Compton *et al.*, 2018) [19]. Regarding PEG's function in the fusion process, different viewpoints are present (Compton *et al.*, 2018, Parray *et al.*, 2020) [19, 48]. PEG may cause dehydration, which would cause the plasma membrane to compress and fold. Strong polarity and a slight ionic charge of the chemical may facilitate the incorporation of groups on the lipids and proteins of antagonistic barriers.

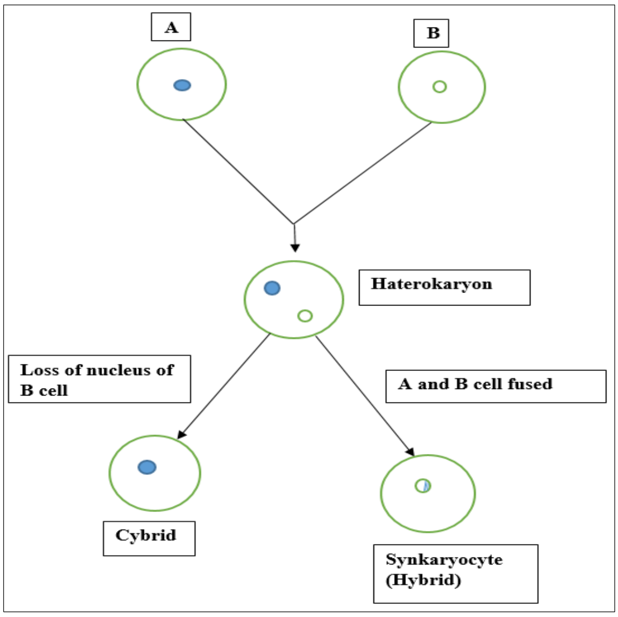

Fig 2: Technique of Protoplast fusion

Hybrid Cells and Heterokaryons

It is possible to combine protoplasts from many plant species and families using the PEG approach. Soybean-barley, soybean-Vicia haj Astana, soybean-pea, carrot-barley, and various other pairings have produced heterokaryons that produce dividing cells containing chromosomes from both the parent plants (Lentz, 2007) [37]. The presence of mitochondria from the developed cell the protoplasts and chloroplasts from their parental leaf the chlorophyll protoplasts visibly identifies these hybrid cells. In this particular instance that undifferentiated plastids seen under a microscope in the grown cells confirms the hybrid character. Fusion could happen before mitosis, or nuclear mixing could happen during the first mitosis. Approaches for differentiating colours have been developed used in investigations using soybean-pea or carrot-barley hybrids to obtain evidence indicating nuclear fusion. It is not known whether fusion-formed synkaryons undergo mitosis. For the accurate isolation thereof hybrid cells and the removal of those derived from parental protoplasts, existing techniques have proven insufficient. Hybrid cell populations of up to 100 have been reported, and current efforts are concentrated on inventing methods that might facilitate these hybrid cells to be isolated.

Chloroplasts, Nuclei, and Assimilation of DNA

The assimilation of cellular constituents, such as chromosomes, nuclei, mitochondria, or chloroplasts, offers a fresh and exciting opportunity to study nuclear-cytoplasmic relationships and the control of plant metabolism. Polyethylene-glycol to enable the integration of algal the chloroplasts into protoplasts that were obtained from carrot cell cultures. When 28% polyethylene glycol was present, this procedure regularly happened. Several of chloroplasts that found a way to reach the cytoplasm were present in around 16% among the viable protoplasts. The chloroplast membranes did not appear to have an impact on the PEG's activation of the plasma membranes. The research provided no details regarding the behaviour of the integrated algal chloroplasts, despite the event that the carrot protoplasts remained viable. It indicates that the double-stranded genetic material has been assimilated (Bengochea, 2012) [8]. The presence of poly-L-lysine or poly-L-ornithine increases the uptake efficiency. The protoplast is negatively impacted by both the polymers and the genetic material. The development of walls and cell division of bacteria are inhibited at concentrations of bacterial DNA greater than 0.25 mg/ml. Protoplast-assimilated bacterial DNA is prone to deterioration. A related study using DNA from plants or similar organisms has been published. (Kanazawa *et al.*, 2011) [33] used labelled homologous DNA to discover that DNA taken in by Petunia protoplasts bonded with the nucleus. The lack of particular nucleases with many more parameters affect how well transformation works in bacterial cells (Blokesch, and Schoolnik, 2008) [10]. Characterising the nucleases found in plant cells and determining possible variations among different plant genera and genetic lineages are urgently needed (Cheng *et al*., 2018) [16]. It's possible that the enzymes in plants that identify and break down non-homologous DNA vary from one another. Seeds and other plant organs can also be used to investigate DNA uptake and genetic changes (Chupeau, *et al.*, 2013) [17]. One benefit of these systems is that they guarantee full plant development following DNA assimilation. The development of cell selection methods must come before the wider use of protoplasts to research genetic information transmission. Classification of reported findings of genetic alteration in higher plants is a topic of considerable controversy. Although a few multiple states data to support DNA uptake, questions remain about how donor DNA integrates and how stable as it stands at the moment cells that receive it.

Vegetative Propagation in Plants

Acquired hybrid and genetically modified cells can be exposed to environments that support plant development and morphogenesis. According to reports, interspecific hybrid cells formed by the fusing of protoplasts from two different tobacco species can regenerate plants (Mwangangi *et al.*, 2019) [44]. The development of intergeneric hybrid crops is inevitable given the advances in tools for hybridization cell selection and the growing capacity to create plants from protoplasts. Determining the precise requirements for initiating organ development in cells rejuvenated from the protoplasm species utilized in the genetic alteration process is essential.

Potato (*Solanum tuberosum*)

Due to its autotetraploid nature, growing potato (*S. tuberosum*) requires a substantial amount of pressure within a population to geared towards combine advantageous agronomic features into just one nucleotide. These kinds of situations, chromosomal doubling (to restore ploidy) after diploid breeding been recommended as an alternative substitute strategy for producing superior cultivars. The effective integration of multiple desirable features from a related or wild species into farmed potatoes has highlighted the potential benefits of this approach. For example, chromosomal doubling (SH) was employed to move the ability to resist bacterial wilt between *S. phureja* and *S. stenotomum* to *S. tuberosum* (Fock *et al.*, 2001) [23]. They have successfully created somatic crossings from *S. tuberosum* as well as *S. commersonii* to get around such incompatibilities resulting from changes in fertility and endosperm balance number (Gaiero *et al.*, 2017) [24]. The majority of the generated fusion hybrids were resistant to bacterial wilt, and upon crossing confident fertile, resistant hybrids with *S. tuberosum*, viable seeds were produced (Andino *et al.*, 2022) [3].

Tomato (*Lycopersicon esculentum*)

Within the vast and varied Solanaceae family comes the genus Lycopersicon, which is somewhat compact. It includes eight related wild Lycopersicon species and the domestic tomato, *L. esculentum*. It is known that the wild members of the Lycopersicon species are important sources of agronomic features. However, uni- or bilateral intolerance makes many sexual crossings between the domestic tomato and these indigenous species difficult to carry out, leading to an abundance for barren F1 hybrids. The cultivated tomato (*L. esculentum*) was successfully crossed with *L. chilense*, *L. pennellii*, and *L. peruvianum*to manufacture a harmonious interspecific hybrid plants. Furthermore, an array of organisms, particularly *Nicotiana tabacum*, *S. tuberosum*, *S. tuberosum* × *S. brevidens*, *S. lycopersicoides, S. muricatum, S. nigrum, S. rickii, and S. tuberosum* have successfully undergone intergeneric fusion of soma by tomatoes. Despite a detailed examination of the morphological and organellar contents of these hybrids, no data on the publication of agronomic features to the somatic crosses is yet available. Growing tomato and L. *peruvianumin* embryonic crossbreeds particularly interesting because this combination belongs among the few times that fertility is noted, as opposed to the documented sterility of the equivalent identical sexual combination.

Brinjal (*Solanum melongena*)

Transferring agriculturally beneficial features from closely associated wild species, like tolerance to pest pests and diseases, to aubergine (*S. melongena*) presents a substantial challenge. While progenies they created by interspecific protoplast fusion, for some combinations the outcome of fusion in reproduction is essential. (Tamura *et al.*, 2002) [54] noticed this general principle in crosses between *S. melongena* and *S. aethiopicum*, *S. melongena* and *S. torvum*, *S. melongena* and *S. khasianum*, and *S. aethiopicum* and *S. violaceum*. In the relationship involving S. melongena and *S. sisymbriifolium*, an uncommon situation is seen where sexual hybridization failed as well as anatomic combination (SH) produced a viable, albeit sterile, hybrid (Collonnier *et al.*, 2003) [18]. Too far, no cultivar of aubergine has been successfully created using interspecific protoplast fusion, despite significant advancement in this field sector. To determine the ancestry of dihaploid organisms that originate from *S. melongena*-*S. aethiopicum* somatic crosses carrying Fusarium wilt opposition (inherited from the *S. aethiopicum* parent), isozymes were first used, then randomly amplified polymorphic DNAs, and finally inter simple sequence repeat markers (Rizza *et al.*, 2002) [51].

Citrus (*Citrus* sp.)

Innovative genotypes have been allowed for legislation the successful resolution of many issues related to Citrus reproductive traits by transformation of placenta via protoplast merger. Harmonic fusion inside citrus has improved fruit quality and output while strengthening rootstock resilience to a variety of living and nonliving stressors (Soriano *et al.*, 2012) [53]. Significantly, symmetric fusions involving premier diploid cultivars have produced better allotetraploid parent with possible uses in breeding programmes, and an amalgam of haploid and diploid protoplasts. These has produced new without seeds triploid Citrus cultivars (Grosser and Gmitter. 2005) [28]. The seeds of *Citrus sinensis* and *Poncirustrifoliata* fused protoplastically to produce the first symmetric hybrid of citrus (Bona *et al.*, 2011) [12]. In 1988, *C. sinensis* L. Osb. cv. 'Hamlin' protons united with *Severiniadisticha* (Blanco) Jolly protoplasm were the first recorded examples of crossbred species between sexually unsuitable Citrus species (Grosser and Gmitter., 2005) [28]. Somatic hybridization (SH) subsequently developed into a recognised method for citrus enhancement. For example, regenerated plants with early blooming were produced when 'Bonanza' naval orange (*C. sinensis*) and 'Red Blush' grapefruit (*C. paradisi*) protoplasts were fused (Guo *et al.*, 2000) [31]. Additionally, plants were produced by protoplast fusions of 'Caipira' in sweet orange (*Citrus sinensis*) and 'Rangpur' lime (*Citrus limonia* L. Osb.), with the goal of fusing the vigour and drought resistance of the former with the blight resistance of the latter (Mendes *et al.*, 2000) [41]. To ensure that specifically increase immunity to citrus decay, Citrus tristeza virus, and Phytophthora-induced illnesses, 'Hamlin' sweet oranges and 'Singapura' pummelo (*Citrus grandis* L. Osb.) were fused (Calixto *et al.*, 2004) [14]. Furthermore, interactions across intergeneric reproductive domains the 'Morita' navel orange and the round kumquat (Fortunella japonica Swingle) were developed. This was done in an effort to produce novel parents through interploid crosses, with the final objective of creating new cultivars that resemble seedless kumquats. Citrus species are incapable of undergoing traditional hybridization owing to the situation existence of apomixis plants and a long juvenile phase. The effective use of the protoplasm fusion-mediated somatic hybridization has enabled the generation of somatic hybrids comprising of among the founders of small-fruited acid lemon (*Citrus micrantha*) and sweet orange (*C. sinensis*). Lime developed resistance to the witches broom disease due to this of this hybridization. Likewise, (Grosser *et al.*, 2007) [27] used a somatic crossing of mandarin (Citrus reticulate Blanco) and pummelo (*Citrus grandis* L. Osbeck) to create rootstock tolerant of venomous nematodes (*Belonolaimus longicaudatus* Rau). By combining leaf protoplasts that are from pummelo seedlings that had previously been chosen for their resistance to sting nematodes with protoplasts obtained from embryogenic suspension-cultivated specimens of certain mandarins, four unique mandarin + pummelo parental pairs were created as combination sympathetic organisms. Citrus asymmetric hybrids show great potential because they provide the benefit of partial population transfer, and these could be more palatable than full nucleotide exchange. Citrus asymmetric hybrids were first reported to regenerate when Liu and Deng used X-rays to successfully produce lopsided crosses with 'Dancy' mandarin and 'Page' tangelo. Another attempt involved electrofusion by protoplasm UV-irradiated "Satsuma" mandarin with "Jincheng" (*C. sinensis*) to produce asymmetric shoots, however rooting induction did not work (Xu *et al.*, 2007) [60]. Although Citrus lopsided hybrid plantlets were produced, no further study was recently conducted to determine their usefulness.

Mango (Mangifera indica)

Among the highest popular fruit harvests in tropical and subtropical areas of the world is the mango. Mango breeding using conventional methods is confronted with difficulties due to low seed production, complex flowering patterns, significant fruit loss, prolonged juvenile life, high inbreeding and polyembryony (Auxcilia, and Shabha, 2017) [7]. Somatic hybridization (SH) provides a workable way to introduce desirable features from wild species or well-regarded cultivars into mango rootstocks, such as resilience to both abiotic and biotic stressors (Litz., 2004) [38]. The mango has lagged behind other fruit crops whilst on the use of single-cell and protoplasm culture methods because it has shown to be included among the most difficult species for *in vitro* studies. However, a few scientists have started studying somatic hybridization, or SH, in mangos. An example of this is the proven efficient regrowth of plants from protons of proembryonic aggregates (PEMs) of the mango cultivar 'Amrapali' (Ara *et al.*, 2000) [5].

Table 1: Somatic Hybridization Methods Key Finding

Crop	Somatic Hybridization Methods	Key Finding	References
Potato	Protoplast Fusion	Protoplast fusion between different potato species has resulted in somatic hybrids with improved traits such as disease resistance and yield.	Thieme, and Rakosy-Tican, (2017) [55].
	Chloroplast Transfer	Electrofusion of leaf mesophyll protoplasts has been utilized to generate somatic hybrids with novel genetic combinations.	Ondrej, & Kormutak, (2000) [46].
	Hybrid Cell Formation	Somatic hybridization has been explored as a strategy to introgress traits like late blight resistance from wild potato species into cultivated varieties.	Borhan, and Hadi, (2017) [13].
Brinjal	Protoplast Fusion	Somatic hybridization studies in Brinjal have demonstrated the potential to combine desirable traits from different cultivars, enhancing crop resilience.	Khawale, & Chandel, (2010) [35].
	Chloroplast Transfer	Interspecific somatic hybrids involving Brinjal have been created, offering opportunities for novel trait combinations and genetic diversity enhancement.	Saini, and Kaushik, (2019) [52].
	Hybrid Cell Formation	Protoplast fusion techniques have been employed to facilitate somatic hybridization, broadening the genetic base of Brinjal cultivars.	Nanda, and Sahoo, (2012).
Tomato	Protoplast Fusion	Somatic hybridization in tomato has led to the development of hybrids with improved vigor, disease resistance, and fruit quality attributes.	Dangi, and Agrawal, (2015) [20].
	Chloroplast Transfer	Challenges and limitations exist in somatic hybridization of tomato, including low fusion efficiencies and regeneration rates.	Daunay, *et al.*, (2019) [21].
	Hybrid Cell Formation	Somatic hybridization offers a promising avenue for tomato breeding, enabling the incorporation of novel traits from wild relatives into cultivated varieties.	Narayanan, and Mohan, (2006) [45].
Citrus	Protoplast Fusion	Protoplast fusion has been utilized for the production of tetraploid and triploid citrus plants, offering potential applications in rootstock and scion breeding.	Grosser, and Gmitter Jr, (2011) [28].
	Chloroplast Transfer	Intraspecific somatic hybridization has been achieved in citrus through protoplast fusion techniques, facilitating the creation of novel genetic combinations.	Ali, S. *et al.*, (2013) [2]
	Hybrid Cell Formation	Somatic hybridization in citrus has provided insights into the anatomy and physiology of hybrid plants, aiding in understanding their potential for crop improvement.	Kumar *et al.*, (2018) [36]
Mango	Protoplast Fusion	*In vitro* culture studies and somatic hybridization have been explored in mango, offering opportunities for genetic improvement and novel trait incorporation.	Maldonado-Celis *et al.*, (2019) [40]
	Chloroplast Transfer	Somatic hybridization has been identified as a potential tool for crop improvement in mango, enabling the introgression of desirable traits from related species.	Rajan, and Hudedamani, (2019) [49].
	Hybrid Cell Formation	Somatic hybridization holds promise for enhancing mango cultivars by incorporating traits such as disease resistance and improved fruit quality.	Khan, (2005) [34].

Application of SH in other significant fruit crops

Apart from the previously mentioned use of SH, other important agricultural traits that are impacted by SH include Actinidia's ability to withstand chilling (Xiao *et al.*, 2004) [62], Gossypium's ability to respond to photoperiod, Ipomoea's ability to form storage roots (Yang *et al.*, 2009) [58], and the creation of new genome/cytoplasm combinations that produce CMS (Cai *et al.*, 2006). (Yamagishi *et al.*, 2008) [59] demonstrated the effectiveness of mitochondrial crossover as a method for bringing CMS in cabbage, while (Fitter *et al.*, 2005) [22] demonstrated the possibility of introducing CMS by mtDNA through a wild species.

Breeding programmes are limited by the intrinsic difficulties presented by varying ploidy rates and the dioecious character of *Actinidia spp*. Conversely, though the use of SH offers a chance to retrieve healthy and productive hybrids, which will support breeding programmes and the ultimate blending of biological lineages inside the same gender. *Actinidia deliciosa* (6x) protoplasm were united by *Actinidia chinensis* (2x), as well as *A. chinensis* (2x) protoplasm were united with *Actinidiakolomikta* (2x).*A. deliciosa* and *A. chinensis* encountered being regeneration-competent protoplasts from cotyledon callus lines, while *A. kolomikta* was recognised to be non-regenerative protoplasts from the young, fully developed leaves of micropropagated shoots. attempted to fuse protoplasts to induce reproductive hybridization (SH) in avocados (*Persea americana*). In order to do this, avocado protoplasts from embryogenic cultures were combined with leaf protoplasts from species resistant to phytophthora root rot (PRR). Due of graft and/or behavioural mismatch restrictions connecting *Persea* spp. for Eriodaphane and genera in subgenus *Persea*, avocado breeders are unable to achieve the susceptibility to PRR seen in many species within the subgenus Eriodaphane. By combining non-morphogenic protoplasm of Persea spp. of the subgenus Eriodaphane with avocado embryonic protoplasts, (Litz and Deng. 2000) [31] successfully recovered somatic hybrids.

Cultivars of banana that flourish in the dry monsoon areas of Thailand, the Philippines, India, and Myanmar originates from blends with the chromosomal and haploid *Musa acuminate* (A gene) and *Musa balbisiana* (B gene). Offspring using this breeding strategy have genomes that are AB, AAB, and ABB. The sterility barriers connected to banana species have been successfully broken down by somatic hybridization (SH). Crossed tetraploid plants-created by the combination of elite diploid clones-with other diploids to reconstitute triploid clones. Furthermore, somatic hybridization has contributed significantly to the increase in genetic diversity among bananas.

Somatic hybridization (SH) allows for exchange of genes between the farmed octoploid (2n=2x=56) strawberry and the diploid strain Fragariavesca (2n=2x=14) in strawberries (*Fragaria spp*.).

Somatic hybrids of sexually incompatible rootstocks-wild pear (*Pyrus communis* var. *pyraster* L.; 2n=2x=16) the Colt cherry (*Pyrusavium* × *pseudocerasus*; 2n=4x=32)-were created, according to (Angers Cédex, 2012) [4]. Different heterokaryons were formed by independently electroporated with chemically fused mesophyll protoplasts from wild pear and protoplasm from cultures in suspension of Colt cherry. For most morphological markers, the hybrids showed traits in the middle range. Notably, the total somatic chromosomal count of the mother species was represented by the 58 chromosomes found in all autologous hybrid plants that were recovered.

Helianthus maximiliani, *H. giganteus*, or *H. Nuttallii* protoplasts they ought to be amalgamated with the ones from the *Helianthus annuus* to create fertile hybrids, as demonstrated by (Miladinović *et al.*, 2019) [43]. Further genetic markers have been utilised in research that characterise straight and asymmetric somatic hybrids produced among sunflower (*H. annuus*) and *H. maximiliani* (Binsfeld and Schnabl., 2002) [11]. Different degrees of linkage among the parental genomes were shown by these two groups of hybrids, making some of them outstanding choices to being an integral component of conventional breeding programmes.

(Mezzetti *et al.*, 2001) [42] argued that temperate soft fruit species ought for it to be contained within the statement somatic hybridization (SH) technology. Protoplasts from Rubusidaeus (raspberry; 2n=2x=14) and R. fruticosus (the blackberry; 2n=4x=28) were successfully fused (caused by PEG) (Mezzetti *et al.*, 2001) [42]. Significant karyotic rearrangements were revealed by fluorescent in situ hybridization in hybrid cells, which may help to explain why it is difficult to regenerate shoots from such tissues. Diospyrusglandulosa and *D. kaki* (persimmon) hybrids are another example of SH technology in fruit tree species in the Ebenaceae family; in these hybrids, solely chloroplasts from the former parent were found (Tamura *et al.*, 2002) [54].

Incredibly taken possible to alter the oil composition of Mentha species through somatic hybridization, which has commercial potential, particularly within the dietary supplement and flavour sectors. To provide peace of mind ensuring perform somatic hybridization, (Maffei *et al.*, 2007) [39] combined gingermint (*Mint gentilis* cv. *variegata*) among spearmint (Mentha piperita cv. Blackmint). Major volatile oil components, such as menthone, menthol, and linalool-the latter two being from spearmint and gingermint, respectively-were synthesised in the ensuing somatic hybrids. (Maffei *et al.*, 2007) [39] then combined the superior oil qualities of spearmint with the illness resistance inherited from spearmint by fusing the peppermint plant protoplasts (*Mentha piperita* cv. Black Mitcham) and peppermint (*Mint spicata* cv. Nature Spearmint).

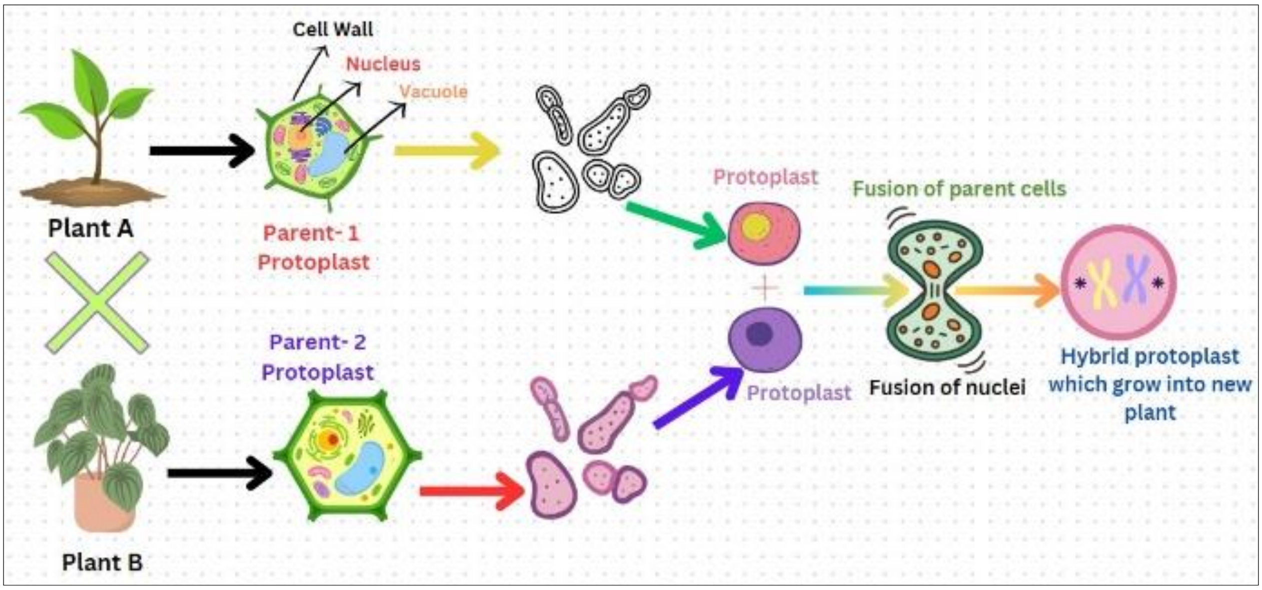

Fig 3: Schematic Diagram Illustrating the Protoplast Fusion Process in Somatic Hybridization for Crop Improvement

Prospects for the Future

Techniques like protoplast merger and somatic hybridization offer asexual pathways for genetic modification, deviating from conventional plant breeding. With these techniques, it is possible to bypass traditional breeding obstacles by directly introducing nuclear and cytoplasmic genomes into plant cells. Somatic hybridization increases the germplasm base and makes it easier to transmit many uncloned genes than transgenic techniques. Moreover, it may produce products that constitute not subject the identical thing regulatory restrictions that control genetically modified crops (Grosser and Gmitter, 2005) [28]. Furthermore, according to (Thieme *et al.*, 2004) [56], it permits both of them were swapped monogenic and polygenic features. Somatic hybrid (SH) has become a well-liked substitute in recent years to deal with issues brought on by incompatible sexual crossover. When contrasted against their sexual counterparts, somatic hybrids frequently have a greater number of chromosome rearrangements, polyploidization, and other genomic consequences (Blasio *et al*., 2022) [9]. Molecular marker systems offer improved means of identifying and monitoring the foreign DNA transferred into the genetic material of the living thing. This method offers an improved comprehension of the historical factors driving hybrid selection all through the combination of genetic process and makes it easier to conduct comprehensive research on genome stability.

Conclusion

Somatic hybridization has emerged as a valuable technique in the realm of horticulture, offering a novel approach to address challenges and unlock opportunities for crop improvement. How embodied hybridization is used in various horticultural crops has proven effective in overcoming issues related to sexual incompatibility, ploidy differences, and conventional breeding barriers. The potential for mitotic fusion to have the capacity to capable induce chromosomal rearrangements, polyploidization, and other genomic effects provides a platform for creating unique genetic combinations, expanding the germplasm base, and introducing desirable traits. This plan of action has proven successful employed in diverse horticultural agriculture, from trees full of pomegranates to soft fruits and herbs, showcasing its versatility and adaptability across different plant species. As we continue investigating in addition refine somatic hybridization techniques, the outlook in the years ahead is filled with hope for enhancing crop traits, improving disease resistance, and expanding the lineage within horticultural crops. The ongoing research and application of assimilation glandular underscore its significance as a transformative tool in modern horticulture, paving the way for sustainable and resilient agricultural practices.

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