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Somatic Hybrids and Cybrids: Innovations in Vegetable Improvement

Kanala Prajwalitha [a++*], Vijay Bahadur [a#] and Saket Mishra [a†]

[a] *Department of Horticulture, Naini Agricultural Institute, SHUATS, Prayagraj, Uttar Pradesh, India.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Somatic hybridization through protoplast fusion is indeed a promising method for producing asymmetrical and unbalanced polyploid somatic hybrids in various plant species. This technique involves merging protoplasts from different plant species to create hybrids that possess desirable traits for both scion and rootstock improvements. By circumventing the limitations of sexual hybridization, such as male/female sterility and sexual incompatibility, somatic hybridization enables the incorporation of beneficial genes from closely related or even distantly related species. The success of somatic hybridization in horticulture is evident in various crops such as citrus, potato, brinjal (eggplant), tomato, mango, avocado, banana, strawberry, pear, and cherry. It facilitates the transfer of numerous uncloned genes that confer resistance to biotic and abiotic stresses, thereby enhancing crop resilience and productivity. Unlike transgenic technology, which is often subject to regulatory constraints, somatic hybridization allows for the exchange of genetic material without the same legal formalities. Despite its potential benefits, somatic hybridization faces challenges and constraints compared to sexual hybridization. These include technical

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[++] *Ph.D Scholar;*
[#] *Associate Professor;*
[†] *Assistant Professor;*
**Corresponding author: E-mail: amrutharao.somavarapu@gmail.com;*

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difficulties in protoplast isolation and fusion, as well as limitations in generating fertile hybrids and maintaining genome stability. However, advancements in genomic technologies provide optimism for overcoming these challenges. Improved understanding of plant genomes enables more precise manipulation and selection of desired traits through somatic hybridization. In conclusion, somatic hybridization is a crucial tool in modern plant breeding and crop improvement efforts. It expands the gene pool available for breeding programs by incorporating genetic variability from diverse sources, thus offering new opportunities to enhance agricultural productivity and sustainability. As research and technology continue to advance, somatic hybridization holds promise for addressing current and future challenges in global agriculture.

Keywords: Somatic hybrids; cybrids; gene cloning; protoplast fusion.

1. INTRODUCTION

“Somatic hybridization is a crucial tool in plant breeding and crop improvement, facilitating the production of inter-specific and inter-generic hybrids. This technique involves fusing the protoplasts of two different genomes, selecting the desired somatic hybrid cells, and regenerating hybrid plants” (Evans DA & Bravo JE, 1988, Shuro, 2018). “It efficiently produces hybrids by merging protoplasts from different plants, species, or varieties, resulting in somatic hybrids. This non-conventional genetic procedure entails the fusion of isolated protoplasts in vitro and the subsequent development of a hybrid plant. Protoplast fusion offers an effective means of transferring desired traits from one species to another, significantly impacting crop improvements” (Brown DCW & Thorpe TA, 1995, Shuro, 2018).

“Once purified protoplasts are obtained from any two different sources—be it different tissues, plants, species, or genera—they can be fused to form somatic hybrids. This non-conventional method of genetic recombination, known as somatic hybridization, involves protoplast fusion in vitro and the development of the resulting product into a hybrid plant” (Kaushal, et al., 2024). The goal of somatic hybridization is to enhance crop plants, medicinal plants, or other plant types. Improvements through somatic hybridization can aim for disease resistance, improved quality, increased quantity, or other traits. For instance, somatic hybridization has been used to make potato plants (Solanum tuberosum) resistant to potato leaf roll disease (Ali et al., 2013).

“Somatic hybridization involves three key aspects: the fusion of protoplasts, the selection of hybrid cells, and the identification of hybrid plants. This technique has enabled the transfer of numerous desirable genetic traits among plants. The potential of somatic hybridization in important crop plants is exemplified by the creation of intergeneric hybrid plants within the Brassicaceae family” (Toriyama, et al., 1987, Shuro, 2018). Protoplasts can be induced to fuse using various fusogens or electrical manipulations that induce membrane instability. Common fusion-inducing agents include sodium nitrate (used by Carlson), high pH/Ca^{2+} concentrations, and Polyethylene Glycol (PEG) treatment (Ara et al., 2000).

This paper provides an overview of somatic hybridization as a method for transferring alien genes to crop species. It discusses the potential of somatic hybridization to restore ploidy levels in polyploid species after breeding at reduced ploidy levels and the challenge of resynthesizing allopolyploid species. Generally, somatic hybridization allows for the transfer of cytoplasmic organelles in a single generation, offering unique opportunities to combine the mitochondria of one species with the chloroplasts of another in a single hybrid. This capability may improve certain characteristics of cytoplasmic male sterile lines, leading to their commercial exploitation. Additionally, even non-flowering and non-tuber-bearing species can be utilized in breeding programs.

“Somatic hybrids can express both desirable and undesirable traits from their fusion parents, leading to unpredictable phenotypic performance. Consequently, these hybrids may not always be directly useful, as they contain the genomes of both parents” (Xu, et al., 2007; Assani et al., 2001). “Somatic hybridization, a significant application of protoplast culture, is particularly useful for hybridization between species or genera that cannot be crossed by conventional sexual hybridization methods” (Schenk and Hildebrandt, 1969; Yang et al., 2009). “Although somatic hybridization was first successfully achieved in animals, its full significance has been realized in plants, where hybrid cells can be induced to regenerate into whole plants” (Evans & Bravo, 1983; Yamagishi et al., 2008).

Morrison et al., (1988) recommended focusing protoplast fusion efforts on four key areas: (1) agriculturally important traits, (2) achieving combinations that can only be accomplished by protoplast fusion, (3) integrating somatic hybrids into conventional breeding programs, and (4) extending protoplast regeneration to a wider range of crop species. The somatic hybridization process involves several steps, including sourcing protoplasts, isolating them, plating, regenerating plants, fusing protoplasts, and conducting selection procedures, along with the identification and characterization of the somatic hybrid plants. Protoplasts can be isolated from almost any plant species and cultured to produce callus. Protoplasts from two different species can be fused using agents like polyethylene glycol, high calcium pH solution, or electrical stimulation to improve the efficiency of protoplast fusion (Blokesch & Schoolnik., 2008; Binsfeld & Schnabl., 2002).

2. CONCEPTS AND ASPECTS

“For many years, the conventional method to improve the characteristics of cultivated plants has been sexual hybridization. However, the major limitation of this method is that it can only be performed within a plant species or between very closely related species, restricting the potential improvements in plants. These species barriers in sexual hybridization can be overcome by somatic cell fusion, which can create viable hybrids (Calixto et al., 2004). Somatic hybridization involves the development of hybrid plants through the fusion of somatic protoplasts from two different plant species or varieties. This process broadly includes the in vitro fusion of isolated protoplasts to form a hybrid cell, followed by its development into a hybrid plant. Protoplast fusion is a relatively new and versatile technique to induce or promote genetic recombination in various prokaryotic and eukaryotic cells” (Bhojwani, et al., 1977).

The term "protoplast" was introduced by Hanstein in 1880. A protoplast is a cell with its cell wall removed, either mechanically or enzymatically. The first isolation of protoplasts was achieved by Klercker in 1892 using a mechanical method. In 1909, Küster described the process of random fusion in mechanically isolated protoplasts. The real beginning of protoplast research came in 1960 when Cocking used an enzymatic method for cell wall removal (Cai et al., 2006). Plant protoplasts are extremely useful in somatic plant cell research, genetic manipulations, and crop improvement. They provide a novel opportunity to create cells with new genetic constitutions and offer a solution to sexual incompatibility between different plant species (Cheng et al., 2018). Takebe et al., (1971) successfully regenerated a whole tobacco plant from protoplasts. Somatic hybridization (fusion of protoplasts) is a versatile technique to induce or promote genetic recombination in various prokaryotic and eukaryotic cells (Bhojwani, et al., 1977).

2.1 Techniques of Somatic Hybridization

Protoplast fusion is a method used to make significant changes in the genetic composition of plants. Protoplasts are released from plant tissues after incubation in cell wall-degrading enzymes (Cocking, 1960; Chupeau & Davey., 2013). Several procedures can induce protoplasts from different plants to fuse, including incubation in Polyethylene Glycol (PEG) (Kao & Michayluk, 1974) or treatment with electrical pulses (Zimmerman, 1982). Somatic hybrid plants can then be regenerated from cultures of these fusion products. The basic technique applied for hybridization is given in Fig. 1.

2.2 Isolation of Protoplast

The term "protoplast" refers to the spherical, plasmolyzed content of a plant cell enclosed by the plasma membrane, essentially a naked cell without its cell wall. Before culturing protoplasts, it is crucial to isolate viable and uninjured protoplasts. Protoplasts can be isolated from almost all plant parts, including roots, leaves, fruits, tubers, root nodules, endosperm, pollen mother cells, callus, and suspension cultures (Cocking, 1960; Cui et al., 2009; Fitter et al., 2005).

Specific examples include:

Leaves: Spongy and palisade mesophyll tissue from mature leaves of Nicotiana and Petunia.
Anthers: Anthers of Pelargonium (Abo El-Nil and Hilderbrandt, 1976).
Callus: Callus from Gossypium hirsutum (Bhojwani, Cocking, and Power, 1977).
CAM Plants: Protoplasts from Crassulacean acid metabolism (CAM) plants (Dodds, 1985).

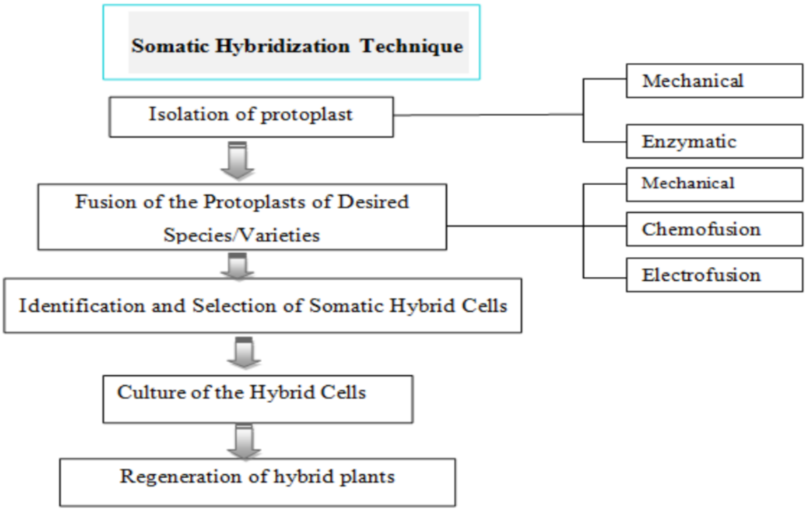

Fig. 1. Schematic representation of production of hybrid plant via protoplast fusion

C3 and C4 Plants: Protoplasts from C3 and C4 plants (Kanai & Edwards, 1973).
Tubers: Protoplasts from *Solanum tuberosum* (Upadhya, 1975).

These examples demonstrate the wide range of plant tissues and species from which protoplasts can be isolated for various research and agricultural applications.

2.3 Protoplast Fusion

Since isolated protoplasts lack cell walls, thier *in vitro* fusion is relatively easy (Gamborg et al., 1974; George et al., 2007). Protoplast fusion does not face barriers of incompatibility, allowing fusion at inter-specific, inter-generic, and even inter-kingdom levels (Fig. 2). To effectively use protoplast fusion technology, two criteria must be met:

1. Protoplasts must be isolated in large quantities.
2. The isolated protoplasts must be totipotent, meaning they should have the ability to proliferate and regenerate into new plants.

Protoplast fusion, which involves the mixing of protoplasts from two different genomes, can be achieved through spontaneous, mechanical, or induced fusion methods (Grosser et al., 2007).

Spontaneous fusion: Protoplasts often fuse spontaneously during the isolation process due to physical contact, a phenomenon known as spontaneous fusion (Grosser & Gmitter., 2011). Cell fusion is a natural process, as observed in egg fertilization. During the enzymatic degradation of cell walls, some adjoining protoplasts may fuse to form homokaryocytes (homokaryons), which are fused cells that can contain a high number of nuclei (2-40). This occurs mainly due to the expansion and subsequent coalescence of plasmodesmal connections between cells. The frequency of homokaryon formation is particularly high in protoplasts isolated from dividing cultured cells (Grosser et al., 2000). However, spontaneously fused protoplasts typically cannot regenerate into whole plants and only undergo a few cell divisions.

Intra-Specific Protoplast Fusion: Intra-specific protoplast fusion refers to the fusion of protoplasts from the same species. This technique provides a unique method for performing crosses and conducting genetic analysis within the same species (Guo et al., 2000).

Inter-Specific Protoplast Fusion: “Inter-specific protoplast fusion involves the fusion of protoplasts from two different species. This type of fusion is particularly important for producing new products. Due to the novel genetic combinations, many new secondary metabolites, such as antibiotics, can be produced. It is well established that pre-zygotic sexual incompatibilities in plants can be overcome using somatic protoplast fusion, coupled with plant regeneration from the heterokaryons formed by interspecies protoplast fusions” (Kumar & Cocking, 1987).

Both intra-specific and inter-specific protoplast fusion techniques offer valuable tools for plant breeding and genetic research, enabling the creation of new genetic combinations and the production of valuable metabolites (Kanazawa et al., 2011; Kao & M.R., 1974).

Induced fusion: The fusion of freely isolated protoplasts from different sources with the aid of fusion-inducing chemical agents is known as induced fusion (Khan, 2005). Normally, isolated protoplasts do not fuse spontaneously because the surface of isolated protoplasts carries negative charges (-10mV to 30mV) around the outside of the plasma membrane. This negative charge creates a strong repulsion between protoplasts, hindering their fusion. To overcome this electrostatic repulsion and facilitate fusion, fusion-inducing chemicals, also known as fusogens, are used. Fusogens work by reducing the electronegativity of the isolated protoplasts, thereby allowing them to overcome their natural repulsion and fuse with each other (Narayanswamy S., 1994). This method is crucial for achieving successful protoplast fusion in experimental settings and is employed extensively in biotechnological applications involving plant cell fusion and genetic modification (Kumar et al., 2018).

Chemo Fusion: “Chemo fusion involves the use of various chemicals to induce protoplast fusion. Several chemicals have been utilized for this purpose, including $NaNO_3$, Polyethylene Glycol (PEG), and Calcium ions (Ca^{2+}). Chemical fusogens work by causing isolated protoplasts to adhere to each other, leading to tight agglutination and subsequent fusion” (Pasha, et al., 2007; Jogdand, 2001).

Mechanical Fusion: Mechanical fusion involves physically bringing isolated protoplasts into intimate contact under a microscope using techniques such as a micromanipulator or perfusion micropipette (Lentz, 2007; Litz, 2004). In this method, protoplasts are pushed together mechanically to induce fusion. However, mechanical fusion carries the risk of damaging protoplasts due to potential injuries caused during the process.

Electro Fusion: Electro fusion is a technique where fusion is induced by electrical stimulation. Protoplasts are exposed to a high-strength electric field (approximately 100 kV m^{-1}) for a few microseconds. This electrical stimulation causes the protoplasts to fuse together, facilitating the formation of hybrid cells (Liu et al., 1999).

Each of these fusion methods—chemo fusion, mechanical fusion, and electro fusion—offers distinct advantages and considerations in protoplast fusion experiments. Chemo fusion is effective due to the ability of chemicals to reduce protoplast surface charge and promote adhesion, while mechanical and electro fusion methods provide direct physical means to bring protoplasts into fusion.

During the protoplast isolation process, spontaneous fusion is partially attributed to plasmodesmata (Liu et al., 2005). However, deliberate fusion of protoplasts from different sources requires creating conditions that promote broad transmembrane contact and activation. Recent studies have highlighted polyethylene glycol (PEG) as a highly effective agent for inducing protoplast fusion (Compton, et al., 2018). PEG, particularly at concentrations between 2.8% and within a molecular mass range of 1500–6000, facilitates protoplast fusion by bringing opposing membranes into close contact (Maffei et al., 2007). As PEG concentration is gradually diluted, the fusion process progresses towards completion. Utilization of PEG has shown success rates in producing heterokaryons ranging from 20% to 30%. Protoplasts exhibit resilience to PEG treatment, and the fusion products regenerate and divide as cells in subsequent cultures (Compton, et al., 2018). The mechanism of PEG's action in protoplast fusion is subject to various perspectives (Compton, et al., 2018, Parray, et al., 2020). PEG is believed to induce dehydration, causing compression and folding of the plasma membrane. Its strong polarity and slight ionic charge likely aid in integrating with lipid and protein groups across opposing membranes, facilitating fusion (Maldonado-Celis et al., 2019).

Role of Somatic hybridization in crop improvement: Improving crop plants through conventional breeding is often time-consuming and expensive. In contrast, unconventional techniques like protoplast fusion, developed and successfully used by Melchers and Labib (1974), can significantly shorten the breeding process. Somatic hybridization, a key application of protoplast fusion, is especially valuable for heterozygous crops with varying ploidy levels, such as potatoes. Potato breeding programs often require pre-breeding to facilitate easier and faster selection at the diploid level. Efficiently utilizing genetic diversity found in economically important plants and their wild relatives and

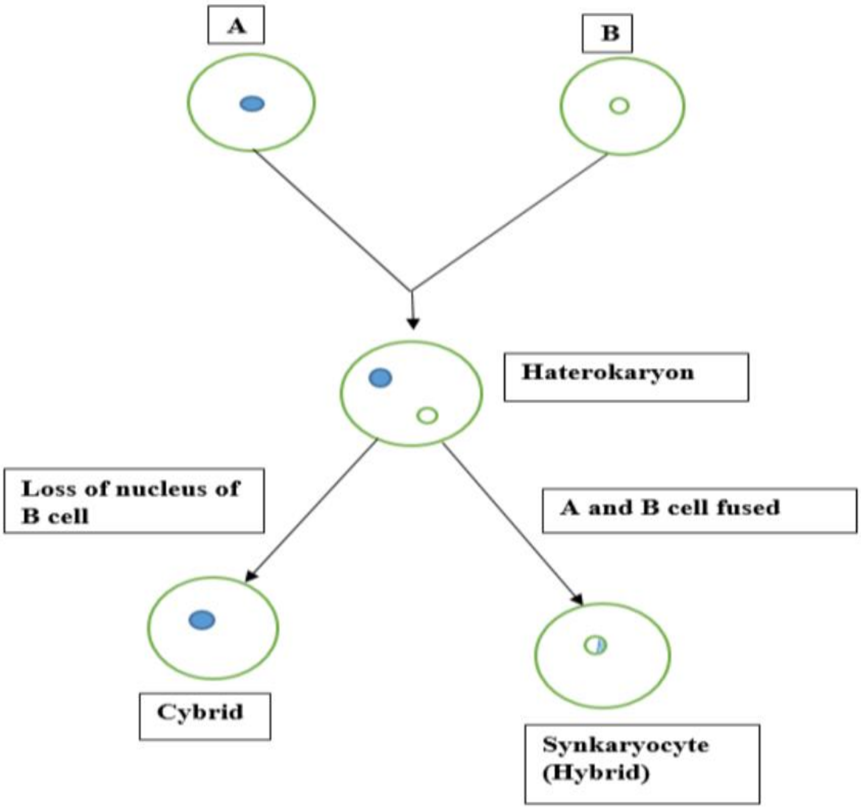

Fig. 2. Techniques of protoplast fusion

introducing stable disease resistance through somatic hybridization and/or molecular biological techniques, can lead to crops with improved traits and contribute significantly to sustainable food security (Mendes et al., 2001). Novel inter-specific and inter-generic crosses, which are challenging to achieve through conventional methods, can be more readily obtained through protoplast fusion (Mezzetti et al., 2001; Wang et al., 2013; Xiang et al., 2010).

Somatic hybridization also offers the opportunity to introduce important traits such as disease resistance, tolerance to abiotic stresses, and other quality characteristics into hybrid plants by fusing protoplasts from a plant with desired traits to another plant that may lack these attributes. Furthermore, protoplasts from sexually sterile haploid, triploid, and aneuploid plants can be utilized to obtain fertile diploids and polyploids, thereby broadening breeding possibilities and enhancing genetic diversity in cultivated crops. These advancements highlight the potential of protoplast fusion and somatic hybridization in accelerating crop improvement and addressing global food security challenges (Miladinović et al., 2019; Newman et al., 1994).

Somatic hybridization (Fig. 3), facilitated by protoplast fusion, offers significant advantages in plant genetic improvement, particularly for traits that are cytoplasmically encoded, such as cytoplasmic male sterility, antibiotic resistance, and herbicide resistance (Ohgawara et al., 1985). Here are some key points highlighting its utility:

1. Transfer of Cytoplasmic Traits: Agronomically important traits like cytoplasmic male sterility and various resistances (antibiotic, herbicide) encoded in the cytoplasm can be efficiently transferred to other plant species through somatic hybridization.
2. Hybridization of Juvenile Plants: Somatic hybridization allows for hybridization of plants in their juvenile stages, which is advantageous compared to traditional methods that require mature plants for sexual hybridization.
3. Autotetraploid Production: It serves as a method for producing autotetraploid, which can be beneficial for enhancing plant vigour and improving certain traits.

4. Interspecies and Intergeneric Hybridization: Somatic hybrids can be generated between species that cannot be crossed sexually, thus expanding the genetic diversity available for breeding programs.
5. Transfer of Resistance Genes: It is particularly useful for transferring genes conferring resistance to diseases and abiotic stresses from wild relatives or other species to cultivated crops.
6. Combination of Organelle Genomes: Somatic hybridization allows for the combination of mitochondria from one species with chloroplasts from another, a feat not achievable through sexual means even between closely related species.
7. Recombinant Organelle Genomes: Recombinant genomes of organelles, especially mitochondria, can be generated in somatic hybrids, potentially leading to organelles with novel and useful features.
8. Use in Asexual and Sterile Plants: It is beneficial for plants that are asexual or sterile, as well as for species with sexual incompatibilities with other plants.
9. Shortened Breeding Time: Compared to traditional backcrossing methods, somatic hybridization significantly reduces the time required for cytoplasm transfer, from several years to just one year.
10. Overcoming Incompatibility Barriers: It circumvents the barriers of sexual incompatibility at interspecific and intergeneric levels, allowing for the creation of hybrids that are otherwise impossible through conventional breeding methods. Somatic hybridization through protoplast fusion has revolutionized plant genetic manipulation, offering new avenues for crop improvement by leveraging genetic diversity and overcoming breeding limitations (Rajan & Hudedamani., 2019; Ramulu et al., 1996b; Reed & Bargmann., 2021).

Protoplast fusion, known as somatic hybridization (SH), offers a pathway to overcome reproductive barriers and enhance traditional breeding efforts. These barriers include conflicting bloom phases, male and female sterility, nucellar development, and polyembryony, which hinder conventional breeding techniques in transferring desirable traits such as disease resistance and cytoplasmic male sterility (CMS) (Aleza, et al., 2010). Somatic hybridization enables the fusion of protoplasts from distinct species, creating novel hybrids that combine traits from both parental species (Mwangangi, et al., 2019). This technique plays a crucial role in crop improvement by facilitating the creation of hybrids across different plant species and genera (Soriano et al., 2012). According to Imandi and Bahadur (2023), somatic hybridization involves merging protoplasm cultures from different species or varieties,

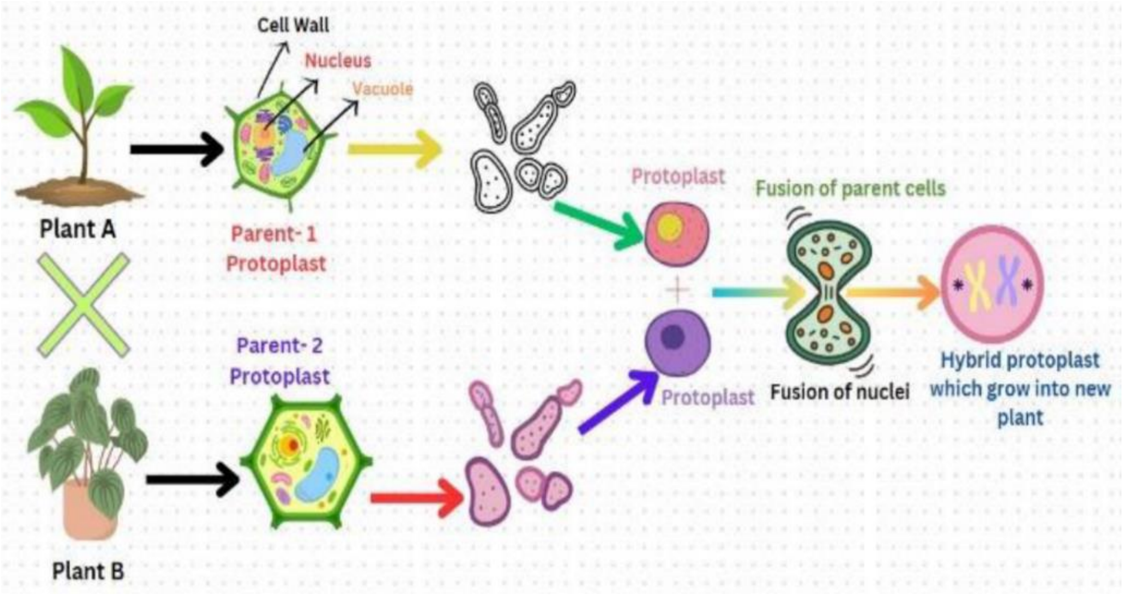

Fig. 3. Schematic Diagram Illustrating the Protoplast Fusion Process in Somatic Hybridization for Crop Improvement

selecting desired somatic hybrid cells, and growing them into hybrid plants. It serves as an effective method for developing hybrids with desired genetic traits, significantly advancing agricultural practices. Protoplast fusion is instrumental in transferring genes with desirable traits from one species to another, impacting agricultural advancements. This method involves the in vitro fusion of isolated protoplasts and subsequent growth into hybrid plants, known as somatic hybrids. It has been extensively used to enhance various plants, including both agricultural and medicinal species, by modifying traits such as quality, quantity, disease resistance, and other characteristics.

2.4 Few Major Advancements in Improvement

Disease and Insect Resistance: Disease resistance genes against various pathogens such as tobacco mosaic virus (TMV), potato virus X, and club rot disease have been successfully transferred from wild species to cultivated plants. For instance, resistance against diseases like TMV and spotted wilt virus has been introduced into tomato through somatic hybridization. Asymmetric somatic hybridization allows for the transfer of specific genomic regions or traits from one species to another. This approach has been successfully utilized to transfer bacterial blight resistance from wild Oryza meyeriana to cultivated Oryza sativa ssp. japonica, demonstrating its promise in targeted trait introgression (Yan, et al., 2004). Asymmetric hybridization offers the advantage of transferring only desired genomic segments, which may be more easily integrated and tolerated compared to whole-genome transfers. Studies have shown that partial genome transfers can be effective in introducing new traits while minimizing potential negative effects (Derks, et al., 1992, Trick, et al., 1994, Liu & Deng, 2002). Wild species of the genus Nicotiana have been particularly valuable in providing disease resistance and other beneficial traits to cultivated tobacco. Traits from over 13 different Nicotiana species have been successfully transferred into tobacco, highlighting the broad applicability and genetic diversity available through somatic hybridization (Lewis, 2011).

Environmental Tolerance and Wider Adaptation: The in vitro fusion of protoplasts represents a breakthrough in overcoming sexual incompatibility barriers, facilitating the development of unique hybrid plants. This technique has been instrumental in introducing genes responsible for cold, frost, and salt tolerance through somatic hybridization. For example, cold tolerance genes have been successfully introduced into tomato varieties using this method. In agriculture, somatic hybrids have been created by electrofusion of protoplasts from rice and ditch reed, specifically targeting salt tolerance traits. This approach has found practical application in the horticultural industry, where it has been used to breed new hybrids with enhanced fruit yield and improved disease resistance. Notably, successful viable hybrid plants have been produced by fusing protoplasts from citrus with other related species within the citrinae group, demonstrating the versatility and efficacy of somatic hybridization in broadening genetic diversity and enhancing desirable traits in cultivated plants (Motomura, et.al, 1997).

Overcoming Barriers of Sexual Incompatibility: Somatic hybridization serves as a powerful tool to overcome the inherent barriers of sexual incompatibility between different species and genera, enabling the creation of novel hybrids that are not achievable through conventional breeding methods. For instance, fusion of protoplasts from potato (*Solanum tuberosum*) and tomato (*Lycopersicon esculentum*) has led to the development of the pomato (*Solano persicon*), a hybrid genus combining traits from both parent species. In another example, inter-specific fusion involving four different species of rice (*Oryza brachyantha, O. eichingeri, O. officinalis,* and *O. perrieri*) has been successfully conducted to enhance crop traits, demonstrating the versatility of somatic hybridization in combining genetic diversity for agricultural improvement. These examples underscore how somatic hybridization expands the genetic possibilities in plant breeding, contributing to the development of new crop varieties with desirable characteristics.

Transfer of Cytoplasm: Cytoplasmic traits play a crucial role in certain plants, influencing characteristics such as male sterility, antibiotic resistance, and herbicide tolerance. Cybridization emerges as a valuable technique for transferring desired cytoplasmic traits in a single step. It is particularly significant for introducing cytoplasmic male sterility (CMS), antibiotic resistance, and herbicide resistance into agriculturally important plants. Notably, cybridization has successfully transferred CMS in rice and created cybrids of *Brassica raphanus*

Table 1. Somatic Hybridization Methods Key Finding in vegetables

Crop	Somatic hybridization methods	Key findings	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 & Rakosy-Tican, (2017)
	Chloroplast Transfer	Electrofusion of leaf mesophyll protoplasts has been utilized to generate somatic hybrids with novel genetic combinations.	Ondrej & Kormutak, (2000)
	Hybrid Cell Formation	Somatic hybridization has been explored as a strategy to introgressive traits like late blight resistance from wild potato species into cultivated varieties.	Borhan & Hadi, (2017)
Tomato	Protoplast Fusion	Somatic hybridization in tomato has led to the development of hybrids with improved vigour, disease resistance, and fruit quality attributes.	Dangi & Agrawal, (2015)
	Chloroplast Transfer	Challenges and limitations exist in somatic hybridization of tomato, including low fusion efficiencies and regeneration rates.	Daunay, et al., (2019)
	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 & Mohan, (2006)
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)
	Chloroplast Transfer	Interspecific somatic hybrids involving Brinjal have been created, offering opportunities for novel trait combinations and genetic diversity enhancement.	Saini & Kaushik, (2019)
	Hybrid Cell Formation	Protoplast fusion techniques have been employed to facilitate somatic hybridization, broadening the genetic base of Brinjal cultivars.	Nanda & Sahoo in 2012 (Kaushal et al., 2024).

with a nucleus from *B. napus*, chloroplasts resistant to atrazine from *B. campestris*, and male sterility traits from *Raphanus sativus*. Somatic hybridization, facilitated by protoplast fusion, has significantly contributed to the study of cytoplasmic genes and their functions. This knowledge is effectively utilized in plant breeding programs to enhance desirable traits. By combining mitochondria and chloroplasts through protoplast fusion, somatic hybridization enables the creation of unique nuclear-cytoplasmic genetic combinations. Importantly, somatic hybridization can be applied to plants in their juvenile phase, allowing for early integration of beneficial traits. Additionally, protoplast transformation, incorporating exogenous DNA such as genes for nitrogen fixation, followed by somatic hybridization, holds promise for generating innovative plant varieties with enhanced characteristics. Thus, these techniques represent powerful tools in modern agriculture for manipulating cytoplasmic traits and advancing crop improvement efforts.

Vegetative Propagation in Plants: Hybrid and genetically modified cells obtained through protoplast fusion can be cultivated in environments conducive to plant development and morphogenesis. Studies (Table 1) have shown that interspecific hybrid cells, formed by fusing protoplasts from two different tobacco species, are capable of regenerating into plants (Mwangangi et al., 2019). As tools for hybrid cell selection advance and the ability to regenerate plants from protoplasts grows, the development of intergeneric hybrid crops becomes increasingly feasible. It is crucial to understand the specific conditions required to initiate organ development in cells rejuvenated from the protoplasts used in genetic modification processes.

Potato (*Solanum tuberosum*): Due to its autotetraploid nature, cultivating potatoes (*Solanum tuberosum*) requires significant efforts to combine beneficial agronomic traits within a single genotype. In such scenarios, chromosomal doubling (auto tetraploidization) following diploid breeding has been proposed as an alternative strategy to develop superior cultivars. This approach has shown potential benefits by effectively integrating multiple desirable traits from related or wild species into cultivated potatoes. For instance, chromosomal doubling via somatic hybridization (SH) was employed to transfer resistance to bacterial wilt from *Solanum phureja* and *Solanum stenotomum* to *Solanum tuberosum* (Fock, et al., 2001). They successfully generated somatic hybrids between *S. tuberosum* and *S. commersonii*, overcoming compatibility issues related to fertility and endosperm balance number (Gaiero, et al., 2017). Most of these hybrid plants exhibited resistance to bacterial wilt, and crossing fertile, resistant hybrids with *S. tuberosum* resulted in viable seed production (Andino, et al., 2022).

Tomato (*Lycopersicon esculentum*): Within the extensive and diverse Solanaceae family, the genus *Lycopersicon* stands out with its compact nature, comprising eight related wild species and the cultivated tomato, *Lycopersicon esculentum*. The wild members of the *Lycopersicon* species are renowned for their valuable agronomic traits. However, significant unilateral or bilateral incompatibilities often hinder sexual crosses between domestic tomatoes and these indigenous species, resulting in predominantly sterile F_1 hybrids. Efforts have successfully crossed cultivated tomatoes (*L. esculentum*) with species like *L. chilense, L. pennellii*, and *L. peruvianum* to create compatible interspecific hybrids. Additionally, various other organisms such as *Nicotiana tabacum, Solanum tuberosum, Solanum tuberosum* × *Solanum brevidens, Solanum lycopersicoides, Solanum muricatum, Solanum nigrum, Solanum rickii,* and *Solanum tuberosum* have undergone successful intergeneric somatic fusion with tomatoes. Despite extensive morphological and organellar studies on these hybrids, detailed agronomic data from these somatic crosses remains scarce. Particularly intriguing is the successful embryonic hybridization between tomatoes and *L. peruvianum,* noted for its fertility—a rare occurrence compared to the documented cases of sterility in such crosses.

Brinjal (*Solanum melongena*): Transferring agriculturally beneficial traits from closely related wild species to eggplant (*Solanum melongena*) poses a significant challenge. Studies on interspecific protoplast fusion have highlighted varying outcomes in reproductive success. Tamura et al., (2002) observed this phenomenon in crosses involving *S. melongena* with *S. aethiopicum*, *S. torvum, S. khasianum,* and between *S. aethiopicum and S. violaceum*. In the case of S. melongena and S. sisymbriifolium, sexual hybridization failed, but somatic hybridization (SH) produced a viable, albeit sterile, hybrid (Collonnier, et al., 2003). Despite significant advancements in the field, creating a successful eggplant cultivar through interspecific protoplast fusion remains elusive. Attempts to trace the ancestry of Di haploid organisms resulting from *S. melongena-S. aethiopicum* somatic crosses, particularly those carrying Fusarium wilt resistance inherited from the *S. aethiopicum* parent, have employed isozymes, randomly amplified polymorphic DNAs (RAPDs), and inter-simple sequence repeat markers (Rizza, et al., 2002).

3. LIMITATIONS

Somatic hybridization was initially anticipated to revolutionize crop improvement by overcoming barriers like pre-fertilization or genomic incompatibility. However, experimental findings have shown mixed results, tempering early enthusiasm. Currently, techniques for selecting and manipulating somatic hybrid cells, as well as regenerating viable hybrid plants, are largely restricted to specific cases that can be effectively managed in culture conditions. As a result, the production of somatic hybrids of agriculturally important plants remains challenging and often impractical. The original ambition of somatic hybridization to achieve wide crosses through protoplast fusion, thereby resolving many crop improvement challenges, has not fully materialized. Inter-generic crosses between distantly related plants that are not sexually compatible remain largely unattainable. Successful inter-specific somatic hybridization is limited to cases where natural reproductive isolation is absent or can be overcome. Another significant limitation of somatic hybridization is the potential loss of chromosomes in hybrid cells, which can hinder the development of desirable traits in the offspring. Efforts to increase the efficiency of cell fusion have been made, but achieving consistent success remains a challenge. Moreover, the lack of standardized methods for identifying, selecting, and isolating hybrids at the culture level further complicates the process.

4. FUTURE PROSPECTS

Techniques such as protoplast fusion and somatic hybridization provide asexual pathways for genetic modification, diverging from traditional plant breeding methods. These techniques enable the direct introduction of nuclear and cytoplasmic genomes into plant cells, bypassing conventional breeding barriers. Somatic hybridization expands the germplasm base and facilitates the transfer of numerous uncloned genes more efficiently than transgenic techniques. Additionally, products derived from somatic hybridization may not be subject to the same regulatory restrictions as genetically modified crops (Grosser & Gmitter, 2005). Moreover, according to Thieme et al., (2004), somatic hybridization allows for the exchange of both monogenic and polygenic traits. In recent years, somatic hybrids (SH) have gained popularity as an alternative to overcome challenges posed by incompatible sexual crosses. Compared to their sexual counterparts, somatic hybrids often exhibit higher levels of chromosome rearrangements, polyploidization, and other genomic alterations (Blasio, et al., 2022). Molecular marker systems provide enhanced tools for identifying and tracking foreign DNA integrated into the organism's genetic material. This approach enhances understanding of the genetic factors influencing hybrid selection throughout the genetic manipulation process and facilitates comprehensive research on genome stability.

5. CONCLUSION

Genetic variability within species has traditionally been a cornerstone of crop improvement efforts, but modern plant breeding demands expanding gene pools beyond existing variability. Sexual crosses between closely related species have been the primary method for introducing new traits, yet reproductive barriers often limit gene transfer to compatible species. This constraint restricts access to potentially valuable traits found in distantly related or unrelated species. Advancements in somatic cell genetics have revolutionized gene transfer by enabling the crossing of sexual barriers and taxonomic distances. When specific genes are known and isolated, transformation is preferred; however, for traits where genes remain unidentified, somatic hybridization emerges as a viable alternative. Beyond gene transfer, somatic hybridization facilitates modification of polygenic traits and organellar genetic material, leveraging the fusion of protoplasts from different species or varieties. Somatic hybridization involves the fusion of protoplasts to produce hybrids known as somatic hybrids. This method is applicable when two criteria are met: abundant isolation of protoplasts and their totipotency. It proves especially valuable for breeding plants that are asexual, sterile, or sexually incompatible with other species. In summary, somatic hybridization stands as a crucial tool in modern plant breeding and crop improvement, facilitating the production of inter-specific and inter-generic hybrids. It enhances the genetic diversity available for breeding programs, overcoming traditional barriers to gene transfer, and offering new avenues for enhancing agricultural productivity and resilience. Somatic hybridization has emerged as a valuable technique in horticulture, offering a novel approach to address challenges and unlock opportunities for crop improvement. This method effectively overcomes issues related to sexual incompatibility, ploidy differences, and conventional breeding barriers across various horticultural crops. The potential of somatic fusion to 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 approach has been successfully employed in diverse sectors of horticulture, from tree fruits like pomegranates to soft fruits and herbs, highlighting its versatility and adaptability across different plant species. As we continue to refine somatic hybridization techniques through ongoing research, the future holds promise for enhancing crop traits, improving disease resistance, and broadening the genetic diversity within horticultural crops. The persistent research and application of somatic hybridization underscore its significance as a transformative tool in modern horticulture, paving the way for sustainable and resilient agricultural practices.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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