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ROLE OF SOMATIC CELL HYBRIDIZATION FOR CONTRIBUTION TO CROP IMPROVEMENT

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Abstract *Breeders have successfully leveraged genetic heterogeneity within the species to enhance crop quality. Large efforts have been made to expand the current crop gene pool since, for modern plant breeding objectives, the variability found in a breeding population may not be sufficient. Mostly, sexual crossovers between distinct genotypes within or across closely related species have provided the basis for the introduction of novel features. The potential to alter and enhance food plants has been limited, though, as gene transfer has only been possible among sexually compatible species because of multiple reproductive hurdles. Only closely related species or even unrelated creatures may possess several desirable and intriguing agronomic traits. Much work has gone into locating, isolating, and transferring these genes into crops because they represent a potential genetic resource. Thanks to the quick advancement of somatic cell genetics, there are now ways to transmit genes across taxonomic boundaries and beyond sexual boundaries. Somatic hybridization is useful not just for the transfer of unknown genes but also for modifying and enhancing polygenic features. Furthermore, since a hybrid cell with a mixture of the two fusion partners is produced, somatic hybridization allows for the change of genetic material. Somatic hybridization is the process of creating hybrid plants by fusing the protoplasts of two distinct plant species or kinds; these hybrids are referred to as somatic hybrids. Thus, only in cases when all of the following two requirements are met—i) large-scale protoplast isolation and ii) totipotency of the separated protoplasts—can somatic hybridization be used. Generally speaking, somatic hybridization is a useful technique for improving crops and plant breeding by creating interspecific and intergeneric hybrids. Asexual and sterile plants can benefit from it, as can those whose sexual compatibility with other species is compromised.*

Keywords*:* *Hybrid; Hybridization; Somatic; Crop improvement; Protoplast fusion*

Introduction

Importation of species from one region into another In order to produce inter-specific and inter-generic hybrids and better crops, somatic hybridization is a crucial strategy in plant breeding (Mwangangi et al., 2019). The process entails fusing the protoplasts of two distinct genomes, choosing the desired somatic hybrid cells, and then regenerating hybrid plants (Eeckhaut et al., 2013). Fusion of two distinct protoplasts from different plants, species, or varieties is an effective method of producing hybrids; these hybrids are known as somatic hybrids (Guo et al., 2013; Javed et al., 2024; Junaid and Gokce, 2024). This unconventional genetic process involves the union of isolated protoplasts in an in vitro environment, leading to the growth of the resultant hybrid plant (Ranaware et al., 2023). Protoplast fusion has a growing influence on agricultural advancements and offers an effective method of transferring genes with desired traits from one species to another (Evans, 1983). Somatic hybrids can be created by fusing purified protoplasts from two distinct origins, which can be separate tissues, plants, species, or genera (Grosser et al., 2010). Somatic hybridization is the term for this unconventional genetic recombination process that involves protoplast fusion in vitro and the subsequent growth of their product into a hybrid plant. Somatic hybridization is used to enhance different kinds of plants, medicinal plants, and crop plants. Somatic hybridization can be used to improve a species by changing its quality, quantity, resistance to illness, or other characteristics (Shuro, 2018). Solanum tuberosum, the potato plant, for instance, develops resistance to the disease known as potato leaf rolling. The three steps of somatic hybridization are the fusion of protoplasts, the selection of hybrid cells, and the identification of hybrid plants (Thieme and Rakosy-Tican, 2017). Multiple desired genetic traits can now be transferred between the plants thanks to the method (Javed et al., 2024; Naeem et al., 2024; Tanksley and Nelson, 1996). The creation of intergeneric hybrid plants among members serves as the best example of the possibility for somatic hybridization among significant agricultural plants (Shuro, 2018). Many fusogens and electrical manipulations that cause membrane instability can cause protoplasts to fuse (Compton et al., 2018). The three most frequently mentioned fusion-inducing agents are treatment with polyethylene glycol (PEG), high pH/Ca2+ concentration, and sodium nitrate.

An overview of the use of somatic hybridization to introduce foreign genes into crop species was provided in this work (Glimelius et al., 1991). We talked about the difficulty of re-synthesising allopolyploid species and the possibility of somatic hybridization to restore the ploidy level in polyploidy species after breeding at a reduced ploidy level (Hegarty and Hiscock, 2008; Rasheed et al., 2026; Raza et al., 2025). In general, Somatic hybridization has special possibilities for fusing mitochondria from one species with chloroplasts from another species in a single hybrid, as well as the transfer of cytoplasmic organelles in a single generation (Rose et al., 1990). This potential could enable the enhancement of specific cytoplasmic male sterile line traits, potentially resulting in their commercial utilization (Bohra et al., 2016). Furthermore, species that do not blossom or grow tubers can still be used in breeding programs.

Therefore, this paper's goals are to:

1. Review the function that somatic hybridization plays in crop improvement.
2. Learn about the processes involved in somatic hybridization and some of its limitations.

The idea behind somatic hybridization and its aspects

For many years, the traditional approach to enhancing the traits of domesticated plants has used sexual hybridization (McKey et al., 2010). The fact that sexual hybridization can occur within a plant species or among closely related species is its main drawback (Rieseberg, 1997). This limits the advancements that plants can undergo. Somatic cell fusion has the potential to generate a viable hybrid, hence overcoming the species barriers to plant improvement that arise during sexual hybridization (Begna, 2021). It is the process by which two distinct plant species or varieties' somatic protoplasts fuse to form hybrid plants (Shuro, 2018). In general, somatic hybridization refers to the in vitro union of distinct protoplasts to create a hybrid cell, which then develops into a hybrid plant. Protoplast fusion is a relatively recent and adaptable method for stimulating or inducing genetic recombination in a range of bacterial and eukaryotic cells (Davey et al., 2005). A protoplast is a cell that has had its cell wall removed through mechanical or enzymatic means (Lionetti et al., 2015). Protoplast isolation we mechanically isolated protoplasts for the first time. When Cocking employed an enzymatic technique to remove the cell wall in 1960, it marked the true beginning of research on protoplasts (Cocking, 1972). The use of plant protoplasts in somatic plant cells, genetic engineering, and agricultural enhancement is extremely beneficial. Therefore, protoplasts offer a unique way to produce cells with a different genetic makeup (Davey et al., 2005). We were able to successfully regenerate the entire tobacco plant from protoplasts, which is a fantastic method of overcoming sexual incompatibility between various plant species (Eeckhaut et al., 2013). A relatively recent and adaptable method for stimulating or inducing genetic recombination in a range of prokaryotic and eukaryotic cells is somatic hybridization, or the fusion of protoplasts (Pandey, 1979).

Somatic Hybridization Methodology

One technique for drastically altering a plant's genetic makeup is protoplast fusion (Pental and Cocking, 1985). Plant tissues release protoplasts when they are incubated with enzymes that break down cell walls (Cocking, 1972). A variety of techniques, such as PEG incubation or electrical pulse treatment, can cause the protoplasts of distinct plants to unite (Compton et al., 2018). It is possible to regenerate somatic hybrid plants from cultures of these fusion products (Figure 1).

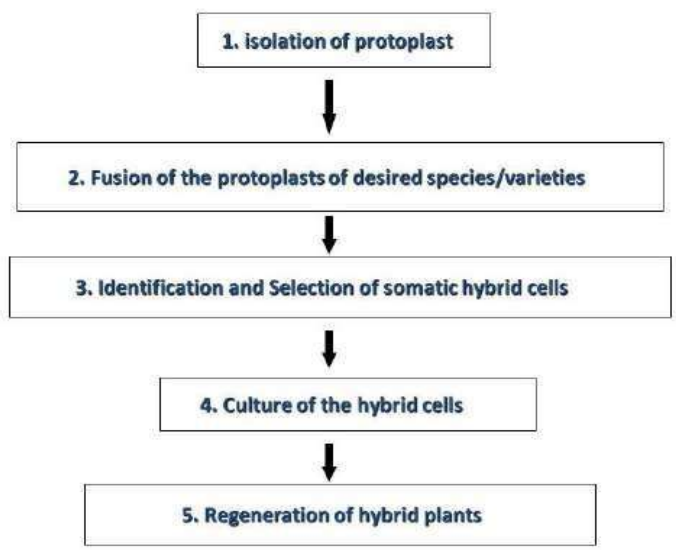

Figure 1: Diagram illustrating the process of creating a hybrid plant by protoplast fusion

Separation of Protoplasts

The word "protoplast" describes the spherically plasmolysed material inside a plant cell that is either naked or surrounded by a plasma membrane (Gupta, 2004). It is critical to separate viable and unharmed protoplasts before culture (Pasternak et al., 2021). Nearly every plant element, including roots, leaves, fruits, tubers, root nodules, endosperm, pollen mother cells, callus, suspension culture, and the fibrous and palisade mesophyll tissue extracted from mature Nicotiana and Petunia leaves, as well as Pelargonium anthers, can be used to isolate protoplasts (Fisher et al., 1994).

Using Protoplast Fusion

Since the isolated protoplasts have no cell walls, fusing in vitro is not too difficult (Potrykus and Shillito, 1986). For the protoplast fusion, there are no obstacles that prevent compatibility (Grosser and Gmitter, 2011). To fully utilize protoplast fusion technology, two requirements must be met: first, a significant amount of protoplasts must be isolated; second, the isolated protoplasts must be totipotent, meaning they must be able to multiply and regenerate into new plants. Protoplast fusion can be accomplished mechanically, spontaneously, or artificially. It entails merging protoplasts from two distinct genomes (Eeckhaut et al., 2013).

Spontaneous Fusion

During the isolation phase, protoplasts spontaneously fuse mostly as a result of physical contact (Cocking, 1972). Spontaneous fusion is the term for the spontaneous fusing that frequently occurs during protoplast isolation (Grosser et al., 2010). Cell fusion occurs naturally, as the fertilization of eggs demonstrates (Russell, 1993). As the enzymatic breakdown of the cell walls proceeds, some of the neighboring protoplasts may unite to form homokaryocytes (Galla, 1975). There may occasionally be a large number of nuclei in these joined cells. This is primarily due to the plasmodermal connections between cells expanding and then coalescing (Shuro, 2018). The homokaryon production frequency in protoplasts extracted from dividing cultured cells was found to be high (Blackhall et al., 1994). However, spontaneously fused protoplasts are limited to going through a few cell divisions and cannot regenerate into entire plants (Tomar and Dantu, 2010).

Intra Specific

When two species cross, it's known as intraspecific protoplast fusion. The sole method available for performing crosses and genetic analysis is this procedure (Shepard et al., 1983).

Inter Generic

Interspecific protoplast fusion refers to the hybridization of two distinct species (Anné and Peberdy, 2020). Interspecific protoplast fusions play a major role in the process of creating new products (Mwangangi et al., 2019). Because of recent genomic configuration, a variety of novel secondary metabolites, including antibiotics, may be generated. It is well known that somatic protoplast fusion in conjunction with plant regeneration from the heterokaryons created by interspecies protoplast fusions can overcome pre-zygotic sexual incompatibility in plants (Bhojwani and Dantu, 2013).

Induced Fusion

Induced fusion is the process of fusing freely separated protoplasts from various sources together with the use of fusion-inducing substances and agents (Naik and Gokul). Because the surface of isolated protoplasts carries negative charges around the outside of the plasma membrane, they normally do not fuse (Abe and Takeda, 1988). Because of their similar charges, protoplasts have a strong tendency to resist one another. Therefore, fusion-inducing chemicals are required for this kind of fusion, which lowers the isolated protoplasts' electro negativity and enables them to fuse (Olivares-Fuster et al., 2005).

Chemo Fusion

Protoplast fusion has been induced by a variety of substances, including NaNO3, polyethylene glycol (PEG), and calcium ions (Ca++) (Anné and Peberdy, 2020). Chemical fusogens make the isolated protoplasts attach, resulting in tight agglutination and protoplast fusion (Verma et al., 2008).

Mechanical Fusion

By utilizing a micromanipulator or perfusion micropipette, the isolated protoplasts are mechanically brought into close physical contact under a microscope in this procedure (Hafke et al., 2007). To fuse, the protoplasts can be mechanically forced together. Protoplasts may suffer harm from mechanical fusion if they become injured (Bengochea, 2012).

Electro Fusion

Electrical stimulation-induced fusion Pearl chain protoplasts fuse when a high-strength electric field (100 kv m-1) is applied for a brief period of time.

Somatic Hybridization's Function in Crop Improvement

Conventional breeding requires a lot of time and money to improve crop plants (Borlaug, 1983). Using unconventional methods helps expedite the procedure. Protoplast fusion is one of these that has been successfully created (Hospet et al., 2023). Potatoes and other heterozygotic crops with varying ploidy levels benefit greatly from somatic hybridization. Pre-breeding is necessary in potato breeding operations to facilitate and expedite diploid selection (Bradshaw, 2017). Crops with improved traits will result from the effective use of the genetic diversity found in both economic plants and their wild relatives, as well as the introduction of stable resistance to diseases through somatic hybridization and/or molecular biological techniques (Jovovic et al., 2020). These actions will greatly contribute to sustainable food security. It is simple to obtain novel inter-specific and inter-generic crosses, which are challenging to construct using traditional approaches (Watts et al., 2018). By fusing the protoplasts of one plant with a certain characteristic to another that can be prone to illness, important traits like disease resistance, the capacity to withstand abiotic stress, and other quality traits can be obtained in hybrid plants. To create fertile diploids and polyploidy, one can employ the protoplasts of sexually sterile haploid, triploid, and aneuploid plants (Eeckhaut et al., 2013). It is possible to create symmetric hybrids between animals that are incapable of sexual hybridization (Lamb et al., 1990). These hybrids may be valuable as new species or easily employed in breeding efforts to pass on beneficial genes to crops (Bradshaw, 2017). This resulted in the first symmetric somatic hybrid of Citrus (Liu and Deng, 2002). Somatic hybrids are typically employed to transfer beneficial genes, such as those for industrial application, abiotic stress resistance, or disease resistance (Begna, 2020). By using somatic hybridization, the 6-7 year duration needed for cytoplasm transfer is reduced to one year in comparison with the backcross approach. Moreover, cytoplasm transfer between sexually incompatible species is made possible by this technique. Somatic hybridization also helps to overcome incompatibility obstacles in sexual recombination at the interspecific or intergeneric levels (Eeckhaut et al., 2013).

On the other hand, somatic hybridization through protoplast fusion has proven to be a potent genetic enhancement technique (Mwangangi et al., 2019).

Disease and Insect Resistance

It is possible for many disease resistance genes, such as those for club rot disease, tobacco mosaic virus, and potato virus X, to be effectively passed from one species to another (Gebhardt and Valkonen, 2001). For instance, tomatoes have been genetically modified to become resistant to pest insects, spotted wilt virus, and tomato mosaic disease (TMV) (Saidi and Warade, 2008). To transfer the bacterial blight resistance trait from wild *Oryza meyeriana* L. to *Oryza sativa* L. ssp. Japonica, asymmetric somatic hybridization was used (Yan et al., 2004).

Environmental Tolerance and Wider Adaptation

Through the process of in vitro protoplast fusion, the obstacle of sexual incompatibility can be overcome, and novel hybrid plants can be created (Zulkarnain et al., 2015). Somatic hybridization can be used to successfully introduce genes that provide resistance to cold, frost, and salt; for example, a tomato's cold tolerance gene can be injected. In order to create somatic hybrids that could withstand salt, ditch reed and rice protoplasts were fused using an electrofusion process (Hasnain et al., 2022). The method has been used in the horticultural sector to develop new hybrids that produce more fruit and are more disease-resistant (Grosser et al., 2010).

Germplasm Diversification

The genomes of two species are combined by somatic hybridization via protoplast fusion, which is then used to convey mono- or polygenic characteristics (Begna, 2020). Additionally, it combines the cytoplasmic genomes of many species or cultivars to produce novel genotypes. This method produced a large number of interspecific, intergeneric, intertribal, and even interfamilial somatic hybrid plants (Tomiczak et al., 2022). There are several accounts of somatic hybrids with grasses in existence. Protoplast fusion somatic hybridization may be a useful technique for grass genetic improvement (Wang et al., 2001).

Cytoplasmic Transfer

Certain plants have cytoplasmically regulated genetic characteristics (Elkonin et al., 2018). This includes resistance to specific medicines and herbicides, as well as various forms of male sterility (Wan et al., 2019). One amazing method for transferring the necessary cytoplasm in a single step is hybridization (Simmons et al., 1989). The transfer of antibiotic and herbicide resistance, as well as cytoplasmic male sterility (CMS), in agriculturally valuable plants is facilitated by hybridization (Kausch et al., 2012). Rice CMS has been effectively transferred by hybridization. It has been possible to create Brassica raphanus hybrids that have the male sterility from Raphanus sativas, the chloroplasts of atrazinc-resistant B. campestris, and the nucleus of B. napus (Mathur et al.). The investigation of cytoplasmic genes and their roles has benefited from somatic hybridization (Ollitrault et al., 2007).

Limitations of Somatic Hybridization

Somatic hybrids were once thought to be very beneficial for crop improvement (Karp, 1995). However, the results of the experiments are not very promising. Currently, there are only a few unique situations in which somatic hybrid cells may be readily manipulated in culture that allow for the selection, modification, and regeneration of hybrid plants from them (Bottino, 1975). It is now impossible to produce somatic hybrids of economically significant plants. The primary goal of somatic hybridization and protoplast fusion was to get past the pre-fertilization barrier that prevents genetic or sexual incompatibility (Shuro, 2018). Therefore, it is reasonable to assume that protoplast fusion will result in very extensive crosses and provide solutions to a number of crop improvement-related issues (Rosati, 2015).

Conclusion

Breeders have made effective use of the genetic variety within the species to enhance crop yields (Tester and Langridge, 2010). Huge efforts have been made to expand the current gene pool of crops since, for modern plant breeding objectives, the variability already present in a breeding population may not be sufficient (Bradshaw, 2017). The primary method used to introduce new traits has been sexual crossings between closely related species or between different genotypes within the same species (Harland, 1936). However, gene transfer has only been possible in sexually compatible species because of a variety of reproductive hurdles, which limit the potential for changing and improving food plants (Limera et al., 2017). Only distantly related species or even unrelated creatures may possess several desirable and agronomically interesting features (Shuro, 2018). Somatic cell genetics has advanced quickly, and as a result, techniques for transposing genes across sexual boundaries and large taxonomic distances currently exist (Bire and Rouleux-Bonnin, 2012). Transformation can transfer intriguing genes that have been found and isolated; however, for the majority of attributes (Lorenz and Wackernagel, 1994). Only in cases when both of the following conditions are met i) large-scale protoplast isolation and ii) totipotency of the isolated protoplasts, can somatic hybridization be used (Davey et al., 2005).

Generally speaking, somatic hybridization is a useful technique for improving crops and plant breeding by creating interspecific and intergeneric hybrids. It is useful for sterile, asexual plants as well as those whose sexual compatibility with other species is compromised (Yang and Kim, 2016).

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Not applicable

Consent to Participate

Not applicable

Authors contribution

MTT and MM conducted research and wrote the initial draft of manuscript. MTT, MM, and QH collected the literature and wrote the manuscript, and edited the manuscript in original. QH, and MM make final editing in the manuscript. All authors have read and approved the final manuscript. The author have read and approved the final manuscript.

Conflict of Interest

The authors state that there is no conflict of interests with regard to this study. There is no conflict of interest in any financial or personal manner concerning the development of this project, the gathering of data, the interpretation of the data, or the writing and publishing of this paper.

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All authenticated data have been included in the manuscript.

Ethics approval and consent to participate

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Consent for publication

Not applicable

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