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Review Paper on the Role of Somatic Hybridization in Crop Improvement

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Abstract: *Genetic variability within the species has been efficiently utilized by breeders in their efforts to improve crops. However, the existing variability in a breeding population may not be sufficient for modern plant breeding purposes, and thus great efforts have been made to broaden the existing gene pool of crops. Introduction of new traits has been based mainly on sexual crosses between different genotypes within or between closely related species. However, due to the presence of various reproductive barriers, gene transfer has been restricted to sexually-compatible species, thus limiting the possibilities of modifying and improving crop plants. Many desirable and agronomically-interesting traits may only be found in distantly related species or even in unrelated organisms. Since they constitute a genetic resource potential, considerable effort has been allocated to identify and isolate these genes and transfer them into crops. Through the rapid development of somatic cell genetics, methods now exist for transferring genes across sexual borders and over wide taxonomic distances. Besides being of value for the transfer of unidentified genes, somatic hybridization is a tool for the modification and improvement of polygenic traits. Furthermore, the modification of organellar genetic material is possible via somatic hybridization since a mixture of the two fusion partners is obtained in the hybrid cell Production of hybrid plants through the fusion of protoplasts of two different plant species/varieties is called Somatic Hybridization, and such hybrids are called Somatic Hybrids. Therefore, somatic hybridization can be resorted to only when the following two criteria are satisfied: i) Isolation of protoplast in large quantity, and ii) Totipotency of the isolated protoplasts. In general, Somatic hybridization is an important tool of plant breeding and crop improvements through the production of inter-specific and inter-generic hybrids. It is valuable for plants which is asexual, sterile and that is also beneficial for those plant which has sexual incompatibility with other species.*

Keywords: *hybrid, hybridization, somatic*

1. INTRODUCTION

Somatic hybridization is an important tool of plant breeding and crop improvements through the production of inter-specific and inter-generic hybrids. The technique involves the fusion of protoplasts of two different genomes followed by the selection of desired somatic hybrid cells and regeneration of hybrid plants (Evans DA and Bravo JE, 1988). It is an efficient way to produce hybrids by fusion of two different protoplast from different plants or species or varieties, and hybrids which produced by this way called somatic hybrids. This is a non-conventional genetic procedure involving fusion between isolated protoplasts under *in vitro* condition and subsequent development of their product to a hybrid plant.Protoplast fusion provides an efficient mean of gene transfer with desired trait from one species to another and has an increasing impact on crop improvements (Brown DCW and Thorpe TA, 1995).

Purified protoplasts once obtained from any two different sources (it can be different tissues, plants, species or different genera), can be fused together to form somatic hybrids. This non-conventional method of genetic recombination involving protoplast fusion under *in vitro* conditions and subsequent development of their product to a hybrid plant is known as somatic hybridization. The aim of somatic hybridization is to improve crop plants, medicinal plant or other types of plants. The purpose of improvement by somatic hybridization is may be to modify species by disease resistance, quality, quantity, or others. For example potato plant *(solanum tuberosum)* making resistant to potato leaf rolling disease.

Somatic hybridization involves three aspects which includes Fusion of Protoplasts, Selection of Hybrid Cells and Identification of Hybrid Plants. The system has made it possible to transfer several desirable genetic characters among the plants. The potential of somatic hybridization in important crop plants is best illustrated by the production of inter generic hybrid plants among the members of Brassicaceae (Toriyama K, et, al 1987).

Protoplasts can be induced to fuse by variety of fusogens or electrical manipulations which induce membrane instability. Most commonly reported fusion inducing agents are sodium nitrate (used by Carlson), high pH/Ca2+ concentration and Polyethylene glycol (PEG) treatment.

In this paper, an overview was given regarding the utilization of somatic hybridization as a method of transferring alien genes to crop species. The potential of somatic hybridization for restoring ploidy level in polyploid species after breeding at reduced ploidy level, as well as the challenge of resynthesising allopolyploid species, was discussed.

Generally Somatic hybridization allows transfer of cytoplasmic organelle in a single generation and offer unique opportunities for combining mitochondria of one species and chloroplast of another species in a single hybrid. This capability may permit improvement of characteristics certain cytoplasmic male sterile line, which may lead to their commercial exploitation. In addition, even non-flowering and non-tuber bearing species can be utilized in breeding programme.

Therefore, the objective of this paper is

- To review the role of somatic hybridization in crop improvement and
- To know procedures and some limitation of somatic hybridization in crop improvement

2. Review of Literature

2.1. Concept and Aspects of Somatic Hybridization

Conventional method to improve the characteristics of cultivated plants, for years, has been sexual hybridization. The major limitation of sexual hybridization is that it can be performed within a plant species or very closely related species. This restricts the improvements that can be done in plants. The species barriers for plant improvement encountered in sexual hybridization can be overcome by somatic cell fusion that can form a viable hybrid. It is the development of hybrid plants through the fusion of somatic protoplasts of two different plant species/varieties. Somatic hybridization broadly involves in vitro fusion of isolated protoplasts to form a hybrid cell and its subsequent development to form a hybrid plant. It (fusion of protoplasts) is relatively a new versatile technique to induce or promote genetic recombination in a variety of prokaryotic and eukaryotic cells (Bhojwani S.S. et al, 1977).

The term “Protoplast” was introduced in 1880 by Hanstein. A cell with its cell wall removed either mechanically or enzymatic ally is named as protoplast. First isolation of protoplast was achieved by Klercker in 1892 by using mechanical method. Küster in 1909 described the process of random fusion in mechanically isolated protoplasts. The real beginning in protoplast research was made by Cocking in 1960 that used enzymatic method for cell wall removal. Plant protoplast is of immense utility in somatic plant cells, genetic manipulations and crop improvement. Thus, protoplasts provide a novel opportunity to create cells with new genetic constitution. It is a wonderful approach to overcome sexual incompatibility between different species of plants. Takebe et al (1971) were successful to achieve the regeneration of whole tobacco plant from protoplasts. Somatic hybridization (fusion of protoplasts) is relatively a new versatile technique to induce or promote genetic recombination in a variety of prokaryotic and eukaryotic cells (Bhojwani S.S. et al 1977).

2.2. Technique of Somatic Hybridization

Protoplast fusion is a method for making large changes in the genetic composition of plants. Protoplasts are released from plant tissues after incubation in cell wall degrading enzymes (Cocking, 1960). Several procedures, including incubation in PEG (Kao & Michayluk, 1974) or treatment with electrical pulses (Zimmerman, 1982), can induce protoplasts from different plants to fuse. Somatic hybrid plants can be regenerated from cultures of these fusion products.

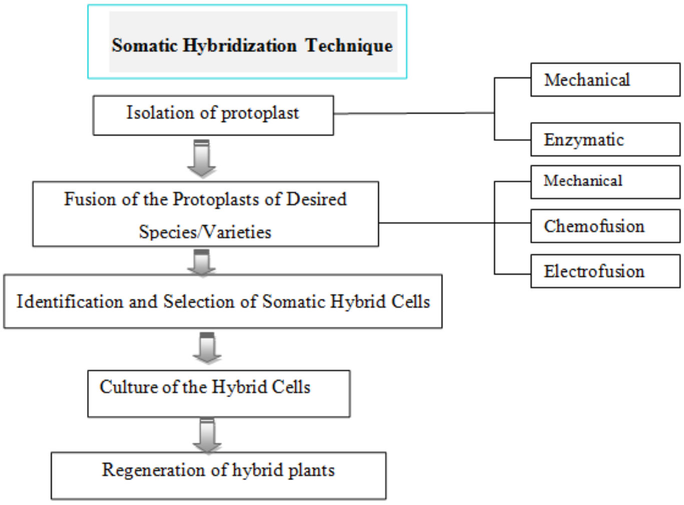

Figure1. *Schematic representation of production of hybrid plant via protoplast fusion*

2.3. Isolation of Protoplasts

The term "Protoplast" refers to the spherical plasmolysed content of the plant cell enclosed by plasma membrane or naked cell without cell wall. Before culturing protoplast, it is important to isolate viable and uninjured protoplasts. Protoplast can be isolated from almost all plant parts; roots, leaves, fruits, tubers, root nodules, endosperm, pollen mother cell, callus and suspension culture, Spongy and palisade mesophyll tissue obtained from mature leaves of Nicotiana and Petunia, Anthers of *Pelargonium* (Abo El-Nil and Hilderbrandt, 1971), Callus of *Gossypium hirsutum* (Bhojwani, Cocking, and Power, 1977), Crassulacean acid metabolism (CAM) plants (Doddds, 1980), C_3 and C_4 plants (Kanai and Edwards, 1973) and *Solanum tuberosum* (Upadhya, 1975)

2.4. Protoplast Fusion

As the isolated protoplasts are devoid of cell walls, there in vitro fusion becomes relatively easy. There are no barriers of incompatibility (at inter-specific, inter-generic or even at inter kingdom levels) for the protoplast fusion. Two criteria have to be fulfilled to exploit the use of protoplast fusion technology; protoplasts must be isolated in large quantities and the isolated protoplasts must be totipotent, i .e. they should have the ability to proliferate and regenerate into new plants .Protoplast fusion that involves mixing of protoplasts of two different genomes can be achieved by spontaneous, mechanical, or induced fusion methods.

2.4.1. Spontaneous Fusion

Protoplast fuse spontaneously during isolation process mainly due to physical contact Protoplast during isolation often fuse spontaneously and this phenomenon is called spontaneous fusion. Cell fusion is a natural process as is observed in case of egg fertilization. During the course of enzymatic degradation of cell walls, some of the adjoining protoplasts may fuse to form homokaryocytes (homokaryons). These fused cells may sometimes contain high number of nuclei (2-40). This is mainly because of expansion and subsequent coalescence of plasmodermal connections between cells. The frequency of homokaryon formation was found to be high in protoplasts isolated from dividing cultured cells. Spontaneously fused protoplasts, however, cannot regenerate into whole plants, except undergoing a few cell divisions.

Intra Specific

Intra specific protoplast fusion is the cross between the same species. This technique offers the only way of carrying out crosses and genetic analysis.

Inter Generic

Inter specific protoplast fusion is the crosses between two different species. Inter specific protoplast fusions are of much importance in the area where new products are to be produced. Due to new genetic set up many noval secondary metabolites such as, antibiotics may be produced.It is well established that pre-zygotic sexual incompatibilities in plants can be overcome using somaticprotoplast fusion coupled with plant regeneration from the heterokaryons formed by interspecies protoplast fusions (Kumar and Cocking, 1987).

2.4.2. Induced Fusion

Fusion of freely isolated protoplasts from different sources with the help of fusion inducing chemicals agents is known as Induced Fusion. Normally isolated protoplast do not fuse with each other because the surface of isolated protoplast carries negative charges(-10mV to 30mV) around the outside of the plasma membrane. And thus there is a strong tendency in the protoplast to repel each other due to their same charges. So this type of fusion needs a fusion inducing chemicals (Fusogens) which actually reduce the electro negativity of the isolated protoplast and allow them to fuse with each other (Narayanswamy S. 1994).

Chemo Fusion

Several chemicals have been used to induce protoplast fusion such as $NaNO_3$, Polyethylene Glycol (PEG) and Calcium ions (Ca^{++}). Chemical fusogens cause the isolated protoplast to adhere (stick) each other and leads to tight agglutination followed by fusion of protoplast (Pasha C.R et al 2007; Jogdand S.N.2001).

Mechanical Fusion

In this method the isolated protoplast are brought into intimate physical contact mechanically under microscope and using Micromanipulator or Perfusion Micropipette. The protoplasts can be pushed together mechanically to fuse. Mechanical fusion may damage protoplasts by causing injuries.

Electro Fusion

Fusion induced by electrical stimulation Fusion of protoplasts of pearl chain is induced by the application of high strength electric field (100kv m^{-1}) for few micro sec.

2.5. Role of Somatic Hybridization in Crop Improvement

Improvement of crop plants by conventional breeding is time consuming and, therefore, expensive. Unconventional techniques can shorten the process. One of these is protoplast fusion, developed and successfully used by Melchers and Labib (1974). Somatic hybridization is especially useful for heterozygotic crops, which possess different Ploidy levels, as for example potato. Breeding programs of potato require pre breeding for easier and faster selection at the diploid level. An efficient utilization of genetic diversity present in economic plants and in their wild relatives, and the introduction of a stable resistance to diseases by somatic hybridization and/or by molecular biological techniques will lead to crops with improved traits and contribute significantly to a sustainable food security.

Novel inter-specific and inter-generic crosses which are difficult to produce by conventional methods can be easily obtained. Important characters, such as resistance to diseases, ability to undergo abiotic stress and other quality characters can be obtained in hybrid plant by the fusion of protoplasts of plant bearing particular character to the other plant which may be susceptible to diseases. Protoplasts of sexually sterile haploid, triploid, aneuploid plants can be used to obtain fertile diploids and polyploidy.

Most of the agronomically important traits, such as cytoplasmic male sterility, antibiotic resistance and herbicide resistance, are cytoplasmically encoded, hence can be easily transferred to other plant. Plants in juvenile stage can also be hybridized by means of somatic hybridization. Somatic hybridization can be used as a method for the production of autotetraploids. Somatic hybrids can be produced between species, which cannot be hybridized sexually. It can be readily used in breeding programme for transfer of resistance genes. Hybrids can be produced even between such clones, which are completely sterile. Cytoplasm transfer can be done in one year, while back crossing may take 5-6 years. Even where backcrossing is not applicable, cytoplasm transfer can be made using this approach. Mitochondria of one species can be combined with chloroplast of another species. This may be very important in some cases, and is not achievable by sexual means even between easily crossable species. Recombinant organelle genomes, especially of mitochondria, are generated in somatic hybrids. Some of these recombinant genomes may possess useful features.

Somatic hybridization is very useful for plants which is asexual, sterile and that is also beneficial for those plant which has sexual incompatibility with other species. Somatic hybridization has opened new possibilities for the in vitro genetic manipulation of plants to improve the crops. Protoplast fusion and somatic hybridization have opened up a new avenue in plant science. It is now a well-known fact that the somatic hybridization in plants can be used in the improvement of plants. One of these is the production of hybrids which is not possible through normal sexual fusion or fertilization process.

Symmetric hybrids can be produced between species, which cannot be hybridized sexually. These hybrids can be readily used in breeding programs for transfer of useful genes to crops or may be useful as new species. The first symmetric somatic hybrid of Citrus was created by protoplast fusion of C. sinens is and Poncirus trifoliata (Ohgawara et al., 1985) and the production of hybrid plants between two sexually incompatible Citrus genera was first reported in 1988, where C. sinens is L. Osb. cv. 'Hamlin' protoplasts were fused with Severinia disticha (Blanco) Swing protoplasts (Grosser et al., 1988).

Generally, somatic hybrids are used for transfer of useful genes such as disease resistance, abiotic stress resistance or genes of industrial use. Somatic hybridization minimizes the time taken for cytoplasm transfer to one year from 6-7 years required in back cross method. Also, this method allows cytoplasm transfer between sexually incompatible species. The incompatibility barriers in sexual recombination at inter specific or inter generic levels are also overcome by somatic hybrization. Somatic hybridization by protoplast fusion, on the other hand, has been a powerful tool in genetic improvement (Mendes *et al*, 2001).

2.5.1. Disease and Insect Resistance

Many disease resistance genes (e.g., tobacco mosaic virus, potato virus X, club rot disease) could be successfully transferred from one species to another. For example, resistance has been introduced in tomato against diseases such as TMV, spotted wilt virus and insect pests. Asymmetric somatic hybridization was exploited for transfer of bacterial blight resistance trait from wild Oryza meyeriana L. to Oryza sativa L. ssp. Japonica (Yan et al. 2004). Asymmetric hybridization is very promising as it allows partial genome transfer (Derks et al., 1992; Trick et al., 1994; Liu & Deng, 2002), which may be better tolerated than a whole-genome transfer (Ramulu et al., 1996a,b). As in other agricultural species, trait introgression from 'wild' species of the genus Nicotiana has been used to improve the cropped species, and characters from at least 13 different species have been transferred into tobacco (Lewis, 2011).

2.5.2. Environmental Tolerance and Wider Adaptation

In vitro fusion of protoplast opens a way of developing unique hybrid plants by overcoming the barriers of sexual incompatibility. The genes responsible for the tolerance of cold, frost and salt could be successfully introduced through somatic hybridization, e.g., introduction of cold tolerance gene in tomato. Somatic hybrids were produced by fusion of protoplasts from rice and ditch reed using electro fusion treatment for salt tolerance. The technique has been applicable in horticultural industry to create new hybrids with increased fruit yield and better resistance to diseases. Successful viable hybrid plants were obtained when protoplasts from citrus were fused with other related citrinae species (Motomura T, et.al, 1997).

2.5.3. Germplasm Diversification

Somatic hybridization via protoplast fusion brings together the genomes of two species and exploited to transfer mono or polygenic traits (Liu et al. 2005). It also creates novel genotypes by combining cytoplasmic genomes of different species or cultivars. Many inter specific, inter generic, intertribal, and even interfamilial somatic hybrid plants generated through this approach. A countable number of reports are available on somatic hybrids involving grasses Somatic hybridization through protoplast fusion can be a relevant tool in genetic improvement of grasses (Mostageer and Elshihy 2003; Cui et al. 2009; Xiang et al. 2010).

2.5.4. Overcoming Barriers of Sexual Incompatibility

Sexual crossing between two different species (inter-specific) and two different genuses (inter-generic) is impossible by conventional breeding methods. Somatic hybridization overcomes the sexual incompatibility barriers. Examples are given hereunder:

Fusion between protoplasts of potato (Solanum tuberosum) and tomato (Lycopersicon esculentum) has created pomato (Solanopersicon, a new genus).

Inter specific fusion of four different species of rice (Oryza brachyantha, O. elchngeri, O. officinalis and O. perrieri) could be done to improve the crop.

2.5.5. Transfer of Cytoplasm

Some of the genetic traits in certain plants are cytoplasmically controlled. This includes some types of male sterility, resistance to certain antibiotics and herbicides. Cybridization is a wonderful technique wherein the desired cytoplasm can be transferred in a single step. Cybrids are important for the transfer of cytoplasmic male sterility (CMS), antibiotic and herbicide resistance in agriculturally useful plants. Cybridization has been successfully used to transfer CMS in rice. Cybrids of Brassica raphanus that contain nucleus of B. napus, chloroplasts of atrazinc resistant B. campestris and male sterility from Raphanus sativas have been developed. Somatic hybridization has helped to study the cytoplasmic genes and their functions. In fact, the information is successfully used in plant breeding programmes. Protoplast fusion will help in the combination of mitochondria and chloroplasts to result in a unique nuclear-cytoplasmic genetic combination. Somatic hybridization can be done in plants that are still in juvenile phase. Protoplast transformation (with traits like nitrogen fixation by incorporating exogenous DNA) followed by somatic hybridization will yield innovative plants.

2.6. Limitations of Somatic Hybridization

It was once suggested that somatic hybrids would be of great value in crop improvement. But the experimental reports are not very encouraging. At present, techniques for selection and manipulation of somatic hybrid cells and regeneration of hybrid plants from them is limited to a few special cases where they can be manipulated very easily in culture. So far the production of somatic hybrid of agro nomically important plants is not possible. The main objective of protoplast fusion and somatic hybridization was to overcome the pre- fertilization barrier to sexual incompatibility or any genomic incompatibility. Therefore, it would be undoubtedly expected to achieve very wide crosses through protoplast fusion and it will solve many problems relating to crop improvement. But practically inter-generic crosses between widely related plants, which are not compatible sexually, are not possible.

Few inter specific somatic hybridization where plants are sexually compatible or incompatible due to natural reproductive isolation, are only achieved. In certain wide crosses, elimination of chromosomes from the hybrid cell is another limitation of somatic hybridization. So, desirable hybrids are no longer available. Although some attempts have been made to increase the percentage of fused cells, still it is also a limitation of somatic hybridization. Lastly, for hybrid identification, selection and isolation at the culture level, there is no standardized method which is applicable for all material.

3. Summary and Conclusion

Genetic variability within the species has been efficiently utilized by breeders in their efforts to improve crops. However, the existing variability in a breeding population may not be sufficient for modern plant breeding purposes, and thus great efforts have been made to broaden the existing gene pool of crops. Introduction of new traits has been based mainly on sexual crosses between different genotypes within or between closely related species. However, due to the presence of various reproductive barriers, gene transfer has been restricted to sexually-compatible species, thus limiting the possibilities of modifying and improving crop plants. Many desirable and agro nomically-interesting traits may only be found in distantly related species or even in unrelated organisms. Since they constitute a genetic resource potential, considerable effort has been allocated to identify and isolate these genes and transfer them into crops.

Through the rapid development of somatic cell genetics, methods now exist for transferring genes across sexual borders and over wide taxonomic distances. Where interesting genes have been identified and isolated, they can be transferred by transformation, but, for most traits the genes have not been identified, and somatic hybridization might then be the method of choice. Besides being of value for the transfer of unidentified genes, somatic hybridization is a tool for the modification and improvement of polygenic traits. Furthermore, the modification of organellar genetic material is possible via somatic hybridization since a mixture of the two fusion partners is obtained in the hybrid cell Production of hybrid plants through the fusion of protoplasts of two different plant species/varieties is called Somatic Hybridization, and such hybrids are called Somatic Hybrids. Therefore, somatic hybridization can be resorted to only when the following two criteria are satisfied: i) Isolation of protoplast in large quantity, and ii) Totipotency of the isolated protoplasts.

In general, Somatic hybridization is an important tool of plant breeding and crop improvements through the production of inter-specific and inter-generic hybrids. It is valuable for plants which is asexual, sterile and that is also beneficial for those plant which has sexual incompatibility with other species.

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