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Applications of Protoplast Fusion in Plant Biotechnology

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Authors' contributions

This work was carried out in collaboration among all authors. Authors GAI and AAK designed the study and wrote the first draft of the manuscript. Authors MA and SI managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Plant biotechnology is the deliberate application of biotechnology tools such as protoplast fusion, DNA extraction, plant bioinformatics, PCR and cloning in creating plants with new, improved and desired traits for human benefit. Protoplasts are often referred to as plant cells which the cell wall has been removed and it has many applications in plant biotechnology. Protoplast fusion has been used for centuries but plant biotechnology is a new technology that gives more realistic results in any plants research. The application of plant biotechnology in protoplast fusion will produce new product in plants with wider applications and more realistic results. In this article different application of protoplast fusion in plant biotechnology was reviewed such as production of useful metabolites, target site mutagenesis, introduction and establishment of disease resistance plants, improvement of food nutrition content, nitrogen-fixation symbioses, production of herbicide resistant plants, insect pest Control and plant-parasitic nematode control in plant for the benefit of humans. The knowledge of protoplast fusion can be use by plant biotechnologist to improve plants trait for human benefits. The application of plant biotechnology is important to any nation's food security and development.

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1. INTRODUCTION

Protoplasts are often referred to as plant cells which the cell wall has been mechanically or enzymatically removed. In theory it is assumed that protoplasts are totipotent, which means they have the ability to dedifferentiate and regenerate into various organs [1] Protoplast fusion has become a very important technique in producing crops with useful agronomic traits and which can also be sold on commercial scale. In the past few decades there have been an increased in research using protoplast fusion due to public antagonism with genetically modified crops [2,3]. Protoplast technology is promising technique that can be exploited by breeders to increase germplasm accessibility and can also bring about improvement in different crop varieties [4]. Plant protoplast can be used as versatile cell-based experimental system which can analyze gene expression during a transient time period [5,6,7] and macromolecules like proteins, RNA and DNA can be delivered into protoplasts using various techniques such as microinjection and PEG-calcium fusion electroporation [8,9].

A biotechnology technique which uses DNA analysis has shown significant advancement for the past year and it has been used continuously in characterizing somatic hybrids. simple sequence repeat (SSR) has been used extensively for somatic hybrids characterization analysis as a biotechnology technique [10,11]. Gancle *et. al.,* 2006 [12] Studied the use of proteomics as better tool in regulation and inheritance rules in somatic hybridization. Eeckhaut *et. al.,* 2013 [7] Suggest the use of next generation sequencing which is cheaper and faster as a tool in somatic hybrid genome screening and stability studies for future scientist. High resolution melting analysis which is also another tool that can be use for screening technique based upon insertions, single nucleotide polymorphisms (SNPs) induce alteration or deletion of double stranded DNA dissociation behavior [13,14]. PCR–RFLP (restriction fragment length polymorphism) and Cyclase-associated proteins (CAP_S) analysis has also been found as efficient and reliable tools in cytoplasmic genome characterization [7].

This article, aimed at reviewing biotechnology application of protoplast fusion such as production of useful metabolites, target site mutagenesis, improvement of food nutrition content, introduction and establishment of disease resistance plants, nitrogen-fixing symbioses, production of herbicide resistant plants, insect pest Control and plant-parasitic nematode control for the benefit of mankind.

2. PLANT BIOTECHNOLOGY APPLICATIONS

2.1 Production of Useful Metabolites

Useful metabolite such as anticancer agents, functional proteins, enzymes and antiviral proteins are found in the cell walls of plants, between cell membrane and the cell walls [15,16,17,18,19].

The major challenge is that the accumulations of these metabolites are usually very low. The use of protoplast fusion allows large amount of the metabolite to the released into the culture [20]. To avoid the regeneration of the cell wall, immobilization matrix together with an inhibitor makes the production of the metabolite to be more efficient [20,21,22].

Catharanthus roseus protoplasts isolated from callus culture was entrapped in alginate gel to study the extracellular production of enzymes (peroxidase and alpha-galactosidase). However, free protoplasts extracellular production of these enzymes was higher than the protoplasts immobilized in 0.7–2.5% alginate gel beads. The use of agarose gel was suggested because of high mass transfer ability and a neutral electric charge instead of the alginate gel as the negative charge of the alginate inhibited the secretion of these enzymes [23].

Enzymes production using protoplasts have been investigated through the efficient production of chitinase by Wasabia japonica protoplasts (isolated from callus culture) entrapped in artificial cell walls (alginate gel) as both elicitor and immobilization matrix. The productivity of chitinase by immobilized protoplasts (2.0 U/mL at 5 days) was much higher than that of cells immobilized in alginate gel (0.36 U/mL at 5 days). The productivity of chitinase by immobilized protoplasts (2.0 U/mL at 5 days) was much higher than that of cells immobilized in alginate gel (0.36 U/mL at 5 days). Cell wall regeneration of immobilized protoplasts was

detected under a light microscope on the third day of cultivation. This implies that the inhibition of cell wall regeneration is necessary for long-term production with protoplasts. 2, 6 dichlorobenzonitrile (2, 6-D; molecular weight = 172.02) was used to inhibit synthesis of the cellulose cell wall in the case of chitinase production by immobilized W. japonica protoplasts. 2, 6-D (2.0 mg/L) maintained active protoplasts for over 30 days without cell wall regeneration when added to the broth[24].

Callus culture protoplasts of *C. roseus* immobilized in alginate gels rich in guluronic acid to study the production of secondary metabolite of indole alkaloids (ajmalicine, catharanthine, and tryptamine), which are synthesized through many enzyme reaction steps. Protoplasts immobilized in alginate produced extracellular ajmalicine much higher than protoplasts immobilized in agarose. Addition of 30 mM $CaCl_2$ to the broth, maintained the active protoplast for 15 days with neither cell wall regeneration nor inhibition of indole alkaloid production [25].

The advantage of using protoplasts to produce useful metabolites is because the product are released readily into the culture medium which has benefits of increasing overall productivity and facilitating downstream processing in situations where the cell wall limits the secretion of useful products. The major challenge is that protoplasts are very fragile and is difficult for long-term production and the cell walls of active protoplasts easily regenerate [26].

2.2 Target Site Mutagenesis

The direct alteration of specific DNA sequence is a vital element of genome editing which is called gene editing. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) which is associated with Protein 9 (CAS9) method is suitable genome editing technique which requires just two reagent; single guide RNA (sgRNA) and Cas9 protein [27,28,29,30,31] Other endonucleases like Cpf1 can cause mutations apart from cas9 [32,33,34,35]. The genome editing reagents can be synthesized and assemble in vitro which can form active ribonucleoprotein (RNP) complexes where the complexes can be delivered into protoplast which can mutagenize the target gene [32,36,37].

In biotechnology, protoplast is usually used to determine target site mutagenesis efficiency and which can also be generated into plants [36]. The regeneration of protoplast is a major challenge when using CRISPR mutagenesis most especially in monocot plants therefore there will be need for high regeneration efficiency protocols which are unavailable [37,38]. CRISPER/Cas9 technology is a very useful tool in research and plant breeding which still a new technology at the infant stage [38]. Using the CRISPER-mediated mutagenesis it is possible to remove the integrated transgenes encoding gene editing reagents from the genome through genetic segregation thereby reducing the fear of the public about genetically modified foods[32,39].

Lin *et. al.,* 2018 [40] reported the development of protocol for analyzing DNA from individual mutagenized protoplasts and also proved that CRISPR-based mutagenesis of protoplasts is a useful technology for polyploid crops.

2.3 Improvement of Food Nutrition Content

The aquatic food chain of fauna aquaculture, microalgae denotes the major natural nutrition. *Chlorella vulgaris* is one example of the sources of aquatic nutrition [41]. The *C. vulgaris* species have been used extensively as nutritional supplements or aquaculture feeds [42]. Microalgae are regarded as one of the organism producing distinct range of bioactive chemical compounds, primarily vitamins, pigments, proteins, minerals, lipids and polysaccharides. The main reasons for their consideration as important source of nutrition for diverse purposes are the high nutritional content of the microalgal species [43,44,45,46]. Lee and Tan 1988 [47] Reported the documented the production of genetically improved strain of algae by somatic fusion and hybridization.

Algae-algae protoplast fusion has been reported as a valuable technique to improve their nutrition. Protoplast fusion technique is an in vitro genetic manipulation process which are considered more effectual compared to the conventional techniques used for strain improvement such as selection and mutation [48,49]. Somatic hybridization put up by this procedure has demonstrated strong efficacy in increasing nutrition and valuable metabolites production [50].

According to Kusumaningrum and Zainuri 2018 [51], the use of protoplast fusion technique for microalgae Chlorella had been carried out to

improve their carotenoid for animal aquatic supplement. During the application of protoplast fusion on interspecific microalgae of *C. vulgaris*, the nutrition content of fusant was subjected for analysis by GCMS methods on *C. vulgaris* powder from 100 L liquid cultivation of the fusant. The study resulted in fusant with high mass production level. 17 amino acid with high concentration of firstly, glutamic acid (14495.52 ppm) secondly, leucine (10856.97 ppm) and thirdly, Aspartic acid (10378 ppm) was showed on the nutrition analysis of fusant. Palmitic acid (1.59%) was showed highest concentration in its lipid acid profile.

2.4 Nitrogen-Fixation Symbioses

Nitrogen which is a compound of many bio-molecules is very important for growth and development of plants. Most nitrogen exist in the atmosphere and the ability fix atmospheric nitrogen through the nitrogenase enzyme complex is restricted to some bacteria and where the bacterian abd plant live in symbiotic relationship provide the reiches sources of nitrogen to plants [52,53]. For the last few decades there have been scientific research on bacteria having nitrogen-fixing symbioses with legumes, which are mostly made of the following genera Sinorhizobium, Mesorhizobium, Bradyrhizobium, and Rhizobium, Azorhizobium [54,55]. Leguminous plants characterization for nitrogen fixation is mainly dependent on their ability in developing nitrogen-fixing nodules through interaction with the symbiotic bacteria [56]. Actinomycete Frankia forms nitrogen-fixing root nodules on non legumes plants thua the genetic basis of the symbiotic interactions that occurs between Frankia strains and host plants is poorly understood [57].

Early works on the genetics of nitrogen fixation was studied on free-living nitrogen-fixing bacteria *Klebsiella pneurnoniae*. The presence of 17 nil (nitrogen fixation) genes that encode nitrogen fixation in K. pneumonia is responsible for nitrogen fixation [58]. Intergeneric transfer of K. pneumoniae nil clusters to other non-nitrogen-fixing bacteria and yeast though nitrogen fixation has only been observed in closely related species [59,60,61].

Prakash and Cummings 1988 [62] reported the successful use of Protoplast fusion to create novel actinomycete capable of fixing atmospheric nitrogen. Protoplasts of Streptomyces griseofuscus, which is a fast-growing actinomycete and Frankia which is a a slow-growing actinomycete were both allowed to fuse and regenerate on media which does not have supply of nitrogen. The regenerated colonies were able to acquire the fast growing characteristics of the streptomyces and tha ability on growon a media lacking nitrogen from Frankia.

Louis and Ensign (1987) [63] also reported the use of four Frankia strains ACN1, EANlpec, CpII and EuIIc for the formation and regeneration of protoplasts of the actinorhizal nitrogen-fixing actinomycete frankia where the protoplast was sandwiched between a layer of a nutritionally rich osmotically stabilizing medium and a layer of low-melting-point agarose. It was observed that the regeneration of the four strains varied widely and the maximum regeneration efficiency was only observed on two strains.

Sabir and El-Bestawy 2009 [64] Also reported an effective role by protoplast fusion in enhancing nodulation of Rhizobial species. The bacteria abilities to produce nodulation were observed on two weak strains (RtI1 and RtI2) and one efficient strain (RtA1) which were selected for protoplast fusion and the numbers of nodules formed by the intra-specific protoplast fusion strains were observed. The Protoplast fusion of the indigenous R. *leguminosarum biovar trifolii* strains resulted in nodulation increases by 1.93- to 5.67-fold when compared to their parent strains. This is an excellent result for agricultural practices for the formation of nitrogen-fixing root nodules on leguminous crops.

The prospect of Green Nitrogen Revolution will be a great achievement in producing stable food crops that has substitute for mineral nitrogen fertilizer which can be achieve by using nitrogen-fixing fertilizers [65].

2.5 Production of Herbicide Resistant Plants

The production of herbicide resistant plant can be achieved through protoplast fusion. Attempts have been made to make plants tolerance herbicides such as bromoxynil, atrazine, sulphonylureas, glyphosate and phosphinothricin [66]. Many herbicides operate by inactivating some plant enzymes (target proteins) which are very important for functions such as the photosynthetic or other biosynthetic pathways which are unique to plants [67].

Rathore *et. al.,* 1993 [68] Reported the use of bar gene in combination with the herbicide Basta to

select transformed rice (*Oryza sativa* L. cv. Radon) protoplasts for the production of herbicide-resistant rice plants. The presences of Phosphinothricin acetyltransferase assays was used to confirmed the expression of the bar gene in plants obtained from phosphinothricin resistant calli. Both the bar and gusA genes were transmitted to progeny as confirmed by Southern analysis, where the progeny having the bar gene was found to be resistant to Basta. Thus Herbiace or Basta can be use as a post-emergence on rice plants transformed with the bar gene.

Menczel *et. al.,* 1986 [69] also reported the use of Terbutryn-resistant plastids of the *Nicotiana plumbaginifolia* TBR2 mutant which was introduced into *Nicotiana tabacum* plants by protoplast fusion following X-irradiation of TBR2 protoplasts where the Cybrid plants was founded resistant to high levels of atrazine (10 kg/ha).The level of atrazine resistance (to 10 kg/ha) is likely to be sufficiently enough to protect the crop under field conditions because atrazine is mostly applied at a rate of 2-4.5 kg/ha.

Datta *et. al.,* 1992 [70] Confirmed that an important Indica rice cultivar *Oryza sativa* cv. IR72 was transformed with the application of direct gene transfer. The transformed rice showed resistance to high dosage level of phosphinothricin.

2.6 Introduction and Establishment of Disease Resistance

Protoplast fusion is a powerful tool to transfer disease resistance genes from different plants [71]. Disease resistance in breeding may come from either more distantly related species or from closely related species. Protoplast fusion is one of the techniques that is used to circumvent problems in introgression genes for resistance [72].

Chen *et. al.,* 2008 [71] Reported the use of protoplast fusion to overcome sexual incompatibility between cultivated potatoes and diploid solanum. They develop a systematic protocol for the isolation of huge number of high quality protoplasts from variety of Mexican wild species of late blight (plant disease). Using the protocol, new somatic hybrids of one Argentina wild species, two Mexican and cultivated potato clones and the successful somatic hybrids were from the cell fusion of *Solanum tuberosum* and the diploid species *Solanum pinnatisectum, Solanum cardiophyllum* and *Solanum chacoense* which shows higher level of resistance to both late blight than was found in *S. tuberosum*.

Cybridizations and Somatic hybridizations in citrus produced rootstocks that is resistant to abiotic and biotech constraints which increased the yield and quality of the fruit [73] also in brown spot resistant scions [74].

Xiao *et. al.,* 2004 [75] also reported the production of the first resistant raphano-brassica asymmetric and symmetric hybrids. This new development showed new resistance types along with multiple resistances which include turnip mosaic virus.

The ability of mycoviruses for managing plant-pathogenic fungi was first confirmed with *Cryphonectria parasitica* [76,77]. The success of plant disease control with hypoviruses has to do with their ability to reduce the virulence (to induce hypovirulence) of the target fungus. Hypoviruses can be transmitted from a hypovirulent strain to a virulent fungal strain by hyphal fusion (anastomosis) when the two strains are vegetatively compatible, but hypoviruses cannot be transmitted when applied by extracellular routes [78].

Lee *et. al.,* 2011 reported the introduction of hypovirulent mycoviruses in fungi as an alternative to fungicides to contol plant diseases. Transmission of hypovirulence-associated double-stranded RNA (dsRNA) viruses between mycelia has become a challenge because it is prevented by the vegetative incompatibility barrier that usually exists between different strains or species of filamentous fungi.They determined whether protoplast fusion could be used to transmit FgV1-DK21 virus, which is associated with hypovirulence on *F. boothii* to *F. asiaticum, F. graminearum*, *F. oxysporum f. sp. lycopersici,* and *Cryphonectria parasitica*. When the result was compared to virus-free strains, the FgV1-DK21 recipient strains had reduced growth rates, reduced virulence and altered pigmentation. The results showed that protoplast fusion can be used to introduce FgV1-DK21 dsRNA into C. parasitica and into other Fusarium species that FgV1-DK21 can be used as a hypovirulence factor and as a plant disease control agent [79].

2.7 Insect Pest Control

For many years there have been search for plants that can produce and survive in spite of insect pests. Advances in biotechnology have

shown numerous insect-resistant plants development. The use of biotechnology as a tool in developing insect-resistant plants will continue to be on the increase. The acceptability of protoplast fusion base insect-resistant plants may be greater along with the increase in the understanding the processes [80].

Chen *et. al.,* 2008 [71] reported the use of protoplast fusion in overcoming sexual incompatibility between diploid cultivated potatoes and *Solanum species*. They developed an effective protoplast fusion system for production of new potatoes insect resistance with the use of Mexican wild potato species as gene pools. They designed a systematic procedure for the isolation of a large number of high quality protoplasts from various insect (Colorado potato beetle) that carries high levels of resistance. Assessment of insect reactions demonstrated that several of the protoplast derived clones and somatic hybrids showed a higher level of resistance to Colorado potato beetle than was found in *Solanum tuberosum*. The result from this study is the first for successful transfer of Colorado potato beetle resistance from a wild Mexican species into cultivated potato by protoplast fusion.

Fungi which is found in the genus Lecanicillium (formerly classified as the single species Verticillium lecanii) are very vital pathogens of insects and some of them have been developed as commercial biopesticides. *Lecanicillium spp*. uses both hydrolytic enzymes and mechanical forces to penetrate the insect integument directly and the cell wall of the fungal plant pathogen. Futher more to mycoparasitism of the plant pathogen, the mode of action is linked to colonization of host plant tissues, triggering an induced systemic resistance [81]. Protoplast fusion was done using three strains of Lecanicillium spp. (as V. lecanii) to get new strains having useful characteristics as insect contol agents. The combination of three strains are; B-2 with Vertalec, B-2 with Mycotal and Vertalec with Mycotal, where new hybrid strains were gotten. They started with 43 hybrid strains which were used in bioassays against the cotton aphid, Aphis gossypii. of these out of which 30 strains induced mortality equal to or higher than Vertalec (42%). Again, 50 hybrid strains were equally used in bioassays against Trialeurodes vaporariorum (greenhouse whitefly) out of which 37 strains exhibited an equal or higher infection rate as compared to that of Mycotal (36.2%) [82]. The results suggest that strains from Lecanicillium spp. can be used as a potential for developing single microbial control agent that can be effective against insects pest due to its antagonistic and parasitic resistance inducing characteristics [81].

Bacillus thuringiensis (Bt) is a ubiquitous Gram-positive and spore forming bacterium that produces parasporal crystals during the stationary phase of its growth cycle [83]. The crystals is made up of one or more crystal proteins (encoded by cry or cyt genes) that has specific toxicity against several orders of insects such as Diptera and Lepidoptera [84,83]. Protoplast fusion was done between *B. thuringiensis* UV-resistant mutant 66/1a and *B. sphaericus* to get a new Bacillus strains with wider spectrum of action against many insects. The results showed the expression of some cry genes encoded for insecticidal crystal proteins from *B. thuringiensis* and the binary toxin genes from *B. sphaericus* in all fusant strains. SDS–PAGE protein analysis confirmed that all some fusant strains acquired and expressed specific larvicidal proteins to both lepidopteran and dipteran species. The recombinant fusants have more efficient potential values insecticidal against *Culex pipiens* and *Spodoptera littoralis* larvae, respectively [85].

2.8 Plant-Parasitic Nematode Control

Plants interact with different types of organisms, most time leading to various pathologies. Among these organisms are nematodes that has intimate interactions with different plant hosts [86,87]. There are over 4100 species of plant-parasitic nematode currently [88] and damages that is caused by plant nematodes has been projected at $US80 billion per year. They represent an important constraint on the delivery of global food security [89]. The management of Nematode depends mainly on chemical nematicides, but due to their negative impact on the environment and nematologists are looking for innovate safer and eco-friendly control methods [90].

Root-knot nematodes are considered as one of the main constraints to vegetable farming worldwide. The use of bacteria such as rhizospher as an antagonistic to nematodes is eco-friendly, Chitinase production is a vital factor in improving the nematicidal activity of this kind of microorganisms. The nematicidal activity of Lysinibacillus sphaericus Amira strain and *Bacillus amyloliquefaciens* subsp. plantarum SA5 against root-knot nematodes, Meloidogyne *incognita,* using protoplast technique was

developed, their fusants were tested for nematicidal and chitinase activity using greenhouse experiments and bioassay. The selected fusants from the two bacterial strains were seen to be more effective in killing *M. incognita* J2 under laboratory conditions.The production of Chitinase from the fusant was much higher under solid-state fermentation than submerged fermentation conditions. The recorded chitinase produced by *B. amyloliquefaciens* is 0 units (µg NAG/ml enzyme/h), *L. sphaericus* is 1393 units (µg NAG/ml enzyme/h), and Bas8 is 3399units (µg NAG/ml enzyme/h, under solid-state fermentation and 90, 85, and 143 units (µg NAG/ml enzyme/h), under submerged fermentation conditions. The result showed that fusant from *L. sphaericuand* and *B. amyloliquefaciens* can be used as biological control agents against root-knot nematode M. incognita [91].

Verticillium lecanii is universally distributed in soils, even though this fungus is mostly isolated from insects. It has a broad host range such as plant-parasitic nematodes, insects and phytopathogenic fungi, and [92,93]. The strains of *V. lecanii* was screened to check the relative potential of hybrid strains of *V. lecanii* against soybean cyst nematode with that of the parental strains in greenhouse pots and to determine the efficacious strains using protoplast fusion as biological control agents. Three parental strains Vertalec®, B-2 and Mycotal® and their 162 hybrid strains were screened in greenhouse pot tests. Few of the hybrid strains reduced the density of soybean cyst nematode in the soil and damage on soybean plants. The hybrid strain AaF42 was seen to reduce the nematode egg density by 93.2% when compared to the control. In addition, this strain significantly reduced egg density and the cyst as compared with the parental strains. Some of the hybrid strains developed by protoplast fusion exhibited higher level of nematode control efficacy against soybean cyst nematode than the parental strains [94].

3. DISCUSSION

Protoplast fusion is a major breeding tool that is used to produce new genetic combination which is different from other scientific tools and it also transfer mono and polygenic traits [95,96]. Protoplast fusion has successfully been used as a tool to produce useful metabolites, target site mutagenesis, improvement of food nutrition content, introduction and establishment of disease resistance plants, creating nitrogen-fixing symbioses, production of herbicide resistant plants, insect pest contol and plant-parasitic nematode control through the application of plant biotechnology techniques. Genomic variation is of important interest in most plants for yield and quality improvement, disease resistance, cytoplasmic male sterility (CMS) transfer, Salt tolerance, rootstock improvement and seedless triploids are the most frequent goals of protoplast fusion [97].

Other researchers have also report important use of protoplast fusion in plant biotechnology. Kao *et. al.,* 1974 [98] Reported the protoplast fusion for intergeneric hybrid cells. Matthew *et. al.,* 2016 [99] Studied the microbead encapsulation of living plant protoplasts which is seen as a new tool for the handling of single plant cells. Miiller-Gensert *et. al.,* 1987 [100] reported the interspecific T-DNA transfer through plant protoplast fusion. Grosser and Gmitte 2011 [101] Examined the Protoplast fusion for production of triploids and tetraploids which is used for rootstock and scion breeding in citrus. Li *et. al.,* 2018 [102] Describe plant adenine base editor based on an evolved tRNA adenosine deaminase fused to the nickase CRISPR/Cas9 which enable A•T to G•C conversion in protoplasts and regeneration in rice and wheat plants.

Many countries have the fears about the application of plant biotechnology but have also forgotten about the two major challenges of plant biotechnology which are; the continuous increase in population at geometric rate and the current climatic changes which are posing serious threat to the human population and the growth of our plants (crops). If the challenges of the plant biotechnology will be solved then the application of plant biotechnology has become very necessary. Since protoplast fusion allows the introduction of new genes into plants without genetically modified plants, which is the fear of common man then protoplast fusion, offers an option. Therefore protoplast fusion has become essential for plant (crops) improvement for the future.

4. CONCLUSION

The use of protoplast fusion will go a long way to remove the fear of genetically modified crops (foods) in the mind of the common man. The fear of increase in population and climatic change has left us with no other option in feeding our

population than the use of plant biotechnology. Protoplast is a useful technology in plant biotechnology not just for food production alone but for other products that are useful to humans.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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