Review Article

Applications of Protoplast Fusion in Plant Biotechnology

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. The applications of protoplast fusion range from the following in plant biotechnology production of useful metabolites, gene editing (crispr mutagenesis), improvement of food nutrition content, creation nitrogen-fixing symbiosis, introduction and establishment of disease resistance, production of herbicide-resistant plants, etc. The knowledge of protoplast fusion can be used by plant biotechnologist to improve plants trait for human benefits. The application of plant biotechnology is also very important to any nation that will achieve food security.

Keywords: [Disease Resistance, Food Security, Herbicide Resistant, Nitrogen-Fixing Symbioses, Gene Editing and Nutrition Content}

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 can 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 a commercial scale. In the past few decades, there have an increased in research using protoplast fusion due to public antagonism with genetically modified crops [2,3]. Protoplast technology is a 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 a versatile cell-based experimental system which can analyze gene expression during a transient 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].

Biotechnology techniques which use DNA analysis have shown significant advancement for the past year and it has been used continuously in characterizing somatic hybrids. SSR has been used extensively for somatic hybrids characterization analysis as a biotechnology technique [10,11,]. [12] Studied the use of proteomics as better toll in regulation and inheritance rules in somatic hybridization. [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 a future scientist. High resolution melting analysis which is also another tool that can be used for screening technique based upon insertions, SNPs induce alteration or deletion of double-stranded DNA dissociation behaviour [13,14]. PCR–RFLP and CAPS analysis has also been found as efficient and reliable tools in cytoplasmic genome characterization [7].

This study aims to determine the biotechnology application of protoplast fusion such as the production of useful metabolites, gene editing (crispr mutagenesis), improvement of nutrition

production, introduction and establishment of disease resistance plants, creating nitrogen-fixing symbioses and production of herbicide-resistant plants.

2.0 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 [15,16,17,1819].

The major challenge is that the accumulations of these metabolites are usually very low. The use of protoplast fusion allows a 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 metabolite to the 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. Callus culture protoplasts of *C. roseus* immobilized in alginate gels rich in guluronic acid to study the production of a 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 [23].

2.2 Gene Editing (Crispr 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 [24,25,26,27,28,] Other endonucleases like Cpf1 can cause mutations apart from cas9 [29,30,31,32,]. The genome-editing reagents can be synthesized and assembled in vitro which can form active ribonucleoprotein (RNP) complexes where the complexes can be delivered into protoplast which can mutagenize the target gene [29,33,34].

In biotechnology, protoplast is usually used to determine target site mutagenesis efficiency and which can also be generated into plants [33]. 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 [34,35]. CRISPER/Cas9 technology is a very useful tool in research and plant breeding which still a new technology at the infant stage [35]. 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 [36].

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 [37]. The *C. vulgaris* species have been used extensively as nutritional supplements or aquaculture feeds [38]. Microalgae are regarded as one of the organism producing a distinct range of bioactive chemical compounds, primarily vitamins, pigments, proteins, minerals, lipids and polysaccharides. The main reasons for their consideration as an important source of nutrition for diverse purposes are the high nutritional content of the microalgal species [39,40,41]. [43] Reported the documented production of the 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 is considered more effectual compared to the conventional techniques used for strain improvement such as selection and mutation [44,45]. Somatic hybridization put up by this procedure has demonstrated strong efficacy in increasing nutrition and valuable metabolites production [46].

According to [47], 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 the highest concentration in its lipid acid profile [46].

2.4 Creating Nitrogen-Fixing Symbioses

Nitrogen which is a compound of many bio-molecules is very important for the growth and development of plants. Most nitrogen exist in the atmosphere and the ability to fix atmospheric nitrogen through the nitrogenase enzyme complex is restricted for some bacteria where the bacterian plant live in symbiotic relationship provide the richest sources of nitrogen to plants [48,49]. 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 [50,51]. Leguminous plants characterization for nitrogen fixation is mainly dependent on their ability in developing nitrogen-fixing nodules through interaction with the symbiotic bacteria [51]. Actinomycete Frankia forms nitrogen-fixing root nodules on non legumes plants thus the genetic basis of the symbiotic interactions that occur between Frankia strains and host plants is poorly understood [53].

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 [54]. Intergeneric transfer of *K. pneumoniae* nil clusters to other non-nitrogen-fixing bacteria and yeast through nitrogen fixation has only been observed in closely related species [55,56,57].

[58] 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 S*treptomyces* and that ability on grow on a media lacking nitrogen from Frankia.

[59] Louis and Ensign (1987) also reported the use of four Frankia strains ACN1, EANlpec, Cpll and Eullc 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.

[60] 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 [61].

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 [62]. Many herbicide 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 [63].
[64] 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 (PAT) assays were used to confirm 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 used as a post-emergence on rice plants transformed with the bar gene.
[65] also reported the use of Terbutryn-resistant plastids of the *Nicotiana plumbaginifolia* TBR2 mutant which was introduced into *N. tabacum* plants by protoplast fusion following X-irradiation of TBR2 protoplasts where the Cybrid plants were founded resistant to high levels of atrazine (10 kg/ha). The level of atrazine resistance (to 10 kg/ha) is likely to be sufficient enough to protect the crop under field conditions because atrazine is mostly applied at a rate of 2-4.5 kg/ha.
[66] Confirmed that an important Indica rice cultivar *Oryza sativa* cv. IR72 was transformed with the application of direct gene transfer to protoplasts. The transformed rice showed resistance to high dosage level of phosphinothricin.

2.6 Introduction and Establishment of Disease Resistance

[67] 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 that has high levels insect [Colorado potato beetle (CPB)] resistance and disease (late blight). 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 S. tuberosum and the diploid species *S. pinnatisectum, S. cardiophyllum,* and *S. chacoense* which shows higher level of resistance to both late blight and CPB than was found in S. tuberosum. [68] reported Camelina sativa and *Brassica napus* hybrids which has increased linolenic acid content when compared to the B. napus partner.
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 [69] also in brown spot resistant scions [70]. [71] 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.

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 tansfere mono and polygenic traits [72,73].
Genomic variation is of important interest in most plants for yield and quality improvement. Quality improvement, disease resistance, cytoplasmic male sterility (CMS) transfer, Salt

tolerance, rootstock improvement and seedless triploids are the most frequent goal of protoplast fusion [74].

Other researchers have also report important use of protoplast fusion in plant biotechnology. [75] reported the protoplast fusion for intergeneric hybrid cells. [76] Studied the microbead encapsulation of living plant protoplasts which is seen as a new tool for the handling of single plant cells. [77] reported the interspecific T-DNA transfer through plant protoplast fusion. [78] Reported the Protoplast fusion for production of triploids and tetraploids which is used for rootstock and scion breeding in citrus. [79] 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 especially some of the developing countries have many fears about the application of plant biotechnology but have forgotten also 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 a very 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 then protoplast fusion, offers an option.

Plant biotechnology tool such protoplast fusion has therefore become essential for plant (crops) improvement for the future. The use of protoplast fusion will go a long way to remove the fear of genetically modified food in the mind of the common man.

4. CONCLUSION

The fear of increase in population and climatic change has left us with no other option in feeding ourselves than the use of plant biotechnology for improved food production to meet our ever increasing population. Plant biotechnology tool such protoplast fusion has therefore become essential for plant improvement for the future. The use of protoplast fusion will go a long way to remove the fear of genetically modified food in the mind of the common man

CONSENT

Not applicable

ETHICAL APPROVAL

Not applicable

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