(REVIEW PAPER)

THE ROLE OF BIOTECHNOLOGY IN CROP IMPROVEMENT

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ABSTRACT

Biotechnology is currently playing a vital role in the improvement of crop plants generally. This is because of its ability to overcome the shortcomings of other conventional practices of crop improvement. This paper therefore, considered the two broad aspects of Biotechnology in crop improvement namely; Genetic Engineering and Bio culture and the various practices under each aspect. In genetic engineering, Virus and Bacterium gene vectors, microinjection, macro injection, Biolistics, Pollen tube-pathway, Electroporation, Liposome fusion were considered. While in Bio culture Techniques, Embryo Rescue, Tissue Culture, Anther/Pollen Culture and Protoplast Culture were looked at. The Author also offers suggestions that will improve access to Biotechnology in Nigeria.

INTRODUCTION

Biotechnology represents the latest front in the on going scientific progress of the 20th century. It has opened a wide view for development of new approaches towards the improvement of Agriculture generally in all countries, because of its ability to directly modify plants, animals and agricultural processes in respond to new needs (Monti, 1992). He further observed that productivity - growth in agriculture and industry was overtaken by the petrochemical and transport industries after World War II, with the maturing, of these industries the potential for further growth in productivity from these sources has diminished. The rapid rise in energy consumption and heavy dependency on fossil fuel due to high population increase has lead to the growing concern about loss in genetic diversity, under nourishment, desertification, deforestation, (resource depletion) and environmental pollution.

Kadams (2000), also pointed out that, although established conventional breeding methods have been successful in producing crop varieties with increased yields, disease and pest resistance, salt, drought and stress tolerance, to sustain increased growth in agriculture, these methods still have a lot of shortcomings, including linkage problem, longer time span for crop maturity, incompatibility, mapping of specific genes and sterility problems among others.

Biotechnology therefore, should not only be seen as a means of solving these problems where other techniques have failed, but also as way of generating understanding of agriculture generally through the cooperation of scientists from different disciplines

such as molecular-biologist, virologist, entomologist, geneticist, plant breeders. physiologist etc. forming research team capable of transforming agriculture to be more productive and at the same time more sustainable (Monti, 1992).

Biotechnology consists of cluster of techniques of genetic manipulation of living organisms at the cellular and molecular levels (Cardwell, 1992). In other words. biotechnology is define as any technique that use living organisms or substances from these organisms to make or modify a product, to improve plant and animal or to develop micro organism for specific use, (OTA, 1989).

Biotechnology is indeed a frontier technology fast emerging from the laboratory to bring under its spell agriculture - the world's largest and oldest organized economic activity (Cardwell, 1992). The two major areas of biotechnology that deals with crop improvement are; Genetic Engineering (Gene Splicing/Recombinant DNA technology) and Bio-culture commonly referred to as Tissue Culture, Gautheret, (1985).

OBJECTIVE

These techniques hold great potentials for the improvement of crops grown in Nigeria. This paper therefore, considered the different aspect of Genetic Engineering and bio-culture and their various roles in crop improvement and as well, offers suggestions to improve access to biotechnology in Nigeria.

1.0 GENETIC ENGINEERING

For the improvement of crop plants, involving specific genes, breeders usually employ a back crossing program. This enables one or few genes to be transferred from one specie to another. This procedure of hybridization involves several generation which makes gene transfer through back crossing slow and time consuming (Covey, 1985). The discovery of TP "Transforming Principle" (Avery *et al* 1944) eventually led to a fascinating field of genetic engineering which entails the isolation, restructuring of DNA and it's insertion into a different cellular environment (Scowcroft, 1977). In a broad sense genetic engineering involves an intentional genetic manipulation of specie (crops) to produce special forms, (Mohammed *et al* 1977). It is the non sexual transfer of genetic information coded on the DNA, from one organism to another, thus bringing about genetic transformation (transgenesis) of the recipient organism. This is possible because every living cell is "Totipotent" and has the capacity to uptake a purified DNA molecules. virus or bacterium (Fitch, 1990). Further they reported that genetic transformation is consummated by successful;

- uptake of foreign DNA
- expression of encoded genetic information
- it's assimilation and integration into the host genome, where in the imported DNA along with he hosts DNA will undergo normal replication, transcription and translation.

Persley (1990), observed that these transfers can be perfected along two lines.

i. Vector mediated transfer of DNA (Transduction)
ii. Direct transfer of purified DNA or physical gene transfer (Transformation).

1.1 VECTOR MEDIATED TRANSFERS OF DNA (TRANSDUCTION)

1.1.1 Viruses as Gene Vectors.

Transfer through appropriate molecular vector of genetic information from one organism to the other is referred to as vector mediated transfer or transaction, (Davies and Stanley, 1989). This phenomenon they pointed out, was discovered in bacteria where in the DNA of an attacking Bacterial virus (episome or bacteriophage) gets integrated into the host cell. The virus remains in prophase (Dormant stage) and replicates along with the host genome. The virus inadvently serve as carrier for the transfer of genetic information from one bacterial cell to another. In addition to bacteriophage some molecular vectors of plant origin have also been discovered, these include cauliflower mossaic virus (CaMV) and potato leaf roll virus (Boulcombe, 1989).

The use of plant virus dates back to 15 years or more (Covey, 1985). Some plant viruses cause serious crop losses. However not all plant viruses are harmful to their host. some such as the *"cryptic"* viruses induce no outward signs of their presence, others induce dramatic colour changes in their host but produce no detrimental effect. These later plants are often intentionally propagated as ornamentals. Over 700 plant-viruses have been identified, of these 75% have genomes of single stranded (ss) messenger RNA (MRNA), as their genetic material and lack any DNA intermediate in their Natural life cycle (Zaitlin and Hull, 1987). The largest and economically most important of the 34 plant virus groups and families currently recognized are the RNA *polyviruses* with 189 members (Ward and Shukler, 1991). In contrast plant virus with genomes of DNA is few, the two main groups being *caulimoviruses* with 12 members and *geminivirus* with 50 members. Historically it is the DNA virus that has received most attention as potential gene vectors.

Davies and Stanley (1989), also pointed out that DNA is more stable and far less prone to error during replication, further more DNA viruses have a nuclear phase where as RNA are normally cytoplasmic and are highly prone to mutation during replication. However the recent advent of efficient invitro transcription system for RNA (Melton *et al.* 1984), there have been significant success using RNA viruses as gene vectors.

A major disadvantage of viruses as gene vector is that they have limited host range of plant species and cell types.

1.1.2 *Agrobacterium* Mediated Transformation

Another great possibility of DNA transfer into plants lies in the exploitation of tumor inducing field bacterium - *Agrobacterium tumefaciens* (Beavan and Chilton, 1982). This bacterium has been doing he same thing the plant genetic engineers would like to master (Grimsley and Bisaro, 1987). Further, it was stated that *Agrobacterium tumefaciens* is a soil borne gram negative bacterium, causing plant disease *"crown gall"*. Cells isolated from crown gall grown invitro without any external source of

hormones show tumourous or neoplastic behaviour, even in the complete absence of bacteria (Binns and Bock, 1989). It was also reported that during infection *Agrobacterium t.* transfer part of a large plasmid, (the Pti) tumour inducing plasmid in the nuclear genome of the infected cell. The Pti fragment transferred (T-DNA) is flanked by two regions "left border" and right border" (Zambryski *et al* 1989). These boarders contribute the signals that is recognized by the transfer system of *Agrobacterium t.* The T-DNA transfer contains genes known as *"Onco genes"* for the synthesis of plant hormones such as *cytokines* and *auxins* and also genes for *opins* synthesis that are used by the bacteria for feeding. Binns (1990), reported that the co-cultivation or co-culture of *Agrobacterium t.* With plant tissues has been seen as a powerful system for transferring genes into plants after having cloned those in the T-DNA. The Ti-plasmid of *Agrobacterium t.* has been extensively studied and use in gene transfers but the actual process by which T-DNA transverses the bacteria and plant cell walls and become integrated into the host genome is not understood. Also the limited host range of *Agrobacterium t.* has prevented its wide spread use for the transformation of other important crops.

The host range of *Agrobacterium t.* include *Glycine max, Gossypium hirsutum, Lycopersicon lycopersicum, Ipomoea batatas,* and many other dicotyledonous crops. *Agrobacterium t.* cannot yet be used to transform *cereals* or other species that are recalcitrant to regeneration (Filippone *et al,* 1992). The 4 major indirect techniques of gene transfers by *Agrobaterium t.* are:

1. Wounding and inoculation of intact or decapitated plant - Here wounding stems or leaves induces response that plays a role in the specific bacterium/tissue attachment.
2. Co cultivation of growing protoplast (naked cell) derived cells, with the bacteria - that is, growing of plant tissues (leaves, roots, stems etc) with the bacteria.
3. Inoculation of explants invitro to induce growth of transformed shoot that can be grown to maturity.
4. Leaf disc transformation; this consist of co-cultivation of leaf mesophyl cell that are still within the tissue of leaf slice. This is due to the fact that leaves provides an abundant source of genetically uniform cells with the capacity to regenerate whole plant for a wide range of species.

Table 1. List of some crops transformed by co-cultivation with *Agrobacterium t.*

Specie	Common name	Improvement
Vigna unguiculata	Cowpea	Disease/pest resistant
Pisium sativum	Field beans	Disease/pest resistant
Nicotiana tabacum	Tobacco	Disease/pest resistant
Gossypium hirsutum	Cotton	Disease/pest resistant
Mangifera indica	Mango	Disease/pest resistant
Dioscorea bulbifera	Aerial yam	Storage longevity

Source: IAPTC 1990.

Grimsley *et al* (1986), Boulton *et al* (1989), Hayes *et al* (1988), reported that viral gene vectors give cell infections with a high copy number compared to the low number achieved by *Agrobacterium t.* mediated transfers.

1.2 Direct or Physical Gene Transfer (Transformation)

Vector mediated gene transfer techniques are restricted to certain plant species and cell types. To overcome such limitation, physical methods of gene transfer have been developed, these are based on the transformation of protoplast or intact cells from which new plants regenerates. The most promising of which are out lined as follows:

1.2.1 Macro Injection

This is carried out using needles with diameter far greater than the cell diameter and allows the delivery of DNA into wound sites, within tissues. The solution is further absorbed by cells surrounding the wounded site. Transgenic progeny of rye for instance was obtained by injecting aph into immature inflorescence to confer antibiotic resistance (Delapena *et al,* 1987).

1.2.2 Micro Injection

This involves the use of fine capillary tubes to deliver DNA directly into the nucleus of a cell under a high powered microscopy. Micro injection has been successfully used to obtain stable transformation of crops such as tobacco (Crossway *et al* 1986), alfalfa (Reich *et al* 1986).

1.2.3 Biolistics

This is a recently developed technique that is used to deliver DNA into intact plant cells. This method uses acceleration of small metal particles covered with DNA, to deliver into plant tissues. It is a technique for gene transfer into a variety of crop species (Sanford, 1990). This system is also referred to as, the use of gene gun or particle gun. In general the technique uses a small (0.3 - 0.5 micron) high density metal (tungsten or gold) particles coated with DNA. These micro projectiles are accelerated by the use of a macro projectile (a large bullet like object of metal or plastic) under a slight vacuum. The macro projectile can be accelerated to a very high velocity by a variety of means, including decompressed air or explosion. The DNA particle is unloaded as the projectile pass through nuclei or organelle (Sanford, 1990). The first demonstration of Biolistics in plant transformation was shown in onion (Klein *et al* 1987). Others include soybean (Mc. Cabe *et al,* 1988), maize (Fromm *et al,* 1990, Gordon Kamn *et al* 1990, Spencer *et al* 1990), and tobacco (Klein *et al* 1988). This process is independent of tissue type and is simple or relatively easy to handle, one shot can yield many hits and cells survives the intrusion of the particles (Singh and Shaw,1992).

1.2.4 Pollen Tube Pathway

Immediately after pollination, pollen tube have been used as a conduit to deliver DNA under the catalytic effect of *CaMv35s* into fertilized ova in rice (Luo and Wu 1988), this method have potential of rapid gene transfer but kaepler *et al* (1990) pointed out that results are yet to be repeated on other crops.

1.2.5 Electroporation

A short pulse of high voltage electricity facilitates the uptake of molecules into protoplast, this process is known as electroporation. It has been used produced stable transformation in barley caryopses (Ahokas, 1989).

1.2.6 Liposome Fusion With Protoplast

This is an established method for the production of transgenic plants. This is because liposome help the DNA to enter through the plasmodemata of the cell (Gade *et al.* 1986). Stable transformation have been demonstrated in tobacco (Deshayes *et al* 1985).

Other Potential Direct Gene Transfer Methods

Several other innovative approaches have been attempted to produce transgenic plants including laser micro bean (Weber *et al* 1988), micro laser driven genetic transformation (Maheswari and Balder, 1961). Pollen transformation (Ohta, 1986) Vortex mixing (Kaeppler *et al* 1990), Seed imbibitions (Topfer *et al* 1989). All these methods have potentials but at present, Singh and Shaw (1992) pointed out that they are not supported by enough evidence of transformation beyond reasonable doubt.

2.0 BIO-CULTURE

Bio-culture, commonly referred to as tissue culture owes it's origin to the ideas of Haberlandt, a German plant physiologist in 1902. Haberlandt based his ideas on the concept of Totipotency that is all living plant tissues have the possibility to regenerate into a mature plant or even a tree from an isolated single cell or callus containing normal chromosome complement (Winton, 1970, Sibi *et al* 1984; Martin, 1985 and Gauthered 1985). Individual achievements were later carried on roots of tomato (White, 1934) eradication of virus by Meristem culture (Morel and Martin 1952); Somatic embryogenesis (Reinert, 1958); protoplast culture (Cocking, 1960), anther culture (Guha and Maheshwari, 1964); DNA uptake by plant cell (Lodoux, 1965), etc.

Bio-culture techniques which are essentially microbiological techniques comprises the following cultures (Bhojwani *et al*, 1977);

- Embryo culture
- Tissue Culture
- Anther/pollen culture

- Protoplast culture

The isolation of cell or protoplast, culture media and regeneration of plants under each kind of culture are discussed in this paper with their utility in the genetic improvement of some economic plants.

Table 2. Basic procedure for invitro propagation of plants.

Stage I	Selection of small pieces of conventionally grown plant, their Sterilization and inoculation on to medium
Stage II	Transfer the explants to a medium or sequence of media that Promote induction and proliferation of shoot (increase concentration Of *cytokine* or *kinetin).*
State III	Transfer to shoot to media or conditions that induce root formation (Increase *Auxin* concentration) followed by planting out in green House.

Source: Hu and Wang, 1983.

2.1 Embryo Culture/Rescue

Interspecific hybridisation is often attended by hybrid sterility due to the failure of embryo or endosperm development after fertilization and to the failure of normal seed development due to unfavourable interaction between embryo and endosperm. However embryos which normally fail to survive can successfully be cultured to maturity on suitable media, specific for each crop (Filippone *et al,* 1992).

Isolation of embryo; the fertilized egg cells are usually excised after one to two weeks following fertilization and are cultured on nutrient agar media until well established then they are transplanted into small pots and kept in a well lighted glass house for two weeks. The plants are then transplanted to larger pots

Culture media; in general culture media for embryo culture essentially contain inorganic salts, micro elements, vitamins, carbon source, auxin, yeast, casein, hydrolysate, coconut liquid etc. (Scowcroft, 1977) Embryo culture overcomes sterility barriers erected by unbalance endosperm on inter-specific hybridization and thus facilitates wide crossing (Williams *et al.* 1987)

Embryo culture has been use for example in IITA (Ng and Hahn, 1985) to increase yield and confer disease and pest resistance on some *Manihot spp.* ½ ms medium with 3% sucrose and agar was used to germinate embryos of several *Manihot* species while, Ng (1989) also reported the use of ovule culture to confer disease resistance on white yam at IITA.

2.2 Tissue culture

Like embryo culture, Cell/Tissue culture of virtually any part of a plant (such as, root, stem, hypocotyle etc) can be cultured and induce to form a callus (Bhojwani, *et al* 1977). It has been observed that stem or roots segments give great success after being germinated on the culture medium

Culture media

The nutritional requirements for cell tissue culture are not different from those of embryo culture although the culture media can be standardized for different genotypes, since there may be different nutritional requirement for genotypes (Hooykaas *et al,* 1984).

Regeneration

It takes 15 hours to several days in a suitable culture medium when cell starts multiplying. This follows formation of mass callus tissue which differentiate into roots and shorts, thus regeneration of plantlets. The culturing of plant cells in synthetic media and developing them into mature plants enhance faster and rapid multiplication of parental genotypes such as slow growing medicinal plants trees. These include, the shoot Tip of pyrethrum *chrysanthemum cinerariefolium* (Sharma, 1978), *Eucalyptus citrodora* (Grewal, 1978)

Other aspects where tissue culture play significant role include somaclonal variation, that is selection of superior stocks at cellular level or the sorting out of highly productive cell lines (Scowroft, 1984) This was demonstrated in sugarcane to improve cane and sugar yield with 32% and 34% respectively (Winthers *el al,* 1990) Success with respect to increased yield have also been reported in maize (Karp and Maddock, 1984) and rice (Nishi *et al,* 1968), using somaclonal variation, somatic hybridization, chromosome doubling etc. The tissue culture technique can be employed for many more purposes like elimination of viral diseases from economic plants etc.

2.3 Anther/pollen Culture

This involves regeneration of plants from cultured anthers or immature pollen graing (microspore) in suitable media which are basically not different from those for tissue culture (Filippone *et al* 1992). The greatest usefulness of anther culture lies in the rapid production of haploid plants which is of great value in plant breeding and genetics. Isolated ovules are also being used, although the microspore culture has advantage over the ovule culture, due to the fact that many thousands of haploid cells are available per plant and can be obtained easily by removing the anthers, example in barley 2000-3000 pollen grains per anther. The chances of obtaining haploid plants by anther or pollen culture depends on pollen viability, choice of correct stage of pollen development and effect of culture media (Kadams, 2000) Further it was observed that stable improvement on various crops have been reported by use of anther or pollen culture. This include disease resistance in tobacco, yield increased in barley, rice, potato and tomato (Yin *et al.*

1976) At IITA anther and un-pollinated ovary culture of cassava for massive regeneration of plantlets have also been reported (Ng. 1989).

2.4 Protoplast culture

Large quantities of protoplast (naked cell) can be isolated, cultured and directed to develop into callus and shoot differentiation. This follows the works of Klercker (1892) who isolated for the first time from a water plant (*Stratiotes aloides*) by mechanical means. Since then the technique for isolation culture and regeneration of protoplast have been successful (Filippone *et al* 1992). They further reported that protoplast culture maintain an edge over other system of bio-culture, in that the impact of any treatment on the protoplast is directed. They also observed that, the characteristic of protoplast that makes it suitable for genetic manipulation for crop improvement include.

i. Protoplast, regardless of their origin tend to fuse, when brought together into intimate contact with each other

ii. Protoplast can uptake DNA, chloroplast, and virus. Nuclei, or even whole bacterium.

iii. Protoplast like its container, the cell, have enormous advantage of Totipotency

Protoplast isolation

Protoplast can be isolated from cells in two ways

1. by mechanical means
2. by enzymic method

The mechanical method is no longer in use due to shattering of cell and low yield of protoplast. (Filippone *et al* 1992). The enzymic method yield large quantity of uniform and intact cells due to low shrinkage. Protoplast can be obtained from plant organ that do not undergo secondary thickening such as roots, petals, pollen, fruits etc. (Ng, 1989) A typical protoplast isolation from tobacco leaf through enzymic method is described by Nagata and Ishir (1979), it involves the following basic procedures;

i. Sterilization of leave: Dipping of fully expanded healthy leaves (form about 60 days old seedling) in 70% alcohol for about one minute, followed by sodium hypochloride 2pc solution treatment for 1½ hours and rinsing the leaves trice in distilled water

ii. Pealing the epidermis: this is done by either of the following methods:

- Physical stripping of the lower epidermis with a pair of forceps
- Epidermal cell can be damage by *carborundun*
- Maceration of leaves.

iii. Enzymic treatment and incubation: This involve direct incubation of pealed leaf segments in a mixture of *pectinase* and *cellulase*

Isolation and cleaning of protoplast

The isolated protoplast is treated with distilled water. The protoplast can be cultured in both liquid suspension or in agar medium for further growth and differentiation, Nagata and Ishir (1979) also suggested that in protoplast culture medium (Macro and micro elements, vitamins, EDTA, Sugar and growth regulators), since protoplasts are highly vulnerable to osmotic damage, an extra amount of osmotic stabilizer such as glucose or sucrose is necessary and to keep pathogens off, antibiotics should be added. Further they reported that during regeneration ,cell wall is formed around the protoplast, more rooting and shooting medium are added to induce quicker planlet regeneration.

Under this remarkable method plants have been generated from protoplast in diverse crop species such as cassava, yam, sweet potato etc. with induced disease/pest resistance and increased yield, in laboratories such as IITA (Ng, 1989)

2.5 Bio-culture as conservation technique of non-seeded crop

The clonal germplasm of crops such as yam, cassava, potato and other non seed crops are conserved by the " in vitro reduced growth storage method" plantlets can be kept in same tube for 2 -5 years while seed germs are stored *ex situ or in situ*(Ng and Hahn, 1985).

BIO-SAFETY

A safe and efficient regulatory process that ensure public health and environmental safety is an added advantage in biotechnology. In most countries the existing legislation is adequate to regulate the use of most agricultural products which are likely to be produced using biotechnology.

Recent studies (Persley, 1990; and Shaw, 1992, OTA, 1989 and Monti, 1992) have shown that there are guidelines which monitor the production of genetically engineered organisms at experimental stage and for the assessment of any risk association with the commercial released of new organisms into the environment. These studies also show that the benefits from the use of new biotechnologies, out-weight the risk. Persley (1990), observed that over 200 small scale genetically modified plants in 17 countries have been released with no outward effects.

Further he stated that current work in several countries is concerned with developing procedures for handling large-scale commercial release of new products. In agriculture, these products will primarily be new plants varieties with novel characteristics.

CONCLUSION/SUGGESTIONS

Biotechnology is a rapidly developing area of science that had brought new ideas and tools as solution to the obvious and unmanageable problems of food crops

improvement in term of quality and quantity, to meet current consumer need in all countries of the world. To facilitate this improvement in response to the mounting population pressure and increasing poverty, there is need to explore this novel technology in order to make a head ways in crops improvement in Nigeria, and other developing countries

The following strategies are suggested so as to improve access to biotechnologies in Nigeria.

1. Links between international agricultural research centre (IARs) in developed and developing countries should be strengthen, so as to identify and transfer high priority technologies from biotechnology into developing country like Nigeria.
2. Companies such as seed company, Agrochemical Company etc. Should invest in modern biotechnology research in agriculture such as in crop breeding programs
3. Courses leading to the awards of various degrees in biotechnology should be taught in Nigerian universities, or candidates (Qualified) Be sponsored in foreign Universities so as to generate the manpower Need of the new biotechnology in Nigeria.
4. Adequate funding of conventional agricultural researches in crop breeding and crop protection in biotechnology by universities, research institutes and other bodies should be carried out probably by allocating substantial amount of research budget to Biotechnologies.

ACRONYMS

ADH-Alcohol dehydrogenase
APH-Aminoglycoside phosphotransferase
DNA-De-oxyribonucleic acid-Genes contain two complex molecules
Protein and DNA-how DNA contain and convey essential life information remains a mystery
MS medium-Murashige and Skoog's medium
Pti Tumour inducing plasmids
RNA-Ribonucleic acid-Aids in protein synthesis of transfer and messenger RNA, usually in the cytoplasm and not the cell nucleus
OTA-Office of Technology Assessment (US congress)
IAPTC-International Association of plant Tissue Culture.

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