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Role of biotechnology in crop and animal improvement for sustainable agriculture

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Abstract
Agriculture now a day is suffering from lots of problems such as changing climate, pressure for large scale production of grain, pest out break etc. Plant breeding have come out to mitigate these problems but limitation in variation, cross incompatibility etc limits its success which can be overcome by genetic engineering. The potential of gene transfer from any source to a plant and animal is its strength. Besides increasing the crop production, it has also been exploited in the field of animal biotechnology. The strength of biotechnology is increasing day by day with the advent of new technologies such as sequencing methods, transcriptome and proteome analysis techniques. Besides plants animals are also an important component of agriculture. Cloning, artificial insemination, embryo transfer, vaccine and pharmaceuticals production, organ development for transplantation etc are some of the applications in the field of animal biotechnology.

Keywords: Biotechnology, Totipotency, Somatic embryogenesis, Genetic engineering, Recombinant DNA Technology, Omics, etc

Introduction
Agriculture is the backbone of our country. It does not only involve the raising of crops but forestry, pisciculture and animal husbandry are also its part. The land and climate of our country is so much rich and diverse that we are in a position to produce agricultural products relatively cheaply. The variety of agro climatic region enables India to produce a large array of products.

The burden on agriculture to feed the population is increasing with the increase in the population. To meet the ever increasing demand of food grains farmers grows the crop on the undesirable lands like salt affected areas. Current agriculture practices and technologies are not able to meet the requirement in a sustainable ways.

Enhancing the agricultural produce, decreasing the post harvest losses, preventing yield losses due to diseases, pest attack, drought stress, cold stress, salt stress and also enhancing the nutritional value of the agricultural produce are some of the major target which scientist are willing to achieve in the modern agriculture.

Agronomy and soil science the core subject of agriculture deals with field-crop production and their soil management can only exploit the potential of the field and crop but up to a certain level only. We have already exploited these factors and any further development in these cannot increase the yield such to meet the current demand. Also, development of new varieties with increased yield depends on the available variation in the particular crop and almost all of the variation present in the nature so further improvement in yield by breeding methods is also a losing battle.

Biotechnology has evolved as a science whose scope is limited by our imagination only. Extraordinary success in the field of chemistry, molecular biology and plant sciences has leaded it to a new height. The applications of biotechnology to agriculture have been persistent, both within the agriculture sector and outside it. The term “biotechnology” was first use by Karoly Ereky in 1919. Biotechnology is the use of living systems and organisms to develop or make products, or "any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" (UN Convention on Biological Diversity). Increasing the food grain production and thereby increasing the profit of farmer is the primary target of the biotechnology.

Applications of agricultural biotechnology
1. Marker assisted breeding
Typical breeding programs usually grow hundreds or even thousands of populations, and many thousands or millions of individual plants (Witcombe & Virk, 2001) [14].

The selection in conventional breeding is totally based on the phenotypic evaluation of the crop. Performance for a particular trait is also based on its heritability. In conventional breeding we cannot go for off season nursery analysis, also we have to for full crop if we want to take data regarding yield and other maturity traits. Also selection done on the basis of morphological characters are not accurate, there are chances of error due to environment influence. Along with progress in molecular biotechnology, various types of molecular markers in crop plants were developed during the 1980s and 1990s (Xu, 2010) [15]. All these limitation of breeding can be overcome by the use of molecular marker technology.

Marker assisted selection is a process in which a particular marker is used for the indirect selection of a plant with trait of interest. It has completely replaced the phenotypic evaluation and its limitations. The extensive use of molecular markers in various fields of plant science, e.g. germplasm evaluation, genetic mapping, map-based gene discovery, characterization of traits and crop improvement, has demonstrated that molecular technology is a powerful and reliable tool in genetic manipulation of agronomical important traits in crop plants (Xu, 2010; Jiang, 2013) [15]. Marker-assisted selection (MAS) has been widely applied in breeding programs for targeted transferring and pyramiding resistance loci in different crops (Kolmer, 1996; Foolad *et al*., 2002; Singh *et al*., 2005; Liu *et al*., 2004, Richardson *et al*., 2006; Asea *et al*., 2009 and Moloney *et al*., 2009) [5, 2, 10, 6, 9, 1, 8].

Examples of where MAS would be advantageous include selection for traits that are difficult or expensive to measure (e.g., salt tolerance, restorer genes); pyramiding multiple genes that confer a similar or identical phenotype (e.g., multiple genes for resistance to blast or bacterial blight); or selecting against the donor chromosomal segments in a backcrossing scheme. Many rice scientists are now beginning to use markers for these types of applications. Many research groups have pyramided two to four resistance genes against bacterial blight, *Xa4*, *xa5*, *xa13*, and *Xa21*, using PCR-based markers in different rice varieties. Molecular markers are particularly useful for accelerating the backcrossing of a gene or QTL into an elite cultivar or breeding line. Markers linked to the gene can be used to select plants possessing the desired trait, and markers throughout the genome can be used to select plants that are genetically similar to the recurrent parent (background selection). This approach is thought to be promising in rice because a number of rice cultivars are widely grown for their adaptation, stable performance, and desirable grain quality.

Some of the advantages of MAS are:-

a) Time saving as selection can be practiced at seedling stage so the desirable cross can be made in the same season.
b) Is an effective strategy for selection in early segregating generations.
c) Threshold characters can be selected without onset of particular environment.
d) Recessive traits as well as heterozygote progenies can also be identified.
e) It can be used for the assessment of purity of breeding material.

2. Plant tissue culture

Plant cells are unique because they possess the property of totipotency and developmental plasticity in the differentiated state and have the ability to dedifferentiate, proliferate, and subsequently regenerate into mature plants under appropriate culture conditions in a hormone-dependent manner (Skoog and Miller 1957; Steward *et al*. 1964) [11, 12]. Gottlieb Haberlandt was first to initiate the work on tissue culture in 1898. The theoretical basis of tissue culture lies in the cell theory given by Schleiden and Schwann (1838–1839). Practically, this technique stands on the concept of "totipotency," i.e., each cell has the ability to regenerate into a new plant. plant cell cultures have evolved as *in vitro* experimental models for studying cell division, differentiation and morphogenesis, which are important in key developmental processes such as meristem formation and embryogenesis (Zimmerman, 1993) [16] and stress-related genome plasticity in plants. The field finds a wide range of applications starting from mass clonal propagation to plant improvement, molecular biology, bio-processing as well as a basic research tool. It has advanced the production in forestry and agriculture to many folds.

With the decreasing arable land Plant tissue culture technique offers an excellent opportunity for mass propagation of plants in laboratory test tubes which can be transferred to the field later on. It is an *in vitro* cultivation of plants under artificial media and aseptic condition. Tissue culture has evoked a path for crossing between to noncompatible plant species. Embryos produced by incompatible crosses are rescued and grown to obtain viable plant. This technique is combined with genetic engineering to regenerate plants with novel characters and combine two or more beneficial characters into a single plant. Several application of plant tissue culture are listed below.

a. Micropropagation

It stands for propagation of any plant part under aseptic conditions, rapid clonal propagation is one of type which is being extensively used in horticultural crop for their propagation. Micropropagation is the application of tissue culture technique to the propagation of plants starting with very small parts grown aseptically in a test tube or other suitable containers. Initially, small plant explants is surface sterilized and inoculated into a culture vessel containing a nutrient medium. The inoculated culture vessel is incubated at room temperature. From it a large number of shoots develop from the axillary bud in a process known as axillary bud proliferation. Each growing point is sub-cultured to give rise to shoot. This phenomenon is known as adventitious shoot formation. Auxin plays an important role in development of root. The new plantlet is transferred to the field.

Although, micropropagation has many successfully applications in large number of crop plants but there are still problems associated with its commercial applications. The major limitations include high input cost of energy and manpower. These limitations have been circumvented to some extent in apple with the advent of bioreactor technology. Further, to obtain clonally uniform healthy plants, few other problems need to be addressed while following the micropropagation protocol: a) manipulation and regeneration of woody explants taken from adult trees; b) presence of exogenous and endogenous microbes; c) browning due to the exudation of phenolics and tannins into the medium as a response to wounding at excision; d) shoot necrosis and mortality of the explant/cultures due to the absorption of these substances; e) hyperhydricity; f) production of offtypes/somaclones, largely through organogenesis alarms the use of micropropagated plants by nursery; g) the loss of

plants during hardening and transplantation as a shift from heterotrophic mode of nutrition to autotrophic.

b. **Organogenesis**

It refers to differentiation of organs, such as shoot and root from an undifferentiated mass of cells. When an explant is placed in an artificially enriched nutrient medium, it's de-differentiate and form a mass of unorganized cells known as callus. The callus cells then re-differentiate and produced the desired tissue and then an organ or organs under the influence of specific growth regulators.

c. Somatic embryogenesis

An zygotic embryo is the result of sexual reproduction. However, plant tissue culture technique offers a method of producing; embryos from somatic cells bypassing sexual reproduction such embryos a known as somatic embryos. The process of formation of somatic embryos is known as somatic embryogenesis. This method can be used for rescue of zygotic embryos formed by incompatible crosses and overcoming seed sterility and dormancy.

d. **Protoplast culture and fusion**

In this method protoplast of two different species of plants have been successfully fused to produce a single protoplast containing the genetic material and the cytoplasm of both protoplasts. This fusion is facilitated by some agents (both chemical and electrical), known as fusogens e.g. PEG. When two protoplasts are fused a cell containing cytoplasm and nucleus of both parent cell is formed which is also called as heterokaryon. Later on the two nuclei fuse forming a synkaryon. This process is also known as somatic hybridization and the products as somatic hybrids. However, sometimes, due to cellular incompatibility, two nuclei cannot co-exist. Consequently, one nucleus is eliminated and a protoplast containing the nucleus of one species and the cytoplasm of both results. This type of hybrid is known as a cytoplasmic hybrid or cybrid. Somatic hybridization is attempted in plant species, which are sexually incompatible. The well known example of a somatic hybrid is 'pomato' obtained from the protoplasts of potato and tomato.

Table 1: Applications of tissue culture in crop improvement

S. No.	Improvement	Approach
1.	Homozygous and heterozygous plant	Rapid clonal propagation
2.	Changing ploidy of plant	Anther and ovary culture Endosperm culture
3.	Novel traits	Disease resistance ('ono' variety of sugarcane) Yield (pusa jaikisan variety of brassica) Colour (scarlet variety of sweet potato)
4.	Secondary metabolites	Suspension culture
5.	Germplasm conservation	Micropropagation and slow culture
6.	Distant hybridization	Somatic hybridization; protoplast fusion and embryo rescue
7.	Molecular breeding	DH lines
8.	Regeneration and selection of transgenic plants	Selection media
9.	Basic studies	Bioreactors
10.	Creation of variation	Somaclonal variation

3. Recombinant dna technology or genetic engineering

Recombinant DNA is produced by joining together of two or more pieces of DNA segment from different organism using genetic engineering tools. Organisms that are produced by transfer of a transgene from a distinct organism by non breeding methods are called as transgenic organism. Various approaches are used to transfer the transgene from one organism to other are used such as *Agrobacterium* method, gene gun method, PEG mediated DNA transfer, microinjection, macroinjection and silicon fiber mediated transformation. Transgenic plants were first developed around 1980's. The first genetically modified crops were soybean and corn, and appeared on the US market in 1996. Since then transgenic plants have been commercialized in many countries and now a day's 28 countries are growing transgenic crop including the latest one Bangladesh.

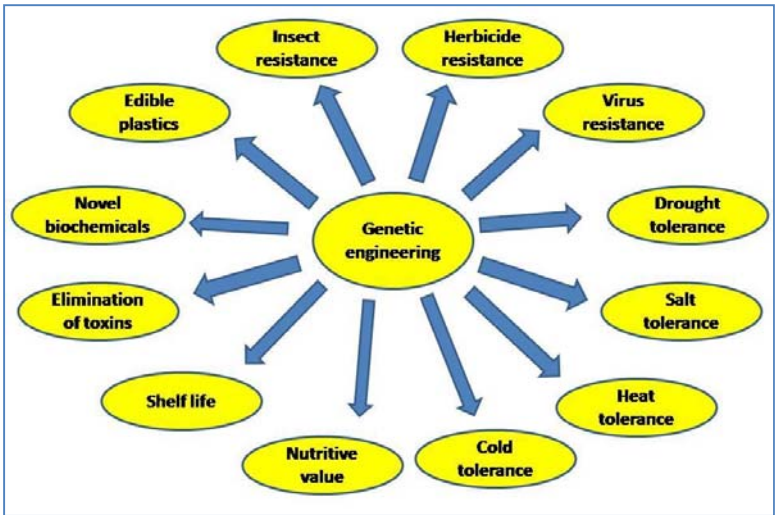

Fig 1: Application of transgenic approach

Many successful examples of the use of recombinant DNA technologies are there.

a) Flavr Savr is a classical example of genetic engineering scientists target to slow down the ripening process of the tomato and thus prevent it from softening, while still allowing the tomato to retain its natural colour and flavor. This was done by adding an antisense gene which interferes with the production of the enzyme polygalacturonase. The enzyme normally degrades pectin in the cell walls and results in the softening of fruit which makes them more susceptible to damaged. The intended effect of slowing down the softening of Flavr Savr tomatoes would allow the vine-ripe fruits to be harvested like green tomatoes without greater damage to the tomato itself.
b) Golden rice produced through genetic engineering to biosynthesize beta-carotene, a precursor of vitamin A, in the endosperm of rice. Golden rice was created by transforming rice with two beta-carotene biosynthesis genes i.e. *psy* (phytoene synthase) from daffodil (*Narcissus pseudonarcissus*) and *crtI* (carotene desaturase) from the soil bacterium *Erwinia uredovora*. The *psy* and *crtI* genes were transferred into the rice nuclear genome and placed under the control of an endosperm-specific promoter, so that they are only expressed in the endosperm. The end product of the engineered pathway is lycopene.
c) *Bt* cotton was created through the addition of genes encoding endotoxin (called as cry toxins) for a particular group of organism. When insects attack and eat the cotton plant the endotoxins are dissolved due to the high pH level of the insect's midgut. Interaction between this protein with receptor opens cation selective pores which allow the flow of potassium. Osmolytic lysis occurs in the midgut of the insect. Also, regulation of potassium concentration is essential and, if left unchecked, causes death of cells. Due to the formation of Cry ion channels sufficient regulation of potassium ions is lost and results in the death of epithelial cells. Septicemia caused by enteric bacteria ilso contributes to this.

4. OMICS

Genome encodes the complete set of genetic information in an organism i.e. provides necessary information vital for the organism to function. It includes both chromosomal as well as orgenalle DNA. Gene is a set of nucleotide sequence containing coding sequences along with its regulatory units, it codes for the RNA and protein molecules required by the organism. In eukaryotes, each cell's genome is enclosed within a membrane-bound structure called the nucleus. Prokaryotes lacking inner membranes, store their genome in a region of the cytoplasm called the nucleoid. The full range of RNA molecules expressed by a genome is known as its transcriptome, and the full assortment of proteins produced by the genome is called its proteome.

a) Genomics

Its a branch of bioinformatics which focus on genome. It aims in the characterization and quantification of genes, which direct the production of proteins with the help of enzymes and mRNA. Proteins in turn make up the body structures like organs and tissues as well as control chemical reactions (as an enzyme) and carry signals (as signaling molecules) between cells. If mutation occurs in DNA it leads to formation of abnormal protein which can disrupt the body's usual processes and in some cases lead to significant visible effects e.g. diseases such as cancer. In contrast to genetics, which is the study of genes and their roles in inheritance, genomics is the study of genes, their functions and related techniques (applications of recombinant DNA, DNA sequencing methods and bioinformatics) to sequence, assemble and analyze the function and structure of genome.

b) Transcriptomics

It is the study of the complete set of RNAs (transcriptome) encoded by the genome of a specific cell of an organism at a specific time under a specific set of conditions. Different subsets of genes are expressed at different time and condition in a particular cell. So, if we compare the transcriptome of cell in two different condition say control and salt stress, we will get two different set of RNA expressing. These RNA which are only found in salt stressed cell may be hypothesized to involve in survival of cell under salt stress. Likewise we can isolate RNA expressing at any particular condition, using which we can isolate the gene behind it and go for its exploitation through transgenic technology.

c) Proteomics

It is the study of complete set of proteins encoded by a cell at a specific time under specific conditions. Concept behind proteomics in finding the causal protein is same as Transcriptomics. It involves the systematic study of proteins in order to provide a comprehensive view of the structure, function and role in the regulation of a biological system.
Understanding the transcriptome and proteome is essential for interpreting the functional elements behind any particular trait. These may helps in understanding the mechanism of cell differentiation and understanding the mechanism of disease.

Application of animal biotechnology

Impressive strides have been developed in the area of plant biotechnology by enhancing production, defense mechanism against various deceases and adaptation to various stresses. Livestock's are been used as a source for production of medicines since long time. Production of insulin for diabetic patient, heparin as anti coagulant, various serum, antiserum organs for human transplantation are some of the core example of this feild. By using recombinant DNA technology many animals are genetically engineered to produce various pharmaceuticals.

1. Artificial insemination

Artificial insemination leads to a mode of selective breeding in case of animals also. Embryo transfer is another excellent example of animal biotechnology to enhance selective breeding. Progress in semen collection and dilution and cryo preservation techniques now enables a bull to be used simultaneously in several countries for up to 100,000 insemination a year (Gibson and Smith, 1989. In this method a superior breeding female is first chemically induced to super ovulate. The egg is then fertilized within the donor which later on developed into embryo. Embryo is then transplanted into recipient female.

2. Cloning

Cloning i.e. production of genetically identical cells, animals etc are another fascinating example of this field. Creation of a clone is doneby inserting a donor cell into an egg which is already enucleated. The egg developed into an embryo which is then transferred into a surrogated mother for gestation.

Sheeps, gaur, pigs, cattles have been produced by this method.

3. Cattle breeding

The formation of new crossbred cattle breeds to increase milk production was started in India because of crossbred cattle were more economical and gave higher milk yield than the indigenous cows and increase the income of a farmers, dairy entrepreneurs and provide beneficial and round the year employment to them. Therefore, population of crossbred cows should be increased simultaneously with Artificial Insemination programme to increase profitability to the farmers and dairy industry. Crossbreeding is mating of animals from different established breeds. The progeny produced is called crossbred. Crossbreeding programmes initiated during the 1950s in India between indigenous and exotic cattle mainly with Holstein Friesian (HF) and Jersey for increase in milk production. During this crossbreeding experiments evolution of a new strains of crossbred cattle, viz., Taylor, Jersind, Jerthar, Karan Swiss, Karan Fries, Sunandini, Frieswal, Phule-Triveni and Vrindavani cattle capable of producing more milk than native breeds

Increase in crossbred cattle population, milk production and per capita of milk availability, lactation length, growth rate, decrease in age at puberty, age at first calving and calving interval (Tomar, 2009) [13], higher birth weight of calves, better growth rates, better reproductive efficiency, advantage of breed complementarity and non-additive effects (dominance and epistatic) thus leading to heterosis (hybrid vigor). Heterosis tends to be most important for lowly heritable traits such as fertility and survival. Heterosis makes crossbred animals more productive and better than either of the parental breeds. Crossbred animals are docile, can be easily handled and more suited for machine milking, Heat detection and artificial insemination is easier in cows. Price of crossbred cow milk is less in comparison to native breeds.

Many genetical and physiological similarities exist between human and animals. Hence research using animals are key to most medical biology research done for humans. Recombinant vaccines are produced using recombinant DNA technology. Recombinant DNA vaccines are those vaccines in which genes for desired antigens of a microbes are inserted into a vector ant transformed into a production system such as *Pichia pastoris.* DNA vaccines, subunit vaccines, synthetic peptides and gene deleted vaccines are some of the examples of recombinant DNA vaccines.

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