Review Paper

Biotechnology: An Advanced Tool for Crop Improvement

Abstract

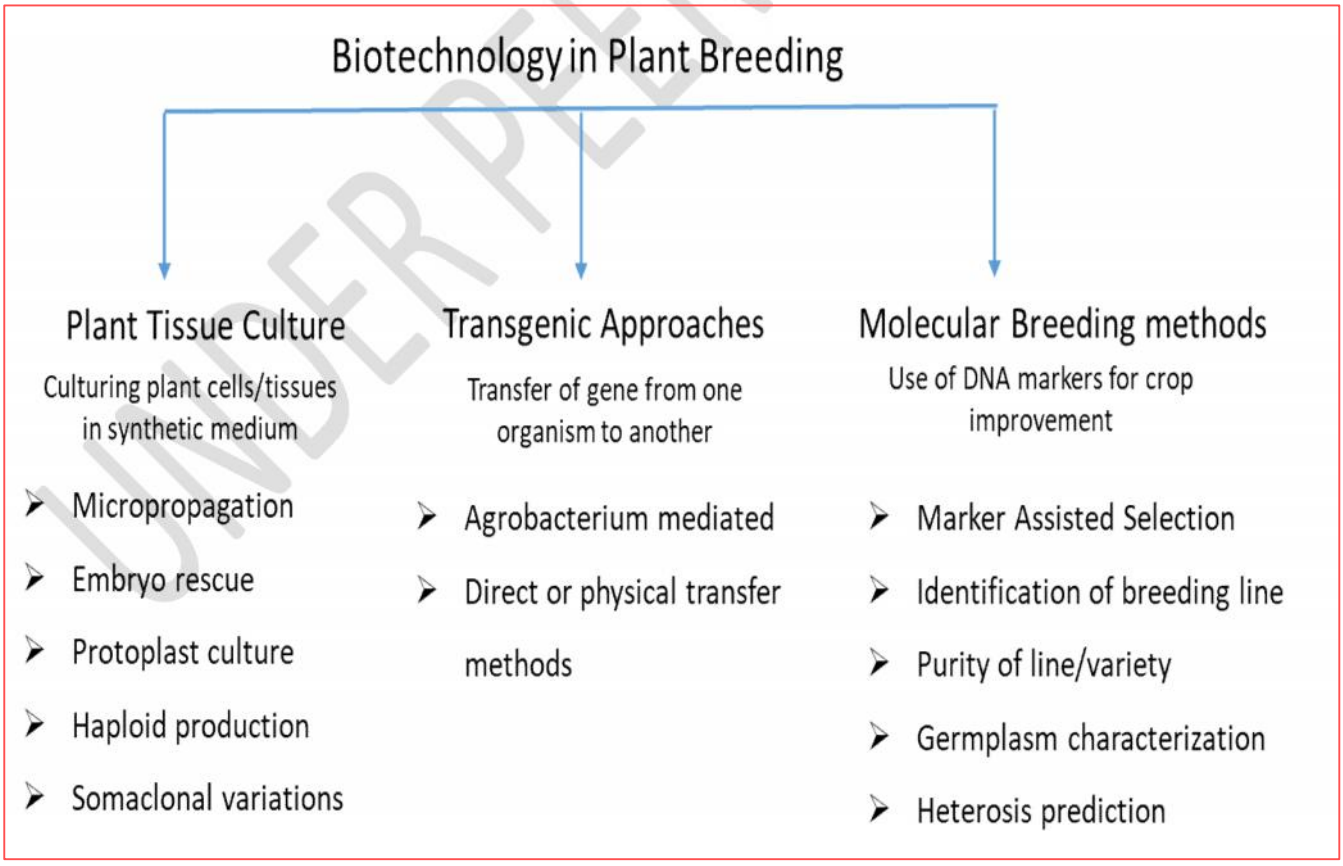
Plant breeding is mainly concerned with genetic improvement of crops through hybridization, screening and selection of advance lines. The conventional methods give advance varieties with desirable traits but take consume more time (6 to 12 years) to achieve. Biotechnology tools makes breeding methods more advance by reducing the time to get improved varieties. Other than conventional methods varietal advancement can be achieved by applying plant tissue culture, transgenic approaches and molecular breeding methods. Crop improvement by using biotechnology approaches is mostly concerned with protoplast fusion to get somatic hybrids, gene transfer to get genetically modified plants and use of DNA markers to select trait of interests. Variety with improved biotic and abiotic stress resistance can be developed in less time and more accuracy using recent biotechnological approaches. Several new tools are being utilized for that purpose including, nanotechnology, bioinformatics offers new era of genomics assisted molecular breeding. Next Generation Sequencing and high throughput genotyping approaches are increasing efficiency and output of biotechnological tools in agriculture. Current review focused on overview of biotechnological tools being applied for crop improvement.

Key words: Plant tissue culture, Transgenic, Molecular Breeding, Crop Improvement

Introduction

Plant breeding plays a major role in increasing crop yield for over a century. Continue efforts have been made to inculcate desirable trait like diseases tolerant, higher yield, abiotic stress tolerant etc. in a single line or genotype. Crop improvement is based on the criteria novelty,

stability, uniformity and utility; which a breeder achieve by combined application of conventional breeding and tools of biotechnology, this emphasis of plant biotechnology supplements breeding for crop improvement. Thus, the integration of both plant breeding and biotechnology, overcome the increasingly sophisticated, and staggering breeding procedure in easiest way. Continuous varietal improvement through conventional breeding needs biotechnology to maximise the probability of success. Tissue culture and genetic engineering are the two major approaches dealing with crop improvement via biotechnology. In plant breeding, biotechnology is more than genetic engineering which address problems in all areas of agricultural production and processing (Fig. 1). This includes raise and stabilize yields; to improve resistance to pests, diseases and abiotic stresses such as drought and cold; and to enhance the nutritional content of foods like protein in pulses, etc.



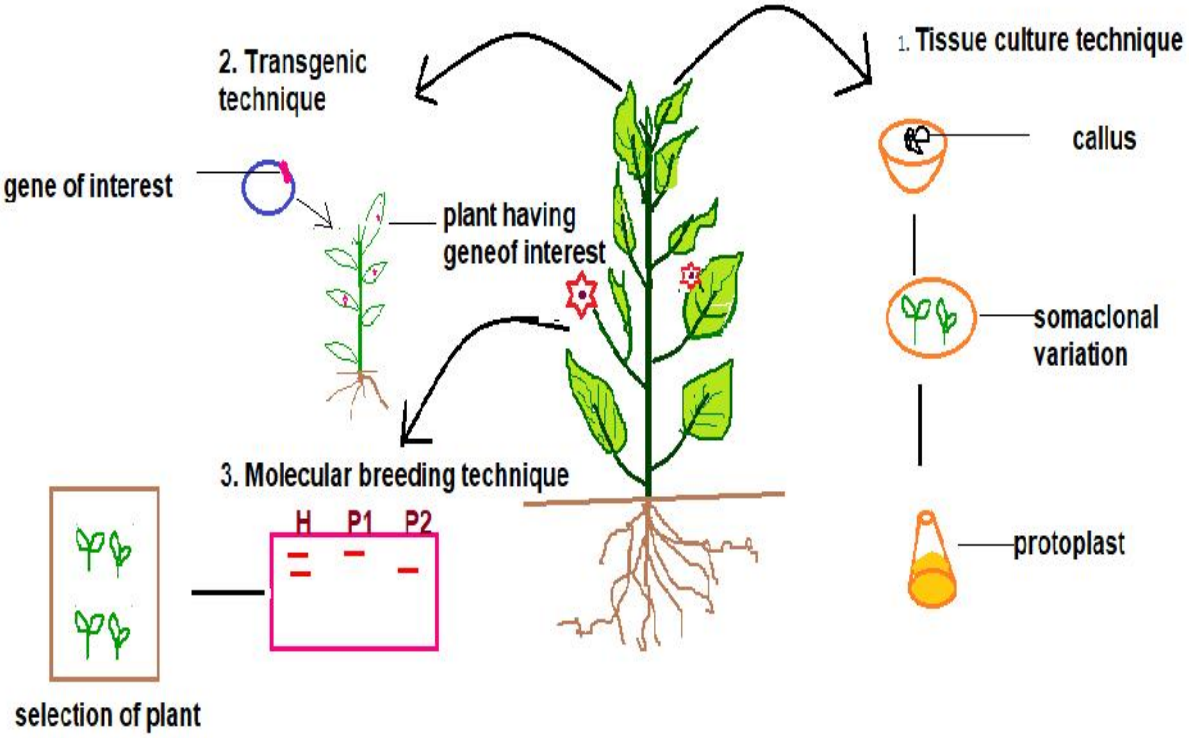


Figure 1. Applications of Biotechnology in Plant Breeding Practices

Global scenario on crop improvement

The broad applications of biotechnology in agriculture, specifically in crops, include the development of disease diagnostic kits, biofertilizers, biopesticides, use of molecular markers, tissue culture, and genetic engineering for varietal development, helped supply the increasing needs of a growing world population estimated to reach 9 billion by 2050 (Teng, 2008; Ortiz, 2010). Research and development (R&D) activities in genetics (1960) has wide practical application of transgenic crops started only during the 1980s with the success of experiments done in tobacco (Vines, 2002). Several transgenic crops were later developed and commercialized starting in tomato with delayed ripening, on agronomic and field crops such as canola, cotton, maize, soybean, sugar beet, papaya, and squash rendering with traits such as herbicide tolerance, virus and insect resistance. In 2004, it was estimated that more than 50 other species of transgenic fruits, vegetables, field crops, and other plants were under research in the laboratory and confined facilities with a long term goal of eventual commercialization (Runge and Ryan, 2004). It is likely that there will be over 120 different transgenic events in biotech crops worldwide, which is about a four-fold increase in the number of current transgenic events found in commercially cultivated genetically modified (GM) crops.

India is the second largest producer of food grains globally & houses numerous varieties of cereals and pulses that are largely consumed domestically. As per 3rd advance estimates, the production of food grains during 2016-17 is 273.38 million tonnes (Hindustan Times, New Delhi). According to International service for the Acquisition of agri-biotech application (ISAAA), INDIA has the forth position under area for genetically modified crop planting. Nearly 96 % of the country‘s cotton area is now covered by *Bt* hybrids.

Plant Tissue Culture

Plant tissue culture broadly refers to the *in vitro* cultivation of living plant cells, tissues or organs (seeds, embryos, single cells, protoplasts) on nutrient media under closely controlled and aseptic environment. Depending upon the plant part used as explant (part of plant used for regeneration), plant tissues culture techniques are micropropagation, somatic embryogenesis, somaclonal variation, meristem culture, anther culture, embryo culture, protoplast culture, cryopreservation, and production of secondary metabolites.

Micropropagation is mass production of clonal progeny from very small plant parts (0.2-10 mm) in the laboratory, followed by their establishment in soil under greenhouse conditions. An estimate of more than 500 million plants belonging to different species are now being annually produced through micropropagation in the world. Banana, strawberries, citrus, timber trees like *Delbergia sisso*, planting material can certainly improve the yield potentials of vegetatively propagated. Micropropagated plants are true to type, disease free, high quality and super elite planting material for further seed production. This technology possesses tremendous potential for making environment clean and green.

Somaclonal variation is the variation among callus-derived plants, is a potent force for broadening the genetic base. Several interesting and potentially useful novel traits have been recovered that either do not exist or are rare in the natural gene pool using this technique —for example, atrazine resistance in maize, glyphosate resistance in tobacco, improved lysine and methionine contents in cereals, increased seedling vigor in lettuce, jointless pedicels in tomato are much significant recovered traits. In India, a somaclonal variant of a medicinal plant, *Citronella java* has been released as a commercial variety, B-3, which gives higher yield and oil content than the original variety. Likewise, Pusa Jai Kishan is a variety of *Brassica juncea* released as a somaclonal variant of Varuna variety.

Haploids production through anther or pollen culture is an attractive method, where pollen grains incubate under optimum conditions leads to growth of microspores into sporophytes. Wide crossing, irradiation, chemical treatment is other principal methods for haploid production.

List of tissue cultured crop in India (listed by Agri-farming)

Fruit crops	Apple, banana, fig, grape, pineapple, strawberry, citrus
Spice crops	Turmeric, ginger, vanilla, large cardamom, small cardamom, blackpepper
Cash crops	Potato and sugarcane
Medicinal crops	Stevia, patchouli, neem, aloe vera, geranium
Ornamental crops	Gerbera, syngonium, lily
Bio fuel	Jatropha, pongamia
Woody plants	Teak, populous, bamboo, eucalyptus

Transgenic approaches

Transgenic technology is a gene transfer process from same or unrelated species to desired crop plant species for genetic analysis and direct manipulation of DNA. This gene technology is also known as recombinant DNA technology or genetic engineering. During the past 15 years, the combined use of recombinant DNA technology and tissue-culture techniques has led to the efficient transformation and production of transgenics in a wide variety of crop plants (Chahal and Gosal, 2002). In fact, transgenesis has emerged as an additional tool to carry out single-gene breeding or transgenic breeding of crops. Rapid and remarkable achievements have been made in the production, characterization, and field evaluation of transgenic plants in several field crop, and fruit and forest plant species.

Genetic engineering for insect resistance: Insect resistance was first reported in tobacco and tomato. Today insect resistant transgenes, whether of plant, bacterial or other origin can be introduced into plants to increase the level of resistance.

Genetic engineering of male sterility (Williams, 1995) have emerged as tangible options for the development of male sterile and restorer lines for hybrid seed production. The barnase gene, from the bacterium Bacillus amyloliquefaciens, encodes the enzyme barnase (ribonuclease), which is produced in the transgenic plant/line during the development of the anthers have been used with greater success.

Engineering for improved nutritional quality: Introduction of provitamin A and β carotene genes have resulted in the production of golden rice (Burkhardt *et al*., 1997; Ye *et al*., 2000; Beyer *et al*., 2002). Vitamin producing transgenic plants have also been developed (Herbers, 2003) and emphasis is being laid on multigene engineering (Daniell and Dhingra, 2002). The main objective of these crops is to add value to agri-foods (Newell-McGloughlin, 2008).

Molecular Breeding

Depending on application and species involved, ideal DNA markers for efficient use in marker-assisted breeding should meet the following criteria:

- ✓ High level of polymorphism
- ✓ Even distribution across the whole genome (not clustered in certain regions)
- ✓ Co-dominance in expression (so that heterozygotes can be distinguished from homozygotes)
- ✓ Clear distinct allelic features (so that the different alleles can be easily identified)

- ✓ Single copy and no pleiotropic effect
- ✓ Low cost to use (or cost-efficient marker development and genotyping)
- ✓ Easy assay/detection and automation
- ✓ High availability (un-restricted use) and suitability to be duplicated/multiplexed (so that the data can be accumulated and shared between laboratories)
- ✓ Genome-specific in nature (especially with polyploids)
- ✓ No detrimental effect on phenotype

Since Botstein *et al*. (1980) first used DNA restriction fragment length polymorphism (RFLP) in human linkage mapping, substantial progress has been made in development and improvement of molecular techniques that help to easily find markers of interest on a largescale, resulting in extensive and successful uses of DNA markers in human genetics, animal genetics and breeding, plant genetics and breeding, and germplasm characterization and management. Selection of desirable plant species is the basic principle of plant breeding; which involves evaluation of agronomic traits, biotic and abiotic stress resistance / tolerance and response towards chemicals. Marker assisted selection a new discipline of molecular breeding helps to evaluation traits using molecular marker that are based on banding pattern of linked DNA marker. Several types of DNA markers that have been developed and are being used in plants include: restriction fragment-length polymorphism (RFLP), amplified fragment-length polymorphism (AFLP), randomly amplified polymorphic DNA (RAPD), sequence-tagged sites (STS), expressed sequence tags (ESTs), simple sequence repeats (SSRs) or microsatellites, sequence-characterized amplified regions (SCARs), and single nucleotide polymorphisms (SNPs) (Paterson *et al*., 1991; Hoisington *et al*., 1998; Joshi *et al*., 1999; Bernardo, 2008). {The gene specific marker are efficiently in use nowdays. QTL mapping or dectection of genes helps in genetic linkage

analysis. Gene pyramiding, backcrossing early generation selection are the broad areas of MAS. An application of MAS in developing disease resistant in rice against Bacterial blight, blast and rice tungro virus; in wheat against leaf rust, powdery mildew and loose smut}-(**Need revision**)

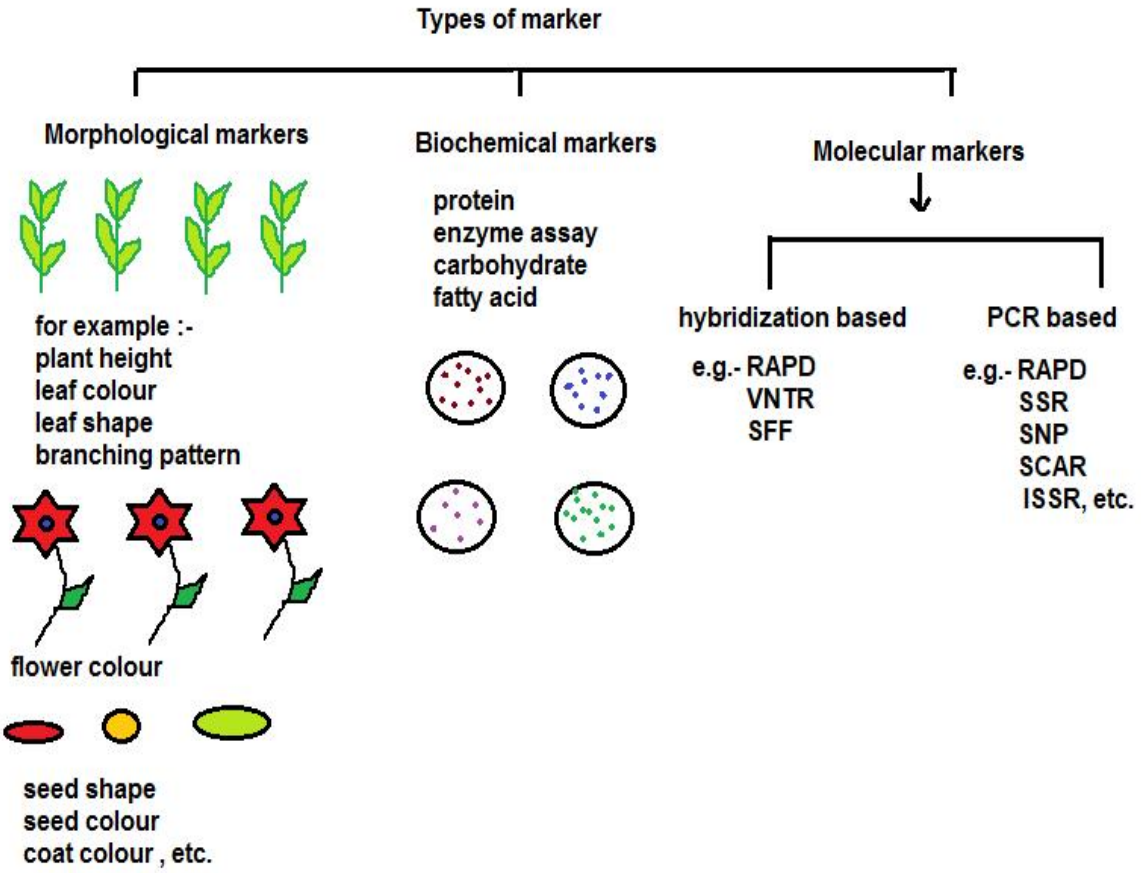


Figure 2. Different Types of Molecular Markers

Table 1. Comparative of different types of molecular markers

Traits	RFLP	RAPD	SSR	CAPS	ISSR	EST	STMS	SNP
Level of polymorphism	Medium	Very high	high	Moderate	High	High	High	High
Cost	Expensive	Cheap	Expensive	Cheap	Cheap	Costly then SSR	High	Variable
Allelism	Co-dominant	Dominant	Co-dominant	Mostly co-dominant	Dominant	Co-dominant	Co-dominant	Co-dominant
Time	Time consuming	Quick working	Quick working	Quick	quick	Time Consuming	quick	quick
Banding pattern	Locus specific	Multi locus	Locus specific	Locus specific	Multi locus	Locus specific	Locus specific	Locus specific
Probe / primer	Probe	Primer	primer	Primer	Primer	primer	Primer	Primer
DNA required (ng)	10000	20	10-20	30-100	20-50	20-50	20-50	5-20
Advantage	Co-dominant and no need of prior sequncing	Less DNA required , easy to use and polymorpic	Less DNA required , high reproductive	Versatile , easily scored and interpreted	Highly polymorphic, , no need of prior sequncing	Rapid and inexpensive	Reproducible	Widely distributed in genome , co-dominant , highly reproductive
Disadvantage	Use of radioactive probe & southern blotting step involve	Low reproducibility . dominant ,highly purified DNA is required	High developing cost , presence of more null allele	Restriction enzymes must be tested for polymorphisms	Non-homology of similar sized fragments, low reproducibility	Lack of prime specificity, labour oriented		High developing cost

reference	(Bostein *et al*.,1980	(Williams *et al*., 1990)	(Jeffreys, 1985)	(Michaels and Amasino, 1998)	(Meyer *et al*., 1993; Gupta *et al*., 1994)	Adams *et al*., 1991)	(Olson *et al*., 1989)	(Batley *et al*., 2003)

Future prospects:

The genome sequences of organisms are fundamentally important for understanding the functions of individual genes and defining evolutionary relationships. The identification of genes and molecular markers underlying agronomic traits will help to accelerate the breeding process and lead to improved varieties with improved yield and quality, tolerance to unfavourable environmental conditions and resistance to diseases. DNA sequencing is a functional assay, and as it gets faster and cheaper, there will be more and more applications and uses for it in plant breeding. Next-generation sequencing has revolutionized our ability to study the variations occurring in whole genomes of organisms in a very short period of time at far lesser costs. Sequencing of crops provides valuable information on genome structure and organization. It opens up an excess of opportunities for research related to the life sciences including evolutionary biology, developmental biology, biochemistry, genetics and molecular biology. In recent years, agricultural sciences have been in the middle of a second technological revolution in DNA sequencing. Although conventional breeding techniques have significantly increased crop production and yield, new approaches are required to further improve crop production in order to meet the global growing demand for food. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 (CRISPR-associated protein9) genome editing technology has shown great promise for quickly addressing emerging challenges in agriculture. Recently

Haque *et al*. (2018) has reported potentials of CRISPR/Cas9 for improvement of crops cultivated in tropical climates to gain resiliency against emerging pests and abiotic stresses. It can be used to precisely modify genome sequence of any organism including plants to achieve the desired trait. In order to improve plant transformation through CRISPR/Cas9, several approaches such as optimization of the promoters to drive and express Cas9 and utilization of different fluorescent reporters and selection markers (Wang *et al*., 2015; Yan *et al*., 2015; Kaur *et al*., 2018) have recently been evaluated. The CRISPR/Cas gene-editing system is able to generate heritable, targeted mutations and also to address concerns over the presence of foreign DNA sequences as it can generate transgene-free plants. The most studied crop is rice (*Oryza sativa*) followed by other major crops: maize (*Zea mays*), tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*). Day to day advancement of biotechnology approaches will definitely help in increment of crop production with sustainability.

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