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 organisms 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 advance tools are being utilized for that purpose including, nanotechnology, bioinformatics tools 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: Crop Improvement, Genomics, Molecular Breeding, Plant tissue culture, Transgenics

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 maximize 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. 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. There are three major aspects of biotechnology in crop breeding i.e., Plant tissue culture, transgenic approaches and molecular breeding methods. Culturing of plant cell/ tissue in synthetic medium is known as plant tissue culture and it may be applied for micropropagation, embryo rescue, protoplast culture, haploid production, somaclonal hybridization or somaclonal variations. Another major application of biotechnology is transfer of gene from one organism to another which could be done by direct method (physical or chemical transfer) or indirect method (agrobacterium mediated gene transfer). Most popular and used method for crop improvement is molecular breeding method, where we use DNA markers and improve variety by marker assisted selection. Agriculture

biotechnological aspects may help in getting improved varieties according to changing climate (Bernardo 2008, Varshney *et al*., 2011) and for biotic and abiotic stress resistant variety development (Sharma *et al*., 2002). Musse and Mumm (2008) emphasize how the application of molecular plant breeding is now contributing to discoveries of genes and their functions which could be helpful for new avenues for basic plant biology research. Recently, Watson *et al*., (2018) focused on integration of speed breeding with other modern crop breeding technologies, including high-throughput genotyping, genome editing and genomic selection for accelerating the rate of crop improvement. Crossa *et al.,* (2017) emphasized on Genomic selection (GS) and said that it facilitates the selection of superior genotypes in less time and thus accelerates the breeding cycle. Crop improvement applying biotechnological tools could be done in faster way by high throughput phenotyping, high throughput genotyping, genomics assisted breeding and genome editing. Figure 1 and 2, clearly indicating different approaches of biotechnology which are being applied in plant breeding practices of crop improvement.

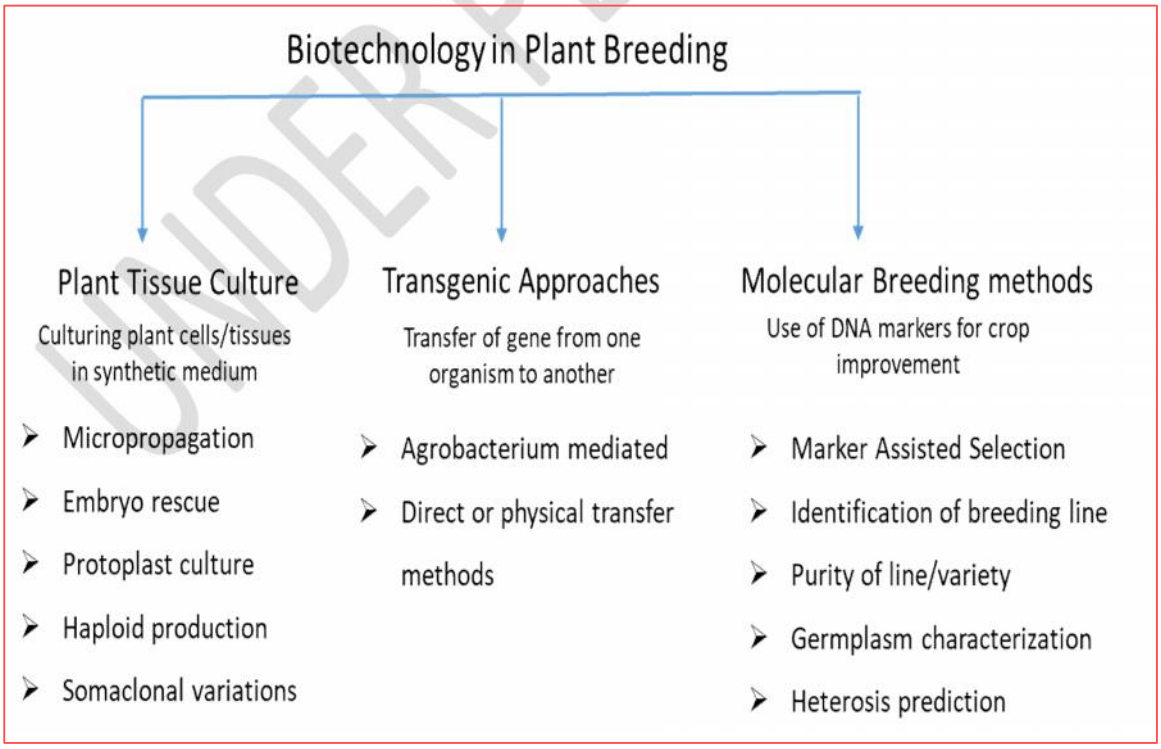

Fig 1. Different approaches of crop improvement using biotechnological tools

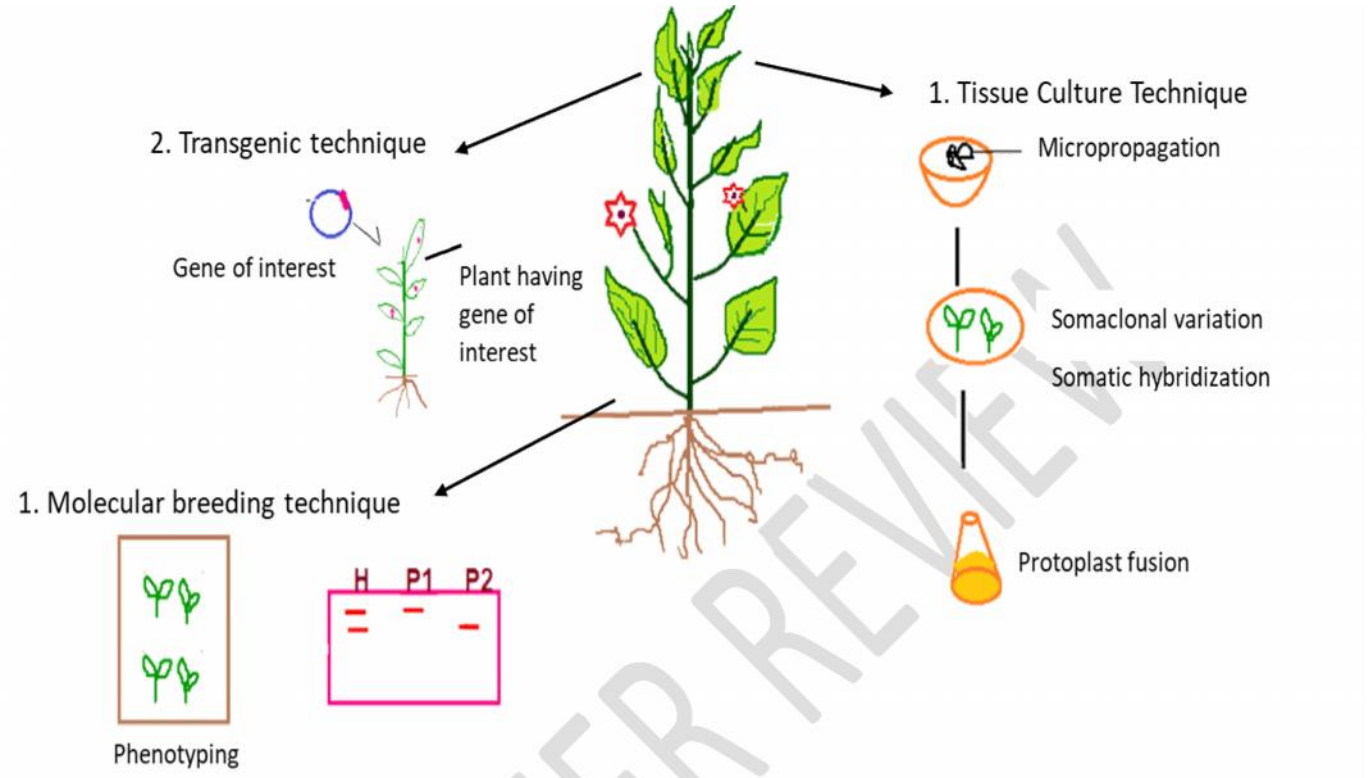

Fig 2. Applications of Biotechnology in Plant Breeding Practices

Global scenario on crop improvement

The broad applications of biotechnology in agriculture, specifically in crops, include the development of gene based markers, biofortification, nanotechnology, use of molecular markers, tissue culture, and genetic engineering. These tools would help in supplying the increasing needs of food to continuously growing world population which is estimated to reach 9 billion by 2050 (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. 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. 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. According to International service for the Acquisition of agri-biotech application (ISAAA), India has the forth position under area for genetically modified crop planting. Field trials for 21 GM food crops, including GM vegetables and cereals have been approved by the many countries but commercial cultivation of GM food has not been permitted by any State government in India till now (Venkat Vidya, 2016).

Plant Tissue Culture

Plant tissue culture broadly refers to the *Invitro* 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. Table 1 is indicating list of crops being studied by different research groups in plant tissue culture area.

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. Nowadays more than 500 million plants belonging to diverse 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.

Table 1. 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, cardamom, black pepper
Cash crops	Potato and sugarcane
Medicinal crops	Stevia, patchouli, neem, aloevera
Ornamental crops	Gerbera, syngonium, lily
Biofuel	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 transgenic in a wide variety of crop plants (Stein *et al*., 2010). 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 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). To add value to agri-foods vitamin producing transgenic plants have also been developed and emphasis is being laid on multigene engineering.

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

Markers are small fragments of DNA which are responsible for specific traits. They can broadly be categorized into three types; morphological (visible phenotypic traits), biochemical (protein, phenolics, enzymes etc) and molecular markers (Figure 3). Molecular markers are piece of DNA which code for specific traits and their inheritance could be detected. They have been categorized into hybridization based and PCR based (Jeffreys *et al.,* 1985, Gupta *et al*., 1999). 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), and simple sequence repeats (SSRs) or microsatellites, sequence-characterized amplified regions (SCARs), and single nucleotide polymorphisms (SNPs) (Joshi *et al*., 1999, Nadeem *et al*., 2018). Table 2 is clearly highlighting comparative study of some of the molecular markers, which are being used in broad spectrum in molecular breeding approaches. Day to day advances in molecular marker technology is being applied in crop improvement for successful breeding applications. Gene based markers are being used widely in basic molecular

biology labs, as they are specific to particular gene and fewer in number for screening of biotic and abiotic stress resistance. Most advance molecular markers are SNPs, but due to requirement of technical expertise and costly machines and reagents, they are limited to well-developed laboratories only. Molecular markers are being rapidly applied for marker assisted foreground and background selection, gene pyramiding, QTL mapping, fine mapping, gene tagging, association mapping, TILLING and Eco-TILLING etc.

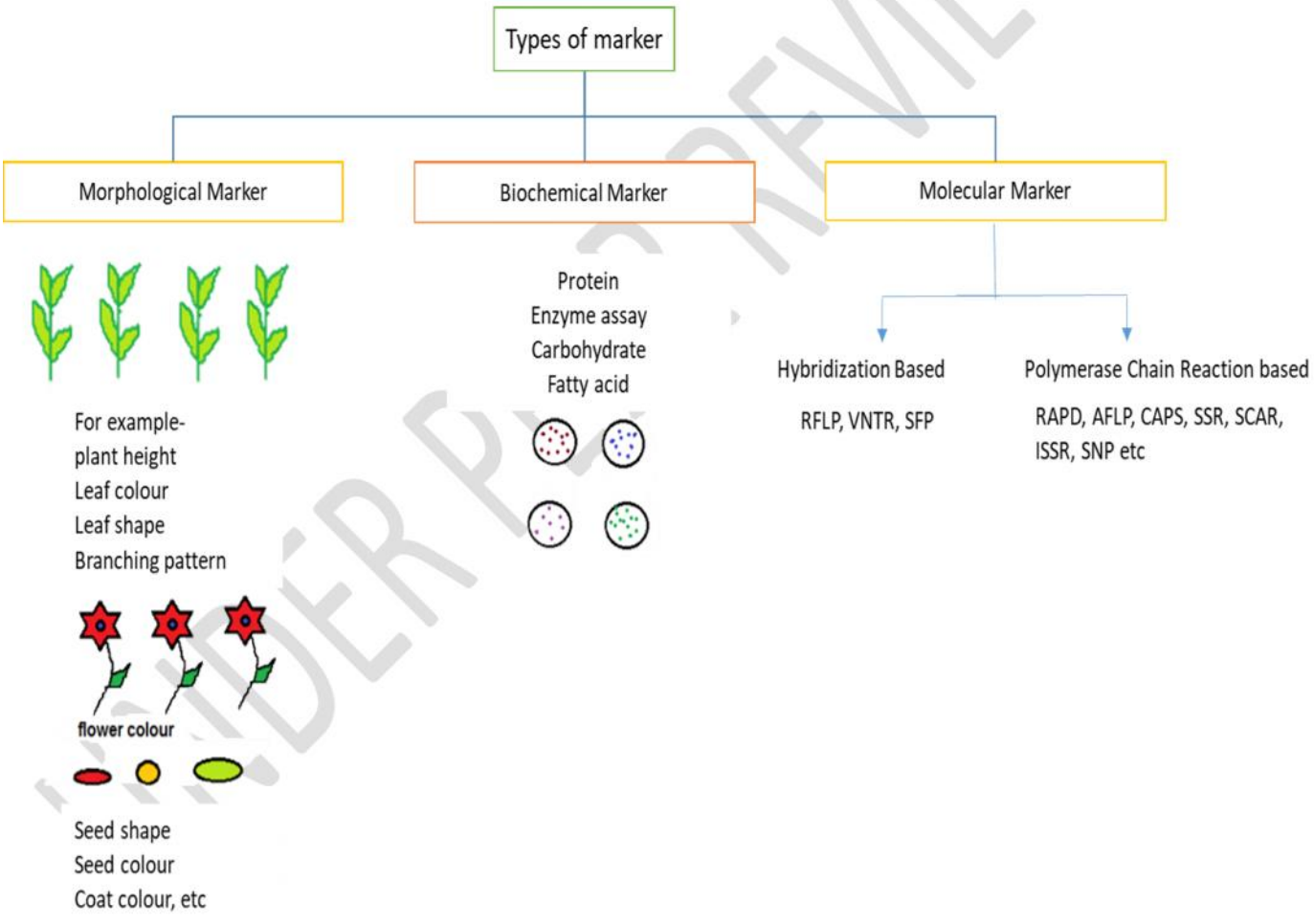

Figure 3. Different Types of Molecular Markers

Table 2. Comparative of different types of molecular markers

Characteristics	**Restriction Fragment Length Polymorphism (RFLP)**	**Randomly Amplified Polymorphic DNA (RAPD)**	**Simple Sequence Repeats (SSR)**	**Cleaved Amplified Polymorphic Sequence (CAPS)**	**Inter Simple Sequence Repeats (ISSR)**	**Expressed Sequence Tags (EST)**	**Single Nucleotide Polymorphism (SNP)**
Level of polymorphism	Medium	Very high	high	Moderate	High	High	High
Cost	Expensive	Cheap	Expensive	Cheap	Cheap	Costly then SSR	Variable
Allelism	Co-dominant	Dominant	Co-dominant	Mostly co-dominant	Dominant	Co-dominant	Co-dominant
Time	Time consuming	Quick working	Quick working	Quick	quick	Time Consuming	quick
Banding pattern	Locus specific	Multi locus	Locus specific	Locus specific	Multi locus	Locus specific	Locus specific
Probe / primer	Probe	Primer	primer	Primer	Primer	primer	Primer
DNA required (ng)	10000	20	10-20	30-100	20-50	20-50	5-20
Advantage	They are first DNA marker discovered. Co-dominant and no need of prior sequencing	Less DNA require, easy to use and polymorpic	Less DNA required, high reproductive	Versatile, easily scored and interpreted	Highly polymorphic, no need of prior sequencing	Rapid and inexpensive	Widely distributed in genome, co-dominant, highly reproductive
Disadvant	Use of radioactive	Low reproduc	High developin	Restriction enzymes	Non-homology	Lack of prime	High developing

age	probe & southern blotting step involve	ibility. dominant ,highly purified DNA is required	g cost, presence of more null allele	must be tested for polymorphisms	of similar sized fragments, low reproducibility	specificity, labour oriented	cost
References	Botstein *et al*. (1980)	(Willians *et al*., 1990, Welsh et al 1990.)	(Tautz *et al*., 1984)	(*Konieczny et al*., 1993)	(Gupta *et al*., 1994, Godwin *et al*., 1997)	Adams *et al*., 1991, 1993)	(Michaels *et al*., 1998, Batley *et al*., 2003, Wiltshire *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, Gao *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, followed by other major crops: maize, tomato, potato, barley and wheat. Day to day advancement of biotechnology approaches will definitely help in increment of crop production with sustainability.

References:

Adams MD, Kelley JM, Gocayne JD *et al* (1991) Complementary DNA sequencing: expressed sequence tags and human genome project. Science 252: 1651-1656.

Adams MD, Soares MB, Kerlavage AR, Fields C, Venter JC (1993) Rapid cDNA sequencing (expressed sequence tags) from a directionally cloned human infant brain cDNA library. Nat. Genet 4: 373-380.

Batley J, Barker G, 'Sullivan HS, Edwards KJ and Edwards D (2003) Mining for single nucleotide polymorphisms and insertions/deletions in maize expressed sequence tag data. Plant Physiology (132) 1: 84-91.

Beyer P, Babili S, Ye X, Lucca P, Schaub P, Welsch R and Potrykus I (2002) Golden Rice: Introducing the β-Carotene Biosynthesis Pathway into Rice Endosperm by Genetic Engineering to Defeat Vitamin A Deficiency. The Journal of Nutrition, (132) 3: 506S-510S

Bernardo R (2008) Molecular markers and selection for complex traits in plants: learning from the last 20 years. Crop Science: 48 (5) 1649-1664.

Botstein D, White RL, Skolnick M, Davis RW (1980) Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am J Hum Genet: 32 (3): 314-31.

Burkhardt PK, Beyer P, Wünn J, Klöti A, Armstrong G, Schledz M, Von JL, and Potrykus I. (1997) Transgenic rice (*Oryza sativa*) endosperm expressing daffodil (*Narcissus pseudonarcissus*) phytoene synthase accumulates phytoene, a key intermediate of provitamin A biosynthesis. Plant J. 11:1071-1078.

Crossa J, Pérez-Rodríguez P, Cuevas J *et al* (2017) Genomic selection in plant breeding: methods, models, and perspectives. Trends Plant Sci 22: 961-975

Gao C (2018) The future of CRISPR technologies in agriculture. Nature Reviews Molecular Cell Biology (19): 275-276

Godwin ID, Aitken EAB and Smith LW (1997) Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis 18: 1524-1528.

Gupta M, Chyi YS, Romero-Severson, J and Owen JL (1994) Amplification of DNA markers from evolutionarily diverse genomes using single primers of simple-sequence repeats', Theoretical and Applied Genetics 89: 998-1006.

Gupta PK, Varshney RK, Sharma PC and Ramesh B (1999) Molecular markers and their applications in wheat breeding. Plant Breeding: 118 (5) 369-390.

Haque E, Taniguchi H, Hassan M M, Bhowmik P *et al* (2018) Application of CRISPR/Cas9 Genome Editing Technology for the Improvement of Crops Cultivated in Tropical Climates: Recent Progress, Prospects, and Challenges. Front Plant Sci 9: 617.

Jeffreys AJ, Wilson V and Thein SL (1985) Individual-specific 'fingerprints' of human DNA. Nature 316: 76-79.

Joshi SP, Ranjekar PK and Gupta VS (1999) Molecular markers in plant genome analysis. Curr. Sci. 77:230-240.

Kaur N, Alok A Shivani, Kaur N, Pandey P, Awasthi P *et al* (2018) CRISPR/Cas9-mediated efficient editing in phytoene desaturase (PDS) demonstrates precise manipulation in banana cv. rasthali genome. Funct. Integr. Genomics 18: 89-99.

Konieczny A, Ausubel FM. (1993) A procedure for mapping arabidopsis mutations using co-dominant ecotype-specific PCR-based markers Plant Journal 4: 403-410.

Michaels SD and Amasino RM (1998) A robust method for detecting single-nucleotide changes as polymorphic markers by PCR. Plant J 14: 381-385.

Moose S and Mumm R (2008) Molecular Plant Breeding as the Foundation for 21st Century Crop Improvement. Plant physiology 147: 969-77

Nadeem MA, Nawaz MA *et al* (2018) DNA molecular markers in plant breeding: current status and recent advancements in gemoic selection and genome editing. biotechnology and biotechnological equipment 32 (2): 261-285.

Ortiz Rodomiro (2010) Agricultural Biotechnologies in Developing Countries: Options and Opportunities in Crops, Forestry, Livestock, Fisheries and Agro-Industry to Face the Challenges of Food Insecurity and Climate Change. Background document for the FAO International Technical Conference on Agricultural Biotechnologies in Developing Countries (ABDC-10), March 1-4, 2010. Guadalajara, Mexico.

Sharma HC, Crouch JH, Sharma KK, Seetharama N and Hash CT (2002) Applications of biotechnology for crop improvement: prospects and constraints. Plant Science 163 (3): 381-395

Stein A and Emilio RC (2010) International Trade and the Global Pipeline of New GM Crops. Nature Biotechnology 28: 23-25.

Tautz D and Renz M (1984) Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res 12: 4127-4138.

Varshney RK, Bansal KC, Aggarwal PK, Datta SK and Craufurd PQ (2011) Agricultural biotechnology for crop improvement in a variable climate: hope or hype? Trends in plant science 16 (7): 363-371

Venkat, Vidya (2016, July 8) All you need to know about the GM food controversy. The Hindu

Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV (1990) DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res 18: 6531-6535

Wiltshire T, Pletcher M T, Batalov S, Barnes S W, Tarantino L M (2003) Genome-wide single-nucleotide polymorphism analysis defines haplotype patterns in mouse. Proc Natl Acad Sci 100(6): 3380-3385.

Welsh J, McClelland M (1990) Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Res 18: 7213-7218.

Watson A, Ghosh S, Williams MJ, Cuddy WS, Simmonds J, Rey MD *et al* (2018) Speed breeding is a powerful tool to accelerate crop research and breeding. Nat. Plants 4: 23-29

Wang ZP, Xing HL, Dong L, Zhang HY, Han CY, Wang XC, *et al* (2015) Egg cell-specific promoter-controlled CRISPR/Cas9 efficiently generates homozygous mutants for multiple target genes in Arabidopsis in a single generation. Genome Biol. 16: 144

Yan L, Wei S, Wu Y, Hu R, Li H, Yang W *et al* (2015) High-Efficiency genome editing in Arabidopsis using YAO promoter-driven CRISPR/Cas9 system. Mol Plant 8: 1820-1823.

Ye X, Al-Babili S, Klöti A, Zhang J, Lucca P, Beyer P and Potrykus, I (2000) Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science 287:303-305.