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Biotechnology: An Advanced Tool for Crop Improvement

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Authors' contributions

This work was carried out in collaboration between all authors. Authors TA and ST wrote the first draft of the manuscript. Author ST checked and corrected final manuscript. Author NG managed the literature search and references. In joint efforts all authors MKT, PB and VSK structured the final version of the manuscript. All authors read and approved the final manuscript.

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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.

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1. 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 [1,2] and for biotic and abiotic stress resistant variety development [3]. Moose and Mumm [4] 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. [5] 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. [6] 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. Figs. 1 and 2, clearly indicating different approaches of biotechnology which are being applied in plant breeding practices of crop improvement.

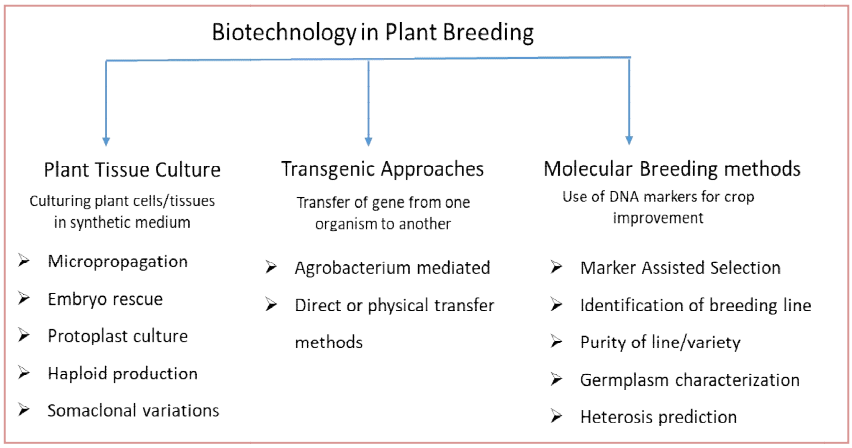

Fig. 1. Different approaches of crop improvement using biotechnological tools

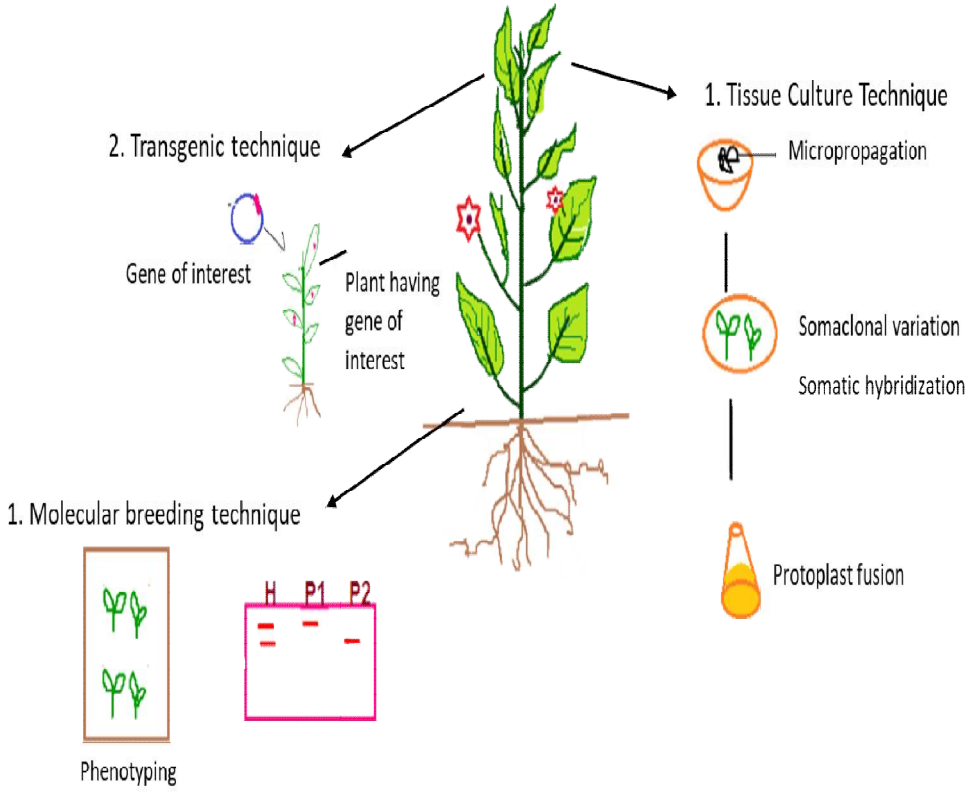

Fig. 2. Applications of biotechnology in plant breeding practices

2. 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 [7]. 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 [8]. Transgenic approach can be a valid alternative for the development of biofortified crops (nutritionally enhanced food crop) when there is a limited or no genetic variation in nutrient content among plant varieties [9]. Among micronutrients, minerals, vitamins, several essential amino acids, and desirable fatty acids have been targeted by genes from different sources to enhance

nutritional level of food crops. Some of the ssuccessful examples of transgenic food crops are maize (high lysine), soybean (high unsaturated fatty acid), cassava (high provitamin A and iron rich), and Golden rice (high provitamin A). Biofortified cereals, legumes, vegetables, fruits, oilseeds, and fodder crops have also been reported. Molecular breeding approaches are most efficient in enhancing the biotic and abiotic stress adaptation of crop plants, and recent advances in high throughput genotyping, sequencing and phenotyping platforms (phenomics) have transformed molecular breeding to genomics assisted breeding (GAB). Most commonly used approaches for genomics assisted breeding are marker assisted selection (MAS) and genomic selection (GS). MAS, includes marker assisted backcrossing, gene pyramiding, mapping for associated targeted traits by specific genes or QTLs, fine mapping of QTL region etc. GS, on the other hand, uses all available marker data for a population to predict breeding value. The development of improved breeding lines for commercial crop cultivation by traditional methods is time consuming and expensive task. With the deployment of genomics assisted breeding, the generation of improved breeding lines has become easier and faster.

3. 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.

3.1 Micropropagation

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.

3.2 Somaclonal Variation

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.

3.3 Haploids Production

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

4. TRANSGENIC APPROACHES

Biotechnology is well known branch of science which deal with the use of living organisms and bioprocess to make or modify a product, to get improve plants or animals or develop new products for specific uses. Biotechnology is widely being used for generation of genetically modified (GM) crops, where one or several genes coding for desirable traits have been inserted through the process of genetic engineering (GE). The gene to develop transgene is originate from the same or other species and organisms unrelated to the recipient organism. 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 [10]. In fact, transgenesis has emerged as an additional tool to carry out single-gene breeding or transgenic breeding of crops. Two methods are being used to transfer foreign genes into plants. The first method involves the use of a plant pathogen called *Agrobacterium tumefaciens*, soil-borne, Gram-negative bacterium which causes crown gall disease in many species. This bacterium has a plasmid that contains tumor-inducing genes (T-DNA), along with additional genes that help the T-DNA integrate into the host genome. This is done by removing most of the T-DNA while leaving the left and right border sequences (24 bp), which integrate a foreign gene into the genome of cultured plant cells. The second delivery method is a "gene gun," which bombard gold particles carrying the foreign DNA into plant cells. Some of these particles pass through the plant cell wall and enter the cell nucleus, where the transgene integrates itself into the plant chromosome. 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. First generation transgenic crops were insect resistant (IR) maize, cotton, canola and herbicide tolerant (HT) soybean, cotton, maize, sugar beet, alfalfa plants, expressing bacterial genes CRY and CP4 EPSPS, respectively. These crops were later joined by those with a combination of the two traits in the same plant (IR/HT), known as stacked IR/HT cotton and maize. In addition to this, coat protein mediated resistance (CPMR) has been successfully applied to generate virus resistant fruits and vegetables such as papaya, plum and squash. Recently, the United States Department of Agriculture allowed Monsanto Company to sell drought tolerant maize MON 87460. Other than that, genetically engineered tomato and sweet pepper have been developed for longer shelf life that prevent them from rotting and degrading. The crops have since been released for commercial production by farmers in the USA and China. Introduction of provitamin A and β carotene genes have resulted in the production of golden rice [11,12,13]. To add value to agri-foods vitamin producing transgenic plants have also been developed and emphasis is being laid on multigene engineering.

5. 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 (Fig. 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 [14]. Since Botstein et al. [15] 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) [16,17]. 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. The markers have made selection independent of the phenotype, the desirable plants can be selected in the seedling stage, and the selected plants can be used for hybridization in the same season. In addition, MAS allows easy pyramiding of oligogenic resistance and combining of horizontal resistance with vertical resistance which is considerably difficult on the basis of disease tests. Closely linked molecular markers have been used for positional cloning of a number of plant genes. Molecular markers have stimulated the development of novel breeding schemes like Genomic selection (GS) and Genomics Assisted Breeding (GAB) Scheme to develop varieties with superior adaptation.

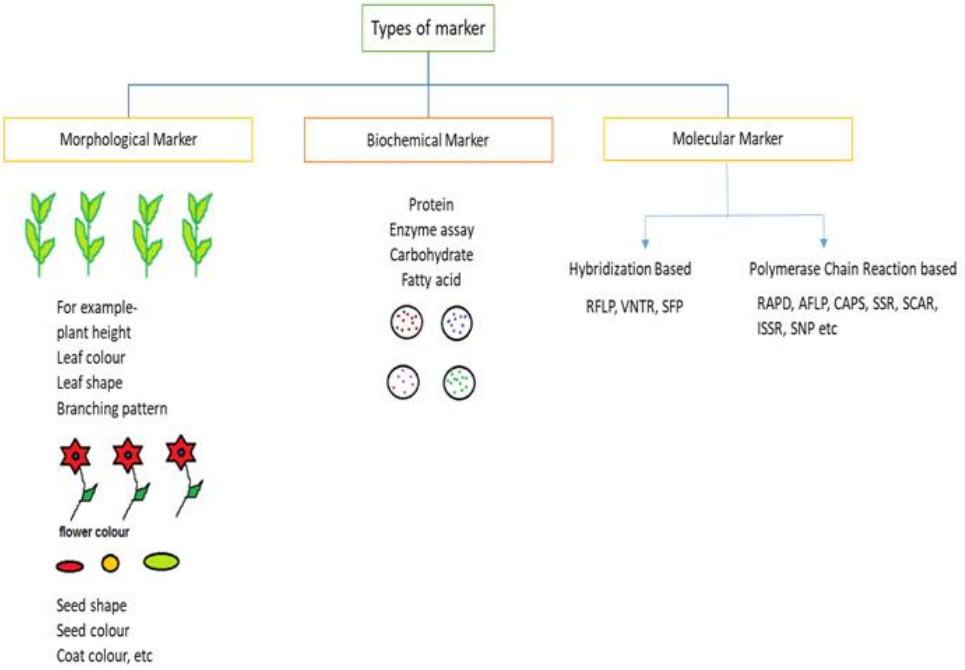

Fig. 3. Different types of molecular markers

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
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
References	Botstein et al. [15]	Willians et al., Welsh et al. [18, 19]	Tautz et al. [20]	Konieczny et al. [21]	Gupta et al. , Godwin et al. [22, 23]	Adams et al., [24, 25]	Michaels et al. Batley et al. Wiltshire et al. [26, 27, 28]

6. GENOMICS ASSISTED BREEDING AND HIGH THROUGHPUT GENO-TYPING

Functional molecular markers and advances in bioinformatics is generating new tools gradually in genomics research that could increase the efficiency and precision of crop improvement. Eventually, relative values of targeted alleles at specific locus in a segregating population could allow the breeder to improve any genotype for particular trait *in silico* and to do whole genome selection. Genomics is most powerful tool for deciphering the stress responsiveness of crop species with adaptation traits or to identify underlying genes, alleles or quantitative trait loci. Molecular breeding approaches are most efficient in enhancing the biotic and abiotic stress adaptation of crop plants, and recent advances in high throughput genotyping, sequencing and phenotyping platforms (phenomics) have transformed molecular breeding to genomics assisted breeding (GAB). Most commonly used approaches for genomics assisted breeding are marker assisted selection (MAS) and genomic selection (GS). High throughput genotyping is being applied by using SNP array, which is a relatively cost-efficient, and automatically genotyping assay. It has been widely used in genetic studies of crops, including genome-wide association studies (GWAS), linkage map construction, genomic selection, population structure analysis and gene mapping. Currently, the most popular high throughput genotyping platforms are the hybridization based SNP array and various NGS enabled genotyping such as GBS and GS [29,30]. SNP array is a type of DNA microarray containing designed probes focusing on the SNP positions, which is hybridized with fragmented DNA or cDNA to determine the specific alleles of all SNPs on the array for the hybridized DNA sample of targeted trait. Many SNP arrays have been successfully applied in diploid species genotyping, such as the Apple 480K SNP array, the Maize 600K SNP array, 58K in tetraploids (peanut), 820K in hexaploids (wheat), 90K in octoploids (strawberry), 345K in dodecaploids (sugarcane) and the Rice 700K SNP array). For high-throughput genotyping of crops, SNP array has several advantages over traditional marker based genotyping and NGS approaches. Some of the points includes, SNP array data is relatively easy to analyze compared to data generated using NGS-based methods, labor intensive NGS library preparation and bioinformatics data analysis investment for accurate SNP calling. However, SNP array has its own shortcomings, it required prior genomic information, and in some case manual dosage scoring. Efforts have been taken to reduce these limitations such as adopting whole genome sequencing with high coverage and updating the markers on the SNP array. For SNP marker selection in development of the array, Illumina and Affymetrix platform are being applied widely. SNP Array selection should include some of the general and important features as SNP depth, SNP types, SNP frequency, additional variations within probe sequence of target SNPs, and probe sequence parameters. Specifically, (1) accuracy of SNPs called can be relate with the average SNP read depth, or single genotype SNP depth. If the depth is too low, sequence errors could be considered for SNP call. If the depth is too high, the SNPs may be called from repetitive sequences. (2) There are two types of SNPs: transition SNPs (purine/purine or pyramidine/pyramidine i.e., A/G, T/C) and transversion SNPs (purine/pyramidine or pyramidine/purine i.e., A/T, C/G, A/C, and T/G). For SNP array development, the transition SNP type is preferred and transversion SNPs, multiple allelic SNPs and INDELs, are excluded to make array more reproducible [31]. The availability of high-density SNP markers has opened a way for genome wide association study (GWAS), an approach using natural populations. GWAS could overcome several constraints of conventional linkage mapping and provide a powerful complementary strategy for dissecting complex traits. Genomic selection (GS) predicts genomic estimated breeding values of lines by analyzing traits and high-density marker scores within an artificially created population at the whole-genome level [6]. GS is another promising breeding strategy for rapid improvement of complex traits. Although still costly, GS has been proved to be superior to marker assisted recurrent selection for improving complex traits in crops, as it can effectively avoid issues associated with the number of QTL that control a trait.

7. 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. [32] 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 [33,34,35,36] 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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