Review Article

Applied genetics and plant breeding in 21st century for crop improvement – status and prospects

ABSTRACT

The foundation for molecular plant breeding is an interdisciplinary science that has revolutionized the 21st century with incorporation of genomic research, biotechnology and molecular marker applications with conventional plant breeding practices for crop improvement. To meet the food demand of the ever-increasing population of the 21st century, conventional breeding methods like ideotype breeding, participatory plant breeding with combination of recent breeding approaches like Genetically Modified crops, Marker Assisted Selection, Next Generation Sequencing and precise genotyping tools with sufficient bioinformatics knowledge play a demanding role. Co-operation among farmers, plant breeders, researchers and other plant scientists should exist to develop quality crops with increased nutritional value. The challenge to feed the ever-increasing population can be met by integrating different research disciplines and activities that form the core of molecular plant breeding. Success of genomics depends on the ability to improve crop phenotypes and reduce the phenotype gap which will be a major concern of the 21st century.

KEY WORDS: *Ideotype breeding, Genetically modified crops, Marker-assisted selection, Marker-assisted molecular breeding.*

ABBREVIATION

Genetically modified- GM, Marker Assisted Selection- MAS, Marker-assisted backcrossing - MABC, Marker-assisted recurrent selection- MARS, Donor Parent- DP, Backcross- BC, Improved Recurrent Parent- IRP, Recurrent Parent- RP, Next generation sequencing –NGS, Targeting induced local lesions in genomes –TILLING, plant genetic resources for food and agriculture- PGRFA, Food and Agriculture Organization- FAO, Participatory Plant Breeding –PPB, Genetically modified organisms –GMOs, Quantitative trait loci – QTL, Genomic selection –GS, Targeting Induced Local Lesions in Genomes - TILLING , International Maize and Wheat Improvement Center- CIMMYT.

1. INTRODUCTION

In the first three decades of the 1900s, principles of theoretical genetics was incorporated into tangible and widely accepted applied plant breeding outcomes which led to agriculturally transformative breeding science. Molecular markers were applied as early as the 1990s for selection of better lines in plant breeding while genomics-assisted breeding was advocated to enhance precision and efficiency in crop improvement. The next decade of plant improvement aims at exploiting genome editing to help scientists to apply their knowledge and skills for crop improvement [1]. Improvement in the production of yield of crops may take more time through conventional breeding approaches like ideotype breeding and Participatory Plant Breeding (PPB) [2]. Therefore, molecular markers have extensively been used for tagging and mapping of genes and Quantitative Trait Loci (QTLs) conferring resistance to biotic and abiotic stresses and also for fingerprinting, screening of germplasm and marker-assisted breeding in crop systems.

2. CONVENTIONAL BREEDING APPROACHES

Conventional breeding approaches like pre breeding, pure line selection, mass selection are used to exploit the variation which already exists in the breeding material. Hybridization methods like pedigree, bulk, backcross, single seed decent method are used to generate recombinants. Distant hybridization methods play a great role in producing novel combination by hybridization with land races which is crucial for biofortification. Back cross breeding and wild hybridization have a significant role for developing biotic and abiotic stress resistance cultivars. Mutation breeding is the ultimate source for generating genetic variation by using mutagens. Ideal plant types are produced through ideotype breeding. Participatory Plant Breeding (PPB) is the involvement of the growers and other investors such as vendors, extensionists, consumers, industry and rural cooperatives for crop improvement to develop new varieties [2].

Population improvement also has great importance to generate an improved population by repeated cycle of selfing and intercrossing of the base population. Generation of new genetic combinations by sexual hybridization and the successive application of selection procedures on phenotypically assessed individuals and populations are a key source for conventional plant breeding. The basics of conventional breeding is based on (a) the wide spectrum of diversity existing in landraces and closely associated species (primary and secondary gene pools) and (b) the improvement of breeding procedures using Mendelian and quantitative genetics methodologies for an effective selection. High yielding varieties, superior hybrids, multiple biotic and abiotic stress resistance varieties with improved nutritional quality varieties is possible through conventional breeding. Suitable biometrical methods are used to analyse genetic diversity, genetic variability, genetic gain, heritability and selection efficiency which provide basis of selection in qualitative and quantitative trait in genotypes. The genetic makeup of plant genetic resources for food and agriculture (PGRFA) provide genetic gain which leads to 70% increase in food production. Illustrations of the histrionic effects of genetic gains on crop yields take account of the progress and considerable distribution of high yielding and resilient cereal crop varieties all over the world in the late 1960s. Researchers are attentive of the inadequacies in the genetic diversity- and hence, risk of vulnerabilities of crops. Wild lineages of crops, indigenous races and other non-adapted genetic resources, even if typically low yielding and concealing undesirable traits, should be used more regularly in genetic enhancement as means to fulfil these deficiencies [3]. A set of multi-location test is conducted to enhance the improved lines before a variety could be identified for cultivation by the farmers. Conventional breeding takes five to six generations for transmission of a trait within a species into the high yielding landraces and requires a large number of offspring to select plants having a suitable combination of characters (Fig.1). One of the main drawbacks of conventional breeding is lack of effective selection scheme during early segregating generations, highly heritable characters unlike yield only effective for selection, tedious phenotype assessment in some traits.

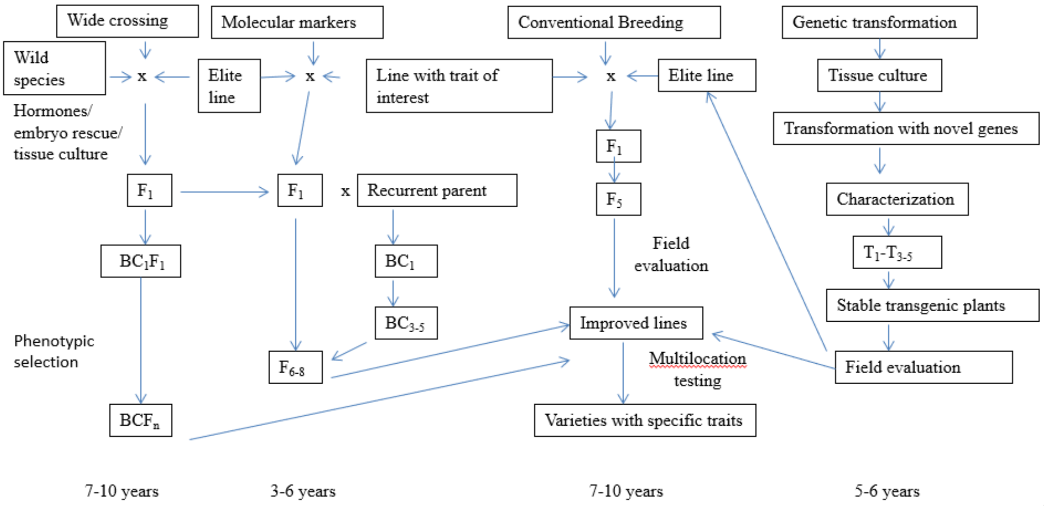

Fig. 1. A schematic outline of biotechnological approaches in crop improvement [4].

3. IDEOTYPE MANIPULATION

Varieties adapted to organic farming systems are required for better yield and quality. Selection for traits such as adaptation to organic soil fertility management requires organic soil conditions. Establishment of breeding programmes for organic farming is hampered by limited area under organic agriculture. The organic crop ideotypes benefit not only organic farming systems, but also conventional systems in the long run [5]. Traditional breeding focuses on yield per se whereas ideotype breeding focuses on several traits to produce a model plant suitable for various agro ecological conditions [6].

Introduction of genes from elite *indica* parents to first-generation New Plant Type (NPT) lines in rice led to improvement of yield. Yield improvement occurred as a result of increase in panicle number per square meter and improved grain-filling percentage. First generation NPT lines had poor yield due to several factors such as low harvest index due to small sink size, poor translocation of biomass and low grain-filling percentage [7].

Tropical *japonica* was crossed with *indica* to develop second generation NPT which resulted in increase in yield potential, resistance to disease and insect and improvement in grain quality [8]. Several F_1 hybrid varieties were developed by China's ‘‘super’’ rice breeding project using a combination of the ideotype approach and intersubspecific heterosism [9].

High-yielding wheat ideotypes were designed to help breeders to identify traits for yield improvements under climate change conditions at two different sites in Europe. It was observed through simulation studies that yield potential of wheat can be increased substantially for new ideotypes than the existing varieties under climate change. Improvement in light conversion efficiency, optimal phenology and increased grain filling duration resulting in a higher harvest index were identified as factors that contributed to increase in yield [10].

A crop ideotype is necessary to select traits for improvement in organic systems. An ideotype includes plant morphology or physiology which may have various benefits. Ideotype should be selected under optimum conditions and trials should be carried out in organic systems for better evaluation of the importance of different traits [11]. The goal for organic breeding should include both grain yield and environmentally safe production while maintaining production cost and quality. Ideotype suitable for organic wheat-cropping systems should have fast early growth, lodging resistance, resistance to pests and diseases and adaptation to local environmental conditions [12].

4. GM CROPS

Recombinant DNA technology, including the use of molecules comprising DNA sequences obtained from several sources to generate innovative genetic variation, has become a potential crop improvement alternative. This is called genetic modification (or transformation) with the novel variants denoted as transgenic or genetically modified organisms (GMOs). The methods involve the integration of exogenous DNA or ribonucleic acid (RNA) sequences, using biolistics or vectors into the genome of the recipient organism. Development and deployment of transgenic plants in an effective pattern is a vital need for sustainable and commercialization of biotechnology for crop improvement. Genetic transformation affords the right to use of genes from elite species, which are capable of producing genetically modified crops, alter the level of gene expression and modify the spatial and temporal form of gene expression. The genes of concern can be incorporated into the target crops in a single occurrence while it takes five to six years to generate cultivars with stable gene expression (Fig.1) [4]. It provides a chance for crop enhancements in the 21st century through the following landmark achievements.

4.1 GM CROP IN INCREASED CROP YIELD

Improvement of quality and chemical composition in plants through advance Genetic Engineering lead to higher agricultural productivity, minimal usage of farm acreage and resource and reduced environmental hazardous. Higher production of GM crops will have a positive global impact by improving the dietary staples (such as cereals, legumes, tubers, vegetables and fruits) of many regions of the world [13].

4.2. GM CROP IN ABIOTIC STRESS RESISTANCE

Through the action of ABF3 gene, an increase in drought tolerance in Arabidopsis was observed Transgenic rice was developed through Mt1D gene for the synthesis of mannitol for salt tolerance [14].

4.3. GM CROP IN NUTRITIONAL FACTORS

Several quality characters (carbohydrates, proteins, fatty acids, vitamins, iron and amino acids content) can be aimed to enhance the nutritional status of crop production. GM rice popularly known as golden rice produces beta-carotene to overcome deficiency of vitamin A [15].

4.4. GM TECHNOLOGY IN GENE STACKING

Multigene transfer (MGT) is a gene stacking approach that enables import of entire metabolic pathways, expression of entire protein complexes, development of transgenic crops in order to produce a wide spectrum of added-value compounds [16].

5. MARKER ASSISTED SELECTION (MAS) SCHEMES IN PLANT BREEDING

Marker Assisted Selection involves selection of plants carrying genomic regions involved in expression of traits using molecular markers [17]. Markers may be morphological, which is used to distinguish qualities visually. Cytological markers are associated with variations present in the chromosome numbers, shape, size, banding patterns, position and order. Biochemical markers are used to estimate gene and genotypic frequencies. Molecular markers are nucleotide sequences which are identified by the polymorphism present between the nucleotide sequences which may be insertion, deletion, duplication, translocation and point mutations [18]. Molecular markers are time saving, stable and reliable with high performance enabling precise selection of complex traits [19]. An ideal molecular marker should be highly polymorphic, co dominant, highly reproducible, abundant, easily available, and should be selectively neutral with easy and fast assay and exchange of data between laboratories [20].

5.1 Early generation selection:

Marker-assisted selection is used in early generation selections to eliminate undesirable gene combinations as molecular markers can be utilized at any stage of a crop. Early generation selection through MAS not only selects suitable gene combinations but also ensures a high probability of retaining superior breeding lines. Large populations and low heritability of the selected traits is necessary for successful early-generation selection with marker-assisted [21]. Early generation selection not only decreases the amount of material to be handled in the segregating generations but also helps to retain the desired recombinant lines. Effectiveness of early generation selection is affected by genotype and environmental interaction. Early generation selection in rice for grain yield should be done at several sites simultaneously during early growth stage [22].

Early generation selection data in 200 progenies in F_3 generation in rice was analysed to estimate selection gain and selection intensity and crosses with highest and lowest selection intensity was identified. When comparing selection intensity in between F_2 and F_3, generations it was increased for all crosses studied in the experiment, indicating that the selections were progressed towards yield improvement [23]. Two segregating populations of rice were used in each of F_3, F_4 and F_5 generations to study the response to selection for high and low grain yields and yield components, estimation of heritabilities and to determine correlation of grain yield with yield components. It was observed that selections in early generations for grain weight and number of grains per panicle were effective. Positive and significant associations were observed between grain yield and number of grains per panicle in the studied populations which could be used as selection criteria in early generations [24].

5.2 MARKER-ASSISTED BACKCROSSING (MABC):

Backcrossing is used to transfer favourable traits from a donor parent into an elite genotype (recurrent parent). The F_1 hybrid is then backcrossed to the recurrent parent and backcrossing is done until most of the genes stemming from the donor are eliminated. Markers can be used in MABC in foreground selection to control the target gene or in background selection to accelerate the reconstruction of the recurrent parent genotype. It can also be used in recombinant selection to select backcross progeny having the target gene with tightly-linked flanking markers to minimize linkage drag. MABC is useful when a single allele is to be transferred into a different genetic background, for example to improve an existing agronomically superior variety for a specific trait [21]. β-carotene level in maize kernel was enhanced in elite maize genotypes by marker-assisted backcross breeding by introgression of a favourable allele (543 bp) of the β-carotene hydroxylase (crtRB1) gene in seven elite inbred parents having low β –carotene. An increase in β-carotene was observed among the crtRB1-introgressed inbreds over the recurrent parent. Increase in β-carotene level was also observed in the reconstituted hybrids in comparison to the original hybrid [25].

5.3 MARKER-ASSISTED RECURRENT SELECTION (MARS):

MARS helps to integrate multiple favourable genes or QTLs from different sources by recurrent selection. Use of markers accelerates recurrent selection and therefore several selection-cycles can be done within one year [21]. Marker Assisted Recurrent Selection (MARS) was used to estimate genetic gains in grain yield in ten biparental tropical maize populations. Study on genetic gain was carried out in test crosses of lines from the different cycles of F_1 and founder parents under water-stressed and well-watered conditions. An increase in grain yield using MARS across the 10 populations was observed under water-stressed and well-watered conditions. Drought tolerance and grain yield was improved in a biparental cross of two elite drought tolerant lines in maize through Marker Assisted Recurrent Selection (MARS). It was observed that out of the three selection cycles (C_0, C_1, C_2) of the MARS population, significantly higher grain field was produced under drought stress condition in C_2 than C_0. Frequency of favourable marker alleles increased whereas the effective number of alleles (Ne) per locus decreased in C_2 [26].

5.4 COMBINED MARKER-ASSISTED SELECTION:

The combination of MAS and phenotypic screening is known as combined marker-assisted selection. This scheme can be followed when a large number of QTLs need to be manipulated or additional QTLs controlling a trait remain unidentified [21].

6. MARKER ASSISTED GENE PYRAMIDING:

Crop production is affected by biotic stresses leading to yield loss. Use of chemicals to control bacteria, fungi, nematodes, insect pests and weeds has several constraints like multiple application, high cost and adverse effects on human health and the environment. It may also lead to the development of chemical-resistant pathogen races, insects and weeds. It is therefore necessary to develop cultivars by assembling genes from different parents into a single genotype so that it can provide broad spectrum resistance against different races of pathogens or pests or combination of stresses. This phenomenon is known as gene pyramiding [27].

Gene pyramiding can be done by conventional methods or by molecular technique using marker assisted selection or transgenic method. Marker assisted backcross based gene pyramiding can be performed in three ways.

6.1 STEPWISE TRANSFER: In this method, the recurrent parent (RP1) is crossed with the donor parent (DP1). The F_1 hybrid produced is backcrossed up to third backcross generation (BC3). The improved recurrent parent (IRP1) produced is then crossed with other donor parent (DP2) to pyramid multiple genes. This strategy is time taking but very precise as it involves one gene at a time.

6.2 SIMULTANEOUS TRANSFER: In this method, the recurrent parent (RP1) is crossed with donor parents (DP1, DP2, etc.). The F_1 hybrids are then intercrossed to produce improved F_1 (IF1) which is then backcrossed with the recurrent parent to get the improved recurrent parent (IRP).

6.3 STEPWISE AND SIMULTANEOUS TRANSFER: The third method is a combination of first two and involves simultaneous crossing of the recurrent parent (RP1) with many donor parents followed by backcrossing them up to the BC_3 generation. The backcross populations are then intercrossed with each other to get the pyramided lines. This method requires less time and fixation of genes is fully assured [28]. Gene pyramiding has been carried out in several crops (Table 1).

Table 1: Pyramided genes and their traits of crop plants.

Sl. No.	**Crops**	**Traits**	**Pyramided Genes**	**Reference**
1.	Rice	Bacterial leaf blight resistance	Xa4, xa5, xa13 and Xa21	[29]
			Xa 5, Xa13 and Xa21	
		Blast resistance	Pi46 and Pi2	[30]
		Gall-midge resistance	Gm2,Gm6t	[31]
2.	Wheat	Leaf rust resistance	Lr24, Lr28 and Lr9	32]
		Powdery mildew resistance	Pm2+Pm4a, Pm2+Pm21, Pm4a+Pm21	[33]
		Cereal cyst nematode	CreX and CreY	[34]
3.	Sunflower	Rust resistance	R4, R5, R13b, R15, R12	[35]
4.	Pea	Nodulation ability	Sym9, Sym10	[36]
5.	Soybean	Soybean mosaic virus resistance	RSC4,RSC8 and RSC14	[37]
		Lepidopteran resistance	cry1Ac +corn ear worm QTL	[38]
6.	Chickpea	Lepidopteran resistance	cry1Ac + cry1Ab	[39]

Genes *Pi46* and *Pita* for resistance against blast disease of rice were introgressed from a resistant accession H4 into an elite restorer line Hang-Hui-179 (HH179) by marker-assisted backcross breeding (MABB). Three improved lines were developed carrying *Pi46* and *Pita* alone and in combination which had significant genetic similarities with the recurrent parent. The resistance spectrum of the improved lines was found to be higher than HH179 which was tested at seedling stage but shared similar agronomic traits such as tillers per plant, plant height, spikelet fertility, panicle length and 1000-grain weight [40].

Four QTLs for blast resistance in rice located on chromosomes 1, 2, 11 and 12 were pyramided in cultivar RD6 and selection was carried out in $BC_2F_{2:3}$ lines. It was observed that RD6 introgression lines which had a higher number of QTLs for blast resistance exhibited higher levels of blast resistance [41].

7. RECENT TREND IN MARKER ASSISTED BREEDING

Development in genomics, proteomics, metabolomics and bioinformatics facilitates storage and analysis of the huge volume of data generated through sequencing approaches. Molecular markers have extensively been used for tagging and mapping of genes and Quantitative trait loci (QTLs) conferring resistance to biotic and abiotic stresses and also for fingerprinting, screening of germplasm and marker-assisted breeding in crop systems. Single Nucleotide Polymorphisms is a potent tool for several genetic applications, including linkage mapping, quality control (QC) analysis, germplasm characterization, allele mining, linkage-based and linkage disequilibrium based quantitative trait loci (QTL) mapping, marker-assisted backcrossing (MABC), marker- assisted recurrent selection (MARS), and genomic selection (GS) [42].

In case of advances in genomics for plant breeding, a huge contribution comes from MAS, which depend on the linkage of the marker and gene or QTL of interest [43]. Individual or multiple genes or QTLs introgression is possible through Marker Assisted Back Cross breeding, gene pyramiding and gene staking with the help of available trait associated markers spread throughout the genome. Speedy transfer of major genes and QTLs into existing high-yielding varieties is possible due to Marker-assisted backcrossing (MABC) and QTL pyramiding (Fig.2).

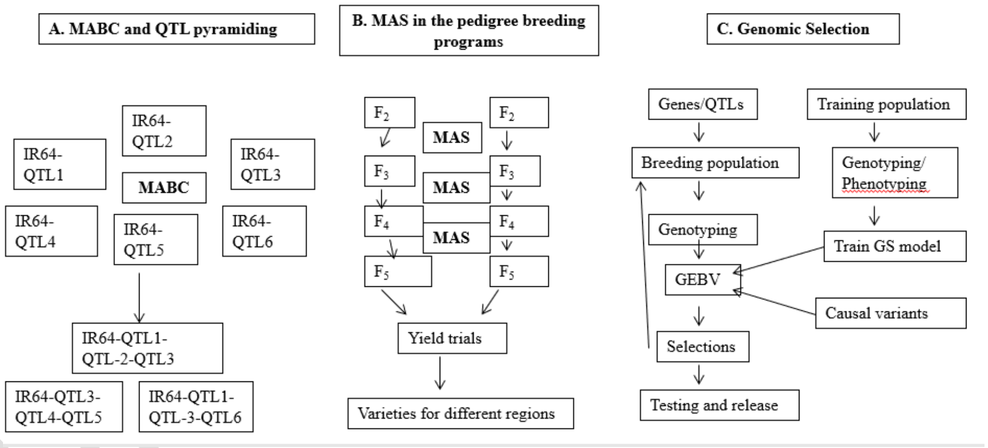

Fig.2 Breeding schemes for integrating SNP genotyping into molecular breeding programmes [44].

Next Generation Sequencing projects overcome the possibility of recombination between the functional gene or QTL and the marker by producing large collections of markers, including intra-genic (functional) markers. NGS has several important areas of applications (Fig.2) such as association mapping, large-scale development of molecular markers for linkage mapping [45], alien introgression and wide crosses [46], epigenetic modifications [47] population genetics, transcript profiling and de novo genome/organellar genome assembly [48] leading to crop enhancement [49].

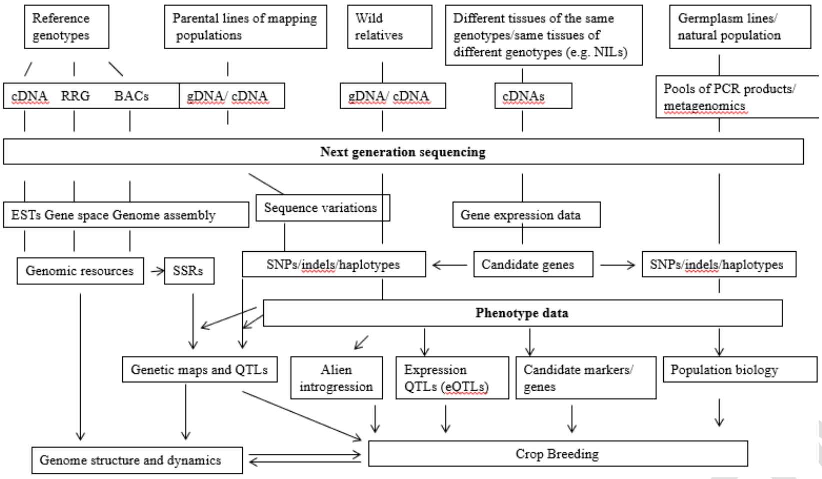

Fig.3 Overview of NGS applications in crop genetics and breeding [49].

Use of candidate gene association and genome-wide association (GWA) generates large number of recombination events in the germplasm collection giving a better resolution of the genetic maps [50]. Detection of novel alleles to understand the function of gene of interest is done using genomic approaches either by direct sequencing and comparison with reference sequences [49] TILLING (Targeting Induced Local Lesions in Genomes). TILLING recognizes mutation events in pre-programmed genome regions which holds great potential and uses induced mutations to widen the genetic base of crops from TILLING or ecotype TILLING (EcoTILLING) methodologies [51][52]. The concerned phenotypic data for which inferences are made must be accurate through the use of molecular breeding [53].

Genomic selection (GS) a new platform of MAS, relies on the simultaneous estimation of effects on the phenotype of all available loci, haplotypes and markers without previous identification of markers [54]. GS requires availability of phenotypic and genotypic data and the establishment of a genetic model [55].

Microarray technology provides immediate expression and analysis of large numbers of genes that will reveal gene function and different interactions among the genes that leads to development of different phenotypes under several environmental conditions [56]. To tackle the problems in fine-mapping and/or validating minor QTL, Marker Assisted Recurrent Selection and genome-wide (genomic) selection [57] have been suggested as methods for gathering desirable alleles from several QTL for highly polygenic characters.

Genomic estimated breeding values (GEBVs) is the key parameter to Genome-wide selection approaches having higher gains of selection through the use of high-density genome wide data instead of being limited to a few known loci [54]. The use of elite genotypes or natural populations in association mapping can be improved for higher resolution to identify genes responsible for difference in a quantitative trait [58] [59].

8. RECENT ADVANCES IN GENOTYPING-

Genotyping is the method of estimating variation in the genetic make-up of an individual by sequencing the individual's DNA using molecular assays and comparing it to another reference sequence. It indicates the alleles an individual has transmitted from their parents. For genotyping, several molecular marker techniques are used, among them SNPs is the most powerful, high throughput, most efficient genotyping technology as it refers to a single base change in a DNA sequence [60]. Due to its low assay cost, potential for high-throughput analysis, locus specificity, co-dominant inheritance, high genomic abundance, simple documentation and relatively low genotyping error rates [42], SNPs have appeared as potent tools for several genetic applications including linkage mapping, quality control (QC) analysis, germplasm characterization, allele mining, linkage-based and linkage disequilibrium based quantitative trait loci (QTL) mapping, marker-assisted backcrossing (MABC), marker- assisted recurrent selection (MARS) and genomic selection (GS).

SNPs have successful conversion rates (the ability to design an assay) varying from 50 to 97 % on the basis of transfer from one platform to another [61] [62] [63]. CIMMYT (International Maize and Wheat Improvement Center) uses KASP for QC analysis, QTL mapping, allele mining and MARS applications that need SNP data extending from a few to several hundred data per sample and also rely on other platforms, such as genotyping-by-sequencing [64] and Maize SNP50 Bead Chip from Illumina, for genome-wide association mapping [65].

9. BREEDING FOR ORGANIC SITUATION

Breeding objectives of organic agriculture include yield, disease resistance and weed competitiveness. Yield has lesser priority in organic breeding compared to conventional agriculture whereas weed competitiveness is important in organic breeding but is not a priority in conventional agriculture. Techniques such as genetically modified (GM) crop varieties, Cytoplasmic Male Sterility hybridization with restorer gene, protoplast fusion etc are not used [66]. Hybrids, marker assisted selection, meristem culture can be used in organic breeding programmes [67].

Propagation of the best suitable existing varieties organically by integrating organic traits helps in improvement of organic seed production in breeding programmes in the long run. Organic propagation of the most suitable, existing varieties is the first step in plant breeding for organic farming systems. Conventionally produced but (post-harvest) chemically untreated seed is used in organic farming due to the lack of organically produced seeds. It is therefore necessary to develop efficient schemes for producing adequate quantities of organically produced seeds and planting materials [68].

Organic farming is a sustainable agricultural model that involves biological pest management, organic fertilizers, green manures and crop rotations and avoids the use of chemical pesticides and herbicides. An important prerequisite for organic farming is to select varieties adapted to organic system which can be done by breeding programs based on direct selection within organic systems. A major bottleneck to develop varieties suited to organic farming is the limited number of public and private plant breeding programs addressing the specific needs of organic agriculture. The increase in damage due to pests and severity of diseases due to climate change poses an additional challenge to organic agriculture, affecting both crop yield and quality. Therefore, it is necessary to develop varieties with tolerance to both abiotic and biotic stress [69].

Organically produced food is high in nutrients and free from harmful chemical pesticides, fungicides and herbicides. The reduction in the use of chemicals in organic farming decreases the cost of production. Organic farming maintains soil fertility due to exclusion of chemical fertilizers and incorporation of crop rotation, inter-cropping techniques and use of green manure. Organic farming being labour intensive provides a source of employment. However, the productivity of organic farming is less compared to conventional farming. It requires time for an area to be certified as organic. Organic farming is labour and time intensive [70]. Organic agriculture differs from conventional farming in the techniques and approaches used by the growers. Organic farming maintains biological diversity and increase the population of natural enemies by exclusion of broad-spectrum pesticides [71].

10. CONCLUSION

Integration of conventional breeding with genetic engineering and marker assisted molecular breeding with precise genotyping platform will help in improving the nutritional status of food. Multiple resistance genes can be transferred from wild varieties to extant varieties through gene pyramiding and gene staking to minimise the cost of production and improvement of elite varieties. Standardization of molecular techniques with proper phenotyping and high throughput genotyping and sequencing may fulfill the food demand of the 21st century to some extent. With the increased awareness and focus on health, people prefer organic products and so there is need to focus on this area to develop organically enriched crop varieties.

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