J. Plant Biochemistry & Biotechnology Vol. 18(2), 127-138, July 2009

Review Article

Novel Genomic Tools and Modern Genetic and Breeding Approaches for Crop Improvement

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In recent past, genomic tools especially molecular markers have been extensively used for understanding genome dynamics as well for applied aspects in crop breeding. Several new genomics technologies such as next generation sequencing (NGS), high-throughput marker genotyping, -omics technologies have emerged as powerful tools for understanding genome variation in crop species at DNA, RNA as well as protein level. These technologies promise to provide an insight into the way gene(s) are expressed and regulated in cell and to unveil metabolic pathways involved in trait(s) of interest for breeders not only in model-/major- but even for under-resourced crop species which were once considered "orphan" crops. In parallel, genetic variation for a species present not only in cultivated genepool but even in landraces and wild species can be harnessed by using new genetic approaches such as advanced-backcross QTL (AB-QTL) analysis, introgression libraries (ILs), multi-parent advanced generation intercross (MAGIC) population and association genetics. The gene(s) or genomic regions, responsible for trait(s) of interest, identified either through conventional linkage mapping or above mentioned approaches can be introgressed or pyramided to develop superior genotypes through molecular breeding approaches such as marker-assisted back crossing (MABC), marker assisted recurrent selection (MARS) and genome wide selection (GWS). This article provides an overview on some recent genomic tools and novel genetic and breeding approaches as mentioned above with a final aim of crop improvement.

Key words: genomic tools, genetic approaches, breeding methodologies, crop improvement.

Plant genomes have been subjected to both structural and functional genomics research, which during the last two decades covered both basic and applied aspects. Fast evolution of novel technologies in the recent past has deepened our understanding from genome to gene level and has facilitated understanding about gene networks for plant development and agronomy in many model or major crop species. These technologies included molecular markers, trait mapping, physical mapping, transcriptome/genome sequencing and functional genomics (1). Further, comparative genomics studies especially in the species of the families Poaceae, Brassicaceae and Solanceae showed colinearity in different genomes of corresponding species of a particular family (2-4). It has been suggested and demonstrated that the information gained from one plant species also benefits the improvement of syntenous species. More interestingly, genomics tools and approaches are revolutionizing the breeding methodology, a procedure referred as 'genomics-assisted breeding' (5), through molecular breeding and directed mutagenesis that significantly enhances the efficiency of breeding for improvement of agronomical traits. In addition, genomics accelerates plant biotechnology by providing more native target genes. Many agronomical traits are under control of genes with unknown functions, which can be mapped and cloned based on their position on genetic maps (map-based or positional cloning) (6,7). The cloned genes, containing their own exons, introns and regulatory elements, are good resources for transformation into other varieties of the same crop or into other related crop species without additional modification.

In addition to above mentioned approaches, some novel technologies e.g. next generation sequencing (NGS) and high-throughput marker genotyping technologies have emerged during last five years that are considered to have

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Abbreviations: eQTLs-Express QTLs, NGS-next generation sequencing, ILs-introgression libraries, MAGIC-multi-parent advanced generation intercross, AB-QTL-advanced-backcross QTL, MABC-marker-assisted back crossing, MARS-marker assisted recurrent selection, GWS-genome wide selection, SNP -single nucleotide polymorphism

greater impact on plant genetics research and breeding programmes. Similarly, several modern genetic approaches have been suggested to harness natural variation. Moreover in addition to routinely used marker-assisted selection (MAS), marker-assisted backcrossing (MABC), marker-assisted recurrent selection (MARS) and genome-wide selection (GWS) approaches are becoming popular nowadays. This article provides an overview on some selected genomics technologies and modern genetic and breeding methodologies along with their potential and limitations for crop improvement programmes.

Novel Genomics Technologies

For genomics-assisted breeding it is very important to enhance our ability to broadly interview the nucleic-acid-based information in the cell. In this context, molecular markers have been proven very useful genetic tools for developing the genetic as well as physical maps and trait mapping in several crop species (8). Indeed in several temperate cereal species, these markers have been used in breeding programmes and improved varieties and/or superior lines have been developed. Among different molecular marker technologies available, microsatellite or SSR (simple sequence repeat) markers have been found the markers of choice for breeding applications. In recent years, however, due to advent of next generation sequencing technologies (9) and high-throughput genotyping technologies (10), SNP (single nucleotide polymorphism) markers are expected to phase out the SSR markers in next five years or so.

Next Generation Sequencing (NGS) Technologies

Detection and utilization of genetic variation has been a major task for plant breeders. Though, classical molecular markers such as RFLPs (restriction fragment length polymorphisms), RAPDs (random amplified polymorphic DNA), AFLPs (amplified fragment length polymorphisms) and SSRs have been used extensively for this purpose, the SNP marker system that has capability to detect the variation at single base level, however have not been used in many crop species. One of the major limiting factor in this direction has been the higher costs involved in sequencing the genes/ transcriptomes/ part of genomes of related individuals for SNP discovery. Because of the race in re-sequencing the human genome in US$1000 (11), several companies have developed an array of new generation of sequencing technologies that are popularly referred as next generation sequencing (NGS) technologies (12). These NGS technologies hold great potential to impact plant genetics and breeding in addition to impact human health and microbial biology (9).

Three major sequencing platforms that are currently being used in plant species include Genome sequencer FLX (Roche/454 Life Sciences, http://www.454.com/), Applied Biosystems SOLiD (http://www3.appliedbiosystems.com) and Illumina Genome Analyzer (http://www.illumina.com/). Details about mechanism and chemistry of these platforms have already been discussed in details in several reviews (13, 14). These three platforms provide thousands of million sequence reads in a single run in reduced time and less costs as compared to conventional Sanger sequencing technology (15). Among these three approaches, FLX/454 platform is superior in terms of read length (about 400 bp) but is rather expensive in terms of cost when compared with the Solexa and AB SOLiD (9). Yet another approach based on single molecule synthesis is gaining attention and is termed as 3rd generation sequencing. Apart from this many new sequencing technologies are emerging and/or are at their infant stages to facilitate genome wide marker discovery in both model/major and orphan crop species. A number of laboratories and companies like Biotage, Helicos, Li-Cor, Microchip Biotechnologies, Nanofluidics, Nanogen, Network Biosystems and Visigen are working on development of 3rd generation sequencing platforms (12, 16).

Sequence data generated for parental genotypes of the mapping populations by using NGS technologies can be used for mining the SNPs at large scale. While in case of model plant species or major crop species, it is easier to align the NGS data from individuals to the reference genome sequence data, if available or the transcript sequence data available through EST sequencing projects. In case of under-resourced crop species where appropriate or adequate sequence data are not available, the best possible strategy is to sequence the cDNAs with NGS technologies and then align with the transcript data of the species, if available or of the related major/ model crop species. These approaches have been discussed in a separate review article (10). In summary, it is possible now to mine large scale SNPs in major as well as under-resourced crop species and to undertake molecular breeding (17). In case, these SNPs have been derived from genes or genic regions, the corresponding markers

are also referred as functional markers/FMs (18, 19) or genic molecular markers/GMM (20). NGS technologies have been applied for identification of SNPs in several crops including maize (21) and soybean (22) as well as under resourced crops like chickpea (23) and pigeonpea (unpublished results). Apart from developing SNP markers, NGS technologies can be and are being used for other applications such as *de novo* sequencing, association mapping, alien introgression, transcriptome expression and polymorphism, population genetics, evolutionary biology and genome-wide assembly in several crop species (9).

High-throughput Marker Genotyping Technologies

As NGS technologies can provide a larger number of SNPs, development of high-throughput and cost effective genotyping platforms for these SNPs is yet another important task. Although there are several high-throughput SNP genotyping platforms are available, each of them has its own merits and demerits. For large scale SNP genotyping, in our opinion, following two platforms are in wider use:

Illumina's GoldenGate assay — This assay involves activation of genomic DNA using paramagnetic particles and PCR based amplification of activated DNA using three oligos and a universal PCR primer pair for each SNP. Two of the oligos used are allele specific oligos which on ligation to DNA containing target allele extends and ligates to the third locus specific oligo (LSO) which contains SNP specific tag and sequence complementary to the universal primer. The universal primer carries allele specific fluorescent label and contains an address sequences which helps in binding of the amplified product to the beads of fiber optic array. Data analysis is done using scatter plots. These beads are present in micro-titer plate which facilitates the genotyping in multiple of 96. GoldenGate assays have been developed for several crop species such as barley (24), wheat (25), soybean (22), cowpea (Tim Close, personal commun) and chickpea (Doug Cook, personal commun) *etc.* SNP genotyping based on GoldenGate assay has been found very successful in constructing genetic map, undertaking trait mapping and association mapping (24, 26). Genetics and breeding communities for several other crops such as pigeonpea (Doug Cook, personal commun), peanut (Steve Knapp, personal commun), pea (Judith Burstin, personal coommun), *etc* are in progress of developing the first generation of GoldenGate assays for SNP genotyping. It is anticipated that such GoldenGate assays should be available for majority of crop species in next five years or so. Once these assays are available, they are expected to phase out the SSR genotyping as SNP genotyping, compared to SSR genotyping, is cost effective and faster.

Whole genome genotyping Infinium assay — This assay is based on comparative genomic hybridization. It facilitates measurement of signal intensity variation and changes in allele composition simultaneously. This assay includes whole genome amplification to increase the amount of DNA followed by fragmentation and capturing on to bead array through SNP specific primer. The primer anneals adjacent to SNP and extension takes place which involves incorporation of hapten labeled nucleotide corresponding to SNP allele. Incorporated hapten labeled nucleotides is detected by adding fluorescent labeled antibodies during various steps to amplify the signal. Data analysis of Infinium assay is done through scatter plot. Illumina Inc. has recently developed Infinium HD Human 1M-Duo (two samples/chip) and the Human 610-Quad (four samples/chip) system featuring highest genome coverage, multi sample format, low sample input, powerful cytogenetics, and streamlined assay per bead chip. Although the use of such assay has not been reported in plant systems so far, some crop community such as soybean and maize are in process of developing Infinium assay for undertaking genotyping of circa. 30,000-100,000 SNPs.

It is also important to note that while high-throughput SNP genotyping platforms are suitable for diversity characterization, genome mapping or association genetics, molecular breeding strategies do not essentially need marker genotyping at that large scale that GoldenGate assay or Infinium assays provide. Though the costs per marker datapoint using GoldenGate or Infinium assay will be cheaper, the costs per genotype will be quite expensive that can not be afforded in molecular breeding programmes. For such cases, Illumina has recently launched BeadXpress array system. This is available in 96-plex and 384-plex which is suitable for analyzing high number of sample with low-plex assay having 1- 384 SNPs at very low cost not only per marker (SNP) but per genotype also. It also allows allele specific primer extention (ASPE) for 1-plex to 72-plex. Several crop communities (e.g. cowpea, Tim Close, pers. commun.) are moving towards developing the second generation SNP genotyping platform (BeadXpress system) after selecting the informative SNPs based on GoldenGate assay.

-omics Approaches

A new era of system biology has evolved in the recent past, known as -omics. The term -omics refers to the comprehensive analysis of biological systems and has transformed cell biology study from one gene or protein analysis to understanding of whole organelle and pathway simultaneously. The -omics technologies involve high-throughput measurements of collection of protein in a cell (the proteome), the collection of RNA transcribed from a gene (the trancriptome) or collection of metabolites (the metabolome). In proteomics the protein identification is done in serial fashion and it is an excellent measure for early identification of disease. On the other hand in transcriptomics the gene expression level which has a direct influence on trait is assessed simultaneously.

Transcriptomics — This area of research was greatly facilitated in late 1990s due to the establishment of EST sequencing projects in major plant species (27, 28). Transcript profiling based on micro/macro-arrays provides the candidate genes responsible for different developmental stages and/or agronomically important traits. Identified candidate genes can be used in marker-assisted selection (MAS) after converting them into suitable marker assays or to produce transgenic plants after manipulating them accordingly. However, in general, transcript profiling provides a larger number of genes (in the range of 100s to 1000s) up-/ or down-regulated for the given trait, so it becomes difficult to pinpoint genes involved in the trait. With an objective of identification of 'candidate genes', gene expression analysis has been suggested to combine with genetic or QTL mapping and the procedure has been referred as 'genetical genomics' (29) or expression genetics (5). In this approach, total mRNA or cDNA of the organ/tissue from each individual of a mapping population is hybridized onto a microarray carrying a high number of cDNA fragments representing the species/tissue of interest and quantitative data are recorded reflecting the level of expression of each gene on the filter (30) . Under the presumption, that every gene showing transcriptional regulation is mapped within the genome of the species of interest, the expression data can be subjected to QTL analysis, thus making it possible to identify the so-called 'ExpressQTLs' (eQTLs). Based on segregating populations, eQTL analysis identifies gene products influencing the quantitative trait (level of mRNA expression) in *cis* (mapping of the regulatled gene within the QTL) or *trans* (the gene is located outside the QTL). The latter gene product (second order effect) is of specific interest because more than one QTL can be connected to such a *trans*-acting factor (genes acting on the transcription of other genes) (31). The mapping of eQTLs allows multifactorial dissection of the expression profile of a given mRNA/cDNA, protein or metabolite into its underlying genetic components, and also allows locating these components on the genetic map (29, 32). Initially this approach was used in human and mice, however, recently this approach has become very popular in plant systems (33). For instance, genetic regulatory network construction by combining e-QTL and mapping and regulatory candidate gene selection was done for studying genes associated with flowering in *Arabidopsis thaliana* (34). Higher costs associated with expression analysis using conventional microarray, however, have been major bottlenecks in wider use of 'genetical genomics' approach in common crop species. Advent of NGS technologies and high-throughput genotyping technologies however would overcome this problem as genome-wide expression analysis and mapping with new technologies mentioned will be cost effective and facilitate wider use of functional/ genetical genomics.

Metabolomics — This approach provides the instantaneous snapshot of physiology of the cell, produced in response to any stimuli or genetic modifications. It quantitatively measures the complete set of small molecule metabolite such as hormones, signaling molecules, metabolic intermediates and secondary metabolites, to produce a metabolic profile which is present within the biological sample. Over 50,000 metabolites have been characterized from plant kingdom and at the same time there are thousands of metabolites identified or characterized for a single plant. There are several studies that employed metabolomics approaches to undertake gene identification (35), genotype discrimination (36), and other applications such as characterization of metabolism like identification of regulated key sites in networks and investigation of gene function (37, 38). Metabolomics can be hence applied alone and in combination with other technologies of functional genomics to understand or to predict the behaviour of complex systems such as plants (37).

Proteomics — Proteomics deals with study of the structure and function of entire set of proteins, present in an organism and hence is essential for studying the whole metabolic

pathway. This involves separation, identification, and determination of function and functional network of proteins allowing the integral study of many proteins at the same time (39). There has been extensive research over last few year to study the technical aspects of proteomics in plants (40, 41) and studies have been conducted in several plant species e.g. rice and *Arabidopsis* (42), maize (43) and chickpea (44, 45). Proteomics enable not only the study of protein–protein interaction but also helps in identification of multisubunit complexes. Furthermore, proteomics can act as a powerful approach to organize and identify the proteome through development of 2-DE gel protein reference maps of sub-proteomes in different plant species.

New Genetic Approaches for Harnessing the Natural Variation

The domestication of the plant species for food, fodder or any commercial purpose for mankind is one of the very ancient practices. However, while carrying out domestication or breeding of any crop species, the genepool has been narrowed with number of alleles (46). Therefore, in general, breeders work with a limited number of alleles available in the cultivated genepool and are unable to utilize the natural variation present in the germplasm collection of a particular species. In this context, wild species can serve as a reservoir of useful alleles to use them in breeding programme (47). Conventional methods of breeding, however, have limited scope as they render the transfer of only a fraction of the genetic variation from wild to cultivated species. Some selected approaches have been described below that have potential to utilize the alleles from wild species to breeding lines.

Introgression of exotic germplasm — As mentioned above, wild species together with landraces represent natural variation within the species. Domestication of these landraces which are highly heterogeneous in nature is the first step to produce cultivars. Extensive studies have been done on the natural variation in crop species to study both evolutionary and ecological potential of the genes. It has been demonstrated that quantitative trait modification which includes phenotypic and compositional changes can not be achieved by mutagenesis or transgenic but can be introgressed through wide genetic variation studies using molecular marker assisted breeding (48). Indeed, in several cases, introgression of important gene(s) from exotic species to the cultivated ones has been successfully done (49). For instance, wild species have facilitated the introduction of single-gene-controlled traits enhancing yield in rice (50), resistance to blight in potato (51), increase in starch content in potato and rice (52), and has also shown an increased nutritional value by introduction of genes for higher protein content in potato and vitamin-C content in tomato (51).

Several other strategies have been used for introgression of favorable gene/QTL/chromosomal-segment by developing isogenic lines using wild species and the variety /genotype of interest (53). Based on the protocol used for the development, the generated lines are referred as introgression lines (ILs), back-cross recombinant inbred lines (BCRIL) (54), recombinant chromosome substitution lines (RCSLs) (55), chromosome segment substitution lines (CSSLs) (56) and stepped aligned inbred recombinant strains (STAIRS) (57). Some crops where these lines have been developed include tomato (58-60), barley (61, 62) and rice (63). Introgression/ exotic libraries are constructed using introgression lines each of which carries a fragment of defined homozygous chromosomal segment form donor exotic parent with a homozygous genetic background of elite parent. These exotic libraries have been used for identification of QTLs controlling tomato aroma (64), fruit nutrition and antioxidant content (65). In rice, a large set of CSSL libraries were constructed which resulted in the transfer of brown plant-hopper (BPH) and the white-backed plant-hopper (WBPH) resistance in the line (66). Hence, this approach can be employed to enrich the genetic variation which was lost during the domestication of crop plant.

Advanced-backcross (AB-QTL) analysis — AB-QTL analysis is an approach for simultaneous discovery and transfer of QTLs from a wild species to a crop variety which was proposed earlier by Tanksley and Nelson (67). In this approach, a wild species is backcrossed to a superior cultivar, and during backcrosses, the transfer of desirable gene/QTL is monitored by employing molecular markers. The segregating BC_2F_2 or BC_2F_3 population is then used not only for recording data on the trait of interest, but also for genotyping using polymorphic molecular markers. These data are then used for QTL analysis, leading to simultaneous discovery of QTLs, while transferring these QTLs by conventional backcrossing. Many AB-QTL studies concluded that wild species contain favourable alleles for enhancement of quantitative traits for cereal crops.

Transfer of agronomic traits like yield and yield components has been successfully conducted through AB-QTL analysis in several vegetable and field crop species like tomato (68), rice (69-71) wheat (72,73), maize (74, 75) and barley (76-84). Availability of high-throughput genotyping platforms should facilitate AB-QTL approach further in other crop species also.

***Association genetics* —** Association genetics is an approach that utilizes natural variation and linkage disequilibrium (LD) existed in natural population to identify the gene(s)/ genomic regions associated with trait. In general, conventional linkage analysis using a bi-parental mapping population such as F_2 lines, back cross (BC) population and recombinant inbred lines (RILs) is a commonly used method for trait mapping. However, such mapping populations are derived from a few cycles of recombination events, hence limit the resolution of genetic maps and localize QTLs from 10 to 20 cM intervals (85) and also do not essentially use the germplasm that is being actively used in breeding programs. In contrast, association mapping, based on LD measures the degree of non-random association between alleles at different loci. It does not require a segregating population and in some cases more powerful than linkage analysis for identifying the genes responsible for the variation in a quantitative trait (86, 87). Conventional mapping provides pertinent information about traits that tends to be specific to the same or genetically related populations, while results from association mapping are more applicable to a much wider germplasm base.

Association mapping offers three advantages over traditional linkage analysis– (i) increased mapping resolution, (ii) greater allele number, and (iii) reduced research time (88). Mapping based on LD allows for large scale assessment of allele/trait relationship when combined with a correction for population structure (89). Under this approach, association between marker and trait is only expected when a QTL is tightly linked to the marker because the accumulated recombination events occurring during the development of the lines will prevent the detection of any marker/trait association in any situation where the QTL is not tightly linked to a molecular marker.

Based on the scale and focus of a particular study, association mapping employs one of following two approaches: (i) candidate-gene association mapping, which relates polymorphisms in selected candidate genes that have purported roles in controlling phenotypic variation for specific traits; and (ii) genome-wide association mapping, or genome scan, which surveys genetic variation in the whole genome to find signals of association for various complex traits (90). Although candidate gene sequencing across several hundreds of genotypes for selected genes using Sanger sequencing was an expensive task in past, use of pools of amplicons for a range of genotypes and/or genes through NGS technologies is expected to reduce the costs significantly (9). Similar will be the case for the projects that employ whole genome scanning approach as high-throughput genotyping platforms such as GoldenGate or Infinium assay should provide genome wide marker data in relative reduced costs and less time. Nevertheless, association genetics has been used to detect the functional/causal polymorphism or markers associated in several crop species. Some examples include mapping of *Dwarf8* gene involved in flowering time (91) and yellow endosperm colour (92), carotenoid content (93), sweet taste (94) in maize, yield and yield components (95, 96), drought related traits (97) in barley, traits related to flowering time (98) and disease resistance (99) in Arabidopsis. In rice this approach has been used for studying association between *WAXY* locus and glutinous phenotype which is commonly known as sticky rice (100) while broad spectrum stem rust resistance (101, 102), grain size (103) and resistance to several diseases (104) have been targeted in wheat. Similarly, leaf traits, flowering time, and phytate content have been dissected in *Brassica* sp. using association genetics approach (105).

As mentioned above recently developed high-throughput sequencing and genotyping will facilitate both candidate gene sequencing as well as whole genome scanning approaches for association genetics. It seems that association genetics approaches will be the approach of choice for trait mapping in coming future.

***Multi-parent advanced generation inter-cross (MAGIC) population* —** MAGIC population is a second generation mapping resources for crop improvement which is constructed using multiple parents. This mapping population strategy involves linkage and association methodology for mapping genetic variation in population segregating for multiple QTLs. Such an approach was initially used in mice where multiple parent RILs were used for mapping many QTLs controlling complex traits (106), however the plant community has dubbed this approach

as MAGIC population (107). In past studies, the 8-parent RIL population of 1000 progenies (MAGIC population) allowed degree of mapping resolution of sub-centromere range in mice (108).

The MAGIC approach can be considered superior to association and linkage mapping as it allows both coarse and fine mapping of RILs developed from multiple parents by sampling seed of any generation with greater genetic variation. It has been demonstrated that if a large set of RILs are produced, the complex architecture of many traits which are associated with crop yield and quality can be studied using epistatic interactions (107). However, it is important to note that large sample size plays an important role to facilitate the screening and characterization of genes responsible for complex traits. Furthermore, the MAGIC lines may show extensive segregation for plant developmental traits like plant height, maturity that may limit the use of MAGIC population in dissection of complex traits. Although there is no published reports on developing MAGIC population in a crop species, work is in quite advanced stage in developing MAGIC populations in some crop species like rice (Hei Leung, personal commun), sorghum (Tom Hash, personal commun), chickpea (Pooran Gaur and Hari Upadhyay, personal commun). It is anticipated that MAGIC populations should provide an important means for the discovery, isolation and transfer of essential genes to facilitate crop improvement.

Modern Breeding Methodologies

Molecular plant breeding aims to improve crop variety in context to its yield, quality and resistance by the means of latest innovations made in genetics and genomics (109). Our understanding about the association between genotype and phenotype has been growing with the help of genomics tools (17, 110). One of the major applications of genomics directly related to breeding has been identification of molecular markers associated with the trait of interest for breeders. Such markers have greatly helped the breeding communities for several crops in overcoming the constraints of phenotypic selection which sometimes is unable to identify individual with highest breeding value. This section presents some selected breeding methodologies that use genomics tools to facilitate breeding for developing the superior lines or genotypes.

Marker-assisted selection and marker- assisted back-crossing — Once the markers associated with a trait of interest is identified through linkage mapping, association mapping, AB-QTL or transcriptomics approach, etc., the next step is to use these markers in the breeding programme (111). In this context, the selection of one or a few genes (QTLs) through molecular markers using backcrossing is a highly efficient technique (112). There are three levels of MABC or MPS: (i) foreground selection (113) which includes screening of target gene or QTL using molecular markers, this step can also be used for selection of recessive allele for backcrossing as recessive alleles require one generation of selfing for its expression, (ii) recombinant selection involves selection of the BC progeny containing the target gene and recombination events (between the target locus and linked flanking markers. The purpose of this selection step is to minimize the 'linkage drag' by using markers that flank the target gene. This linkage drag poses a big problem during selection through conventional breeding methods. Furthermore this recombination selection event is usually carried out using two BC generations (114), (iii) background selection involves use of markers that are unlinked to the target locus for the selection of BC progeny containing highest proportion of recurrent parent (RP). In summary, the MABC employs linked markers to select the target gene/QTL from the donor parent and the unlinked markers to recover RP. Traditional approaches of recovery of RP genome take upto six BC generations but the use of markers enables to achieve the same in even in BC_2.

MABC approach has been very popular approach in molecular breeding community (17). While MABC approaches are in routine in many multi-national companies, MABC has been successfully utilized in some breeding programmes in public sector. Success stories of MABC leading the development of improved varieties and superior genotypes have been reviewed in both cereals (17, 110) as well as legumes (115). In recent years, MABC approach is becoming more popular in developing countries like India, China, Bangladesh and Thailand where public sector in collaboration with the international agriculture research centres have been working towards developing the improved varieties in their targeted environments.

Marker-assisted recurrent selection — There are cases where quantitative variation is controlled by many genes (QTLs) with minor effect; in such cases the previous approach (MABC) has certain limitations in the introgression

of the target trait. Moreover, the markers identified linked with a trait to be used in MABC are generally identified bi-parental mapping populations. This limits the study of allelic diversity and genetic background which are very essential in crop breeding program. Limited statistical tools for studying polygenic traits controlled by many small effect loci are yet another drawback of MAS (116-119). Furthermore, minor QTLs show an inconsistent QTL effect. Even though the effect of these minor QTLs is consistent, introgression of these QTLs through MABC approach becomes extremely difficult as a larger number (sometimes unmanageable) of progenies, depending on the number of QTLs, are required to select appropriate lines in MABC.

In cases as mentioned above, marker assisted recurrent selection (MARS) can be used for pyramiding of several genes/QTLs (of minor effect) in a single genotype (120-122). MARS is based on *ad hoc* significance test which include the identification of trait associated markers and estimation of their effect. The approach involves multiple cycles of marker based selection that includes, (i) identification of F_2 progeny which contain favorable alleles for most if not all QTLs, (ii) recombination of the selected progenies to the selfed ones, and (iii) repetition of these cycles. However, development of publicly available appropriate statistical tools for calculating the selection indices to select the progeny lines for the selection cycles is still an issue.

According to the recent studies, the response of MARS is larger in case of prior knowledge of the QTLs and the response decreases as the knowledge of the number of minor QTL associated with the trait decreases (122). In sweet corn, MARS was employed to fix six marker loci in two different F_2 populations which showed an increase in the frequency of marker allele from 0.50 to 0.80 (120). Similarly in a separate study, enrichment of rust resistance gene (*Lr34*/*Yr18*) with the increase in frequency from 0.25 to 0.60 was reported in wheat BC_1 through MARS (123). MARS, becoming a popular approach, can thus be effectively utilized for selection of traits associated with multiple QTLs by increasing the frequency of favorable QTLs or marker alleles. Several multinational companies such as Syngenta and Monsanto use MARS in the breeding programmes of several crops such as maize, soybean etc. (124, 125). Recently, some international agricultural research centres in collaboration with Generation Challenge Program (GCP) have also initiated MARS in crops like chickpea, sorghum, rice, cowpea, *etc* for pyramiding favorable drought tolerant alleles.

***Genome-wide selection* —** In addition to MARS, the genome-wide selection is another approach that can be used to pyramid favourable alleles for minor effect QTLs at whole genome level (126). Unlike MABC or MARS, the GWS calculates the marker effects across the entire genome that explains entire phenotypic variation. The genome wide marker data (marker loci or haplotypes) available or generated on the progeny lines, therefore, are used to calculate genomic estimated breeding values (GEBV) (126). It is important to note that the GEBVs are calculated for individuals based on genotyping data using a model that was 'trained' from individuals having both phenotyping and genotyping data. These GEBVs are then used to select the progeny lines for advancement in the breeding cycle. In summary, the GWS provides a strategy for selection of an individual without phenotypic data by using a model to predict the individual's breeding value. However, to maximize the GEBV accuracy in GWS, it is very critical to select the appropriate training population (used to develop the model for calculating GEBVs) that is representative of selection candidates in the breeding programme to which GWS will be applied.

Although, to the best of our knowledge, there is no published report on deploying GWS in a crop breeding programme, availability of high-throughput genotyping platforms in several crops makes it feasible to generate genome wide marker data and undertake the GWS in breeding pogramme. It is anticipated that at least a few breeding programmes for major crops like maize, rice or soybean should be using GWS soon.

Summary and Future Prospects

Recent advances in sequencing and genotyping technologies have made it possible to develop molecular markers as well as undertake genotyping at large scale in both major as well as minor (or so called orphan crop species) that can be used not only for developing high-density genetic and physical maps but also for generating transcriptome or sequence data. These approaches together with –omics approaches such as transcriptomics, genetical genomics, metabolomics and proteomics can be used to identify the genomic regions or genes involved in expression of trait(s) that are of interest to the breeding community. In parallel, the high-throughput sequencing and genotyping approaches can be used to detect genetic

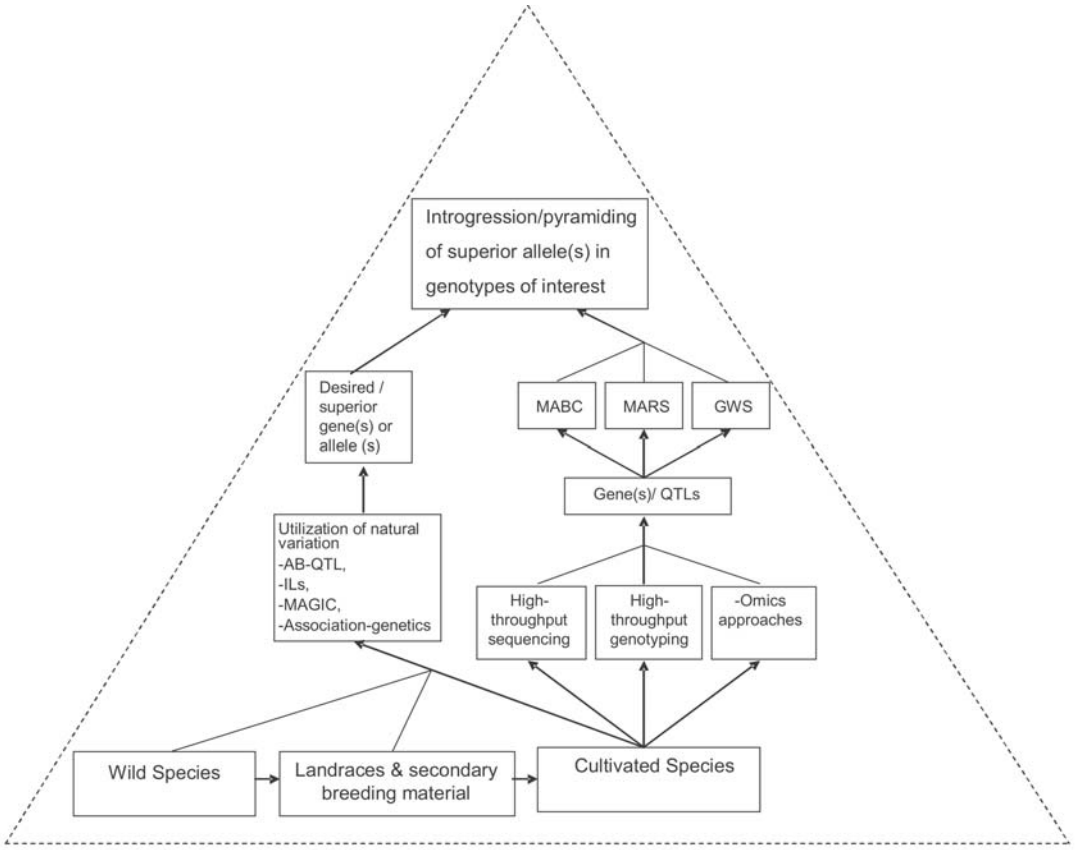

Fig. 1. A hypothetical pyramid showing the use of novel genomics technologies together with modern breeding methodologies in an integrated way. AB-QTL- Advanced backcross QTL; ILs- Introgression libraries; MAGIC- Multi-parent Advanced Generation Inter-Cross; MABC- Marker-Assisted Back crossing; MARS- Marker Assisted Recurrent Selection; GWS- Genome Wide Selection.

variation existed in germplasm collection not only in cultivated gene pool but also in landraces and wild species. Such kind of genetic variation (or favourable alleles) can be introgressed in elite variety or genotype of interest by using AB-QTL approach or developing introgression libraries. Furthermore, the QTLs or genes or superior alleles for the trait of interest identified through linkage mapping, association mapping, AB-QTL approach or –omics approach can be introgressed or pyramided in elite varieties or genotype of interest by using MAGIC, MABC, MARS or GWS approaches. An integrated view of using different genomic tools and genetic/breeding strategies has been shown in the Figure 1.

In summary, the presented tools and approaches in this article are ready to be added in the plant breeders' tool box that have a great potential to impact crop breeding. However, it is really important at this stage that different technologies/approaches, in integrated way, should be brought in practice from theory; then only the potential of genomics-assisted breeding can be realized. Use of these approaches together with conventional breeding methodologies should prove very useful for enhancing the genetic gain leading to crop improvement. Due to reduced costs on sequencing and genotyping technologies combined with advances in biometrics and bioinformatics, we envisage a bright future on application of these novel tools/approaches in breeding programmes.

Acknowledgements

Authors are thankful to Jean-Marcel Ribaut, Generation Challenge Programme (GCP), Mexico; Jean-Christophe Glaszmann, CIRAD, France; Michel Ragot, Syngenta, France and Pooran Gaur, ICRISAT, for useful discussions on different technologies and approaches. AD is thankful to Dr Ragini Gothalwal, Barkatullah University, Bhopal, India for her kind support. Thanks are also due to Generation Challenge Programme (GCP) for financial support.

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