Plant genetic resources management and pre-breeding in genomics era

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Abstract

Plant Genetic Resources (PGR) conserved in gene bank provide genetic variability for efficient utilization in breeding programmes. Pre-breeding is required for broadening the genetic base of the crop through identification of useful traits in un-adapted materials and transfer them into better adapted ones for further breeding. So, pre-breeding is a promising alternative (due to use of un-adapted materials) to link genetic resources and breeding programs. Utilization of PGR in crop improvement programmes including pre-breeding have been very limited. Advances in genomics have provided us with high-quality reference genomes, sequencing and re-sequencing platforms with reduced cost, marker and QTL assisted selection, genomic selection and population level genotyping platforms. Further, genome editing tools like, CRISPR/Cas9 and its latest modification base editing technology can be used to generate target specific mutants and are important for establishing gene functions with respect to their phenotypes through developing knockout mutations. These new genomic tools can be used to generate, analyse and manipulate the genetic variability for designing cultivars with the desired traits. The genomic tools have not only accelerated the utilization of PGR but also assisted pre-breeding through rapid selection of trait-specific germplasm, reduced periods in breeding cycle for confirming gene of interest in intermediate material and validation of transfer of gene of interest in the cultivated gene pool. In crops, where limited genetic and genomic resources are available, pre-breeding becomes very challenging. We can say that genomics assisted utilization of PGR and pre-breeding has accelerated the pace of introgression of complex traits in different crop cultivars.

Key words: Exotic germplasm, QTL, GBS, genome sequencing, genotyping, GWAS, marker assisted selection, NGS

Introduction

Plant breeders deal with production of superior cultivars with desired traits by changing the genetic constitution of plants. They are under tremendous pressure to raise the overall food production by 70% to feed the increasing population which is expected to reach 9.7 billion by 2050 (FAO 2009; United Nations, Department of Economic and Social Affairs, Population Division 2015). Other factors like changing environmental conditions and newly emerging insect-pests and diseases, new emerging insect-pest infestations are also hindering the overall grain production (Zhao et al. 2017). Further, the process of domestication has narrowed down the genetic base in modern cultivars and yield plateau has already been achieved in these cultivars (Chen et al. 2014a). Under these circumstances, use of Plant Genetic Resources (PGR) in crop improvement programs provides an avenue to solve the problem.

Management of Plant Genetic Resources includes conservation of the available variability for future and utilization of trait-specific germplasm in national crop improvement programme. Most suitable method for the PGR management for sustainable food security is through their utilization in breeding programs, followed by adoption and cultivation (Haussmann et al. 2004). PGR also includes landraces and wild relatives of crop species which constitute an important source for broadening the genetic base of modern cultivars, for developing cultivars with higher production to withstand changing environmental conditions as well as with resistance to biotic and abiotic stresses and high nutritional qualities.
Pre-breeding is a link between genetic resources and breeding (Nass and Paterniani 2000). Indigenous (primitive landraces and, wild species and their relatives) along with exotic germplasm possesses high levels of genetic diversity for valuable traits. However, only a small fraction of this naturally occurring genetic diversity has been utilized for crop improvement. So, increased efforts are needed to use this available potential germplasm and to identify beneficial genes particularly controlling traits like higher yield, resistance against biotic, abiotic stresses and high nutritional qualities and for their introgression in modern cultivars (Wang et al. 2017). Moreover, the wide yield gap between elite and exotic germplasm in many crops including maize and rapeseed, highlights the importance of exotic germplasm in pre-breeding programme in the country. The success of pre-breeding in the last century has relied in the utilization of natural variation and in the efficient selection, by using suitable conventional breeding methods, of the favourable genetic combinations where selection have largely been based on the phenotypic evaluation. These conventional methods of pre-breeding are laborious and time consuming.

Genomics era has provided several new tools to generate, analyse and manipulate the genetic variability for designing cultivars with the desired traits. The combination of conventional plant breeding and genomics has already revolutionized the science of plant breeding since beginning of 21st century. Now, it has also been used in pre-breeding which has reduced the time period for transfer of desired trait in modern cultivar (Riar et al. 2012) and cultivar development in many crops is underway. Availability of quality reference genomes, high-throughput sequencing, automated and cost-effective high throughput genotyping platforms have made utilization of PGR and pre-breeding more productive and efficient (Varshney et al. 2018). Genomics has brought a paradigm shift by facilitating the direct study of genotype and its relationship with the phenotype (Tester and Langridge, 2010). These advances paved the way for a new genomics assisted breeding era which is becoming a standard practice in many pre-breeding programmes particularly for biotic stresses (Enciso-Rodriguez et al. 2018), abiotic stresses (Dwivedi et al. 2017) and quality traits (Heffner et al. 2011).

Genomics assisted utilization of PGR and pre-breeding, both has great potential for overcoming the challenges agriculture faces today for ensuring sustainable food production through development of cultivars with higher yield, adapted to biotic and abiotic stresses with novel nutritional traits. This article reviews various genomic technologies and their potential role in management of plant genetic resources and pre-breeding.

Management of plant genetic resources in changing scenario

Plant Genetic Resources are plant genetic materials of actual or potential use available for change in genetic constitution of a plant species for the production of an improved cultivar. They are valuable natural variants in form of exotic and indigenous collections including advance cultivars, released varieties, RILs, NILs, mutants, genetic stocks, landraces, wild and weedy relatives etc. Various natural mechanisms including open pollinated reproductive systems, introgression from wild relatives, and mutations act to generate genetic diversity over a range of environments and time (Mercer and Perales 2010). Management of plant genetic resources includes augmentation (exotic as well as indigenous collection), conservation, characterization, evaluation and documentation as essential steps. Maintenance of genetic diversity and PGR utilization are possible mainly through synchronising among these activities (Nass and Paterniani 2000). Management of Plant Genetic Resources aims for fulfilling present as well as future agricultural needs as per changing scenario. For example, Neelam et al. (2016) screened 1176 rice accessions, comprising different species including wild relatives against two most Bacterial blight (BB) Xoopathotypes viz., PbXo-10 and PbXo-8, and identified accessions with immune and different resistance levels. Oryza longistaminata accessions IRGC92624 and IRGC92644 had resistance against both the Xoopathotypes indicating presence of BB resistance gene other than Xa21. So, characterization and evaluation of germplasm is necessary to identify new sources for various desirable traits.

Haussmann et al. (2004) reported that the most effective method for the PGR management for sustainable food security is through their utilization in breeding programs, followed by adoption and cultivation. Despite widely recognized importance, low utilization of PGR may be due to scanty documentation, characterization and evaluation, difficulty to identify useful genes, accessions with restricted adaptability and low seed availability. The low utilization of PGR has resulted in narrow genetic base of commercial hybrids (Lu et al. 2009). PGR
utilization is also required to break the performance plateaux in major crops (FAO, 1996a). There are about 30,000 edible plant species, and out of these only 30 ‘feed the world’, with the three major crops being maize (Zea mays), wheat (Triticum aestivum) and rice (Oryza sativa) providing at least 30% of the food calories to more than 4.5 billion people in 94 developing countries (FAO 1996b). PGR utilization is further advocated by changing climate scenario with emergence of new diseases/stresses. Development of core collections is an efficient way to manage PGR for their utilization (Nass and Paterniani 2000). CIMMYT has developed efforts to establish maize cores, for example, Tuxpeño core collection were reported by Taba (1994). Mega characterization of PGR conserved in national genebank by ICAR-NBPGR has resulted in development of core sets in many crops including mung bean (Bisht et al. 1998), sesame (Mahajan et al. 2007), brinjal (Gangopadhyay et al. 2010), global wild annual Lens collections (Singh et al. 2014), rice (Roy et al. 2014), wheat (Dutta et al. 2015) and chickpea (Archak et al. 2016).

Role of pre-breeding in management of plant genetic resources

Process of domestication has narrowed down the genetic base of modern cultivars in comparison to progenitor species which results in loss of many allelic variants of genes controlling traits undesirable for cultivation (Lu et al. 2009; Chen et al. 2014b). The best studied example is maize where domestication has affected 1,200 genes and so the genetic diversity during the process as identified through comparison of modern cultivars, early-domesticated maize, and wild teosinte (Bevan et al. 2017). Pre-breeding constitutes a crucial step between conservation of PGR and their utilization in breeding programs. The Global Partnership Initiative for Plant Breeding Capacity Building (GIPB)/FAO and Biodiversity International use the term ‘pre-breeding’ to describe the various activities of plant breeding research that have to precede the stages involved in cultivar development, testing and release (Biodiversity International and GIPB/FAO 2008). Further, the Global Crop Diversity Trust defined pre-breeding as ‘the art of identifying desired traits, and incorporation of these into modern breeding materials’.

The aim of pre-breeding is to broadening the genetic base of the crop through identification of useful traits in non-adapted materials and transfer them into better adapted ones (cultivated ones) for further breeding. It is required in crop species where sufficient variability is not present for target trait in available germplasm or the available variability has already been exploited up to maximum limit. Landraces and wild relatives have been described as a vast genetic resource for introduction of novel traits into tomato breeding programmes (Miller and Tanksley 1990). These breeding goals of enhanced genetic base of modern cultivars for desired traits would be easier to address if the vast genetic variation of progenitor populations would be accessible to breeders in a form they could use in their breeding programs (Sood et al. 2014). The knowledge of characterization and evaluation, genetic diversity and inter species relationship is required to initiate a pre-breeding program. Pre-breeding programs have been initiated at global level for maize at CIMMYT (Taba 1994), wheat by ICARDA in 1994/1995 (Valkoun 2001). Some other examples of different crops include rice (Brar and Khush 2002), wheat (Riar et al. 2012) and lentil (Singh et al. 2017). Singh et al. (2017) compared agronomic performance of lentil (Lens culinaris subsp. culinaris), inter-sub-specific (L. culinaris subsp. orientalis) and interspecific (L. ervoides) derivatives and obtained high level of heritability estimates.

Need for genomics assisted management of PGR and pre-breeding

Genomics is branch of science dealing with structure, function, evolution, mapping, and editing of genomes. Integration of modern genomics approaches, for example, next generation sequencing (NGS), cost effective high-throughput genotyping together with high throughput phenotyping (phenomics), and bioinformatics and statistical decision support tools
can accelerate genetic gains over time (Varshney et al. 2014). The actual and potential application of genomics in management of PGR and pre-breeding include providing identity to an individual accession, inhibit the evading of insects-pests of quarantine significance, identification of genetic value of germplasm through trait-specific characterization, and finally ensuring availability of trait specific germplasm to the breeders.

Pre-breeding is a difficult to execute and time-consuming activity. Pre-breeding based on conventional methods have some limitations related to phenotypic evaluation including masked environmental effect and polygenic nature of key traits, crossing barriers, linkage drags and negative correlations between traits etc. (Prohens 2011). Breakdown of blockage of favourable alleles requires a series of back crosses to reconstitute the recipient parent which is time consuming. Introduction of linkage drag can be avoided by using genomics assisted breeding (GAB). Further, in case of complex traits, it is difficult to identify desirable allelic variants and genetic combinations. Genomics approaches help in the selection of superior haplotypes/alleles to be used in pre-breeding and latter transfer of these useful alleles to the modern cultivars. Genomics-assisted pre-breeding approaches are contributing to the more efficient development of climate-resilient crops (Varshney et al. 2018).

Molecular markers, QTL mapping, association mapping etc. have been used extensively for utilization of PGR and pre-breeding (Riar et al. 2012; Neelam et al. 2016; Zhou et al. 2017). In genomics era, availability of quality reference genomes, high-throughput sequencing and re-sequencing platforms, automated and cost-effective high throughput genotyping platforms has made utilization of PGR and pre-breeding more productive and efficient (Kim et al. 2016; Zhou et al. 2017). Whole genome provides information required for genomics-assisted pre-breeding in the form of best haplotypes or combinations of alleles, optimal gene networks, and specific genomic regions (Xu et al. 2012). Short breeding cycle, high accuracy and selection efficiency, and direct improvement are the key features of genomics assisted pre-breeding (Tuberosa 2013). Varshney et al. (2018) describe how climate-change ready crops can be developed through pre-breeding using genomic tools. Pre-breeding in minor crops or non-crop plants require different strategies as enough genomic resources are not available. Translational genomics-derived genome annotations based approach can be used in these crops in studying the phenotypic expressions and to select trait-specific genetic markers to perform marker-assisted breeding and genome selection (Kang et al. 2016).

Genomics approaches to enhance utilization of PGR and empower pre-breeding

Genomics has provided various technologies including sequencing and re-sequencing platforms, availability of genome sequences as references, high-throughput genotyping platforms, SNP arrays, genome editing tools etc. These technologies are described here in details:

**Genome sequencing and re-sequencing**

The inexpensive sequencing and resequencing technologies are the major driving forces behind increased number of assembled plant genomes of different crops including wild relatives (Brozynska et al. 2016). A single reference genome doesnot represent the total diversity within a species, hence, re-sequencing of cultivars, landraces and wild accessions is required to harness the total genetic variation and to identify the superior alleles for the target traits. Genome information availability has generated many next-generation sequencing-based platforms for allele mining and candidate genes identification. Next-generation sequencing and whole-genome re-sequencing is required for discovery, validation, and assessment of diagnostic markers in different crops and it provides genome-wide markers. The draft genome sequences are now available in a number of crops through different genome sequencing consortia for rice (IRGSP 2005), sorghum (Paterson et al. 2005), maize (Schnable et al. 2009), pigeonpea (Varshney et al. 2012), chickpea (Varshney et al. 2013), wheat (IWGSC 2018) etc. The genome sequencing using NGS has resulted in large collections of functional markers which enhance gene assisted breeding, reducing the possibility of losing the desirable trait variation due to recombination. Sequencing and re-sequencing of populations developed in crossing programs or of natural population (germplasm) along with high-throughput phenotyping helps in identification and linking of variations in gene sequences to their phenotypes (Abdurakhmonov and Abdukarimov 2008). Kim et al. (2016) reported the whole-genome resequencing of the 137 rice mini core collection, potentially representing 25,604 rice germplasms in the
Korean genebank of the Rural Development Administration (RDA) based on the Nipponbare reference genome, and resequencing data yielded more than 15 million (M) SNPs and 1.3 M INDELs. Further study of this rice mini core with phylogenetic and population analysis using 2,046,529 high-quality SNPs successfully assigned accessions to the relevant subgroups, suggesting that the SNPs capture evolutionary signatures present in rice subpopulations. Similarly, a population structure analysis of 300 rapeseed accessions (278 representative of Chinese germplasm, plus 22 outgroup accessions of different origins and ecotypes) was carried out based on the 201,817 SNPs obtained from sequencing, divided accessions in nine subpopulations (Zhou et al. 2017). However, hierarchical clustering and principal component analysis showed intermingle of spring type accessions with semi-winter types pointing out towards frequent hybridization between spring and semi-winter ecotypes in China.

Sequence-based markers associated with rare elite alleles facilitate positional cloning and pre-breeding. In case of PGR including landraces and wild relatives, screening of collection to be used for genomic analysis can be done based on passport data (collection site, specific traits etc.) in combination with evaluation data. Sequencing based approaches provide opportunity to identify novel variations for a large number of genes through genotype-phenotype associations. Re-sequencing of large number of genotypes helps in determining process of origin, domestication, population structure and identifies lines with deleterious mutations in the genomes that can be eliminated to minimize the genetic load in the crop species as observed in case of maize (Bevan et al. 2017). NGS technologies together with precise phenotyping have been used for identification of marker trait associations in several crops, for example, rare wheat haplotypes effective against abiotic or biotic stresses were developed through introgression of useful and novel stress and quality traits’ alleles to lines derived from crosses of exotics with CIMMYT’s best elite germplasm under CIMMYT’s Seeds of Discovery (SeeD 2011) initiative (Vikram et al. 2016). Singh et al. (2018) used next-generation sequencing, together with multi-environment phenotyping to study the contribution of exotic genomes to 984 three-way-cross-derived (exotic/elite1/elite2) pre-breeding lines (PBLs) for accelerating grain yield gains using exotic wheat genetic resources.

**Genotyping by sequencing**

Genome complexity reducing methods like genotype by sequencing (GBS) has become the method of choice for genotyping of a large number of recombinant progenies or natural PGR for rapid, high-throughput identification of genetic mechanisms underlying various trait variations (Elshire et al. 2011; Deschamps et al. 2012; Poland and Rife 2012; Davey et al. 2011). Genotyping by sequencing (GBS) approach in combination with phenotyping can be used for identification of QTLs controlling various traits. Availability of SNP markers based on whole genome re-sequencing data and cost effective automated genotyping platforms have made genome-wide genotype-to-phenotype associations (GWAS) very popular (Prohens 2011). SNPs are heritable, abundantly distributed across the genome, and allow single base resolution, facilitating the detection of causal, or ‘perfect’, markers. SNPs are currently the most popular markers in breeding programs because of their abundance and our ability to detect them with high throughput methods (Shirasawa et al. 2010; Mammadov et al. 2012).

GBS methods can be divided into whole genome re-sequencing (WGR) which provides high SNP densities adequate for accurate SNP calling in recombinant populations based on a high quality reference genome and reduced representation sequencing (RRS) which provides lower SNP densities to narrow the focus to only a fraction of the genome (Huang et al. 2009; Andrews et al. 2016). The GBS has been used extensively in pre-breeding, for example, ICRISAT initiated pigeonpea hybrid program to enhance yield using A4 cytoplasm with cytoplasmic-nuclear male sterility (CMS) system derived through the introgression from the wild relative Cajanus cajanifolius. Out of 34 mitochondrial genes, nad7 gene was found to be associated with A4 CMS and nad7 gene specific markers were used for detection of CMS seed purity (Huang et al. 2014).

Genotyping derived SNPs implemented in arrays known as genotyping arrays or SNP arrays have been used extensively for targeting of alleles of interest, timely data generation and simple computational analysis in different crops including rice (Zhao et al. 2011; Yu et al. 2014), canola (Snowdon and Luy 2012), maize (Chen et al. 2014a), and wheat (Wang et al. 2014; Winfield et al. 2015). SNP array is useful for pre-breeding programs as these provide valuable data for genetic mapping, association studies and genomic selection (Ganal et al. 2012).
Pangenomics

Initially Pangenome was introduced for describing a bacterial species (Tettelin et al. 2005). Pangenome is sum of all the genes of a particular species rather than single sample reference and it allows a better representation of diversity by reducing sampling bias (Golicz et al. 2016). It is based on genomic structural diversity and can be used for differentiating genomes via presence and absence of sequences known as presence/absence variants (PAV) and differences in copy numbers known as copy number variants (CNV) (Schatz et al. 2014). Links of copy number variations to the key traits in crops has accelerated the pre-breeding programs (Prohens, 2011). Pangenomes are now available in various crops including rice (Schatz et al. 2014), maize (Hirsch et al. 2014), Brassica rapa (Lin et al. 2014b) and soybean (Li et al. 2014a) for responses to biotic stresses in several species including muskmelon (Gonzalez et al. 2013).

Application of genomics in management of PGR and pre-breeding

Molecular marker, QTL and genomic maps assisted utilization of PGR and pre-breeding

Recent developments in genome sequencing and re-sequencing has resulted in development of large number of molecular markers in different crops. Availability of molecular markers linked to specific traits enhances pre-breeding efficiency and effectiveness through marker assisted selection (MAS). Molecular markers that are linked to the genes of a desired trait known as diagnostic markers can be indirectly used for selection of target traits (Xu and Crouch 2008). A major earlier success for crop breeding using genomic markers was the marker-assisted introgression of the ethylene response factor, known as Submergence 1A (Sub1A) gene, for submergence-tolerance into high-yielding commercial rice varieties which acts by limiting shoot elongation during the inundation period (Septiningsih et al. 2009; Bailey-Serres et al. 2010). Riar et al. (2012) used polymorphic D-genome-specific SSR markers for analysing the co-segregation of the SDS anchored markers (Xcfd187, Xcfd78, Xd81 and Xcfd189) with the rust resistance in an F2 population, and mapped the leaf rust resistance gene (LrAC, a novel homoeoallele of an orthologue Lr57) on the short arm of wheat chromosome 5D. Vikal et al. (2014) used SSR markers for pyramiding of candidate genes for Xa8, the resistance gene against Bacterial blight disease in elite rice varieties. Ellur et al. (2016) incorporated a novel Bacterial blight resistance gene Xa38 in variety PB1121 from donor parent PR114-Xa38 using a modified marker-assisted backcross breeding (MABB) scheme.

Genomics has provided powerful approaches to understand interaction between many genes and complex signalling pathways in case of polygenic traits like resistance to abiotic and biotic stresses (Sakuma et al. 2006). In rice breeding, high-density genome maps are being effectively used in background selection integrated with foreground selection of bacterial blight resistance (xa13 and Xa21 genes), amylose content (waxy gene) and fertility restorer gene in order to identify superior lines with maximum recovery of Basmati rice genome along with the quality traits and minimum non-targeted genomic introgressions of the donor chromosomes (Gopalakrishnan et al. 2008). Quantitative trait loci (QTL) analysis of the genome linked to quantitative phenotypic traits, has yielded climate governed QTL in diverse crop species (Scheben et al. 2016). Rodrigues et al. (2017) determined protein content and genetic divergence of 29 soybean genotypes using 39 microsatellite markers from QTL regions of the trait grain protein content for plant breeding purposes. The pairs of genotypes with greater genetic distances and protein contents were selected to produce populations with higher means and genetic variances and greater gains with selection.

Genome wide association studies (GWAS)

Genome wide association studies (GWAS) could overcome several constraints of conventional linkage mapping and provide a powerful complementary strategy for dissecting complex traits. GWAS make use of past recombinations in diverse association panels to identify genes linked to phenotypic traits at higher resolution than QTL analysis. GWAS has become a powerful tool for QTL mapping in plants because a broad range of genetic resources may be accessed for marker trait association without any limitation on marker availability. Different approaches used for GWAS include:

SNP marker arrays or SNP chips approach

Discovery and tagging of new genes using genome wide association studies (GWAS) or QTL analysis have now become much easier. The availability of high-density SNP marker arrays has opened a way for cost effective genome wide association studies
using natural populations. Wang et al. (2016) developed a high-throughput NJAU 355K SoySNP array and conducted GWAS in 367 soybean accessions, (including 105 wild and 262 cultivated) across multiple environments and reported a strong linkage disequilibrium region on chromosome 20 significantly correlated with seed weight. Zhao et al. (2019) carried out meta-analysis GWAS using 775 tomato accessions (including wild accessions) and 2,316,117 SNPs from three GWAS panels and discovered 305 significant associations for the contents of sugars, acids, amino acids, and flavor-related volatiles.

**Genotyping by sequencing (GBS) approach**

As the cost of sequencing is continuously declining, genotyping by sequencing also known as next generation genotyping method, is becoming more common for discovering SNPs in novel plants and used them for GWAS studies (Arruda et al. 2016). Kim et al. (2016) reported the whole-genome resequencing of 137 rice mini core collection and conducted genome-wide association studies (GWAS) on four agriculturally important traits including 'grain pericarp colour', 'amylose content', 'protein content', and 'panicle number, and identify some novel alleles. Similarly, Arora et al. (2017) genetically characterized 177 *A. tauschii* accessions using GBS to study the variation for grain size using GWAS.

**Genomic selection**

One of the major limitations of marker assisted pre-breeding particularly in case of highly polygenic traits is that only a limited portion of genetic variance is explained (Meuwissen et al. 2001). Further, in MAS approach, markers with effects on phenotype are required (Heffner et al. 2011). Genomics assisted breeding approach known as genomic selection (GS) is a better approach which simultaneously uses large genotypic data (genome wide) (exceeding phenotypic data), phenotypic data and modelling using statistical tools to predict the genomic estimated breeding values (GEBVs) for each individual (Meuwissen et al. 2001; Crossa et al. 2017). In genomic selection, a statistical model is generated using a representative population of the breeding population known as training population. This model is subsequently used to calculate the allelic effects of all marker loci i.e. genomic assisted breeding values without having phenotypic data and these values can be used for preselection of trait-specific genotypes (Heffner et al. 2011). Xu et al. (2012) and Spindel et al. (2016) highlighted that coupling of genome wide data with genomic selection offered great specificity and predictability which can be used to accelerate pre-breeding. Using GS, complex traits can be improved rapidly through generation of reliable phenotypes by shortening the selection cycle. GS application in pasture grass *Lolium perenne* resulted in four-year reduction in the breeding cycle (Lin et al. 2016). In genomic selections, genomic estimated and true breeding values were found to be closely correlated, even for polygenic traits with low heritability (Jia and Jannink 2012). GS can facilitate selection of complex traits, e.g., grain yield (Saint Pierre et al. 2016) tolerance to abiotic and biotic stresses.

Genomic selection has great potential to be used as a selection tool in pre-breeding programs particularly to enrich the starting germplasm with favourable polygenic variation. It has been used for initiating pre-breeding programs in maize (Gorjanc et al. 2016) and wheat (Poland et al. 2012). The potential of GBS based genomic prediction to harness variation in maize landraces pre-breeding has been proved in Seeds of Discovery (SeeD; http://seedsofdiscovery.org) funded mostly by the Mexican government through the Sustainable Modernization of Traditional Agriculture program (MasAgro; http://masagro.mx) (Crossa et al. 2013; Gorjanc et al. 2016). Gorjanc et al. (2016) used genomic selection for initiating pre-breeding programs in maize under SeeD and developed and evaluated various designs. They observed that maize landraces can be used directly to initiate a pre-breeding program and testcrosses led to a rapid reconstruction of the elite donor genome. In genomic selection approach, maximum marker density is not necessarily required; instead it requires representative markers for every QTL across the genome. Recently, Werner et al. (2018) demonstrated that low-density marker sets in *B. rapa* enabled high prediction accuracies in breeding populations with strong LD for cost-efficient genomic selection comparable to those achieved with high-density arrays. A QTL with a low frequency of the favourable allele or having a small effect may not be of immediate gain but potentially contributes towards long-term genetic gain by maintaining genetic variance over time (Liu et al. 2015).

In genomic selection, genetic diversity specific to the population or family (species) of interest is captured through markers developed through GBS which minimized the ascertainment bias. GS is superior in respect of fixing all the genetic variation
and to select individuals with higher GEBV without any phenotyping (Bhat et al. 2016). In case of polyploid species, polysomic inheritance and possibility of double reduction requires specific consideration while using for genomic selection. Different softwares are available for assigning marker genotypes (estimating dosage of marker alleles in heterozygous condition), establishing chromosome-scale linkage phase among marker alleles, constructing (short-range) haplotypes, and simulating polyploid populations. These softwares also elucidate the mode of inheritance whether it is disomic, polysomic or a mixture of both as in segmental allopolyploids. These tools also revealed the occurrence of double reduction and multivalent chromosomal pairing (Bourke et al. 2018). Using GBS, good prediction models for breeding in polyploidy wheat (Poland et al. 2012) and tetraploid potato (Sverrisdottir et al. 2017) have been developed and used successfully for genomic selection.

Integration of genome sequencing technologies along with proteomics and metabolomics known as chemical genomics has enriched genomic selection to accelerate the introgression of complex traits. Chemical genomics is genome analysis coupled with metabolomics which helps in studying the genetic response for identification of specific genotypes producing higher amount of a particular biomolecule or metabolite of industrial or pharmaceutical importance like growth regulators, hormones, drugs etc.

**Genome editing**

Recent advancements in genomics have also made feasible the editing of genomes and their use in crop improvement programs. Pre-breeding involves genetic transformation through recombination and genome editing (GE) tools provides an alternative. To replace conventional genetic engineering, a number of genome editing technologies have been developed during last two decades including antisense, RNA interference (RNAi), virus-induced gene silencing (VIGS), oligonucleotide directed mutagenesis (ODM), zinc-finger nuclease (ZFN), transcription activator-like effects nucleases (TALENs), and clustered regularly interspaced short palindromic repeats/Cas9 (CRISPR/Cas9) (Sauer et al. 2015). These genome editing technologies can accelerate pre-breeding programs through beneficial knockout mutations, e.g. identification of genes for disease resistance or suppressing of unwanted traits linked with desired traits in wild species, as products of these technologies are not considered a genetically modified organism (GMO) (Huang et al. 2016). Most of these genome editing tools except RNAi, act by inserting, removing or replacing specific regions of genome with the help of specific nucleases known as “molecular scissors” (Esvelt and Wang 2014).

GE approaches can be used to modify genes with defined quantitative trait nucleotides (QTNs) that cause a sizeable phenotypic effect. Further, GE tools can be used for broadening the allele pool through generating targeted variations useful for genomic selection (Scheben and Edwards 2017; Scheben et al. 2017). Using GE tools, desired traits can be physically linked to ensure their co-segregation known as “trait stacking” (Urnov et al. 2010). For example, ZFN-assisted gene targeting helped insertion of heritably insert herbicide-resistant genes (SuRA/SuRB and PAT) in the Z. mays genome (Shukla et al. 2009). Zhang et al. (2016) recently used CRISPR/Cas9 system for production of homoygous transgene free wheat mutants. Jenko et al. (2015) showed that GE and GS, both can be combined and referred as the promotion of alleles by genome editing (PAGE) which also has a great potential for pre-breeding in near future. Recently, Gupta (2019) reviewed the latest modification of CRISPR/Cas9 system, a base editing technology applicable to DNA as well as RNA, has revolutionized GE and demonstrated in several crops including rice, maize, wheat etc. will be highly useful for base broadening to be used for genomic selection and relating phenotypes to genes through mutant development, particularly in primitive landraces and wild species and will provide a new direction to pre-breeding programmes.

GE provides promising tools for a rapid site-specific editing of the genomes. Using GE tools, target traits can be improved faster than traditional or even molecular breeding. There are some aspects need not to be done in pre-breeding as these can be addressed more effectively by GE tools e.g. knocking out genes negatively affecting the target trait. Three major rice negative regulators of grain weight (GW2, GW5, and TGW6) were knocked out using a CRISPR/Cas9 system with a significant increase in thousand-grain weight in mutants (Xu et al. 2016). Recently, genome editing tool CRISPR/Cas9 has been used extensively to enhance yield through development of knockout mutants (Braatz et al. 2017).
Can we say advances in genomics has revolutionized the area of management of PGR and pre-breeding?

Undoubtedly, advancements in genomics has revolutionized the area of PGR management and pre-breeding by enabling crop curators of developing molecular core sets conserving all allelic variability without phenotyping, rapid trait mapping and so the rapid breeding, reduced gestation period required for transfer of target trait as mentioned below:

**Availability of different tools for assessing genetic variability, identification of markers and QTLs**

Sequencing platforms resulted in sequencing and resequencing of whole genome can be used for identification of new molecular markers (SNPs) and development of high-density map of traits. SNP arrays and genotyping by sequencing have been used extensively in GWAS studies.

**Reduction of long gestation period in the process of transfer of useful genes from wild or un-adapted germplasm to agronomically superior genepool**

With the availability of cost-effective sequencing platforms, sequencing and resequencing of whole genome has significantly reduced the time period required for mapping a trait. SNPs generated through WGRS and GBS of mapping populations or natural population (germplasm) can be used for rapid high resolution mapping of traits. Prebreeding involves interspecific crosses generally associated with introduction of linkage drag from wild species to the cultivated gene pool. Introgression through use of molecular markers linked to target traits which minimizes linkage drag also reduces the gestation period. In case of prebreeding of complex traits, combined use of GE and GS popularly known as PAGE also favours rapid pre-breeding.

**Genome editing tools with no transgene to broadening the genetic base and knockout negatively affecting genes**

Base editing tools which is a modification of CRISPR/Cas9 system can be used to generate the site-specific modifications in the genome to generate mutants with broadened genetic base. Further, knockout of negatively affecting genes have been used in crops like wheat and rice to get the benefit of target traits.

**Availability of over 56 whole genome assemblies in different crop species for trait-mapping, initiating need-based pre-breeding or improvement through GE**

With availability of over 56 whole genome assemblies in different crop species, whole genome resequencing and GBS of bulk or natural populations including landraces and wild relatives can be used for rapid species-specific trait mapping and so the rapid pre-breeding. GS based on total marker variability will be helpful in identifying rare frequency alleles contributing significantly towards complex trait in these crop species. A need based prebreeding programme may be initiated in these crop species to improve the complex traits like yield, climate resilience etc.

**Conclusion**

Genomics era has provided various technologies including sequencing and re-sequencing platforms, high-throughput trait-associated markers, cost-effective genotyping platforms and genome editing which can result in effective management of PGR with enhanced utilization along with efficient pre-breeding. No doubt, application of genomics tools has made management of PGR and pre-breeding more effective and efficient but still there are some bottlenecks in harnessing the full potential of genomic tools particularly the availability of high-throughput phenotyping platforms. We believe, marker/QTL assisted selection and genomic selection either alone or in combination will be used extensively in breeding/pre-breeding programs which will further enhance PGR utilization. The genomic tools will help conventional pre-breeding in broadening the genetic base of modern cultivars using landraces or wild relatives for various traits including higher yield, resistance to various biotic/abiotic factors and improved nutritional qualities.

**Declaration**

The authors declare no conflict of interest.

**References**


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