

REVIEW

Wheat genetic resources in the post-genomics era: promise and challenges

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Received: 26 May 2017 Returned for revision: 17 July 2017 Editorial decision: 5 September 2017 Accepted: 13 October 2017
Published electronically 12 December 2017

• **Background:** Wheat genetic resources have been used for genetic improvement since 1876, when Stephen Wilson (*Transactions and Proceedings of the Botanical Society of Edinburgh* 12: 286) consciously made the first wide hybrid involving wheat and rye in Scotland. Wide crossing continued with sporadic attempts in the first half of 19th century and became a sophisticated scientific discipline during the last few decades with considerable impact in farmers' fields. However, a large diversity of untapped genetic resources could contribute in meeting future wheat production challenges.

• **Perspectives and Conclusion:** Recently the complete reference genome of hexaploid (Chinese Spring) and tetraploid (*Triticum turgidum* ssp. *dicoccoides*) wheat became publicly available coupled with on-going international efforts on wheat pan-genome sequencing. We anticipate that an objective appraisal is required in the post-genomics era to prioritize genetic resources for use in the improvement of wheat production if the goal of doubling yield by 2050 is to be met. Advances in genomics have resulted in the development of high-throughput genotyping arrays, improved and efficient methods of gene discovery, genomics-assisted selection and gene editing using endonucleases. Likewise, ongoing advances in rapid generation turnover, improved phenotyping, envirotyping and analytical methods will significantly accelerate exploitation of exotic genes and increase the rate of genetic gain in breeding. We argue that the integration of these advances will significantly improve the precision and targeted identification of potentially useful variation in the wild relatives of wheat, providing new opportunities to contribute to yield and quality improvement, tolerance to abiotic stresses, resistance to emerging biotic stresses and resilience to weather extremes.

Key words: Wheat genetic resources, genomics, single nucleotide polymorphism (SNP), wild species, wide-hybridization

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) belongs to the tribe Triticeae (syn. Hordeae) and is one of the most important food crops, cultivated on about 220 million ha, providing food to one-third of the global population and providing 20 % of the global caloric requirements. Triticeae has more than 150 different species of various ploidy, among which bread wheat, durum wheat (*T. turgidum* L.), einkorn wheat (*T. monococcum* L.), rye (*Secale cereale* L.) and barley (*Hordeum vulgare* L.) are cultivated for food and commercial purposes (Ortiz *et al.*, 2008). Together, these species and their close and distant relatives constitute an important reservoir of genetic resources that include 434 358 accessions collected and stored in 23 gene banks and harbouring many beneficial alleles for wheat improvement. The knowledge of and manipulation of these resources has shaped a discipline called 'wheat wide crosses' or 'wide hybridization', which like other scientific disciplines, evolved significantly in recent decades due to significant advances in cytogenetics, genomics and other allied disciplines (Mujeeb-Kazi *et al.*, 2013; Dolezel *et al.*, 2014). This discipline has potential to bring about

'super-domestication' because it could lead to a domesticate with dramatically increased yield that could not be selected in natural environments from naturally occurring variations without resource to new technologies (Vaughan *et al.*, 2007).

Five decades ago we witnessed the demonstrable impact of the 'green revolution' (1967–1970) based on dwarfing genes, photoperiod insensitivity and stem rust resistance catalysed through the work in the International Maize and Wheat Improvement Center (CIMMYT) by conventional breeding methods; this was led by late Nobel laureate Dr Norman Borlaug and colleagues with international partnerships involving the National Agriculture Research Systems (NARS) in developing countries. CIMMYT wheat germplasm has impacted almost every wheat improvement programme globally. However, it was sensed very early after the Nobel Prize was awarded to Dr Borlaug in 1970 that there was a need to accelerate more efficiently the use of unique genetic diversity in wheat collections and wheat relatives for future yield improvement. This heralded the wide crossing programme at CIMMYT (Mexico), Kansas State University (USA) and a few other centres of wheat research and development, with emphasis on wheat

progenitor species, such as *Triticum monococcum*, *Aegilops tauschii* and *T. dicoccoides*, as well as more distant relatives including *Agropyron elongatum*, *Haynaldia villosa* and cultivated cereal rye (*S. cereale*). Until recently progenies of one derivative of cereal rye (1BL.1RS translocation) were grown on millions of hectares worldwide. Significant outcomes such as this encouraged donors in public and corporate sectors to invest in pre-breeding, and specific international projects like the Synthetic Evaluation Project in Australia (www.caigeproject.org.au), Wheat Initiative Strategic Partnership (WISP; www.wheatisp.org) in the UK, Seeds of Discovery (SeeD) in Mexico and DivSeek (www.divseek.org) were initiated to introduce genes of wild relatives into elite germplasm, making such resources available to wheat breeding programmes. Pay-offs from the wide crossing are quite apparent, but much greater effort must now be made if we are to harness the enormous but untapped potential genetic diversity that can be used not only to improve the yield and nutritional quality of wheat, but also to maintain and protect hard-fought yield increases from the many biotic and abiotic stresses that threaten the crop worldwide.

We are now facing the challenge of the two-fold increase in productivity target for a projected human population of 9.2 billion by 2050. This is further compounded by the challenges of climate change, stagnant wheat yields in many countries, outbreaks of new pathogen/pest races, and invasive weed species that delay progress and pose serious threats to world food security. It is

therefore prudent to approach the challenges of food security by placing emphasis on outcome-based research approaches. This should embrace strategies that focus on rapid returns by stringent targeting of alien genetic resources to be used ‘anew’ or exploitation of current user-friendly genetic stocks. The scientific community should be well aware of bandwagons to be avoided (Bernardo, 2016), and should adapt a realistic approach in using appropriate genomics and phenotyping technologies and analytical tools to harness actual outputs (Fig. 1).

We provide here an overview on the use of genomics to exploit wheat genetic resources (WGRs) and discuss the bottlenecks in utilizing those resources.

HISTORICAL ACHIEVEMENTS ARE HOPE FOR THE FUTURE: KEY CONSIDERATIONS

Moore (2015) argued that we are now equipped with new marker tools that provide increased efficiency for breeding, so various issues come to mind in discussing pragmatic approaches that are outcome-focused. The strict requirements for modern plant varieties has meant that breeders are reluctant to go back to wild relatives or even land races for new variation. However, the diversity present in the unadapted gene pool has provided many useful traits now deployed widely in elite germplasm, and expanded use of this germplasm resource is seen as

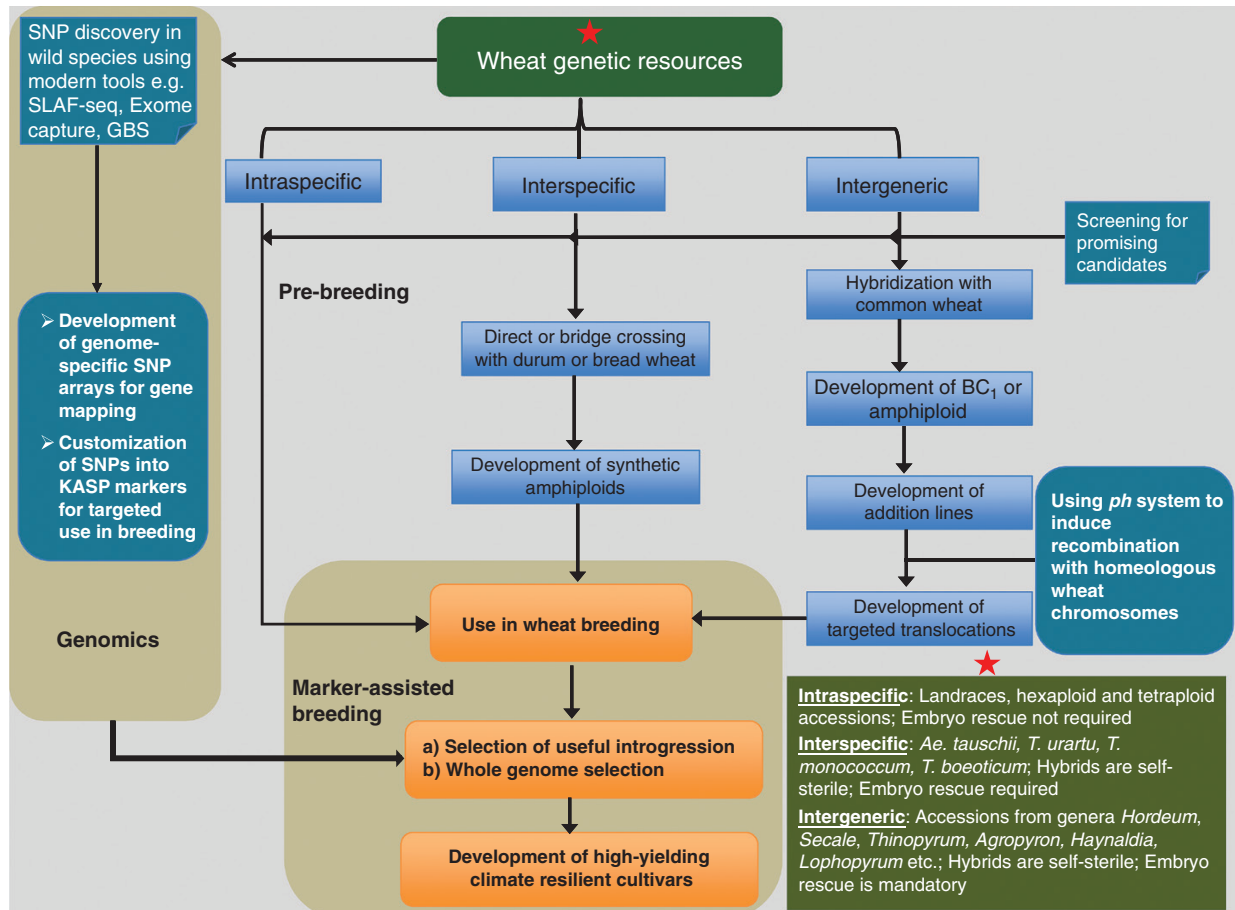


FIG. 1. A workflow depicting types of wheat genetic resources, pre-breeding and genomics-assisted breeding strategies to exploit in crop improvement.

crucial to accelerating the rate of genetic gain in plant breeding (McCouch *et al.*, 2013). This raises an important question of whether pre-breeding should restart with new hybrids of distant wild relatives or should we attempt to exploit what is currently available and set priorities that will result in early practical returns? We posit that the major impact will come from ‘homologous’ gene transfer reliant on genetic recombination involving the primary gene pool of wheat relatives. The potential impact is exemplified by the success of using the D genome (*Aegilops tauschii*)-derived user friendly genetic stocks (synthetic wheats, SH or SHW) produced at CIMMYT and elsewhere (Ogbonnaya *et al.*, 2013). Advanced derivatives of these materials have positively impacted yield and yield components, enhanced micronutrient content, provided new genes for resistance to major biotic and abiotic stresses, and improved the processing quality of elite varieties (Ogbonnaya *et al.*, 2013). Additional to the D genome donor there are A genome synthetics ($2n = 6x = 42$, AABBA^uA^vA^mA^m), the AABB tetraploids (*T. dicoccum*, *T. dicoccoides*, *T. polonicum* and *T. carthlicum*) and less used AABBDD hexaploids (e.g. *T. spelta* and *T. sphaerococcum*) representing unharnessed variability of the primary gene pool (Xie and Nevo, 2008; Mujeeb-Kazi *et al.*, 2013). Many leading varieties in Europe (e.g. ‘Robigus’) are derived from unknown introgressions from *T. dicoccoides*. Similarly, an introgression from *Ae. umbellulata* saved US wheat production from leaf rust in 1960; and a gene from *Ae. ventricosa* conferring resistance to eyespot has been exploited in breeding programmes (Garcia-Olmedo *et al.*, 1977). More recently, early results from whole genome re-sequencing of four elite cultivars suggest that two of the cultivars carry a 20-Mbp translocation on chromosome 2A representing the *Vpm-1* introgression from *Ae. ventricosa* (Pozniak *et al.*, 2017). Beyond these, there is the extensive diversity of the tertiary gene pool (species of distant relatives of wheat that tend to produce anomalous, lethal or completely sterile hybrids when crossed with wheat; embryo rescue may be necessary to obtain viable hybrids) (Fig. 1).

The most significant measurable output from a tertiary germplasm source was the fortuitous spontaneous wheat-alien chromosome translocation 1BL.1RS that occurred in the 1930s, and greatly contributed to world wheat production after its release in Eastern and Western Europe, Russia, China and CIMMYT. Various varieties were distributed by CIMMYT from the mid-1970s, initially with VEERY ‘S’, and national selections of it such as Pakistan 81 (Pak-81) in Pakistan and elsewhere. Varieties carrying the 1BL.1RS translocation occupied more than 50 % of the wheat area in China and a significant proportion across Europe, Asia, Africa and other wheat growing regions in the 1990s, and continue to cover around 25 % of the present area in China. Another example is Xiaoyan 6, an alleged derivative of a cross between wheat and the 70 chromosome grass species *Thinopyrum ponticum*, and its derivatives have also occupied millions of hectares in China. Besides these, three genera further exemplify the complexity of using distant relatives despite having the advantage of being diploids and preferred for wide hybridization programmes: *Haynaldia villosa*, *Thinopyrum elongatum* and *Thinopyrum bessarabicum*. However, we assert that the true measure of success in pre-breeding using distant relatives can only be measured by their contribution to improved or maintained wheat productivity in

farmer fields; that is, the magnitude of the practical impact on yield improvement measured by the tonnes/hectare of output.

ADVANCES IN CYTOGENETICS TO FACILITATE ALIEN INTROGRESSIONS AND RECOMBINATION

Continuing advances in wheat improvement will rely on effective mining of genetic variation, which is dependent on how efficiently we can make genetic recombination and exploiting the biological mechanisms controlling recombination and meiosis. Wheat has a long history of cytogenetics research, and is also one of the best suited crops for cytogenetics studies due to the huge array of aneuploid stock (Sears, 1954; Law *et al.*, 1987; Endo and Gill, 1996). The outcomes of advances in wheat cytogenetics could be categorized, but not limited to (1) development of precise wheat–alien genetic stocks for gene discovery and breeding (Mujeeb-Kazi *et al.*, 2013; Khlestkina, 2014), (2) advancements in molecular cytology to study polyploidization, genome evolution and structure (Ali *et al.*, 2016; Patokar *et al.*, 2016), (3) initiation, control and enhancing the rate and distribution of homoeologous recombination between wheat and alien chromosomes (Moore, 2014) and (4) advances in chromosome flow-sorting to develop chromosome-specific libraries as a conduit to reducing complexity for further genomics and sequencing applications (Dolezel *et al.*, 2014). Previously, an extensive review was published on the protocols and list of genetic stocks, for example amphiploids, chromosome addition, and substitution lines and translocations carrying genes of breeding interest (Mujeeb-Kazi *et al.*, 2013). These genetic stocks were developed by key centres such as Kansas State University (<http://www.k-state.edu/wgrc/>), Kyoto University, Japan (<https://shigen.nig.ac.jp/wheat/komugi/>), John Innes Centre (<https://www.jic.ac.uk/GERMPLASM/Wheat-Precise-Genetic-Stocks.htm>), CIMMYT wide crossing programme, University of Adelaide, Australia (<http://www.agwine.adelaide.edu.au/research/germplasm/>), and Nanjing Agriculture University, China. Therefore, here we briefly discuss the genomics interventions for homoeologous recombination and use of flow cytometry for discovery of single nucleotide polymorphisms (SNPs) from wild species facilitating gene discovery.

Bread and durum wheat are tetra- and hexaploid but behave as diploid because synapsis and crossover only occurs between homologous chromosomes, despite highly similar gene order and content between homoeologous chromosomes. Both Riley and Sears observed that homeologous recombination is possible in the 5B chromosome deletion line (Riley and Chapman, 1958; Sears and Okamoto, 1958), which was later defined as the *Ph1* locus present on the long arm of chromosome 5B. Moore and co-workers presented a series of experiments on molecular characterization of the *Ph1* gene and localized the *Ph1* locus to a 2.5-Mb region in smaller chromosomal deletions (Griffiths *et al.*, 2006) containing a cluster of *cdc2*-like genes (also known as *Hyp3* gene), and a segment of heterochromatin within the cluster (Griffiths *et al.*, 2006; Al-Kaff *et al.*, 2008). Greer *et al.* (2012) also suggested that the *cdc2* cluster may alter chromatin structure. Consistent with these results is that treatment with okadaic acid, an inhibitor of phosphatase activity, increases cdk2-type phosphorylation and phenocopies the *ph1b* allele by inducing crossovers (Knight *et al.*, 2010). Contrary to these reports, Bhullar *et al.* (2014) proposed *C-Ph1* as a candidate

for *Ph1*, as they used virus-induced gene silencing (VIGS) and RNA interference (RNAi) to suppress expression from all *C-Ph1* homoeologous copies in wheat, observing meiotic metaphase I chromosome clumping and chromosome associations, similar to the effects occurring in the absence of *Ph1*. Recently, *Hyp3* was annotated as ZIP4 by EMBL-EBI, which is known as a major controller of crossovers in rice (Shen et al., 2012). Sears (1977) developed a single *Ph1*-deleted mutant referred to as CS *ph1B*, which has been extensively used globally to introgress chromosome segments from wild relatives into bread wheat. Several other *Ph1* mutants have been described, such as *ph1c* in tetraploid wheat (Giorgi et al., 1978), and the *Ph1* gene from *T. speltoides* transferred to ‘Chinese Spring’ to develop genetic stock ‘CS *Ph1*’, which has shown remarkable crossover between wheat and *H. villosa* chromosomes (Chen et al., 1994). Recently, two *TaZIP4-B2 (Ph1)* mutant lines (Cadenza1691 and Cadenza0348) were selected from a Cadenza-mutant population and these exhibited high levels of homeologous crossovers and are claimed to be more suitable than *Ph1* locus deletion lines (CS *ph1b*) (Rey et al., 2017). As the sequence underpinning variation in the ZIP4 gene within the *Ph1* locus is clearly known, this demonstrated the potential use of modern genomics tools to precisely develop desired genetic stocks for wheat pre-breeding.

Besides *ph1*-mediated homeologous recombination of wheat with wild species, understanding the rate and distribution of recombination in conventional wheat hybridization remains a major challenge. However, little is known about the variation of the genome-wide recombination rate within plant species. Such information could assist in manipulating those loci to break unfavourable linkage blocks and create desirable recombination. Recently, a nested association mapping (NAM) population comprising 60 bi-parental populations from Watkin’s landraces and ‘Paragon’ was characterized and used to build a consensus map, and 114 quantitative trait loci (QTLs) for crossover counting were identified. About 50 % of QTLs with increasing effects were from non-reference parents, and landrace alleles of QTL regions on chromosomes 2D, 3B, 5A and 7A could be good candidates to increase recombination rates (Wingen et al., 2017). However, no practical example to manipulate these QTLs for increasing the rate of recombinations in breeding programmes has yet been demonstrated.

Since Wang et al. (1992) reported the first successful wheat chromosome-enriched library using flow cytometry, it has witnessed remarkable progress due to its promise in reducing the complexity associated with genomics and sequencing application. The analysis of sequences at the single chromosome level has provided new insights into the structure of the complex and polyploid wheat genome, where comparisons between homeologous chromosomes were the main bottleneck to sequencing and assembly of the genome. Efforts by the International Wheat Genome Sequencing Consortium were based largely on the construction of ready-to-sequence chromosome arm-specific BAC libraries, which indicated that chromosome genomics can contribute materially to the analysis of genomes lacking a high-quality reference sequence (Dolezel et al., 2014). Sequencing single chromosomes has been highly productive in the context of marker development and validation, especially in wheat–alien hybrids. In some cases, alien chromosome differs in DNA content from those of host species; its peak should be recognizable and can therefore be sorted, or chromosome addition

or substitution lines are used to sort alien chromosome. Tiwari et al. (2014) provide a classic example, in which a wheat/*Ae. geniculata* disomic substitution line [DS5M^S S(5D)] was used to sort the 5M^S chromosome and then subjected to next generation sequencing (NGS) for discovery of SNPs specific to the 5M^S chromosome. These 5M^S-specific SNP markers were then used to screen translocations with smallest introgressions carrying genes resistance to leaf rust (*Lr47*) and stripe rust (*Yr40*). Hence, powerful molecular marker tools with least ascertainment bias were developed and then used to track the smallest useful introgressions to avoid linkage drag.

MODERN GENOTYPING PLATFORMS FOR WHEAT GENETIC RESOURCES

In the genomics era, rapid advances in biotechnological techniques should be better targeted to mine genetic diversity as part of pre-breeding and be more closely integrated with conventional breeding programmes to achieve better and faster breeding outcomes. Effective genotyping tools should cover complete genomes and should be high-throughput and cost-effective. The key question remains as to what extent have advances in genomics dealt with the two biggest constraints in exploiting genes from distant relatives, namely ascertainment bias and linkage drag? Solving this is only possible if the genotyping platforms are unbiased to structural variations [large insertions or deletions (indels) and copy number variations] and SNPs in WGRs and abundant enough to target the recombination points in introgression lines.

The NGS of mRNA (RNA-seq) in diverse wheat germplasm produces transcript assemblies permitting development of high-density SNP arrays amenable for genotyping large populations for gene mapping and discovery (Cavanagh et al., 2013). However, SNPs in those arrays were biased to the genetic backgrounds of accessions used for RNA-seq, which were predominantly improved varieties and few landraces. Earlier SNP arrays such as the 9K (Cavanagh et al., 2013) and 90K (Wang et al., 2014) arrays had very few SNPs to detect polymorphism in WGRs (Wang et al., 2013). Hence these genotyping arrays remain ineffective in capturing rare variants among diverse alien genetic resources due to ascertainment bias, and result in hampered identification of introduced chromosomal segments and/or alleles from distantly related genetic resources. Winfield et al. (2016) used a different approach by targeted re-sequencing of wheat exomes for identification of SNPs from 43 bread wheat and wild relatives. They were able to develop a wheat 820K Axiom SNP array consisting of polymorphic SNPs from bread wheat varieties, *T. monococcum*, *Ae. tauschii*, *Th. bessarabicum*, *Th. ponticum*, *Th. intermedium*, *Th. elongatum*, *S. cereale*, *Ae. speltoides*, *Ae. markgrafii*, *Ae. mutica*, *Ae. variabilis*, *T. timopheevii* and *T. dicoccoides*. King et al. (2016) later developed a 35K wheat relative array from a subset of the 820K Axiom array and demonstrated its functionality by developing high-density linkage maps of introgression lines from wheat/*Amblyopyrum muticum* derivatives. The marker array and millions of data points available through WISP (www.wheatisp.org) are huge resources (Moore, 2015), of which the Axiom 820K and Axiom 35K wheat relative arrays are

capable of tracking introgressions from a range of species in the secondary and tertiary gene pools (Winfield *et al.*, 2016).

In addition to fixed array-based genotyping platforms there are *de novo* genotyping-by-sequencing (GBS)-based platforms that are applicable for various crops regardless of prior knowledge of genomics, genome size, organization or ploidy. In comparison with whole-genome sequencing, reduced representational sequencing has many advantages, such as reducing genome complexity, avoiding inherent ascertainment bias in current fixed SNP arrays, and lower cost. It has been applied in studies on evolutionary genomics, genome-wide association studies (GWAS) and marker-assisted breeding. Parallel efforts have been made to exploit specific locus amplified fragment sequencing (SLAF-seq) in *Agropyron* (Zhang *et al.*, 2015) and *Thinopyrum* (Chen *et al.*, 2013) for genome-specific SNP marker discovery. These efforts have demonstrated that successful strategies do exist to identify and exploit rare diversity present in wheat relatives. However, these academic demonstrations should be translated on an applied scale for practical outcomes. We now have high-throughput, cost-effective uniplex genotyping platforms such as Kompetitive Allele-specific PCR (KASP), which can enhance the selection process among wide cross progenies; these capabilities were previously impossible (Rasheed *et al.*, 2016).

These new genotyping platforms have facilitated genetic mapping and gene discovery in wheat (Table 1). Their major application areas are for association of available natural diversity with traits of agronomic importance and improved understanding of the genetics of important traits, but gaps remain in the integration of phenotype–genotype–environment cues, as do the challenges of appropriate statistical design and analysis models to optimize outcomes.

NEW INSIGHTS INTO GENOME EVOLUTION AND GENETIC DIVERSITY

There is a consensus that bread wheat evolved through two polyploidization events between *T. urartu* and *Ae. speltoides*-related species 0.5 Mya forming *T. turgidum*, and between *T. turgidum* and *Ae. tauschii* 10 000 years ago forming modern hexaploid wheat. However, the phylogeny and evolutionary history of progenitor species and *Aegilops–Triticum* are more complex than our current understanding from nuclear (Marcussen *et al.*, 2014) and chloroplast genome assemblies (Li *et al.*, 2015a). The genome assemblies of bread wheat and five diploid relatives provided novel insight and helped to estimate the evolutionary relatedness and divergence times of ancestral components of bread wheat (Marcussen *et al.*, 2014). Divergence of the A and B genomes from a common ancestor is estimated to have occurred almost 7 Mya, and these genomes gave rise to the D genome through homoploidy 5–6 Mya. These findings laid the foundation for a new framework to understand bread wheat genome as a multi-level phylogenetic mosaic.

This initiated a debate on the origin of hexaploid wheat. Marcussen *et al.* (2014) proposed a scenario of a homoploid origin of *Ae. tauschii* through hybridization of ancient A- and B-genome species 5 Mya. Gornicki *et al.* (2014) later analysed the 25 chloroplast genomes from 13 species, but they did not address the origin of the D genome. Li *et al.* (2015b)

re-evaluated the origin of *Ae. tauschii* based on data from nuclear and chloroplast genomes and concluded that the homoploid origin of *Ae. tauschii* was far more complex than envisaged by Marcussen *et al.* (2014). The nested topology of *Ae. tauschii* suggested its origin involved multiple rounds of both ancient and recent hybridizations. Sandve *et al.* (2015) responded to the findings and reported that the disagreement between Marcussen *et al.* (2014) and Li *et al.* (2015b) was due to the differences in the nomenclature used to describe the major *Triticum/Aegilops* clades and that the homoploid hybridization reported by Marcussen *et al.* (2014) gave rise to an ancestor of all D+S*+M clade species, including *Ae. tauschii* and *Ae. sharonensis*, contrary to just *Ae. tauschii* as reported by Li *et al.* (2015b). Similarly, the origin of the B genome remains a subject of ongoing debate (Feldman and Levy, 2015), and numerous phylogenetic studies have been carried out since the early work of Zohary and Feldman (1962). Taken together, the findings of these studies suggested two hypotheses. The first was that the progenitor of the B genome is a unique, ancient *Aegilops* species that remains unknown [i.e. monophyletic origin and ancestor closely related to *Ae. speltoides* (*Sitopsis* section)]. The second hypothesis was that the B genome resulted from introgression of several unknown parental *Aegilops* species (i.e. polyphyletic origin) from the *Sitopsis* section. Furthermore, El Baidouri *et al.* (2017) proposed a reconciled evolutionary scenario based on transposable elements which complemented the earlier studies. They concluded that more complete genome sequences from diploid, tetraploid and hexaploid wheat will offer long-term opportunities to improve upon the currently proposed evolutionary scenario to explain how the modern bread wheat genome evolved from its diploid progenitors (El Baidouri *et al.*, 2017).

The past three years have also witnessed an increasing number of reports on using modern genomics technologies to characterize the wheat germplasm collections. SNP arrays and *de novo* GBS platforms have been used in characterizing landraces (Wingen *et al.*, 2014; Sehgal *et al.*, 2015; Vikram *et al.*, 2016; Riaz *et al.*, 2017), and these offer great potential. If these technologies were integrated with choice of germplasm and appropriate analytical approaches, they could provide solutions to the problems of linkage drag. Vikram *et al.* (2016) investigated ‘Creole’ wheat accessions held in CIMMYT’s gene bank using the GBS platform, and identified opportunities to harness these materials in developing the next generation of high-yielding wheat varieties. Most of the 8416 ‘Creole’ landraces were genetically similar, but some were adapted to extreme environments, and a reference set of 1133 accessions captured 89 % of the rare alleles present in the whole collection. Parallel studies on characterization of the century-old Vavilov (Riaz *et al.*, 2017) and Watkins (Wingen *et al.*, 2014; Jordan *et al.*, 2015) collections, and of the CIMMYT landrace collection (Sehgal *et al.*, 2015) aimed at mobilizing unused diversity in wheat breeding have also been recently reported.

The A. E. Watkins global landrace collection, consisting of 826 landraces from 32 countries, was established to develop resources for wheat research and breeding (Wingen *et al.*, 2014). The Watkins collection is also a core pillar of the WISP project initiated to capture useful diversity from exotic sources. More insightful analysis of diversity in the Watkins collection using the 35K SNP Breeder’s array identified substantial numbers

of novel SNP variants which either have not been captured in current breeding programmes or have been lost through previous selection pressure (Winfield *et al.*, 2017). Genetic diversity studies on this collection led to a core set of 120 accessions capturing most of the inherent diversity. Each of these lines was crossed with variety ‘Paragon’ to generate segregating populations amenable to NAM. These populations, comprising over 9000 unique individuals, were genotyped, to produce more than three million data points (Moore, 2015). These efforts enabled identification of 130 loci for nitrogen-use efficiency and biomass accumulation, and marker-assisted selection (MAS) will allow some of these genes to be deployed in wheat breeding programmes. The Watkins collection was also assessed for diversity at the *Pina-D1* and *Pinb-D1* loci (Qamar *et al.*, 2014), and for resistance to rusts (Bansal *et al.*, 2013), eyespot (Burt *et al.*, 2014) and root-lesion nematode (Thompson and Seymour, 2011). Similarly, the Vavilov landrace collection of 295 accessions from 25 countries was assessed for diversity using DArT-seq markers and later screened for leaf rust reaction (Riaz *et al.*, 2016). Accessions resistant to leaf rust and phenotyped for other useful characters were hybridized with selected varieties to exploit useful variations through speed-breeding (L. Hickey, University of Queensland, personal communication). Manickavelu *et al.* (2014) analysed genetic diversity in 446 landraces from Afghanistan using GBS markers and similarly highlighted the importance of exotic alleles present in these resources.

With regard to other species in the Triticeae, Ren *et al.* (2013a) analysed genetic diversity in wild emmer wheat from Israel and Turkey, and found genetic diversity to be correlated with ecological factors. Similarly, Ren *et al.* (2013b) evaluated genetic diversity in a worldwide collection of 150 durum accessions and concluded that the richest genetic diversity occurred in South America, North America and Europe. Wang *et al.* (2013) analysed diversity in global *Ae. tauschii* collections using the 10K Infinium SNP array and established two major lineages with little genetic contact. Each of the two lineages has two sub-lineages and it was concluded that lineage 2 in the south-western and southern Caspian region is the main source of the wheat D genome. Winfield *et al.* (2016) developed a 820K SNP array specific for wheat secondary and tertiary gene pool species, but did not assess diversity in large collections of these species. Thus, the 820K SNP array and its subsequent version, the ‘wheat relative 35K SNP array’, hold promise for studies of genetic diversity to target useful variation in large gene bank collections, and derivatives of wild relatives such as amphiploids and partial amphiploids, chromosome addition lines and translocation lines. These reports on genetic diversity studies are timely and necessitate discussion on prioritization of WGRs, especially landraces, and the use of available innovative genomics technologies to more effectively harness diversity in ancient and close relatives of wheat.

GENOME-WIDE ASSOCIATION STUDIES AND GENOMIC PREDICTIONS IN WGR

Conventional linkage mapping using bi-parental mapping populations is the most common method to detect QTLs for complex traits in plants. However, GWAS is considered a powerful tool

for resolving complex trait variations and preliminary identification of loci for novel traits in natural populations. Compared to classical linkage mapping, GWAS provides a more representative gene pool and a higher mapping resolution because all historical meiotic events that have occurred in ancestors of a diverse panel of germplasm can be assessed. Furthermore, GWAS mapping bypasses the expense and time of developing mapping populations, and enables the mapping of many traits in one set of genotypes, making the method more efficient and less expensive than linkage mapping (Huang and Han, 2014). Thus, GWAS mapping is now routinely used in genetic studies.

While linkage mapping and GWAS are the most widely used approaches to understand the genetic architecture of quantitative traits in WGRs, very few examples have led to gene discovery of complexly inherited traits and/or variety development through MAS. For example, the wheat grain gene database lists 1527 QTLs as at the time of this study (<https://wheat.pw.usda.gov>, accessed 30 April 2017). This is consistent with the opinion expressed by Bernardo (2016) that this gap persists despite early optimism that increased knowledge on genetic architecture of quantitative traits through gene mapping will increase our understanding of underlying genes for exploitation in breeding. Successful examples of genes or QTLs from WGR studies being used in breeding include, but are not limited to, the *Fhb1* gene from Sumai 3, *Gpc-B1* from *T. dicoccoides* and *Pm21* from *Hyalida villosa*. Others such as *Sr2* and *Lr34* were selected in conventional breeding by phenotypic markers before the underlying genetics and function were discovered.

The number of studies using GWAS and genomic predictions continue to increase rapidly. SHWs and landraces have been central to such studies (Börner *et al.*, 2015; Jordan *et al.*, 2015), but there have been reports on derivatives of tertiary gene pools such as advanced lines derived from *Th. intermedium* and *Ae. sharonensis*. GWAS have been conducted in hexaploid landraces for rust resistances (Jordan *et al.*, 2015; Pasam *et al.*, 2017), agronomic traits and grain mineral contents (Manickavelu *et al.*, 2017). Similarly, tetraploid landraces were studied for agronomic traits (Hu *et al.*, 2015). SHWs were studied for rust resistance (Zegeye *et al.*, 2014), root architecture and multiple disease resistances (Mulki *et al.*, 2013), insect resistances (Joukhadar *et al.*, 2013), boron tolerance (Emebiri and Ogbonnaya, 2015), pre-harvest sprouting response (Imtiaz *et al.*, 2008) and grain morphology (Rasheed *et al.*, 2014) using DArT and SNP markers.

Among primary gene pool species, *Ae. tauschii* collections have been widely targeted for GWAS of morphological traits (Liu *et al.*, 2015b), phosphorus deficiency (Liu *et al.*, 2015a) and drought tolerance (Qin *et al.*, 2016), and several genomic regions were identified. Spelt wheat (*T. spelta*), now gaining favour in several parts of the world (Longin and Wurschum, 2016), was also used in GWAS for agronomic and disease resistance traits using a 15K SNP array (Würschum *et al.*, 2017). Among tertiary gene pool species, 125 accessions of the diploid species *Ae. sharonensis* were used to identify stem rust resistance genes by GWAS, with mapping populations used to validate two *Sr* genes (Yu *et al.*, 2017). Intermediate wheatgrass (*Th. intermedium*) has high grain yield, large seed weight and several other desirable agronomic traits. Genomic analysis in this grass species using GBS markers indicated high prediction ability for biomass and grain weight (Zhang *et al.*, 2016). This

could be helpful to increase the efficiency of recurrent selection and accelerate domestication and improvement in this species. It has also been argued that associations between SNP alleles and environment of origin in crop landraces reflect adaptation, and these could be used to predict phenotypic variation for adaptive traits. Thus, envirotyping is essential, as evidenced from several recent studies which detected population genetic signatures of adaptation around GWAS loci. Examples can be found in other crops such as African rice, where Meyer *et al.* (2016) identified 11 significant loci, four of which are within ~300 kb of genomic regions that possess signatures of positive selection for six salt tolerance traits as identified from whole-genome re-sequencing of 93 landraces. Similarly, Lasky *et al.* (2015, and references therein) reported that genomic studies in sorghum landraces have demonstrated that genome–environment associations (i.e. associations between SNP alleles and environment of origin of accessions) can be used to identify adaptive loci and predict phenotypic variation.

In contrast to GWAS, genomic prediction or genomic selection (GS) studies have gained more importance in breeding, because unlike GWAS and QTL mapping it is not used to identify trait-associated genomic regions, but rather to predict the performance of germplasm based on genomics-estimated breeding values (GEBVs) (Heffner *et al.*, 2009). GS has emerged as a valuable tool for improving complex traits controlled by QTLs with small effects. Various simulation models for predicting selection accuracy depend largely on marker density, size of training populations (a population subset used to train a best prediction model) and trait heritability. A detailed GS experiment on 97 synthetically derived introgression populations indicated several candidates with higher GEBVs than the respective recurrent bread wheat parents with a clear contribution of synthetic parents in improving grain yield in heat-stressed environments (Jafarzadeh *et al.*, 2016). Similar results were reported by Duncel *et al.* (2017) in a GS study on synthetic derivatives from a cross with the bread wheat parent ‘Opata M85’. Optimization of GS in WGRs is now becoming routine in many breeding programmes focused on harnessing new diversity from alien species, and has been explored for domesticating new crops such as *Th. intermedium* (Zhang *et al.*, 2016), and landraces for rust resistance (Daetwyler *et al.*, 2014; Pasam *et al.*, 2017), mineral contents (Manickavelu *et al.*, 2017), and heat and drought stress adaptation (Crossa *et al.*, 2016). Compared to QTL mapping and GWAS, GS has more promise in harnessing the genetic gains from WGRs for quantitative traits and is seen as a more reliable and useful approach (Bernardo, 2016). However, the key challenges in successful practice of GS depend on: cost-effectiveness and less biased approaches for genotyping; software for handling, quality control and joint analysis of genotypic, phenotypic and environment data; and a streamlined work flow for using GS within the overall breeding pipeline.

GENE DISCOVERY AND ISOLATION USING MODERN GENOMICS TOOLS

Gene discovery in WGRs is a relatively long process compared to gene discovery in adapted germplasm. This is mainly due to the time required for introgression into adapted wheat

backgrounds, followed by QTL mapping, validation, fine mapping and subsequent gene cloning (Fig. 2). Given previous reviews in the use of WGRs (Mujeeb-Kazi *et al.*, 2013; Longin and Reif, 2014), we will focus here on recent advances in genomics that have significantly shortened the gene discovery process in WGRs. Due to affordable, high-throughput sequencing technologies innovative approaches are emerging to discover genes in wild relatives. For example, well-annotated genes with distinctive functions and sequences can be captured using gene family-specific oligonucleotide probes, which are then sequenced and assembled to provide the genetic information of the gene in wild relatives. This strategy has been successfully used to discover and clone genes underlying stem rust resistance in *Ae. tauschii* (Steuernagel *et al.*, 2016). This recently developed technology is fast and does not rely on recombinant populations or fine mapping. It combines mutagenesis and genome complexity reduction and is referred to as resistance-gene enrichment sequencing (RenSeq or MutRenSeq). The stem rust resistance gene *Sr50* derived from *S. cereale* was also cloned using mutant populations and sequencing of BAC clones (Mago *et al.*, 2015). Thind *et al.* (2017) reported ‘targeted chromosome-based cloning via long range assembly’, which combines complexity-reduction via chromosome flow sorting and also works in chromosome regions with reduced recombination rates. They cloned the broad-spectrum leaf rust resistance gene *Lr22a* in wheat with *de novo* genome assembly, marker information and mutant population. Another strategy that holds promise for gene discovery in WGRs is through RNAseq, which involves SNP discovery in bulks with contrasting phenotypes (referred to as bulked segregant analysis) and has recently been used to fine map the *Gpc-B1* (Trick *et al.*, 2012) and *Yr15* (Ramirez-Gonzalez *et al.*, 2015) genes from *T. turgidum* subsp. *dicoccoides* and *T. dicoccoides*, respectively.

GENOME EDITING TOOLS HOLD PROMISE FOR GENE DISCOVERY IN WGR

The recent developments in genome editing based on site-specific nucleases offer exciting potential to precisely edit targeted genes with greater speed. These methods include zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN) and clustered regulatory interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), which were originally developed in non-plant systems and are now being adopted to modify genes in crop plants (Lozano-Juste and Cutler, 2014). These technologies make use of sequence-specific designer nucleases that cleave targeted loci to create small indels of novel DNA, and even replacement of individual alleles. ZFN and TALEN suffer from difficulties in design, construction, cost and uncertain success rates. By contrast, CRISPR-Cas9 is robust, affordable and easy to use and has proved useful across a range of plant species (Jiang *et al.*, 2013). Although reports on the use of CRISPR-Cas9 are limited, it is expected that large-scale germplasm-characterization efforts in conjunction with CRISPR-based genome-editing technologies will herald a new era whereby crop plants can be precisely modified without necessarily needing to use physical seed samples that contain important traits. Starting with genetically less complex

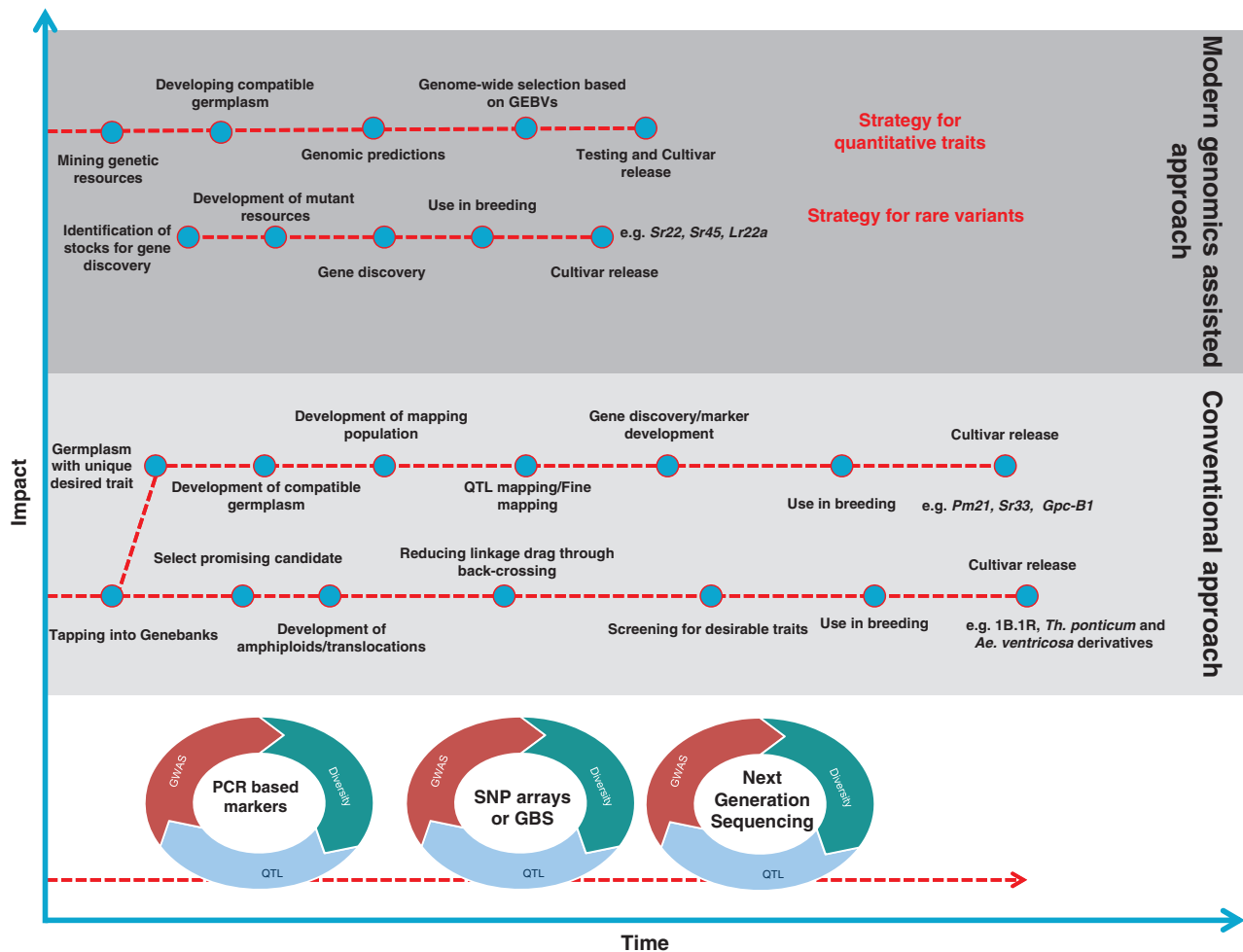


FIG. 2. Research and breeding activities surrounding wheat genetic resources plotted against time (x-axis) and impact on wheat improvement (y-axis). QTL mapping, GWAS and genetic diversity studies will continue with discovery of new technologies and have given low impact as very little has been converted into resources that are actually used in breeding and gene discovery. The major impact of genomics technologies will be in reducing the time to characterize and deploy useful variation with reduced linkage drag.

traits and large-effect alleles, breeders will increasingly be able to ‘overwrite’ undesirable allelic variants in otherwise elite material using beneficial variants discovered through genetic analyses of other plant genetic resources. It is relatively simple to demonstrate correlative associations between sequence variants and phenotypic traits, but it can take years to demonstrate causation using laborious approaches such as fine mapping, expression analysis, biochemical modelling and other functional genomics tools. CRISPR-Cas9 offers a shortcut to establish causation quickly by editing candidate causal variants (Li *et al.*, 2013). As ‘digitalization’ of genetic resources unfolds, the role and significance of gene banks will grow in that they will transform into biological discovery platforms and enhance the role of crop diversity as a source of haplotypes encoding desirable traits. Another area where gene editing could hold promise is in inactivating undesirable dragged genes, a major impediment in the use of wild relatives in breeding because introduced chromosome segments come as variably sized linkage blocks that may carry genes

conferring negative impacts on traits of economic interest. A classic example is the 1RS component of the T1BL.1RS translocation carrying a gene(s) for sticky dough.

REGULATIONS ON GERMLASM ACCESS AND DATA SHARING

Ownership of genetic resources and genes, and better access to genomics database resources in an era of information technology must be resolved to ensure easier access to genetic resources worldwide. A fundamental principle for granting access to genetic resources must be an assurance of fair and equitable sharing of the benefits arising from their use. The huge investments made by private companies in recombinant DNA technologies in the 1980s and release of commercial genetically modified (GM) crops greatly contributed to strengthening intellectual property rights (IPR), a trend that has gained strength since adoption of the Trade-Related Aspects of

TABLE 1. Modern genomics platforms for wheat genetic resources with different breeding and genetics objectives

Method	Species	Genomics technology/strategy	Reference
Draft or whole-genome sequencing	<i>Aegilops tauschii</i>	Illumina HiSeq2000, Roche 454	Jia <i>et al.</i> (2013)
	<i>Triticum turgidum</i> (subsp. <i>dicoccoides</i>)	Whole genome sequencing	Avni <i>et al.</i> (2017)
	<i>T. urartu</i> <i>T. monococcum</i> , <i>T. urartu</i> , <i>Ae. sharonensis</i> , <i>Ae. speltoides</i> , <i>Ae. tauschii</i>	Illumina HiSeq2000, Roche 454 Illumina HiSeq2000	Ling <i>et al.</i> (2013) Marcussen <i>et al.</i> (2014)
Transcriptome sequencing	<i>Ae. tauschii</i>	Roche 454	Iehisa <i>et al.</i> (2014)
	<i>T. urartu</i>	Roche 454	Ling <i>et al.</i> (2013)
	<i>Ae. sharonensis</i>	Roche 454	Bouyioukos <i>et al.</i> (2013)
	<i>T. urartu</i>	Illumina HiSeq2000	Krasileva <i>et al.</i> (2013)
	<i>T. monococcum</i>	Illumina HiSeq2000	Fox <i>et al.</i> (2014)
	<i>Ae. variabilis</i>	Illumina HiSeq2000	Xu <i>et al.</i> (2012)
	<i>T. turgidum</i> subsp. <i>dicoccoides</i>	Illumina HiSeq2000	Akpinar <i>et al.</i> (2015)
Genotyping platforms for diversity studies	<i>Ae. tauschii</i>	10K SNP infinium	Wang <i>et al.</i> (2014)
	10 wild species	820K Affymetrix	Winfield <i>et al.</i> (2016)
	<i>Amblyopyrum muticum</i> but applicable to many species	35K wheat relative array from Affymetrix	King <i>et al.</i> (2016)
	Landraces	35K wheat breeder's array	Allen <i>et al.</i> (2017)
	<i>Thinopyrum elongatum</i>	SLAF-seq	Chen <i>et al.</i> (2013)
	<i>Agropyron cristatum</i>	SLAF-seq	Zhang <i>et al.</i> (2015)
	Creole landraces	DARtseq	Vikram <i>et al.</i> (2016)
	CIMMYT landraces collection	Genotyping-by-sequencing	Sehgal <i>et al.</i> (2015)
	Watkins landrace collection	Illumina 90K SNP	Jordan <i>et al.</i> (2015)
	Vavilov landrace collection	DARtseq	Riaz <i>et al.</i> (2016)
	Watkins landrace collection	Exome capture	Shi <i>et al.</i> (2017)
	Wild emmer	Illumina GoldenGate	Ren <i>et al.</i> (2013a, b)
	Wheat/ <i>Aegilops geniculata</i>	Chromosome-specific SNP discovery	Tiwari <i>et al.</i> (2014)
	<i>Ae. tauschii</i>	<i>Sr33</i>	Periyannan <i>et al.</i> (2013)
	<i>T. urartu</i>	<i>Sr22</i>	
<i>Secale cereale</i>	<i>Sr50</i>	Mago <i>et al.</i> (2015)	
<i>T. dicoccoides</i>	<i>Gpc-B1</i>	Trick <i>et al.</i> (2012)	
Watkins landrace collection	<i>Pin-D1</i>	Qamar <i>et al.</i> (2014)	
Sumai 3 landrace	<i>Fhb1</i>	Rawat <i>et al.</i> (2016)	
Genome-wide association studies	Synthetic hexaploids	Illumina 9K for stripe rust response	Zegeye <i>et al.</i> (2014)
	Synthetic hexaploids	Illumina 9K for tolerance to boron toxicity	Emebiri and Ogbonnaya (2015)
	Watkins landrace collection	Illumina 90K SNP array for rust resistance	Jordan <i>et al.</i> (2015)
	<i>Ae. tauschii</i>	10K Infinium array/agronomic traits	Liu <i>et al.</i> (2015b)
	<i>Ae. tauschii</i>	10K Infinium array/drought tolerance	Qin <i>et al.</i> (2016)
	<i>Ae. tauschii</i>	10K Infinium array/phosphorus deficiency traits	Liu <i>et al.</i> (2015a)
	<i>T. spelta</i>	15K SNP array/agronomic traits	Würschum <i>et al.</i> (2017)
	<i>Ae. sharonensis</i>	Stem rust resistance	Yu <i>et al.</i> (2017)
	Wild emmer	Illumina SNP GoldenGate/agronomic traits	Hu <i>et al.</i> (2015)
	Mexican and Iranian landraces	DARtseq genotyping/agronomic traits	Crossa <i>et al.</i> (2016)
Genomic predictions	<i>Th. intermedium</i>	Genotyping-by-sequencing	Zhang <i>et al.</i> (2016)
	Synthetically derived wheats	GBS/heat and drought adaptability	Jafarzadeh <i>et al.</i> (2016)
	Landraces	Minerals/GBS	Manickavelu <i>et al.</i> (2017)
	Landraces	Rust resistance	Pasam <i>et al.</i> (2017), Daetwyler <i>et al.</i> (2014)

Intellectual Property Rights (TRIPS) Agreement. In response to concerns that IPR were being detrimental to global progress in plant improvement, new policy frameworks emerged to regulate access to, and use of, genetic resources. The first legally binding intergovernmental agreement was the convention of biological diversity (CBD) in 1993, which established access and benefit-sharing principles by which anyone wishing to access genetic resources from a particular country must obtain prior informed consent (PIC) from relevant authorities and establish mutually agreed terms (MAT) specifying the conditions under which the resources can be used. The CBD was seen as a significant treaty for consolidating efforts to conserve biodiversity, but was also seen as a barrier to sharing genetic material, including domesticated plants and their wild relatives.

The Plant Treaty also known as the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPFGFR) that came into force in 2004 was designed to operate in harmony with the CBD, and was the first agreement to recognize farmers' rights. It established a multilateral system of access and benefit sharing regulated by a standard material transfer agreement (SMTA) that defines non-negotiable MAT. The Plant Treaty's objectives are the conservation and sustainable use of PGRFAs and the fair and equitable sharing of benefits arising out of their use for sustainable agriculture and food security.

With current advancements in the genomics of wheat genetic resources, we can now predict agronomic potential using genome-wide marker scans or select plants with specific genes for biotic and abiotic resistances without exposing them to the relevant

stresses. The need for wheat genetic resources will grow as a result of these technologies, which will enable breeders to eliminate 70–80 % of individuals in any generation without having to invest in laborious multi-environmental field testing. Therefore, a more efficient regulatory framework for better access to germplasm for both public and commercial breeding purposes will facilitate the deployment of recent and emerging advances so that global impacts that were previously not possible could be catalysed. Initiatives such as WISP and SeeD are classical examples where germplasm resources are freely available on request along with genotyping data. For example, a toolkit comprising 40 lines derived from landrace/‘Paragon’ crosses, and capturing diversity in specific traits, has been provided to breeders, and is freely available to all on request in the WISP project. These resources could be particularly useful for less developed countries that lack sophisticated genotyping facilities. Out-sourcing is also an increasingly common practice that is both time- and cost-effective (Rossetto and Henry, 2014). As the germplasm and genomic data could be easily available, more investment is needed to train scientists from developing countries in data analysis and decision support tools to enable global exploitation of WGRs. Considerable investment in data management and informatics systems will be required to ensure that gene banks can fulfil their roles as custodians of the expanding genetic knowledge linked to their physical resources. This view was echoed by Shaw *et al.* (2017), who reported that the ability to effectively manage genetic resource collections and integrate unique and diverse data types is crucial in exploring, understanding and exploiting the diversity contained within gene banks. A ‘one shoe fits all’ approach that promotes a common platform may negate the achievement of that goal. Instead we posit that there is some sense of urgency in directing efforts towards ensuring that the disparate gene bank databases are interoperable, ontologies for collection and characterization are harmonized, and ego-geographical, edaphic and biotic information for the accessions be strengthened at points of collection. While recent advances in genotyping technologies will enhance the use of genetic resources, that by itself is not enough, and must be coupled with advances in phenotyping tools integrated with advances in sensing technologies that link environmental cues with phenotypic plasticity and genotypic (DNA, proteomic and metabolic) signatures if the goals of achieving food security and improving current agricultural output are to be met.

TIME FACTOR IN EXPLOITATION OF GENETIC RESOURCES: MAKING LONG JOURNEY SHORTER

A major constraint in using WGRs in breeding programmes is the time factor associated with difficulties in evaluating WGRs with widely different phenologies of growth habit, flowering time and height. These differences make accurate assessments and comparisons difficult, if not impossible. Even if useful characteristics can be identified the difficulties of transferring desirable traits into cultivated species are considerable, often requiring embryo rescue and cytological expertise. The time required to transfer traits from WGRs and to enable their use in breeding often may exceed that in conventional breeding programmes. The most important impact from innovative genomics technologies in utilizing WGRs will be reducing the time to harness beneficial alleles with reduced linkage drag and increased capacity to do so (Fig. 2).

The recent advances in the development of high-throughput, time-saving methods fall into five categories:

1. *Rapid generation advancements*: CIMMYT introduced the ‘shuttle breeding’ strategy, which moves germplasm between contrasting environments: Ciudad Obregon in north-western Mexico and Toluca in the highlands of Central Mexico, enabling two generations per year. This has enabled screening for a range of traits such as photo-period sensitivity, heat tolerance and arrangement of important biotic and abiotic stresses (Ortiz *et al.*, 2007). Double haploids (DHs) and single seed descent rapidly bring about homozygosity, but usually without selection. A new generation advancement method called ‘speed breeding’ uses constant light and precisely controlled temperature to accelerate plant growth and development (Hickey *et al.*, 2012, 2017). Screening protocols can be combined with speed breeding (Riaz *et al.*, 2016; L. Hickey, personal communication). Several advantages in using speed breeding include: (a) 7 weeks to complete one cycle, (b) controlled environmental factors for homogenous treatment of populations for screening, and (c) can be practised year-round. Based on these points, speed breeding holds significant promise to accelerate exploitation of WGRs in a time-efficient manner. Similarly, Zheng *et al.* (2013) reported a procedure which combines embryo culture with management of watering regimes, lighting intensity and duration, temperature and quantity of potting mixture that allows the production of up to eight generations of wheat and nine generations of barley per year. However, a drawback may be its limitation in mimicking diverse environmental factors attainable under field conditions.
2. *Rapid gene cloning through mutation and genomics*: WGRs are largely seen as sources of rare variants controlled by major genes, not specifically for quantitative traits. The discovery of such genetic factors in WGRs is now a rapid process due to the advancements in genomics, and we have seen discovery of several rust resistance genes from *Ae. tauschii* and *T. monococcum* within a few years (Periyannan *et al.*, 2013; Steuernagel *et al.*, 2016). The three-step method (MutRenSeq) that combines mutagenesis with exome capture and sequencing provided a rapid way to clone R genes from wheat wild relatives, a classical example being discovery of *Sr22* from *T. monococcum* and *Sr45* from *Ae. tauschii*. MutRenSeq is fast, cheap and takes <24 months to complete because it is independent of fine mapping, uses the generation of a physical contig across the map interval and is easily scalable. This approach can be applied to most crops or their wild relatives, and will allow the cloning of R genes that could be used in multi-R gene pyramids, a strategy that promises more durable disease resistance in crops. Thind *et al.* (2017) reported ‘targeted chromosome-based cloning via long read assembly (TACCA)’, which offers great flexibility with respect to gene validation and could be used for traits with partial phenotypes such as partial disease resistance or abiotic stress tolerance. They also compared various rapid gene cloning methods and concluded that TACCA holds significant promise and is equivalent to positional cloning. Unfortunately, gene discovery for quantitative traits is still lagging behind because it depends on heritability of trait, number of genes, extent of quantitative variation, and size and position of recombinants in the genome. Bulk-segregation analysis by making bulks of contrasting phenotypes followed by exome-capture or NGS could be an effective strategy to underpin genes for quantitative traits in WGRs, and it has been successfully used

to identify the genetic basis of kernel numbers per row in maize (Yang *et al.*, 2015) and holds promise for underlying gene discovery for quantitative traits in WGRs.

3. *High-throughput genotyping and phenotyping platforms:* In recent years, there have been rapid advances in high-throughput genotyping arrays or GBS. It is now possible to genotype hundreds of samples for high-density markers within a couple of days (Rasheed *et al.*, 2017). The 820K and 35K wheat SNP chips, GBS and DArTseq platforms are classical examples. However, accurate phenotyping of growth, yield and stress responses in both the field and controlled environments remains a major bottleneck in both pre-breeding and breeding.

Recent developments in high-throughput phenotyping based on imaging (Fahlgren *et al.*, 2015), spectral reflectance and remote-sensing (Araus and Cairns, 2014) will make significant impacts with regard to characters such as variation for leaf surface temperature associated with osmotic components of salinity stress and quantified by high-throughput infrared thermography (Z. Khan *et al.*, Quaid-i-Azam University, Pakistan, unpublished data). Although there is still no demonstration on the application of high-throughput phenomics on WGRs or wild species, they are likely to bridge the phenome–genome gap. Recent and continuing advances in computing, robotics, machine vision and image analysis to the wider field of plant biology will make significant contributions in phenotyping (Furbank and Tester, 2011). The ultimate benefit will be to what extent they contribute to achieving improved rates of genetic gain by significantly influencing the key components: selection intensity, selection accuracy, and identification and introgression of desirable genetic variation within a short period of time.

4. *High-throughput marker-assisted selection strategies:* Progress in developing high-throughput single marker genotyping was relatively slow compared to current high-density genotyping platforms. Single marker genotyping is very important for wheat pre-breeding programmes because breeders are much more interested in deploying specific alleles from WGRs: for example, screening for the presence of alien translocations, or new rust resistance genes. A high-throughput KASP marker toolkit for wheat breeding that includes markers for the 1B.1R translocation, *Lr37*, *Yr15* and several other alleles for agronomic traits was recently developed (Rasheed *et al.*, 2016). KASP markers have scalable flexibility without compromising data throughput and it is possible to obtain 150K data points in one day. KASP is a commercial technology involving chemical reagents from LGC Genomics, and it is a relatively expensive procedure. A step change in lowering costs of single marker genotyping is semi-thermal asymmetric reverse PCR (STARP) markers, which unlike KASP markers, can be used with many types of commercial chemical reagents (Long *et al.*, 2016).

5. *Rapid gene editing technologies:* The establishment of efficient and specific CRISPR/Cas9-mediated gene editing methods with decreased off-target mutations in a short period of time is a major focus for researchers. Liang *et al.* (2017) recently established a DNA-free gene editing method using CRISPR/Cas9 ribonucleoproteins (RNPs), taking only 7–9 weeks with 4–5 independent mutants produced from 100 immature wheat embryos. The main steps of this method include RNP preparation, RNP

functional validation, RNP coating and delivery, plantlet regeneration and mutant identification. The most important advantage of CRISPR/Cas9 RNP-mediated genome editing is elimination of transgene integration and the small DNA insertions that can be generated. This is highly desirable for public acceptance of genome-edited plants and holds significant promise for exploitation of WGRs within reduced time periods.

CONCLUSION AND FUTURE PROSPECTS

The success of a programme, in our view, is global impact using new diversity that can easily be manipulated to generate user-friendly genetic stocks that are unique, readily accessible to both public and private sectors, amenable to immediate use in breeding, and globally distributed, as originally occurred with synthetic wheats produced at CIMMYT and elsewhere. It was those collective features that catalysed their worldwide use and subsequent release of more than 30 high-yielding varieties in Mexico, China, Pakistan, USA and other countries. Synthetically derived germplasm currently constitutes about 30 % of entries in international nurseries distributed annually to over 150 countries by CIMMYT and ICARDA. Despite the proven evidential value of synthetic hexaploid wheats and their derivatives in contributing novel alleles for wheat improvement, such materials have scarcely been characterized using current genomics resources and statistical genetic methods. In addition, there are also few extensive wide crossing programmes at present, and most scientists previously trained in cytogenetics have moved to other areas. In a world that is reluctant to accept transformation technologies in our major food crops, training in cytogenetics could give rapid returns in translating academic research into breeding outcomes. The value of deploying advances in genomics, phenotyping, envirotyping and statistical tools to optimize the benefits in SHW as a valuable genetic diversity for wheat improvement also lies in the fact that they can be directly hybridized with cultivated wheat. Widely shared germplasm derived from wide crosses and open-access databases for WGRs will enhance more expanded collaborations including developing countries. It is timely to undertake a critical appraisal of the prioritization of WGRs that will be appropriate to meet production targets set by policy-makers around current production constraints within reasonable time lines by harnessing and optimizing existing resources. The urgency is now more compelling if the sustainability goals are to be attained.

ACKNOWLEDGEMENTS

We are grateful to Prof. Bob McIntosh (University of Sydney, Australia) and Dr Peter Langridge (University of Adelaide, Australia) for reviews of the manuscript.

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