

# Crop Breeding Chips and Genotyping Platforms: Progress, Challenges, and Perspectives

Awais Rasheed<sup>1,2</sup>, Yuanfeng Hao<sup>1</sup>, Xianchun Xia<sup>1</sup>, Awais Khan<sup>3</sup>, Yunbi Xu<sup>1,2</sup>, Rajeev K. Varshney<sup>4</sup> and Zhonghu He<sup>1,2,\*</sup>

<sup>1</sup>Institute of Crop Sciences, National Wheat Improvement Center, Chinese Academy of Agricultural Sciences (CAAS), Beijing 100081, China

<sup>2</sup>International Maize and Wheat Improvement Center (CIMMYT), c/o CAAS, Beijing 100081, China

<sup>3</sup>Department of Plant Pathology and Plant-Microbe Biology, Cornell University, Geneva, NY, USA

<sup>4</sup>International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502324, India

\*Correspondence: Zhonghu He ([zhhecaas@163.com](mailto:zhhecaas@163.com))

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## ABSTRACT

There is a rapidly rising trend in the development and application of molecular marker assays for gene mapping and discovery in field crops and trees. Thus far, more than 50 SNP arrays and 15 different types of genotyping-by-sequencing (GBS) platforms have been developed in over 25 crop species and perennial trees. However, much less effort has been made on developing ultra-high-throughput and cost-effective genotyping platforms for applied breeding programs. In this review, we discuss the scientific bottlenecks in existing SNP arrays and GBS technologies and the strategies to develop targeted platforms for crop molecular breeding. We propose that future practical breeding platforms should adopt automated genotyping technologies, either array or sequencing based, target functional polymorphisms underpinning economic traits, and provide desirable prediction accuracy for quantitative traits, with universal applications under wide genetic backgrounds in crops. The development of such platforms faces serious challenges at both the technological level due to cost ineffectiveness, and the knowledge level due to large genotype-phenotype gaps in crop plants. It is expected that such genotyping platforms will be achieved in the next ten years in major crops in consideration of (a) rapid development in gene discovery of important traits, (b) deepened understanding of quantitative traits through new analytical models and population designs, (c) integration of multi-layer -omics data leading to identification of genes and pathways responsible for important breeding traits, and (d) improvement in cost effectiveness of large-scale genotyping. Crop breeding chips and genotyping platforms will provide unprecedented opportunities to accelerate the development of cultivars with desired yield potential, quality, and enhanced adaptation to mitigate the effects of climate change.

**Key words:** Single nucleotide polymorphisms (SNPs), Genotyping-by-sequencing (GBS), SNP arrays, Crop breeding, Genotyping platforms

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## INTRODUCTION

The current yield gain trends in major crops are insufficient to feed a global population of 9 billion by 2050 (Ray et al., 2012). Feeding such a huge population is further challenged by climate change, which is predicted to get worse in the future with altered rainfall patterns (leading to floods or drought), extreme weather events, and changing patterns of pathogens and pests in terms of severity and distribution (Abberton et al., 2015). Nobel Peace Prize Laureate Dr. Norman E. Borlaug pointed out that modern genomics and biotechnological tools have had a great impact on medicine and public health, but

have also provided potential for a second Green Revolution that would come about by combining the invaluable scientific methodologies and products with conventional breeding techniques (Borlaug, 2000).

Development and application of molecular markers in crop genetics have gained remarkable attention in the last three decades. Molecular markers started with low-throughput

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restriction fragment length polymorphisms (RFLPs) (Tanksley et al., 1989) and recently culminated in single nucleotide polymorphism (SNP) markers based on next-generation sequencing (NGS) technologies (Varshney et al., 2009). A major landmark was reached with crop-specific simple sequence repeat (SSR) markers that were easily used, abundant in number, and highly polymorphic. These markers facilitated the development of high-density maps at that time for common wheat (Somers et al., 2004), rice (McCouch et al., 2002), barley (Varshney et al., 2007), maize (Smith et al., 1997), potato (Sharma et al., 2013), apple (Khan et al., 2012), pear (Wu et al., 2014), and other crops (Varshney et al., 2005). Despite the frequent use of SSRs for gene mapping and tagging, there was limited potential for use in practical plant breeding for the following four reasons (Xu and Crouch, 2008). Firstly, it is challenging to properly identify precise information in terms of multiple alleles per locus. Secondly, it is difficult to integrate or compare SSR data from different platforms or populations. Thirdly, SSR motifs are finite in a genome and not evenly distributed. Last but not least, gel-based SSR analysis is cost-ineffective as genotyping is laborious and time consuming. Therefore, it is of paramount importance to establish simple, accurate, high-throughput platforms for marker-assisted breeding. SNPs are abundant in crop genomes, and are ideal markers for genetic discovery research and molecular breeding. Likewise, SNPs from cloned genes and genome-wide linkage and association analyses using array-based platforms complemented with genotyping-by-sequencing (GBS) permit development of robust tool kits for application in breeding.

The current genomics landscape of crop plants has been revolutionized due to the NGS technologies, which provides a plethora of sequencing information with great improvements in coverage, time, and costs (Bevan and Uauy, 2013). These technologies profoundly facilitate the development of chip-based marker platforms for genotyping in an ultra-high-throughput fashion. Although sophisticated genotyping platforms have substantially improved genetic mapping and gene discovery studies, and reduced the time and cost to genotype large populations, their applications in practical crop improvement are very limited. Molecular diagnostics in crop improvement programs still heavily rely on conventional gel-based markers or other low-throughput platforms that hinder application of large-scale marker-assisted selection due to increased cost and time. Progress in gene isolation has provided diagnostic markers for use in breeding, but its application in high-throughput assays continues to be slow in many crops.

Several high-throughput multiplex and single-plex marker platforms in rice (Masouleh et al., 2009), wheat (Berard et al., 2009; Bernardo et al., 2015; Rasheed et al., 2016), legumes (Varshney et al., 2016), and other crops have been proposed for marker-assisted selection (MAS), but the implementation of such platforms in crop breeding is hindered due to the lower efficiency of customization, higher cost, desired flexibility, and costly equipment needed for application. The development of ultra-high-throughput, cost-effective genotyping platforms for practical breeding still has a long way to go, but is an ultimate objective. We defined such platforms that could genotype tens of thousands of breeding accessions with tens to hundreds of

markers in a short duration for actual breeding use. We anticipate that our review and perspectives on several aspects of genotyping platforms are invaluable in opening further discussion of various development and application opportunities in crop breeding.

## POTENTIAL OF MOLECULAR BREEDING IN DEVELOPING NEXT-GENERATION CROP CULTIVARS

### Field Crops

The rate of historical genetic gains has indicated that reliance on conventional breeding methods alone is unsustainable to fulfill the need of the burgeoning population (Ray et al., 2012), and we need innovative breeding strategies to accelerate the rate of genetic gains in crop breeding (Barabaschi et al., 2016; Bevan et al., 2017; Xu et al., 2017b). For this reason the scientific community has made heavy investment in developing genomic resources and intelligent decision support systems that would likely reduce the genotype–phenotype gap and provide the effective tools to develop next-generation cultivars (Batley and Edwards, 2016; Varshney et al., 2016). The most commonly applied molecular tools for crop breeding are molecular markers, which are used for parental selection, genetic diversity estimation, and reducing the linkage drags and genomics-assisted breeding. There is much debate on appropriate genomics tools that have actual impact on crop improvement and do not turn into the so-called “bandwagons” after heavy investment of time and resources (Bernardo, 2016). Fortunately, “genomic selection” and MAS facets of molecular breeding are considered a research area that has become an impactful and rewarding discipline. The lower cost, high read accuracy, and competing sequencing systems are increasing the availability of markers for crop breeding (Kang et al., 2016). Today we have several successful examples whereby application of molecular tools has effectively contributed to developing cultivars in rice (Miah et al., 2013; Rao et al., 2014), millet (Goron and Raizada, 2015), maize (Prasanna et al., 2010), several legumes (Pandey et al., 2016), and horticultural crops (Kole et al., 2015; Iwata et al., 2016). The other application area of these array- and NGS-based platforms is for association of available natural diversity with traits of agronomic importance, which have improved our understanding of the genetics of important traits. Similarly, heterotic patterns in polyploid crops such as wheat (Zhao et al., 2015), and hybrid vigor in rice (Xu et al., 2014a) and maize (Riedelsheimer et al., 2012) have been widely elucidated by genotyping arrays. The availability of high-quality whole-genome reference sequences and subsequent pan-genome sequences data accelerate gene mapping and discovery, potentially allowing the application of MAS (all types of selection methods during crop breeding including genomic selection).

### Trees and Long Juvenile Species

Horticultural trees that produce fruits and nuts divvy a remarkable market share for agricultural products. These include woody perennials that usually have a very long juvenile phase ranging from at least 3 years (almonds) to >15 years (avocado). The benefits of MAS to a breeder are greatest when the targeted species takes a long time to reach maturity and is expensive to grow and maintain. Thus, MAS holds particular promise in perennials since

they are often costly and time consuming to grow until maturity for evaluation (van Nocker and Gardiner, 2014). For example, apple breeders wait as long as 7 years from seed to identifying the tree as potential parent. As the first step, foreground selection of seedling progenies for major genes “must-have traits,” e.g., pest and disease resistance, flesh color, and root-stock dwarfing ability, using molecular markers could significantly reduce the number of seedlings to be raised for breeding and population development. Kumar et al. (2012) used genomic selection by 8K SNP array and demonstrated further reduction in the time and cost for developing potential parents for apple breeding. Desirable germplasm was developed in ~2 years instead of 5–7 years in the case of conventional phenotypic-based selection (Kumar et al., 2013).

Several fixed SNP arrays have been developed in fruit trees including apple (Bianco et al., 2014, 2016), pear (Montanari et al., 2013), peach (Verde et al., 2012), and grape (Le Paslier et al., 2013), but one issue associated with SNP arrays is the constraint posed by the number of SNPs on the array, which limits the application of genome-wide association studies (GWAS) for association of candidate markers with trait-specific alleles that can be used for screening. This limitation is being addressed by the subsequent development of arrays with higher numbers of SNPs, such as in apple from 20K to 480K (Table 1); however, this also increases the genotyping costs of screening. We discuss this aspect below in detail, as this is a general constraint in the development of fixed SNP arrays. A step change in throughput is offered by GBS and its use in horticultural crops is rapid, with a report of its use for genetic map construction in *Rubus* (Ward et al., 2013), as well as several reports on its development and application in apple, grape, pear, and kiwifruit (van Nocker and Gardiner, 2014).

### Genotyping Scenarios and Decision Support Tools

As shown in Figure 1, the molecular markers derived from modern genomics tools should be high density, cost effective, and high throughput and are able to be used for GWAS, quantitative trait locus (QTL) mapping, and gene discovery. This information is then used to manipulate trait variation for several of the breeding objectives. The successful application of molecular markers in crop breeding programs is not as common as it should be, although the effective practice and implementation of genomic- and marker-based selections could be predicted as a routine activity in breeding programs. The development of high-throughput, cost-effective platforms for crop breeding is not only necessary but is now more applicable as most genotyping requirements can be outsourced commercially at affordable prices. Breeding programs, especially in developing countries, can now apply MAS, including marker-assisted backcrossing (MABC), marker-assisted gene pyramiding, marker-assisted recurrent selection (MARS), and genome-wide or genomic selection (GS), to breed crop cultivars without large capital investments, technological upgrading, or training (Rossetto and Henry, 2014). Different molecular breeding approaches usually need different genotyping platforms. The genotyping scenarios can be categorized as follows: (a) hundreds of samples for few target markers as in gene tagging, gene transfer, or introgression breeding; (b) hundreds of samples for several to hundreds of markers as in quality control (QC) analysis for

hybrid purity; (c) hundreds of samples for few targets and hundreds of background markers as in MABC; and (d) hundreds to thousands of samples for up to thousands of markers as in GWAS, GS, and linkage mapping experiments. Among genotyping platforms, array- and NGS-based platforms are suitable for genotyping hundreds to thousands of samples with many markers such as required in gene mapping experiments and GS, or a few samples with many markers such as for genetic diversity analysis or background selection, whereas molecular breeding activity such as gene tagging, MABC, and QC analysis needs flexible platforms with relatively fewer markers, and array-based platforms are not suitable for such studies.

The initiation of the genomic open-source breeding informatics initiative (GOBii; <http://gobiiproject.org/>) and other initiatives such as the integrated breeding platform (IBP; <http://www.integratedbreeding.net>) would help in aligning MAS with conventional crop breeding in developing countries where the real impact of these innovative technologies could significantly increase genetic gains in key food crops. For example, the Breeding Management System (BMS), the core product of IBP, is an integrated statistical analysis tool that supports different stages of crop breeding processes and is useful for analyzing phenotypic and genotypic datasets and managing day-to-day activities through all phases of breeding programs. This is an open-source, one-stop shop for all of the tools required for genomics-assisted breeding programs. One such tool for MAS experiments is the marker-assisted backcross breeding tool (MBDT) (<https://www.integratedbreeding.net/179/training/bms-user-manual/marker-assisted-backcross-breeding-tool>). This tool comprises six modules including data validation, phenotyping, linkage map building, QTL analysis, genome display, and MABC sample size. Apart from these modules, several other analytical and decision support tools for data analysis software, data storage, and data management will usher crop breeding programs into a modern, knowledge-based crop breeding era, leading to sustainable crop production (Varshney et al., 2016).

## GENOTYPE-TO-PHENOTYPE GAP IN CROPS: A MAJOR LIMITATION FACTOR IN MOLECULAR BREEDING

A complete understanding of gene networks underlying the important traits, for example yield, quality, and traits associated with resilience to climate change, would revolutionize our ability to breed next-generation crop cultivars. However, the lack of access to such information is not fully attributed to the limited genomics interventions but also to the phenotyping bottlenecks (Furbank and Tester, 2011) and genotype–environment interaction (Xu, 2016). Here we briefly discuss the strategies and tools to bridge the genotype–phenotype gap, which is a key step toward the development of practical breeding chips.

### Genetic Architecture for the Economically Important Traits

It is prudent that bridging the genotype–phenotype gap requires us to simultaneously record genotype, phenotype, and environment. The most widely used strategies to identify underlying genetics are performing linkage mapping studies in family-based populations,

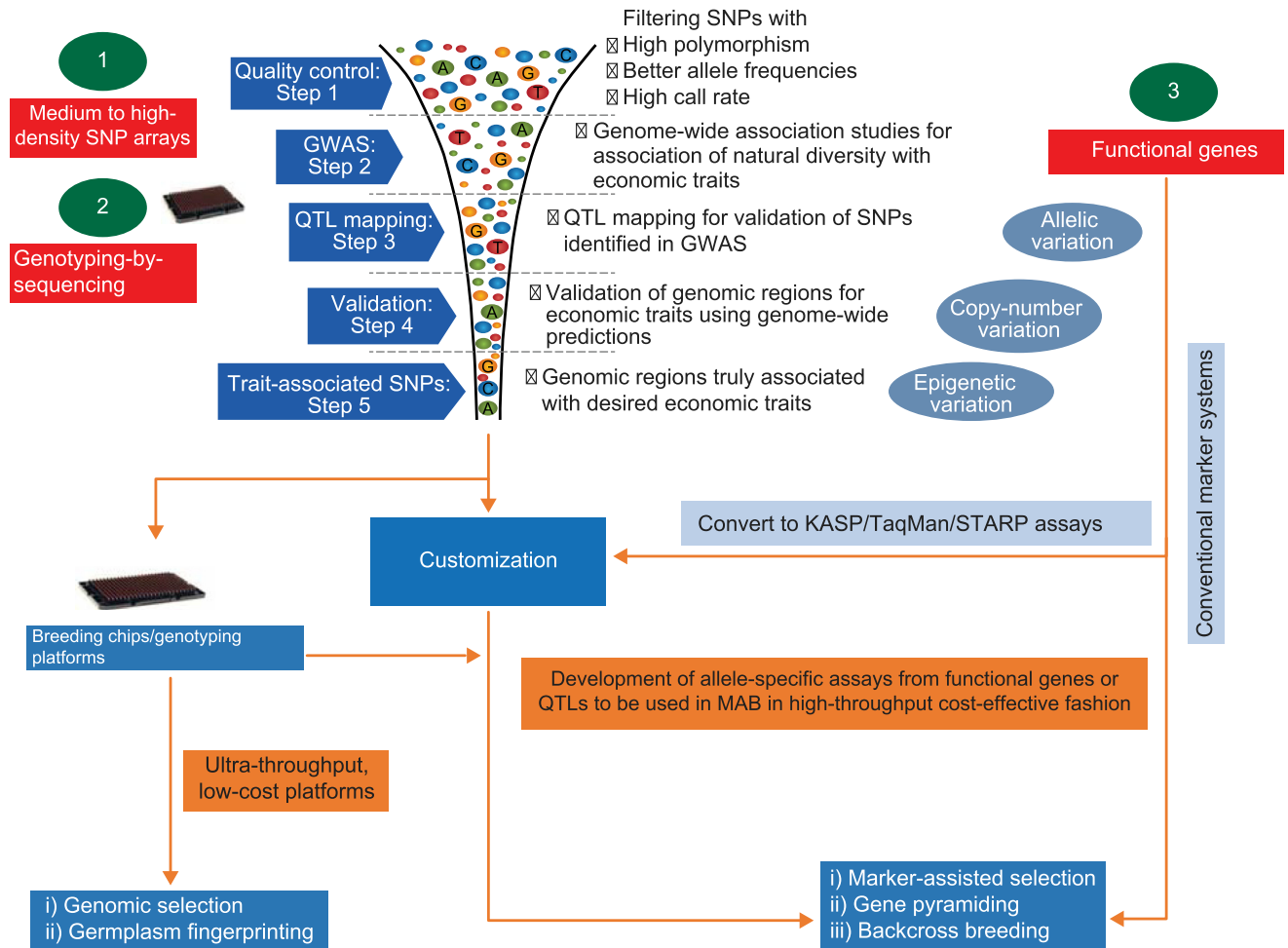
Crop	Size	Technology	Information/resource	Reference
Apple	20K	Illumina Infinium BeadChip		<a href="#">Bianco et al. (2014)</a>
Apple	480K	Affymetrix Axiom	Axiom Apple480K	<a href="#">Bianco et al. (2016)</a>
Apple	8K	Illumina Infinium BeadChip		<a href="#">Chagné et al. (2012)</a>
Barley	9K	Illumina Infinium BeadChip		<a href="#">Comadran et al. (2012)</a>
Brassica	60K	Illumina Infinium BeadChip	International Brassica SNP Consortium	<a href="#">Clarke et al. (2016)</a>
Brassica	15K	Illumina Infinium BeadChip	TraitGenetics	Unpublished
Cherry	6K	Illumina Infinium BeadChip	RosBREED 6K SNP	<a href="#">Peace et al. (2012)</a>
Chickpea	50K	Affymetrix Axiom	Axiom <i>Cicer</i> SNP Array	
Cotton	63K	Illumina Infinium BeadChip	In development	<a href="#">Hulse-Kemp et al. (2015)</a>
Cotton	35K	Affymetrix Axiom	Axiom cotton Genotyping array	
Cowpea	60K	Illumina Infinium BeadChip		<a href="#">Close et al. (2015)</a>
Grape	18K	Illumina Infinium BeadChip	GrapeReSeq 18K Vitis	<a href="#">Le Paslier et al. (2013)</a>
Grape	9K	Illumina Infinium BeadChip	Vitis9KSNP	<a href="#">Myles et al. (2010)</a>
Lettuce	35K	Affymetrix GeneChip		<a href="#">Stoffel et al. (2012)</a>
Maize	50K	Illumina Infinium BeadChip	MaizeSNP50 BeadChip	<a href="#">Ganal et al. (2011)</a>
Maize	3K	Illumina Infinium BeadChip	Subset of MaizeSNP50	<a href="#">Rousselle et al. (2015)</a>
Maize	600K	Affymetrix Axiom	Axiom 600K	<a href="#">Unterseer et al. (2014)</a>
Maize	50K	Affymetrix Axiom	Maize 55K Axiom	<a href="#">Xu et al. (2017a)</a>
Oat	6K	Illumina Infinium BeadChip	Infinium 6K Oat array	<a href="#">Tinker et al. (2014)</a>
Peach	9K	Illumina Infinium BeadChip		<a href="#">Verde et al. (2012)</a>
Pear	1K (9K)	Illumina Infinium BeadChip	8K apple chip + 1K Pear	<a href="#">Montanari et al. (2013)</a>
Peanut	58K	Affymetrix Axiom	Axiom_Arachis array	<a href="#">Pandey et al. (2017)</a>
Pepper	16K	Illumina Infinium BeadChip		<a href="#">Ashrafi et al. (2012)</a>
Pepper	640K	Affymetrix GeneChip	<a href="https://pepchip.genomecenter.ucdavis.edu">https://pepchip.genomecenter.ucdavis.edu</a>	In progress
Poplar	12K	Illumina Infinium BeadChip		<a href="#">Favre-Rampant et al. (2016)</a>
Potato	20K	Affymetrix Axiom	SolSTW array	<a href="#">Vos et al. (2015)</a>
Potato	8K	Illumina Infinium BeadChip		<a href="#">Hamilton et al. (2011)</a>
Rice	1M	Affymetrix		<a href="#">McCouch et al. (2010)</a>
Rice	50K	Illumina Infinium BeadChip	RiceSNP50	<a href="#">Chen et al. (2014)</a>
Rice	6K	Illumina Infinium BeadChip	RICE6K	<a href="#">Yu et al. (2014)</a>
Rice	50K	Affymetrix Axiom	OsSNPnks	<a href="#">Singh et al. (2015)</a>
Rice	44K	Affymetrix GeneChip		<a href="#">Tung et al. (2010)</a>

Table 1. Array-Based and NGS-Based Platforms for Genome-wide High-Throughput Genotyping in Crop Species.

(Continued on next page)

Crop	Size	Technology	Information/resource	Reference
Rose	68K	Affymetrix Axiom	WagRhSNP68K	<a href="#">Koning-Boucoiran et al. (2015)</a>
Rye	600K	Affymetrix Axiom	Rye600K	<a href="#">Bauer et al. (2017)</a>
Ryegrass	9K	Illumina Infinium BeadChip		<a href="#">Blackmore et al. (2015)</a>
Soybean	50K	Illumina Infinium BeadChip	SoySNP50K	<a href="#">Song et al. (2013)</a>
Soybean	180K	Affymetrix Axiom	SoyaSNP180K Axiom	<a href="#">Lee et al. (2015)</a>
Strawberry	90K	Affymetrix Axiom	IStraw90	<a href="#">Bassil et al. (2015)</a>
Sunflower	25K	Illumina Infinium BeadChip		<a href="#">Livaja et al. (2016)</a>
Sunflower	10K	Illumina Infinium BeadChip		<a href="#">Bachlava et al. (2012)</a>
Tomato	10K	Illumina Infinium BeadChip		<a href="#">Sim et al. (2012)</a>
Wheat	9K	Illumina Infinium BeadChip	Wheat 9K iSelect	<a href="#">Cavanagh et al. (2013)</a>
Wheat	90K	Illumina Infinium BeadChip	Wheat 90K iSelect	<a href="#">Wang et al. (2014)</a>
Wheat	660K	Affymetrix Axiom	Wheat 660K Axiom	<a href="#">Jia Jizeng (personal communication)</a>
Wheat	820K	Affymetrix Axiom	Wheat HD genotyping array	<a href="#">Winfield et al. (2016)</a>
Wheat	35K	Affymetrix Axiom	Wheat Breeder's genotyping array	<a href="#">Allen et al. (2017)</a>
Crop specific	Scalable	Exome capture	Exome sequencing	<a href="#">Allen et al. (2013)</a> ; <a href="#">Henry et al. (2014)</a>
<i>De novo</i> (applicable to multiple crops)	50–300K	GBS	Genotype-by-sequencing	<a href="#">Elshire et al. (2011)</a>
	~50K	DArT-seq	DArT sequencing	<a href="http://www.diversityarrays.com">http://www.diversityarrays.com</a>
	1-2K	rAmpSeq	Repeat amplification sequencing	<a href="#">Buckler et al. (2017)</a>
	Depend on genome size, sequencing depth and technology	SLAF-seq	Specific length amplified sequencing	<a href="#">Sun et al. (2013)</a>
		RAD-seq	Restriction site-associated DNA sequencing	<a href="#">Baird et al. (2008)</a>
		Two-enzyme GBS		<a href="#">Poland et al. (2012)</a>
		ddRAD	Double digest RAD	<a href="#">Peterson et al. (2012)</a>
		SBG	Sequencing-based genotyping	<a href="#">Truong et al. (2012)</a>
		REST-seq	Restriction fragment sequencing	<a href="#">Stolle and Moritz (2013)</a>
		RAD capture	Rapture	<a href="#">Ali et al. (2016)</a>
MSG	Multiplexed shotgun genotyping	<a href="#">Andolfatto et al. (2011)</a>		
ezRAD		<a href="#">Toonen et al. (2013)</a>		

Table 1. Continued



**Figure 1. A Workflow System to Develop Practical Breeding Chips with Possible Genetic Information from Linkage Mapping, GWAS, and Functional Genes.**

Step-wise selection of trait-associated markers and their subsequent customization is mentioned to allow flexibility and high throughput in several Tier 2 breeding objectives.

GWAS in natural diversity panels, and joint linkage-association mapping using both bi-parental and natural populations. Linkage mapping is still the predominant strategy to discover the genetic basis of quantitative traits. Recently, GWAS reports have been exponentially increasing since the availability of reference genome sequences of crop plants and the availability of high-density automated genotyping platforms (Xiao et al., 2017). Other strategies are also used sometimes for complexity reduction, such as bulk segregation analysis to unravel the genetic basis of quantitative traits. The genetic basis of kernel row number in maize diversity panels was determined by making extremely contrasting bulks followed by exome sequencing (Yang et al., 2015). Likewise, the QTL sequencing (QTL-seq) approach in contrasting bulks was used for understanding the seedling vigor in rice (Takagi et al., 2013) and 100-seed weight in chickpea (Singh et al., 2016). Zou et al. (2016) thoroughly reviewed the usefulness, applications, and strategies for bulk sample analysis for crop genomics and breeding, and presented several examples across the crop species.

In contrast to the quantitative traits, gene discovery for traits controlled by single or major effect genes of quantitative traits

are relatively straightforward. Such genes can be easily identified through QTL and fine mapping. Several other innovative strategies emerged recently for the cloning of such genes. For example, the 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 genes underlying late blight resistance in *Solanum americanum* (Witek et al., 2016) and stem rust resistance in *Aegilops tauschii* (Steuernagel et al., 2016). This recently developed technology is referred as resistance-gene enrichment sequencing (RenSeq or MutRenSeq), which does not rely on recombinant populations or fine mapping and combines mutagenesis and genome complexity reduction. Thind et al. (2017) reported another rapid gene-cloning strategy in wheat referred as “targeted chromosome-based cloning via long-read assembly,” which uses chromosome flow sorting followed by long-read sequencing to assemble complex genomes. This is a rapid and cost-effective approach that can be used to clone any gene and could be effective in cloning genes in reduced recombination regions in the genome.

Development and refinement of the appropriate genotyping tools to practice the aforementioned strategies are of extreme importance. The first step is to determine the genetic polymorphism that exists among the individuals of a population. As whole-genome sequencing (WGS) is too costly to sequence all the individuals of the population, several genotyping platforms are used alternatively. However, in some cases WGS has been used to perform GWAS for salinity tolerance in soybean (Patil et al., 2016), agronomic traits in rice (Yano et al., 2016), and stress adaptive traits in *Arabidopsis* (Thoen et al., 2017).

Despite the significant achievements in understanding genomics of crop phenotype plasticity, the progress is not on a par with that demonstrated in health and biomedical sciences. For example, the genetics of wheat bread-making quality has been an area of research over the last 30 years comprising extensive genetics and genomics studies of major genes involved in bread-making quality, yet still a 30%–40% knowledge gap persists (Rasheed et al., 2014). Here we highlight the key areas in crop genomics where the improvements could significantly bridge genotype–phenotype gaps in crop species.

- (a) The availability of pan-genome could pave the way for bridging the genotype–phenotype gap. Pan-genome refers to the full complement of the genes in a gene pool, consisting of a core genome shared by all individuals, and a dispensable genome partially shared by individuals. Pan-genome is usually achieved through resequencing coupled with *de novo* assembly of the sequences not matching the reference sequence to identify a potentially large number of structural variants. It has been successfully used to discover genes for flowering time in *Glycine soja* accessions (Li et al., 2014), morphotypes in *Brassica rapa* and *Brassica oleracea* accessions (Cheng et al., 2016), and adaptive traits in maize using pan-genome and pan-transcriptome of 503 inbred lines (Hirsch et al., 2014). This is also helpful in improving genotyping arrays, with more representation from the gene pool and inclusion of selectively neutral loci.
- (b) As the genotype–phenotype gap is somewhat less in model crops such as rice and *Arabidopsis*, more efforts are needed to translate this information for non-model species. One of the prominent examples is genes for grain size and weight in wheat that are mostly identified using translation biology approaches between wheat and rice (Valluru et al., 2014).
- (c) There is a huge need for deploying genomics-assisted breeding strategies in parallel with genomics studies to understand quantitative traits. GS, which can be performed with GWAS simultaneously, holds significant promise. The favorable haplotypes identified by GWAS can be used to identify promising breeding lines with high genomics estimated breeding values for desired traits.
- (d) Integration of multiple -omics from RNA (transcriptome), protein (proteomics), and metabolite (metabolomics) in genomics can further elucidate the biological role or process that determines gene effect. Chen et al. (2014) used GWAS to identify 36 candidate gene modulating levels of metabolites that are of potential physiological and nutritional importance. Such types of -omics information can complement and saturate the genetic

information to manipulate the biological processes in crop breeding. Although higher cost is the key limitation in generating -omics data in large populations, as the technologies for -omics continue to decrease in cost a whole new era of crop molecular breeding will emerge that has a potential to fill the knowledge gaps in understanding important breeding traits.

- (e) Gene mapping information from most of the studies could not be compared due to the inconsistencies in genotyping platforms, germplasm used for mapping, and different statistical procedures. Genome-wide meta-analysis with support from new statistical procedures and availability of reference genomes in many crop species is becoming a powerful tool to integrate mapping information from different studies, thus reducing information redundancy. This will help to decipher the genetic variations between and within different populations, and predicts a more holistic overview of haplotype structure at a given locus.

### Structural Variations

Structural variations (SVs) including copy number variations (CNVs) and presence–absence variations (PAVs) are the most important polymorphisms in humans after SNPs and small In-Dels. SVs are abundant in crop species and significantly affect phenotypes, but are less pursued in crop genomics while their effects on genotypic variation are still unknown (Saxena et al., 2014). GWAS in *Arabidopsis* showed that while CNVs are there, only a few true CNV polymorphisms cause differentially expressed genetic variation, leading to a conclusion that CNVs are likely to have only a small impact on the phenotype (Gan et al., 2011). At the individual gene level, CNV are known to significantly affect tolerance to abiotic stresses in barley (Sutton et al., 2007), aluminum tolerance in maize (Maron et al., 2013), resistance to soybean cyst nematode in soybean (Knox et al., 2010), and heading time in wheat (Diaz et al., 2012; Wurschum et al., 2015). Likewise, genome-wide analyses in maize (Springer et al., 2009; Belo et al., 2010), rice (Ma and Bennetzen, 2004; Yu et al., 2011), sorghum (Zheng et al., 2011), and soybean (McHale et al., 2012) identified 400, 641, 234, and 267 CNVs across the respective genomes. However, less effort has been made to associate genome-wide CNVs and PAVs with phenotypic variations in economically important traits (Saxena et al., 2014), such as reproductive morphology traits in cucumber (Zhang et al., 2015b). Also there has been almost no effort to develop automated platforms that can type CNVs in crops following the example from Human SNP array 6.0 having both SNPs and CNVs. There are some reports on detecting SVs by micro-array-based comparative genomics hybridization, but this technology can only detect SVs with sequences that are homologous to the probes and cannot determine the exact copy number or breakpoints.

### Epigenetic Variations

Factors affecting gene function include not only DNA sequence variation but also epigenetic modification, including DNA methylation and histone modifications. Epigenetic information plays a role in developmental gene regulation, environment response, and natural variation of gene expression level. Because of these central roles, epigenetics has the potential to play important roles

in crop improvement strategies including the selection for favorable epigenetic states, creation of novel epialleles, and regulation of transgene expression (Springer, 2013). Our understanding of epigenetic variation and its phenotypic effects are very limited in crop plants. For example, it was demonstrated that identical isogenic populations in *Brassica napus* had distinct agronomic characteristics for energy use efficiency despite their identical DNA sequences (Hauben et al., 2009). Some QTLs may represent the epigenetic rather than genetic variation. Recently, the first genome-wide DNA methylation patterns were mapped in wheat (Gardiner et al., 2015); however, association of these variations with phenotypic difference and parallel epiallelic discovery remains a long-term goal.

Array-based platforms are available for genome-wide CNV and epigenetic analysis in humans, such as HumanMethylation450 (450K) BeadChip (Nishida et al., 2008; Ting et al., 2015), but no such examples are available in crop plants. Therefore, it is very important to deal with these bottlenecks to obtain maximum benefits from the technologies, reduce phenotype–genotype gaps, and make array-based genotyping more fruitful in plants.

## ARRAY- AND SEQUENCING-BASED GENOTYPING IN CROPS: ACHIEVING HIGH THROUGHPUT

NGS techniques have proved to be exceptional tools for the discovery, validation, and assessment of genetic markers, and thus have facilitated the development of genome-wide SNP markers and array-based genotyping platforms (Davey et al., 2011). An overview of available genome-wide, high-density genotyping platforms are briefly discussed here because they are reservoirs of SNP markers likely to be associated with important traits and can be used in breeding. There are many array-based genotyping platforms available in major crops (Table 1). The benefits of these platforms include, but are not limited to: (a) a range of multiplex levels providing rapid high-density genome scans; (b) robust allele calling with high call rates; and (c) cost-effectiveness per data point when genotyping large numbers of SNPs and samples. The main disadvantages are that arrays are non-flexible and, despite the reduced cost per data point, the overall cost to genotype one sample is quite high, making them still inaccessible for most of the crop genetics and breeding programs. Given the various reviews on the technological comparison of these platforms (Gupta et al., 2008; Thomson, 2014), this aspect will be described here only briefly.

All of these genotyping arrays are based on technologies from Illumina and Affymetrix, which have revolutionized the genome-wide genotyping concept. Despite their similarity in size, format, and application, the two technologies differ substantially. Illumina BeadArray technology uses beads covered with specific oligos that fit into patterned microwells allowing for highly multiplexed SNP detection, initially employing BeadXpress and then the GoldenGate assay that incorporates locus and allele-specific oligos for hybridization, followed by allele-specific extension and fluorescent scanning of 48–384 and 384–3072 SNPs per sample (Shen et al., 2005). The BeadArray technology was expanded to higher-density arrays

with Infinium assays, which are based on a two-color single base extension from a single hybridization probe per SNP marker with allele calls ranging from 3K to over 5 million per sample (Steemers and Gunderson, 2007). In contrast, Affymetrix implemented GeneChip arrays using photolithographic printing of oligos on an array, followed by hybridization to overlapping allele-specific oligos consisting of perfect match and mismatch probes for SNP calling (Matsuzaki et al., 2004). More recently, Affymetrix Axiom technology based on a two-color, ligation-based assay with 30-mer probes allowed simultaneous genotyping of 384 samples with 50K SNPs, or 96 samples × 650K SNPs (Hoffmann et al., 2011).

In addition to the crop-specific SNP arrays, there are NGS-based platforms that are applicable to various crops regardless of prior genomics knowledge, genome size, organization, or ploidy. The term “GBS” is a generalized description for all platforms using a sequencing approach for genotyping. Scheben et al. (2016) enlisted 13 different GBS techniques that have been used in crop plants (see Table 1), each of which has some distinctive features. This includes GBS (Elshire et al., 2011; Poland et al., 2012; Kim et al., 2016), diversity array technology sequencing (DArT-seq) (Cruz et al., 2013; Li et al., 2015), sequence-based genotyping (SBG) (Truong et al., 2012; van Poecke et al., 2013), restriction fragment sequencing (REST-seq) (Stolle and Moritz, 2013), and restriction enzyme site comparative analysis (RESCAN) (Kim and Tai, 2013). Elshire GBS and DArT-seq are the most widely used platforms in crop genomics. GBS simply makes use of restriction enzyme digestion, followed by adapter ligation, PCR, and sequencing. While the original Elshire GBS protocol employed a single enzyme protocol, a two-enzyme modification has been successfully employed in barley, wheat (Poland et al., 2012), oat (Huang et al., 2014), and chickpea (Jaganathan et al., 2015). A further step change in reducing the cost of GBS is the development of repeat amplification sequencing (rAmpSeq) which combines the novel bioinformatics and robust genotyping to score hundreds to thousands of markers for less than US\$5 per sample (Buckler et al., 2017). Despite several advantages such as low cost and genotyping polymorphisms in low-copy intervening sequences, rAmpSeq produces fewer markers than conventional GBS, and knowledge of the reference genome sequence is extremely important in designing a quality assay.

In comparison with WGS, reduced representational sequencing has many advantages, such as reducing genome complexity, avoiding inherent ascertainment bias in the fixed SNP arrays, and lower cost. GBS has been applied in studies on evolutionary genomics, GWAS, and marker-assisted molecular breeding. One such platform, specific locus amplified fragment sequencing (SLAF-seq), was recently developed (Sun et al., 2013) and implemented in soybean (Han et al., 2015), cucumber (Xu et al., 2014b), tea (Ma et al., 2015), *Agropyron* (Zhang et al., 2015a), and *Brassica* (Geng et al., 2016) to study domestication, construct high-density linkage maps, and undertake GWAS of agronomic traits. Likewise, exome capture can help to focus genic regions and has the advantages of *de novo* SNP discovery in crops with large genome and highly repetitive DNA, such as maize (Fu et al., 2010; Yang et al., 2015), wheat (Jordan et al., 2015), rice (Saintenac et al., 2011; Henry et al., 2014), and barley (Mascher et al., 2013).



Despite several advantages, there are serious shortfalls on broader application of genotyping platforms in major crops. These shortcomings are described in the following subsections.

### Ascertainment Bias

Crop wild relatives and landraces are important reservoirs of new genes for yield, climatic resilience, and food quality, with nutritional and health benefits (Brozynska et al., 2015). The existing genotyping arrays remain ineffective to capture rare variants in diverse genetic resources due to ascertainment bias, and ultimately result in hampered identification of introduced segments from distantly related genetic resources. This was realized very early during the earlier phase of SNP arrays development in maize, when the allele frequencies based on SSR and SNP markers were compared in global maize inbred lines (Hamblin et al., 2007; Yan et al., 2009). This ascertainment bias is not only observed in interspecific populations but is also a major problem in intraspecific populations from the same species. For example, inbred lines used for the maize reference genome, including B73 and Mo17, are adapted to temperate conditions. High-density genotyping based on the currently available SNP chips and resequencing strategies may have significant ascertainment bias when used for analyzing tropical maize germplasm (Xu et al., 2017b). As a result, favorable alleles hidden in tropical maize (in tropical-specific genomic regions) could be missed. To develop unbiased SNP chips and reference maize genomes, information hidden in tropical maize should be unlocked. Such an effort is ongoing through collaboration between CIMMYT, Chinese Academy of Agricultural Sciences (CAAS), and Beijing Genomics Institute (BGI). This includes large-scale resequencing of tropical maize inbred lines and high-density genotyping of tropical maize populations and inbred lines through unbiased SNP discovery strategies (Prasanna et al., 2014). Although the use of *de novo* NGS-based platforms in combination with fixed arrays could be effective to some extent in avoiding ascertainment bias, such strategies not only increase the cost but also bring complexities associated with *de novo* platforms described below. Consequently, breeders hesitate to use these platforms.

The marker support for wheat wild relatives has recently been documented by development of 820K and 35K SNP chips in wheat, which resolve the issues of ascertainment bias to a great extent when using wild relatives in breeding (King et al., 2016; Winfield et al., 2016). Another example is development of the cost-effective KWS 15K wheat Infinium array used in European winter wheat germplasm (Boeven et al., 2016), which is a subset of high-quality and polymorphic markers from wheat 90K Infinium array that are highly informative in European winter wheat. These examples represent the current efforts to deal with ascertainment bias, namely the customization of SNP chips, replacement of markers on the chips, and combining markers from more than one chip to apply in germplasms with broader genetic backgrounds. Due to the design bias in most of the available genotyping chips, the concept of “one chip for all purposes” is far beyond the reality. There are many chips for single crops (e.g., six in wheat and seven in rice), and the high-quality contents from all the chips can be chosen to develop a “second-generation chip” which would facilitate the wider application.

### General Limitations in NGS-Based Genotyping Platforms

NGS-based platforms are a promising tool for cost-effective genotyping, especially in orphan crops because SNP discovery and genotyping can be done simultaneously with less bias toward genetic backgrounds. General limitations of GBS include genotyping errors due to the low coverage of GBS reads, leading to misidentification of homozygotes from heterozygotes. This problem could be intense in outcrossing species or in mapping populations at early generation levels (e.g., F2:3). The absence of reference genome and polyploidy also increase the intensity of genotyping errors because the paralogs may be recognized as the same reads when similarities are very high. Such problems can be solved by increasing sequence depth by using rare cutters, reducing the number of multiplexed samples during library preparation, or sequencing the library with improved NGS equipment. Accurate allele calling in allopolyploids is more complicated than in autopolyploids because each subgenome is expected to show disomic inheritance. If a good reference genome is available the genotypes can be separated, otherwise genotypes in highly similar subgenomes may not be readily distinguished. On the other hand, allelic dosage of each locus in autopolyploids cannot be evaluated with low-coverage NGS data, but all the mixed-allele loci are genotyped as heterozygous. It is necessarily advised to remove all heterozygous loci in autopolyploids to obtain fairly high coverage of GBS data, which usually results in wasting a number of NGS reads.

Another drawback is the allele dropout due to the polymorphism in the restriction enzyme recognition site, which inhibits enzyme action and leads to genotyping error (Davey et al., 2013). Sometimes genotyping errors caused by stochastic uneven PCR duplication during library preparation leads to allele bias in all GBS-based methods except ezRAD, which is a PCR-free method (Toonen et al., 2013). Another common problem could be the differences in coverage due to the amplification bias toward fragments of shorter length and with higher GC content. Beyond these common errors, the frequent use of methylation-sensitive enzymes in GBS could also cause ascertainment bias, harboring almost half of trait-associated SNPs (Hindorf et al., 2009).

Other problems include labor-intensive library preparation, high level of missing data, absence of perfect bioinformatics tools for data imputation models, and higher complexity in data analysis and storage. Apart from these drawbacks, GBS has become increasingly popular in crop genetics. This is mainly due to the enormous developments in the sequencing chemistry, availability of the long-read sequencing platforms, and enrichments in the existing reference genomes, resulting in genotyping methods to make more use of this technology.

### Genotyping in Polyploidy Crops

Polyploidy, or whole-genome duplication, is an important driving force in eukaryotic evolution and success of many crop plants. The crop plants can be either ancient polyploids (paleo-polyploids), such as maize, soybean, and poplar in which the genome duplication event was followed by the gene loss and subsequent diploidization, or can be neo-polyploids, such as wheat (6 $\times$ ),

oilseed rape (4×), potato (4×), sugarcane (8×), cotton (4×), and strawberry (8×). Polyploidy generally leads to high sequence conservation between homoeologous genes, increased gene and genome dosage, and large genome size, which form different layers of complexities for gene discovery and ultimately affect the efficient use of markers during breeding. For example, the key challenge in wheat genome sequencing was correct genome assembly due to polyploidy and high repeat content. This challenge was dealt with by using flow-sorting technology (Dolezel et al., 2014) to separate individual chromosomes from “Chinese Spring” aneuploidy stocks and then sequencing individually. Parallel to genome-sequencing efforts, the NGS of mRNA (RNA-seq) in diverse wheat germplasms produced transcript assemblies and led to the development of high-density fixed chips amenable to genotyping of large populations for gene mapping and discovery (Cavanagh et al., 2013). Although the rates of SNP validation in polyploid SNP arrays were lower when compared with diploids, they still achieved success rates above 61% (Tinker et al., 2014; Wang et al., 2014; Hulse-Kemp et al., 2015). However, overall success rate varied across SNP identification strategies in cotton (Hulse-Kemp et al., 2015). Gene-enriched sequence-supplied SNPs (SNPs from RNA-seq data) had a higher success rate than genomic resequencing data-supplied SNPs (87% versus 49%), and genomic SNPs identified between species had a higher success rate than SNPs identified within species (59% versus 49%) (Hulse-Kemp et al., 2015). Although not all of the SNPs in the first version of these chips could be assigned to individual chromosomes, the subsequent versions of these chips have shown significant improvement in homoeolog-specific chromosome assignments to SNPs. Together, these developments are making a huge impact on availability of genotyping resources, and availability of high-density genotyping platforms is no longer a limitation in polyploid crops (Table 1). Since Kaur et al. (2012) provided an in-depth review of SNP discovery and SNP-calling techniques and resources in polyploid crops, we solely focus here on how the expression of different patterns of homoeologous genes affect the use of markers in breeding crops.

The homoeologous copies of a certain gene in polyploid crops result in multiple phenotypic consequences, thus determining the number of subgenome-specific (homoeologous-specific) markers to be used to manipulate a locus underpinning a phenotypic trait (Borrill et al., 2015). There are various possible scenarios influencing the use of markers in polyploids during trait breeding. (a) Functional redundancy is the case when a single copy of a gene compensates for the other deleted homoeologous copies. The loss-of-function mutation (*mlo*) induces resistance to powdery mildew in barley, while the expression of a single copy of its ortholog *Mlo* gene is sufficient to induce susceptibility to powdery mildew in wheat, even when the other two homoeologs have null mutations (Wang et al., 2014). (b) The dosage of the different homoeolog chromosomes additively affects the phenotype; e.g., the mutation at each of reduced height (*Rht*) gene in wheat significantly decreases the plant height as compared with wild-type alleles at *Rht* genes (Kim et al., 2003). (c) Homoeolog dominance is the case when mutation in any of the homoeolog copies eliminates the dosage effect in other homoeologs; e.g., three recessive homoeologous *Vrn1* genes confer vernalization requirement in wheat, while dominant mutation in any homoeologous copy eliminates vernalization

requirement and confers spring growth habit. Likewise, other interactions among homoeologs may occur at regulatory or transcription level, adding a further layer of complexity in phenotypic expression of important developmental traits (Borrill et al., 2015). Thus, it requires *a priori* understanding of different dosage effects of homoeologous genes in polyploid crops before using markers for any given trait. In case of additive dosage effect and homoeolog dominance, there is a need to use markers from all available homoeologous genes to fine-tune the underpinning traits; thus the number of markers to be used will be significantly high compared with diploid crops, ultimately increasing the cost and time.

## CUSTOMIZATION OF TRAIT-ASSOCIATED SNPS: ACHIEVING FLEXIBILITY

The use of GBS and SNP chips in mapping and gene discovery is important for identification of trait-associated markers, which are then used for gene tagging and gene pyramiding during crop breeding (Figure 1). Flexibility is extremely important for such markers as it gives choice in using any number of markers and any number of samples, and largely depends on conversion of markers (SNPs) on the chip to a stand-alone format. It has long been a challenge to visualize SNPs with traditional molecular techniques for applications in breeding. Various single-marker methods have been developed for SNP genotyping, including allele-specific PCR (AS-PCR), cleaved amplified polymorphic sequences (CAPS), temperature-switch PCR (TS-PCR), and gene resequencing (Gaudet et al., 2009; Tabone et al., 2009). All these methods have common limitations of low throughput, high cost, and labor intensiveness; therefore, actual applications in crop breeding programs occur only on a small, limited scale. Rapidly evolving genotyping technologies have made molecular diagnosis more high throughput and cost effective in all aspects, but large-scale transfer of the technologies to breeding programs has been very limited, at least in public breeding programs. Several high-throughput technologies for SNP genotyping are available. The important factors in choosing an appropriate genotyping platform include the number of data points that can be generated in a short time period, ease of use, data quality (sensitivity, reliability, reproducibility, and accuracy), flexibility (genotyping few samples with many SNPs or many samples with few SNPs), assay development requirements, and genotyping cost per sample or data point. Sufficient recent reports indicate that LGC's KASP (Kompetitive Allele Specific PCR) is an evolved global benchmark technology for such genotyping requirements in terms of both cost-effectiveness and high throughput (Semagn et al., 2014; Thomson, 2014). We recently used this platform to convert conventional PCR markers for functional genes in wheat with >95% assay conversion success, and it proved to be 45-times higher in throughput and 30%–45% cheaper than other available conventional methods (Rasheed et al., 2016). As sample size increases to 1536 genotypes, it can be 4-fold high-throughput and cost-effective (due to the low mastermix volume used) in 1536-well plates.

Most recently, Long et al. (2016) introduced a novel SNP genotyping method designated semi-thermal asymmetric

reverse PCR (STARP), and successfully validated it in rice, sunflower, and *A. tauschii*. STARP uses unique PCR conditions with two universal priming element-adjustable primers (PEA primers) and one group of three locus-specific primers: two asymmetrically modified allele-specific primers (AMAS primers) and their common reverse primer. The two AMAS primers each were substituted one base in different positions at their 3' regions to significantly increase the amplification specificity of the two alleles and tailed at 5' ends to provide priming sites for PEA primers. The two PEA primers were developed for common use in all genotyping assays to stringently target the PCR fragments generated by the two AMAS primers with similar PCR efficiencies and for flexible detection using either gel-free fluorescence signals or gel-based size separation. The state-of-the-art primer design and unique PCR conditions endowed STARP with all the major advantages of high accuracy, flexible throughput, simple assay design, low operational costs, and platform compatibility. In addition to SNPs, STARP can also be employed in genotyping of InDels. Contrary to the KASP and TaqMan assays, which use the specific commercial master-mix, the STARP can be used with any commercial PCR master-mix.

KASP, TaqMan, and STARP currently appear to be the most promising techniques offering genotyping, with exceptional chemistry and scalable flexibility without compromising data throughput. They are an excellent choice for adoption in crop breeding programs for single-plex genotyping.

## THROUGHPUT VERSUS FLEXIBILITY: THE BREEDER'S PERSPECTIVE TO ACHIEVE GOALS

The choice of genotyping methodology largely depends upon the nature of the study. For instance, there are generally two applications for genotyping: (1) first-tier applications for genome-wide association, linkage analysis, and genetic diversity analysis; and (2) second-tier application for validating hits in the first-tier application. Ultra-high throughput and low cost are paramount. Automated chip-based platforms do facilitate achievement of objectives in first-tier applications and are widely used in crop genomics. Unfortunately, breeders, in most of the public sector and in developing countries, still have to rely on conventional PCR/gel-based methods for second-tier applications, which are low throughput and hinder large-scale marker application.

There are four different general usages for second-tier applications, namely major gene selection, backcross breeding, recurrent selection, and QC. As discussed above, arrays are too expensive to be used for low-density genotyping in large breeding populations for backcross breeding, gene tagging, and recurrent selection, and such objectives can be accomplished with the single-plex high-throughput platforms such as KASP and TaqMan, which offer scalable flexibility (Semagn et al., 2014). Recently, there have been many efforts to develop such assays in wheat (Rasheed et al., 2016), peanut (Leal-Bertioli et al., 2015), soybean (Patil et al., 2016), lupin (Yang et al., 2012), legumes (Varshney, 2016), and several other agronomic and horticultural crops. These single-plex technologies could be very costly and time consuming for the other two objectives, i.e., germplasm fingerprinting for diversity anal-

ysis, gene mapping and GS. The most important question is, what should be the break point in terms of cost and marker density to opt for chip-based genotyping? In our experience, routine genotyping for 150 KASP assays (either on 384 or 1584 samples) is effective in terms of cost and throughput; however, above that number automated chip-based genotyping platforms are feasible (Table 2). The second question is, what are the alternative multiplex or array-based key technologies available for low- to medium-density genotyping requirements in breeding? In these scenarios multiplexing is a very important variable because a shift from single-plex to multiplex assays will allow simultaneous analyses of multiple markers and increase MAS efficiency to implement GS. Recently, an attempt was made to multiplex 24 functional wheat markers using an Ion Torrent Proton-based NGS method. This system can perform KASP assays in nanoliter volumes to reduce genotyping costs (Bernardo et al., 2015). However, broad-scale application of this technology is yet to be demonstrated to fulfill the needs of breeders. The Sequenom MassARRAY platforms were used earlier in rice and wheat (Berard et al., 2009; Masouleh et al., 2009) for multiplex low-density SNP genotyping. Sequenom MassARRAY uses a matrix-assisted laser desorption/ionization mass spectrometer, which processes the reactions, and can accommodate thousands of SNPs in almost 1000 samples per day, leading to half a million data points per day. In human genome applications, genotyping up to 500 SNPs is more effective in balancing cost and throughput when done by Sequenome (Perkel, 2008). Affymetrix recently introduced Eureka, an NGS-based platform for low-density genotyping. This platform is currently available only as a service and involves a barley panel of 400 SNPs associated with malt characteristics, sucrose synthase, disease resistance (*Mla*), vernalization (*Vrn-H1* and *Vrn-H3*), photoperiod (*Ppd-H1*), and row type. A genotyping service for these genes is available at cost of as low as US\$15 per sample (<https://www.eurekagenomics.com/ws/products/barley.html>). The general shortcomings in these platforms are the unavailability of trait-associated markers in required formats and data calling, especially in polyploidy species, which are the main reasons that these technologies are not widely pursued by crop breeding programs.

## DEVELOPMENT OF ULTRA-HIGH-THROUGHPUT PRACTICAL BREEDING CHIPS: THE ULTIMATE OBJECTIVE

We are experiencing a huge shortfall in underpinning genomics in order to target breeding traits in all major crops, without which the development of functional breeding chips cannot be achieved. The landscape of crop genomics is changing rapidly, and the current important question is can all the genomics information encompassing SNPs, InDels, CNVs, and epigenetics be embedded in a single chip? For example, the Affymetrix Human SNP array 6.0 can detect 1.8 million polymorphisms, of which 960K are CNV (Nishida et al., 2008). Similarly, the Eureka platform offers a low-density genotyping assay for simultaneous detection of SNPs, InDels, CNVs, and methylation, but the downstream data analysis pipeline for allele calling could be a challenge in polyploid crops. With many chips available for genome-wide genotyping in any crop, there is a certain amount

Platform	Technology <sup>a</sup>	Provider	Cost per sample	Cost per data point	Analysis complexity	Prior genomic knowledge	Throughput <sup>b</sup>	Flexibility <sup>c</sup>	Application <sup>d</sup>
Array based	GoldenGate	Illumina	High	Moderate	Moderate	Yes	172 × 1.5K	No	Tier 1
	Infinium XT	Illumina	Moderate	Low	Moderate	Yes	96 × 50K	No	Tier 1
	Infinium iSelect HD/HTS	Illumina	High	Low	Moderate	Yes	24 × 90K/24 × 700K	No	Tier 1
	Axiom	Affymetrix	Moderate to high	Low	Moderate	Yes	96 × 1000K or 384 × 55K	No	Tier 1
NGS based	GBS	Non-commercial	Moderate	Low	Difficult	No	Depend on sample multiplex	Low	Tier 1
	RAD-seq	Non-commercial	Moderate	Low	Difficult	No	-do-	Low	Tier 1
	SLAF-seq	Biomarker Tech	High	Low	Difficult	No	-do-	Low	Tier 1
	Exome capture	Agilent/ NimbleGen	High	Low	Difficult	Yes	-do-	Low to moderate	Tier 1
	DArT-seq	DiversityArray	Moderate	Low	Commercial support available	No	96 × 50-100K	Low	Tier 1
	rAmpSeq	Non-commercial	Very low	Very low	Difficult	Yes	Multiplex	Low	Tier 1
Targeted GBS/Low density arrays	FluidgM	FluidgM	Moderate	Moderate	Moderate	Yes	96 × 96/ 24 × 192/ 48 × 48	Moderate	Tier 2
	Sequenom MassARRAY	Agena Bioscience	Moderate	Moderate	Moderate	Yes	96 × 48	Low	Tier 2
	Eureka	Affymetrix	Moderate	Moderate	Moderate	Yes	At least 5K × 3K	Low	Tier 2
	AmpliSeq	Thermo Fisher	Moderate	Moderate	Moderate	Yes	Customizable	Moderate	Tier 2
Single markers	KASP	LGC Group	Depend on reaction volume and assay number	High	Easy	Yes	Single-plex (up to ~150K data points/day)	Scalable	Tier 2
	TaqMan	Roche Molecular System	-do-	High	Easy	Yes	-do-	-do-	Tier 2
	STARP	Non-commercial	-do-	Moderate	Easy	Yes	-do-	-do-	Tier 2

**Table 2. Features of Modern Genotyping Technologies Available for Gene Discovery and Molecular Breeding in Crop Species.**

<sup>a</sup>See Table 1 for abbreviations.

<sup>b</sup>Throughput: Maximum data points that could be generated from a single run, and are presented here as 'number of samples × number of markers'. All NGS-based technologies are high-throughput but depends on ability of number of samples that can be multiplexed.

<sup>c</sup>Flexibility refers to independence in choosing any number of samples and markers. Most of the NGS based methods are more flexible in choosing number of samples.

<sup>d</sup>Tier 1: GWAS; QTL mapping; genomic selection; genetic diversity. Tier 2: gene tagging; marker-assisted backcrossing (MABC); background selection.

of bias in each, and scientists have to decide on the best one based on the genetic background of the germplasm and objectives. On the contrary, the concept of breeding chips is more universal because such chips are based on functional genes that have been validated across the genetic backgrounds. Therefore, the choice of technology for second-tier applications will have wider acceptability and is actually the choice of technologies to harness benefits from the post-genomics era.

The future landscape of crop genotyping is debatable; either sequencing will eventually replace all genotyping platforms as sequencing becomes cheaper, and as data management and analysis becomes easier with huge numbers of markers, samples, and tools that are available and accessible to all geneticists and breeders, or genotyping platforms will continue to be updated and remain as effective alternatives to WGS. In our opinion, the use of whole-genome resequencing data for genetics studies will be routinely used in major crops such as wheat, rice, maize, soybean, cotton, and some legumes in the coming years because WGS from a much larger gene pool is now becoming accessible. However, genotyping platforms still have to make progress in minor crops, and crops with complex and large genomes. The development of genotyping platforms solely for crop breeding could be realized in the next 10 years in consideration of: (1) availability of functional markers for important quantitative traits such as yield and quality; (2) enhancing the value of genotyping platforms by reducing ascertainment bias; (3) reducing redundant polymorphism and good genome coverage; and (4) reducing the cost of whole-genome genotyping of large populations in parallel with improvement in reducing the cost per data point.

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