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## Exploring Genetic Diversity in Plants Using High-Throughput Sequencing Techniques


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**Abstract:** Food security has emerged as an urgent concern because of the rising world population. To meet the food demands of the near future, it is required to improve the productivity of various crops, not just of staple food crops. The genetic diversity among plant populations in a given species allows the plants to adapt to various environmental conditions. Such diversity could therefore yield valuable traits that could overcome the food-security challenges. To explore genetic diversity comprehensively and to rapidly identify useful genes and/or allele, advanced high-throughput sequencing techniques, also called next-generation sequencing (NGS) technologies, have been developed. These provide practical solutions to the challenges in crop genomics. Here, we review various sources of genetic diversity in plants, newly developed genetic diversity-mining tools synergized with NGS techniques, and related genetic approaches such as quantitative trait locus analysis and genome-wide association study.

**Keywords:** Genetic diversity, NGS technology, Genotyping, QTL analysis, GWAS, Crop improvement.

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### 1. INTRODUCTION

Food security is an urgent concern because of the growing world population. A recent estimate suggests that the world population will reach 9 billion or more by 2050 [1], and meeting the demands of this estimated global population is expected to require a 70% increase or at least a doubling in food production [2-4]. Furthermore, the Intergovernmental Panel on Climate Change has recently provided unequivocal evidence of global warming [5], indicating that climate change is a risk factor that might trigger shortfalls in crop production due to an increase in adverse environmental conditions.

To fulfill the food demands of the near future, it is critical to improve the productivity of not only staple food crops, but also crops used for livestock fodder [6, 7]. Beyond ensuring the success of the first green revolution initiated by Norman Borlaug [8-10], improvement of the tolerance of abiotic and biotic stresses and the efficiency of water and nutrient use for each crop must be included as a key approach for preventing a food crisis [11, 12]. Discovering genes that are associated with useful biological functions and rationally integrating these genes and allele in designing crops that exhibit various upgraded functionalities could help improve the sustainability of the global agricultural system.

For this purpose, comprehensive genome-scale and population-scale explorations of genetic diversity in various plant species have gained increasing attention.

The genetic diversity in a given species allows the plants to adapt to various environmental conditions, such as fluctuation in climate and soil conditions. The genetic diversity in a population of a plant species, including cultivars, landraces, and wild individuals, is a crucial resource for increasing food production and for development of sustainable agricultural practices [13]. Therefore, assessment of genetic and phenotypic diversities in the available genetic resources is the primary step to launch a research project to discover useful genes in crops. A dataset of genome-scale and population-scale DNA polymorphisms provides the genetic basis for the characteristics of a population of a species of interest, such as the population diversity, population structure, and linkage disequilibrium. This information essential to efficiently identify associations between genetic polymorphisms and phenotypic differences using genetic map-based approaches such as quantitative trait locus (QTL) analysis and genome-wide association study (GWAS).

The emergence of NGS technologies has enabled drastic innovations in the field of genomics [14]. NGS technologies have substantially reduced the cost and time required to obtain information on several gigabases of nucleotide sequence. Currently, the complete genomes of even plant species that possess very large or complex genomes can be sequenced. For example, the genomes of 142 species of land plants have been sequenced (as of 07 May 2015) (<http://www.ncbi.nlm.nih.gov/genome/browse/>). Web accessible databases for plant genome information are also avail-

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able: Phytozome (<http://phytozome.jgi.doe.gov/pz/portal.html>) and Ensembl Plants (<http://plants.ensembl.org/index.html>). A whole-genome sequence and a well-annotated genome would serve as essential information resources and would facilitate the discovery of useful genes, determination of their physical location in the genome, and prediction of their biological functions. Furthermore, the use of NGS technologies coupled with bioinformatics techniques would allow the development of high-throughput genetic markers and genome-scale investigation of genotypes in crops.

In this review, we address the recent advances in methodologies for comprehensive exploration of the genetic diversity of plants. A major focus here is on introducing the significance of the new genetic diversity-mining tools used in NGS techniques that are widely available.

## 2. SOURCES OF GENETIC DIVERSITY

The sources of genetic diversity in a species can be classified into 3 types according to the mechanisms underlying the diversity: (1) cultivars, i.e., crops that are artificially selected by humans based on useful phenotypic traits; (2) natural variations selected by nature over a long period; and (3) mutants produced using transgenic technologies or chemical/physical mutagens.

### 2.1. Crop Accessions (Artificial Selection)

For various crop plants, numerous cultivars have been produced, selected by breeders for their desirable characteristics based on the various demands of society. For example, >124,000 accessions of rice, including modern and traditional varieties, and wild relatives of rice are stored at the International Rice Gene Bank (<http://irri.org/our-work/research/genetic-diversity/international-rice-genebank>), which is maintained by the International Rice Research Institute (<http://irri.org/>), and these are used by researchers worldwide. Furthermore, >30,000 and >10,000 accessions of maize (*Zea mays*) and wheat (*Triticum aestivum*), respectively, are stocked and maintained at the International Maize and Wheat Improvement Center (<http://www.cimmyt.org/en/>). 1,746 accessions of potato (*Solanum tuberosum*), 8,177 accessions of tomato (*Solanum lycopersicum*), 20,183 accessions of soybean (*Glycine max*), and 30,604 accessions of barley (*Hordeum vulgare*) are stocked and widely available from the National Plant Germplasm System of USDA-ARS (as of 13 July 2015) (<http://www.ars-grin.gov/npgs/>).

The crop accessions potentially possess genes or alleles governing traits selected by breeders. These genes are classified as the following: (1) Genes and/or alleles associated with important traits in wild ancestors that were selected during domestication activities in the old world. These genes and/or allele are important from the view point of human history, as they indicate the change in human lifestyle from hunter-gatherer to farmer. (2) The genes and/or alleles conserved in landraces, which are potentially associated with traits developed as adaptation to local environments. Landraces are established when the cultivated area of domesticated crops is extended in adaptation to local environmental conditions and human cultural changes. Therefore, traits of landraces are selected over the course of the demographic history of the crop. (3) Finally, genes and/or alleles pos-

sessed by modern varieties as the result of genetic admixtures due to modern breeding processes. The patterns of genome-wide polymorphisms observed in these crop accessions will provide clues to discover genes involved in important traits linked with past activities of humans as well as with adaptation to environmental changes. Furthermore, assessment of genetic polymorphisms in a crop species would help strategic management of breeding programs to avoid the loss of genetic diversity.

### 2.2. Natural Accessions (Natural Selection)

Intraspecific natural variation (hereafter referred to as natural variation) in plants is defined as the genetic diversity in a single species that has been maintained in nature by an evolutionally conserved process such as natural selection. Plants adapt to various environments under the pressure of natural selection and thus acquire adaptive traits over several generations. Typically, natural accessions are originally collected from around the world and then selected as pure lines, and these natural accessions vary considerably in terms of morphology (e.g., flower shape or root architecture), and physiology (e.g., stress tolerance or flowering time) [15-29]. Therefore, natural accessions contain valuable genetic resources that can serve as beneficial traits for crop breeding. For certain species, natural accessions are stocked and maintained at several organizations.

For example, 5,695 stocks of *Arabidopsis thaliana*, a model species, are available (as of 08 May 2015; [https://www.arabidopsis.org/abrc/catalog/natural\\_accession\\_1.html](https://www.arabidopsis.org/abrc/catalog/natural_accession_1.html)) from the Arabidopsis Biological Resource Center (<https://abrc.osu.edu/home>). For studies on the whole-genome sequence of natural accessions, the 1001 Genome Project (<http://1001genomes.org/>) was launched in 2008 in order to elucidate genetic variations among 1,001 natural accessions in *A. thaliana* [30]. The complete genome sequences of 80 accessions have been released [31] and several more accessions have been added. As of 08 May 2015, the genomes of 818 out of 1,051 accessions have been re-sequenced and made available through the 1001 Project website. In the case of the model temperate grass *Brachypodium distachyon*, 179 natural accessions are stocked and available from the National Plant Germplasm System of USDA-ARS (<http://www.ars-grin.gov/npgs/>). In a review, Alonso-Blanco *et al.* reported that nearly 100 genes and polymorphisms, which are related to seed dormancy, flowering, root growth, reproduction, or morphology, were identified from natural variations of *A. thaliana* and major crop species such as tomato, wheat, barley, rice, and maize [15]. Natural accessions of a model plant that is phylogenetically related to a crop species would offer the advantage of allowing the transfer of useful knowledge from laboratory to crop breeding sites.

### 2.3. Artificially Produced Mutants

Artificial mutants are produced using transgenic technologies or chemical/physical mutagens. In the case of mutants generated using transgenic technology, T-DNA tagging has been widely used for large-scale analyses [32, 33]. Transposon tagging has also been used to identify gene function using the Ac/Ds element in various plant species such as

*Arabidopsis*, tomato, tobacco, and rice [34, 35]. Because both these insertional mutagenesis approaches typically generate recessive loss-of-function mutations, the resulting mutants are not suitable for studying redundant gene functions. In contrast, in activation tagging [36], the insert carries a constitutive promoter such as the cauliflower mosaic virus 35S enhancer of promoter sequences [37], and it can be used for overexpressing tagged genes to obtain dominant gain-of-function mutations. Furthermore, in the Full-length cDNA Over-eXpressing-hunting system, full-length cDNAs are used for ectopic gene expression and can also be used for producing gain-of-function mutations [38]. Using these systems, researchers can identify causal mutations more readily than using non-physically tagged mutants (described below). More recently, gene-editing systems such as TALEN and CRISPR/Cas9, which are based on the generation of a target-gene-specific double-strand break in the DNA, have become available for knocking out genes of interest in a genome [39-41]. However, in the case of plants, these methods can be used only in transformable species.

Artificially induced chemical or physical mutagenesis introduces various mutations throughout a genome [42], and this approach is widely used in molecular genetics studies and breeding of both model plants and crops. Chemical mutagenesis, such as mutagenesis performed using ethyl methane sulfonic acid (EMS), is widely used for inducing nucleotide changes (point mutations) through the alkylation of specific nucleotides [43-50]; this results mostly in G/C to A/T transitions [44] that are distributed almost randomly throughout a genome. Another chemical mutagen is sodium azide, a recognized respiratory inhibitor. Since sodium azide was first reported to act as an effective mutagen in barley when applied under acidic conditions [51], the chemical has been widely used for inducing mutations in various plants [52-56]. Moreover, sodium azide was found to generate point mutations and did not induce chromosomal aberrations in either embryonic shoots or microspores in barley [57].

In addition to the chemical mutagens, ionizing radiation (e.g., gamma-rays and heavy-ion beams) has been used as a physical mutagen in plant research. Gamma-rays induce DNA damage mostly randomly and generate several types of mutations, including base substitutions, deletions, and structural changes in chromosomes. Gamma-rays were used for inducing mutations in rice [58] and wheat [59]. Heavy-ion beams—accelerated ions that are produced by an ion accelerator such as a cyclotron or synchrotron—are a new tool for inducing mutations [60, 61]. The physical characteristics of heavy-ion beams are considered to allow the accelerated ions to densely deposit their energy in a localized region. Heavy-ion beams have been suggested to predominantly induce double-strand breaks [62-65], and higher linear energy transfer ( $113 \text{ keV } \mu\text{m}^{-1}$ ) carbon ions have been reported to also induce base substitutions, small insertions/deletions (<100 bp), translocations, inversions, and large insertions/deletions (>100 bp) [66]. Heavy-ion beams have been used for breeding several cultivars in *torenia* [67] and *verbena* [68].

For a long period in the past, when an interesting phenotype was identified, considerable effort was required to identify the causal genetic mutation induced by the chemical or physical mutagens; this was because of (1) the high cost and

low throughput of traditional sequencing technologies; (2) the absence of physical tagging of mutations; and (3) the limited availability of technologies for surveying genetic diversity at genome-wide level. The emergence and development of NGS technologies have enabled high-throughput screening of casual mutations from a large population.

### 3. DETERMINATION OF GENETIC DIVERSITY BY USING NEXT-GENERATION SEQUENCING

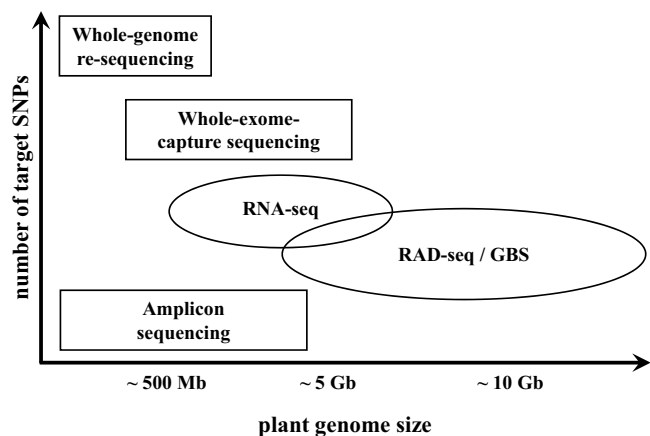
Following the wide-scale availability of NGS technologies, the cost and time required for sequencing have decreased drastically from those required using conventional sequencing methods based on the Sanger method. The main commercially available “second-generation” NGS technologies have included GS FLX from Roche, Genome Analyzer and HiSeq from Illumina, and SOLiD from Life Technologies [14]. Moreover, personalized benchtop NGS instruments, such as GS Jr from Roche, MiSeq from Illumina, and Ion PGM and Ion Proton from Life Technologies, have accelerated sequencing efforts in small research centers and laboratories [69]. In 2011, PacBio RS II from Pacific Biosciences became the first commercially available third-generation Single-Molecule Real-Time sequencer [69, 70]. The data produced by the “third-generation” PacBio RS II has significantly greater read length but relatively lower accuracy than that yielded by “second-generation” platforms. To improve read accuracy, a hybrid genome assembly approach called the PacBio corrected Reads algorithm was developed [71]. This algorithm of the hybrid assembly is based on computational construction of accurate longer consensus sequence by mapping higher-accuracy short reads from “second generation” NGS to longer PacBio reads. Recently, the PacBio sequencing achieved an NG50 of 4.3 Mb in assembling the haploid human genome and could produce *de novo* near-complete eukaryotic assemblies that are 99.99% accurate when compared with available reference genomes [72]. This third-generation NGS technology in combination with the Irys system, a DNA linearization and imaging platform based on the NanoChannel Array technology available from BioNano Genomics [73], would be a powerful tool to generate better quality *de novo* assemblies and to detect chromosomal structural variations for large genomes. The MinION nanopore sequencer is another new platform that can produce long sequencing reads on a palm-sized device plugs into the USB port of a laptop [74-76].

In this section, we provide an overview of determination of genetic diversity using NGS technologies (Fig. 1) and describe several newly developed genotyping methods that are based on NGS technology.

#### 3.1. Whole-genome Re-sequencing

Whole-genome re-sequencing enables determination of genetic diversity at a genome-wide level at a high resolution [77, 78]. This method provides unbiased information of genetic variation. For instance, the complete genomes of 80 natural accessions of *A. thaliana*, from 8 regions, were re-sequenced, and about 5 million single nucleotide polymorphisms (SNPs) were identified across these natural accessions [31]. In *A. thaliana*, whole genome re-sequencing was used to identify a causal SNP of a circadian clock mutation

induced by EMS [79]. In rice, 6.5 million SNPs were obtained by re-sequencing across 50 accessions (40 cultivated and 10 wild accessions), and the candidate regions related to domestication were identified [80]. In *japonica* rice, 132,462 SNPs were identified between the standard cultivar Nipponbare and a sake-brewing cultivar [81]. In tomato, 11.6 million SNPs were identified from the re-sequencing data of 360 diverse accessions collected from around the world [82]. Whole-genome re-sequencing has also been performed in other crops such as chickpea [83], pepper [84], and sesame [85]. After the *Arabidopsis* genome was sequenced in 2000 [86], the number of species for which genome sequences have been released has increased, for both model and non-model crop plants, because of the prevalence of NGS technologies [87]. Currently, the complete genomes of several species of interest, particularly those with small genomes, can be re-sequenced at any laboratory, and technologies with greater throughput and more useful bioinformatics techniques are being developed rapidly. However, several challenges remain when dealing with large populations of a species that possess a complex and large genome more than 1 Gb long. Major cereal crops, for example, Triticeae crops such as common wheat and barley, have 17 Gb and 5 Gb genomes, respectively, and the genomes contain highly repetitive sequences [88, 89]. For avoiding such genomic complexity, several solutions are available that help reduce the challenges associated with whole-genome re-sequencing.



**Fig. (1).** Characteristics of representative methods for determining genetic diversity using NGS technologies. A scatter plot representing the relationship between plant genome size and number of target SNPs. Boxed and circled approaches must be used with and without reference-genome sequences, respectively.

### 3.2. Whole-exome-capture Sequencing

Exome capture—a hybridization-based enrichment method for targeted re-sequencing of entire protein-coding regions (exons)—enables cost-effective identification of nearly all of the genetic variation present in protein-coding genes [90, 91]. Briefly, genomic DNA is randomly sheared and hybridized with oligonucleotide probes specific to target exons, and then only hybridized fragments are recovered and sequenced. Thus, the number of fragments targeted for sequencing is enriched substantially and sequence coverage is increased from that in whole-genome re-sequencing. Exome-

capture sequencing has been effectively applied in the study of rare Mendelian diseases in human [91]. In maize, whole-exome capture was used to verify copy-number variation among 2 lines of Recombinant Inbred Lines (RILs) and their inbred parents [92]. A whole-exome-capture platform has also been developed for barley [93]. Pankin *et al.* successfully identified the causal gene and candidate mutation that control long-day flowering [94] by the mapping-by-sequencing approach combined with whole-exome capture [93, 95]. They applied the combined sequencing approach to the parental lines (Bowman and its introgression line eam5) and a backcross population selected for early flowering in barley.

### 3.3. RNA-sequencing (RNA-seq)

RNA-seq is a rapidly growing application of NGS technology that is used for studying gene expression [96]. RNA-seq used with a reference genome is a powerful tool for identifying transcription units and splice isoforms together with their expression profiles [97, 98]. For non-model organisms, reference-genome-free RNA-seq, a *de novo* RNA-seq method, has become a widely used method for obtaining transcribed sequences and information on genetic variations together with quantified gene expression [99–101].

In a number of cases, although genome sizes vary among different crops, the number of genes or the total gene sizes do not differ substantially. Because intergenic regions are ignored based on the principle of RNA-seq method, genotyping performed using RNA-seq is more cost effective than genotyping based on whole-genome re-sequencing. For example, >1 million SNPs were genotyped by RNA-seq in 368 maize inbred lines [102]; in rye, >5,000 SNPs were identified from RNA-seq data of 5 winter inbred lines [103]; and in cassava, 675,559 SNP markers were identified through RNA-seq of 16 cassava accessions [104]. However, RNA-seq-based genotyping has some limitations: (1) the SNP density in genic regions is considerably lower than that in intergenic regions and (2) several genes exhibit extremely low or even no expression in specific tissues or at specific time points.

### 3.4. Restriction-site-associated DNA Sequencing (RAD-seq)

RAD-seq can concurrently provide information on thousands of genetic markers from several hundreds of individuals [105, 106]. RAD-seq involves 3 simple molecular biology techniques: (1) shearing of DNA by restriction enzymes; (2) size selection (300–700 bp); and (3) PCR amplification of only fragments containing restriction sites. Because RAD-seq data can also be analyzed without reference-genome sequences, this method facilitates genetic screening in non-model plant species [107]. RAD-seq has been used for identifying genotypes and constructing linkage maps of barley [108], chickpea [109], and ryegrass [110]. Recently, a RAD-seq genotyping dataset, containing 55,052 SNPs from 286 genotyped accessions, was generated and used for identifying 48 loci related to domestication in soybean [111].

### 3.5. Genotyping-by-sequencing (GBS)

GBS was developed as a technically simple and highly multiplex genotyping approach, and was first applied to

maize [112], GBS has now been extended to diverse organisms, because it does not include a size-selection step and is simpler than the RAD-seq method [106]. In GBS, complexity is reduced by using a methylation-sensitive enzyme, *ApeKI* [112]. Moreover, a 2-enzyme GBS protocol, which can suitably and uniformly reduce complexity, has been developed and applied in barley and wheat [113]. GBS approach has also been extensively used for mining genetic diversity and for high-throughput genotyping at a genome-wide scale in various crops. For instance, GBS has been used to identify 47,702 SNPs across a diverse set of 304 lines in soya bean [114], 44,844 SNPs across 93 diverse cultivated and wild accessions in chickpea [115], 265,487 SNPs across 971 accessions in sorghum [116], and 30,984 SNP markers across 176 RILs (*indica* IR64 × *japonica* Azucena) in rice [117].

**3.6. Amplicon Sequencing**

Recently, a combination of multiplex PCR amplification and NGS technologies, termed “AmpliSeq,” has been used for detecting genetic diversity [118]. In AmpliSeq, multiplex PCR is used for simple and fast library construction for enrichment of targeted sequences. Furthermore, because only 10 ng of input DNA per reaction is required for this method, it can be used for analyzing minute amounts of samples. For example, 10 ng of input DNA from formalin-fixed paraffin-embedded samples of tumor tissue were successfully amplified and sequenced using this method [119, 120]. Moreover, AmpliSeq has been receiving increasing attention in clinical studies because a commercially available system, Ion AmpliSeq Cancer Hotspot Panel v2, has been designed to amplify 207 amplicons covering approximately 2,800 mutations, in 50 cancer-related genes, listed in the Catalogue of Somatic Mutations in Cancer (<http://www.lifetechnologies.com/order/catalog/product/4475346>). Furthermore,

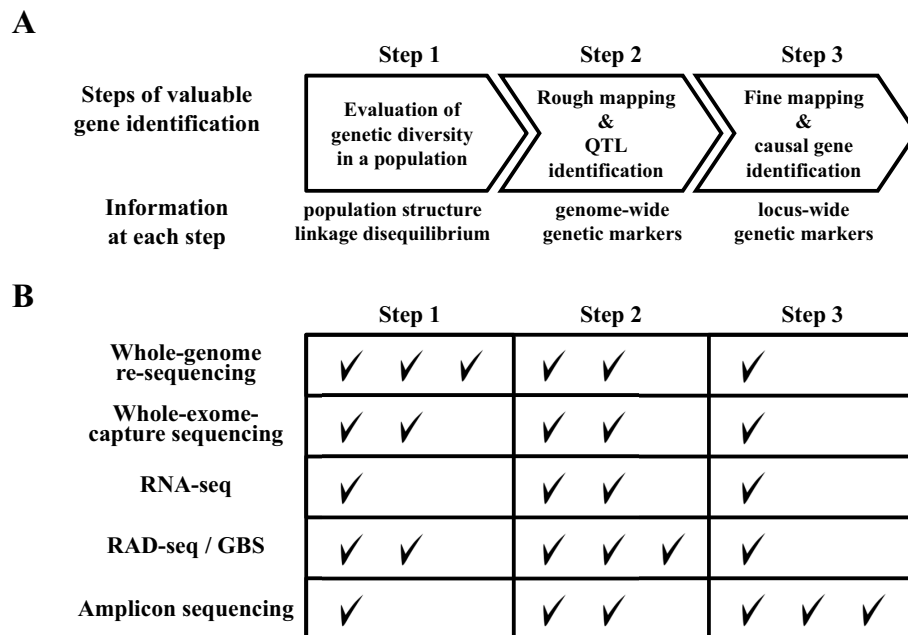
AmpliSeq can be used not only for clinical studies but also for studies on crop plants: Ion AmpliSeq Custom Panels can create primer pairs at genomic regions of interest in any organism by using a reference genome, and can increase the number of primer pairs used in a multiplex PCR reaction to 6,144; thus, thousands of target genes can be analyzed quickly and easily. The online Ion AmpliSeq Designer tool (<https://www.ampliseq.com/>) is preloaded with the reference genome of crops (e.g., maize, rice, soybean, and tomato) and is freely available for creating custom panels.

**4. ANALYSIS OF THE RELATIONSHIP BETWEEN PHENOTYPE AND GENOTYPE**

Understanding the relationship between genetic diversity and the phenotypic differences observed within a species is not only important biologically but is also a crucial first step in improving agriculturally useful traits. Because the newly developed methods for mining genetic diversities allow genetic markers to be identified quickly and inexpensively at a high density and randomly in a genome, forward genetics approaches can serve as a straight-forward method for uncovering the causal genetic polymorphisms that underlie phenotypic differences (e.g., growth rate, yield, and abiotic/biotic stress tolerance) (Fig. 2). Here, we showcase examples of genetic approaches such as QTL analysis and GWAS that have been significantly accelerated by synergistic uses of NGS-based methods for genotyping.

**4.1. Quantitative Trait Locus Analysis**

A Quantitative trait locus analysis(QTL) analysis is a genetic approach that is used in an attempt to describe the genetic basis of variation in quantitative traits [121]. QTL analysis has provided a solution for identifying genomic regions



**Fig. (2). Conceptual framework for identification of valuable genes.**

**A.** Schematic diagram of gene identification from genetic polymorphisms. **B.** Eligibility at each step of NGS-based approaches used for identification of genetic polymorphisms.

that cosegregate with a trait in mapping population such as  $F_2$  or RILs. The NGS-based genome-scale genotyping methods to identify genetic polymorphisms have been carried out in various plant species including crops. In *Arabidopsis*, approximately 40 quantitative traits produced through natural variation have been investigated and mapped by means of QTL analysis as reviewed in [122]. The traits of 9 distinct morphologies in barley [108], stem rust resistance in perennial ryegrass [110], and fiber strength and rust resistance in cotton [123] were mapped using the genetic markers created and assessed using the NGS utilizing method of RAD-seq.

#### 4.2. Genome-wide Association Study

A GWAS is a genetic approach used for evaluating the association between genomic diversity and a phenotype of interest that could be scored in a large population. This method was developed about 10 years ago for studying genetic diseases and complex traits in human [124]. The GWAS approach has been applied in plant science recently, and it provides advantages particularly for investigations in inbred plant species. Because genotyping data can be reused in a GWAS panel, phenotyping data obtained for several traits of interest can be analyzed repeatedly without genotyping [30, 125]. GWASs have been conducted successfully for numerous crops such as in maize genotyped by RNA-seq [102], rice genotyped by whole-genome re-sequencing [126], wheat genotyped by whole-exome capture and GBS [127], foxtail millet genotyped by whole-genome re-sequencing [128], *Miscanthus sinensis* genotyped by RAD-seq [129], and sorghum genotyped by GBS [116]. The ability of GWAS for detecting rare alleles is low, but this limitation could be overcome by using a large sample size or creating multiple mapping populations, as in the case of nested association mapping [130] or Multiparent Advanced Generation Inter-Cross [131].

#### 4.3. SHOREmap and MutMap

SHOREmap [95] is based on bulked-segregant analysis of phenotyped  $F_2$  progeny [132]. In this approach, an artificially induced mutant is crossed into its wild-type line to obtain an  $F_2$  mapping population, and the DNA of  $F_2$  individuals are bulked and the whole genome is re-sequenced by NGS to identify the genomic region harboring the causal mutation. Since SHOREmap was originally performed in the  $F_2$  population of *A. thaliana*, it has been extended to other types of mapping population such as isogenic back cross population [133, 134] and has been extended to crop plants such as maize [135].

MutMap [136] is also an NGS-based method that is technically similar to SHOREmap; however, this approach uses SNPs incorporated by mutagenesis as markers to search for the regions including causal mutation for a phenotype. Because MutMap needs fewer SNPs than SHOREmap, reliable alignment of genome sequences and SNP calling with lower noise can be achieved [136]. Recently, the MutMap method was used to identify a loss-of-function mutation responsible for salt tolerance and to breed a salt-tolerant variety quicker than by using conventional breeding methods (only two years were required) [137]. This breakthrough will dramatically increase the efficiency of identification of genes responsible for valuable phenotypes in crop breeding.

## 5. CONCLUSIONS

The rapid diffusion of high-throughput sequencing technologies has revitalized the exploration of genetic diversities. Even in crop species with large and complex genomes, various scales of sequencing applications enable cost-effective genotyping at each step of genetics studies. Sequencing of the biogeographic samples will provide insights into the demographic history and local adaptation of the species. Genome-scale polymorphisms analysis of artificially designed mapping populations in various crop species will be beneficial for identifying genetic variations and would thereby accelerate the process of identifying genes associated with agronomically important traits. Furthermore, the sequencing power of the current technologies allow for the determination of the relationship between genetic diversity and the physiological response of a plant species in a fluctuated environment. Integrated approaches of population genomics with other sequencing-based omics spectrums, such as transcriptome and epigenome, in a field condition is still a challenging area. These approaches based on high-throughput sequencing will allow identification of the systems evolved in a plant species as adaptations to various conditions, and will provide clues to enhance plant productivity.

#### LIST OF ABBREVIATIONS

NGS	=	Next-generation sequencing
QTL	=	Quantitative trait locus
GWAS	=	Genome-wide association study/studies
EMS	=	Ethyl methane sulfonic acid
SNP	=	Single nucleotide polymorphism
RILs	=	Recombinant Inbred Lines
RNA-seq	=	RNA-sequencing
RAD-seq	=	Restriction-site-associated DNA sequencing
GBS	=	Genotyping-by-sequencing

#### CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflict of interest.

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#### REFERENCES

- [1] United Nations. *World Population Prospects: The 2012 Revision*, United Nations, New York, 2013.

- [2] Smith, P.; Gregory, P.J. Climate change and sustainable food production. *Proc. Nutr. Soc.*, **2013**, *72*, 21-28.
- [3] World Bank. World Development Report 2008: Agriculture for Development, World Bank, Washington, DC, **2008**.
- [4] Royal Society. Reaping the benefits: Science and the sustainable intensification of global agriculture, Royal Society, London, **2009**.
- [5] IPCC. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change, IPCC, Geneva, Switzerland, **2014**.
- [6] Godfray, H.C.J.; Beddington, J.R.; Crute, I.R.; Haddad, L.; Lawrence, D.; Muir, J.F.; Pretty, J.; Robinson, S.; Thomas, S.M.; Toulmin, C. Food security: the challenge of feeding 9 billion people. *Science*, **2010**, *327*, 812-818.
- [7] Tester, M.; Langridge, P. Breeding technologies to increase crop production in a changing world. *Science*, **2010**, *327*, 818-822.
- [8] Evenson, R.E.; Gollin, D. Assessing the impact of the green revolution, 1960 to 2000. *Science*, **2003**, *300*, 758-762.
- [9] Nelson, G.C.; Rosegrant, M.W.; Koo, J.; Robertson, R.; Sulser, T.; Zhu, T.; Ringler, C.; Msangi, S.; Palazzo, A.; Batka, M.; Magalhaes, M.; Valmonte-Santos, R.; Ewing, M.; Lee, D. *Climate change: Impact on agriculture and costs of adaptation*, International Food Policy Research Institute (IFPRI), Washington, DC, **2009**.
- [10] Pingali, P.L. Green revolution: impacts, limits, and the path ahead. *Proc. Natl. Acad. Sci. U. S. A.*, **2012**, *109*, 12302-12308.
- [11] Du, T.; Kang, S.; Zhang, J.; Davies, W.J. Deficit irrigation and sustainable water-resource strategies in agriculture for China's food security. *J. Exp. Bot.*, **2015**, *66*, 2253-2269.
- [12] Baker, A.; Caesar, S.A.; Palmer, A.J.; Paterson, J.B.; Qi, W.; Muench, S.P.; Baldwin, S.A. Replace, reuse, recycle: improving the sustainable use of phosphorus by plants. *J. Exp. Bot.*, **2015**, doi: 10.1093/jxb/erv210.
- [13] Esquinas-Alcázar, J. Protecting crop genetic diversity for food security: political, ethical and technical challenges. *Nat. Rev. Genet.* **2005**, *6*, 946-953.
- [14] Metzker, M.L. Sequencing technologies — the next generation. *Nat. Rev. Genet.*, **2010**, *11*, 31-46.
- [15] Alonso-Blanco, C.; Aarts, M.G.M.; Bentsink, L.; Keurentjes, J.J.B.; Reymond, M.; Vreugdenhil, D.; Koornneef, M. What has natural variation taught us about plant development, physiology, and adaptation? *Plant Cell*, **2009**, *21*, 1877-1896.
- [16] Barboza, L.; Effgen, S.; Alonso-Blanco, C.; Kooke, R.; Keurentjes, J.J.B.; Koornneef, M.; Alcázar, R. *Arabidopsis* semidwarfs evolved from independent mutations in GA20ox1, ortholog to green revolution dwarf alleles in rice and barley. *Proc. Natl. Acad. Sci. U. S. A.*, **2013**, *110*, 15818-15823.
- [17] Brock, M.T.; Kover, P.X.; Weinig, C. Natural variation in GA1 associates with floral morphology in *Arabidopsis thaliana*. *New Phytol.*, **2012**, *195*, 58-70.
- [18] Kellermeier, F.; Chardon, F.; Amtmann, A. Natural variation of *Arabidopsis* root architecture reveals complementing adaptive strategies to potassium starvation. *Plant Physiol.*, **2013**, *161*, 1421-1432.
- [19] Luo, N.; Liu, J.; Yu, X.; Jiang, Y. Natural variation of drought response in *Brachypodium distachyon*. *Physiol. Plant*, **2011**, *141*, 19-29.
- [20] Khan, N.; Kazmi, R.H.; Willems, L.A.J.; van Heusden, A.W.; Ligterink, W.; Hilhorst, H.W.M. Exploring the natural variation for seedling traits and their link with seed dimensions in tomato. *PLoS One*, **2012**, *7*, e43991.
- [21] Ream, T.; Woods, D.; Schwartz, C.; Sanabria, C.; Mahoy, J.; Walters, E.; Kaeppeler, H.; Amasino, R. Interaction of photoperiod and vernalization determine flowering time of *Brachypodium distachyon*. *Plant Physiol.*, **2014**, *164*, 694-709.
- [22] Ren, Z.; Zheng, Z.; Chinnusamy, V.; Zhu, J.; Cui, X.; Iida, K.; Zhu, J.-K. RAS1, a quantitative trait locus for salt tolerance and ABA sensitivity in *Arabidopsis*. *Proc. Natl. Acad. Sci. U. S. A.*, **2010**, *107*, 5669-5674.
- [23] Schwartz, C.J.; Doyle, M.R.; Manzaneda, A.J.; Rey, P.J.; Mitchell-Olds, T.; Amasino, R.M. Natural Variation of Flowering Time and Vernalization Responsiveness in *Brachypodium distachyon*. *BioEnergy Res.*, **2010**, *3*, 38-46.
- [24] Bloomer, R.; Juenger, T.; Symonds, V. Natural variation in GL1 and its effects on trichome density in *Arabidopsis thaliana*. *Mol. Ecol.*, **2012**, *21*, 3501-3515.
- [25] Wingler, A.; Juvany, M.; Cuthbert, C.; Munné-Bosch, S. Adaptation to altitude affects the senescence response to chilling in the perennial plant *Arabidopsis alpina*. *J. Exp. Bot.*, **2015**, *66*, 355-367.
- [26] Kwon, C.-T.; Yoo, S.-C.; Koo, B.-H.; Cho, S.-H.; Park, J.-W.; Zhang, Z.; Li, J.; Li, Z.; Paek, N.-C. Natural variation in Early flowering1 contributes to early flowering in *japonica* rice under long days. *Plant Cell Environ.*, **2013**, *37*, 101-112.
- [27] Juenger, T.E. Natural variation and genetic constraints on drought tolerance. *Curr. Opin. Plant Biol.*, **2013**, *16*, 274-281.
- [28] Tang, J.; Yu, X.; Luo, N.; Xiao, F.; Camberato, J.J.; Jiang, Y. Natural variation of salinity response, population structure and candidate genes associated with salinity tolerance in perennial ryegrass accessions. *Plant Cell Environ.*, **2013**, *36*, 2021-2033.
- [29] Abraham, M.C.; Metheerairut, C.; Irish, V.F. Natural variation identifies multiple loci controlling petal shape and size in *Arabidopsis thaliana*. *PLoS One*, **2013**, *8*, e56743.
- [30] Weigel, D.; Mott, R. The 1001 genomes project for *Arabidopsis thaliana*. *Genome Biol.*, **2009**, *10*, 107.
- [31] Cao, J.; Schneeberger, K.; Ossowski, S.; Günther, T.; Bender, S.; Fitz, J.; Koenig, D.; Lanz, C.; Stegle, O.; Lippert, C.; Wang, X.; Ott, F.; Müller, J.; Alonso-Blanco, C.; Borgwardt, K.; Schmid, K.J.; Weigel, D. Whole-genome sequencing of multiple *Arabidopsis thaliana* populations. *Nat. Genet.*, **2011**, *43*, 956-963.
- [32] Feldmann, K.A. T-DNA insertion mutagenesis in *Arabidopsis*: mutational spectrum. *Plant J.*, **1991**, *1*, 71-82.
- [33] Jeon, J.S.; Lee, S.; Jung, K.H.; Jun, S.H.; Jeong, D.H.; Lee, J.; Kim, C.; Jang, S.; Yang, K.; Nam, J.; An, K.; Han, M.J.; Sung, R.J.; Choi, H.S.; Yu, J.H.; Choi, J.H.; Cho, S.Y.; Cha, S.S.; Kim, S.I.; An, G. T-DNA insertional mutagenesis for functional genomics in rice. *Plant J.*, **2000**, *22*, 561-570.
- [34] Sundaresan, V. Horizontal spread of transposon mutagenesis: new uses for old elements. *Trends Plant Sci.*, **1996**, *1*, 184-190.
- [35] Izawa, T.; Ohnishi, T.; Nakano, T.; Ishida, N.; Enoki, H.; Hashimoto, H.; Itoh, K.; Terada, R.; Wu, C.; Miyazaki, C.; Endo, T.; Iida, S.; Shimamoto, K. Transposon tagging in rice. *Plant Mol. Biol.*, **1997**, *35*, 219-229.
- [36] Walden, R.; Fritze, K.; Hayashi, H.; Miklashevichs, E.; Harling, H.; Schell, J. Activation tagging: a means of isolating genes implicated as playing a role in plant growth and development. *Plant Mol. Biol.*, **1994**, *26*, 1521-1528.
- [37] Odell, J.T.; Nagy, F.; Chua, N.H. Identification of DNA sequences required for activity of the cauliflower mosaic virus 35S promoter. *Nature*, **1984**, *313*, 810-812.
- [38] Ichikawa, T.; Nakazawa, M.; Kawashima, M.; Iizumi, H.; Kuroda, H.; Kondou, Y.; Tsuchida, Y.; Suzuki, K.; Ishikawa, A.; Seki, M.; Fujita, M.; Motohashi, R.; Nagata, N.; Takagi, T.; Shinozaki, K.; Matsui, M. The FOX hunting system: an alternative gain-of-function gene hunting technique. *Plant J.*, **2006**, *48*, 974-985.
- [39] Sprink, T.; Metje, J.; Hartung, F. Plant genome editing by novel tools: TALEN and other sequence specific nucleases. *Curr. Opin. Biotechnol.*, **2015**, *32*, 47-53.
- [40] Bortesi, L.; Fischer, R. The CRISPR/Cas9 system for plant genome editing and beyond. *Biotechnol. Adv.*, **2014**, *33*, 41-52.
- [41] Osakabe, Y.; Osakabe, K. Genome editing with engineered nucleases in plants. *Plant Cell Physiol.*, **2014**, *56*, 389-400.
- [42] Alonso, J.M.; Ecker, J.R. Moving forward in reverse: genetic technologies to enable genome-wide phenomic screens in *Arabidopsis*. *Nat. Rev. Genet.*, **2006**, *7*, 524-536.
- [43] Segal, G.A. A review of the genetic effects of ethyl methanesulfonate. *Mutat. Res.*, **1984**, *134*, 113-142.
- [44] Greene, E.A.; Codomo, C.A.; Taylor, N.E.; Henikoff, J.G.; Till, B.J.; Reynolds, S.H.; Enns, L.C.; Burtner, C.; Johnson, J.E.; Odden, A.R.; Comai, L.; Henikoff, S. Spectrum of chemically induced mutations from a large-scale reverse-genetic screen in *Arabidopsis*. *Genetics*, **2003**, *164*, 731-740.
- [45] Botticella, E.; Sestili, F.; Hernandez-Lopez, A.; Phillips, A.; Lafiandra, D. High resolution melting analysis for the detection of EMS induced mutations in wheat SBEIIa genes. *BMC Plant Biol.*, **2011**, *11*, 156.
- [46] Cooper, J.L.; Till, B.J.; Laport, R.G.; Darlow, M.C.; Kleffner, J.M.; Jamai, A.; El-Mellouki, T.; Liu, S.; Ritchie, R.; Nielsen, N.; Bilyeu, K.D.; Meksem, K.; Comai, L.; Henikoff, S. TILLING to detect induced mutations in soybean. *BMC Plant Biol.*, **2008**, *8*, 9.
- [47] Xin, Z.; Wang, M.L.; Barkley, N.A.; Burrow, G.; Franks, C.; Pederson, G.; Burke, J. Applying genotyping (TILLING) and phenotyping analyses to elucidate gene function in a chemically

- induced sorghum mutant population. *BMC Plant Biol.*, **2008**, *8*, 103.
- [48] Okabe, Y.; Asamizu, E.; Saito, T.; Matsukura, C.; Ariizumi, T.; Brès, C.; Rothan, C.; Mizoguchi, T.; Ezura, H. Tomato TILLING technology: development of a reverse genetics tool for the efficient isolation of mutants from Micro-Tom mutant libraries. *Plant Cell Physiol.*, **2011**, *52*, 1994-2005.
- [49] Zhu, Q.; Smith, S.M.; Ayele, M.; Yang, L.; Jogi, A.; Chaluvadi, S.R.; Bennetzen, J.L. High-throughput discovery of mutations in tef semi-dwarfing genes by next-generation sequencing analysis. *Genetics*, **2012**, *192*, 819-829.
- [50] Kurowska, M.; Daszkowska-Golec, A.; Gruszka, D.; Marzec, M.; Szurman, M.; Szarejko, I.; Maluszynski, M. TILLING: a shortcut in functional genomics. *J. Appl. Genet.*, **2011**, *52*, 371-390.
- [51] Nilan, R.; Sideris, E.; Kleinhofs, A.; Sander, C.; Konzak, C. Azide — a potent mutagen. *Mutat. Res.*, **1973**, *17*, 142-144.
- [52] H, H.; M, I. Mutagenic effect of sodium azide in barley. *Japanese J. Breed.*, **1980**, *30*, 20-26.
- [53] Engvild, K.C. Mutagenesis of the model grass *Brachypodium distachyon* with sodium azide, Risø National Laboratory, Roskilde, Denmark, **2005**.
- [54] Al-Qurainy, F.; Khan, S. Mutagenic effects of sodium azide and its application in crop improvement. *World Appl. Sci. J.*, **2009**.
- [55] Konzak, C.; Niknejad, M.; Wickham, I.; Donaldson, E. Mutagenic interaction of sodium azide on mutations induced in barley seeds treated with diethyl sulfate or N-methyl-N'-nitrosourea. *Mutat. Res. Mol. Mech. Mutagen.*, **1975**, *30*, 55-61.
- [56] Hadwiger, L.; Sander, C.; Eddyvean, J.; Ralston, J. Sodium azide-induced mutants of peas that accumulate pisatin. *Phytopathology*, **1976**, *66*, 629-630.
- [57] Sander, C.; Nilan, R.A.; Kleinhofs, A.; Vig, B.K. Mutagenic and chromosome-breaking effects of azide in barley and human leukocytes. *Mutat. Res.*, **1978**, *50*, 67-75.
- [58] Morita, R.; Kusaba, M.; Iida, S.; Yamaguchi, H.; Nishio, T.; Nishimura, M. Molecular characterization of mutations induced by gamma irradiation in rice. *Genes Genet. Syst.*, **2009**, *84*, 361-370.
- [59] Chono, M.; Matsunaka, H.; Seki, M.; Fujita, M.; Kiribuchi-Otobe, C.; Oda, S.; Kojima, H.; Kobayashi, D.; Kawakami, N. Isolation of a wheat (*Triticum aestivum* L.) mutant in ABA 8'-hydroxylase gene: effect of reduced ABA catabolism on germination inhibition under field condition. *Breed. Sci.*, **2013**, *63*, 104-115.
- [60] Phillips, T.L.; Ross, G.Y.; Goldstein, L.S.; Ainsworth, J.; Alpen, E. *In vivo* radiobiology of heavy ions. *Int. J. Radiat. Oncol. Biol. Phys.*, **1982**, *8*, 2121-2125.
- [61] Kraft, G.; Kraft-Weyrather, W.; Blakely, E.A.; Roots, R. Heavy-ion effects on cellular and subcellular systems: inactivation, chromosome aberrations and strand breaks induced by iron and nickel ions. *Int. J. Radiat. Oncol. Biol. Phys.*, **1986**, *6*, 127-136.
- [62] Ward, J.F. The complexity of DNA damage: relevance to biological consequences. *Int. J. Radiat. Biol.*, **1994**, *66*, 427-432.
- [63] Goodhead, D.T. Molecular and cell models of biological effects of heavy ion radiation. *Radiat. Environ. Biophys.*, **1995**, *34*, 67-72.
- [64] Höglund, E.; Blomquist, E.; Carlsson, J.; Stenerlöw, B. DNA damage induced by radiation of different linear energy transfer: initial fragmentation. *Int. J. Radiat. Biol.*, **2000**, *76*, 539-547.
- [65] Yokota, Y.; Yamada, S.; Hase, Y.; Shikazono, N.; Narumi, I.; Tanaka, A.; Inoue, M. Initial yields of DNA double-strand breaks and DNA Fragmentation patterns depend on linear energy transfer in tobacco BY-2 protoplasts irradiated with helium, carbon and neon ions. *Radiat. Res.*, **2007**, *167*, 94-101.
- [66] Shikazono, N.; Suzuki, C.; Kitamura, S.; Watanabe, H.; Tano, S.; Tanaka, A. Analysis of mutations induced by carbon ions in *Arabidopsis thaliana*. *J. Exp. Bot.*, **2005**, *56*, 587-596.
- [67] Miyazaki, K.; Suzuki, K.; Iwaki, K.; Kusumi, T.; Abe, T.; Yoshida, S.; Fukui, H. Flower pigment mutations induced by heavy ion beam irradiation in an interspecific hybrid of *Torenia*. *Plant Biotechnol.*, **2006**, *23*, 163-167.
- [68] Kanaya, T.; Saito, H.; Hayashi, Y.; Fukunishi, N.; Ryuto, H.; Miyazaki, K.; Kusumi, T.; Abe, T.; Suzuki, K. Heavy-ion beam-induced sterile mutants of verbena (*Verbena × hybrida*) with an improved flowering habit. *Plant Biotechnol.*, **2008**, *25*, 91-96.
- [69] Miyamoto, M.; Motooka, D.; Gotoh, K.; Imai, T.; Yoshitake, K.; Goto, N.; Iida, T.; Yasunaga, T.; Horii, T.; Arakawa, K.; Kasahara, M.; Nakamura, S. Performance comparison of second- and third-generation sequencers using a bacterial genome with two chromosomes. *BMC Genomics*, **2014**, *15*, 699.
- [70] Eid, J.; Fehr, A.; Gray, J.; Luong, K.; Lyle, J.; Otto, G.; Peluso, P.; Rank, D.; Baybayan, P.; Bettman, B.; Bibillo, A.; Bjornson, K.; Chaudhuri, B.; Christians, F.; Cicero, R.; Clark, S.; Dalal, R.; Dewinter, A.; Dixon, J.; Foquet, M.; Gaertner, A.; Hardenbol, P.; Heiner, C.; Hester, K.; Holden, D.; Kearns, G.; Kong, X.; Kuse, R.; Lacroix, Y.; Lin, S.; Lundquist, P.; Ma, C.; Marks, P.; Maxham, M.; Murphy, D.; Park, I.; Pham, T.; Phillips, M.; Roy, J.; Sebra, R.; Shen, G.; Sorenson, J.; Tomaney, A.; Travers, K.; Trulson, M.; Vieceli, J.; Wegener, J.; Wu, D.; Yang, A.; Zaccarin, D.; Zhao, P.; Zhong, F.; Korfach, J.; Turner, S. Real-time DNA sequencing from single polymerase molecules. *Science*, **2009**, *323*, 133-138.
- [71] Koren, S.; Schatz, M.C.; Walenz, B.P.; Martin, J.; Howard, J.T.; Ganapathy, G.; Wang, Z.; Rasko, D.A.; McCombie, W.R.; Jarvis, E.D.; Adam M Phillippy. Hybrid error correction and de novo assembly of single-molecule sequencing reads. *Nat. Biotechnol.*, **2012**, *30*, 693-700.
- [72] Berlin, K.; Koren, S.; Chin, C.-S.; Drake, J.P.; Landolin, J.M.; Phillippy, A.M. Assembling large genomes with single-molecule sequencing and locality-sensitive hashing. *Nat. Biotechnol.*, **2015**, *33*, 623-630.
- [73] Reifemberger, J.G.; Dorfman, K.D.; Cao, H. Topological events in single molecules of *E. coli* DNA confined in nanochannels. *Analyst*, **2015**, *140*, 4887-4894.
- [74] Check Hayden, E. Pint-sized DNA sequencer impresses first users. *Nature*, **2015**, *521*, 15-16.
- [75] Ashton, P.M.; Nair, S.; Dallman, T.; Rubino, S.; Rabsch, W.; Mwaigwisya, S.; Wain, J.; O'Grady, J. MinION nanopore sequencing identifies the position and structure of a bacterial antibiotic resistance island. *Nat. Biotechnol.*, **2014**, *33*, 296-300.
- [76] Jain, M.; Fiddes, I.T.; Miga, K.H.; Olsen, H.E.; Paten, B.; Akesson, M. Improved data analysis for the MinION nanopore sequencer. *Nat. Methods*, **2015**, *12*, 351-356.
- [77] Michael, T.P.; VanBuren, R. Progress, challenges and the future of crop genomes. *Curr. Opin. Plant Biol.*, **2015**, *24*, 71-81.
- [78] James, G.V.; Patel, V.; Nordström, K.J.; Klansen, J.R.; Salomé, P.A.; Weigel, D.; Schneeberger, K. User guide for mapping-by-sequencing in *Arabidopsis*. *Genome Biol.*, **2013**, *14*, R61.
- [79] Ashelford, K.; Eriksson, M.E.; Allen, C.M.; D'Amore, R.; Johansson, M.; Gould, P.; Kay, S.; Millar, A.J.; Hall, N.; Hall, A. Full genome re-sequencing reveals a novel circadian clock mutation in *Arabidopsis*. *Genome Biol.*, **2011**, *12*, R28.
- [80] Xu, X.; Liu, X.; Ge, S.; Jensen, J.D.; Hu, F.; Li, X.; Dong, Y.; Gutenkunst, R.N.; Fang, L.; Huang, L.; Li, J.; He, W.; Zhang, G.; Zheng, X.; Zhang, F.; Li, Y.; Yu, C.; Kristiansen, K.; Zhang, X.; Wang, J.; Wright, M.; McCouch, S.; Nielsen, R.; Wang, J.; Wang, W. Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. *Nat. Biotechnol.*, **2012**, *30*, 105-111.
- [81] Arai-Kichise, Y.; Shiwa, Y.; Nagasaki, H.; Ebana, K.; Yoshikawa, H.; Yano, M.; Wakasa, K. Discovery of genome-wide DNA polymorphisms in a landrace cultivar of *japonica* rice by whole-genome sequencing. *Plant Cell Physiol.*, **2011**, *52*, 274-282.
- [82] Lin, T.; Zhu, G.; Zhang, J.; Xu, X.; Yu, Q.; Zheng, Z.; Zhang, Z.; Lun, Y.; Li, S.; Wang, X.; Huang, Z.; Li, J.; Zhang, C.; Wang, T.; Zhang, Y.; Wang, A.; Zhang, Y.; Lin, K.; Li, C.; Xiong, G.; Xue, Y.; Mazzucato, A.; Causse, M.; Fei, Z.; Giovannoni, J.J.; Chetelat, R.T.; Zamir, D.; Städler, T.; Li, J.; Ye, Z.; Du, Y.; Huang, S. Genomic analyses provide insights into the history of tomato breeding. *Nat. Genet.*, **2014**, *46*, 1220-1226.
- [83] Varshney, R.K.; Song, C.; Saxena, R.K.; Azam, S.; Yu, S.; Sharpe, A.G.; Cannon, S.; Baek, J.; Rosen, B.D.; Tar'an, B.; Millan, T.; Zhang, X.; Ramsay, L.D.; Iwata, A.; Wang, Y.; Nelson, W.; Farmer, A.D.; Gaur, P.M.; Soderlund, C.; Penmetsa, R.V.; Xu, C.; Bharti, A.K.; He, W.; Winter, P.; Zhao, S.; Hane, J.K.; Carrasquilla-Garcia, N.; Condie, J.A.; Upadhyaya, H.D.; Luo, M.-C.; Thudi, M.; Gowda, C.L.L.; Singh, N.P.; Lichtenzevig, J.; Gali, K.K.; Rubio, J.; Nadarajan, N.; Dolezel, J.; Bansal, K.C.; Xu, X.; Edwards, D.; Zhang, G.; Kahl, G.; Gil, J.; Singh, K.B.; Datta, S.K.; Jackson, S.A.; Wang, J.; Cook, D.R. Draft genome sequence of chickpea (*Cicer arietinum*) provides a resource for trait improvement. *Nat. Biotechnol.*, **2013**, *31*, 240-246.
- [84] Qin, C.; Yu, C.; Shen, Y.; Fang, X.; Chen, L.; Min, J.; Cheng, J.; Zhao, S.; Xu, M.; Luo, Y.; Yang, Y.; Wu, Z.; Mao, L.; Wu, H.; Ling-Hu, C.; Zhou, H.; Lin, H.; González-Morales, S.; Trejo-Saavedra, D.L.; Tian, H.; Tang, X.; Zhao, M.; Huang, Z.; Zhou, A.; Yao, X.; Cui, J.; Li, W.; Chen, Z.; Feng, Y.; Niu, Y.; Bi, S.; Yang,



- X.; Li, W.; Cai, H.; Luo, X.; Montes-Hernández, S.; Leyva-González, M.A.; Xiong, Z.; He, X.; Bai, L.; Tan, S.; Tang, X.; Liu, D.; Liu, J.; Zhang, S.; Chen, M.; Zhang, L.; Zhang, L.; Zhang, Y.; Liao, W.; Zhang, Y.; Wang, M.; Lv, X.; Wen, B.; Liu, H.; Luan, H.; Zhang, Y.; Yang, S.; Wang, X.; Xu, J.; Li, X.; Li, S.; Wang, J.; Palloix, A.; Bosland, P.W.; Li, Y.; Krogh, A.; Rivera-Bustamante, R.F.; Herrera-Estrella, L.; Yin, Y.; Yu, J.; Hu, K.; Zhang, Z. Whole-genome sequencing of cultivated and wild peppers provides insights into *Capsicum* domestication and specialization. *Proc. Natl. Acad. Sci. U. S. A.*, **2014**, *111*, 5135-5140.
- [85] Wang, L.; Yu, S.; Tong, C.; Zhao, Y.; Liu, Y.; Song, C.; Zhang, Y.; Zhang, X.; Wang, Y.; Hua, W.; Li, D.; Li, D.; Li, F.; Yu, J.; Xu, C.; Han, X.; Huang, S.; Tai, S.; Wang, J.; Xu, X.; Li, Y.; Liu, S.; Varshney, R.K.; Wang, J.; Zhang, X. Genome sequencing of the high oil crop sesame provides insight into oil biosynthesis. *Genome Biol.*, **2014**, *15*, R39.
- [86] The Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. *Nature* **2000**, *408*, 796-815.
- [87] Michael, T.P.; Jackson, S. The first 50 plant genomes. *Plant Genome*, **2013**, *6*, 1-7.
- [88] Mayer, K.F.X.; Rogers, J.; Dole, J.; Pozniak, C.; Eversole, K.; Feuillet, C.; Gill, B.; Friebe, B.; Lukaszewski, A.J.; Sourdille, P.; Endo, T.R.; Kubalaková, M.; Ihalikova, J.; Dubska, Z.; Vrana, J.; Perkova, R.; Imkova, H.; Febrer, M.; Clissold, L.; McLay, K.; Singh, K.; Chhuneja, P.; Singh, N.K.; Khurana, J.; Akhunov, E.; Choulet, F.; Alberti, A.; Barbe, V.; Wincker, P.; Kanamori, H.; Kobayashi, F.; Itoh, T.; Matsumoto, T.; Sakai, H.; Tanaka, T.; Wu, J.; Ogihara, Y.; Handa, H.; Maclachlan, P.R.; Sharpe, A.; Klassen, D.; Edwards, D.; Batley, J.; Olsen, O.-A.; Sandve, S.R.; Lien, S.; Steuernagel, B.; Wulff, B.; Caccamo, M.; Ayling, S.; Ramirez-Gonzalez, R.H.; Clavijo, B.J.; Wright, J.; Pfeifer, M.; Spannagl, M.; Martis, M.M.; Mascher, M.; Chapman, J.; Poland, J.A.; Scholz, U.; Barry, K.; Waugh, R.; Rokhsar, D.S.; Muehlbauer, G.J.; Stein, N.; Gundlach, H.; Zytynicki, M.; Jamilloux, V.; Quesneville, H.; Wicker, T.; Faccioli, P.; Colaiacovo, M.; Stanca, A.M.; Budak, H.; Cattivelli, L.; Glover, N.; Pingault, L.; Paux, E.; Sharma, S.; Appels, R.; Bellgard, M.; Chapman, B.; Nussbaumer, T.; Bader, K.C.; Rimbart, H.; Wang, S.; Knox, R.; Kilian, A.; Alaux, M.; Alfama, F.; Couderc, L.; Guillhot, N.; Viseux, C.; Loaec, M.; Keller, B.; Praud, S. A chromosome-based draft sequence of the hexaploid bread wheat (*Triticum aestivum*) genome. *Science*, **2014**, *345*, 1251788.
- [89] The International Barley Genome Sequencing Consortium. A physical, genetic and functional sequence assembly of the barley genome. *Nature*, **2012**, *491*, 711-716.
- [90] Mamanova, L.; Coffey, A.J.; Scott, C.E.; Kozarewa, I.; Turner, E.H.; Kumar, A.; Howard, E.; Shendure, J.; Turner, D.J. Target-enrichment strategies for next-generation sequencing. *Nat. Methods*, **2010**, *7*, 111-118.
- [91] Bamshad, M.J.; Ng, S.B.; Bigham, A.W.; Tabor, H.K.; Emond, M.J.; Nickerson, D.A.; Shendure, J. Exome sequencing as a tool for Mendelian disease gene discovery. *Nat. Rev. Genet.*, **2011**, *12*, 745-755.
- [92] Liu, S.; Ying, K.; Yeh, C.-T.; Yang, J.; Swanson-Wagner, R.; Wu, W.; Richmond, T.; Gerhardt, D.J.; Lai, J.; Springer, N.; Nettleton, D.; Jeddeloh, J.A.; Schnable, P.S. Changes in genome content generated via segregation of non-allelic homologs. *Plant J.*, **2012**, *72*, 390-399.
- [93] Mascher, M.; Richmond, T.A.; Gerhardt, D.J.; Himmelbach, A.; Clissold, L.; Sampath, D.; Ayling, S.; Steuernagel, B.; Pfeifer, M.; D'Ascenzo, M.; Akhunov, E.D.; Hedley, P.E.; Gonzales, A.M.; Morrell, P.L.; Kilian, B.; Blattner, F.R.; Scholz, U.; Mayer, K.F.X.; Flavell, A.J.; Muehlbauer, G.J.; Waugh, R.; Jeddeloh, J.A.; Stein, N. Barley whole exome capture: a tool for genomic research in the genus *Hordeum* and beyond. *Plant J.*, **2013**, *76*, 494-505.
- [94] Pankin, A.; Campoli, C.; Dong, X.; Kilian, B.; Sharma, R.; Himmelbach, A.; Saini, R.; Davis, S.J.; Stein, N.; Schneeberger, K.; von Korff, M. Mapping-by-sequencing identifies HvPHYTOCHROME C as a candidate gene for the early maturity 5 locus modulating the circadian clock and photoperiodic flowering in barley. *Genetics*, **2014**, *198*, 383-396.
- [95] Schneeberger, K.; Ossowski, S.; Lanz, C.; Juul, T.; Petersen, A.H.; Nielsen, K.L.; Jørgensen, J.-E.; Weigel, D.; Andersen, S.U. SHOREmap: simultaneous mapping and mutation identification by deep sequencing. *Nat. Methods*, **2009**, *6*, 550-551.
- [96] Strickler, S.R.; Bombarely, A.; Mueller, L.A. Designing a transcriptome next-generation sequencing project for a nonmodel plant species. *Am. J. Bot.*, **2012**, *99*, 257-266.
- [97] Loraine, A.E.; McCormick, S.; Estrada, A.; Patel, K.; Qin, P. RNA-seq of *Arabidopsis* pollen uncovers novel transcription and alternative splicing. *Plant Physiol.*, **2013**, *162*, 1092-1109.
- [98] Filichkin, S.A.; Priest, H.D.; Givan, S.A.; Shen, R.; Bryant, D.W.; Fox, S.E.; Wong, W.-K.; Mockler, T.C. Genome-wide mapping of alternative splicing in *Arabidopsis thaliana*. *Genome Res.*, **2010**, *20*, 45-58.
- [99] Hamilton, J.P.; Robin Buell, C. Advances in plant genome sequencing. *Plant J.*, **2012**, *70*, 177-190.
- [100] Onda, Y.; Mochida, K.; Yoshida, T.; Sakurai, T.; Seymour, R.S.; Umekawa, Y.; Pirintsos, S.A.; Shinozaki, K.; Ito, K. Transcriptome analysis of thermogenic *Arum concinatum* reveals the molecular components of floral scent production. *Sci. Rep.*, **2015**, *5*, 8753.
- [101] Martin, J.A.; Wang, Z. Next-generation transcriptome assembly. *Nat. Rev. Genet.*, **2011**, *12*, 671-682.
- [102] Li, H.; Peng, Z.; Yang, X.; Wang, W.; Fu, J.; Wang, J.; Han, Y.; Chai, Y.; Guo, T.; Yang, N.; Liu, J.; Warburton, M.L.; Cheng, Y.; Hao, X.; Zhang, P.; Zhao, J.; Liu, Y.; Wang, G.; Li, J.; Yan, J. Genome-wide association study dissects the genetic architecture of oil biosynthesis in maize kernels. *Nat. Genet.*, **2013**, *45*, 43-50.
- [103] Haseneyer, G.; Schmutzer, T.; Seidel, M.; Zhou, R.; Mascher, M.; Schön, C.-C.; Taudien, S.; Scholz, U.; Stein, N.; Mayer, K.F.; Bauer, E. From RNA-seq to large-scale genotyping—genomics resources for rye (*Secale cereale* L.). *BMC Plant Biol.*, **2011**, *11*, 131.
- [104] Pootakham, W.; Shearman, J.R.; Ruang-Areerate, P.; Sonthirod, C.; Sangsrakru, D.; Jomchai, N.; Yoocha, T.; Triwitayakorn, K.; Tragoonrungs, S.; Tangphatsornruang, S. Large-scale SNP discovery through RNA sequencing and SNP genotyping by targeted enrichment sequencing in cassava (*Manihot esculenta* Crantz). *PLoS One*, **2014**, *9*, e116028.
- [105] Davey, J.W.; Davey, J.L.; Blaxter, M.L.; Blaxter, M.W. RADSeq: next-generation population genetics. *Brief. Funct. Genomics*, **2010**, *9*, 416-423.
- [106] Davey, J.W.; Hohenlohe, P.A.; Etter, P.D.; Boone, J.Q.; Catchen, J.M.; Blaxter, M.L. Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat. Rev. Genet.*, **2011**, *12*, 499-510.
- [107] Schneeberger, K. Using next-generation sequencing to isolate mutant genes from forward genetic screens. *Nat. Rev. Genet.*, **2014**, *15*, 662-676.
- [108] Chutimanitsakun, Y.; Nipper, R.W.; Cuesta-Marcos, A.; Cistué, L.; Corey, A.; Filichkina, T.; Johnson, E.A.; Hayes, P.M. Construction and application for QTL analysis of a Restriction Site Associated DNA (RAD) linkage map in barley. *BMC Genomics*, **2011**, *12*, 4.
- [109] Deokar, A.A.; Ramsay, L.; Sharpe, A.G.; Diapari, M.; Sindhu, A.; Bett, K.; Warkentin, T.D.; Tar'an, B. Genome wide SNP identification in chickpea for use in development of a high density genetic map and improvement of chickpea reference genome assembly. *BMC Genomics*, **2014**, *15*, 708.
- [110] Pfender, W.F.; Saha, M.C.; Johnson, E.A.; Slabaugh, M.B. Mapping with RAD (restriction-site associated DNA) markers to rapidly identify QTL for stem rust resistance in *Lolium perenne*. *Theor. Appl. Genet.*, **2011**, *122*, 1467-1480.
- [111] Zhou, L.; Wang, S.-B.; Jian, J.; Geng, Q.-C.; Wen, J.; Song, Q.; Wu, Z.; Li, G.-J.; Liu, Y.-Q.; Dunwell, J.M.; Zhang, J.; Feng, J.-Y.; Niu, Y.; Zhang, L.; Ren, W.-L.; Zhang, Y.-M. Identification of domestication-related loci associated with flowering time and seed size in soybean with the RAD-seq genotyping method. *Sci. Rep.*, **2015**, *5*, 9350.
- [112] Elshire, R.J.; Glaubitz, J.C.; Sun, Q.; Poland, J.A.; Kawamoto, K.; Buckler, E.S.; Mitchell, S.E. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, **2011**, *6*, e19379.
- [113] Poland, J.A.; Brown, P.J.; Sorrells, M.E.; Jannink, J.-L. Development of high-density genetic maps for barley and wheat using a novel two-enzyme genotyping-by-sequencing approach. *PLoS One*, **2012**, *7*, e32253.
- [114] Sonah, H.; O'Donoghue, L.; Cober, E.; Rajcan, I.; Belzile, F. Identification of loci governing eight agronomic traits using a GBS-GWAS approach and validation by QTL mapping in soya bean. *Plant Biotechnol. J.*, **2015**, *13*, 211-221.
- [115] Kujur, A.; Bajaj, D.; Upadhyaya, H.D.; Das, S.; Ranjan, R.; Shree,

- T.; Saxena, M.S.; Badoni, S.; Kumar, V.; Tripathi, S.; Gowda, C.L.L.; Sharma, S.; Singh, S.; Tyagi, A.K.; Parida, S.K. Employing genome-wide SNP discovery and genotyping strategy to extrapolate the natural allelic diversity and domestication patterns in chickpea. *Front. Plant Sci.*, **2015**, *6*, 162.
- [116] Morris, G.P.; Ramu, P.; Deshpande, S.P.; Hash, C.T.; Shah, T.; Upadhyaya, H.D.; Riera-Lizarazu, O.; Brown, P.J.; Acharya, C.B.; Mitchell, S.E.; Harriman, J.; Glaubitz, J.C.; Buckler, E.S.; Kresovich, S. Population genomic and genome-wide association studies of agroclimatic traits in sorghum. *Proc. Natl. Acad. Sci. U. S. A.*, **2013**, *110*, 453-458.
- [117] Spindel, J.; Wright, M.; Chen, C.; Cobb, J.; Gage, J.; Harrington, S.; Lorieux, M.; Ahmadi, N.; McCouch, S. Bridging the genotyping gap: using genotyping by sequencing (GBS) to add high-density SNP markers and new value to traditional bi-parental mapping and breeding populations. *Theor. Appl. Genet.*, **2013**, *126*, 2699-2716.
- [118] Millat, G.; Chanavat, V.; Rousson, R. Evaluation of a new high-throughput next-generation sequencing method based on a custom AmpliSeq™ library and ion torrent PGM™ sequencing for the rapid detection of genetic variations in long QT syndrome. *Mol. Diagn. Ther.*, **2014**, *18*, 533-539.
- [119] Singh, R.R.; Patel, K.P.; Routhort, M.J.; Reddy, N.G.; Barkoh, B.A.; Handal, B.; Kanagal-Shamanna, R.; Greaves, W.O.; Medeiros, L.J.; Aldape, K.D.; Luthra, R. Clinical validation of a next-generation sequencing screen for mutational hotspots in 46 cancer-related genes. *J. Mol. Diagn.*, **2013**, *15*, 607-622.
- [120] Beadling, C.; Neff, T.L.; Heinrich, M.C.; Rhodes, K.; Thornton, M.; Leamon, J.; Andersen, M.; Corless, C.L. Combining highly multiplexed PCR with semiconductor-based sequencing for rapid cancer genotyping. *J. Mol. Diagn.*, **2013**, *15*, 171-176.
- [121] Mackay, T.F.C.; Stone, E.A.; Ayroles, J.F. The genetics of quantitative traits: challenges and prospects. *Nat. Rev. Genet.*, **2009**, *10*, 565-577.
- [122] Koornneef, M.; Alonso-Blanco, C.; Vreugdenhil, D. Naturally occurring genetic variation in *Arabidopsis thaliana*. *Annu. Rev. Plant Biol.*, **2004**, *55*, 141-172.
- [123] Wang, Y.; Ning, Z.; Hu, Y.; Chen, J.; Zhao, R.; Chen, H.; Ai, N.; Guo, W.; Zhang, T. Molecular mapping of restriction-site associated DNA markers in allotetraploid upland cotton. *PLoS One*, **2015**, *10*, e0124781.
- [124] Hirschhorn, J.N.; Daly, M.J. Genome-wide association studies for common diseases and complex traits. *Nat. Rev. Genet.*, **2005**, *6*, 95-108.
- [125] Atwell, S.; Huang, Y.S.; Vilhjálmsson, B.J.; Willems, G.; Horton, M.; Li, Y.; Meng, D.; Platt, A.; Tarone, A.M.; Hu, T.T.; Jiang, R.; Muliyati, N.W.; Zhang, X.; Amer, M.A.; Baxter, I.; Brachi, B.; Chory, J.; Dean, C.; Debieu, M.; de Meaux, J.; Ecker, J.R.; Faure, N.; Kniskern, J.M.; Jones, J.D.G.; Michael, T.; Nemri, A.; Roux, F.; Salt, D.E.; Tang, C.; Todesco, M.; Traw, M.B.; Weigel, D.; Marjoram, P.; Borevitz, J.O.; Bergelson, J.; Nordborg, M. Genome-wide association study of 107 phenotypes in *Arabidopsis thaliana* inbred lines. *Nature*, **2010**, *465*, 627-631.
- [126] Huang, X.; Zhao, Y.; Wei, X.; Li, C.; Wang, A.; Zhao, Q.; Li, W.; Guo, Y.; Deng, L.; Zhu, C.; Fan, D.; Lu, Y.; Weng, Q.; Liu, K.; Zhou, T.; Jing, Y.; Si, L.; Dong, G.; Huang, T.; Lu, T.; Feng, Q.; Qian, Q.; Li, J.; Han, B. Genome-wide association study of flowering time and grain yield traits in a worldwide collection of rice germplasm. *Nat. Genet.*, **2012**, *44*, 32-39.
- [127] Jordan, K.W.; Wang, S.; Lun, Y.; Gardiner, L.-J.; MacLachlan, R.; Hucl, P.; Wiebe, K.; Wong, D.; Forrest, K.L.; Sharpe, A.G.; Sidebottom, C.H.D.; Hall, N.; Toomajian, C.; Close, T.; Dubcovsky, J.; Akhunova, A.; Talbert, L.; Bansal, U.K.; Bariana, H.S.; Hayden, M.J.; Pozniak, C.; Jeddleloh, J.A.; Hall, A.; Akhunov, E. A haplotype map of allohexaploid wheat reveals distinct patterns of selection on homoeologous genomes. *Genome Biol.*, **2015**, *16*, 48.
- [128] Jia, G.; Huang, X.; Zhi, H.; Zhao, Y.; Zhao, Q.; Li, W.; Chai, Y.; Yang, L.; Liu, K.; Lu, H.; Zhu, C.; Lu, Y.; Zhou, C.; Fan, D.; Weng, Q.; Guo, Y.; Huang, T.; Zhang, L.; Lu, T.; Feng, Q.; Hao, H.; Liu, H.; Lu, P.; Zhang, N.; Li, Y.; Guo, E.; Wang, S.; Wang, S.; Liu, J.; Zhang, W.; Chen, G.; Zhang, B.; Li, W.; Wang, Y.; Li, H.; Zhao, B.; Li, J.; Diao, X.; Han, B. A haplotype map of genomic variations and genome-wide association studies of agronomic traits in foxtail millet (*Setaria italica*). *Nat. Genet.*, **2013**, *45*, 957-961.
- [129] Slavov, G.T.; Nipper, R.; Robson, P.; Farrar, K.; Allison, G.G.; Bosch, M.; Clifton-Brown, J.C.; Donnison, I.S.; Jensen, E. Genome-wide association studies and prediction of 17 traits related to phenology, biomass and cell wall composition in the energy grass *Miscanthus sinensis*. *New Phytol.*, **2014**, *201*, 1227-1239.
- [130] McMullen, M.D.; Kresovich, S.; Villeda, H.S.; Bradbury, P.; Li, H.; Sun, Q.; Flint-Garcia, S.; Thornsberry, J.; Acharya, C.; Bottoms, C.; Brown, P.; Browne, C.; Eller, M.; Guill, K.; Harjes, C.; Kroon, D.; Lepak, N.; Mitchell, S.E.; Peterson, B.; Pressoir, G.; Romero, S.; Oropeza Rosas, M.; Salvo, S.; Yates, H.; Hanson, M.; Jones, E.; Smith, S.; Glaubitz, J.C.; Goodman, M.; Ware, D.; Holland, J.B.; Buckler, E.S. Genetic properties of the maize nested association mapping population. *Science*, **2009**, *325*, 737-740.
- [131] Kover, P.X.; Valdar, W.; Trakalo, J.; Scarcelli, N.; Ehrenreich, I.M.; Purugganan, M.D.; Durrant, C.; Mott, R. A multiparent advanced generation inter-cross to fine-map quantitative traits in *Arabidopsis thaliana*. *PLoS Genet.*, **2009**, *5*, e1000551.
- [132] Michelmore, R.W.; Paran, I.; Kesseli, R. V. Identification of markers linked to disease-resistance genes by bulked segregant analysis: a rapid method to detect markers in specific genomic regions by using segregating populations. *Proc. Natl. Acad. Sci. U. S. A.*, **1991**, *88*, 9828-9832.
- [133] Lindner, H.; Raissig, M.T.; Sailer, C.; Shimosato-Asano, H.; Bruggmann, R.; Grossniklaus, U. SNP-Ratio Mapping (SRM): Identifying Lethal Alleles and Mutations in Complex Genetic Backgrounds by Next-Generation Sequencing. *Genetics*, **2012**, *191*, 1381-1386.
- [134] Hartwig, B.; James, G.V.; Konrad, K.; Schneeberger, K.; Turck, F. Fast isogenic mapping-by-sequencing of ethyl methanesulfonate-induced mutant bulks. *Plant Physiol.*, **2012**, *160*, 591-600.
- [135] Liu, S.; Yeh, C.-T.; Tang, H.M.; Nettleton, D.; Schnable, P.S. Gene Mapping via Bulk Segregant RNA-Seq (BSR-Seq). *PLoS One*, **2012**, *7*, e36406.
- [136] Abe, A.; Kosugi, S.; Yoshida, K.; Natsume, S.; Takagi, H.; Kanzaki, H.; Matsumura, H.; Yoshida, K.; Mitsuoka, C.; Tamiru, M.; Innan, H.; Cano, L.; Kamoun, S.; Terauchi, R. Genome sequencing reveals agronomically important loci in rice using MutMap. *Nat. Biotechnol.*, **2012**, *30*, 174-178.
- [137] Takagi, H.; Tamiru, M.; Abe, A.; Yoshida, K.; Uemura, A.; Yaegashi, H.; Obara, T.; Oikawa, K.; Utsushi, H.; Kanzaki, E.; Mitsuoka, C.; Natsume, S.; Kosugi, S.; Kanzaki, H.; Matsumura, H.; Urasaki, N.; Kamoun, S.; Terauchi, R. MutMap accelerates breeding of a salt-tolerant rice cultivar. *Nat. Biotechnol.*, **2015**, *33*, 445-449.