Review

Embracing the Omics Era for Plant Breeding

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ABSTRACT

The increasing demand for food, feed, fuel, and fiber in modern society calls for urgent crop improvement, especially when faced with challenges such as climate change and decreasing arable land. Therefore, there is a constant need for advances in plant breeding. Over the last two decades, high-throughput techniques, such as next-generation sequencing, have given momentum to multiple omics technologies, including genomics, epigenomics, transcriptomics, proteomics, and metabolomics, generating an immense amount of data daily. These technologies and advanced bioinformatic tools enhance our understanding of agronomically important traits, including yield, nutrient content, and tolerance to biotic/abiotic stresses. For example, research on nucleotide-binding leucine-rich-repeat (NLR) genes, key players in plant immunity, is driven by high-throughput gene discovery, functional annotation, and synthetic design, accelerating disease resistance breeding. Additionally, high-throughput techniques facilitate the generation of valuable tools like molecular markers, which can be utilized in applications such as marker-assisted selection, quantitative trait locus mapping, genome-wide association study, and genomic selection. As regulations on genetically engineered crops move from process-based approaches toward product-based approaches, omics technologies are expected to play a pivotal role in regulating new crop varieties by assessing substantial equivalence. Embracing the omics era in plant breeding requires a paradigm shift in every aspect of the field, and readiness is essential.

KEYWORDS: omics; genomics; transcriptomics; proteomics; metabolomics; plant breeding; NLR; process-based regulation; product-based regulation; substantial equivalence

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INTRODUCTION

Crops cover 40% of the Earth's land area and provide essential resources like food, fuel, and fiber to support human society [1]. By 2050, as the global population approaches 9.6 billion, the demand for crops is projected to rise by 100%–110% compared to 2005 levels [2]. This increased demand is further challenged by climate change and a reduction in arable land. Sustainable intensification has been proposed by many

conservationists as a solution to increase crop yields per hectare without causing environmental harm [3]. This approach is particularly crucial as, in recent decades, new arable land has predominantly been created through deforestation in biodiverse tropical regions, leading to significant biodiversity losses [4,5]. Addressing these challenges necessitates improvements in a wide range of agronomic traits, such as yield, nutritional content, and resistance to biotic and abiotic stresses. Consequently, advances in plant breeding remain critical, requiring both a deeper understanding of the biochemistry, physiology, biology, and agroecology underlying these traits, as well as the development of novel genetic engineering technologies [6]. Plant breeding is mainly carried out through crossbreeding, mutation breeding, and transgenic breeding in modern agriculture. Traditionally, breeding relied solely on phenotypic selection of plant germplasm with desirable traits from progeny produced via crosses or mutagenesis—a labor-intensive, time-consuming process that lacked insights into the genetic composition of selected plants. In the 1980s, the emergence of molecular biology revolutionized plant breeding by enabling the understanding of the genetic basis of traits. This led to the advent of marker-assisted selection (MAS), which uses DNA-based markers to screen plant germplasm for specific alleles. MAS has significantly improved efficiency and reduced costs by permitting the selection of desirable individuals from smaller populations and has been applied extensively in numerous crops [7]. As genomic information has become increasingly available, MAS has become a cornerstone of plant breeding programs [6]. Genetic engineering has introduced transgenic breeding approaches, allowing for the direct insertion of foreign genes into elite crop varieties to confer desired traits. This method bypasses the barriers of reproductive isolation inherent in crossbreeding and avoids the randomness associated with mutation breeding. Since the introduction of genetically engineered (GE) crops in 1994 [8], transgenic breeding has gained increasing prominence in modern agriculture, especially with the advent of the omics era. Advanced genetic engineering techniques, including genome editing and synthetic biology, hold immense potential to revolutionize crop production and plant breeding due to their unparalleled precision and versatility. Nevertheless, transgenic breeding is complementary to traditional breeding, and utilizing both approaches could synergistically enhance progress in crop improvement [6].

Over the past two decades, the development of novel omics technologies has enabled high-throughput analyses of biological molecules, such as the genome (DNA sequence), epigenome (DNA modifications), transcriptome (RNA transcripts), proteome (proteins), and metabolome (metabolites) [9–12]. These tools have provided a comprehensive understanding of the molecular basis of plant breeding, enabling researchers to explore crop biology in unprecedented detail [6,11]. With advances in data acquisition, processing, and accessibility (e.g., lower costs, increased availability of facilities and expertise), multi-omics

approaches have been extensively applied to study traits like yield, growth, and stress responses in economically important crops, including rice (*Oryza sativa*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*), maize (*Zea mays*), cotton (*Gossypium hirsutum*), soybean (*Glycine max*), millet (*Setaria italica*), and tomato (*Solanum lycopersicum*) [10,13–16]. Large-scale omics data have also shed light on crucial topics such as the identification of key genes and events during crop domestication, as well as genetic bottlenecks that limit breeding advancements. This knowledge has greatly enhanced breeding efficiency and strategies employed. For example, breeders are leveraging wild relatives, genome-wide association studies (GWAS), gene editing, and pangenomics to restore lost genetic diversity, enhance disease resistance, and improve yield stability [17–23]. In the context of disease resistance breeding, multi-omics approaches are also driving advancements in the study of nucleotide-binding leucine-rich-repeat (NLR) genes. Additionally, fundamental research into each set of biological molecules, especially DNA and RNA, has led to the development or refinement of essential tools, such as genome sequencing, resequencing, and single-nucleotide polymorphism (SNP) assays, which are now integral to plant breeding programs [24–32]. Moreover, omics technologies are increasingly recognized as valuable tools for evaluating the effects of genetic modifications in new crops, aligning with calls for a shift from process-based to product-based regulatory approaches for crop evaluation [6,33].

OMICS IMPROVES BASIC UNDERSTANDING OF AGRONOMIC TRAITS AND PROVIDES USEFUL TOOLS IN PLANT BREEDING

The Advancement of Various Omics

DNA-genomics

Genomics investigates the genes and genomes' structure, function, and evolution, and the resulting enhanced understanding of genetic variation can significantly boost crop breeding efficiency, thereby enabling genetic enhancements in crop species [9]. Pangenomics extends genomics by examining the genetic diversity within a species, encompassing both core and accessory genomes, providing a more comprehensive view of genetic variation. Further, the study of structural genomics involves examining the polymorphism of genome sequences and the organization of chromosomes. Results lead to the construction of genetic and physical maps to aid in the identification of valuable agronomic traits. Structural genomics depends on DNA-based markers to tag and map genes of interest. Molecular markers generated with genomics approaches, such as genotyping-by-sequencing (GBS), have been widely used in MAS by plant breeders to improve crop quality [30,34]. SNPs are single nucleotide variations in the genome among individuals. Historically, PCR-based techniques for testing SNP molecular markers used methods like amplified fragment length polymorphisms (AFLP), and random amplified

polymorphic DNA (RAPD) [35–37], but the emergence of next-generation sequencing (NGS) has made it feasible to identify and utilize a large number of SNPs simultaneously in the omics era. Quantitative trait loci (QTL) mapping and GWAS are two methods for studying multiple or complex traits in crops. Mapping QTLs is a statistical approach that can link the data of complex phenotypes to that of genotypes. DNA-based markers, including AFLPs and SNPs, are often used to localize traits of interest using QTL mapping [24–31,38–41]. While using GWAS, based on SNPs identified in genome sequence data, the correlation between genetic variants and phenotypes can be determined or it can identify variants associated with traits in a population [39,42–44]. GWAS is an indispensable genomics approach for studying complex agronomic traits such as tolerance to abiotic stress [17,42]. A study using GWAS identified 48 QTLs that are linked to maize yield under water and heat stress [45], and another study demonstrated the impact of several abiotic stressors on the oil content in sunflowers [46]. Numerous studies have used GWAS to identify QTLs associated with drought tolerance in sorghum (*Sorghum bicolor*) [47,48], rice [49], and maize [50]. In addition, the association of structural variants (SVs), which are vital in controlling agronomically essential traits in crops, has been reported in maize [51], soybean [52], and oilseed rape (*Brassica napus*) [53] using GWAS approach. Genomic selection (GS) expands upon GWAS and QTL mapping by utilizing genome-wide markers to predict breeding values, enabling early selection of superior genotypes without the need for full phenotypic evaluations [54–57]. Unlike MAS, which focuses on major-effect loci, GS captures both major and minor QTLs, improving accuracy in selecting for complex traits such as yield, disease resistance, and stress tolerance [58,59]. As a cutting-edge breeding strategy, GS accelerates genetic gain, enhances breeding efficiency, and is increasingly being integrated into modern crop improvement programs to develop resilient and high-yielding cultivars [54].

Functional genomics involves the study of gene functions related to trait regulation. For this, different biotechnological tools and global experimental approaches have been used to identify, clone, and characterize the functions of genes [9]. Gene identification before the omics era was a tedious process that relied on procedures such as expressed sequence tag (EST), suppression subtractive hybridization (SSH), and cDNA-AFLP sequencing. However, the introduction of NGS and the vast resources and data generated by genomics has greatly reduced the tediousness of these approaches [9]. Mutagenesis is a vital technique to develop new crops with desirable traits and is used to determine gene functions [60,61]. Reverse genetic approaches including Virus-induced gene silencing (VIGS) and RNA interference (RNAi) are often utilized to screen/induce mutations to investigate the functions of genes [10,62–64]. VIGS has been used to identify various mutants that are linked to crop yield, growth, and stress tolerance in rice, wheat, barley, and tomato

[10,65]. Some high-throughput genomic technologies such as microarray and Targeted Induced Local Lesions IN Genomes (TILLING) are also used for characterizing overall mutational events in crops for functional analyses [11]. Microarray studies showed that transgene insertion caused fewer changes in gene expression profiles compared with changes induced by mutagenesis [61]. TILLING was initially developed as a functional genomics approach [66], but after its successful application in numerous crops to detect and characterize mutations [60,67–73], it became a powerful tool for crop breeding, serving as an alternative approach to transgenesis [74–79]. To serve functional genomics, genome editing tools such as the transcription activator-like effector nuclease (TALEN) and cluster regularly interspaced short palindromic repeats (CRISPR) systems can be utilized for functional analyses and subsequently crop improvement [80–84]. For example, TALEN and CRISPR/Cas9 technologies were applied on characterized mildew resistance locus in bread wheat (*TaMlo*) or tomato (*SlMlo*), respectively, and novel mutants with resistance to powdery mildew were successfully generated [85,86]. Since its introduction in 2011, the CRISPR/Cas system has successfully edited the genomes of many important crops through targeted genome editing [81,82,87–94].

DNA modification-epigenomics

Epigenetics explores partially heritable modifications that occur without alterations to the DNA sequence, such as DNA methylation and post-translational modifications of histones [95,96]. Epigenomics is a recent omics approach that combines epigenetics and genomics to study gene regulation and its role in cellular growth and responses to stress [97]. Various environmental factors can affect epigenomics, and genomic-level technologies can be utilized to study these epigenetic changes during different developmental stages or in response to different environmental stimuli [9]. As a result, the study and application of epigenomics could contribute to crop improvement by elucidating how plants respond to various environmental stresses. The bisulfite sequencing (BS-seq) technique has been utilized in various crops, including tomato, maize, and soybean, to identify the DNA methylation status of the genome [98–101]. The methylation-sensitive amplified polymorphism method, which is used to quantify DNA methylation in the genome, has been applied in wheat and foxtail millet [102,103]. Chromatin immunoprecipitation sequencing (ChIP-Seq) studies both histone proteins and DNA methylation [104,105], which has been utilized to study epigenetic alterations in rice in response to drought [106]. Various epigenomic studies have identified epigenetic changes related to ripening in tomato [107], photosynthesis in maize [108], mantled phenotype in oil palm [109], and drought response in cotton [110].

RNA-transcriptomics

Transcriptomics investigates the transcriptome, which is the complete set of RNA transcripts produced by an organism's genome. Transcriptome profiling is a promising technique that has emerged to analyze time-dependent gene expression in response to internal or external stimuli [111]. Traditional RNA profiling techniques, such as cDNAs-AFLP and SSH, provide low-resolution data [112], but this has greatly improved with the advent of high-throughput approaches such as microarray, RNA sequencing (RNA-seq), and Serial Analysis of Gene Expression (SAGE) [113,114]. These high-throughput approaches are frequently utilized to examine the variances in expression patterns of genes or regulatory networks at various developmental stages and/or in response to stress. The obtained information can aid in functional analyses and could be used to create molecular markers for linking phenotypes in plant breeding aimed at enhancing crops [11]. For example, numerous transcriptomics studies using microarray or RNA-seq have revealed the differentially expressed genes in soybean, barley, sorghum, maize, rapeseed, foxtail millet, sweet potato, and rice at different developmental stages under various stresses [20,115–127]. Notably, some novel advancements in transcriptomics have brought new insights into this field. Spatially resolved transcriptomics is a technique that allows for the detection of gene expression and its spatial distribution within cells or tissues [128], and in situ RNA-seq aims at profiling RNA in living cells or tissues [129]. Comparative transcriptomics identifies the differential expression profiles across different crops in response to stress, and a total of 16 common stress-responsive genes were identified by comparing transcriptomes of wheat, maize, and rice to that of switchgrass under heat stress [130,131]. Recently, alternative splicing transcriptomics was employed to investigate how splicing factors control abiotic stress responses in key crops such as maize, rice, and sorghum [132,133].

Protein-proteomics

Proteomics involves the analysis of the entire set of proteins expressed in an organism, encompassing sequence, structural, functional, and expression aspects. [134,135]. Sequence proteomics identifies amino acid sequences via high-performance liquid chromatography (HPLC), while structural proteomics investigates protein structure using computer-based modeling, crystallization, electron microscopy, nuclear magnetic resonance, and X-ray diffraction [136,137]. High-throughput techniques like X-ray crystallography and Nuclear Magnetic Resonance (NMR) spectroscopy have recently been developed to determine protein structure [138]. Functional proteomics determines protein functions through methods like yeast-two-hybrids and protein microarray profiling. Expression proteomics identifies differentially expressed proteins (DEPs) in response to stress conditions through techniques like Stable Isotope

Labeling by Amino acids in Cell culture (SILAC), Isotope-Coded Affinity Tag (ICAT) labeling, and Isobaric Tags for Relative and Absolute Quantification (iTRAQ). Proteins can be separated using techniques like Two-Dimensional Gel Electrophoresis (2-DE), Two-Dimensional Difference Gel Electrophoresis (2D-DIGE), and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE), and analyzed for molecular mass via Mass Spectrometry (MS), Ion Trap (IT)-MS, Liquid Chromatography (LC), Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF), Electrospray Ionization (ESI), or Collision-Induced Dissociation (CID) [139–141]. Using functional proteomics approaches, many important proteins, such as reactive oxygen species (ROS) scavengers and molecular chaperones, have been identified in tomato, sunflower, wheat, and sugarcane under various stress conditions [142–145], which depicts their crucial function in the defense response of crops. Quantitative proteomics has employed the iTRAQ technique to discover numerous stress-responsive DEPs in potato and coconut [146,147]. Furthermore, using proteomic techniques such as MALDI-TOF, LC-MS/MS, 2-DE, and SDS-PAGE, numerous studies have been conducted to determine the stress-response pathways in rapeseed, soybean, sugarcane, cotton, and chickpea [145,148–152]. Collectively, these proteomic researches have illustrated the significant role of those identified proteins for relevant crops in response to diverse stress conditions. Advancements in the extraction and separation techniques of protein have improved plant proteomic research at both sample and genome scales [153], but there are still limitations to current techniques. One major limitation is sensitivity as proteomic studies typically can only identify the most abundant proteins [154]. Additionally, a variety of sample-preparation methods must be employed to provide a comprehensive assessment of the proteome [154]. Lastly, interpreting the proteomic differences is challenging due to limited knowledge of the functions of many proteins in a plant cell.

Metabolite-metabolomics

Metabolomics investigates the entire set of metabolites produced by metabolic pathways in a biological system using advanced analytical techniques such as MS and NMR spectroscopy [155,156]. Its importance is particularly evident in plant systems, as plants synthesize more metabolites than animals or microbes. Metabolites form a complex defense system in plants against abiotic stress and pathogens, and numerous studies have identified metabolite changes in response to stress in crops such as rice, wheat, maize, tomato, and soybean through targeted and untargeted metabolomics approaches [157]. Using metabolomics methods such as LC-MS, gas chromatography (GC)-MS, and HPLC coupled with tandem MS, several studies have identified some metabolites in rice in response to pathogens like gall midge biotype 1 (GMB1) pathogen, and rice bacterial blight pathogen (*Xanthomonas oryzae* pv. *oryzae*) [158,159]. Using similar approaches in wheat, phenolic and phenylpropanoid

metabolites were found to be responsive to biotic stress [160]. Additionally, metabolic fingerprinting and metabolic profiling techniques have identified metabolite changes related to drought, cold, and heat stress in wheat, maize, tomato, and soybean [161–167]. Phenotype is bridged with genotype via metabolome [168], thus metabolomics is often integrated with other omics such as genomics, and proteomics to obtain a more comprehensive understanding of biological processes and phenotypes [169]. Under various abiotic stresses, the integrated omics approach has revealed strong correlations among different omics data in crops, providing novel insights into stress response mechanisms [170,171].

Integrated-panomics

Omics technologies could provide “big data” encompassing the entire set of certain biological molecules, but it is still a sectional view with limited information on the complex behaviors of biosystems. To better understand valuable agronomic traits, especially those which are biologically complex (e.g., yield, nitrogen-use efficiency, drought tolerance, etc.), an integration of different omics approaches is beneficial due to their complementary nature. Besides, integrating multi-omics data can help to reduce false positive results and improve the accuracy of genotype-phenotype predictions [172]. To that end, the idea of panomics was proposed [173], which is a platform that enables the integration of diverse omics datasets for the prediction of complex traits [169,174]. For example, the integration of multi-omics datasets has revealed the epigenetic basis of staged single-cell differentiation in cotton fiber [175]. Datasets obtained with different omics approaches need to be merged and analyzed with special tools [176]. For example, PAINTOMICS is an internet-based tool that offers the visual presentation of integrated transcriptomics and metabolomics datasets on Kyoto Encyclopedia of Genes and Genomes (KEGG) maps [177]. Online software tools for multi-omics analysis have been reviewed by Yang et al. [11]. The integration of panomics with GWAS has also been used to study phenotypic variance in crops, potentially leading to the discovery of new genes and functional pathways that underlie valuable but complex agronomic phenotypes [173]. For example, combining GWAS with metabolomics has emerged as an effective method for analyzing the genetic and biochemical mechanisms in model crops like rice, maize, and tomato [178–180]. In recent years, genome editing technologies (such as the CRISPR/Cas9 system) were proposed to be integrated with panomics for precision breeding [173].

The Role of Omics in Advancing NLR Research

Plants possess sophisticated immune systems to combat diverse biotic stresses, with NLR proteins playing a pivotal role in pathogen recognition and defense activation [181,182]. NLR genes encode intracellular immune receptors that detect pathogen-derived effectors, initiating robust immune responses through a process termed effector-triggered immunity (ETI).

This defense mechanism represents the second layer of plant innate immunity, complementing pattern-triggered immunity (PTI), which responds to conserved pathogen-associated molecular patterns (PAMPs) [183–185]. NLR genes are central to plant immunity and have immense potential as targets for crop improvement [186]. The integration of multi-omics approaches has significantly advanced our understanding of their structure, function, and regulation. By combining high-throughput gene discovery, functional annotation, and synthetic design, these approaches are unlocking the full potential of NLR genes for developing resilient and sustainable crops. As these technologies continue to evolve, they will undoubtedly drive further breakthroughs in plant immunity and agricultural biotechnology.

NLR and disease resistance breeding

NLR proteins are modular in nature, consisting of three core domains: an N-terminal domain, a central nucleotide-binding (NB-ARC) domain, and a C-terminal leucine-rich repeat (LRR) domain. The N-terminal domain, often a Toll/interleukin-1 receptor (TIR) or coiled-coil (CC) domain, facilitates downstream signaling. The NB-ARC domain functions as a molecular switch, cycling between inactive (ADP-bound) and active (ATP-bound) states, while the LRR domain mediates effector recognition [183,186–189]. NLR proteins detect pathogen effectors either directly, through physical binding, or indirectly, by sensing effector-mediated modifications of host proteins. For example, the Arabidopsis *Resistance to Pseudomonas syringae 2* (*RPS2*) NLR protein recognizes the bacterial effector *Avirulence protein Rpt2* (*AvrRpt2*) through its interaction with the host protein *RPM1-Interacting Protein 4* (*RIN4*) [190]. Upon activation, NLRs trigger a cascade of immune responses, including ROS production, and transcriptional reprogramming of defense genes, which results in a hypersensitive response (HR) whereby localized cell death restricts pathogen spread.

NLR genes have been widely utilized in plant breeding due to their ability to confer race-specific resistance to pathogens. For instance, the *Resistance to Phytophthora infestans-blb1* (*Rpi-blb1*) gene from wild potato confers resistance to *Phytophthora infestans*, the causative agent of late blight [191]. Similarly, the *Xanthomonas resistance 21* (*Xa21*) gene in rice provides resistance to bacterial blight caused by *Xanthomonas oryzae* [192]. Leveraging NLR genes has been instrumental in enhancing crop resilience; however, traditional introgression of NLR genes can be labor-intensive and may result in linkage drag. Advances in genome editing technologies, such as CRISPR/Cas9, have enabled precise manipulation of NLR genes to enhance resistance while minimizing trade-offs [193,194]. In addition to their utility in conferring resistance, stacking multiple NLR genes or engineering synthetic NLRs with broader specificity holds promise for durable and broad-spectrum resistance [195,196].

Understanding the molecular mechanisms underlying NLR function and diversity is critical for their effective utilization in breeding programs.

Omics and NLR research

The advent of multi-omics approaches, including genomics, transcriptomics, proteomics, and epigenomics, has revolutionized our understanding of NLR genes. These integrative strategies have facilitated high-throughput NLR gene discovery, functional annotation, and elucidation of their regulatory networks.

Genomic studies employing NGS and pan-genome analyses have greatly expanded our knowledge of NLR gene diversity [197–199]. By leveraging comparative genomics, researchers have identified lineage-specific expansions and conserved NLR gene clusters, shedding light on their evolutionary trajectories. For instance, GWAS has pinpointed novel NLR loci associated with disease resistance traits, enabling targeted breeding efforts [200]. Transcriptomic and proteomic studies have been instrumental in characterizing NLR gene expression and activity. RNA-seq analyses under pathogen challenge have revealed dynamic expression patterns of NLR genes, providing insights into their roles in specific defense responses [201]. Proteomic approaches, including co-immunoprecipitation and mass spectrometry, have identified NLR protein complexes and downstream signaling components, further elucidating their mechanisms of action [202]. Epigenomic studies have uncovered regulatory elements that modulate NLR gene expression, such as DNA methylation and histone modifications [203–205]. These findings have highlighted the importance of chromatin accessibility in fine-tuning NLR-mediated defense responses. Multi-omics approaches are also paving the way for the rational design of synthetic NLRs. By integrating structural genomics and computational modeling, researchers have engineered synthetic receptors with enhanced specificity and durability [206]. Directed evolution experiments, coupled with high-throughput screening, are enabling the development of NLR variants with novel resistance capabilities.

OMICS TECHNOLOGIES SERVE A REGULATORY ROLE IN PLANT BREEDING

In addition to facilitating crop improvement through enhanced understanding and the development of valuable tools, omics methods can also play a regulatory role in plant breeding by assessing the health and environmental impacts of new crop varieties developed through both traditional breeding and genetic engineering.

From Process-based to Product-based Regulation of Crops

Over the past four decades, the worldwide safety regulations for GE crops have been crafted and updated to exempt conventionally bred crops

[33]; however, differentiation no longer makes sense in light of the advances in genetic engineering technologies. Convincing arguments have been made that the current “process-based” safety regulations, which discriminate against GE crops simply because they are developed by genetic engineering technologies, are scientifically invalid and unfit for their original purpose [6,33,207,208]. Product-based regulation, which focuses on the characteristics of the final product rather than the method used to develop it, offers a more relevant approach. This approach considers the actual genetic composition and traits of the crop, ensuring that safety assessments are based on the potential risks posed by the product itself, rather than the technique used to create it. Although authorities may recognize the importance of considering the biological characteristics of new plant products in a process-based regulatory regime, the safety regulations applied are typically determined by the specific technology used to develop the plant variety. However, emerging genetic engineering technologies have increasingly blurred the distinction between conventionally bred crops and their genetically engineered counterparts, causing governing authorities to struggle with redefining which plants require regulation. “Process-based” regulations assume the safety of conventional breeding due to its long familiarity. When GE crops were commercialized in the 1990s, concerns arose over the potential risks to the environment and health due to the random insertion of transgenes in the genome. The argument critics raised was that only 70 plant-synthesized chemicals were monitored and limited animal testing was insufficient to establish the safety status of GE food [6]. Nowadays, genetic engineering technologies, such as the CRISPR/Cas system and other site-specific nucleases (SSNs), can modify a plant’s phenotype significantly by altering a single nucleotide or inserting/deleting genetic sequences of different sizes [81,82]. Some regulatory authorities, such as the United States Department of Agriculture (USDA), believe that such changes could happen naturally and, as a result, are safe and not subject to regulations. Additionally, many current and proposed regulations assume that genes from closely related species are less risky to use than those from distant relatives [208–210]. However, as the 2016 National Academies of Sciences, Engineering, and Medicine (NASEM) report pointed out, the biological effect of genetic transformation and thus the safety risk to environment or health is relatively independent of its size and extent [6]. All genetic alterations, no matter large or small, are aimed at changing a plant’s phenotype to develop a new variety, and these alterations have no fundamental differences when it comes to potential safety risks. Therefore, a revised regulatory framework is necessary, where the need for safety testing is determined by modern molecular technologies such as omics that assess the overall physical and biological traits of new crop varieties [33]. This product-based regulatory approach would ensure a more scientifically sound and effective governance structure.

Evaluation of Substantial Equivalence with Omics Technologies

Regulatory authorities worldwide have typically relied on the concept of "substantial equivalence" to ensure that newly developed crop varieties have a similar food composition to existing ones and pose no additional risks. While not mandated by regulators, many academic researchers and commercial entities have adopted omics technologies to evaluate substantial equivalence beyond traditional measures of food composition. Ricroch [211] reviewed 60 omics studies of plants, which mainly used a single omics approach like transcriptomics, proteomics, or metabolomics for evaluation. Since then, there has been an increase in the volume of omics studies, and many have used multiple omics methods demonstrating increasing sophistication in this type of assessment [212]. This tendency also indicates that there is a need to establish integrated and shared networks of omics databases serving the evaluation purpose [6,213,214]. The integration of these omics technologies can offer a comprehensive and non-specific evaluation of numerous plant characteristics, encompassing the levels of mRNA, proteins, and metabolites in the plant of interest. Therefore, it is more effective in detecting changes in a GE crop than the evaluation methods currently suggested by regulatory authorities. Some studies have found almost no changes in the transcriptome of GE plants with an added marker gene [215], but the use of metabolomics methods detected changes in the same plants [216]. Adding a gene for a nonenzymatic protein, like the insecticidal Bt toxin, to a GE plant is predicted to result in few changes in the plant's metabolism. In contrast, when a gene is added to a plant to specifically alter a certain metabolic pathway, several predicted and unpredicted changes can potentially occur. For instance, Shepherd, et al. [217] observed that when the enzymes responsible for the biosynthesis of either of the two toxic glycoalkaloids (alpha-chaconine and alpha-solanine) were downregulated in GE potatoes, the other compound typically increased; while another two compounds, fucosterol and beta-sitosterol, increased with the down-production of both toxic glycoalkaloids. Several studies employing omics methods have identified variations between GE crops and their conventionally-bred counterparts. Nevertheless, in numerous plant characteristics analyzed, the variability among conventionally-bred varieties is greater than that between GE and non-GE varieties. Besides, the developmental stage of the crop and the environmental conditions also affect the findings [211,218].

To regulate new crops in the future based on the core concept of "product-based regulation" and "substantial equivalence", the omics features of any new crop variety could be compared to all the current varieties across a country or reference panel to determine whether they are substantially different. In this way, both GE and non-GE crops could be regulated, and the regulation efforts could be concentrated on the omics features of the plants and products from new potential varieties. A tiered regulatory approach has been proposed based on the comparison of omics

characteristics of a new crop variety with a panel of existing commercial varieties. This panel could consist of conventionally bred varieties representing the genetic and phenotypic diversity of the species. Depending on the results of the omics evaluation with various omics technologies, four paths could be taken [6,33]. In Tier 1, no differences between the new variety and the panel varieties are detected by any omics technologies. In Tier 2, the differences detected by any omics technologies are known to have no adverse effects on human/animal health or the environment. New varieties evaluated as Tier 1 or Tier 2 crops shall require no further testing. In Tier 3, the differences detected are understood to have potential adverse effects. In Tier 4, the detected differences cannot be interpreted. Thus new varieties evaluated as Tier 3 or Tier 4 require further safety testing. For instance, introducing a previously approved GE trait into a new variety of the same species and comparing its omics profile to another deregulated GE variety already in use establishes substantial equivalence and categorizes the new variety as Tier 1. If omics analyses detect differences that may have potential adverse health effects or changes of a protein or metabolite whose consequences are unknown, they are categorized as Tier 3 and 4, respectively, and require further safety testing. The tiered regulatory approach would provide a structured framework for the use of omics evaluation methods in regulatory decision-making [6].

Ideally, panomics data including all omics data available such as genomics, transcriptomics, proteomics, and metabolomics, should be used for the evaluation of substantial equivalence. To leverage the potential of omics technologies for substantial equivalence assessment, it is crucial to develop comprehensive species-specific omics databases that showcase the transcriptome, proteome, and metabolome variations of diverse genotypes grown under various environmental conditions. Currently, state-of-the-art different omics technologies vary considerably. New developments in DNA/RNA sequencing, such as NGS, have made it possible to acquire complete genomes or transcriptomes at acceptable speed and cost. Therefore, transcriptomics and genomics have the potential to play a crucial role in evaluating substantial equivalence. In contrast, proteomic and metabolomics technologies cannot produce a comprehensive catalog of the proteome or metabolome at present [219,220], but they can still contribute to the evaluation. For instance, if a new crop variety has a proteome or metabolome similar to a de-regulated variety, it may indicate substantial equivalence, whereas a difference suggests the need for further evaluation. Scientists understand that the current technical limitations of omics technologies for plants must be overcome by new research [219,220], and much greater investment especially from the public sector will be needed. To avoid the long-term backlash from the public like the first generation of GE crops, scientists, regulators, and diverse members of the public must work together to guide the research, and they should all be included in the deliberations on those important

questions like what differences between new crops and existing ones would be significant enough to warrant safety regulation [6,33]. The most comprehensive evidence of substantial equivalence would involve a complete understanding of the biochemical makeup of a given crop variety in comparison to others, but current technology does not yet allow for the development of extensive species-specific proteome or metabolome databases. Looking into the future, ongoing advances in omics technologies will surely expand basic knowledge of those agronomical traits in plants and thus greatly fuel molecular plant breeding.

SUMMARY AND PROSPECTS

At the time of writing the review, a reference genome was available for almost all major crop species. In addition, an immense amount of multi-omics data is being generated and uploaded to online databases daily. With the help of advanced bioinformatic tools, the omics data are being utilized by scientists to improve our understanding of valuable agronomic traits such as disease resistance, stress tolerance, and yield in crops, and ultimately used in plant breeding and crop improvements. Besides, as the regulation regime of GE crops worldwide shifts towards product-based approaches, omics technologies are expected to play a crucial role in the evaluation of substantial equivalence. With all these clear signs showing a bright future for the vast potential of omics technologies, we should all get ready to embrace the omics era for plant breeding. This could mean changes in every aspect of crop science including our understanding of plant traits, tools we use, and even working logic or concepts. However, it is important to view this paradigm shift as complementary to our current work routine, as we still face several challenges in the omics era. First, omics data are incomplete, with varying quality of reference genomes depending on technical and cost limitations. Therefore, multiple high-quality reference genomes are needed to capture diversity within a crop variety adequately. Second, users may not fully utilize omics data due to the lack of access, computational tools, or analytical skills. These limitations can be overcome with the development of omics and bioinformatics technologies. Finally, phenotyping technologies are still limited compared to fast-evolving omics technologies [221], and in the future, field-based high-throughput phenotyping approaches with increased efficiency and reduced cost are likely to be developed to parallel datasets with omics technologies and aid in plant breeding [222,223].

DATA AVAILABILITY

No data were generated from the study.

AUTHOR CONTRIBUTIONS

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest in relation to this work.

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REFERENCES

1. Foley JA, Defries R, Asner GP, Barford C, Bonan G, Carpenter SR, et al. Global consequences of land use. Science. 2005;309(5734):570-4.
2. Tilman D, Balzer C, Hill J, Befort BL. Global food demand and the sustainable intensification of agriculture. P Natl Acad Sci USA. 2011;108(50):20260-4.
3. Garnett T, Appleby MC, Balmford A, Bateman IJ, Benton TG, Bloomer P, et al. Sustainable intensification in agriculture: premises and policies. Science. 2013;341(6141):33-4.
4. Gibbs HK, Ruesch AS, Achard F, Clayton MK, Holmgren P, Ramankutty N, et al. Tropical forests were the primary sources of new agricultural land in the 1980s and 1990s. P Natl Acad Sci USA. 2010;107(38):16732-7.
5. Phalan B, Bertzky M, Butchart SH, Donald PF, Scharlemann JP, Stattersfield AJ, et al. Crop expansion and conservation priorities in tropical countries. PLoS One. 2013;8(1):e51759.
6. National Academies of Sciences, Engineering, and Medicine. Genetically engineered crops: Experiences and prospects. Washington (US): National Academies Press; 2016.
7. Ru S, Main D, Evans K, Peace C. Current applications, challenges, and perspectives of marker-assisted seedling selection in Rosaceae tree fruit breeding. Tree Genet Genomes. 2015;11(1):8.
8. ISAAA. GM Approval Database. Available from: https://www.isaaa.org/gmapprovaldatabase/. Accessed on 20 Feb 2024.
9. Muthamilarasan M, Singh NK, Prasad M. Multi-omics approaches for strategic improvement of stress tolerance in underutilized crop species: A climate change perspective. Adv Genet. 2019;103:1-38.

10. Talukdar D, Sinjushin A. Cytogenomics and Mutagenomics in Plant Functional Biology and Breeding. In: Barh D, Khan MS, Davies E, editors. PlantOmics: The Omics of Plant Science. New Delhi (India): Springer; 2015. p. 113-56.
11. Yang Y, Saand MA, Huang L, Abdelaal WB, Zhang J, Wu Y, et al. Applications of multi-omics technologies for crop improvement. Front Plant Sci. 2021;12:563953.
12. Wu S, Ning F, Zhang Q, Wu X, Wang W. Enhancing Omics Research of Crop Responses to Drought under Field Conditions. Front Plant Sci. 2017;8:174.
13. Deshmukh R, Sonah H, Patil G, Chen W, Prince S, Mutava R, et al. Integrating omic approaches for abiotic stress tolerance in soybean. Front Plant Sci. 2014;5:244.
14. Muthamilarasan M, Prasad M. Genetic Determinants of Drought Stress Tolerance in Setaria. In: Doust A, Diao X, editors. Genetics and Genomics of Setaria. Cham (Switzerland): Springer International Publishing; 2017. p. 267-89.
15. Shah T, Xu J, Zou X, Cheng Y, Nasir M, Zhang X. Omics Approaches for Engineering Wheat Production under Abiotic Stresses. Int J Mol Sci. 2018;19(8):2390.
16. Yadav CB, Pandey G, Muthamilarasan M, Prasad M. Epigenetics and Epigenomics of Plants. In: Varshney RK, Pandey MK, Chitikineni A, editors. Plant Genetics and Molecular Biology. Cham (Switzerland): Springer International Publishing; 2018. p. 237-61.
17. Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y, et al. Genome-wide association studies of 14 agronomic traits in rice landraces. Nat Genet. 2010;42(11):961-7.
18. Lam HM, Xu X, Liu X, Chen W, Yang G, Wong FL, et al. Resequencing of 31 wild and cultivated soybean genomes identifies patterns of genetic diversity and selection. Nat Genet. 2010;42(12):1053-9.
19. Chia JM, Song C, Bradbury PJ, Costich D, de Leon N, Doebley J, et al. Maize HapMap2 identifies extant variation from a genome in flux. Nat Genet. 2012;44(7):803-7.
20. Hufford MB, Xu X, van Heerwaarden J, Pyhajarvi T, Chia JM, Cartwright RA, et al. Comparative population genomics of maize domestication and improvement. Nat Genet. 2012;44(7):808-11.
21. Jiao Y, Zhao H, Ren L, Song W, Zeng B, Guo J, et al. Genome-wide genetic changes during modern breeding of maize. Nat Genet. 2012;44(7):812-5.
22. Li YH, Zhou G, Ma J, Jiang W, Jin LG, Zhang Z, et al. De novo assembly of soybean wild relatives for pan-genome analysis of diversity and agronomic traits. Nat Biotechnol. 2014;32(10):1045-52.
23. Lin T, Zhu G, Zhang J, Xu X, Yu Q, Zheng Z, et al. Genomic analyses provide insights into the history of tomato breeding. Nat Genet. 2014;46(11):1220-6.
24. Chang A, Lamara M, Wei Y, Hu H, Parkin IAP, Gossen BD, et al. Clubroot resistance gene *Rcr6* in *Brassica nigra* resides in a genomic region homologous to chromosome A08 in *B. rapa*. BMC Plant Biol. 2019;19(1):224.
25. Chu M, Song T, Falk KC, Zhang X, Liu X, Chang A, et al. Fine mapping of *Rcr1* and analyses of its effect on transcriptome patterns during infection by *Plasmodiophora brassicae*. BMC Genomics. 2014;15:1166.

26. Huang Z, Peng G, Gossen BD, Yu F. Fine mapping of a clubroot resistance gene from turnip using SNP markers identified from bulked segregant RNA-Seq. Mol Breed. 2019;39(8):131.
27. Huang Z, Peng G, Liu X, Deora A, Falk KC, Gossen BD, et al. Fine Mapping of a Clubroot Resistance Gene in Chinese Cabbage Using SNP Markers Identified from Bulked Segregant RNA Sequencing. Front Plant Sci. 2017;8:1448.
28. Karim MM, Dakouri A, Zhang Y, Chen Q, Peng G, Strelkov SE, et al. Two clubroot-resistance genes, *Rcr3* and *Rcr9*^{wa}, mapped in *Brassica rapa* using bulk segregant RNA sequencing. Int J Mol Sci. 2020;21(14):5033.
29. Yu F, Zhang X, Huang Z, Chu M, Song T, Falk KC, et al. Identification of genome-wide variants and discovery of variants associated with *Brassica rapa* clubroot resistance gene *Rcr1* through bulked segregant RNA sequencing. PLoS One. 2016;11(4):e0153218.
30. Yu F, Zhang X, Peng G, Falk KC, Strelkov SE, Gossen BD. Genotyping-by-sequencing reveals three QTL for clubroot resistance to six pathotypes of *Plasmodiophora brassicae* in *Brassica rapa*. Sci Rep. 2017;7(1):4516.
31. Yu F, Zhang Y, Wang J, Chen Q, Karim MM, Gossen BD, et al. Identification of Two Major QTLs in *Brassica napus* Lines with Introgressed Clubroot Resistance From Turnip Cultivar ECD01. Front Plant Sci. 2021;12:785989.
32. Fu F, Liu X, Wang R, Zhai C, Peng G, Yu F, et al. Fine mapping of *Brassica napus* blackleg resistance gene *Rlm1* through bulked segregant RNA sequencing. Sci Rep. 2019;9(1):14600.
33. Gould F, Amasino RM, Brossard D, Buell CR, Dixon RA, Falck-Zepeda JB, et al. Toward product-based regulation of crops. Science. 2022;377(6610):1051-3.
34. He J, Zhao X, Laroche A, Lu ZX, Liu H, Li Z. Genotyping-by-sequencing (GBS), an ultimate marker-assisted selection (MAS) tool to accelerate plant breeding. Front Plant Sci. 2014;5:484.
35. Williams JG, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 1990;18(22):6531-5.
36. Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, et al. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 1995;23(21):4407-14.
37. Rabouam C, Comes AM, Bretagnolle V, Humbert JF, Periquet G, Bigot Y. Features of DNA fragments obtained by random amplified polymorphic DNA (RAPD) assays. Mol Ecol. 1999;8(3):493-503.
38. Kearsey MJ. The principles of QTL analysis (a minimal mathematics approach). J Exp Bot. 1998;49(327):1619-23.
39. Dakouri A, Lamara M, Karim MM, Wang J, Chen Q, Gossen BD, et al. Identification of resistance loci against new pathotypes of *Plasmodiophora brassicae* in *Brassica napus* based on genome-wide association mapping. Sci Rep. 2021;11(1):6599.
40. Rahaman M, Strelkov SE, Hu H, Gossen BD, Yu F. Identification of a genomic region containing genes involved in resistance to four pathotypes of *Plasmodiophora brassicae* in *Brassica rapa* turnip ECD02. Plant Genome. 2022;15(3):e20245.

41. Karim MM, Yu F. Identification of QTLs for resistance to 10 pathotypes of *Plasmodiophora brassicae* in *Brassica oleracea* cultivar ECD11 through genotyping-by-sequencing. Theor Appl Genet. 2023;136(12):249.
42. Challa S, Neelapu NRR. Genome-Wide Association Studies (GWAS) for Abiotic Stress Tolerance in Plants. In: Wani SH, editor. Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress Tolerance in Plants. Cambridge (US): Academic Press; 2018. p. 135-50.
43. Fu F, Zhang X, Liu F, Peng G, Yu F, Fernando D. Identification of resistance loci in Chinese and Canadian canola/rapeseed varieties against *Leptosphaeria maculans* based on genome-wide association studies. BMC Genomics. 2020;21(1):501.
44. Dakouri A, Zhang X, Peng G, Falk KC, Gossen BD, Strelkov SE, et al. Analysis of genome-wide variants through bulked segregant RNA sequencing reveals a major gene for resistance to *Plasmodiophora brassicae* in *Brassica oleracea*. Sci Rep. 2018;8(1):17657.
45. Millet EJ, Welcker C, Kruijer W, Negro S, Coupel-Ledru A, Nicolas SD, et al. Genome-Wide Analysis of Yield in Europe: Allelic Effects Vary with Drought and Heat Scenarios. Plant Physiol. 2016;172(2):749-64.
46. Mangin B, Casadebaig P, Cadic E, Blanchet N, Boniface MC, Carrere S, et al. Genetic control of plasticity of oil yield for combined abiotic stresses using a joint approach of crop modelling and genome-wide association. Plant Cell Environ. 2017;40(10):2276-91.
47. Lasky JR, Upadhyaya HD, Ramu P, Deshpande S, Hash CT, Bonnette J, et al. Genome-environment associations in sorghum landraces predict adaptive traits. Sci Adv. 2015;1(6):e1400218.
48. Spindel JE, Dahlberg J, Colgan M, Hollingsworth J, Sievert J, Staggenborg SH, et al. Association mapping by aerial drone reveals 213 genetic associations for Sorghum bicolor biomass traits under drought. BMC Genomics. 2018;19(1):679.
49. Guo Z, Yang W, Chang Y, Ma X, Tu H, Xiong F, et al. Genome-Wide Association Studies of Image Traits Reveal Genetic Architecture of Drought Resistance in Rice. Mol Plant. 2018;11(6):789-805.
50. Shikha M, Kanika A, Rao AR, Mallikarjuna MG, Gupta HS, Nepolean T. Genomic Selection for Drought Tolerance Using Genome-Wide SNPs in Maize. Front Plant Sci. 2017;8:550.
51. Lu F, Romay MC, Glaubitz JC, Bradbury PJ, Elshire RJ, Wang T, et al. High-resolution genetic mapping of maize pan-genome sequence anchors. Nat Commun. 2015;6:6914.
52. Zhou Z, Jiang Y, Wang Z, Gou Z, Lyu J, Li W, et al. Resequencing 302 wild and cultivated accessions identifies genes related to domestication and improvement in soybean. Nat Biotechnol. 2015;33(4):408-14.
53. Gabur I, Chawla HS, Liu X, Kumar V, Faure S, von Tiedemann A, et al. Finding invisible quantitative trait loci with missing data. Plant Biotechnol J. 2018;16(12):2102-12.
54. de Koning DJ. Meuwissen *et al.* on Genomic Selection. Genetics. 2016;203(1):5-7.

55. Meuwissen THE, Hayes BJ, Goddard ME. Prediction of Total Genetic Value Using Genome-Wide Dense Marker Maps. Genetics. 2001;157(4):1819-29.
56. Jannink JL, Lorenz AJ, Iwata H. Genomic selection in plant breeding: from theory to practice. Brief Funct Genomics. 2010;9(2):166-77.
57. Heffner EL, Sorrells ME, Jannink JL. Genomic Selection for Crop Improvement. Crop Sci. 2009;49(1):1-12.
58. Goddard ME, Hayes BJ. Genomic selection. J Anim Breed Genet. 2007;124(6):323-30.
59. Moose SP, Mumm RH. Molecular Plant Breeding as the Foundation for 21st Century Crop Improvement. Plant Physiol. 2008;147(3):969-77.
60. Henikoff S, Till BJ, Comai L. TILLING. Traditional mutagenesis meets functional genomics. Plant Physiol. 2004;135(2):630-6.
61. Varshney RK, Thundi M, May GD, Jackson SA. Legume Genomics and Breeding. Plant Breed Rev. 2010;33:257-304.
62. Dwivedi S, Perotti E, Ortiz R. Towards molecular breeding of reproductive traits in cereal crops. Plant Biotechnol J. 2008;6(6):529-59.
63. Gupta PK, Mir RR, Mohan A, Kumar J. Wheat genomics: present status and future prospects. Int J Plant Genomics. 2008;2008:896451.
64. Tomlekova N. Induced mutagenesis for crop improvement in Bulgaria. Plant Mutat Rep. 2010;2(1):4-27.
65. Saand MA, Xu YP, Li W, Wang JP, Cai XZ. Cyclic nucleotide gated channel gene family in tomato: genome-wide identification and functional analyses in disease resistance. Front Plant Sci. 2015;6:303.
66. McCallum CM, Comai L, Greene EA, Henikoff S. Targeting Induced LocalLesions IN Genomes (TILLING) for Plant Functional Genomics. Plant Physiol. 2000;123(2):439-42.
67. Suzuki T, Eiguchi M, Kumamaru T, Satoh H, Matsusaka H, Moriguchi K, et al. MNU-induced mutant pools and high performance TILLING enable finding of any gene mutation in rice. Mol Genet Genomics. 2008;279(3):213-23.
68. Till BJ, Reynolds SH, Weil C, Springer N, Burtner C, Young K, et al. Discovery of induced point mutations in maize genes by TILLING. BMC Plant Biol. 2004;4:12.
69. Dong C, Dalton-Morgan J, Vincent K, Sharp P. A Modified TILLING Method for Wheat Breeding. Plant Genome. 2009;2(1):39-47.
70. Caldwell DG, McCallum N, Shaw P, Muehlbauer GJ, Marshall DF, Waugh R. A structured mutant population for forward and reverse genetics in Barley (*Hordeum vulgare* L.). Plant J. 2004;40(1):143-50.
71. Minoia S, Petrozza A, D'Onofrio O, Piron F, Mosca G, Sozio G, et al. A new mutant genetic resource for tomato crop improvement by TILLING technology. BMC Res Notes. 2010;3:69.
72. Cooper JL, Till BJ, Laport RG, Darlow MC, Kleffner JM, Jamai A, et al. TILLING to detect induced mutations in soybean. BMC Plant Biol. 2008;8:9.
73. Mba C. Induced Mutations Unleash the Potentials of Plant Genetic Resources for Food and Agriculture. Agronomy. 2013;3(1):200-31.
74. Pathan MS, Sleper DA. Advances in Soybean Breeding. In: Stacey G, editor. Genetics and Genomics of Soybean. New York (US): Springer; 2008. p. 113-33.

75. Kurowska M, Daszkowska-Golec A, Gruszka D, Marzec M, Szurman M, Szarejko I, et al. TILLING: a shortcut in functional genomics. J Appl Genet. 2011;52(4):371-90.
76. Chen L, Hao L, Parry MA, Phillips AL, Hu YG. Progress in TILLING as a tool for functional genomics and improvement of crops. J Integr Plant Biol. 2014;56(5):425-43.
77. Witzel K, Neugart S, Ruppel S, Schreiner M, Wiesner M, Baldermann S. Recent progress in the use of 'omics technologies in brassicaceous vegetables. Front Plant Sci. 2015;6:244.
78. Knoll JE, Ramos ML, Zeng Y, Holbrook CC, Chow M, Chen S, et al. TILLING for allergen reduction and improvement of quality traits in peanut (*Arachis hypogaea* L.). BMC Plant Biol. 2011;11:81.
79. Kumar AP, Boualem A, Bhattacharya A, Parikh S, Desai N, Zambelli A, et al. SMART–Sunflower Mutant population And Reverse genetic Tool for crop improvement. BMC Plant Biol. 2013;13:38.
80. Rinaldo AR, Ayliffe M. Gene targeting and editing in crop plants: a new era of precision opportunities. Mol Breed. 2015;35(1):40.
81. Liu H, Chen W, Li Y, Sun L, Chai Y, Chen H, et al. CRISPR/Cas9 Technology and Its Utility for Crop Improvement. Int J Mol Sci. 2022;23(18):10442.
82. Rao Y, Yang X, Pan C, Wang C, Wang K. Advance of Clustered Regularly Interspaced Short Palindromic Repeats-Cas9 System and Its Application in Crop Improvement. Front Plant Sci. 2022;13:839001.
83. Hu H, Yu F. Studies on the temporal, structural, and interacting features of the clubroot resistance gene *Rcr1* using CRISPR/Cas9-based systems. Hortic Plant J. 2024;10(6):1035-48.
84. Hu H, Yu F. Advances in CRISPR/Cas-based Genome Editing: Break New Ground for Plant Functional Genomics. In: Chen JT, editor. CRISPR and Plant Functional Genomics. 1st ed. Boca Raton (US): CRC Press; 2024. p. 1-19.
85. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, et al. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nat Biotechnol. 2014;32(9):947-51.
86. Nekrasov V, Wang C, Win J, Lanz C, Weigel D, Kamoun S. Rapid generation of a transgene-free powdery mildew resistant tomato by genome deletion. Sci Rep. 2017;7:482.
87. Shan Q, Wang Y, Li J, Zhang Y, Chen K, Liang Z, et al. Targeted genome modification of crop plants using a CRISPR-Cas system. Nat Biotechnol. 2013;31(8):686-8.
88. Nekrasov V, Staskawicz B, Weigel D, Jones JD, Kamoun S. Targeted mutagenesis in the model plant Nicotiana benthamiana using Cas9 RNA-guided endonuclease. Nat Biotechnol. 2013;31(8):691-3.
89. Jiang W, Zhou H, Bi H, Fromm M, Yang B, Weeks DP. Demonstration of CRISPR/Cas9/sgRNA-mediated targeted gene modification in Arabidopsis, tobacco, sorghum and rice. Nucleic Acids Res. 2013;41(20):e188.
90. Lawrenson T, Shorinola O, Stacey N, Li C, Østergaard L, Patron N, et al. Induction of targeted, heritable mutations in barley and Brassica oleracea using RNA-guided Cas9 nuclease. Genome Biol. 2015;16:258.

91. Li Z, Zhang D, Xiong X, Yan B, Xie W, Sheen J, et al. A potent Cas9-derived gene activator for plant and mammalian cells. Nat Plants. 2017;3(12):930-6.
92. Li Z, Liu ZB, Xing A, Moon BP, Koellhoffer JP, Huang L, et al. Cas9-Guide RNA Directed Genome Editing in Soybean. Plant Physiol. 2015;169(2):960-70.
93. Svitashev S, Young JK, Schwartz C, Gao H, Falco SC, Cigan AM. Targeted Mutagenesis, Precise Gene Editing, and Site-Specific Gene Insertion in Maize Using Cas9 and Guide RNA. Plant Physiol. 2015;169(2):931-45.
94. Hu H, Yu F. A CRISPR/Cas9-based system with controllable auto-excision feature serving cisgenic plant breeding and beyond. Int J Mol Sci. 2022;23(10):5597.
95. Strahl BD, Allis CD. The language of covalent histone modifications. Nature. 2000;403(6765):41-5.
96. Novik KL, Nimmrich I, Genc B, Maier S, Piepenbrock C, Olek A, et al. Epigenomics: genome-wide study of methylation phenomena. Curr Issues Mol Biol. 2002;4(4):111-28.
97. Callinan PA, Feinberg AP. The emerging science of epigenomics. Hum Mol Genet. 2006;15(Suppl_1):R95-101.
98. Cokus SJ, Feng S, Zhang X, Chen Z, Merriman B, Haudenschild CD, et al. Shotgun bisulphite sequencing of the Arabidopsis genome reveals DNA methylation patterning. Nature. 2008;452(7184):215-9.
99. Gent JI, Ellis NA, Guo L, Harkess AE, Yao Y, Zhang X, et al. CHH islands: de novo DNA methylation in near-gene chromatin regulation in maize. Genome Res. 2013;23(4):628-37.
100. Gonzalez RM, Ricardi MM, Iusem ND. Epigenetic marks in an adaptive water stress-responsive gene in tomato roots under normal and drought conditions. Epigenetics. 2013;8(8):864-72.
101. Schmitz RJ, He Y, Valdes-Lopez O, Khan SM, Joshi T, Urich MA, et al. Epigenome-wide inheritance of cytosine methylation variants in a recombinant inbred population. Genome Res. 2013;23(10):1663-74.
102. Pandey G, Yadav CB, Sahu PP, Muthamilarasan M, Prasad M. Salinity induced differential methylation patterns in contrasting cultivars of foxtail millet (*Setaria italica* L.). Plant Cell Rep. 2017;36(5):759-72.
103. Zhong L, Xu YH, Wang JB. DNA-methylation changes induced by salt stress in wheat Triticum aestivum. Afr J Biotechnol. 2009;8(22):6201-6.
104. Yamaguchi N, Winter CM, Wu MF, Kwon CS, William DA, Wagner D. PROTOCOLS: Chromatin Immunoprecipitation from Arabidopsis Tissues. Arabidopsis Book. 2014;12:e0170.
105. Zentner GE, Henikoff S. High-resolution digital profiling of the epigenome. Nat Rev Genet. 2014;15(12):814-27.
106. Zong W, Zhong X, You J, Xiong L. Genome-wide profiling of histone H3K4-tri-methylation and gene expression in rice under drought stress. Plant Mol Biol. 2013;81(1-2):175-88.
107. Zhong S, Fei Z, Chen YR, Zheng Y, Huang M, Vrebalov J, et al. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nat Biotechnol. 2013;31(2):154-9.

108. Offermann S, Danker T, Dreymüller D, Kalamajka R, Töpsch S, Weyand K, et al. Illumination Is Necessary and Sufficient to Induce Histone Acetylation Independent of Transcriptional Activity at the C4-Specific Phosphoenolpyruvate Carboxylase Promoter in Maize. Plant Physiol. 2006;141(3):1078-88.
109. Ong-Abdullah M, Ordway JM, Jiang N, Ooi SE, Kok SY, Sarpan N, et al. Loss of Karma transposon methylation underlies the mantled somaclonal variant of oil palm. Nature. 2015;525(7570):533-7.
110. Lu X, Wang X, Chen X, Shu N, Wang J, Wang D, et al. Single-base resolution methylomes of upland cotton (*Gossypium hirsutum* L.) reveal epigenome modifications in response to drought stress. BMC Genomics. 2017;18(1):297.
111. El-Metwally S, Ouda OM, Helmy M. Next generation sequencing technologies and challenges in sequence assembly. New York (US): Springer; 2014.
112. Nataraja KN, Madhura BG, Parvathi M. Omics: Modern tools for precise understanding of drought adaptation in plants. In: Zargar SM, Rai V, editors. Plant Omics and Crop Breeding. 1st ed. New York (US): Apple Academic Press; 2017. p. 263-94.
113. Kawahara Y, Oono Y, Kanamori H, Matsumoto T, Itoh T, Minami E. Simultaneous RNA-seq analysis of a mixed transcriptome of rice and blast fungus interaction. PLoS One. 2012;7(11):e49423.
114. De Cremer K, Mathys J, Vos C, Froenicke L, Michelmore RW, Cammue BP, et al. RNAseq-based transcriptome analysis of Lactuca sativa infected by the fungal necrotroph Botrytis cinerea. Plant Cell Environ. 2013;36(11):1992-2007.
115. Guo P, Baum M, Grando S, Ceccarelli S, Bai G, Li R, et al. Differentially expressed genes between drought-tolerant and drought-sensitive barley genotypes in response to drought stress during the reproductive stage. J Exp Bot. 2009;60(12):3531-44.
116. Le DT, Nishiyama R, Watanabe Y, Tanaka M, Seki M, Ham LH, et al. Differential gene expression in soybean leaf tissues at late developmental stages under drought stress revealed by genome-wide transcriptome analysis. PLoS One. 2012;7(11):e49522.
117. Khan F, Chai HH, Ajmera I, Hodgman C, Mayes S, Lu C. A Transcriptomic Comparison of Two Bambara Groundnut Landraces under Dehydration Stress. Genes. 2017;8(4):121.
118. Dugas DV, Monaco MK, Olsen A, Klein RR, Kumari S, Ware D, et al. Functional annotation of the transcriptome of Sorghum bicolor in response to osmotic stress and abscisic acid. BMC Genomics. 2011;12:514.
119. Johnson SM, Lim FL, Finkler A, Fromm H, Slabas AR, Knight MR. Transcriptomic analysis of Sorghum bicolor responding to combined heat and drought stress. BMC Genomics. 2014;15:456.
120. Jin Y, Yang H, Wei Z, Ma H, Ge X. Rice male development under drought stress: phenotypic changes and stage-dependent transcriptomic reprogramming. Mol Plant. 2013;6(5):1630-45.
121. Kakumanu A, Ambavaram MMR, Klumas C, Krishnan A, Batlang U, Myers E, et al. Effects of Drought on Gene Expression in Maize Reproductive and Leaf Meristem Tissue Revealed by RNA-Seq. Plant Physiol. 2012;160(2):846-67.

122. Huang L, Zhang F, Zhang F, Wang W, Zhou Y, Fu B, et al. Comparative transcriptome sequencing of tolerant rice introgression line and its parents in response to drought stress. BMC Genomics. 2014;15:1026.
123. Bhardwaj AR, Joshi G, Kukreja B, Malik V, Arora P, Pandey R, et al. Global insights into high temperature and drought stress regulated genes by RNA-Seq in economically important oilseed crop *Brassica juncea*. BMC Plant Biol. 2015;15:9.
124. Qi X, Xie S, Liu Y, Yi F, Yu J. Genome-wide annotation of genes and noncoding RNAs of foxtail millet in response to simulated drought stress by deep sequencing. Plant Mol Biol. 2013;83(4-5):459-73.
125. Hittalmani S, Mahesh HB, Shirke MD, Biradar H, Uday G, Aruna YR, et al. Genome and Transcriptome sequence of Finger millet (*Eleusine coracana* (L.) Gaertn.) provides insights into drought tolerance and nutraceutical properties. BMC Genomics. 2017;18:465.
126. Bonthala VS, Mayes K, Moreton J, Blythe M, Wright V, May ST, et al. Identification of Gene Modules Associated with Low Temperatures Response in Bambara Groundnut by Network-Based Analysis. PLoS One. 2016;11(2):e0148771.
127. Lin Y, Zou W, Lin S, Onofua D, Yang Z, Chen H, et al. Transcriptome profiling and digital gene expression analysis of sweet potato for the identification of putative genes involved in the defense response against *Fusarium oxysporum* f. sp. *batatas*. PLoS One. 2017;12(11):e0187838.
128. Burgess DJ. RNA: Putting transcriptomics in its place. Nat Rev Genet. 2015;16(6):319.
129. Ke R, Mignardi M, Pacureanu A, Svedlund J, Botling J, Wahlby C, et al. In situ sequencing for RNA analysis in preserved tissue and cells. Nat Methods. 2013;10(9):857-60.
130. Ding X, Li X, Xiong L. Insight into Differential Responses of Upland and Paddy Rice to Drought Stress by Comparative Expression Profiling Analysis. Int J Mol Sci. 2013;14(3):5214-38.
131. Li YF, Wang Y, Tang Y, Kakani VG, Mahalingam R. Transcriptome analysis of heat stress response in switchgrass (*Panicum virgatum* L.). BMC Plant Biol. 2013;13:153.
132. Zhang C, Yang H, Yang H. Evolutionary Character of Alternative Splicing in Plants. Bioinform Biol Insights. 2015;9:47-52.
133. Laloum T, Martin G, Duque P. Alternative Splicing Control of Abiotic Stress Responses. Trends Plant Sci. 2018;23(2):140-50.
134. Mosa KA, Ismail A, Helmy M. Omics and System Biology Approaches in Plant Stress Research. In: Mosa KA, Ismail A, Helmy M, editors. Plant Stress Tolerance. Cham (Switzerland): Springer; 2017. p. 21-34.
135. Aizat WM, Hassan M. Proteomics in Systems Biology. In: Aizat W, Goh HH, Baharum S, editors. Omics Applications for Systems Biology. Cham (Switzerland): Springer; 2018. p. 31-49.
136. Sali A, Glaeser R, Earnest T, Baumeister W. From words to literature in structural proteomics. Nature. 2003;422(6928):216-25.

137. Woolfson MM. The development of structural x-ray crystallography. Phys Scr. 2018;93(3):032501.
138. Aslam B, Basit M, Nisar MA, Khurshid M, Rasool MH. Proteomics: Technologies and Their Applications. J Chromatogr Sci. 2017;55(2):182-96.
139. Tanaka K, Waki H, Ido Y, Akita S, Yoshida Y, Yoshida T, et al. Protein and polymer analyses up to *m/z* 100 000 by laser ionization time-of-flight mass spectrometry. Rapid Commun Mass Spectrom. 1988;2(8):151-3.
140. McLuckey SA, Stephenson JL Jr. Ion/ion chemistry of high-mass multiply charged ions. Mass Spectrom Rev. 1998;17(6):369-407.
141. Fournier ML, Gilmore JM, Martin-Brown SA, Washburn MP. Multidimensional separations-based shotgun proteomics. Chem Rev. 2007;107(8):3654-86.
142. Shalata A, Mittova V, Volokita M, Guy M, Tal M. Response of the cultivated tomato and its wild salt-tolerant relative Lycopersicon pennellii to salt-dependent oxidative stress: The root antioxidative system. Physiol Plant. 2001;112(4):487-94.
143. Di Baccio D, Navari-Izzo F, Izzo R. Seawater irrigation: antioxidant defence responses in leaves and roots of a sunflower (*Helianthus annuus* L.) ecotype. J Plant Physiol. 2004;161(12):1359-66.
144. Mittova V, Guy M, Tal M, Volokita M. Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species Lycopersicon pennellii. J Exp Bot. 2004;55(399):1105-13.
145. Jangpromma N, Songsri P, Thammasirirak S, Jaisil P. Rapid assessment of chlorophyll content in sugarcane using a SPAD chlorophyll meter across different water stress conditions. Asian J Plant Sci. 2010;9(6):368-74.
146. Liu B, Zhang N, Zhao S, Chang J, Wang Z, Zhang G, et al. Proteomic changes during tuber dormancy release process revealed by iTRAQ quantitative proteomics in potato. Plant Physiol Biochem. 2015;86:181-90.
147. Yang Y, Saand MA, Abdelaal WB, Zhang J, Wu Y, Li J, et al. iTRAQ-based comparative proteomic analysis of two coconut varieties reveals aromatic coconut cold-sensitive in response to low temperature. J Proteomics. 2020;220:103766.
148. Toorchi M, Yukawa K, Nouri MZ, Komatsu S. Proteomics approach for identifying osmotic-stress-related proteins in soybean roots. Peptides. 2009;30(12):2108-17.
149. Nouri MZ, Komatsu S. Comparative analysis of soybean plasma membrane proteins under osmotic stress using gel-based and LC MS/MS-based proteomics approaches. Proteomics. 2010;10(10):1930-45.
150. Deeba F, Pandey AK, Ranjan S, Mishra A, Singh R, Sharma YK, et al. Physiological and proteomic responses of cotton (*Gossypium herbaceum* L.) to drought stress. Plant Physiol Biochem. 2012;53:6-18.
151. Mohammadi PP, Moieni A, Komatsu S. Comparative proteome analysis of drought-sensitive and drought-tolerant rapeseed roots and their hybrid F1 line under drought stress. Amino Acids. 2012;43(5):2137-52.
152. Subba P, Kumar R, Gayali S, Shekhar S, Parveen S, Pandey A, et al. Characterisation of the nuclear proteome of a dehydration-sensitive cultivar

of chickpea and comparative proteomic analysis with a tolerant cultivar. Proteomics. 2013;13(12-13):1973-92.
153. Nakagami H, Sugiyama N, Ishihama Y, Shirasu K. Shotguns in the front line: phosphoproteomics in plants. Plant Cell Physiol. 2012;53(1):118-24.
154. Baerenfaller K, Grossmann J, Grobei MA, Hull R, Hirsch-Hoffmann M, Yalovsky S, et al. Genome-scale proteomics reveals Arabidopsis thaliana gene models and proteome dynamics. Science. 2008;320(5878):938-41.
155. Fiehn O, Kopka J, Dormann P, Altmann T, Trethewey RN, Willmitzer L. Metabolite profiling for plant functional genomics. Nat Biotechnol. 2000;18(11):1157-61.
156. Baharum SN, Azizan KA. Metabolomics in Systems Biology. In: Aizat W, Goh HH, Baharum S, editors. Omics Applications for Systems Biology. Cham (Switzerland): Springer; 2018. p. 51-68.
157. Balmer D, Flors V, Glauser G, Mauch-Mani B. Metabolomics of cereals under biotic stress: current knowledge and techniques. Front Plant Sci. 2013;4:82.
158. Agarrwal R, Bentur JS, Nair S. Gas chromatography mass spectrometry based metabolic profiling reveals biomarkers involved in rice-gall midge interactions. J Integr Plant Biol. 2014;56(9):837-48.
159. Sana TR, Fischer S, Wohlgemuth G, Katrekar A, Jung KH, Ronald PC, et al. Metabolomic and transcriptomic analysis of the rice response to the bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae*. Metabolomics. 2010;6(3):451-65.
160. Gunnaiah R, Kushalappa AC, Duggavathi R, Fox S, Somers DJ. Integrated metabolo-proteomic approach to decipher the mechanisms by which wheat QTL (*Fhb1*) contributes to resistance against *Fusarium graminearum*. PLoS One. 2012;7(7):e40695.
161. Semel Y, Schauer N, Roessner U, Zamir D, Fernie AR. Metabolite analysis for the comparison of irrigated and non-irrigated field grown tomato of varying genotype. Metabolomics. 2007;3(3):289-95.
162. Bowne JB, Erwin TA, Juttner J, Schnurbusch T, Langridge P, Bacic A, et al. Drought responses of leaf tissues from wheat cultivars of differing drought tolerance at the metabolite level. Mol Plant. 2012;5(2):418-29.
163. Silvente S, Sobolev AP, Lara M. Metabolite Adjustments in Drought Tolerant and Sensitive Soybean Genotypes in Response to Water Stress. PLoS One. 2012;7(6):e38554.
164. Witt S, Galicia L, Lisec J, Cairns J, Tiessen A, Araus JL, et al. Metabolic and phenotypic responses of greenhouse-grown maize hybrids to experimentally controlled drought stress. Mol Plant. 2012;5(2):401-17.
165. Sun CX, Gao XX, Li MQ, Fu JQ, Zhang YL. Plastic responses in the metabolome and functional traits of maize plants to temperature variations. Plant Biol. 2016;18(2):249-61.
166. Le Gall H, Fontaine JX, Molinie R, Pelloux J, Mesnard F, Gillet F, et al. NMR-based Metabolomics to Study the Cold-acclimation Strategy of Two Miscanthus Genotypes. Phytochem Anal. 2017;28(1):58-67.

167. Paupiere MJ, Muller F, Li H, Rieu I, Tikunov YM, Visser RGF, et al. Untargeted metabolomic analysis of tomato pollen development and heat stress response. Plant Reprod. 2017;30(2):81-94.
168. Fiehn O. Metabolomics—The link between genotypes and phenotypes. Plant Mol Biol. 2002;48(1-2):155-71.
169. Weckwerth W. Green systems biology—From single genomes, proteomes and metabolomes to ecosystems research and biotechnology. J Proteomics. 2011;75(2):284-305.
170. Urano K, Kurihara Y, Seki M, Shinozaki K. 'Omics' analyses of regulatory networks in plant abiotic stress responses. Curr Opin Plant Biol. 2010;13(2):132-8.
171. Jogaiah S, Govind SR, Tran LS. Systems biology-based approaches toward understanding drought tolerance in food crops. Crit Rev Biotechnol. 2013;33(1):23-39.
172. Ritchie MD, Holzinger ER, Li R, Pendergrass SA, Kim D. Methods of integrating data to uncover genotype-phenotype interactions. Nat Rev Genet. 2015;16(2):85-97.
173. Weckwerth W, Ghatak A, Bellaire A, Chaturvedi P, Varshney RK. PANOMICS meets germplasm. Plant Biotechnol J. 2020;18(7):1507-25.
174. Weckwerth W. Toward a Unification of System-Theoretical Principles in Biology and Ecology—The Stochastic Lyapunov Matrix Equation and Its Inverse Application. Front Appl Math Stat. 2019;5:29.
175. Wang M, Wang P, Tu L, Zhu S, Zhang L, Li Z, et al. Multi-omics maps of cotton fibre reveal epigenetic basis for staged single-cell differentiation. Nucleic Acids Res. 2016;44(9):4067-79.
176. Kuo TC, Tian TF, Tseng YJ. 3Omics: a web-based systems biology tool for analysis, integration and visualization of human transcriptomic, proteomic and metabolomic data. BMC Syst Biol. 2013;7:64.
177. Garcia-Alcalde F, Garcia-Lopez F, Dopazo J, Conesa A. Paintomics: a web based tool for the joint visualization of transcriptomics and metabolomics data. Bioinformatics. 2011;27(1):137-9.
178. Wen W, Li D, Li X, Gao Y, Li W, Li H, et al. Metabolome-based genome-wide association study of maize kernel leads to novel biochemical insights. Nat Commun. 2014;5:3438.
179. Luo J. Metabolite-based genome-wide association studies in plants. Curr Opin Plant Biol. 2015;24:31-8.
180. Matsuda F, Nakabayashi R, Yang Z, Okazaki Y, Yonemaru J, Ebana K, et al. Metabolome-genome-wide association study dissects genetic architecture for generating natural variation in rice secondary metabolism. Plant J. 2015;81(1):13-23.
181. Huang S, Wang J, Song R, Jia A, Xiao Y, Sun Y, et al. Balanced plant helper NLR activation by a modified host protein complex. Nature. 2025;639:447-55.
182. Xiao Y, Wu X, Wang Z, Ji K, Zhao Y, Zhang Y, et al. Activation and inhibition mechanisms of a plant helper NLR. Nature. 2025;639:438-46.
183. Jones JDG, Staskawicz BJ, Dangl JL. The plant immune system: From discovery to deployment. Cell. 2024;187(9):2095-116.

184. Guo BC, Zhang YR, Liu ZG, Li XC, Yu Z, Ping BY, et al. Deciphering Plant NLR Genomic Evolution: Synteny-Informed Classification Unveils Insights into TNL Gene Loss. Mol Biol Evol. 2025;42(2):msaf015.
185. Li SX, Liu Y, Zhang YM, Chen JQ, Shao ZQ. Convergent reduction of immune receptor repertoires during plant adaptation to diverse special lifestyles and habitats. Nat Plants. 2025;11:248-62.
186. Contreras MP, Lüdke D, Pai H, Toghani A, Kamoun S. NLR receptors in plant immunity: making sense of the alphabet soup. EMBO Rep. 2023;24(5):e57495.
187. Chia KS, Kourelis J, Teulet A, Vickers M, Sakai T, Walker JF, et al. The N-terminal domains of NLR immune receptors exhibit structural and functional similarities across divergent plant lineages. Plant Cell. 2024;36(7):2491-511.
188. Shepherd S, Yuen ELH, Carella P, Bozkurt TO. The wheels of destruction: Plant NLR immune receptors are mobile and structurally dynamic disease resistance proteins. Curr Opin Plant Biol. 2023;74:102372.
189. Chia KS, Carella P. Taking the lead: NLR immune receptor N-terminal domains execute plant immune responses. New Phytol. 2023;240(2):496-501.
190. Axtell MJ, Staskawicz BJ. Initiation of RPS2-specified disease resistance in Arabidopsis is coupled to the AvrRpt2-directed elimination of RIN4. Cell. 2003;112(3):369-77.
191. Song J, Bradeen JM, Naess SK, Raasch JA, Wielgus SM, Haberlach GT, et al. Gene RB cloned from Solanum bulbocastanum confers broad spectrum resistance to potato late blight. P Natl Acad Sci USA. 2003;100(16):9128-33.
192. Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, et al. A Receptor Kinase-Like Protein Encoded by the Rice Disease Resistance Gene, *Xa21*. Science. 1995;270(5243):1804-6.
193. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science. 2012;337(6096):816-21.
194. Chen K, Wang Y, Zhang R, Zhang H, Gao C. CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. Annu Rev Plant Biol. 2019;70:667-97.
195. Cesari S. Multiple strategies for pathogen perception by plant immune receptors. New Phytol. 2018;219(1):17-24.
196. Xi Y, Cesari S, Kroj T. Insight into the structure and molecular mode of action of plant paired NLR immune receptors. Essays Biochem. 2022;66(5):513-26.
197. Van de Weyer AL, Monteiro F, Furzer OJ, Nishimura MT, Cevik V, Witek K, et al. A Species-Wide Inventory of NLR Genes and Alleles in Arabidopsis thaliana. Cell. 2019;178(5):1260-72.e14.
198. Thatcher S, Jung M, Panangipalli G, Fengler K, Sanyal A, Li B, et al. The NLRomes of Zea mays NAM founder lines and Zea luxurians display presence-absence variation, integrated domain diversity, and mobility. Mol Plant Pathol. 2023;24(7):742-57.
199. Tong C, Zhang Y, Shi F. Genome-wide identification and analysis of the *NLR* gene family in *Medicago ruthenica*. Front Genet. 2023;13:1088763.
200. Li T, Ma X, Li N, Zhou L, Liu Z, Han H, et al. Genome-wide association study discovered candidate genes of Verticillium wilt resistance in upland cotton (*Gossypium hirsutum* L.). Plant Biotechnol J. 2017;15(12):1520-32.

201. Cui H, Tsuda K, Parker JE. Effector-triggered immunity: from pathogen perception to robust defense. Annu Rev Plant Biol. 2015;66:487-511.
202. Smakowska-Luzan E, Mott GA, Parys K, Stegmann M, Howton TC, Layeghifard M, et al. An extracellular network of Arabidopsis leucine-rich repeat receptor kinases. Nature. 2018;553(7688):342-6.
203. Hannan Parker A, Wilkinson SW, Ton J. Epigenetics: a catalyst of plant immunity against pathogens. New Phytol. 2022;233(1):66-83.
204. Huang CY, Jin H. Coordinated Epigenetic Regulation in Plants: A Potent Managerial Tool to Conquer Biotic Stress. Front Plant Sci. 2022;12:795274.
205. Cambiagno DA, Torres JR, Alvarez ME. Convergent Epigenetic Mechanisms Avoid Constitutive Expression of Immune Receptor Gene Subsets. Front Plant Sci. 2021;12:703667.
206. De la Concepcion JC, Franceschetti M, MacLean D, Terauchi R, Kamoun S, Banfield MJ. Protein engineering expands the effector recognition profile of a rice NLR immune receptor. Elife. 2019;8:e47713.
207. Davison J, Ammann K. New GMO regulations for old: Determining a new future for EU crop biotechnology. GM Crops Food. 2017;8(1):13-34.
208. Hu H. Transgenic Plants. In: Liu D, editor. Handbook of Molecular Biotechnology. Boca Raton (US): CRC Press; 2024. p. 681-94.
209. Hu H, Zhang Y, Yu F. A CRISPR/Cas9-based vector system enables the fast breeding of selection-marker-free canola with *Rcr1*-rendered clubroot resistance. J Exp Bot. 2023;75(5):1347-63.
210. Schneider K, Barreiro-Hurle J, Vossen J, Schouten HJ, Kessel G, Andreasson E, et al. Insights on cisgenic plants with durable disease resistance under the European Green Deal. Trends Biotechnol. 2023;41(8):1027-40.
211. Ricroch AE. Assessment of GE food safety using '-omics' techniques and long-term animal feeding studies. N Biotechnol. 2013;30(4):349-54.
212. Ibanez C, Simo C, Garcia-Canas V, Acunha T, Cifuentes A. The role of direct high-resolution mass spectrometry in foodomics. Anal Bioanal Chem. 2015;407(21):6275-87.
213. Fukushima A, Kusano M, Mejia RF, Iwasa M, Kobayashi M, Hayashi N, et al. Metabolomic Characterization of Knockout Mutants in Arabidopsis: Development of a Metabolite Profiling Database for Knockout Mutants in Arabidopsis. Plant Physiol. 2014;165(3):948-61.
214. Simo C, Ibanez C, Valdes A, Cifuentes A, Garcia-Canas V. Metabolomics of genetically modified crops. Int J Mol Sci. 2014;15(10):18941-66.
215. El Ouakfaoui S, Miki B. The stability of the Arabidopsis transcriptome in transgenic plants expressing the marker genes nptII and uidA. Plant J. 2005;41(5):791-800.
216. Ren Y, Wang T, Peng Y, Xia B, Qu LJ. Distinguishing transgenic from non-transgenic Arabidopsis plants by (1)H NMR-based metabolic fingerprinting. J Genet Genomics. 2009;36(10):621-8.
217. Shepherd LV, Hackett CA, Alexander CJ, McNicol JW, Sungurtas JA, Stewart D, et al. Modifying glycoalkaloid content in transgenic potato--Metabolome impacts. Food Chem. 2015;187:437-43.

218. Ricroch AE, Berge JB, Kuntz M. Evaluation of genetically engineered crops using transcriptomic, proteomic, and metabolomic profiling techniques. Plant Physiol. 2011;155(4):1752-61.
219. Enfissi EMA, Drapal M, Perez-Fons L, Nogueira M, Berry HM, Almeida J, et al. New plant breeding techniques and their regulatory implications: An opportunity to advance metabolomics approaches. J Plant Physiol. 2021;258:153378.
220. Benevenuto RF, Venter HJ, Zanatta CB, Nodari RO, Agapito-Tenfen SZ. Alterations in genetically modified crops assessed by omics studies: Systematic review and meta-analysis. Trends Food Sci Technol. 2022;120:325-37.
221. Gill T, Gill SK, Saini DK, Chopra Y, de Koff JP, Sandhu KS. A Comprehensive Review of High Throughput Phenotyping and Machine Learning for Plant Stress Phenotyping. Phenomics. 2022;2(3):156-83.
222. Arya S, Sandhu KS, Singh J, Kumar S. Deep learning: as the new frontier in high-throughput plant phenotyping. Euphytica. 2022;218(4):47.
223. Kundu S, Saini DK, Meena RK, Bahuguna RN, Jagadish SVK. High-throughput phenotyping and AI technologies for deciphering crop resilience to heat stress. Plant Physiol Rep. 2024;29(4):699-715.

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