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Review

Genomic and Transcriptomic Approaches to Developing Abiotic Stress-Resilient Crops

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Abstract: In the realm of agriculture, a pressing concern remains the abiotic stresses, such as temperature fluctuation, drought, soil salinity, and heavy metal contamination. These adverse growth conditions hamper crop yields and global food security. In this review, we present a comprehensive examination of the recent advancements in utilizing genomics and transcriptomics, tools to enhance crop resilience against these stress factors. Genomics aids in the identification of genes responsive to stress, unravels regulatory networks, and pinpoints genetic variations linked to stress tolerance. Concurrently, transcriptomics sheds light on the intricate dynamics of gene expression during stress conditions, unearthing novel stress-responsive genes and signaling pathways. This wealth of knowledge shapes the development of stress-tolerant crop varieties, achieved through conventional breeding programs and state-of-the-art genetic engineering and gene editing techniques like CRISPR-Cas9. Moreover, the integration of diverse omics data and functional genomics tools empowers precise manipulation of crop genomes to fortify their stress resilience. In summary, the integration of genomics and transcriptomics holds substantial promise in elucidating the molecular mechanisms behind crop stress tolerance, offering a path towards sustainable agriculture and safeguarding food security amidst shifting environmental challenges.

Keywords: abiotic stress; development; genomics; transcriptomics

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1. Introduction

The Food and Agriculture Organization (FAO) of the United Nations reported in its statistical yearbook 2022 that hunger is still on the rise with 770 million people undernourished in the year 2021. In every continent on the earth, there is moderate to severe level of food insecurity. Between 2000 and 2020, agricultural land declined by 134 million ha and water stress is prevalent in most countries [1]. Considering these circumstances, the primary goal must be ensuring nourishment for the growing population. Unfortunately, crop production seems to have reached a plateau and is even decreasing in recent times, primarily because of climate change and the scarcity of arable land for cultivation. In particular, abiotic stresses like temperature extremes (such as heat or cold stress), water availability (drought or flooding), salinity, nutrient deficiency or excess, heavy metal toxicity, pollution, radiation, and other physical or chemical factors account for significant crop yield losses every year all over the world [2]. Abiotic stress can disrupt various physiological and biochemical processes within plants, leading to reduced growth, development, and productivity. It can also make plants more susceptible to diseases, pests, and other biotic stresses [3]. Hence, enhancing agricultural productivity and sustainability holds immense significance on a global scale. There is an urgent requirement for crop improvement to guarantee food security for the world's population [4]. The goal of crop improvement is to develop improved plant varieties that exhibit desirable traits such as increased yield, disease resistance, tolerance to environmental stresses, improved nutritional content, and better post-harvest characteristics [5]. During the green revolution,

biotechnology tools were not widely employed as the field of biotechnology was still in its infancy. The full potential of modern biotechnology, including genetic engineering and genomics was realized later [6]. Since then, these technologies have played a significant role in crop improvement. Multiple "omics" approaches are extensively used for crop improvement. The progress made in next-generation sequencing (NGS) has opened avenues for the development of new omics fields, including genomics, transcriptomics, proteomics, metabolomics, ionomics, etc. [7]). Especially, genomics and transcriptomics play crucial roles in crop improvement by providing valuable insights into the genetic makeup and gene expression patterns of crucial genes and gene families [7]. In this review, we will focus on genomics and transcriptomics approaches employed in crop improvement against abiotic stresses.

2. Genomics for Understanding Abiotic Stress Response in Plants

Genomics focuses on exploring and studying the genomes, i.e., the complete set of DNA or genetic material within an organism, with the help of a range of techniques and approaches [8]. It involves analyzing and interpreting the structure, function, and evolution of genomes. Genomics has been broadly classified into functional, structural, and comparative genomics, based on its methodologies and outcomes [9]. In this section, the three classes of genomics, their methodologies, and their application in crop improvement for abiotic stress tolerance will be discussed.

2.1. Functional Genomics

Functional studies of genomes readily produce information that is applicable to crop improvement. Functional genomics involves the functional characterization of genes and their interactions with other genes in a regulatory network [10]. Functional genomics includes different approaches to identify gene functions, such as sequence- or hybridization-based methodologies, gene inactivation or editing-based approaches, and gene overexpression [10]. These different approaches will be discussed in detail here.

2.1.1. Gene Inactivation and Editing Approaches for Functional Analysis of Abiotic Stress-Related Genes

RNAi and VIGS

RNAi technology can be used for gene inactivation and functional studies [11]. It involves the introduction of small interfering RNA (siRNA) or short hairpin RNA (shRNA) molecules into the cell. These molecules bind to the target mRNA, leading to its degradation or inhibition of its protein translation. Through the utilization of RNAi techniques, the roles of various genes in abiotic stress responses have been uncovered across plant species. In soybean, overexpression of the Tubby-like protein gene *GmTLP8* enhanced the plant's resilience to drought and salt stress, while its suppression decreased tolerance [12]. Similarly, in rice, overexpression of the Aux/IAA gene *OsIAA20* and the nuclear export receptor *OsXPO1* improved plant tolerance to drought and salt stress, but reduced plant height and seed-setting rate in the case of *OsXPO1*. Conversely, suppressing these genes by RNAi decreased plant resilience to stress and induced developmental defects [13]. In tobacco, suppression of the APETALA2/ethylene response factor (AP2/ERF) gene *NtRAV-4* enhanced root development, leaf photosynthetic ability, and drought tolerance [14].

Virus-induced gene silencing (VIGS) is a transient induction of RNAi by using modified viral vectors for plant functional genomics. RNAi and VIGS have been used for improvement of several traits in crop plants. For example, VIGS of *CaWRKY40a* in pepper enhances resistance against Xanthomonas campestris infection [15]. In wheat VIGS of Diketone Metabolism-PKS (DMP), -Hydrolase (DMH) affects β-Diketone biosynthesis and results in increased glaucousness which is associated with yield [16]. In rice, both the General Control Non-derepressible 5 (*GCN5*) and Adenosine Deaminase2 (*ADA2*) RNAi lines produced fewer crown roots and showed reduced primary root length and shoot height compared with the wild type [17]. These examples show that both RNAi and VIGS

are useful tools in improving several important traits in crops through gene silencing [18]. Antisense oligonucleotides (ASO) are short synthetic nucleic acid sequences that are designed to be complementary to the target gene's mRNA. Upon binding to the mRNA, ASOs can prevent mRNA translation or promote degradation of the target mRNA. ASOs can be chemically modified to enhance stability and specificity, and they are being explored as therapeutic agents for various genetic diseases [19]. Conditional gene knockout techniques enable the inactivation of genes in a specific tissue, at a particular developmental stage, or upon induction by external stimuli. This can be achieved using Cre-loxP or Flp-FRT systems by flanking the target gene with loxP sites. When Cre recombinase is expressed under the control of a specific promoter (e.g., tissue-specific promoters or inducible promoters), it mediates the excision of the target gene between the loxP sites, resulting in gene inactivation [20]. Flp recombinase recognizes FRT sites and catalyzes recombination between them, resulting in DNA excision, inversion, or rearrangement, depending on the orientation and arrangement of the FRT sites [21].

ZFN, TALEN, and CRISPR-Cas9

To functionally characterize plant genes, genome editing techniques like targeted mutation, INDEL creation, and genomic sequence modifications can be applied [11]. Common genome editing tools include Zinc finger nucleases (ZFNs), transcriptional activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeat (CRISPR)-Cas9 [11]. ZFNs, for instance, induce double-strand breaks at specific genomic locations, resulting in targeted mutagenesis such as chromosomal deletions, transgene removal, and precise DNA integration [22]. They consist of a non-specific cleavage domain from the FoKI endonuclease fused with custom-designed Cys2-His2 zinc finger proteins, leading to DSB formation. Plant systems employ error-prone non-homologous end joining (NHEJ) for DNA repair (Wada et al., 2022). TALEN- and ZFN-based genome editing for abiotic stress tolerance have not been carried out extensively in crops, yet. But CRISPR-Cas (clustered regularly interspaced short palindromic repeats-CRISPR-associated proteins) has been extensively used in crops for genome editing in this decade. Using CRISPR-Cas9 knockout mutation of miRNAs, the *OsMIR408* and *OsMIR528* lines were developed. These lines were salt-sensitive, and it has been found that these genes were positive regulators of salt stress tolerance [23]. In the past decade, many salt stress-related genes have been identified, for example, *OsRR22*, identified as a salt stress-related gene, encodes a 696-amino acid B-type response regulator transcription factor that is involved in cytokinin signaling. CRISPR/Cas9 editing leading to loss of function mutation in the *OsRR22* gene results in increased salt tolerance [24]. Many genes have been identified using mutation studies and among them, the drought and salt tolerance (*DST*) gene was identified as a non-desirable gene, and is present in the genome due to linkage. Genome editing via CRISPR-Cas technology has caused 366 bp deletion and has generated the loss of function mutation of the *DST* gene. This mutant line shows enhanced leaf water retention under dehydration stress [25]. Hybrid proline-rich proteins (HyPRPs) have been demonstrated to play distinct roles in responses to biotic and abiotic stresses across various plant species. However, in the case of tomato, a specific HyPRP called SlHyPRP1 has been identified as a suppressor of multiple stress responses. Precise elimination of *SlHyPRP1* negative-response domain(s) using CRISPR-Cas9 has led to high salinity tolerance at the germination and vegetative stages [26]. The *ARGOS8* gene, which acts as a negative regulator in the ethylene response pathway, has been found to induce drought tolerance genes in maize [27]. Although numerous natural genotypes displaying drought resistance have been identified, the expression of *ARGOS8* therein has been observed to be very low. To overcome this limitation, CRISPR-Cas9 was used with a less-restrictive constitutive promoter called *GOS2* to enhance the expression of *ARGOS8* [27]. This led to increased drought tolerance in maize. CRISPR-Cas9-mediated editing of genes encoding two enzymes, poly ADP-ribose polymerase (PARP) and ADP-ribose specific Nudix hydrolase (NUDX), has resulted in increased tolerance to oxidative, drought, and genotoxic stresses in maize [27]. *NPR1* (non-

expressor of pathogenesis-related gene 1) serves as a central regulator in the plant defense response against pathogens. While its role in the defense pathway is well understood, its involvement in abiotic stress remains unclear. CRISPR-Cas9-mediated mutagenesis of *SlNPR1* in tomatoes has led to reduced drought tolerance, which was accompanied by decreased expression of key drought-related genes, including *SlGST*, *SlDHN*, and *Sl-DREB* [28]. These findings suggest that NPR1 not only regulates the response to biotic stress but also plays a role in the plant's response to abiotic stress. Overall, these findings demonstrate the potential of CRISPR-Cas9 technology in crop improvement particularly for abiotic stress tolerance traits. All this information aids in the development of transgenic or targeted mutant lines of crops for abiotic stress tolerance.

TILLING

Targeted induced local lesions in genomes (TILLING) is a useful and high-throughput technique to identify single nucleotide mutations in a specific region of a gene of interest. TILLING methods are generally employed for screening of both phenotypic and genotypic variations in crops under abiotic stresses [29]. Its updated variant EcoTILLING has been used for the identification of natural polymorphisms [30]. EcoTILLING is useful particularly for non-model organisms with limited genetic resources and genomic information. In one study, chemically mutated lines were screened using the TILLING approach to identify variants in membrane transport genes and their response to salt stress [31]. Among 2961 mutant lines, 41 mutants with single nucleotide polymorphisms (SNPs) in nine membrane transporters were discovered. Altered sequences found in the exon region of seven genes, and these seven mutants exhibited salt tolerance. Additionally, five mutants with SNPs, i.e., *OsAKT1* (outward rectifying Potassium channel 1), *OsHKT6* (high-affinity Potassium transporter 6), *OsNSCC2* (nonselective cation channel 2), *OsHAK11* (high affinity Potassium transporter 11), and *OsSOS1* (salt overly sensitive 1) showed altered gene expression levels [31]. These mutants hold potential for developing salt-tolerant lines. Similarly, chilling-tolerant lines were identified in a TILLING mutant population of Donganbyeo rice cultivar. Comparative transcriptome analysis revealed that chilling stress tolerance was associated with monosaccharide catabolic processes, which provide the necessary energy for cold adaptation in rice [32]. High-temperature stress during grain filling leads to delayed endosperm formation and grain chalkiness. Multi-omics analysis indicated that the downregulation of starch synthesis enzymes and upregulation of α-amylases could be the possible reason behind this [32]. Targeting the TILLING mutants of α-amylase genes may reduce chalkiness in heat-stressed grains without the need for transgenic approaches. TILLING was also used to create three mutations in the *Brassica rapa* CAX1a transporter. These mutants, along with the original strain (R-o-18), were cultivated in a saline environment, and various parameters were assessed, including biomass, photosynthesis efficiency, glucose-6-phosphate dehydrogenase (G6PDH), and soluble carbohydrates. It was revealed that the *BraA.cax1a-7* mutation negatively impacted alkalinity tolerance, resulting in reduced plant biomass, increased oxidative stress, and partial inhibition of antioxidant responses and photosynthesis. Conversely, the *BraA.cax1a-12* mutation enhanced plant biomass, increased calcium (Ca^{2+}) accumulation, reduced oxidative stress, and improved both antioxidant responses and photosynthesis performance [33]. Thus, *BraA.cax1a-12* emerges as a promising mutation for enhancing alkaline tolerance in plants. Also, a mutant of the tomato *HSBP1* (heat shock binding protein 1) gene was identified in the TILLING population, resulting in a partial loss of protein function. This mutation led to improved resistance to high temperatures in young plants and increased resilience in mature plants under repeated heat stress [34]. A list of tools and databases available for plant gene inactivation or gene editing is given in Table 1.

Table 1. List of tools and database available for plant gene inactivation or gene editing.

Tool/Database	URL	Function	Citation
CRISPR-PLANT	http://omap.org/crispr/ (accessed on 3 October 2023)	Selects suitable CRISPR target sites for gene editing in various plant species.	[35]
Cas-Designer	www.rgenome.net/cas-designer/ (accessed on 3 October 2023)	Chooses CRISPR-Cas9 target sites for various organisms, including plants.	[36]
DESKGEN Cloud	www.deskgen.com/ (accessed on 3 October 2023)	Online platform for gRNA design and CRISPR experiment planning, supporting various CRISPR applications.	[37]
E-CRISP	www.e-crisp.org/E-CRISP/ (accessed on 3 October 2023)	Fast CRISPR target site identification tool.	[38]
GuideScan2	www.guidescan.com/ (accessed on 3 October 2023)	Software for designing CRISPR guide RNA (gRNA) libraries.	[39]
ZiFiT	https://mybiosoftware.com/zifit-4-2-zinc-finger-engineering-tool.html (accessed on 3 October 2023)	Software tool for designing custom zinc finger proteins for gene editing.	[40]
CHOPCHOP	chopchop.cbu.uib.no/ (accessed on 3 October 2023)	Web tool for precise design of CRISPR/Cas9 targets.	[41]
CRISPOR	http://crispor.gi.ucsc.edu/ (accessed on 3 October 2023)	Web tool offering efficiency and specificity scores for CRISPR-Cas9 genome editing.	[42]
CRISPR-ERA	crispr-era.stanford.edu/ (accessed on 3 October 2023)	Tool for evaluating and annotating CRISPR experiment results and designing gRNAs for gene editing.	[43]
CRISPR-P	crispr.hzau.edu.cn/CRISPR2/ (accessed on 3 October 2023)	Web tool for identifying potential CRISPR target sites in input DNA sequences.	[44]
Cas-OFFinder	www.rgenome.net/cas-offinder/ (accessed on 3 October 2023)	Web-based tool for searching potential off-target sites in the genome for CRISPR/Cas-derived RNA-guided endonucleases.	[45]
SSFinder	https://code.google.com/p/ssfinder/ (accessed on 3 October 2023)	Web-based tool for detecting sequences suitable for base editing using CRISPR/Cas-derived base editors.	[46]
JASPAR	jaspar.genereg.net/ (accessed on 3 October 2023)	Database of transcription factor binding profiles, including those for Cre and FLP recombinases.	[47]

2.1.2. Gain-of-Function Approach through Gene Overexpression

The technique of gene overexpression has become a pivotal tool in genomics, offering valuable insights into gene function and potential applications in crop improvement. In the past decades, numerous significant studies have underscored the efficacy of this approach, particularly in enhancing stress tolerance and overall crop performance. For instance, in rice, *OsCYP19-4* was found to be significantly upregulated under cold stress. Overexpression of *OsCYP19-4* in rice demonstrated enhanced tolerance to cold stress and improved grain yield [48]. Tomato plants overexpressing *SlGRAS7* (GAI, RGA, and SCR-like protein 7) exhibited enhanced resistance to drought and salt stress compared to the wild type (WT) [49]. Elongation factor 1 A (EF1A), a crucial regulator for protein synthesis, has been found to participate in plant responses to abiotic stress and environmental adaptation. Overexpression of *MtEF1A1* in *Medicago truncatula* resulted in increased salt stress resistance and reduced levels of reactive oxygen species (ROS) in leaves [50]. Additionally, the expression of abiotic stress-responsive genes (*MtRD22A* and *MtCOR15A*) and calcium-binding genes (*MtCaM* and *MtCBL4*) was upregulated in *M. truncatula* lines overexpressing *MtEF1A1* [50]. These abiotic stress-related genes could be important for developing stress resilient crops. Some important genes could impart tolerance to multiple abiotic stresses in plants when overexpressed. For example, in white birch (*Betula platyphylla Suk.*), overexpression of *Ethylene response factor 1.1 (ERF1.1)* showed increased tolerance to cold, drought, and salt stress compared to WT. RNA-Seq analysis has shown 689 differentially expressed genes (DEGs) in transgenic birch compared to WT. This shows overexpression of single gene results in triggering of cascade of gene networks leading to stress tolerance [51]. Similarly, *Arabidopsis ICE1 (inducer of CBF expression 1)* was overexpressed in Indica rice to improve cold tolerance in cold-sensitive rice. *AtICE1* lines showed lower accumulation of H_2O_2 and higher membrane stability, thus increased seed survival rate under cold stress. Also, *AtICE1* lines had increased grain yield under cold, drought, and salt stresses [52]. In rice group-A PP2Cs, *OsPP108* conferred tolerance to salt and drought stresses when overexpressed in Arabidopsis. Interestingly, this gene rendered plants highly insensitive to ABA and proposed to regulate abiotic stress tolerance possibly in an ABA-independent manner [53]. Similarly, tomato overexpression of the transgenic line of *dwarf and delayed flowering 2 (SlDDF2)* under stress-inducible *RD29a* promoter showed better growth performance and tolerance under abiotic stresses including salinity and drought [54]. Overall, these studies suggested that identification of the key gene inducible under multiple stresses, and its overexpression in plants, is an effective strategy for developing multi-stress resilient crops. Details of several other genes that regulate a plant's abiotic stress response through overexpression or gene knockout/silencing is given in Table 2.

Table 2. Genes involved in regulating abiotic stress response through gene overexpression and gene silencing approaches.

Gene	Plant	Abiotic Stress Tolerance	Functional Analysis Method	Citation
GmNAC10	Soybean	Cold, salt and dehydration	Overexpression	[55]
AtICE1	Rice	Cold and drought	Overexpression	[52]
MP-mi397	Banana	Copper and iron deficiencies, salt and drought	Overexpression	[56]
BPERF1.1	White Birch	Cold, salt and drought	Overexpression	[51]
OsRIP1	Rice	Osmotic stress	Overexpression	[57]
MiMFTs	Mango	Salt and osmotic stress	Overexpression	[58]
JrWOX11	Walnut	Salt and osmotic stress	Overexpression	[59]
SlDDF2	Tomato	Drought, salinity and cold	Overexpression.	[54]

Table 2. *Cont.*

Gene	Plant	Abiotic Stress Tolerance	Functional Analysis Method	Citation
OsHis1.1	Rice	Heat and cold	Overexpression	[60]
ZmSLK1 and ZmSLK2	Arabidopsis	Drought	Overexpression	[61]
GmlncRNA77580	Soybean	Drought	Overexpression	[62]
GhSAMS	Cotton	Drought and salinity	VGIS	[63]
SlWRKY79	Tomato	Salt	VGIS	[64]
GhMYB36	Cotton	Drought	VGIS	[65]
GmAITR	Soybean	Salinity	CRISPR	[66]
OsCCA1	Rice	Salinity, osmotic and drought	CRISPR	[67]
OsmiR535	Rice	Salt and osmotic	CRISPR	[68]
OsPUB7	Rice	Drought and salinity	CRISPR	[69]
NtCycB2	Tobacco	Salinity	CRISPR	[70]
SlGRAS10	Tomato	Osmotic stress	RNAi	[71]
OsIAA20	Rice	Drought and salt stress	RNAi	[72]
Mslea-D34	Alfalfa	Drought and salt	RNAi	[73]

2.2. Structural Genomics

Functional genomics primarily addresses gene functionality, whereas structural genomics focuses on elucidating the physical structure of genomes. Understanding an individual genome's structure is valuable for gene manipulation and DNA segment control [11]. Structural genomics encompasses the creation of high-resolution genetic and physical maps. In the context of crop improvement, genome sequencing and molecular mapping hold significant importance [74]. Molecular markers found on polymorphisms within DNA play a crucial role in assessing genetic diversity in germplasm [75]. These DNA markers find extensive utility in plant breeding, aiding in gene mapping, the identification of quantitative trait loci (QTL), germplasm evaluation, and marker-assisted breeding (MAS). Recent advances in genotyping techniques based on single nucleotide polymorphisms have accelerated MAS. The advent of next-generation sequencing (NGS) has further facilitated genome resequencing and the comparison of various genotypes, leading to the identification of thousands of SNPs [76]. A noteworthy development in molecular markers is insertion site-based polymorphisms (ISBPs), capitalizing on polymorphisms generated by insertion regions at repeat junctions [75].

Molecular Mapping and Marker-Assisted Breeding in Crops for Abiotic Stress Tolerance

Molecular mapping techniques play a pivotal role in traditional breeding, facilitating the development of elite crop varieties. These techniques have unveiled significant structural genomic variations associated with stress conditions, thus aiding in the identification of genotypes resilient to such stressors [11]. These variations serve as valuable indicators for pinpointing the target genes responsible for abiotic stress responses. Furthermore, these advancements have led to the localization of stress-related quantitative trait loci (QTLs). The evolution of next-generation sequencing (NGS) technologies and DNA polymorphism detection techniques, alongside map-based cloning, has further enhanced our ability to identify additional QTLs and develop the associated markers. These methodologies collectively expedite the breeding process, significantly boosting breeding efficiency [75]. For instance, in the context of salinity tolerance in temperate japonica rice accessions, the evaluation of 235 accessions marked with 30,000 SNP markers facilitated a genome-wide association study (GWAS). This study resulted in the identification of 27 QTLs, several of which were closely positioned to genes linked with calcium signaling and kinases. [77]. In

a separate study employing bulk segregant analysis–next generation sequencing (BSA-Seq), four candidate regions were associated with thousand-grain weight (TGW) under alkali stress conditions. Notably, *QTL-qATGW 2-2* was precisely mapped within a 116Kb range between molecular markers RM13592 and Indel3 on Chr.2, encompassing 18 predictive genes. This analysis highlighted *Os02g39884* as the prime candidate gene in *QTL-qATGW 2-2*, representing an alkali-tolerant gene locus in rice [78,79] aiming to enhance stress tolerance in improved white ponni (IWP) rice by pyramiding QTLs against drought (*qDTY 1.1*; *qDTY 2.1*), salinity (Saltol), and submergence (Sub1) through marker-assisted selection (MAS). These QTLs offer promising avenues for developing triple-stress-tolerant crop varieties.

Meta analysis was conducted to refine the locations of 195 major QTLs related to drought, salinity, and waterlogging tolerance in barley from various mapping populations. Identified meta-QTLs (MQTLs) were used to search for candidate genes linked to these tolerances. These refined MQTLs and candidate genes are crucial for successful MAS in barley breeding [13]. A comprehensive meta-QTL analysis on maize revealed the presence of 32 meta-QTLs associated with different abiotic stresses, with a total of 1907 candidate genes identified [80]. Notably, the meta-QTLs designated as MQTL2.1, 5.1, 5.2, 5.6, 7.1, 9.1, and 9.2 were found to control various stress-related traits, contributing to combined abiotic stress tolerance. Recently, a total of 33 QTLs associated with drought tolerance were identified across eight chromosomes in sunflower. Notably, four genes located on chromosome 13 were found to be associated with drought stress in both the germination and seedling stages. These genes, namely *LOC110898128 (aquaporin SIP1-2-like)*, *LOC110898092 (cytochrome P450 94C1)*, *LOC110898071 (GABA transporter 1-like)*, and *LOC110898072 (GABA transporter 1-like isoform X2)* have been annotated and will undergo further functional validation [81]. This study contributes insights into the molecular mechanisms underlying sunflower's response to drought stress, providing a foundation for drought tolerance breeding and genetic improvement in sunflower. Thus, in this decade, many molecular markers and QTLs have been developed and this can help in finding crop accessions with desired traits and faster breeding for the development of new hybrid lines with multiple abiotic stress tolerance. A list of tools and databases related to QTLs and molecular markers in plants are given in Table 3.

Table 3. Database of molecular markers and QTL-based tools in plants.

Tool/Database Name	URL	Description	Citation
Gramene	www.gramene.org (accessed on 3 October 2023)	A resource for comparative analysis of grass genomes, includes a vast array of molecular markers.	[82]
SoyBase	www.soybase.org (accessed on 3 October 2023)	A comprehensive database of soybean genetic and genomic information, including molecular markers.	[83]
CottonGen	www.cottongen.org (accessed on 3 October 2023)	A database of genetic and genomic information for cotton, includes molecular markers.	[84]
SOL Genomics Network (SGN)	solgenomics.net (accessed on 3 October 2023)	A database with genomic, genetic, and taxonomic information for the Solanaceae family and more distantly related species. Includes molecular markers.	[85]
MaizeGDB	https://www.maizegdb.org/ (accessed on 3 October 2023)	The Maize Genetics and Genomics Database, a resource for maize sequence, stock, phenotype, and polymorphism data, including molecular markers.	[86]
MolMarker	https://sourceforge.net/projects/molmarker/ (accessed on 3 October 2023)	This software evaluates plant molecular marker information as well as the related QTL information.	[87]

Table 3. *Cont.*

Tool/Database Name	URL	Description	Citation
The Triticeae Toolbox (T3)	https://triticeaetoolbox.org/ (accessed on 3 October 2023)	A database for Triticeae (wheat and barley) genetic and genomic data, including molecular markers and QTL information.	[88]
Cucurbit Genomics Database (CuGenDB)	http://cucurbitgenomics.org/ (accessed on 3 October 2023)	A centralized platform for cucurbit genomics and genetic data, including molecular markers and QTLs.	[89]
QTL IciMapping	https://isbreedingen.caas.cn/software/qtllcimapping/294607.htm (accessed on 3 October 2023)	A software used for the genetic mapping of QTLs.	[90]
R/QTL	https://rqtl.org/ (accessed on 3 October 2023)	R/QTL is an interactive QTL mapping software, implemented as an R package.	[91]
CottonFGD	https://cottonfgd.org/ (accessed on 3 October 2023)	A functional genomics database for cotton, including molecular markers and QTLs.	[92]
PlantQTL-GE	http://www.scbit.org/qtl2gene/new/ (accessed on 3 October 2023)	A database that stores QTLs and genetically mapped genes in plant species and provides a platform to perform comparative studies on the genetic architecture of complex traits.	[93]
PeanutBase	https://peanutbase.org/ (accessed on 3 October 2023)	A peanut community resource providing genetic, genomic, gene function, and germplasm data, including molecular markers and QTLs.	[94]
GnpIS-Ephesis	https://urgi.versailles.inra.fr/gnpis (accessed on 3 October 2023)	An information system that allows querying and visualizing genotyping data and phenotypic scores for plant species. It includes QTL data.	[95]
Wheat@URGI	https://wheat-urgi.versailles.inra.fr/Seq-Repository/Annotations (accessed on 3 October 2023)	A database providing a complete view of genetic, physical, and functional wheat sequence resources, including molecular markers and QTLs.	[96]

2.3. Comparative Genomic

Comparative genomics involves the comparison of two or more genomes to uncover both their similarities and differences. In this context, gene annotations derived from model plants can be applied to newly sequenced crop species that are yet to undergo functional studies. Essential to this process is knowledge about orthologs, genes that have evolved from a common ancestor and serve similar functions among species descended from that ancestor [97]. Furthermore, comparative genomics finds utility in analyzing the expression profiles of less-studied plants under diverse stress conditions, enabling the identification of stress-related genes and facilitating inter-species expression profile comparisons. Both intra- and inter-specific sequence comparisons rely on a range of computational methods, including multiple sequence alignment, genome-wide comparisons, analyses of orthology and paralogy, as well as the construction of phylogenetic trees [98]. Various tools and databases are available for comparative genomic studies. Ensembl, an extensive database, provides access to numerous annotated genomes and comparative analysis tools, making it a cornerstone for studying genetic variation across species [99,100]. The UCSC Genome Browser offers visualization tools for a diverse range of genomes, simplifying cross-species comparisons and aiding in the exploration of genomic features [101,102]. Another critical resource is OrthoDB, a comprehensive catalog of orthologous genes spanning various species, facilitating the identification of conserved genes with potential roles in evolutionary processes [103]. These tools play a pivotal role in comparative genomics, enabling researchers to uncover evolutionary relationships, conserved genetic elements, and functional insights across genomes. In the context of crop improvement, these resources are invaluable for

identifying candidate genes, regulatory elements, and conserved pathways that can inform breeding strategies, enhance crop resilience, and improve agricultural productivity.

Genome-Wide Identification of Genes Families and Promoter Elements Responsible for Abiotic Stress Response

Genome-wide identification and analysis of gene families in crop genomes typically rely on the sequence homology to known genes. Expression analysis further aids in identifying functional members and pseudogenes [98]. This approach has successfully identified numerous gene families in crops. In rice, numerous gene families have been identified through genome-wide approaches. Moreover, expression analysis helped in marking genes related to specific functions such as biotic stress, abiotic stress, plant development, etc. For instance, genome-wide analysis revealed 491 Pentatricopeptide-repeat proteins (PPRs), categorized into subclass P (246 genes) and subclass PLS (245 genes). Expression analysis showed induction of many PPR genes under both biotic and abiotic stress [104]. *DUF221 domain-containing genes (DDP genes)* play essential roles in plant development, hormone signaling, and stress responses. Comparative genomics in rice identified at least nine *DDP* gene members in both domesticated and wild rice, with various expression analyses showing their upregulation under salt stress [105]. Comparative genomic tools identified 81 Ca^{2+} transport element genes in rice, with their expression established during abiotic stresses and different developmental stages using microarray and qRT-PCR techniques [106]. Similarly, many gene family cell signaling components, such as protein phosphatases [107], phospholipases [108,109], Ca^{2+} dependent protein kinases (CDPKs) [110], and receptor-like cytoplasmic kinases (RLCKs) [111] were identified and analyzed in rice. Additionally, several other gene families, such as, MADS-box, Phytocyanin, BURP, Arabinogalactan, Nuclear-factor Y, ABA repressor, and various transcription factors, have been identified as abiotic stress-responsive genes in rice through comparative genomics approaches [112–118]. Besides rice, the genome-wide approach has been used to identify and analyze of a number of gene families in different crops like soybean [119,120]), cotton [121,122], *Brassica* Spp. [52], *Chickpea* [123–126] and *Maize* [127–129].

Identifying stress-inducible promoter regions is important for deploying transgenes with specific promoters for optimum expression under stress conditions. Studies have shown the effectiveness of promoters from stress-responsive genes, such as *OsABA2*, *RAB16A*, *RD29A*, and *HP1* in driving strong expression of genes under abiotic stress conditions [130]. Transcription profiles of rice, soybean, and Arabidopsis revealed conserved sequences in cold and dehydration-inducible promoters, including the abscisic acid-responsive element (ABRE). Novel cold-inducible cis elements CGTACG and GTAGTA were identified in rice genes promoters [131]. The AL resistance transcription factor1 (ART1), a C2H2 type zinc finger transcription factor with specific cis-acting elements in the promoter, was found to be involved in detoxifying aluminum in rice [132]. Comparative studies in rice and Arabidopsis revealed the occurrence and arrangements of cis-regulatory elements ABRE and CE3 in gene promoters, with ABRE forming ABA-responsive complexes and exhibiting distinct combinations with CE3 in rice [133]. These findings significantly contribute to our understanding of the genetic mechanisms involved in plant response to environmental stresses, paving the way for future research and potential applications in crop improvement. In summary, comparative genomics approaches have successfully identified various gene families involved in abiotic stress responses. These findings provide valuable insights into the genetic mechanisms underlying stress tolerance and can facilitate the development of stress-tolerant crop varieties.

3. Transcriptomics Techniques

Transcriptomics encompasses the comprehensive analysis of all RNAs transcribed by a cell or tissue, including both coding and non-coding RNA at a specific functional state. It involves the study of the type, structure, function, and regulation of gene transcription [134]. Transcriptomics provides valuable insights by quantitatively analyzing changes in plant

gene expression. It enables the exploration of regulatory networks and whole-genome expression patterns, which help in revealing novel stress tolerance-associated genes in crop plants [135].

Advancements in transcript sequencing and analysis technologies have provide a significant upthrust to the field of transcriptomics. Traditional methods like northern blotting and RT-PCR are limited to analyzing single transcripts or small groups of transcripts at a time [136]. However, the introduction of microarrays in the mid-1990s revolutionized transcript profiling, enabling simultaneous analysis of thousands of genes [137]. Subsequently, real-time RT-PCR or RT-qPCR emerged as a sensitive technique for detecting low-abundance transcripts and became popular for both absolute and relative quantification of gene expression [134]. A major breakthrough happened with the advent of next-generation sequencing (NGS), which profoundly impacted gene expression profiling. NGS-based RNA sequencing (RNA-Seq) has widened our horizon of understanding gene regulatory networks and epigenetics. This powerful technology enables the detection and quantification of known, novel, and less abundant transcripts, encompassing both coding and non-coding RNA [136]. The transcriptomics approaches involved in crop improvement against abiotic stress are summarized in Figure 1.

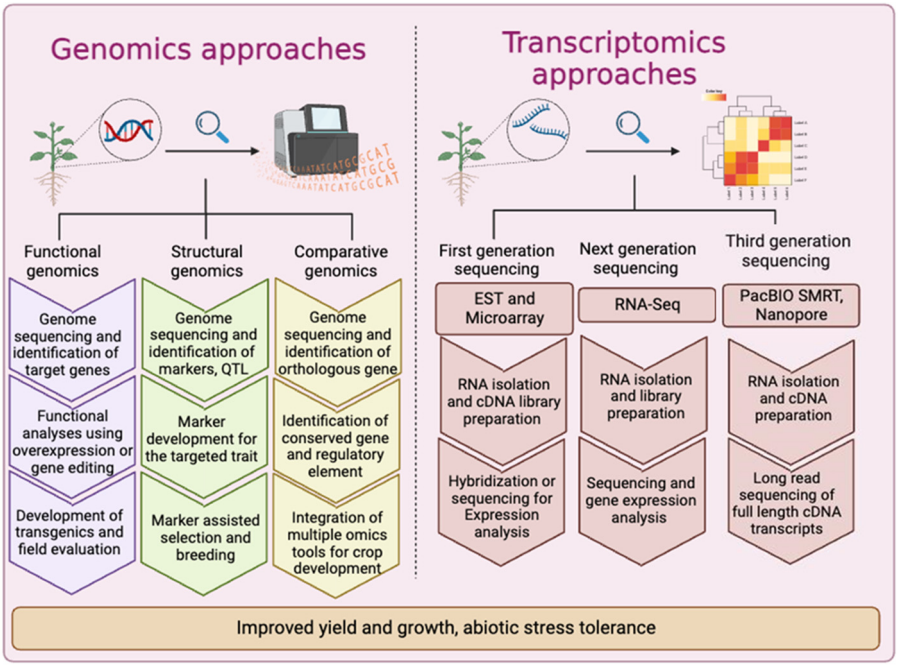

Figure 1. Genomics and Transcriptomics Approaches for Enhancing Abiotic Stress Tolerance in Plants: an overview of the genomics and transcriptomics methodologies in the study of abiotic stress tolerance. Genomics plays a crucial role in the identification and functional analysis of stress-responsive genes, while transcriptomics enables the comprehensive analysis of gene expression patterns. Together, these approaches contribute significantly to crop improvement efforts.

ESTs (expressed sequence tags) are random individual transcripts obtained from cDNA libraries and sequenced using one-time, low-throughput Sanger sequencing. Initially, ESTs were considered an efficient means of determining the gene content of an organism without the need for whole-genome sequencing, making them a valuable tool in early transcriptomics research [134]. Another sequencing-based gene expression analysis technique emerged in 1995, known as serial analysis of gene expression (SAGE). This method involved Sanger sequencing of concatenated random transcript fragments, and quantification is achieved by matching the transcripts with known genes. A variant of SAGE utilizing high-throughput sequencing techniques, called digital gene expression analysis, was also briefly employed [136]. However, these techniques have been overtaken

by contemporary approaches such as microarrays and RNA-Seq, which offer more advanced capabilities and broader applications in transcriptomics research. These modern transcriptomics technologies have significantly advanced our understanding of gene expression regulation and the complexity of cellular processes. They have become essential tools for researchers studying various biological phenomena, including stress responses, development, and disease mechanisms [138]. The continuous development of transcriptomics methods promises to unveil further layers of gene regulation and functional insights in the future.

3.1. ESTs and Microarray for Identification of Abiotic Stress Responsive Genes

Over the years, numerous candidate genes related to abiotic stress in different plant species have been identified using ESTs and microarray techniques. For example, transcript profiling using microarrays and ESTs revealed that Glutathione S-transferases (GSTs) exhibit similar and specific functions during various stages of rice development, while also mediating cross-talk between different stress and hormone response pathways [139]. Microarray expression analysis revealed significant differential expression under abiotic stresses such as drought, salinity, and cold and during reproductive developmental stages in important gene families like protein phosphatases [107], phospholipase A and D [108,140], phospholipase C [110], Calcium transport elements [106], the MADS box family [112], and the C2H2 zinc finger TF family [141] in rice. The expression patterns of most of these differentially expressed genes were validated using qRT-PCR. Heat shock proteins (HSPs) are another important group involved in plant response to heat stress and regulated by heat shock factors (HSFs). In rice, HSFs are categorized into three classes: A, B, and C [142]. Expression profiling through ESTs, microarrays, and qRT-PCR showed that eight *OsHSFs* are upregulated during seed development and six HSFs during abiotic stress in both roots and shoots. *OsHSFA2a* and *OsHSFA3* are upregulated in response to cold and drought stress, while *OsHSFB4a* showed little or no change in expression [143]. Affymetrix microarray technology was used to analyze the expression of the AP2/EREBP gene family in Chaling wild rice and cultivated cold-sensitive rice cultivar Pei'ai64S. Microarray revealed that 36 *AP2/EREBP* genes had a much higher expression level in Chaling wild rice than in Pei'ai64S [144]. Rice is a C3 plant and has become a model organism for studying the genetic engineering of the C4 pathway. Several C4 gene families were identified in the rice genome through sequence homology using maize C4 gene sequences as queries [145]. Expression analysis using EST and FL-cDNA databases indicated the presence of at least one EST or FL-cDNA for all the identified genes. In addition, abiotic stress-related genes were also identified [145].

Using EST and microarray analysis, researchers identified genes associated with drought stress tolerance in cotton. For instance, genes encoding dehydration-responsive element binding (DREB) transcription factors were found to be upregulated under drought conditions, suggesting their role in regulating drought-responsive genes [146]. Additionally, using EST and microarray analysis in cotton, many abiotic stress responsive genes were identified, which included *MYB-related*, *C2H2*, *FAR1*, *bHLH*, *bZIP*, *MADS* [147] and *RTNLB5*, and *PRA1* [148]. Similarly, in barley, a pre-mRNA processing (Prp1) gene was found to be crucial during seed development and abiotic stress [149]. Moreover, in barley, EST databases helped in revealing the roles of HKT (high-affinity K+ transporter) proteins in regulating potassium (K+) uptake under saline conditions [150]. Also, *SbDREB2A*, encoding a DREB transcription factor, was found to be upregulated under drought conditions using ESTs and microarray analysis. Its overexpression enhanced drought tolerance in transgenic sorghum plants [151]. To facilitate such research, there are two large EST repositories, edbEST, and UniGene, both hosted by NCBI, which include EST data from a variety of organisms [152]. These repositories serve as valuable resources for scientists to access and analyze transcriptomic data for various research purposes.

3.2. RNA-Seq for Identification of Genes Involved in Abiotic Stress Responses

RNA-Seq is a powerful technique that combines high-throughput sequencing with computational methods to quantify and analyze transcripts within an RNA pool. The sequencing process generates nucleotide sequences, typically around 100 base pairs in length, although the actual read length can vary depending on the specific sequencing method used [153].

The fundamental principle of RNA-Seq involves the alignment of these generated sequences either to a reference genome or to each other. By mapping the RNA transcripts, one can identify the specific genes present within a genome and also determine which genes are actively expressed at a particular point in time [153]. One of the major advantages of RNA-Seq is its ability to accurately measure gene expression levels. Unlike microarrays, which rely on hybridization to predefine probes, RNA-Seq provides a more comprehensive and quantitative view of gene expression. It can detect both known and novel transcripts, including non-coding RNA, thereby offering a more complete understanding of the transcriptome. Additionally, RNA-Seq is not limited by the predefined nature of microarrays, allowing researchers to explore previously unannotated regions of the genome and discover novel transcripts and isoforms. This capability has been instrumental in identifying alternative splicing events, alternative transcription start sites, and other post-transcriptional regulatory processes [138]. RNA-Seq analysis between imbibed seeds and dry seeds of rice showed that genes related to the cell wall, abiotic stress, and antioxidants were associated with stress response during imbibition and germination [154]. Genes like receptor kinase (e.g., *OsCRK2*), pectinesterase (e.g., *OsPME3*), polygalacturonase (e.g., *OsPGIP1*), cupin-domain protein (e.g., *OsCP1*), methyltransferases (e.g., *OsTRMT1*), SPX domain (e.g., *OsPHR2*), GSTs (e.g., *OsGSTL3*), and peroxidase (e.g., *OsAPX2*) were significantly expressed. GSTs were particularly implicated in preventing H_2O_2 accumulation during the initial imbibition stage, contributing to successful seed germination [154]. RNA-Seq analysis of Aus, a drought and heat-tolerant cultivar of rice, identified 56 differentially expressed genes in developing seeds under combined drought and heat stresses [155]. Among them, B12288 (RAB21), a dehydrin family LEA protein, was significantly induced. Although sequence differences were not large, functional effects were observed, highlighting the role of dehydrins in stress regulation responses. RNA-Seq analysis in tomatoes showed that C2H2-type zinc finger protein genes *C2H2-ZFP3*, *-5*, and *-8* were involved in cold, salt, and drought resistance [156]. RNA-Seq analysis of rice treated with Cd and As revealed genes associated with redox control, stress response, transcriptional regulation, transmembrane transport, signal transduction, biosynthesis, and metabolism of macromolecules and sulfur compounds [157].

In wheat (*Triticum aestivum*), RNA-Seq analysis revealed that *R2R3-MYB* family genes are abiotic stress-responsive [158]. In maize (*Zea mays*), RNA-Seq analysis under abiotic stress showed involvement of various transcription factors like ERF, NAC, ARF and HD-ZIP to initiate abiotic stress response [159]. Soybean (*Glycine max*) experiencing water deficit stress displayed significant changes in gene expression in RNA-Seq analysis, with upregulation of genes involved in ABA signaling, including GmPYLs and GmPP2Cs, both part of the ABA receptor complex [160]. Recently, RNA-seq analysis of two contrasting cultivars of chickpea, i.e., K+ deficiency-sensitive (Pusa362) and -tolerant (Pusa372), identified hundreds of differentially expressed genes in both the cultivars [161]. These genes belonged to different functionally categories and pathways. This analysis provided a significant insight into the K+ deficiency-tolerant mechanism in the important legume crop chickpea.

The wealth of information gained from these RNA-Seq studies contributes to advancing our knowledge of plant stress responses and has laid a foundation for targeted strategies to improve crop resilience and ensure global food security. Various databases that encompass mRNA sequences obtained from crops have been developed over the years. A list of databases related to plant expression is given in Table 4.

Table 4. RNA-Seq-based and gene expression databases.

Database Name	URL	Short Particulars	Citation
NCBI Gene Expression Omnibus (GEO)	https://www.ncbi.nlm.nih.gov/geo/ (accessed on 3 October 2023)	A repository for gene expression data	[162]
ArrayExpress	https://www.ebi.ac.uk/arrayexpress/ (accessed on 3 October 2023)	A public repository for gene expression data	[163]
Plant Expression Database (PLEXdb)	https://www.plexdb.org/ (accessed on 3 October 2023)	A resource for plant gene expression data	[164]
Genevestigator	https://genevestigator.com/ (accessed on 3 October 2023)	A gene expression database and analysis platform	[165]
Rice Expression Database (RiceXPro)	http://ricexpro.dna.affrc.go.jp/ (accessed on 3 October 2023)	A repository for rice gene expression data	[166]
SoyBase	https://www.soybase.org/ (accessed on 3 October 2023)	A repository for soybean genomics data	[83]
MaizeGDB	https://www.maizegdb.org/ (accessed on 3 October 2023)	A database for maize genetics and genomics	[167]
Wheat Expression Browser	https://wheat.pw.usda.gov/ (accessed on 3 October 2023)	A platform for wheat gene expression data	[168]
Tomato Expression Atlas	http://tea.solgenomics.net/ (accessed on 3 October 2023)	A resource for tomato gene expression data	[169]
CottonFGD	https://cottonfgd.net/ (accessed on 3 October 2023)	A functional genomics database for cotton	[83]
SorghumFDB	http://structuralbiology.cau.edu.cn/sorghum/index.html (accessed on 3 October 2023)	A repository for sorghum genomics data	[170]
BarleyBase	https://www.plexdb.org/ (accessed on 3 October 2023)	A database for barley genomics data	[171]
Legum IP V3	https://plantgrn.noble.org/LegumeIP (accessed on 3 October 2023)	A database for legume RNA-Seq data	[172]
Oryza Express	https://rice.plantbiology.msu.edu/ (accessed on 3 October 2023)	A repository for rice gene expression data	[173]
Coexpression Browser (BAR)	https://bar.utoronto.ca/ (accessed on 3 October 2023)	A database for gene coexpression in plants	[174]
PLEXdb	https://www.plexdb.org/ (accessed on 3 October 2023)	A repository for plant gene expression data	[164]

3.3. Third Generation Sequencing for Identification of Abiotic Stress Related Genes

The recent surge in the third-generation sequencing (TGS) technologies has significantly impacted the field of transcriptomics, as TGS offer several advantages over traditional first- and second-generation sequencing [175]. Third-generation sequencing methods, such as Pacific Biosciences (PacBio) and Oxford Nanopore Technologies (ONT) provide longer reads and can directly sequence RNA molecules. This feature significantly facilitates the transcriptional profiling as they enable the identification of full-length transcripts without the need for assembly or the use of sophisticated bioinformatics tools [175]. Importantly, TGS help in identifying novel genes involved in abiotic stress responses in plants. Recently, PacBio sequencing was employed to sequence the transcriptomes of ten rice cultivars belonging to three distinct subspecies under normal and abiotic stress conditions. It was able to reconstruct high-quality, plant-specific isoforms, ranging from 37,500 to 54,600 isoforms per cultivar. To reduce redundancy in the sequences, the isoforms were consolidated and assessed for protein completeness. Approximately 40% of the identified transcripts represented novel isoforms not present in the reference transcriptome of Nipponbare rice. Moreover, for the drought- and heat-tolerant aus cultivar N22, 56 differentially expressed genes were identified in developing seeds under the combined stress of heat and drought [155]. Similarly in poplar PacBio sequencing and RNA-Seq was combinedly used to identify the role of alternative splicing (AS) in cold stress tolerance. It was found that 1261 AS events in *Populus trichocarpa* and 2101 in *P. ussuriensis* among which intron retention, with a frequency of more than 30% was the most prominent type under cold stress [176]. Similarly in cotton, long read transcripts were sequenced using PacBio to identify novel transcripts involved in salt stress. A significant number of DEGs involved in various ion homeostasis, hormone signaling, cell wall modification and transcription factors were found [136]. The *Arachis glabarata* transcriptome was sequenced using PacBio sequencing and several DEGs related to abiotic stress at various organs/tissues were obtained. This study identified 30 polyphenol oxidase (PPO) encoding genes, and most of them were proposed to be involved in biotic or abiotic stresses responses [177].

The cost effectiveness and high-throughput nature of TGS has made it useful for whole genome sequencing of many under-explored crops. *Rehmannia glutinosa*, a medicinal crop was recently sequenced using ONT sequencing. The assembly genome is 2.49 Gb long with a scaffold N50 length of 70 Mb and high heterozygosity (2%). The newly generated reference genome sequence of *R. glutinosa* increases the genomic resources in the Lamiales order [178]. *Cunninghamia lanceolata* (China Fir) belongs to Gymnospermae, which are fast-growing and have desirable wood properties. However, in this species genes involved in stress regulation are little known. Direct RNA sequencing using nanopore technologies has revealed a total of 51 AP2/ERF, 29 NAC, and 37 WRKY transcription factors in *C. lanceolata*. The expression of most of the NAC and WRKY TFs increased under cold stress. These provided preliminary clues about genes involved in stress regulation in *Cunninghamia* [179]. This evidence suggests that TGS has not only simplified transcriptomics studies but also provide valuable and novel insight into stress responses in plants.

4. Comparative Analysis of Tools Employed in Genomics and Transcriptomics Studies

As discussed earlier, genomics and transcriptomics encompasses several tools and techniques, each with its own advantages and disadvantages. For instance, TILLING is used for precise and systematic identification of specific mutations in a target gene, making it a valuable tool for functional analysis. However, TILLING can be labor-intensive and may not be as high-throughput as desired, particularly for large-scale screening [180]. QTL mapping is a genetic analysis approach focused on identifying genomic regions associated with specific traits or quantitative character. Thus, it is instrumental in understanding the genetic basis of complex traits. However, QTL mapping has limited resolution and often relies on the presence of genetic markers, which can restrict its application in cases where markers are not readily available [181]. Comparative genomics, while invaluable for understanding gene function and evolution, may have limitations in comparing vastly

different species or distant evolutionary relatives. The interpretation of observed similarities and differences can be complex and may not always provide straightforward answers about gene function or regulatory elements (Tam et al., 2019). Gene inactivation methods, such as CRISPR-Cas9, ZFNs, and TALENs, are highly precise in gene editing and inactivation, but they may require complex design and validation procedures. Also, there are potential off-target effects, where unintended and untargeted genetic modifications or disruptions can occur, necessitating careful scrutiny and validation of the edited genes [182].

Microarray analysis enables simultaneous analysis of gene expression patterns, making it useful for studying gene regulation on a large scale. However, it is limited to known probes, and there is a risk of cross-hybridization between closely related sequences [183]. ESTs are valuable for gene expression analysis, identifying, and cataloging genes. Nevertheless, they provide only partial gene information, which can be limited in comprehensive studies [184]. RNA-Seq, on the other hand, offers high-throughput transcriptome analysis and is exceptionally valuable for understanding gene expression patterns. However, it requires complex data analysis and can be costly [185]. RT-qPCR is highly sensitive in gene expression analysis, but it necessitates prior knowledge of the target sequence, making it most suitable for studying known genes [186]. When selecting a specific tool for research, one should take into account the scale of their project, including both the cost implications and the number of genes they intend to target. A comprehensive list of tools and techniques involved in genomics and transcriptomics studies, with their year of origin, in chronological order, along with their advantages and disadvantages are given in Table 5.

Table 5. A list of tools involved in genomics and transcriptomics with their advantages and disadvantages.

Technique	Year of Origin	Application	Advantages	Disadvantages	Citation
FISH (Fluorescence In Situ Hybridization)	1969	Gene mapping	Visualizes specific DNA sequences on chromosomes	Limited to fixed cells, labor-intensive	[187]
PCR (Polymerase Chain Reaction)	1971	In vitro DNA amplification	Amplifies DNA quickly	Susceptible to contamination, limited to short sequences	[188]
Restriction Enzyme Mapping	1976	Enzymatic gene mapping	Maps DNA fragments	Limited resolution, labor-intensive	[189]
Sanger Sequencing (DNA Sequencing)	1977	Chain-termination sequencing	Reads DNA base by base	Slow, expensive for whole genomes	[190]
QTL Mapping (Quantitative Trait Loci Mapping)	1988	Genetic linkage analysis	Identifies genomic regions associated with traits	Limited resolution, requires genetic markers	[186]
ESTs (Expressed Sequence Tags)	1991	Gene expression analysis	Identifies and catalogs genes	Provides only partial gene information	[191]
RT-PCR (Reverse Transcription PCR)	1992	Gene expression analysis	Highly sensitive	Requires prior knowledge of the target sequence	[192]
Transposon-Mediated Insertional Mutagenesis	1995	Gene disruption	Random gene disruption	Lack of precise control, potential for multiple insertions	[193]
SAGE (Serial Analysis of Gene Expression)	1995	Quantification of mRNA tags	Quantifies gene expression	Requires a significant amount of starting material	[194]
Antisense Oligonucleotides	1995	Inhibition of gene expression	Specific gene silencing	Transient effect, variable efficiency	[195]
Microarray Analysis	1995	Hybridization-based gene expression analysis	Simultaneous analysis of gene expression	Limited to known probes, cross-hybridization risk	[196]

Table 5. *Cont.*

Technique	Year of Origin	Application	Advantages	Disadvantages	Citation
VIGS (Virus-Induced Gene Silencing)	1995	Gene silencing	Silences gene expression in plants	Limited to plants, viral interactions	[197]
RNAi Technology (RNA interference)	1998	Gene silencing	Specific inhibition of gene expression	Off-target effects, variability in silencing efficiency	[198]
RNA-Seq	2008	Next-Generation Sequencing (NGS)	High-throughput transcriptome analysis	Data analysis complexity, cost	[138]
NGS (Next-Generation Sequencing)	2008	High-throughput sequencing (e.g., Illumina)	High-throughput genome sequencing	Data storage and analysis demands	[199]
ZFNs (Zinc Finger Nucleases)	2009	Genome editing	Precise gene targeting, reduced off-target effects	Design complexity, higher cost	[200]
Single-Cell RNA sequencing	2009	Single-cell analysis	Reveals cellular heterogeneity	Complex data analysis, limited to single cells	[201]
CRISPR-Cas9 Genome Editing	2012	Genome editing	Precise gene editing and inactivation	Off-target effects, ethical concerns	[202]
TALENs (Transcription Activator-Like Effector Nucleases)	2013	Genome editing	Precise gene targeting, reduced off-target effects	Design complexity, higher cost	[203]
Long-Read Sequencing (PacBio)	2019	Long-read sequencing	Sequences longer DNA fragments	Higher error rate, cost	[204]

5. Conclusions and Future Prospects

In conclusion, the synergy between genomics and transcriptomics has ushered in a new era of understanding and enhancing crop responses to abiotic stress conditions. Genomics, encompassing functional, structural, and comparative genomics, empowers researchers with a suite of powerful tools. Functional genomics techniques, including RNAi, VIGS, and genome editing with CRISPR-Cas9, enable precise gene manipulation and identification of pivotal stress-responsive genes. Structural genomics contributes to the development of molecular markers, QTLs, and marker-assisted breeding, while comparative genomics unveils conserved genes and pathways across species. These insights facilitate the selection of candidate genes for crop improvement, thereby expediting the breeding of stress-tolerant varieties crucial for sustainable agriculture in the face of climate change. Transcriptomics techniques, on the other hand, have revolutionized our comprehension of gene expression regulation under abiotic stress. Traditional methods like northern blotting and microarray paved the way for advanced approaches like RNA-Seq and third generation sequencing allowing simultaneous analysis of thousands of genes and the detection of both known and novel transcripts. These modern techniques have uncovered a plethora of abiotic stress-responsive genes, shedding light on the intricate mechanisms underlying stress tolerance. The wealth of information generated through transcriptomics provides invaluable insights into potential targets for crop improvement. However, there has been substantial progress, but certain crucial findings are still lacking. While numerous stress-responsive genes have been identified, the critical functional characterization of these genes remains a significant challenge, hindering our ability to precisely target crop improvement efforts. Most studies have focused on identifying major genes or pathways involved in stress responses. In-depth investigation is needed to understand the quantitative nature of stress tolerance traits and how multiple genes with small effects contribute to overall tolerance. Additionally, understanding the role of epigenetic modifications and non-coding RNAs in stress responses is an emerging area, warranting further investigation. Addressing these research gaps, along with considerations of environmental variability and the validation of findings in real-world field trials, is crucial for realizing the full potential of genomics and transcriptomics in enhancing crop resilience and food security.

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