MicroRNA and cDNA-Microarray against Abiotic Stress Response

Subjects: Agriculture, Dairy & Animal Science

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The miRNAs and cDNA-microarrays are powerful tools to enhance abiotic stress tolerance in plants through multiple advanced sequencing and bioinformatics techniques, including miRNA-regulated network, miRNA target prediction, miRNA identification, expression profile, features (disease or stress, biomarkers) association, tools based on machine learning algorithms, NGS, and tools specific for plants. Such technologies were established to identify miRNA and their target gene network prediction, emphasizing current achievements, impediments, and future perspectives.

salinity stress cold stress miRNAs

1. miRNAs and cDNA-Microarray Associated with Cold Stress

Cold stress (frost and chilling) decreases crop yields worldwide through tissue degradation and delayed growth. Most temperate plants have evolved cold resistance through cold-acclimatization [1]. Signaling pathways were being used in response to winter stress. The functional genes transform reactions, and reposts suggest that the signaling pathways for leaf senescence and plant defense responses may overlap [2]. The most characteristic region of cold-stress responsive genes includes transcription factors, such as *CBF/DREB* and stress-inducible candidate genes, identified as *KIN* (cold-induced), *COR* (cold-regulated), and *LTI* genes (induced by low temperature) or *RD* (dehydration) [3]. Several *HSPs* (heat shock proteins) are also reported for their functions against cold stress. *HSPs*, which perform as molecular chaperons, play an important regulatory function in protecting from stress by restoring normal protein conformation and thus maintaining cellular homeostasis in plants [4]. The number of the miRNA target genes in expression is intricate during stress and plant growth. These miRNAs are co-regulated by both developmental signals and ecological factors (**Table 3**). The cold-responsive miRNAs were detected by microarray analysis in *Arabidopsis thaliana* (miR165, miR31, miR156, miR168, miR171, miR396) and recommended by identifying their expression patterns in their promoter sequences and evaluating the cis-components (**Table 3**, **Figure 1**) [5][6]. Furthermore, high-intensity light (HL) responsive genes were assessed with the drought-inducible genes reported with a similar microarray system, which exposed an impenetrable intersection between drought and HL-induced genes. Moreover, 10 genes were identified as being involved in the regulation by HL, drought, salinity, and cold stress (**Table 1** and **Table 2**). These genes are comprised of *ERD10*, *RD29A*, *KIN1*, *LEA14*, *COR15a*, and *ERD7*, and most of them are considered to be concerned in the defense of cellular components [7][8][9]. Along with the HL-inducible genes, some are also identified and encouraged by other stresses (heat, drought, and cold), including *AtGolS*, *LEA*, *RAB*, *RD*, *COR*, *ERD*, *HSP*, *KIN*, lipid-transfer proteins, and *fibrillins* [10][11][12].

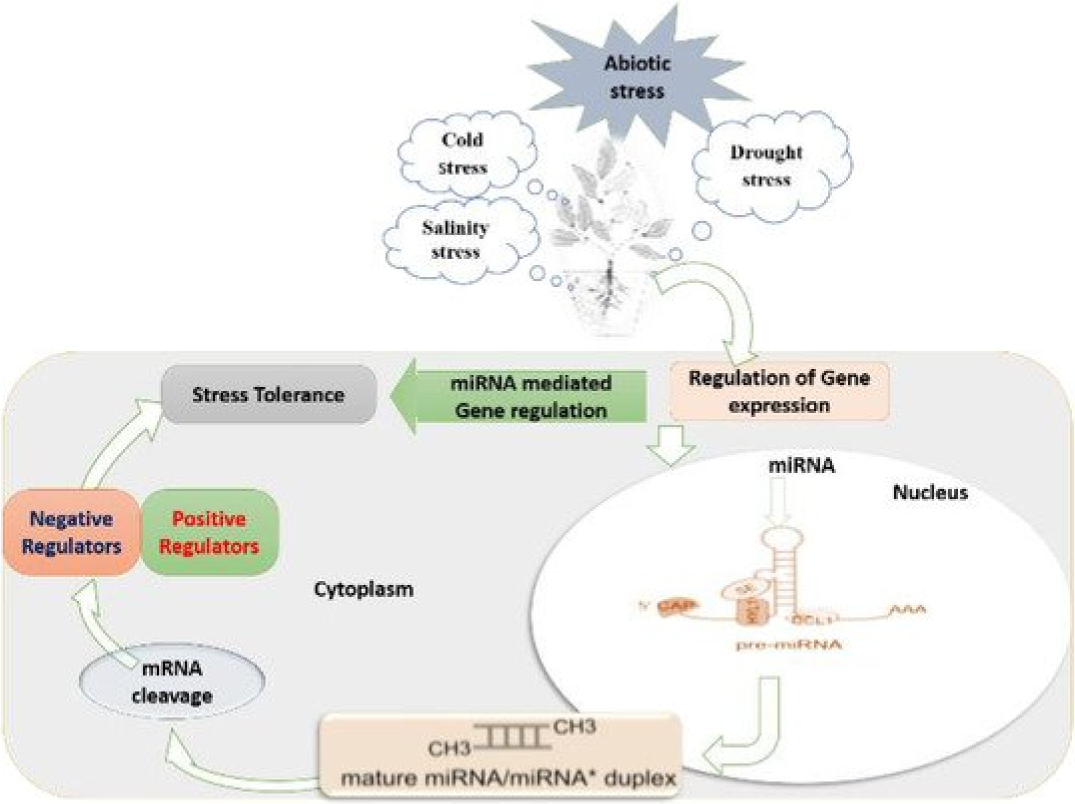

Figure 1. Schematic summary of miRNA-mediated regulatory mechanisms under abiotic stress in plant cells, with the particular formation process of miRNAs and miRNA mediated gene regulation: (1) miRNA gene is transcribed to a long sequence of primary miRNA (pri-miRNA). Primary miRNAs (pri-miRNAs) are transcribed from nuclear-encoded MIR genes by RNA polymerase II (Pol II), leading to precursor transcripts with a characteristic hairpin structure. (2) The pri-miRNA is cleaved to a stem-loop intermediate called miRNA precursor or pre-miRNA.

Table 1. Examples of miRNAs identified in model plants under drought, cold and salinity stresses.

Stress Condition	Plant Species	Inducible Genes	Known Responsive miRNAs	Functions	References
Drought stress	*Arabidopsis thaliana*	Rd29A (At5g52310) *CCAAT-binding transcription factors*	miR164, miR169, miR389, miR393, miR396, miR397, miR402	Pathogen immune response Drought tolerance Oxidative stress tolerance Pathogen immunity response Syncytium	[13][14][15][16]

Stress Condition	Plant Species	Inducible Genes	Known Responsive miRNAs	Functions	References
				formation response to parasitic nematodes	
	Medicago truncatula	*CCAAT Binding Factor (CBF)* *Growth Regulating Factor (GRF)* *Cu/Zn superoxide dismutases (CSD1, CSD2)* *TIR-NBS-LRR domain protein*	miR169, miR396 miR398, miR2118	Drought tolerance Syncytium formation response to parasitic nematodes Oxidative stress tolerance Photoperiod-sensitive male sterility	[13][17]
	Oryza sativa	SalT (LOC_Os01g24710) *TIR1* OsLEA3 (LOC_Os05g46480)	miR393 miR402	Salt/cold tolerance	[18][14][15][19]
Cold stress	*Arabidopsis thaliana*	*Rd29A* (At5g52310) *CBF3* (At4g25480)	*miR165*, *miR172*, *miR169*, *miR396*, *miR397*, *miR402*	Drought/cold tolerance Drought tolerance Heat stress tolerance	[13][14]
	Oryza Sativa	*OsWRKY71* (LOC_Os02g08440) *OsMAPK2*(LOC_Os03g17700) Os05g47550, Os03g42280 Os01g73250, Os12g16350 Os03g19380	*miR319*, *miR389*, *miR393*, miR1320, miR1435 miR1884b, CHY1 CP12-2	Drought/salt tolerance Cold tolerance Pathogen immunity response	[14][19][20][21]
Salinity stress	*Arabidopsis thaliana*	*Rd29A* (At5g52310) *COR15A* (At2g42540)	*miR389*, *miR393*,	Oxidative stress tolerance Heat stress tolerance	[22]
	Populus trichocarpa	Dihydropyrimidinase	miR162, miR164, miR166, miR167, miR168, miR172,	Pathogen immune response Drought tolerance Drought/cold	[23][24][25]

Stress Condition	Plant Species	Inducible Genes	Known Responsive miRNAs	Functions	References
			miR395, miR396	tolerance Sulfate-deficiency response	
	Glycine max		miR1507a, miR395	Sulfate-deficiency response	[26]
	Oryza	*SalT* (LOC_Os01g24710)	miR156, miR158, miR159, miR397,	Drought tolerance Pathogen	[20][27][28]

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Phenotype of Mutants	Genes	Function	AGI Code	Coded Proteins	Microarrays	
Increased tolerance to drought	*AtPARP2*	DNA repair	At2g31320	Poly (ADPribose) polymerase	24K Affymetrix	[30] [31] [32]
Hypersensitive to drought stress	*AHK1/ ATHK1*	positive regulator of drought and salt stress responses	At2g17820	Histidine kinase	22K Agilent	[30] [33] [34]
Increased tolerance to drought stress	*AREB1/ ABF2*	regulate the ABRE-dependent expression	At1g45249	bZIP TF	22K Agilent	[31] [35] [36]
Increased tolerance to salt stress	*AtbZIP60*	encodes a predicted protein of 295 aa	At1g42990	bZIP TF	44K Agilent	[35] [37]
Increased tolerance to drought stress	*AtMYB60*	regulates stomatal movements and plant drought tolerance	At1g08810	MYB TF	7K cDNA	[38]
Increased sensitivity to drought stress	*AtMYB41*	control of primary metabolism and negative regulation	At4g28110	MYB TF	24K Affymetrix	[39] [40]
Increased tolerance to drought and salt stress	*AHK2*	positive regulators for cytokinin signaling	At5g35750	Histidine kinase	Agilent	[33] [34]
Increased tolerance to drought and salt stress	*AHK3*	perception of cytokinin, downstream signal transduction	At1g27320	Histidine kinase	22K Agilent	[33] [34]

Phenotype of Mutants	Genes	Function	AGI Code	Coded Proteins	Microarrays	
Increased tolerance to drought and freezing stress	*DREB1A/ CBF3*	stress-inducible transcription factor	ERF/AP2 TF	ERF/AP2 TF	1.3K cDNA	[41]
Increased tolerance to drought stress	*DREB2A*	heat shock-stress responses.	At5g05410	ERF/AP2 TF	22K Agilent 7K cDNA	[42]
Hypersensitive to salt	*HOS10*	coordinating factor for responses to abiotic stress and for growth and development.	At1g35515	MYB TF	24K Affymetrix	[30] [43]
Increased						

Stress Condition	Plant Species	miRNA	Key Functions	Response	References
Drought stress	*Medicago truncatula*	miR398a,b miR408 miR399k miR2089 miR2111a-f,h-s miR2111g miR4414a	Oxidative stress tolerance Salt/drought/cold/oxidative osmotic-stress responses Phosphate-deficiency response	Up-regulated	[20][44][45][46][47]
		miR398b,c miR2111u,v miR5274b miR1510a-3p, 5p miR1510a	Heat stress tolerance Drought responsive Oxidative-stress tolerance triggering phasiRNA production from numerous NB-LRRs	Down-regulated	[44][46][47]
	Glycine max	miR5554a-c	Drought responsive		[46]
Salinity stress	*Glycine max*	miR169d miR395a miR395b,c miR1510a-5p miR1520d,e,l,n,q	Drought tolerance Sulfate-deficiency response triggering phasiRNA production from numerous NB-LRRs	Up-regulated	[20][48][49]
		gma-miR159b,c gma-miR169b,c gma-miR1520c	Pathogen immune response Drought/Salt tolerance	Down-regulated	[49]
	Phaseolus vulgaris	pvu-miR159.2	Plant–nematode interaction		[31]
Cold stress	*Phaseolus vulgaris*	pvu-miR2118	regulate the expression of genes encoding the TIR-NBS-LRR	Up-regulated	

Stress Condition	Plant Species	miRNA	Key Functions	Response	References
		[50]	resistance protein		

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and 328 temperature-regulated transcripts were reported. *OsMYB3R-2* was studied further and was shown to be a dominant regulator against stress [51]. In this study, there was an attempt to use a 3.1K cDNA-microarray to express the cold-regulated transcripts in the *Capsicum annuum*. Several TFs, including the *EREBP* (*CaEREBP-C1* to *C4*) family of four genes, a protein of the ring domain, a *bZIP* protein (CaBZ1), RVA1, a WRKY (CaWRKY1), and HSF1 protein have been observed among the cold stress-regulated genes. These genes included *CaBZ1*, *CaEREBP-C3*, *NtPRp27*, the *SAR8.2* protein precursor, putative trans-activator factor, malate hydrogenase, putative protein of auxin-repressed, xyloglu-canendo-1, 4-D-gucanase precursor, LEA protein 5 (*LEA5*), homologous *DNAJ* protein, *PR10* and Stns *LTP* [52][53]. cDNA microarray z1300 full-length cDNAs were used in Arabidopsis to identify cold stress-inducing genes and target genes of *DREB1A/CBF3*. Six genes were documented based on microarray and, in RNA gel blot analyses, it was observed that a novel *DREB1A* controls cold- and drought-inducible genes [41][54]. Furthermore, microarray with full-length cDNA was performed by 1300 full-length cDNAs and cDNA microarray to discover cold-induced genes. Previous reposts exhibited the target genes of *DREB1A/CBF3* and stress-inducible gene expressions were controlled by transcription factors [10]; in contrast, stress-sensitive genes' expressions were reported as specific to the growth stage [40]. Full-length cDNA microarray is convenient for analyzing the Arabidopsis gene expression patterns under cold stress, and can also be used to identify the functional genes of stress-related TFs that are likely to act as DNA elements by merging the genomic sequence data with the expression data [10][55]. Additionally, cold stress is also induced by the increase in the proline content in plants (osmoprotectant). Microarray and RNA gel blot research found that the proline can induce the expression of several genes with the proline-responsive elements in their promoters (PRE, ACTCAT) [11][55][56]. Microarray analysis was carried out to detect the cold-inducible *AP2* gene family transcription factor *RAV1* [57], which could control plant growth under stress. *RAV1* is down-regulated by epibrassinolide, and transgenic Arabidopsis overexpressing *RAV1* exhibits a rosette leaf and adjacent root growth retardation, although the early-flowering phenotype showed antisense to RAV1 plants [58][59].

2. miRNAs and cDNA-Microarray Response to Salinity Stress

Salt intrusion from saline soils and irrigation water is one of the most severe and harmful risks to reduce agricultural production and adverse effects on cultivated land and the geographical distribution of plant species [60][61][62], coupled with oxidative stress [63]. The most imperative cations in saline soils are calcium, potassium, magnesium, and sodium, and the main anions in saline soils are chloride, bicarbonate, sulfate, nitrate, and carbonates. Other electrolytes causative to salinity are borane, molybdenum, strontium, silicon dioxide, aluminum cation, and barium ion [64][65]. Higher concentrations of sodium chloride (NaCl) typically affect plant development, metabolism, and physiology at various metabolic phases (ion toxicity, nutrient imbalance, and oxidative stress) [60][66]. Despite such advances in scientific research, it remains unclear about the underlying molecular mechanism of salinity responses in plants. However, based on the combination of microarray and inhibition subtractive hybridization (SSH), changes in the transcriptome profile caused by salt induction were studied and evaluated [67]. Investigation of complete transcriptomics suggests that these processes, such as the synthesis of osmolytes and ion carriers and the regulation of transcription and translation mechanisms, have distinctive reactions under salinity stress. In particular, the introduction of transcripts of specific TFs, ribosomal genes, RNA-binding proteins, and translation initiation and elongation factors has been testified [68][69].

Using cDNA microarray in *Synechocystis*, 19 genes were reported to be instantaneously regulated under salinity stress. The salt- and osmo-regulated genes, and some putative sensor molecules, have been implicated during salinity stress signaling [33]. Several differentially regulated miRNAs have been reported against salinity stress. In *A. thaliana*, several microRNAs are regulated against salinity stress, such as miR156, miR158, miR159, miR165, miR167, miR168, miR169, miR171, miR319, miR393, miR394, miR396, and miR397 (**Table 3**, **Figure 2**) [70]. In *Populus trichocarpa*, miR1445, miR1447, miR1446a-e, miR530a, and miR171l-n were down-regulated (**Table 3**) [71]. Arenas-Huertero et al. [29] reported, in *Proteus vulgaris*, the production of miRS1 and miR159.2 expression in response to salinity. Furthermore, miR169g and family members of miR169n were induced in saline-rich conditions [72]. However, there is a need to discover and annotate novel functional genes which have a probable function against salinity stress. Subsequently, a large number of genes in plants still have unknown functions [73]. Recent studies revealed that specific down-regulation of the bacterial-type *phosphoenolpyruvate carboxylase* (*PEPC*) gene *Atppc4* by artificial microRNA enhanced the salinity tolerance in *A. thaliana*. The increased salinity tolerance might be linked to enhanced PEPC activity [74][75]. Transcript control for salinity-tolerant rice with microarrays, like 1728 cDNAs from salinity-stressed roots libraries, was studied in response to high salinity (**Table 3**) [75][76][77].

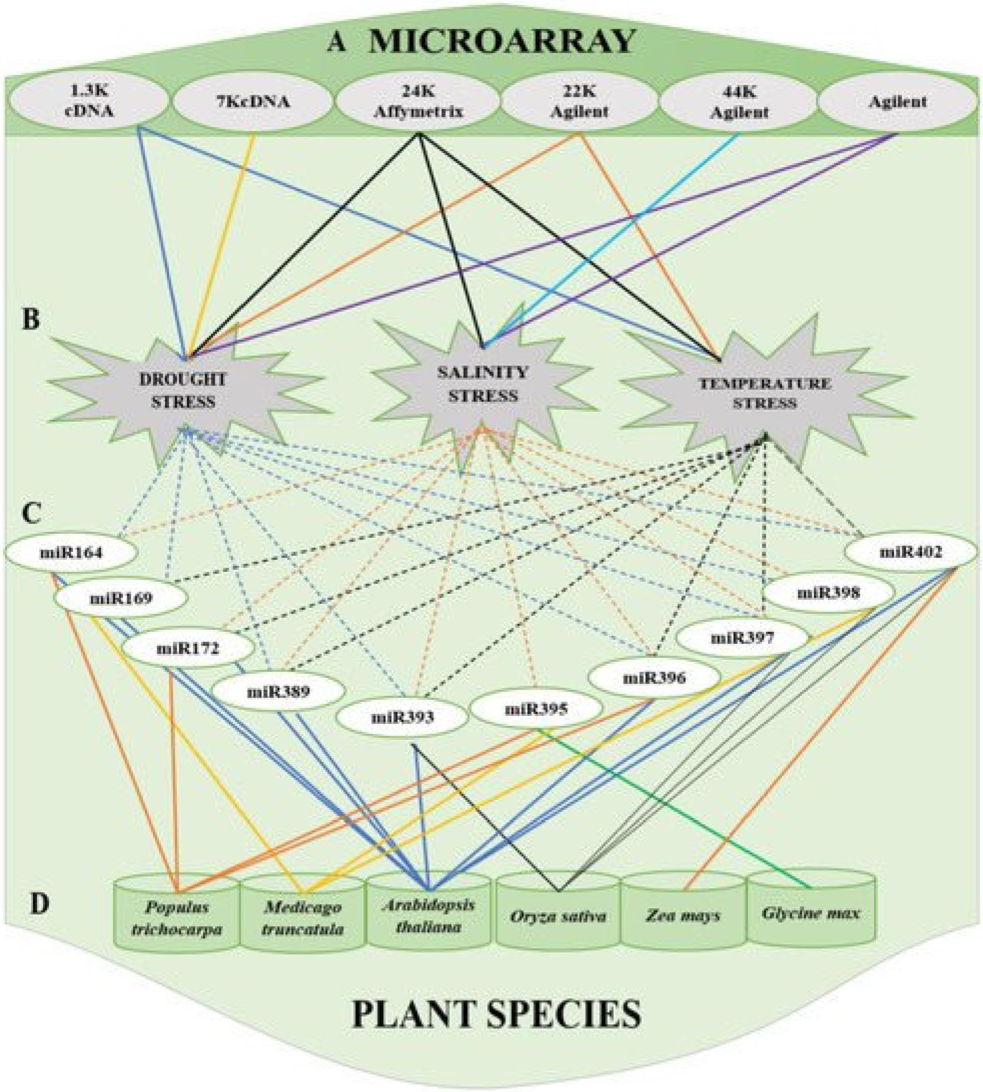

Figure 2. Summary of commonly used (**A**) microarrays (cDNA, Affymetrix, and Agilent) to stress and (**B**) miRNAs, categorized based on the stress, that respond to drought stress, salinity and temperature stress and (**C**) miRNAs reported in (**D**) plant species: *Populus trichocarpa*, *Medicago truncatula*, *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays* and *Glycine max*.

A tiling path microarray was used to examine the high-throughput expression profiling patterns under various environmental stresses for all of the known miRNAs [13][60] (**Table 1** and **Table 4**). The analysis revealed that the effects of miRNAs under low-temperature, drought, and high salinity with miRNA chips represent, approximately, all of the reported miRNAs cloned or recognized in *A. thaliana* (L.). High salinity stress agitates homeostasis in water potential. Extreme changes in water homeostasis and ions lead to molecular breakdown, stunted growth, and even the death of cells or whole plants [13][44].

Table 4. Software and tools used for the detection of plant miRNA and cDNA microarray data analysis.

Software and Tools	Function	Website	Reference	Accessed
Software and tools used for detection of plant miRNA and data analysis				
MiPred	Random forest (RF)-based miRNA predictor, which can distinguish between real and pseudo-miRNA precursors	http://server.malab.cn/MiPred/	[45]	5 November 2021
miBridge	Algorithm and database	http://sitemaker.umich.edu/mibridge/home	[46]	5 November 2021
miRTar	A novel rule-based model learning method for cell line specific microRNA target prediction	http://miRTar.mbc.nctu.edu.tw	[45]	5 November 2021
PolymiRTS	Linking polymorphisms in microRNAs and their target sites	http://compbio.uthsc.edu/miRSNP	[47]	25 November 2021
miRGator	microRNA portal for deep sequencing, expression profiling and mRNA targeting	http://mirgator.kobic.re.kr	[48]	10 November 2021
Bowtie	Aligns efficiently, and short-read aligners	http://bowtie-bio.sourceforge.net	[45]	5 November 2021
miRBase	Provides handy and useful ID conversion tools	http://www.mirbase.org/	[45]	25 November 2021
miRDB	miRNA target databases	http://www.mirdb.org	[49]	25 November 2021

Software and Tools	Function	Website	Reference	Accessed
mirDIP	Integrative database of microRNA target predictions	http://ophid.utoronto.ca/mirDIP	[78]	25 November 2021
miRanda	Predict or collect miRNA targets	http://34.236.212.39/microrna/home.do	[45]	25 November 2021
RNAhybrid	microRNA target prediction	https://bibiserv.cebitec.uni-bielefeld.de/rnahybrid	[45]	8 November 2021
miTALOS	Analyzes tissue specific microRNA function.	http://mips.helmholtz-muenchen.de/mitalos	[79]	5 November 2021
RNA22	microRNA target predictions	https://cm.jefferson.edu/rna22	[80]	5 November 2021
psRNATarget	Small RNA target analysis server	http://plantgrn.noble.org/psRNATarget/	[81]	5 November 2021
miRandola	Curated knowledge base of non-invasive biomarkers	http://mirandola.iit.cnr.it/	[81]	5 November 2021
ChIPBase	Decoding transcriptional regulatory networks of non-coding RNAs and protein-coding genes from ChIP-seq data	http://rna.sysu.edu.cn/chipbase/	[81][82]	1 October 2021
MirGeneDB	Curated miRNA gene database	http://mirgenedb.org/	[83]	28 November 2021
TarHunter	Predicting conserved microRNA targets and	http://tarhunter.genetics.ac.cn	[84]	28 November 2021

Software and Tools	Function	Website	Reference	Accessed
	target mimics in plants			
TissueAtlas	Tissue specificity miRNA database	https://ccb-web.cs.uni-saarland.de/tissueatlas/	[45]	28 November 2021
miRNAme Converter	miRNA ID converter	http://163.172.134.150/miRNAmeConverter-shiny	[85]	28 November 2021
Software and tools used for detection of plant microarray and data analysis				
Array Designer	Design primers and probes for oligo and cDNA expression microarrays.	http://www.premierbiosoft.com/dnamicroarray/index.html	[86]	1 November 2021
Stanford Microarray Database SMD	Stores raw and normalized data from microarray experiments	http://smd-www.stanford.edu//download/	[87]	1 November 2021
eArray	Designing Agilent arrays	http://earray.chem.agilent.com/earray/login.do	[86]	1 November 2021
Significance Analysis of Microarrays	Adjustments for multiple testing, statistical analysis for discrete, quantitative, and time series data, gene set enrichment analysis	http://www-stat.stanford.edu/~tibs/SAM/	[88]	5 November 2021
Visual OMP	Design software for RNA, DNA, single or multiple probe design, microarrays, Taq Manassays, genotyping, single and multiplex PCR, secondary structure	http://www.dnasoftware.com/Products/VisualOMP	[86]	5 November 2021

Software and Tools	Function	Website	Reference	Accessed
	simulation, sequencing, genotyping.			
caArray	Open-source, web and programmatically accessible microarray data management system that supports the annotation of microarray	http://caarray.nci.nih.gov/		5 November 2021
Gene Expression Model Selector	Diagnostic models and biomarker discovery	http://www.gems-system.org/	[89]	18 November 2021
Gene index	Gene Index Project is to use the available EST and gene sequences, along with the reference genomes, to provide an inventory of likely genes and variants.	http://compbio.dfci.harvard.edu/tgi/plant.html	[86]	5 November 2021
Genesis	Java package of tools to simultaneously visualize and analyze a whole set of gene expression experiments	http://genome.tugraz.at/genesisclient/genesisclient_description.shtml		18 November 2021
RMA Express	Standalone GUI program for Windows, OS X and Linux to compute gene expression summary values for Affymetrix	http://rmaexpress.bmbolstad.com http://www.r-project.org http://www.bioconductor.org		18 November 2021

Software and Tools	Function	Website	Reference	Accessed
dCHIP	Model-based expression analysis for Affymetrix gene expression arrays	http://www.dchip.org	[90]	18 November 2021
TM4	Microarray Data Manager (MADAM), TIGR Spotfinder, Microarray Data Analysis System (MIDAS), and Multi experiment Viewer (MeV)	http://www.tm4.org/	[90]	18 November 2021
Able Image Analyser	Software for image analysis. It enables dimensional measurements: distance, area, angle in digital images	http://able.mulabs.com	[86]	18 November 2021
ImaGene	Unique, robust, room-temperature preservation solutions for nucleic acids, biospecimens and bioreagents for in the living ectors	http://www.biodiscovery.com/index/imagene	[86]	13 November 2021
Spotfinder	Custom-designed cDNA array, the chips are scanned using a microarray scanner	http://www.tm4.org/spotfinder.html	[90]	18 November 2021
SNOMAD	Web-based tool and has various normalization options for two-channel and	http://pevsnerlab.kennedykrieger.org/snomadinput.html	[90]	18 November 2021

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8100 *A. thaliana* genes, approximately 2400 genes were reported to have a widespread expression in exposure to salt, oxidative and cold stress [99]. In addition, 23 genes were reported against NaCl stress. This also accounted for a small percentage of DEGs, including encoding transcription factors *WOX2* and *BZIP3*, calcium-binding protein *CML42*, ubiquitin-protein ligase *UBC17*, and *IDA-like 5* protein [99]. Most prominently, synthesized *isiA* encoded a novel chlorophyll (*Chl*)-binding protein [100] (**Table 3**).

References

Software and Tools	Function	Website	Reference	Accessed
	single-channel experiments			
Multiexperimet Viewer	Cloud-based application supporting analysis, visualization, and stratification of large genomic data	http://www.tm4.org/mev.html		18 November 2021
Onto-Express and Pathway-Express	Automatically translates DE gene transcripts from microarray experiments into functional profiles characterizing the impact of the condition studied	http://vortex.cs.wayne.edu/projects.htm	[90]	13 November 2021
DAVID/EASE	Database for annotation, visualization and integrated discovery (DAVID) is an online tool for annotation and functional analysis. Expression analysis systematic sxplorer (EASE)	http://david.abcc.ncifcrf.gov	[90]	13 November 2021

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