

Unravelling genetic mechanisms of drought tolerance in sorghum



Sorghum (www.sorghumsa.co.za)

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Introduction

- Drought is a problem for agricultural productivity
- Genetic mechanisms of drought tolerance
- Sorghum shows resilience
- Genome sequenced (Paterson *et al.*, 2009)
- Reservoir of genetic resources

Identify important candidate genes and key regulatory elements that govern drought tolerance in selected sorghum landraces



(<http://en.wikipedia.org/wiki/Drought>)



Sorghum (www.sorghumsa.co.za)



Mature sorghum (www.sorghumsa.co.za)

Materials and methods

Experimental Approach



Water stress growth room experiment



Transcriptome profiling (microarrays)



qRT-PCR validation

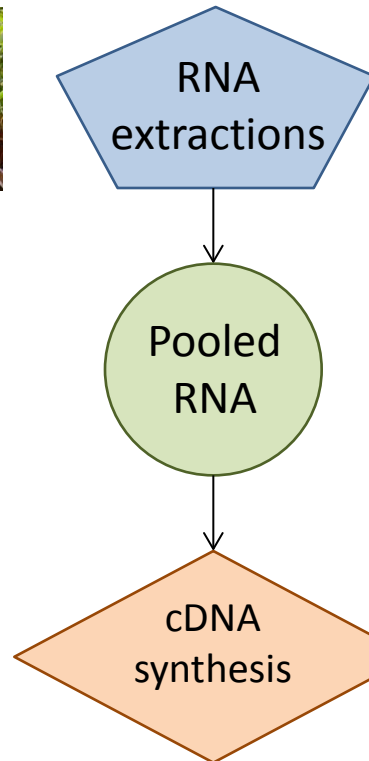


Targeted protein profiling



Elucidation of important drought tolerance genes,
transcription factors & regulatory networks

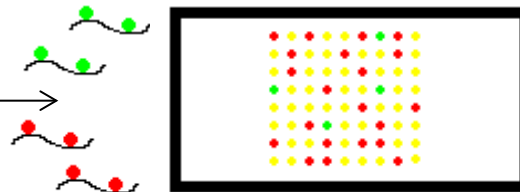
Materials and methods



Plant material – Mild, Severe & Moderate Re-watered
RNA extracted from P898012, LR5, LR6 & LR35 leaves
Qiagen RNeasy kit, Nanodrop
9 biological replicates, 54 extractions per genotype



3 RNA samples pooled = 18 per genotype
Quality checked on Agilent Bioanalyzer



Axon microarray scanner
9 arrays per landrace = 36 arrays
In progress: data analysis

Custom designed microarrays - each array contained probes for 35 899 transcripts

18 cDNA synthesis reactions for each genotype
Cy5/Cy3 labelled, hybridized onto microarray
Direct comparison Treatment vs. Control

Results – Mild stress

Mild stress – four days of water deficit

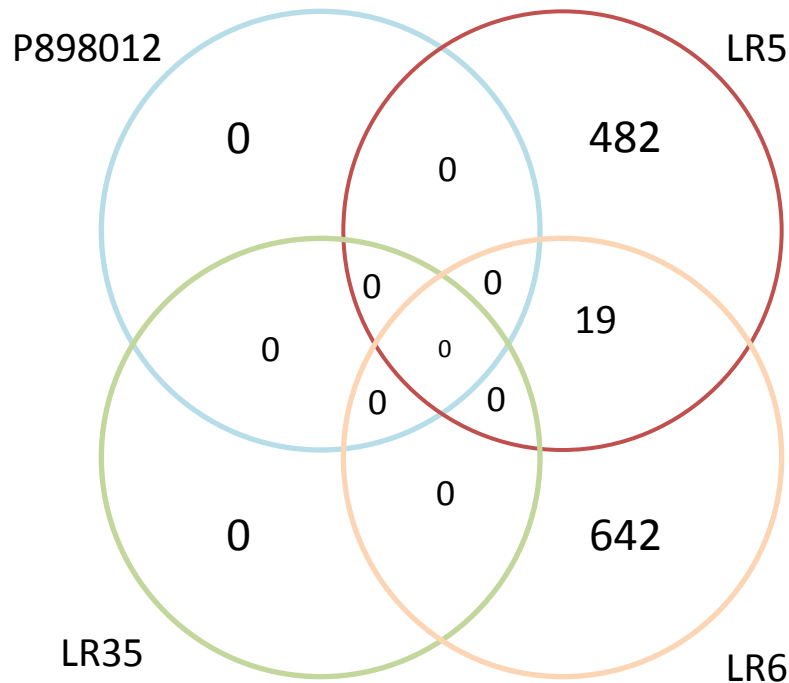


Fig 1: Venn diagram - differentially regulated genes during MS (R-Statistics: LIMMA, Robustspline and Aquantile normalization; P < 0.05, FDR ≤ 5 %, Fold change > 1.5)

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Differentially-regulated genes common across genotypes during MS	P898012	LR5	LR6	LR35
Definition: In phosphorous-containing anhydrides.; Pathway: Folate biosynthesis; Starch and sucrose metabolism		4.76	-2.49	
Definition: UTP--glucose-1-phosphate uridylyltransferase.; Pathway: Nucleotide sugars metabolism; Pentose and glucuronate interconversions;		7.96	2.40	
Definition: Valine--tRNA ligase.; Pathway: Valine, leucine and isoleucine biosynthesis; Aminoacyl-tRNA biosynthesis		2.62	-2.01	
estExt_fgenes1_kg.C_chr_60097; GO: phosphopantetheine binding; metabolism; biosynthesis; catalytic activity; anthranilate synthase activity		3.85	4.51	
estExt_fgenes1_pg.C_chr_101958		4.65	2.97	
estExt_fgenes1_pg.C_chr_13187		2.79	-1.85	
estExt_fgenes1_pg.C_chr_14191; GO: intracellular; nucleic acid binding		-6.26	-2.10	
estExt_fgenes1_pg.C_chr_33912		5.66	3.23	
estExt_fgenes1_pm.C_chr_30631; GO: membrane		-1.67	-1.60	
estExt_Genewise1Plus.C_chr_48768; GO: amino acid transport; membrane; amino acid-polyamine transporter activity		10.35	-5.24	
fgenes1_pg.C_chr_500055		7.74	2.03	
Sb01g037700		2.74	-3.00	
Sb02g043320; RNA regulation of transcription. G2-like transcription factor family GARP; GO: proteolysis and peptidolysis; nucleus; DNA binding; O-sialoglycoprotein endopeptidase activity		7.37	2.63	
Sb03g027131		3.57	8.57	
Sb05g021620		2.42	-5.78	
Sb07g019050; RNA regulation of transcription: unclassified		-17.30	34.59	
Sb09g001360		2.74	2.41	

Results – Severe stress

Severe stress – six days of water deficit

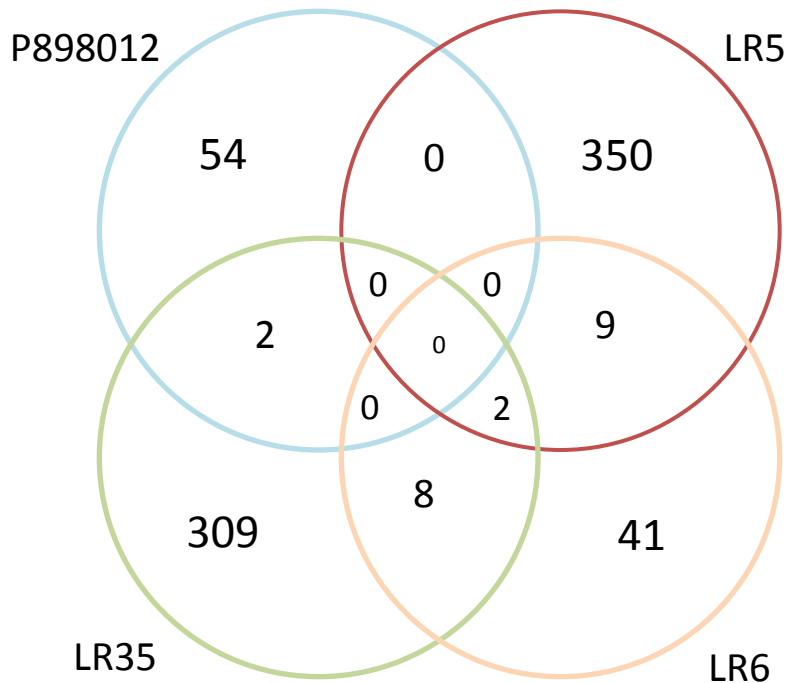


Fig 2: Venn diagram - differentially regulated genes during SS (R-Statistics: LIMMA; Robustspline and Aquantile normalization; P < 0.05, FDR ≤ 5 %, Fold change > 1.5)

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Differentially-regulated genes common across genotypes during SS	P8980:LR5	LR6	LR35
e_gw1.2.646.1; GO: DNA binding	-1.55		-1.64
e_gw1.5.15215.1; GO: protein amino acid phosphorylation; protein-tyrosine kinase activity; protein serine/threonine kinase activity; protein kinase activity; ATP binding		2.66	1.61
estExt_fgenes1_pg.C_chr_61961; GO: nucleus;DNA binding	2.14	2.22	
estExt_Genewise1Plus.C_chr_96573; GO: structural constituent of ribosome; small ribosomal subunit; ribosome; intracellular; protein biosynthesis; nucleic acid binding	1.87	2.09	
fgenes1_pg.C_chr_10001738		2.46	1.71
fgenes1_pg.C_chr_1003406; GO: ubiquitin-protein ligase activity; ubiquitin ligase complex;zinc ion binding; protein ubiquitination	2.34	2.16	1.70
fgenes1_pg.C_chr_2001881		2.82	2.00
fgenes1_pg.C_chr_2003515; GO: nucleus; DNA binding	-1.81	-2.51	
fgenes1_pg.C_chr_4003062	-1.66		-1.89
fgenes1_pg.C_chr_5000677	2.04		-1.92
fgenes1_pg.C_chr_5001502	-1.53	-2.68	-1.67
fgenes1_pg.C_chr_6000661		3.50	1.75
fgenes1_pg.C_chr_6000824	1.83		1.54
fgenes1_pm.C_chr_3001723; GO: cell wall organization and biogenesis (sensu Magnoliophyta); extracellular region	1.78	2.43	
Sb01g003710; development unspecified	2.02	2.18	
Sb03g013310; GO: carbohydrate metabolism; polygalacturonase activity	1.85		-1.52
Sb07g023833; transport misc		2.94	1.64
Sb07g028980; GO: nucleus; zinc ion binding; nucleic acid binding		2.33	1.67
Sb08g002780; GO: tRNA ligase activity; tRNA aminoacylation for protein translation; ATP binding		3.42	1.85
Sb08g022340	1.89	2.11	
Sb10g005170; hormone metabolism,ethylene synthesis-degradation	-3.19		3.37

Results – Moderate re-watered treatment

Moderate re-watered treatment – plants harvested 5 hours after re-watering on day 7 stress

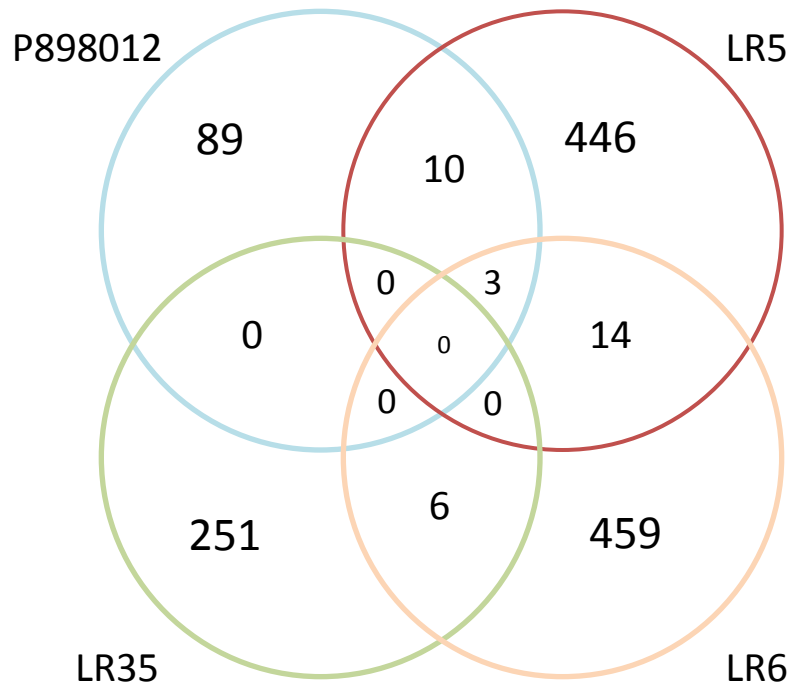


Fig 3: Venn diagram - differentially regulated genes during Mod-RW (R-Statistics: LIMMA; Robustspline and Aquantile normalization; $P < 0.05$, $FDR \leq 5\%$, Fold change > 1.5)

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Differentially regulated genes common across genotypes during Mod-RW	P898012	LR5	LR6	LR35
Definition: Acylglycerol lipase.; Pathway: Glycerolipid metabolism		3.58		6.15
Definition: Dihydrokaempferol 4-reductase.; Pathway: Flavonoids, stilbene and lignin biosynthesis		3.06	4.61	
Definition: Dolichyl-phosphate beta-D-mannosyltransferase.; Pathway: N-Glycans biosynthesis		2.06	1.96	
Definition: Mitochondrial processing peptidase.; Pathway: \N		4.13	-4.14	
Definition: Phosphotransferases with an alcohol group as acceptor.; Pathway: Inositol phosphate metabolism; Nicotinate and nicotinamide metabolism; Starch and sucrose metabolism; Benzoate degradation via CoA ligation; Sphingoglycolipid metabolism	-2.45	-1.87	-1.69	
e_gw1.1.15440.1; GO: integral to membrane			2.32	-10.45
e_gw1.59.172.1		-1.73	-1.56	
estExt_fgenesh1_kg.C_chr_100140	-1.60	-1.62		
estExt_fgenesh1_pg.C_chr_23486; GO: transcription factor activity;nucleus;regulation of transcription, DNA-dependent; DNA binding	-4.33	-6.00		
estExt_fgenesh1_pm.C_chr_30631; GO: membrane		2.75	2.41	
estExt_Genewise1.C_chr_38504		2.32		5.66
estExt_Genewise1Plus.C_chr_11255; GO: GTP binding;molecular function unknown		4.34	-7.35	
estExt_Genewise1Plus.C_chr_12030		-2.60	2.40	
estExt_Genewise1Plus.C_chr_72602; GO: proteolysis and peptidolysis;ubiquitin-protein ligase activity;nucleus;zinc ion binding;ubiquitin cycle;serine-type endopeptidase activity;nucleic acid binding	-2.11	-1.71	-1.62	
fgenesh1_pg.C_chr_2001800		6.47	-3.87	

Results – Moderate re-watered treatment

Differentially regulated genes common across genotypes during Mod-RW	P8980:	LR5	LR6	LR35
fgenes1_pg.C_chr_2002042	-2.53	-1.64		
fgenes1_pg.C_chr_3001340			4.93	3.38
fgenes1_pg.C_chr_4003062		3.97	1.71	
fgenes1_pg.C_chr_4003104	-2.84	-1.81		
fgenes1_pg.C_chr_5000677	1.88	2.21	1.56	
fgenes1_pg.C_chr_6000063			5.38	5.30
fgenes1_pg.C_chr_8000536	-1.74	-1.54		
fgenes1_pg.C_chr_9002545; GO: endoplasmic reticulum;molecular function u			4.24	-4.95
fgenes1_pm.C_chr_6000341		4.60		4.07
gw1.4.16103.1; GO: ubiquitin-protein ligase activity; ubiquitin ligase complex; protein ubiquitination		2.30		-4.33
Sb01g026940; signalling; GO: protein amino acid phosphorylation; protein- tyrosine kinase activity; protein serine/threonine kinase activity; protein			3.16	-4.14
Sb02g001440		3.96		-11.10
Sb03g013310; GO: carbohydrate metabolism;polygalacturonase activity	2.09	1.71		
Sb03g043790			4.23	-6.28
Sb07g019050; RNA regulation of trancription: unclassified		-24.19	-12.09	

From Literature...

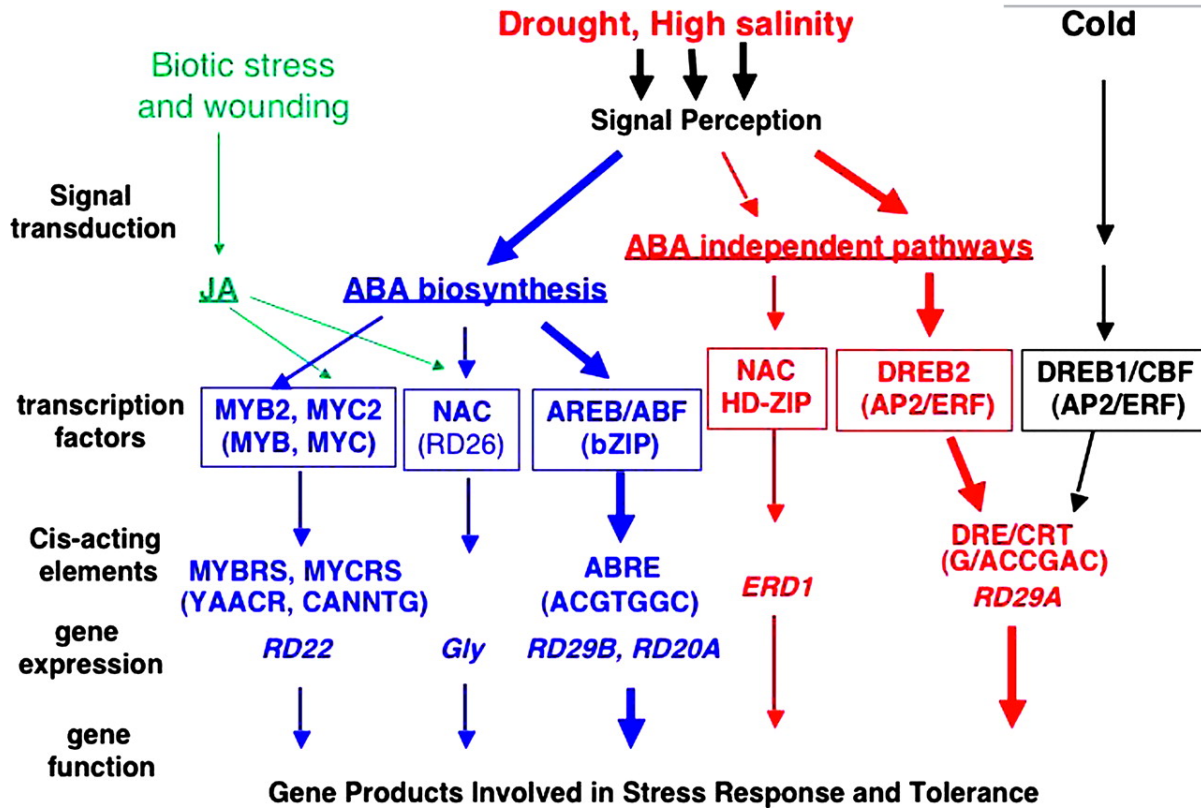


Fig 4: Transcriptional regulatory networks of abiotic stress signals and gene expression.

Shinozaki and Yamaguchi-Shinozaki, 2007. Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany*, 58:221-227

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Thank you



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