Pan-transcriptomes And Gene Regulation Network In Response To Drought And Salt Stresses In Alfalfa

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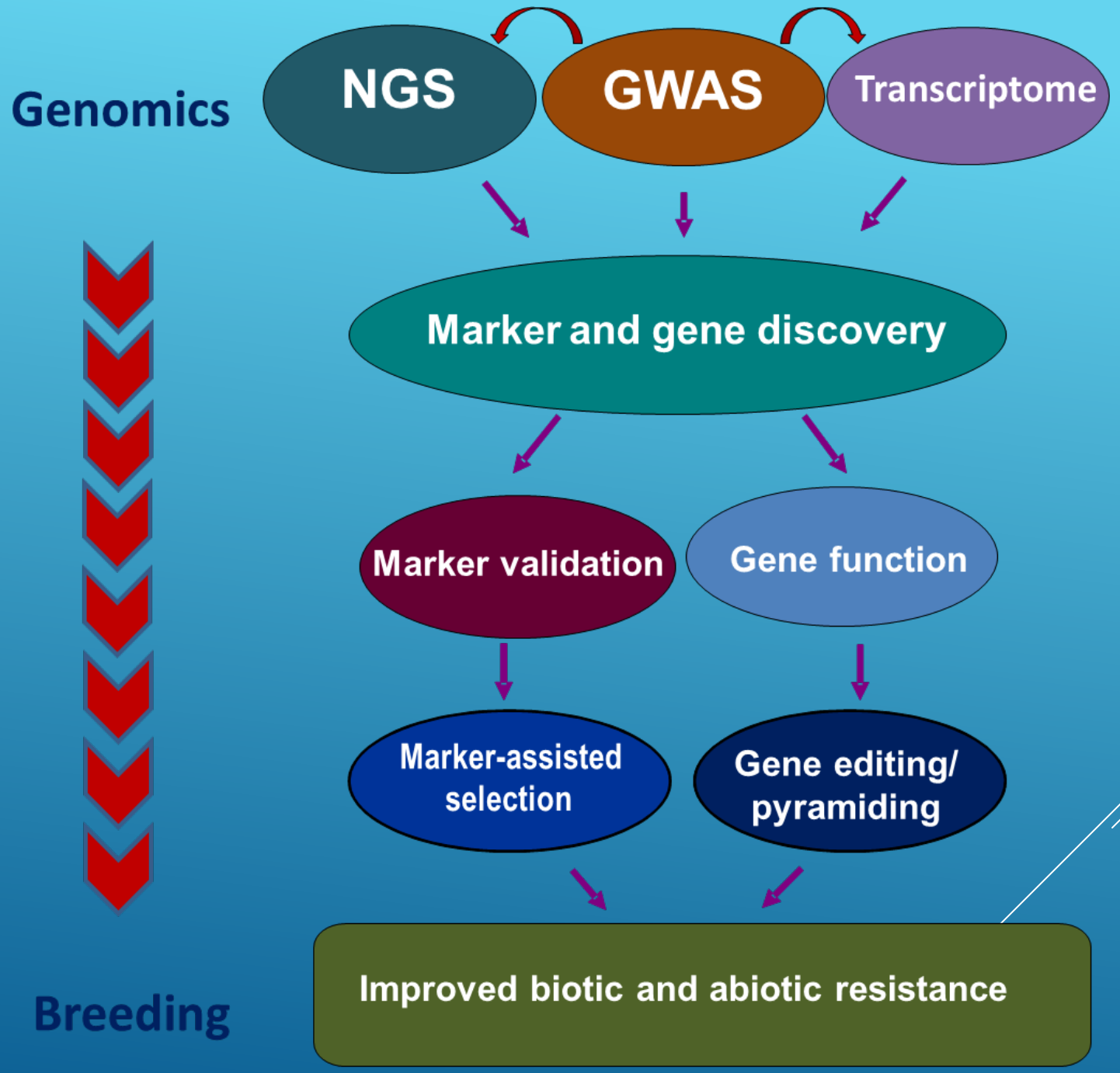
Goals and Strategies



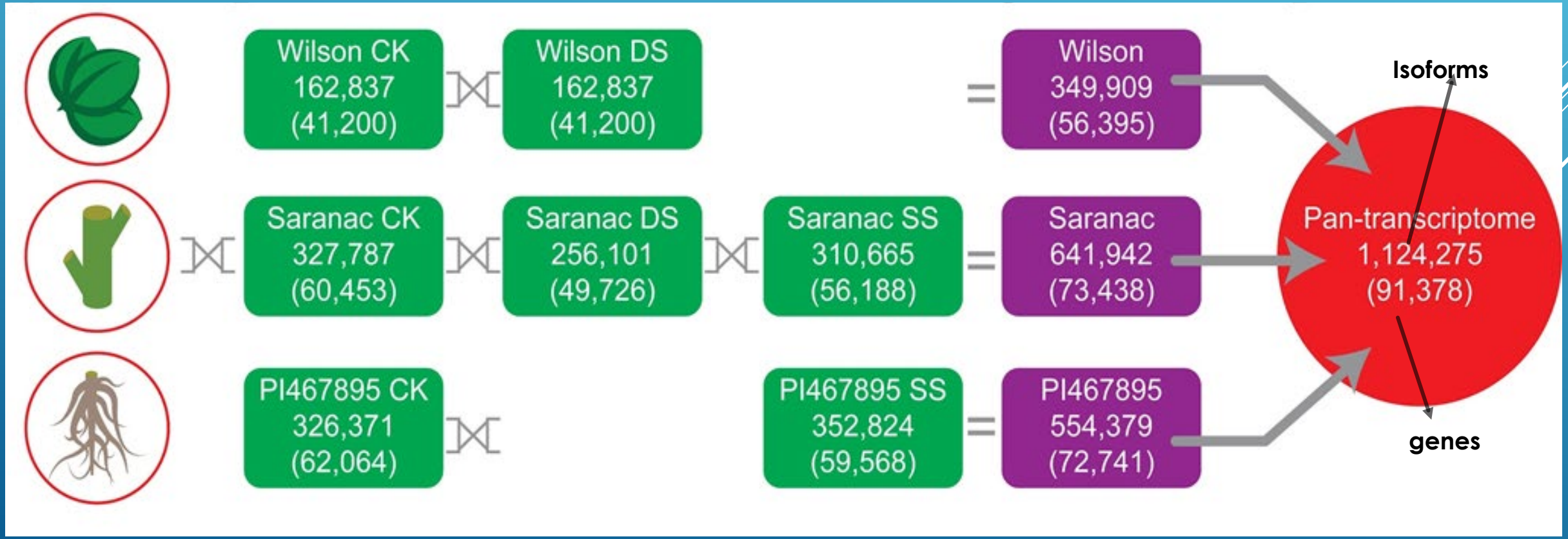
Transcriptomes in response to drought and salt stresses

Three genotypes:

1. Wilson (drought tolerance)
2. PI467895 (salt tolerance)
3. Saranac (non tolerance)

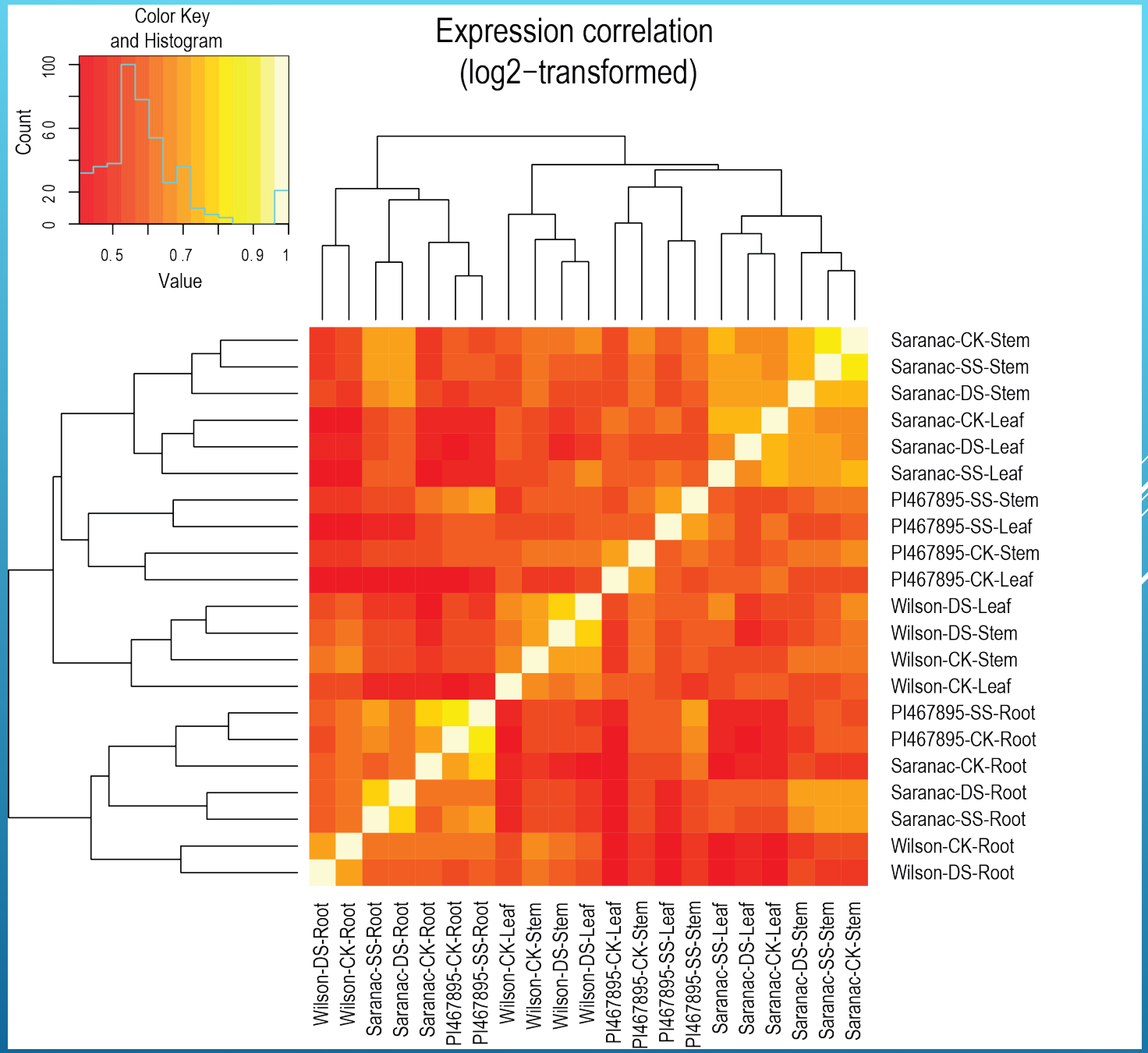
Three tissue resources:

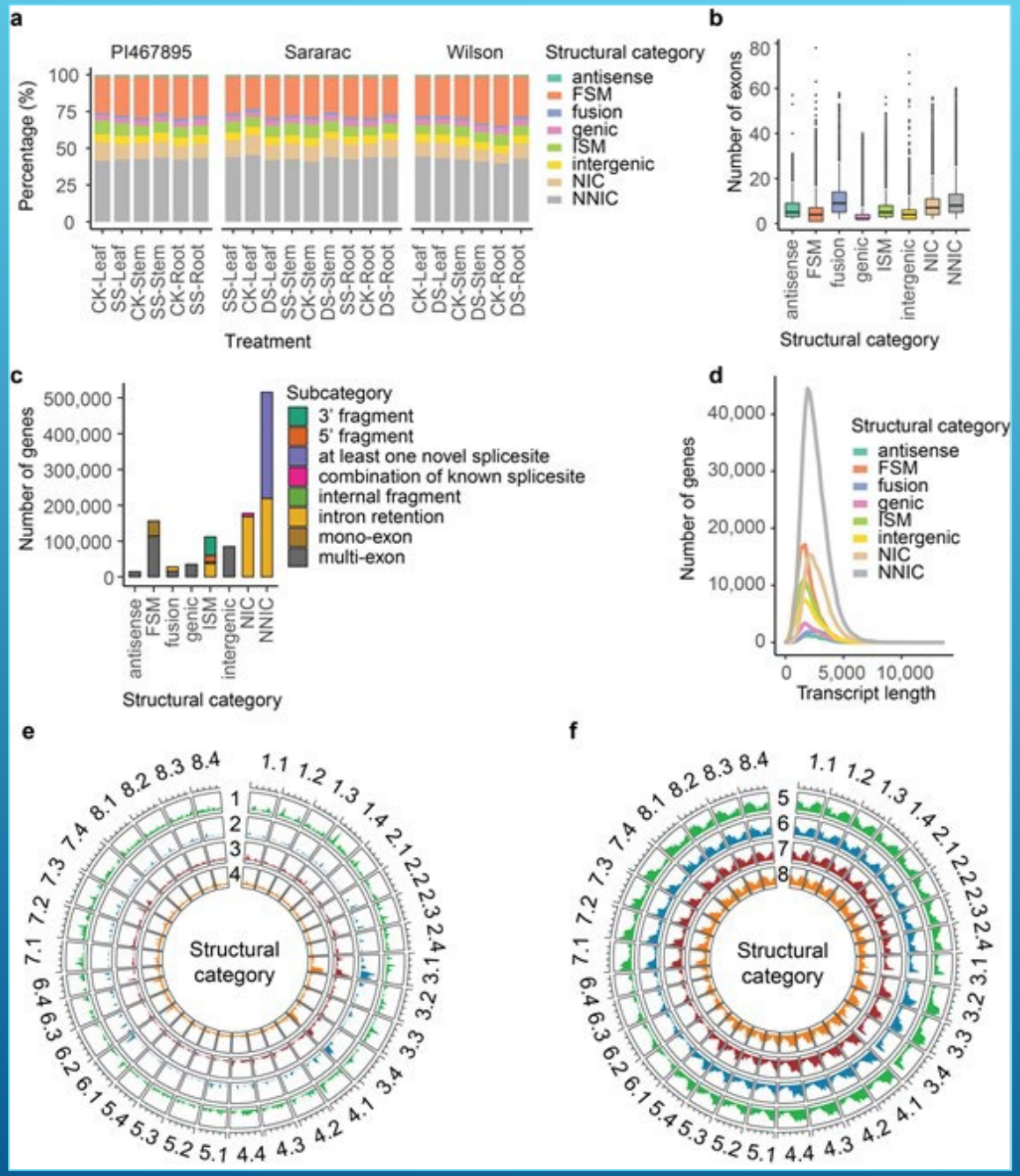
1. Leaf
2. Stem
3. Root

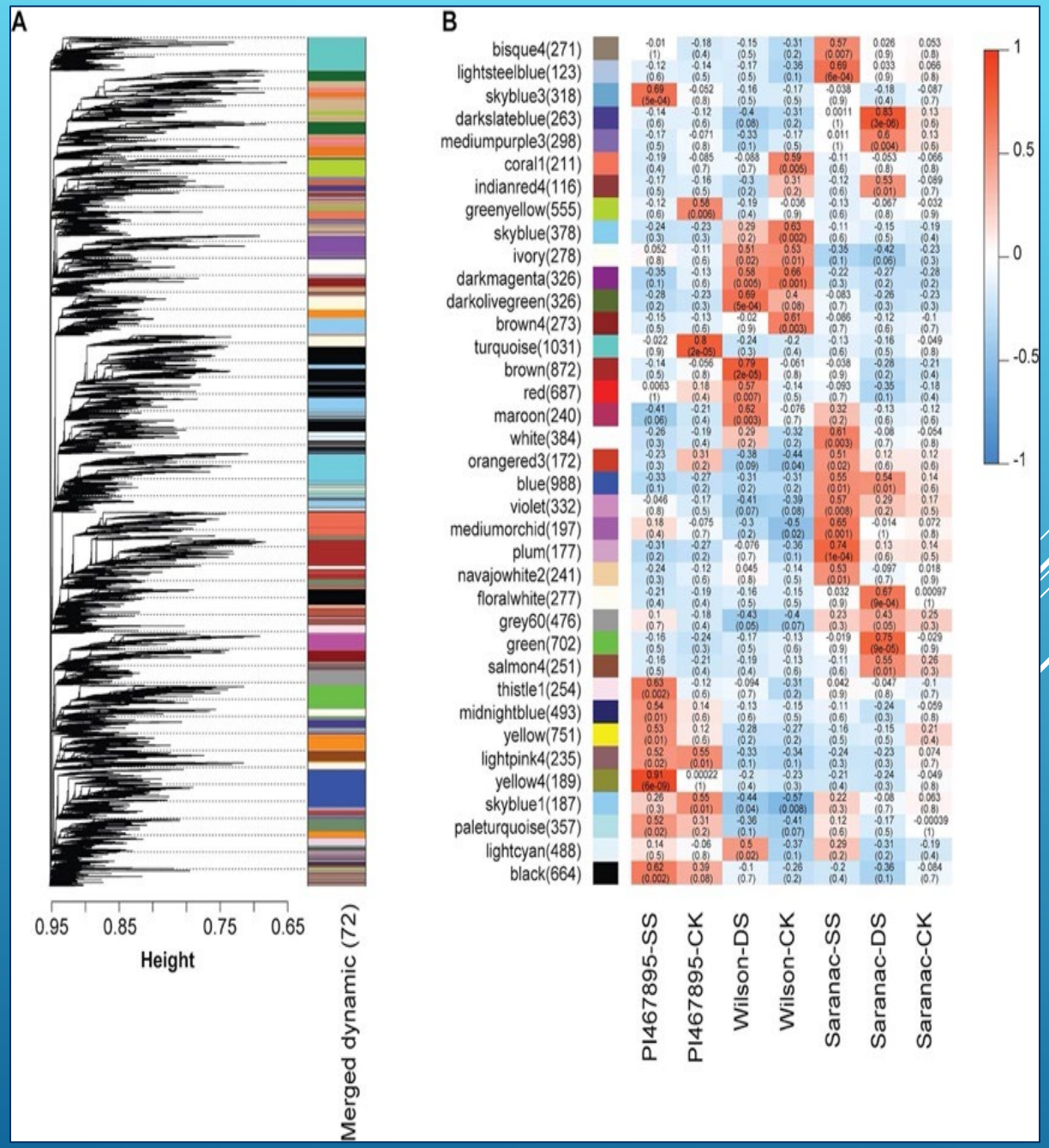


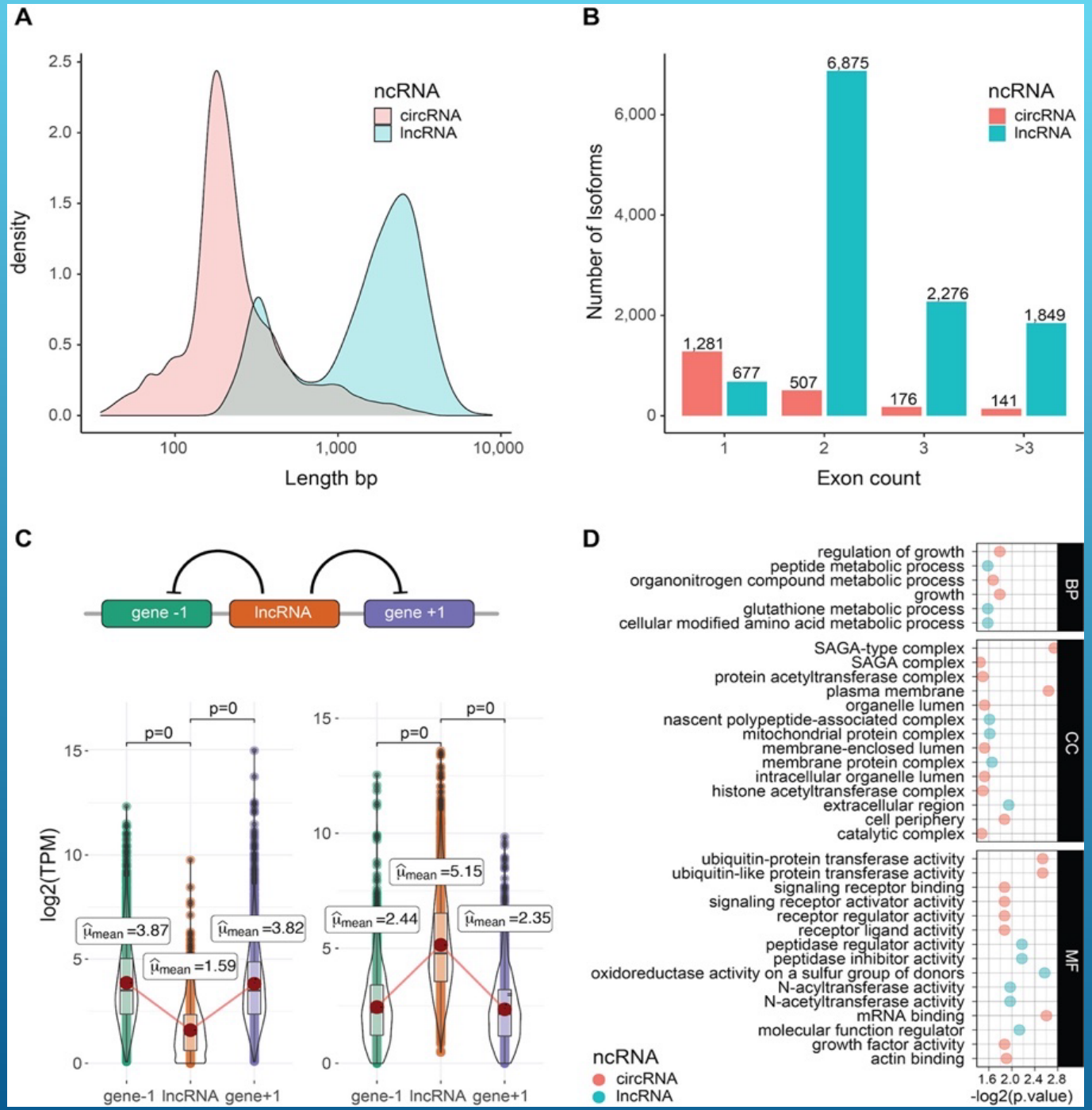
Iso and RNA-seq

- Iso-seq data were used to obtain full-length non-concatemer reads.
- RNA-seq data was used to confirm full-length transcripts by Iso-seq.
- WGCNA was used to obtain DEG and GO.



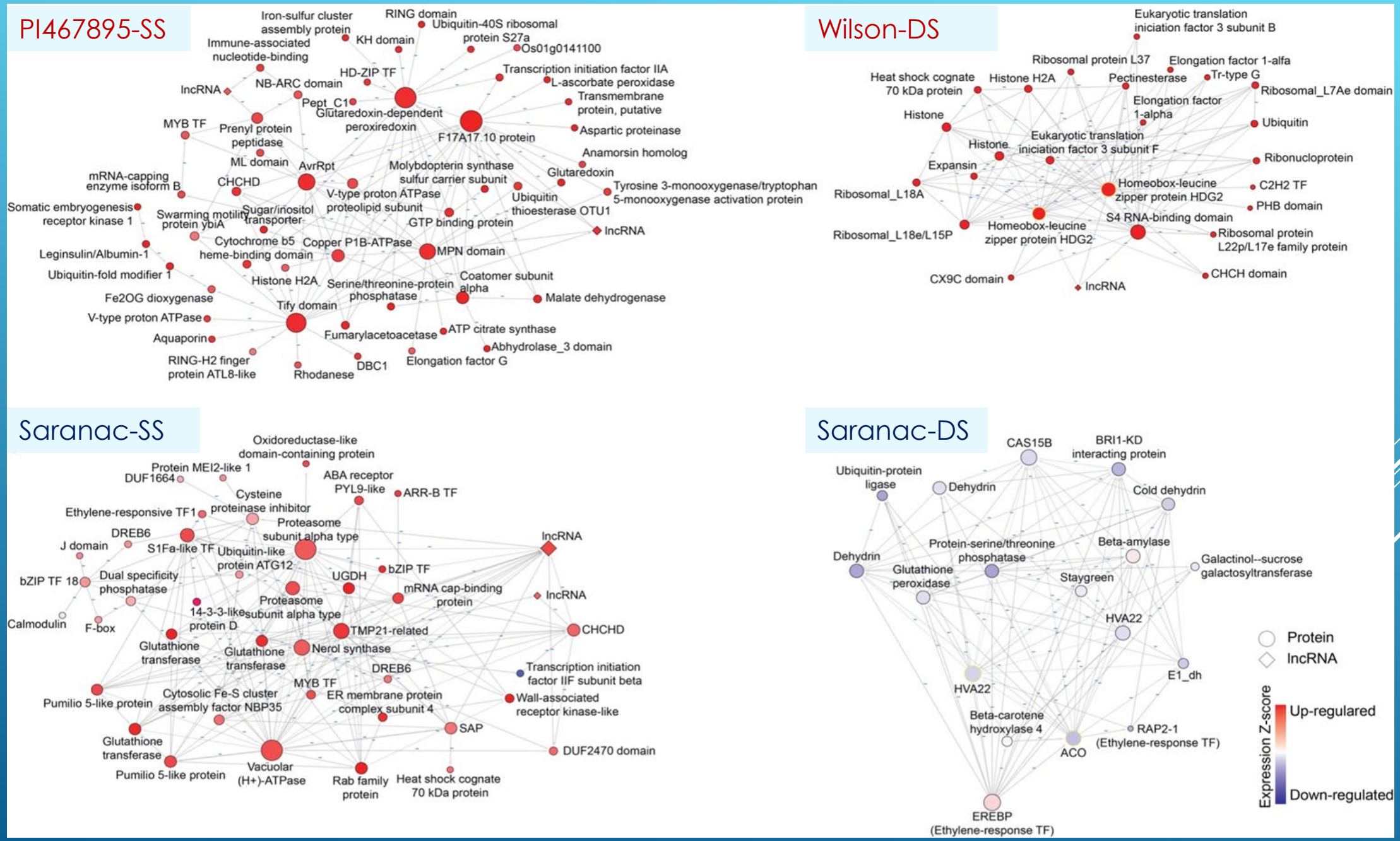

a
PI467895
Sararac
Wilson
Structural category
antisense
FSM
fusion
genic
ISM
intergenic
NIC
NNIC
Percentage (%)
Treatment
b
Number of exons
Structural category
c
Subcategory
3' fragment
5' fragment
at least one novel splicesite
combination of known splicesite
internal fragment
intron retention
mono-exon
multi-exon
Number of genes
Structural category
d
Structural category
Number of genes
Transcript length
e
Structural category
f
Structural category


A
Height
Merged dynamic (72)
B
bisque4(271)
lightsteelblue(123)
skyblue3(318)
darkslateblue(263)
mediumpurple3(298)
coral1(211)
indianred4(116)
greenyellow(555)
skyblue(378)
ivory(278)
darkmagenta(326)
darkolivegreen(326)
brown4(273)
turquoise(1031)
brown(872)
red(687)
maroon(240)
white(384)
orangered3(172)
blue(988)
violet(332)
mediumorchid(197)
plum(177)
navajowhite2(241)
floralwhite(277)
grey60(476)
green(702)
salmon4(251)
thistle1(254)
midnightblue(493)
yellow(751)
lightpink4(235)
yellow4(189)
skyblue1(187)
paleturquoise(357)
lightcyan(488)
black(664)
PI467895-SS
PI467895-CK
Wilson-DS
Wilson-CK
Saranac-SS
Saranac-DS
Saranac-CK



Comparisons by group	Unique lncRNAs	Comparisons by group	Unique lncRNAs	
Sanarac_CK_Leaf	45	Sanarac_CK_Leaf	40	Shared 1
Sanarac_DS_Leaf	45	Sanarac_SS_Leaf	75	
Wilson_CK_Leaf	16	PI467895_CK_Leaf	30	
Wilson_DS_Leaf	156	PI467895_SS_Leaf	97	
Sanarac_CK_Stem	25	Sanarac_CK_Stem	47	Shared 2
Sanarac_DS_Stem	19	Sanarac_SS_Stem	12	
Wilson_CK_Stem	6	PI467895_CK_Stem	98	
Wilson_DS_Stem	38	PI467895_SS_Stem	135	
Sanarac_CK_Root	153	Sanarac_CK_Root	41	Shared 3
Sanarac_DS_Root	234	Sanarac_SS_Root	107	
Wilson_CK_Root	1	PI467895_CK_Root	50	
Wilson_DS_Root	25	PI467895_SS_Root	38	

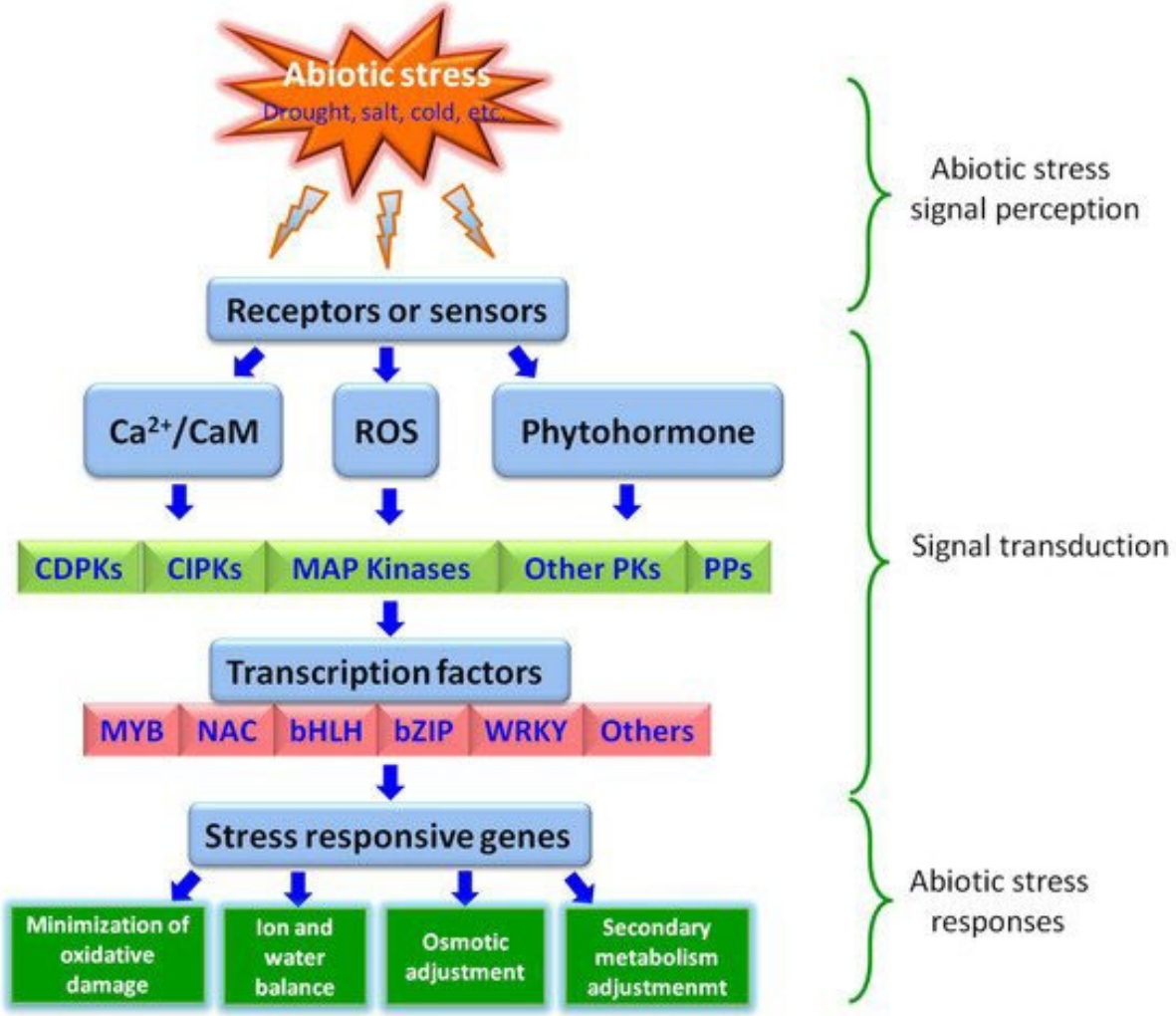
Gene regulation network in response to salt stress (SS) and drought stress (DS)



Master genes identified in alfalfa pan-transcriptome by weighed gene co-expression network analysis.

Isoform	Protein name	Uniprot ID	Reported function	
G1130.10	Nuclear Fusion Defective 6 (NFD6), chloroplastic	A0A1S2XCH5	Required for karyogamy during female gametophyte development	Drought Response
G47352.5	C2H2-type domain-containing protein	C6TJ33	Involved in drought stress response	
G12294.8	ERF TF (RAP2-1-like)	A0A1S3U158	Transcription factor with role in adaptation to drought and salt stress	
G1447.4	Beta-carotene hydroxylase 4	G4Y9K0	Required for biosynthesis of zeaxanthin, a carotenoid precursor of abscisic acid	
G28222.9	HVA22-like protein	A0A498JQ43	Induced by drought and salt stress	
G9711.2	1-aminocyclopropane-1-carboxylate oxidase (ACO)	A0A445LLE1	Involved in ethylene biosynthesis	
G30158.7	MYB TF	A0A4D6MN31	Involved in tolerance to drought and salt stress via an ABA-dependent pathway	
G1134.5	Ethylene-responsive element binding protein (EREBP protein)	D5LMH1	Transcription factor with role in adaptation to drought and salt stress	
G3736.3	Zinc finger A20 and AN1 stress-associated protein 8 (SAP)	A0A371IE91	Involved in abiotic stress tolerance	Salt Response
G10416.2	Vacuolar (H+)-ATPase G subunit	A0A1R3HQE5	Role in sodium sequestration into the central vacuole in response to salt stress	
G7530.16	Glycolipid transfer protein (GLTP)	C6SYQ5	Role in transfer of sphingoid- and glycerol-based glycolipids	
G5353.7	Homeobox protein HAZ1	A0A371FMI3	Transcriptional repressor involved in the regulation of gibberellin	
G16835.10	Tify TF	A0A445DW05	TF induced by salt stress	
G3495.11	Malate dehydrogenase	F5B9G0	Increase salt and cold tolerance modifying the redox state and salicylic acid content	
G63340.1	Glutaredoxin-dependent peroxiredoxin	A0A2G9GS61	Involved in salt stress adaptation by redox signaling	
G27169.8	MPN domain-containing protein (JAMM domain)	A0A5B6ZYU7	Domain present metalloenzymes involved in deubiquitination	
G19941.23	Fumarylacetoacetase	A0A444ZCX7	Involved in synthesizes of acetoacetate and fumarate from L-phenylalanine degradation	
G8059.13	Coatomer subunit alpha	Q70I39	Involved in protein transport from the ER, via the Golgi up to the trans Golgi network	

Medina et al. 2021, Sci Reports



Khan et al. 2018

Conclusion:

- RNA-seq alone does not show all isoform complexity, and the complexity of alfalfa transcriptome has been underestimated.
- The use of combination of Iso-Seq to generate the full-length transcripts and RNA-seq to correct the transcript sequences allowed us to identify full length DEGs and isoforms in alfalfa with high accuracy.
- A pan-transcriptome with 1,124,275 non-redundant isoforms and 91,378 non-redundant genes was obtained and deposited in the NCBI Sequence Read Archive.
- Gene regulation networks allowed us to identify key genes involved in regulation of plant response to salt and drought stresses, including 341 transcription factors and 170 predicted lncRNAs.
- The master genes identified can be used for gene pyramiding, gene editing and developing high-throughput makers that can be used in marker-assisted selection for developing drought and salt tolerant alfalfa.

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