CIHEAM

Universitat de Lleida

Identification of Transcriptional Responses in Root and Leaf of *Prunus persica* Under Conditions of Drought Stress Using RNA Sequencing

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Estación Experimental de Aula Dei (EEAD-CSIC)

CSIC
Consejo Superior de Investigaciones Científicas

Structure of the presentation

- 1- Introduction
- 2- Objectives
- 3- Material and Methods
- 4- Results and Discussion
- 5- Conclusions

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1- Introduction

Family: *Rosaceae*

Subfamily: *Prunoidae*

Subgenus: *Amygdalus*

Genus: *Prunus*

Almond

P. dulcis (Mill) D.A.

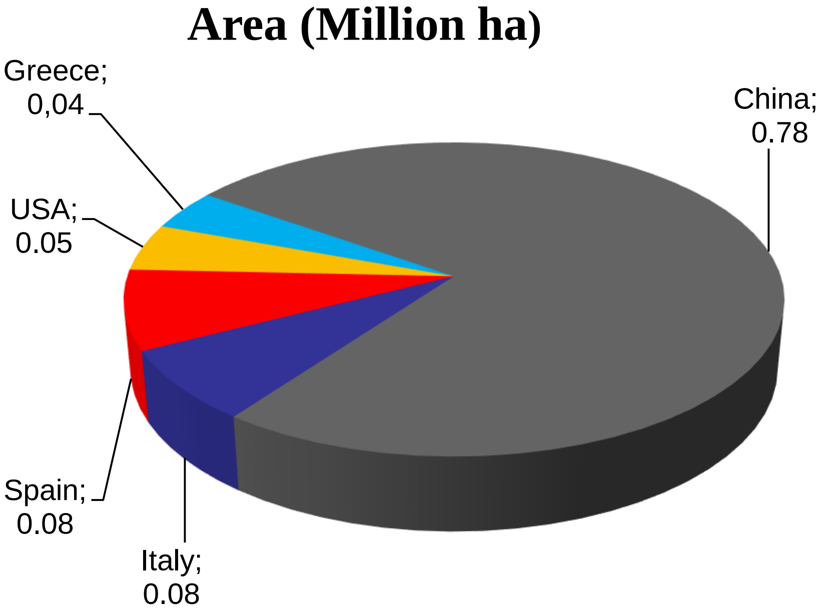
Apricot

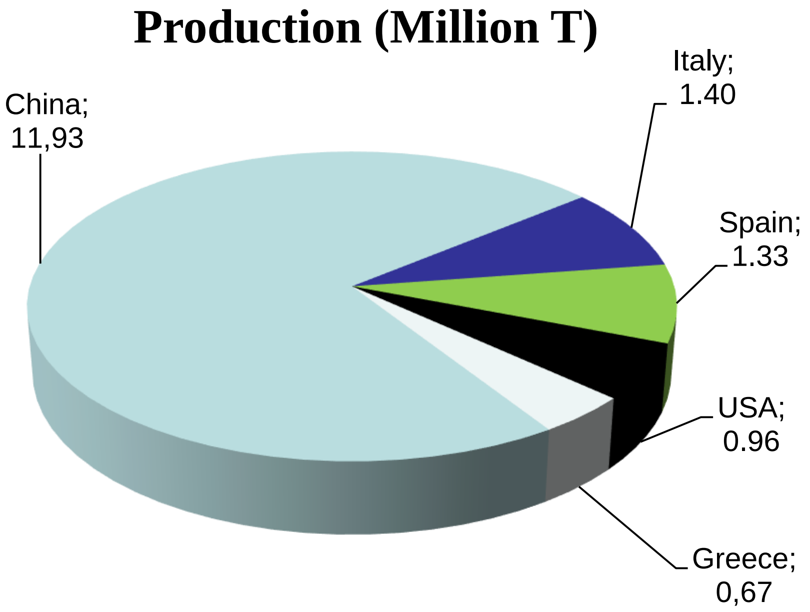
P. armeniaca L.

Plum

P. domestica L.

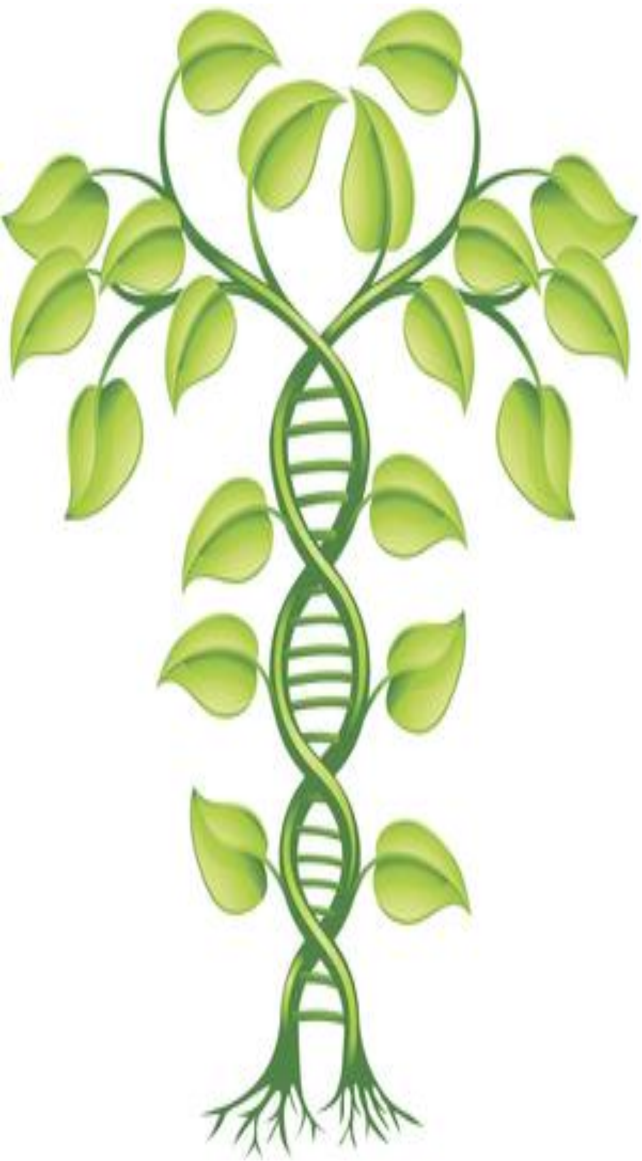
1.1 Economic interest





(FAOSTAT, 2016)

1.2 Genetic interest



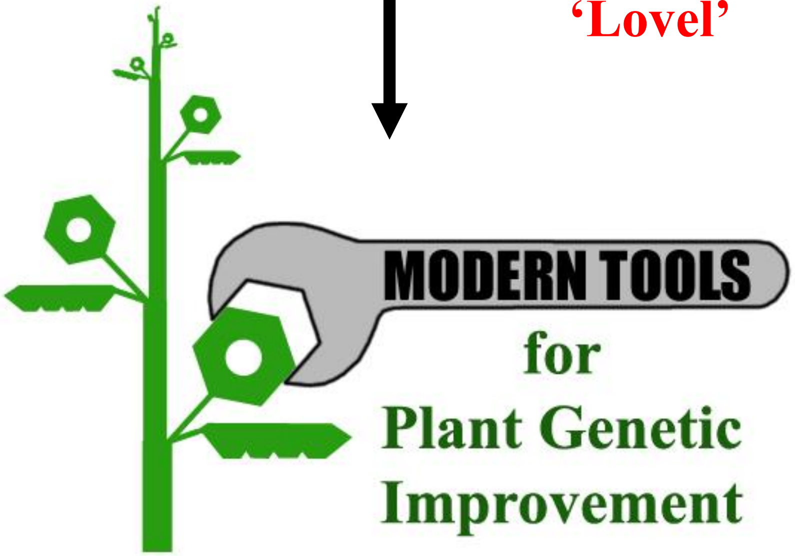
- Diploid species 2x = 2n = 16
- Small genome ≈ 230 Mbp
- Short juvenile phase
- Self-compatibility

Plant model of the *Rosaceae*

Genetic maps

Sequenced Genome

‘Lovel’



1.3 Importance of water for cultivation

87% of water

Report of (IPCC, 2014)

Drought to 30%

1.4 Fruit rootstocks (*Prunus*)

Seedling rootstock

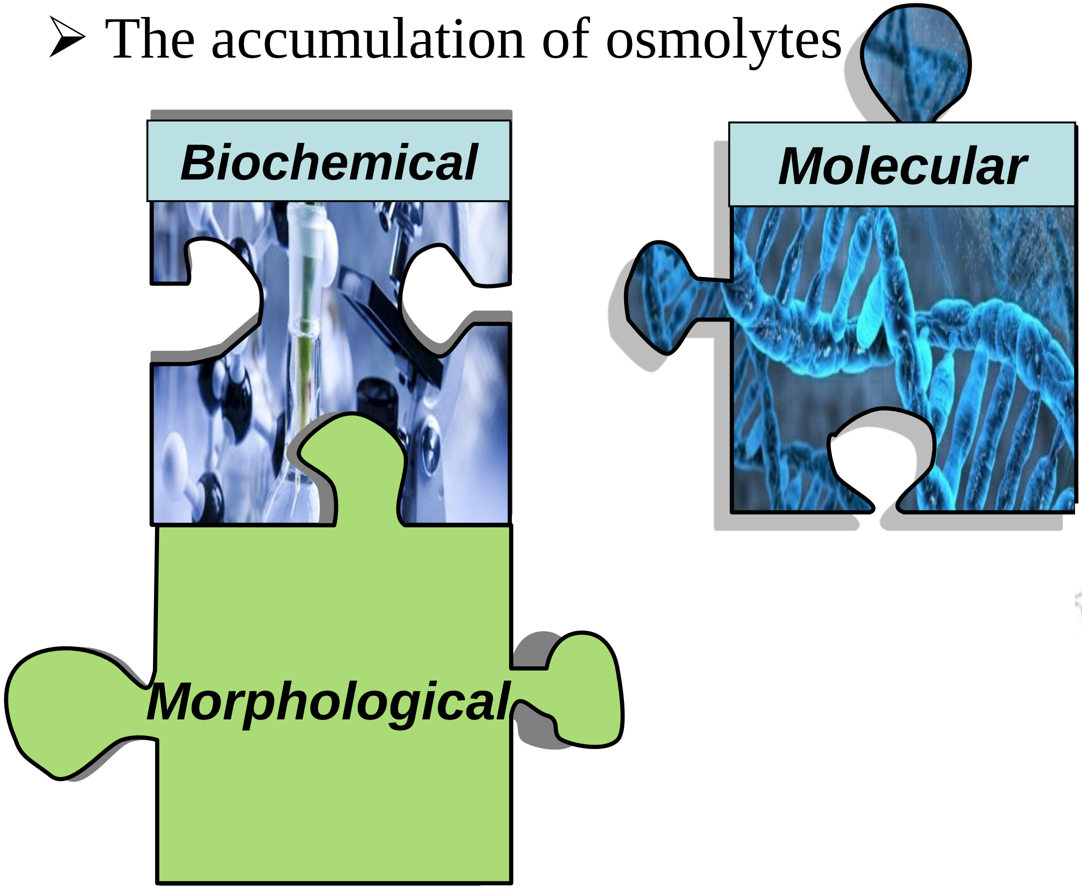
Interspecific hybrids

Peach × Almond

GF677

1.5 Influence of the rootstock on the variety

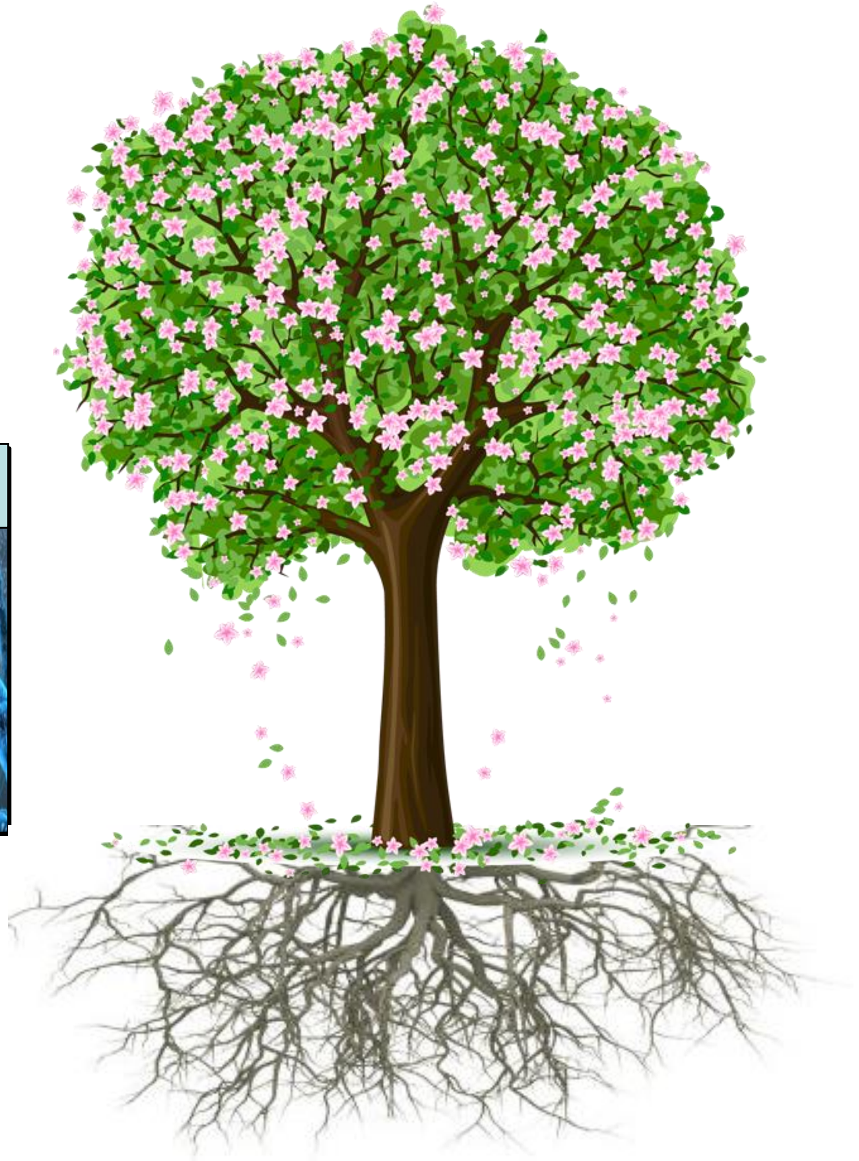
- Vigour of the rootstock
- Root system well developed
- The accumulation of osmolytes



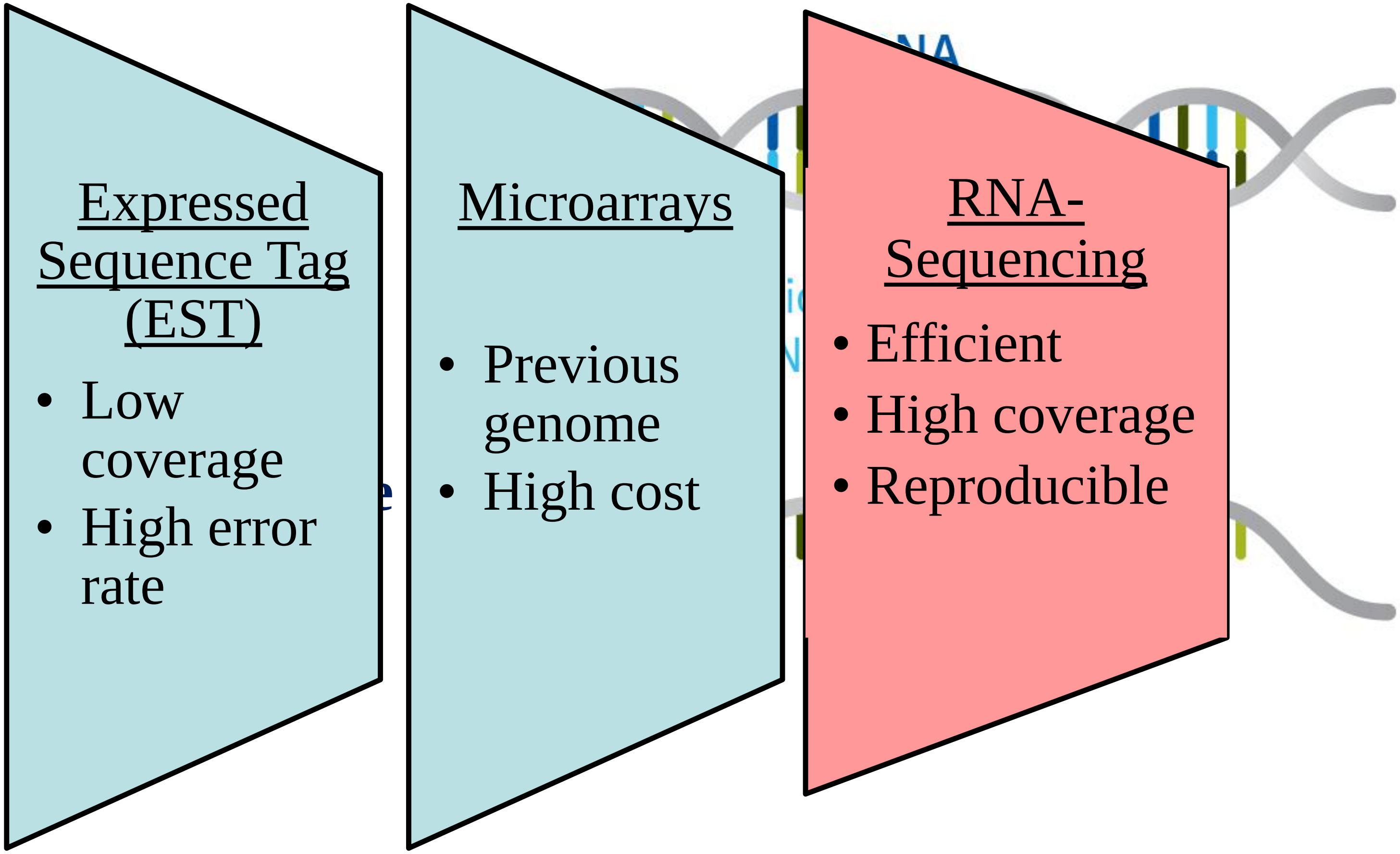
Biochemical

Molecular

Morphological



1.6 Transcriptome analysis



Expressed Sequence Tag (EST)

- Low coverage
- High error rate

Microarrays

- Previous genome
- High cost

RNA-Sequencing

- Efficient
- High coverage
- Reproducible

➢ Infection by *Xanthomonas arboricola* (**Juglard et al., 2013**)

➢ Infection by Plum pox Virus en *P. domestica* **(Rubio et al., 2014)**

➢ Hypoxia in *P. persica* **(Arismendi et al., 2015)**

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2- Objectives

General objective

Investigate the molecular mechanisms involved in the response of *P. persica* to drought and identifying the associated candidate genes.

Specific objectives

- Exploration of the transcriptomic variations between control and drought-affected plants using RNA-seq.
- Identification of the differentially expressed genes (DEGs).
- Annotation and functional classification of DEGs, then investigation of the metabolic pathways involved.
- Validation of the reliability of the RNA-seq analysis by RT-qPCR .

2.1 Originality of the research in peach

Characterization of transcriptional response to drought stress in root and leaf

Variety (Catherina) → **Manages water loss**

Rootstock (GF677) → **Perceives drought stress**

3- Methodology

3.1
- Experimental design and sample collection

3.2
- High throughput sequencing using Illumina Hiseq 2000: RNA-seq analysis

3.3
- Annotation of the DEGs and gene ontology enrichment GOs

3.4
- Validation of RNA-seq reliability using RT-qPCR

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3.1 Plant material: rootstock GF677 and variety Catherina

Micropropagation

2 weeks

pots 300 cm^3
(peat)

Micrografted with 'Catherina'

3 weeks

30 plants

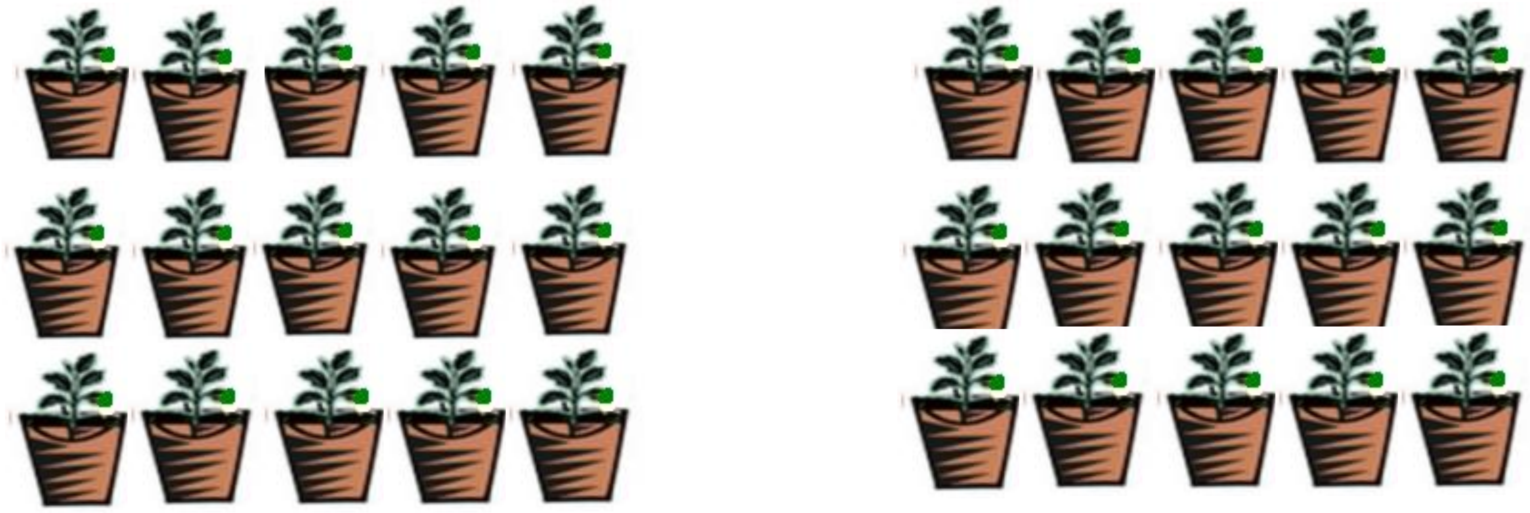
- Temperature 23°C day / 18°C night
- 14 h light / 10 h dark

pots 15 L peat : sand (1:1)

(Jiménez et al., 2013)

3.2 Experimental design and sample collection

Start of Treatment



Drought (80% of evapotranspiration)

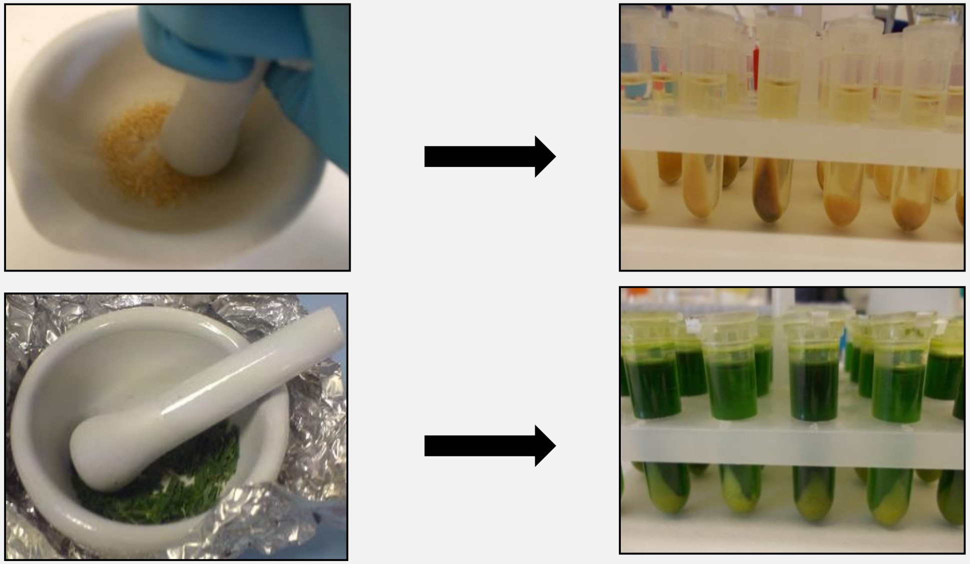
Control

Measurements of physiological parameters
Days (0, 7, 12, 16) (**Jiménez et al., 2013**)

Day 16 of the Treatment

(Jiménez et al., 2013)

3.2 Experimental design and sample collection (II)

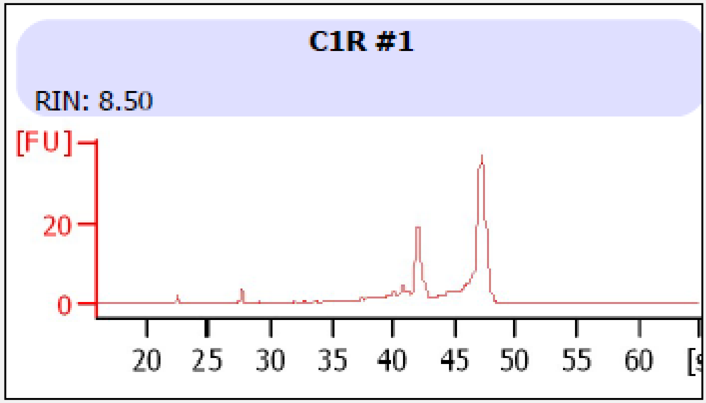


RNA extraction

(Meisel et al., 2005)

A260/280 = 1,9

A260/230 = ≥ 2



3.2 High throughput sequencing using Illumina Hiseq-2000

Sequencing

Illumina

Library 1 ……. Library 12

Quality assess

FastQC

Raw reads

Trimming

ConDeTri

Pre-processed reads

Alignment

TopHat

Post-processed reads

Assembly

Cufflinks

Cuffmerge

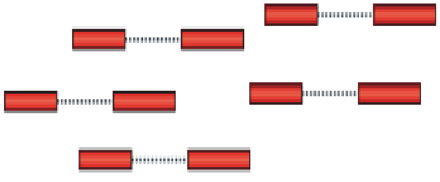
Aligned reads

Quantification

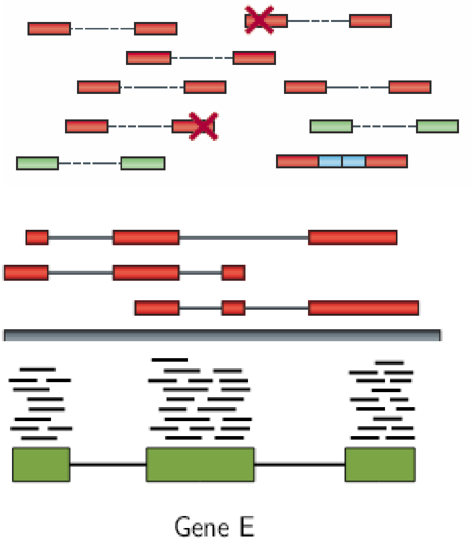
Cuffdiff

Assembled reads

(DEGs) $Q < 0.05$



90-100 bp



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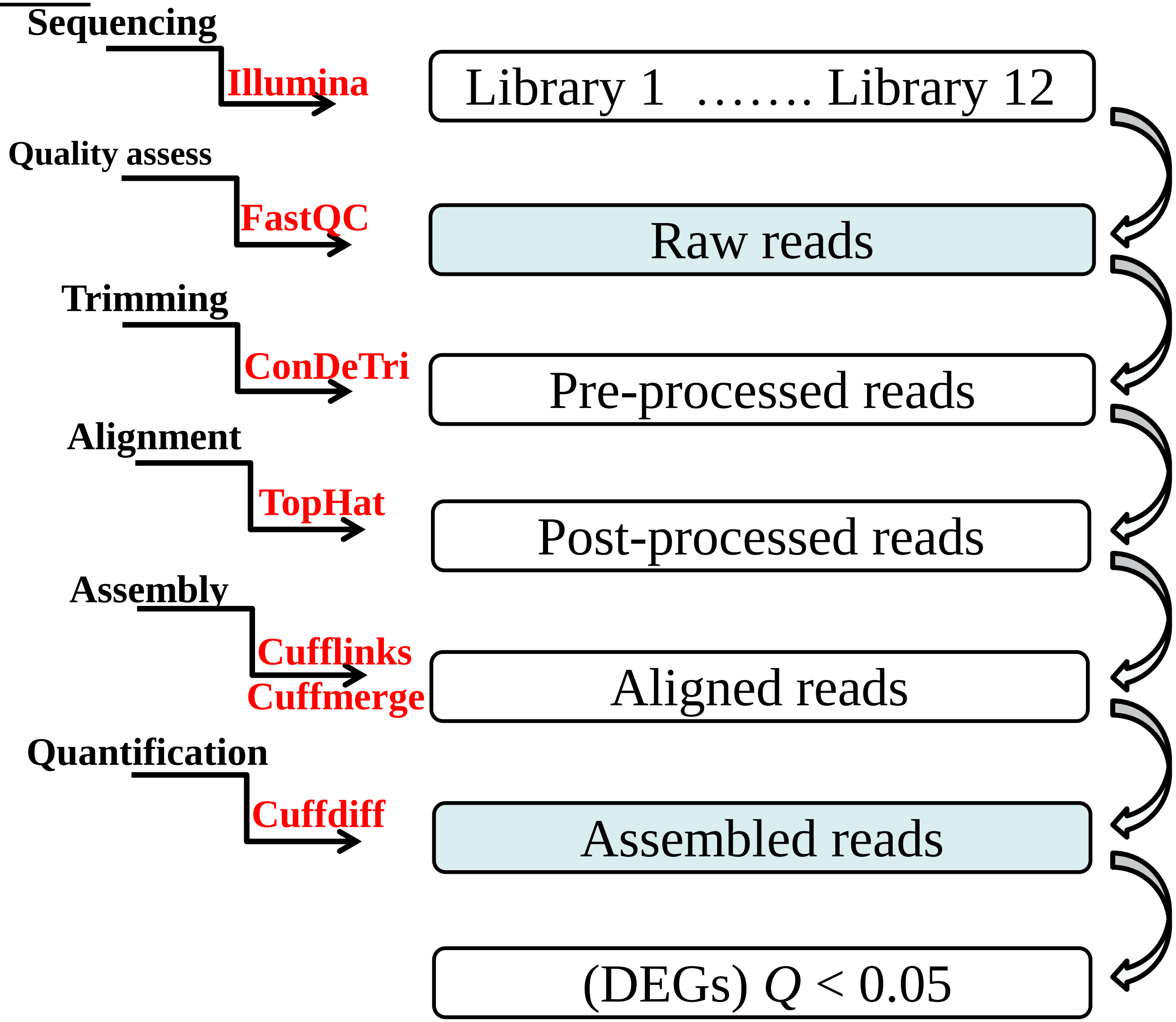

Sequencing

Illumina

Library 1 ……. Library 12

Quality assess

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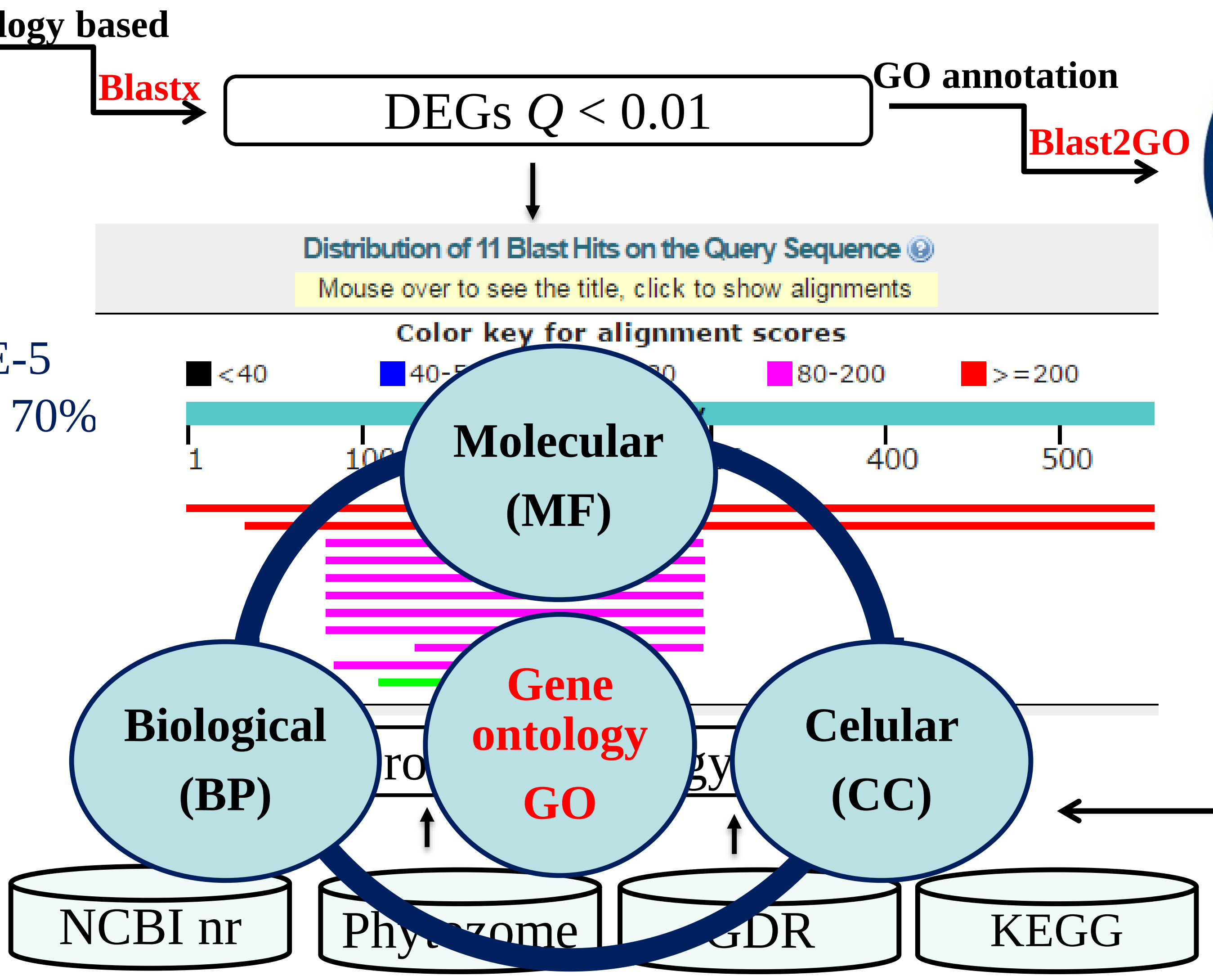
Assembled reads

(DEGs) $Q < 0.05$

(ENA)

PRJEB12334

3.3 Annotation of the DEGs and GO enrichment



Homology based

Blastx

DEGs $Q < 0.01$

GO annotation

Blast2GO

E-valor 1E-5
Coverage 70%

Distribution of 11 Blast Hits on the Query Sequence

Mouse over to see the title, click to show alignments

Molecular (MF)

Gene ontology GO

Biological (BP)

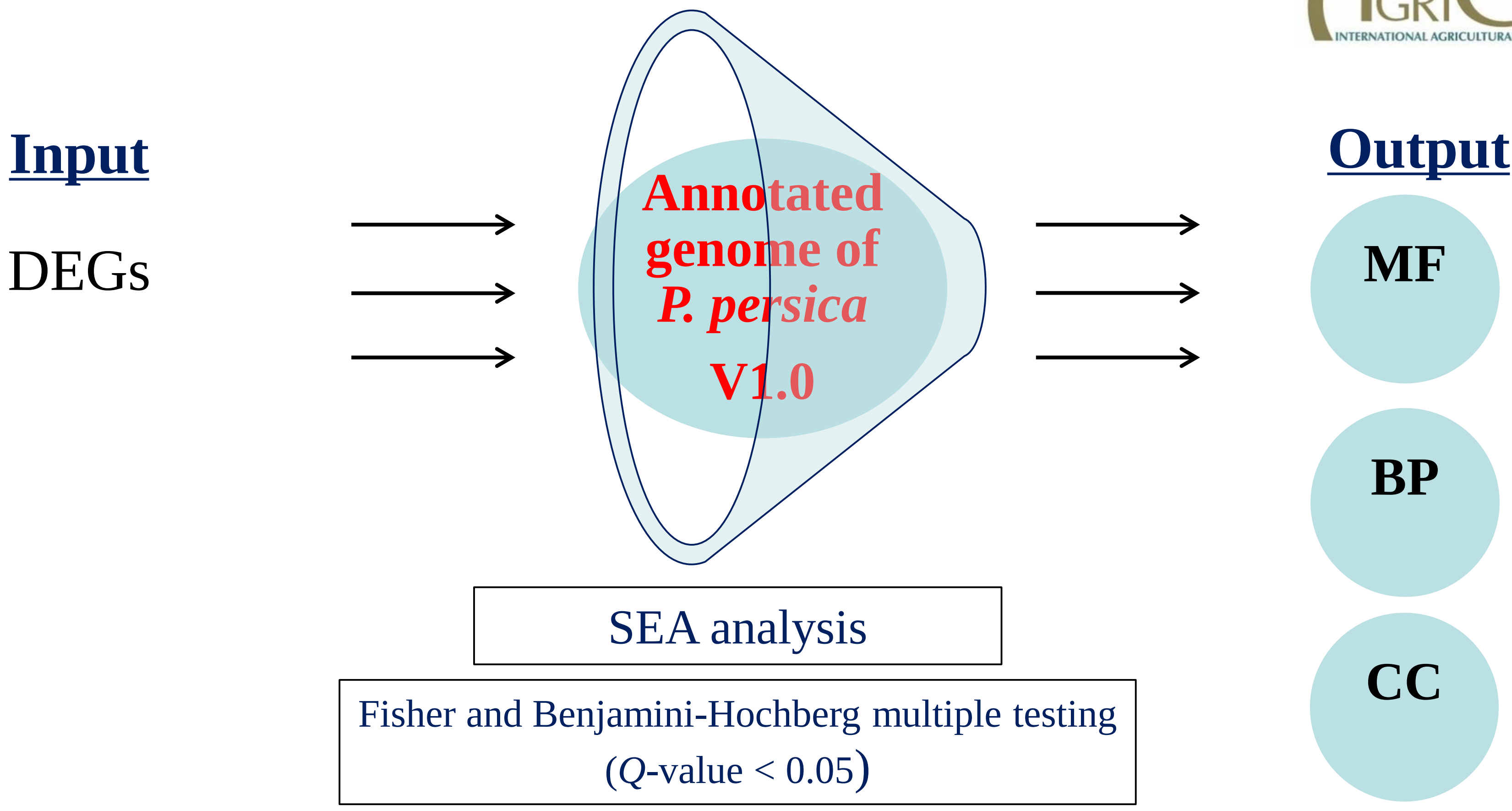
Celular (CC)

NCBI nr

GDR

KEGG

3.3 Annotation of the DEGs and GO enrichment (II)



Input

DEGs

Annotated genome of *P. persica* V1.0

SEA analysis

Fisher and Benjamini-Hochberg multiple testing (Q-value < 0.05)

Output

MF

BP

CC

3.4 Validation of RNA-seq reliability using RT-qPCR

Selection of 33 DEGs

Log_2FC

Function

Primers design

NCBI
primer
BLAST

Control

RNA

cDNA

RT-qPCR

Drought

RNA

cDNA

RT-qPCR

Quantification of gene expression

(Schefe et al., 2006)

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4.1 Physiological responses to drought stress

16 days

of drought

- Wilting
- Defoliation

(Jiménez et al., 2013)

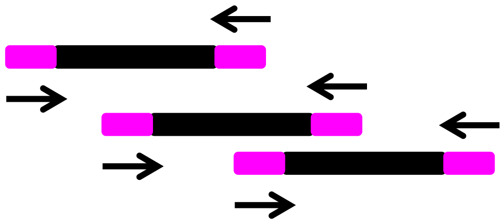
Treatments	**SWC** **%**	**Ψs** **MPa**	***g_s*** **mol H_2O $m^{-2}s^{-1}$**	***Ci*** **µmol CO_2 mol^{-1}**	***A_N*** **µmol CO_2 m^{-2} s^{-1}**
Well-watered	26.63 ± 0.18^b	-0.73 ± 0.05^b	0.56 ± 0.03^b	277.30 ± 2.17^b	20.82 ± 0.54^b
Water-deprived	10.69 ± 0.28^a	-1.08 ± 0.02^a	0.37 ± 0.03^a	261.27 ± 4.27^a	18.35 ± 0.42^a

(Data are mean ± SE of n=6 replicates)

(Rahmati et al., 2015)

4.2 Transcriptomic profile

Sequencing
(Paired-end)



	Libraries	Raw reads (M)	Clean reads (M)			Mapped reads (M)		
			Paired	Unpaired	Total	Paired	Unpaired	Total
Root	**RC 1**	61.30	28.54	12.31	40.85	10.71	4.16	14.78
	RC 2	25.52	10.10	5.15	15.25	7.83	4.07	11.90
	RC 3	22.69	8.48	4.75	13.23	7.03	3.95	10.98
	RD 1	24.97	9.37	5.04	14.41	7.49	4.05	11.54
	RD 2	24.49	7.76	5.47	13.23	5.89	4.29	10.18
	RD 3	20.81	8.14	4.79	12.93	6.51	3.81	10.32
Leaf	**LC 1**	23.12	8.72	5.44	14.16	7.14	4.57	11.71
	LC 2	23.02	9.05	5.30	14.35	7.48	4.51	11.99
	LC 3	22.30	8.01	4.76	12.77	6.63	4.07	10.70
	LD 1	22.00	8.33	4.42	12.75	6.77	3.75	10.84
	LD 2	22.86	6.20	5.51	11.71	5.37	4.93	10.30
	LD 3	22.00	8.63	4.33	12.96	7.20	3.78	10.38
	Total	315.08	121.33	67.27	188.60	86.05	49.94	135.99

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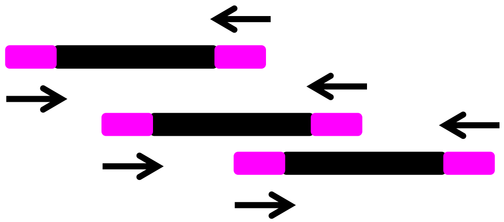

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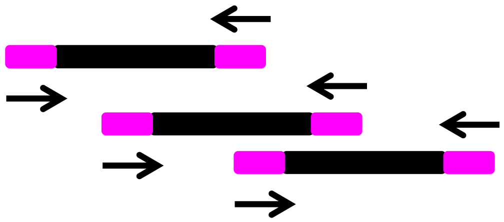

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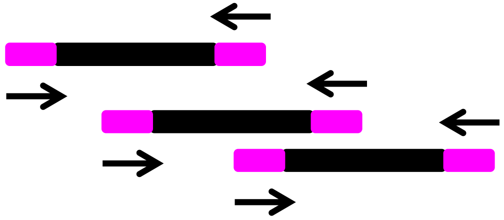

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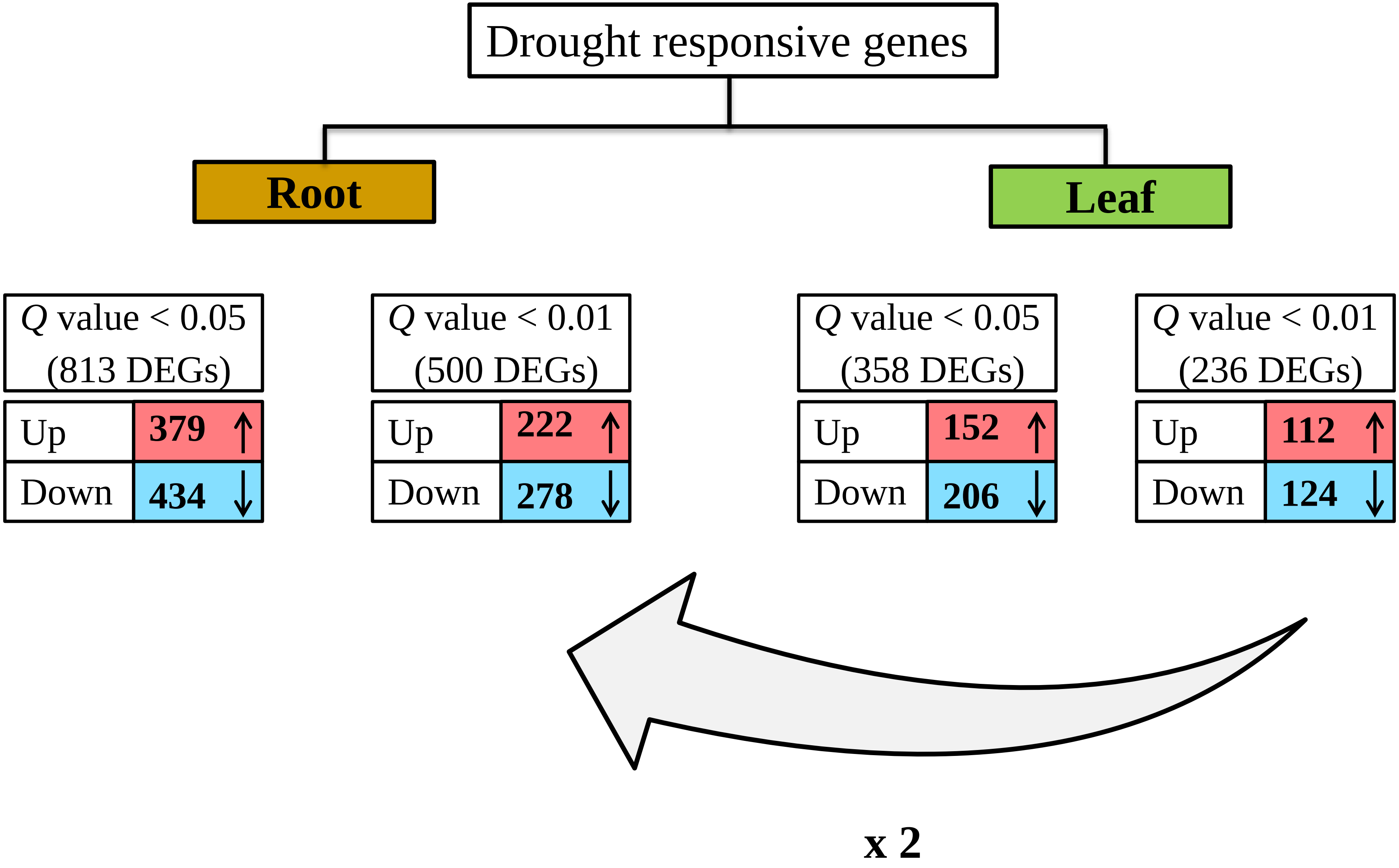
72.34%

4.3 Analysis of differentially expressed genes (DEGs)



Drought responsive genes

Root

Q value < 0.05 (813 DEGs)	
Up	**379** ↑
Down	**434** ↓

Q value < 0.01 (500 DEGs)	
Up	**222** ↑
Down	**278** ↓

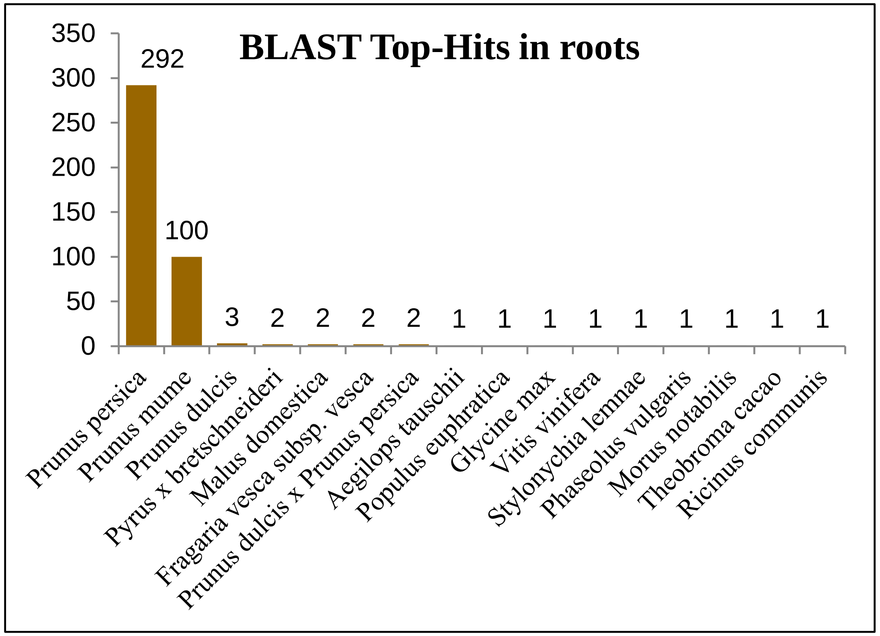
Leaf

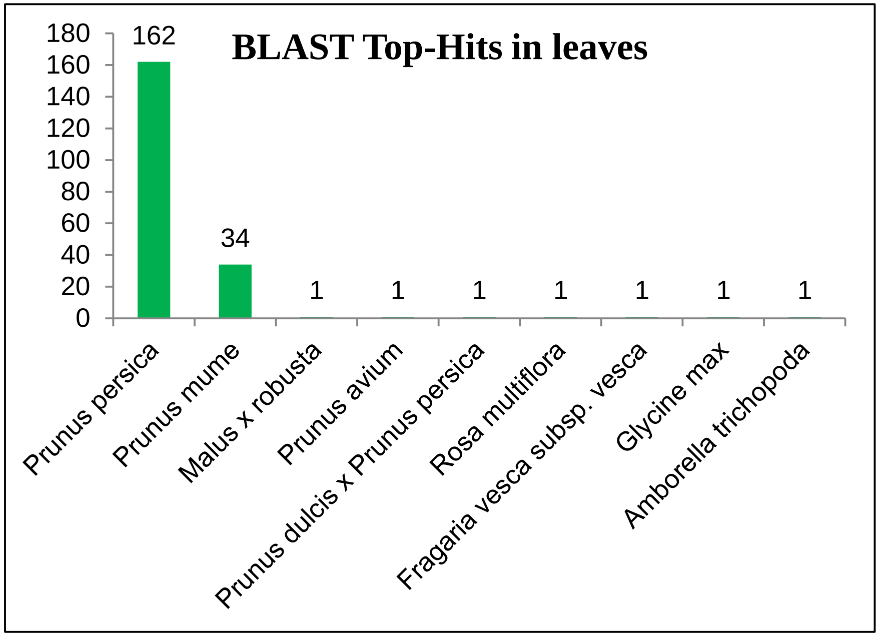
Q value < 0.05 (358 DEGs)	
Up	**152** ↑
Down	**206** ↓

Q value < 0.01 (236 DEGs)	
Up	**112** ↑
Down	**124** ↓

x 2

4.4 Sequence homology searches



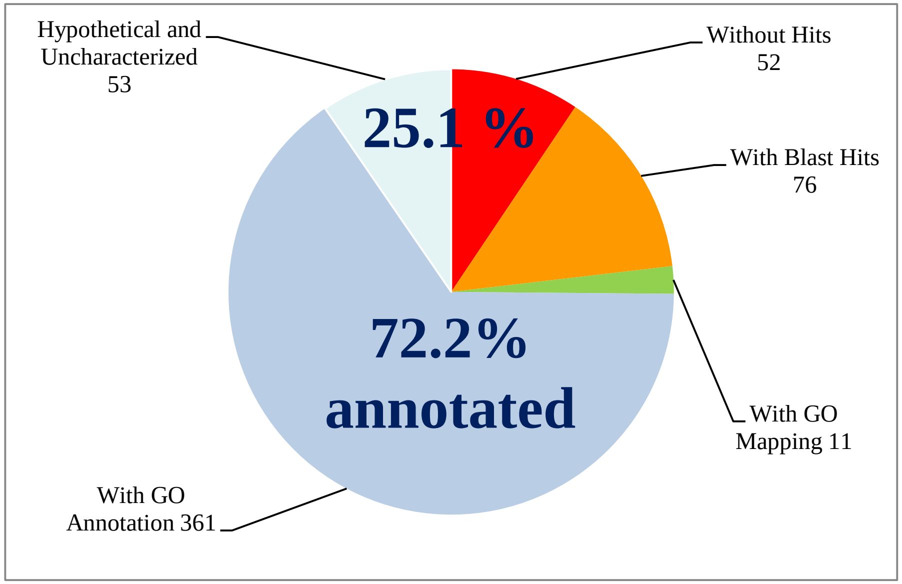


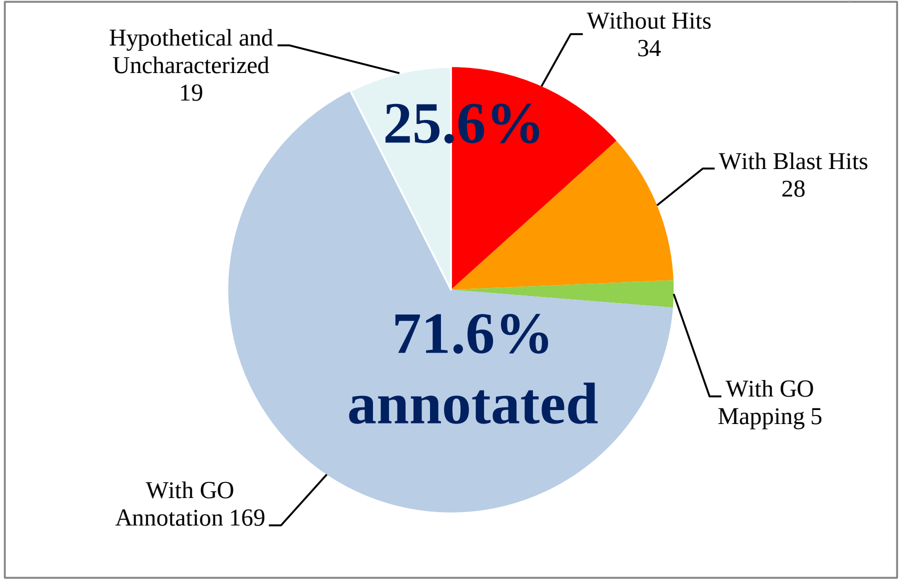
4.5 Functional annotation of DEGs

Q value < 0.01 (DEGs)

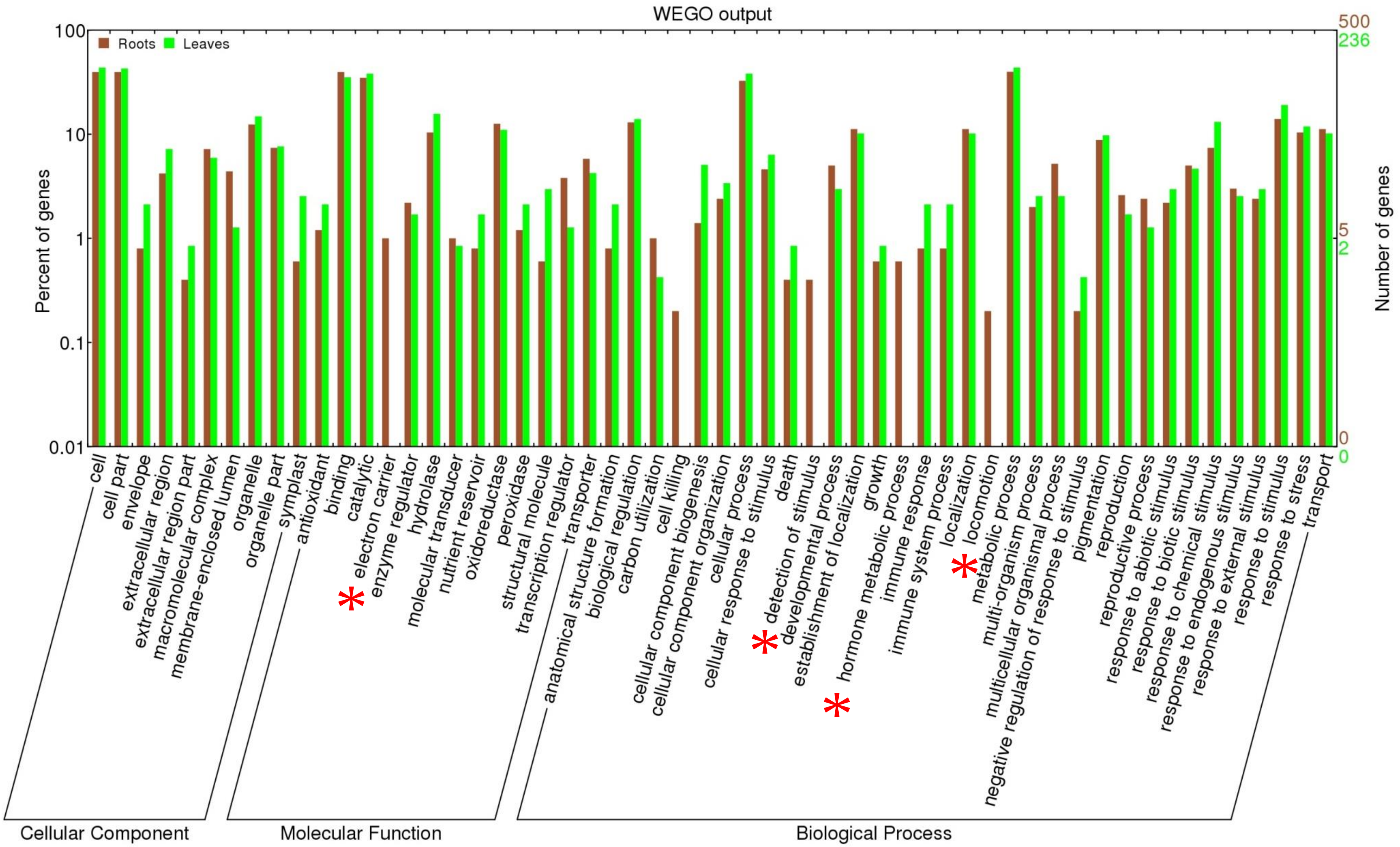
GO annotation

Blast2GO





4.5 Functional annotation of DEGs (56 GOs)

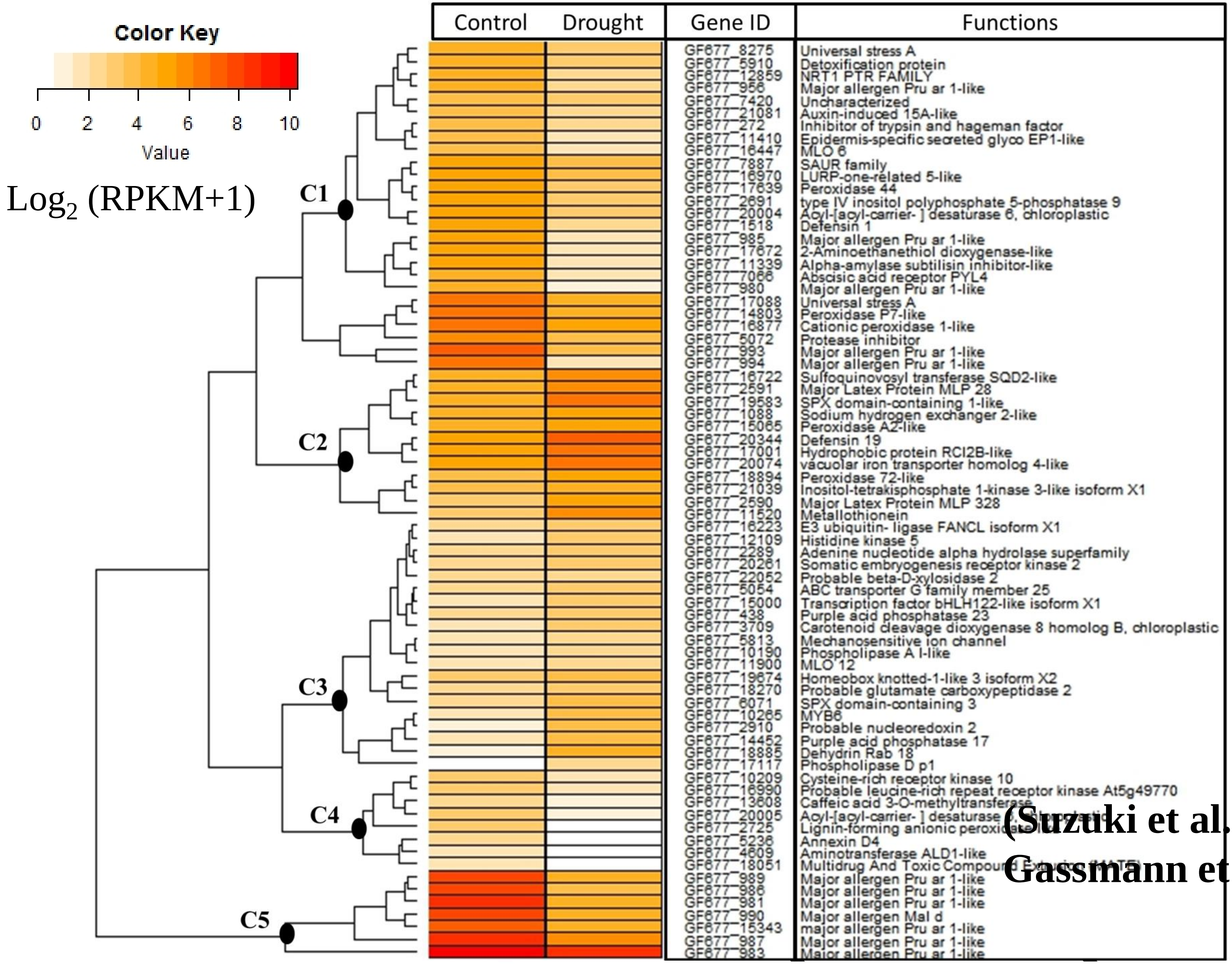


4.6 Gene ontology enrichment GOs

3 GO terms (data not shown)

26 GO terms (Biological process and molecular function)

4.6 Gene ontology enrichment GOs (II)



Gene ID	Functions
GF677_8275	Universal stress A
GF677_5910	Detoxification protein
GF677_12859	NRT1 PTR FAMILY
GF677_956	Major allergen Pru ar 1-like
GF677_7420	Uncharacterized
GF677_21081	Auxin-induced 15A-like
GF677_272	Inhibitor of trypsin and hageman factor
GF677_11410	Epidermis-specific secreted glyco EP1-like
GF677_16447	MLO 6
GF677_7887	SAUR family
GF677_16970	LURP-one-related 5-like
GF677_17639	Peroxidase 44
GF677_2691	type IV inositol polyphosphate 5-phosphatase 9
GF677_20004	Acyl-[acyl-carrier-] desaturase 6, chloroplastic
GF677_1518	Defensin 1
GF677_985	Major allergen Pru ar 1-like
GF677_17672	2-Aminoethanethiol dioxygenase-like
GF677_11339	Alpha-amylase subtilisin inhibitor-like
GF677_7066	Abscisic acid receptor PYL4
GF677_980	Major allergen Pru ar 1-like
GF677_17088	Universal stress A
GF677_14803	Peroxidase P7-like
GF677_16877	Cationic peroxidase 1-like
GF677_5072	Protease inhibitor
GF677_993	Major allergen Pru ar 1-like
GF677_994	Major allergen Pru ar 1-like
GF677_16722	Sulfoquinovosyl transferase SQD2-like
GF677_2591	Major Latex Protein MLP 28
GF677_19583	SPX domain-containing 1-like
GF677_1088	Sodium hydrogen exchanger 2-like
GF677_15065	Peroxidase A2-like
GF677_20344	Defensin 19
GF677_17001	Hydrophobic protein RCI2B-like
GF677_20074	vacuolar iron transporter homolog 4-like
GF677_18894	Peroxidase 72-like
GF677_21039	Inositol-tetrakisphosphate 1-kinase 3-like isoform X1
GF677_2590	Major Latex Protein MLP 328
GF677_11520	Metallothionein
GF677_16223	E3 ubiquitin- ligase FANCL isoform X1
GF677_12109	Histidine kinase 5
GF677_2289	Adenine nucleotide alpha hydrolase superfamily
GF677_20261	Somatic embryogenesis receptor kinase 2
GF677_22052	Probable beta-D-xylosidase 2
GF677_5054	ABC transporter G family member 25
GF677_15000	Transcription factor bHLH122-like isoform X1
GF677_438	Purple acid phosphatase 23
GF677_3709	Carotenoid cleavage dioxygenase 8 homolog B, chloroplastic
GF677_5813	Mechanosensitive ion channel
GF677_10190	Phospholipase A I-like
GF677_11900	MLO 12
GF677_19674	Homeobox knotted-1-like 3 isoform X2
GF677_18270	Probable glutamate carboxypeptidase 2
GF677_6071	SPX domain-containing 3
GF677_10265	MYB6
GF677_2910	Probable nucleoredoxin 2
GF677_14452	Purple acid phosphatase 17
GF677_18885	Dehydrin Rab 18
GF677_17117	Phospholipase D p1
GF677_10209	Cysteine-rich receptor kinase 10
GF677_16990	Probable leucine-rich repeat receptor kinase At5g49770
GF677_13608	Caffeic acid 3-O-methyltransferase
GF677_20005	Acyl-[acyl-carrier-] desaturase 6, chloroplastic
GF677_2725	Lignin-forming anionic peroxidase-like
GF677_5236	Annexin D4
GF677_4609	Aminotransferase ALD1-like
GF677_18051	Multidrug And Toxic Compound Extrusion (MATE)
GF677_989	Major allergen Pru ar 1-like
GF677_986	Major allergen Pru ar 1-like
GF677_981	Major allergen Pru ar 1-like
GF677_990	Major allergen Mal d
GF677_15343	major allergen Pru ar 1-like
GF677_987	Major allergen Pru ar 1-like
GF677_983	Major allergen Pru ar 1-like

(Suzuki et al., 2013; Gassmann et al., 2016)

4.7 Functional characterization of DEGs

Regulatory

- Signaling and transcriptional regulation

Functional

- Cell protection and damage repair

4.7.1 Regulatory role of DEGs

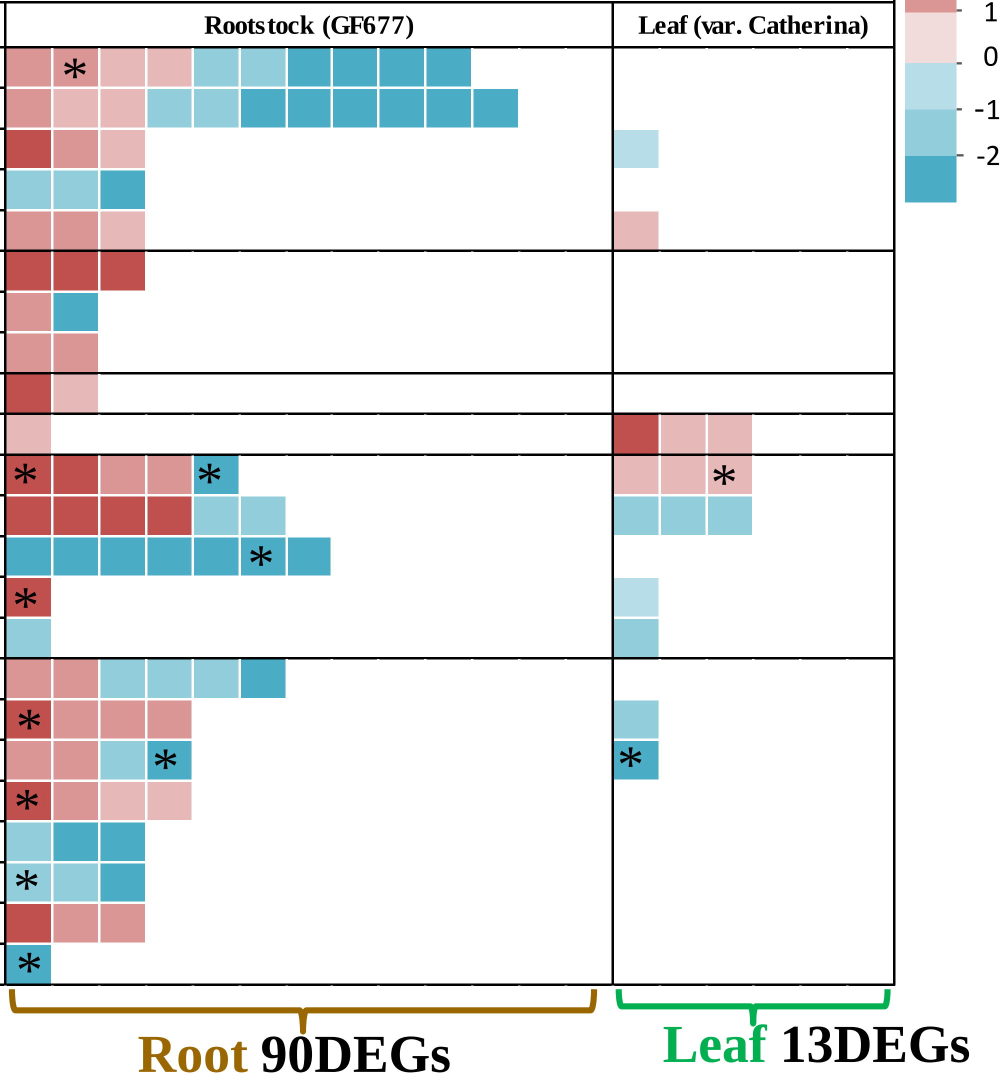
Signal perception

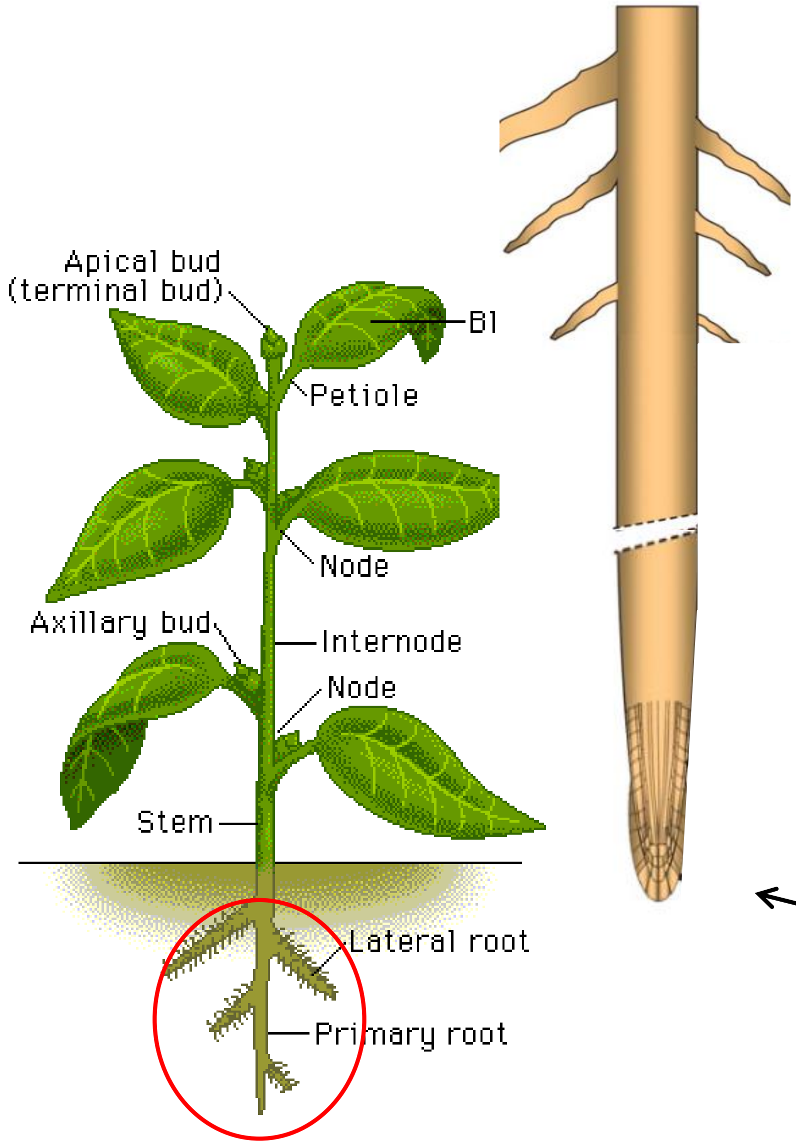
Signal transduction

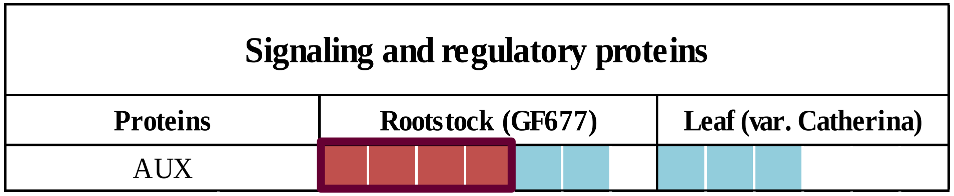
Transcription regulation

Signaling and regulatory proteins

Functions	Proteins
Receptors and proteins kinases	LRR-RLK
	RLK-RK
	STK
	CRK
	PK
Ca^{2+} signaling	EF-hand
	CML
	uniporter 2
Phospholipases	-
Phosphatases	-
Hormone signaling	ABA
	AUX
	ETH
	GA
	BR
Transcription factors	bHLH
	NAC
	ERT
	HD-ZIP
	ORG2-Like
	WRKY
	MYB
	GRF5



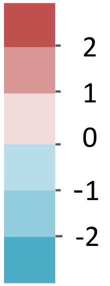


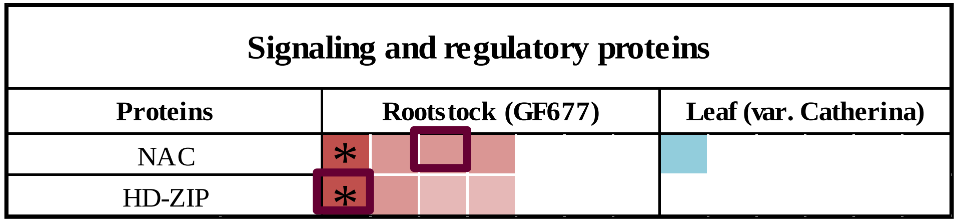


Signaling and regulatory proteins		
Proteins	**Rootstock (GF677)**	**Leaf (var. Catherina)**
AUX	🟥🟥🟥🟥🟦🟦	🟦🟦🟦

(Böttcher et al., 2012)

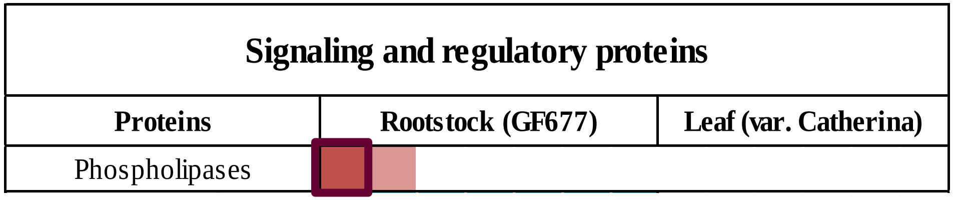
⊢ AUX



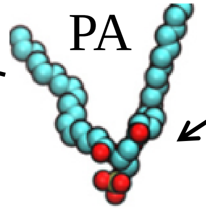


Signaling and regulatory proteins		
Proteins	**Rootstock (GF677)**	**Leaf (var. Catherina)**
NAC	🟥* 🟥🟥🟥	🟦
HD-ZIP	🟥* 🟥🟥🟥	

(Huang et al., 2015; Ré et al., 2014)



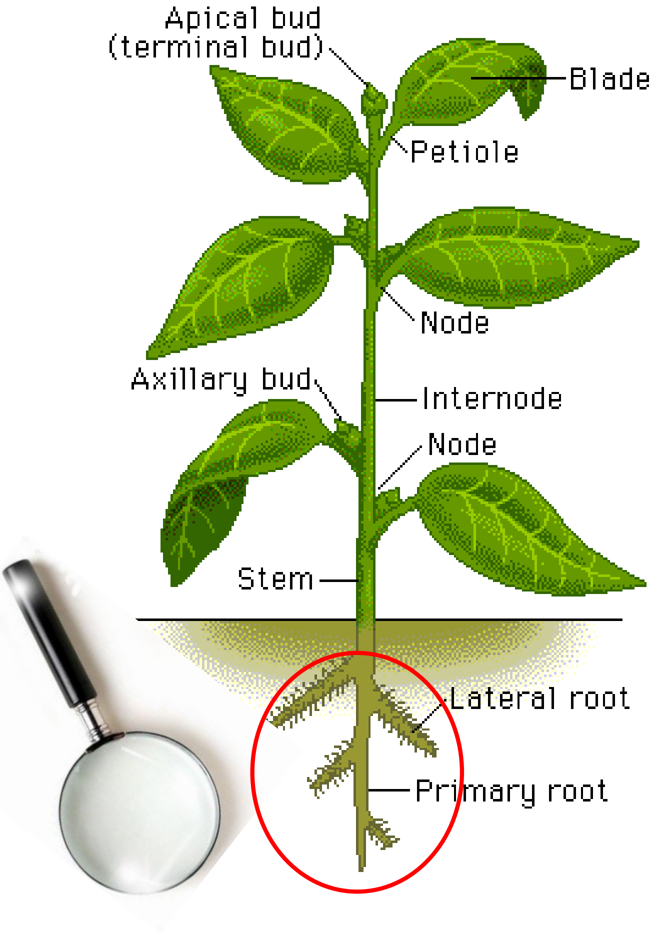
Signaling and regulatory proteins		
Proteins	**Rootstock (GF677)**	**Leaf (var. Catherina)**
Phospholipases	🟥🟥	

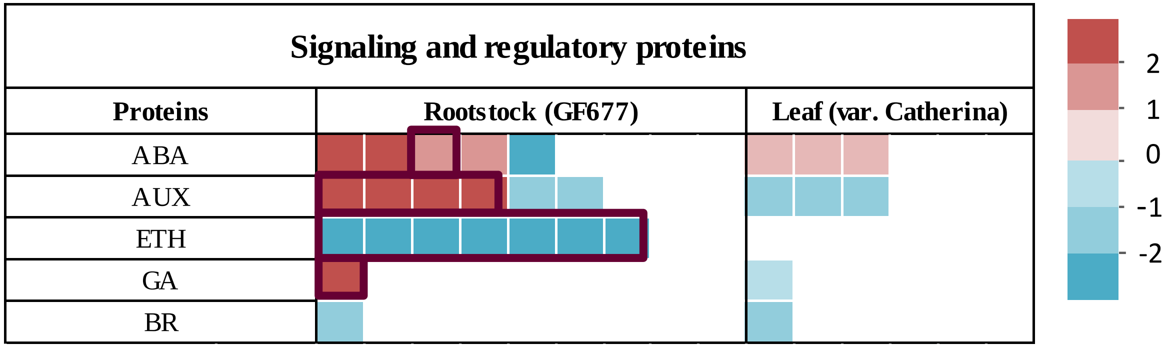


PA

Phospholipid ← PLD

(McLoughlin and Testerink, 2013)





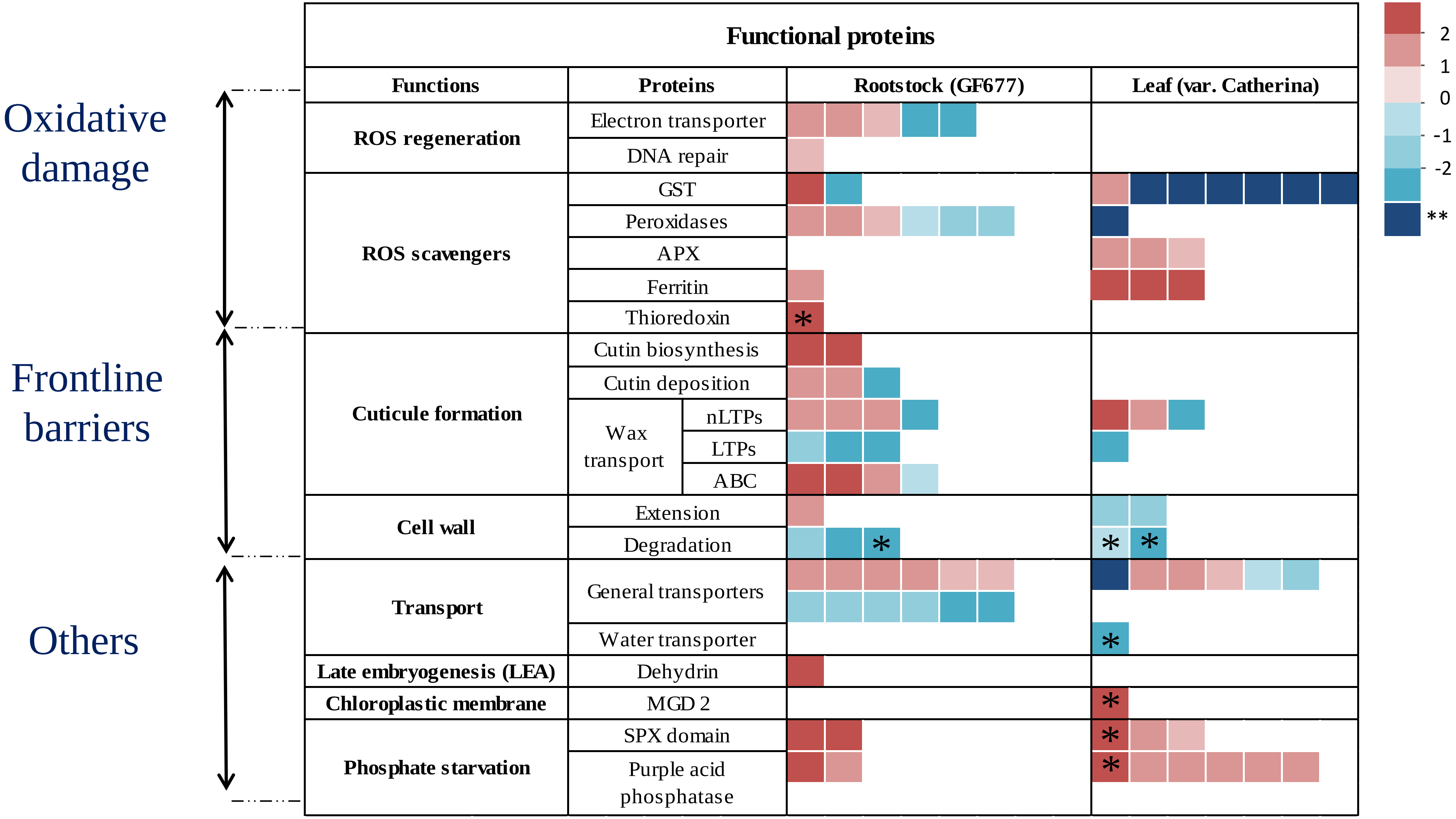
Signaling and regulatory proteins

Proteins	Rootstock (GF677)	Leaf (var. Catherina)
ABA	🟥🟥🟥🟥🟦	🟥🟥🟥
AUX	🟥🟥🟥🟥🟦🟦	🟦🟦🟦
ETH	🟦🟦🟦🟦🟦🟦🟦	
GA	🟥	🟦
BR	🟦	🟦

Hormone metabolism

Biosynthesis	Catabolism
ABA (NCED3) ETH (ACO1)	AUX (GH.3) ⊣ GA (GA2OX2) ⊣

4.7.2 Funcional role of DEGs



Oxidative damage

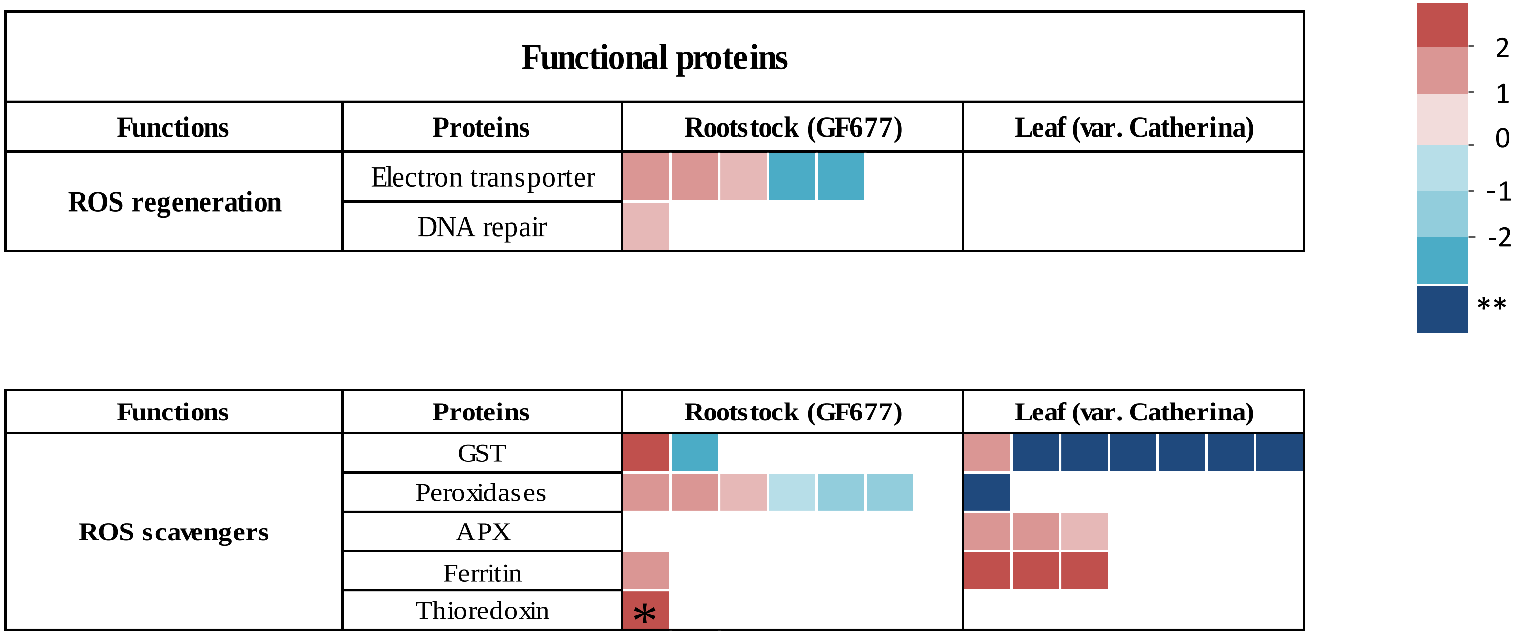
Frontline barriers

Others

Functional proteins

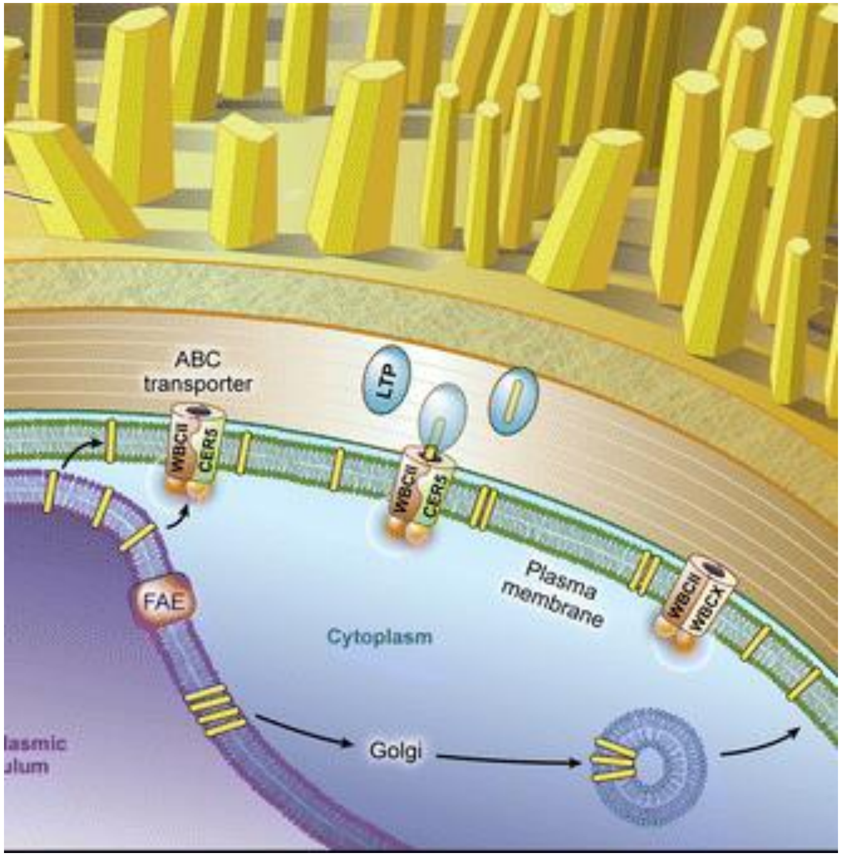
Functions	Proteins		Rootstock (GF677)	Leaf (var. Catherina)
ROS regeneration	Electron transporter			
	DNA repair			
ROS scavengers	GST			
	Peroxidases			
	APX			
	Ferritin			
	Thioredoxin		*	
Cuticule formation	Cutin biosynthesis			
	Cutin deposition			
	Wax transport	nLTPs		
		LTPs		
		ABC		
Cell wall	Extension			
	Degradation		*	* *
Transport	General transporters			
	Water transporter			*
Late embryogenesis (LEA)	Dehydrin			
Chloroplastic membrane	MGD 2			*
Phosphate starvation	SPX domain			*
	Purple acid phosphatase			*

4.7.2 Funcional role of DEGs



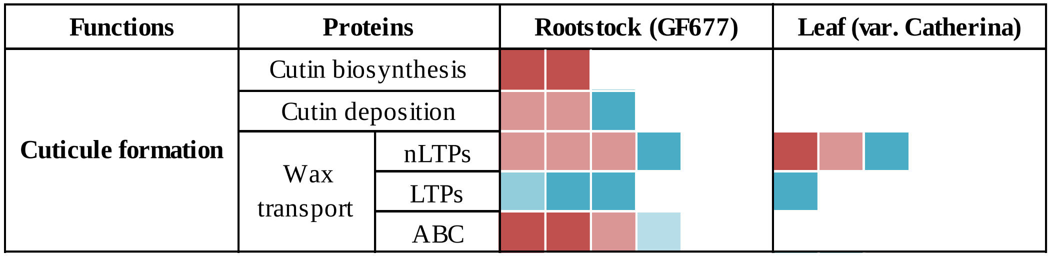
Functional proteins			
Functions	**Proteins**	**Rootstock (GF677)**	**Leaf (var. Catherina)**
ROS regeneration	Electron transporter		
	DNA repair		

Functions	**Proteins**	**Rootstock (GF677)**	**Leaf (var. Catherina)**
ROS scavengers	GST		
	Peroxidases		
	APX		
	Ferritin		
	Thioredoxin	*	

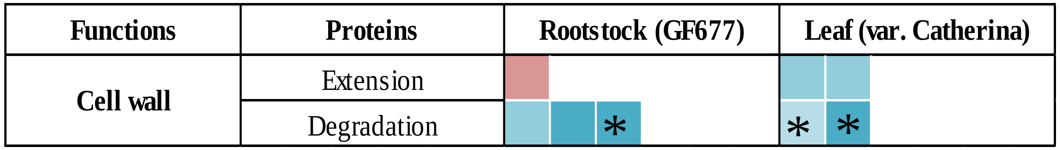


Cuticle

Cell wall



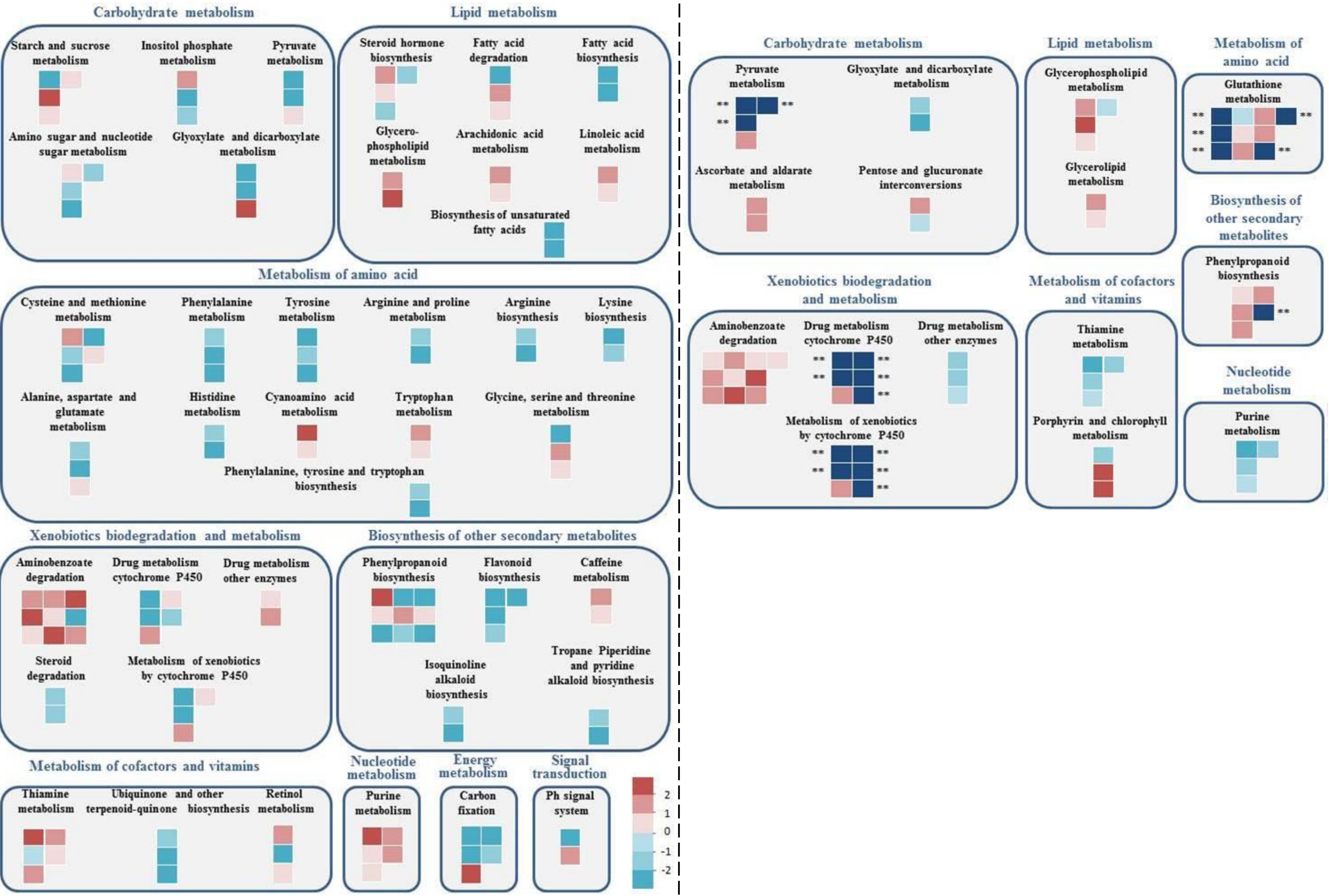
Functions	Proteins		Rootstock (GF677)	Leaf (var. Catherina)
Cuticle formation	Cutin biosynthesis		🟥🟥	
	Cutin deposition		🟥🟥🟦	
	Wax transport	nLTPs	🟥🟥🟥🟦	🟥🟥🟦
		LTPs	🟦🟦🟦	🟦
		ABC	🟥🟥🟥🟦	



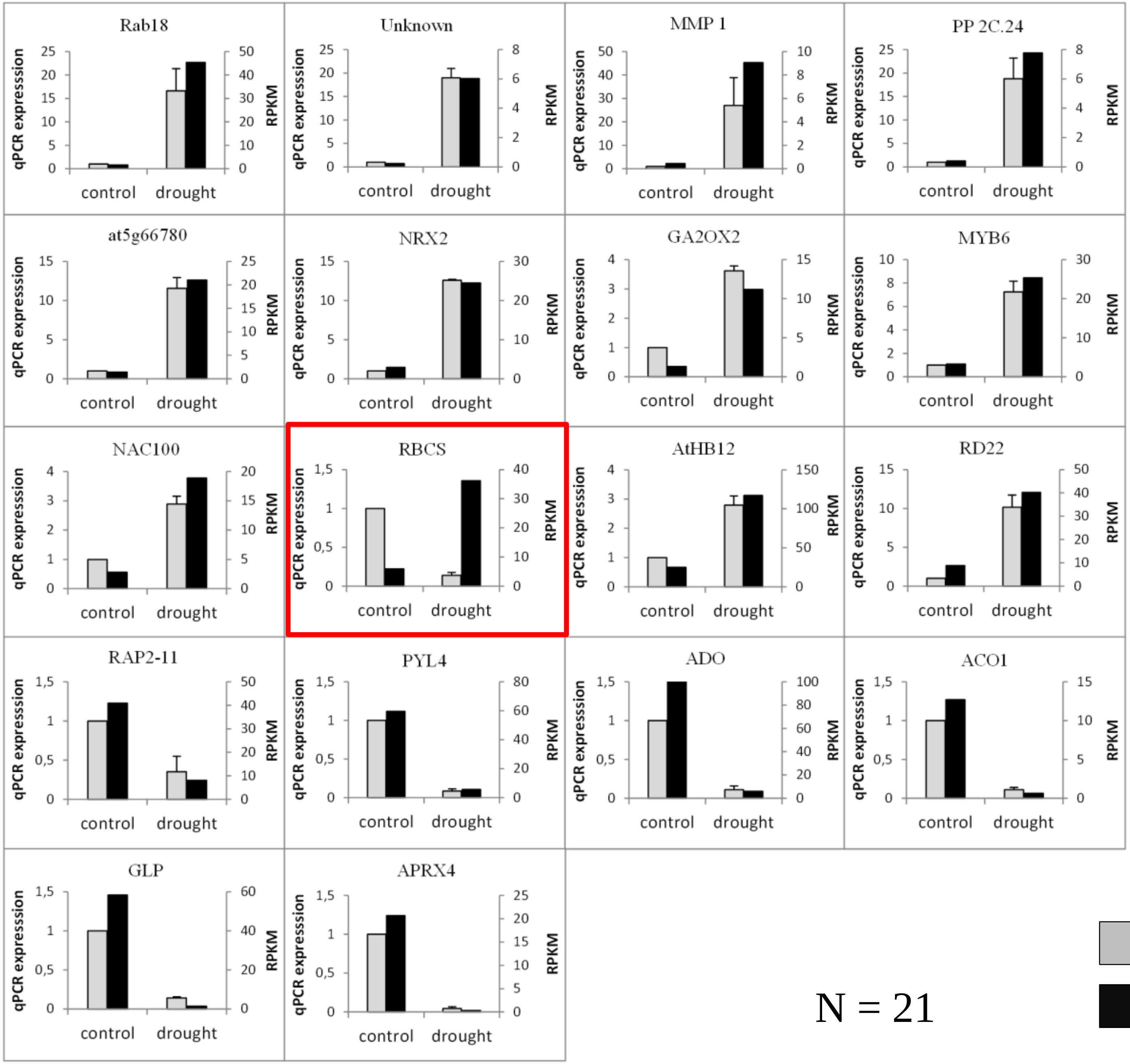
Functions	Proteins	Rootstock (GF677)	Leaf (var. Catherina)
Cell wall	Extension	🟥	🟦🟦
	Degradation	🟦🟦🟦*	🟦*🟦*

4.8 Metabolic pathways

Root **Leaf**



4.9 Validation of RNA-seq reliability using RT-qPCR

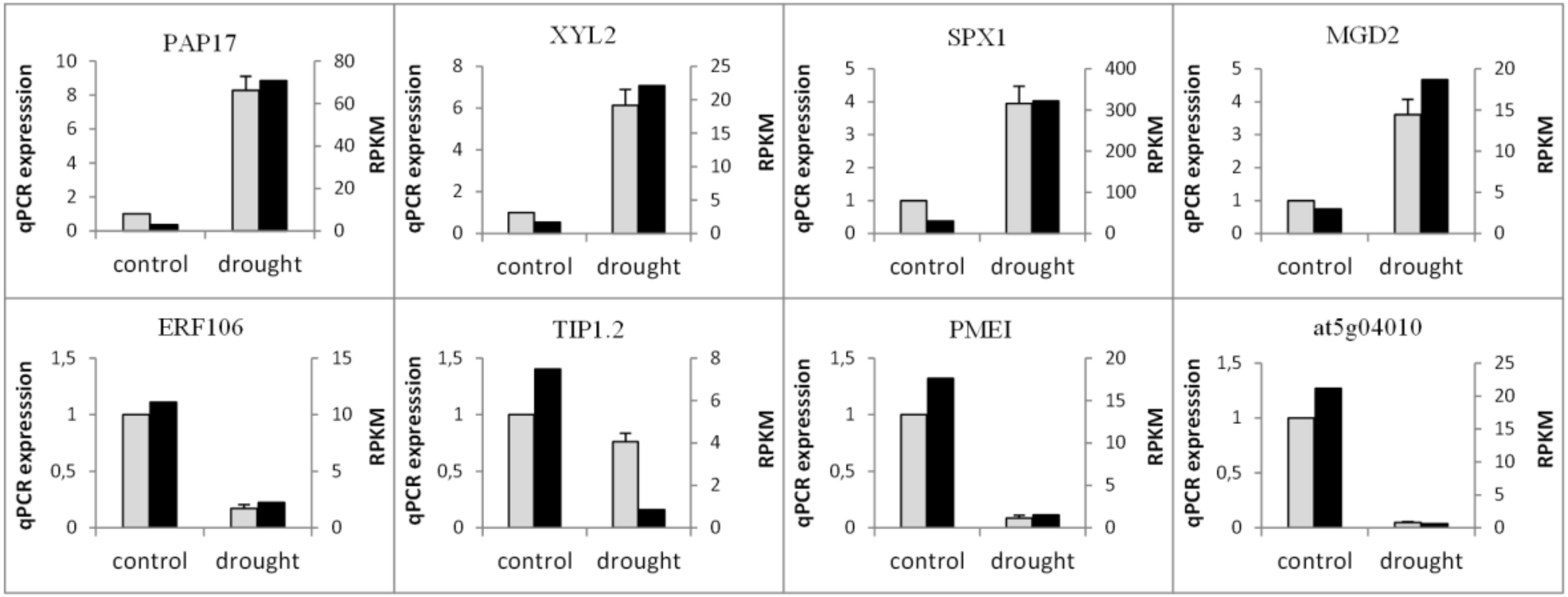


N = 21

▨ RT-qPCR

■ RPKM

4.9 Validation of RNA-seq reliability using RT-qPCR



▨ RT-qPCR

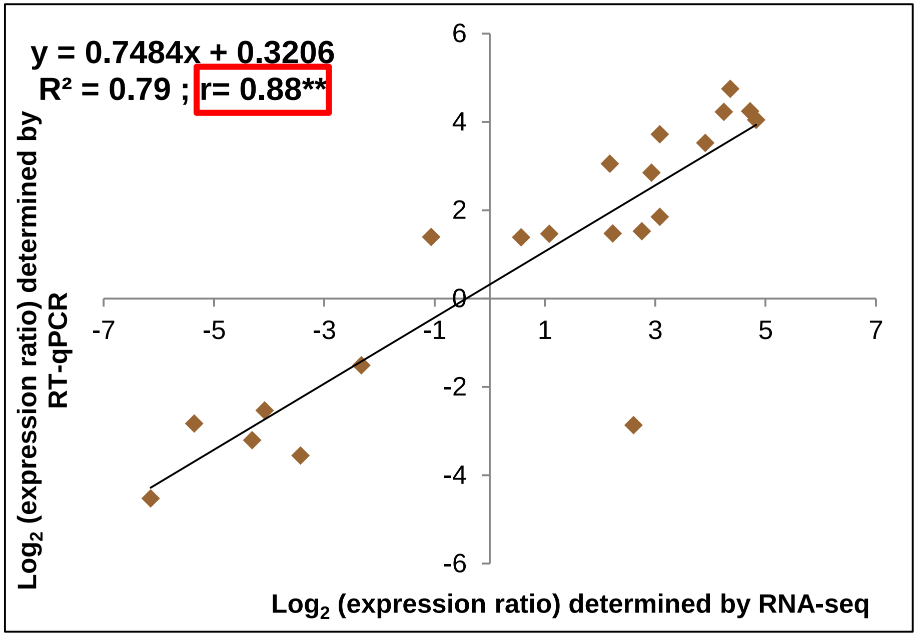
■ RPKM

N = 12

4.9 Validation of RNA-seq reliability using RT-qPCR (II)

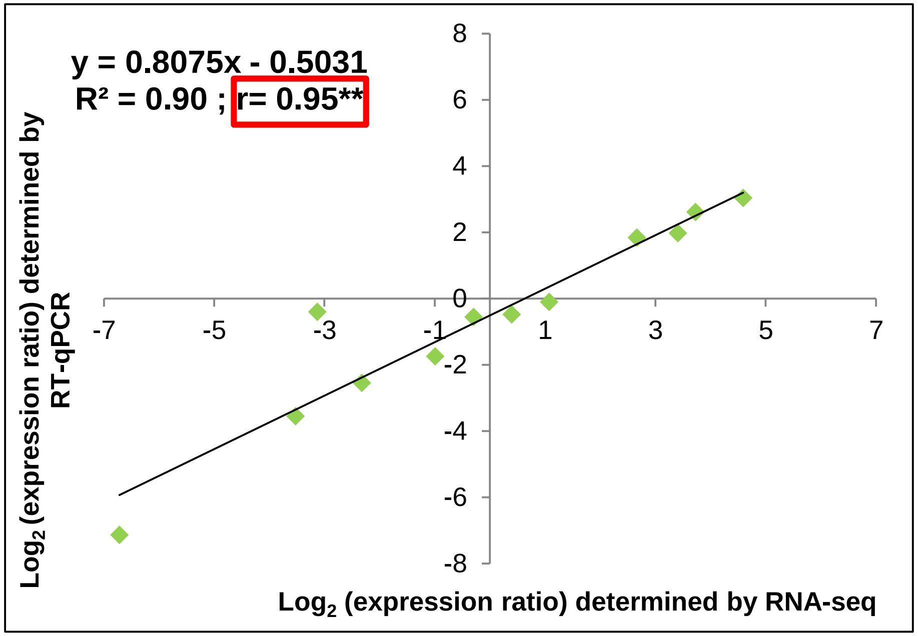
Root

N = 21



Leaf

N = 12



Structure of the presentation

1- Introduction

2- Objectives

3- Material and Methods

4- Results and Discussion

5- Conclusions

5- Conclusions

1. RNA-seq is a powerful tool for exploring the diversity of differentially expressed genes expressed in different tissues, however, the analysis of these results is complex and should be validated through other approaches such as RT-qPCR.

2. This study is the first that simultaneously evaluates the effect of drought on roots and on leaves of a grafted variety of peach, observing changes at the physiological and transcriptional level.

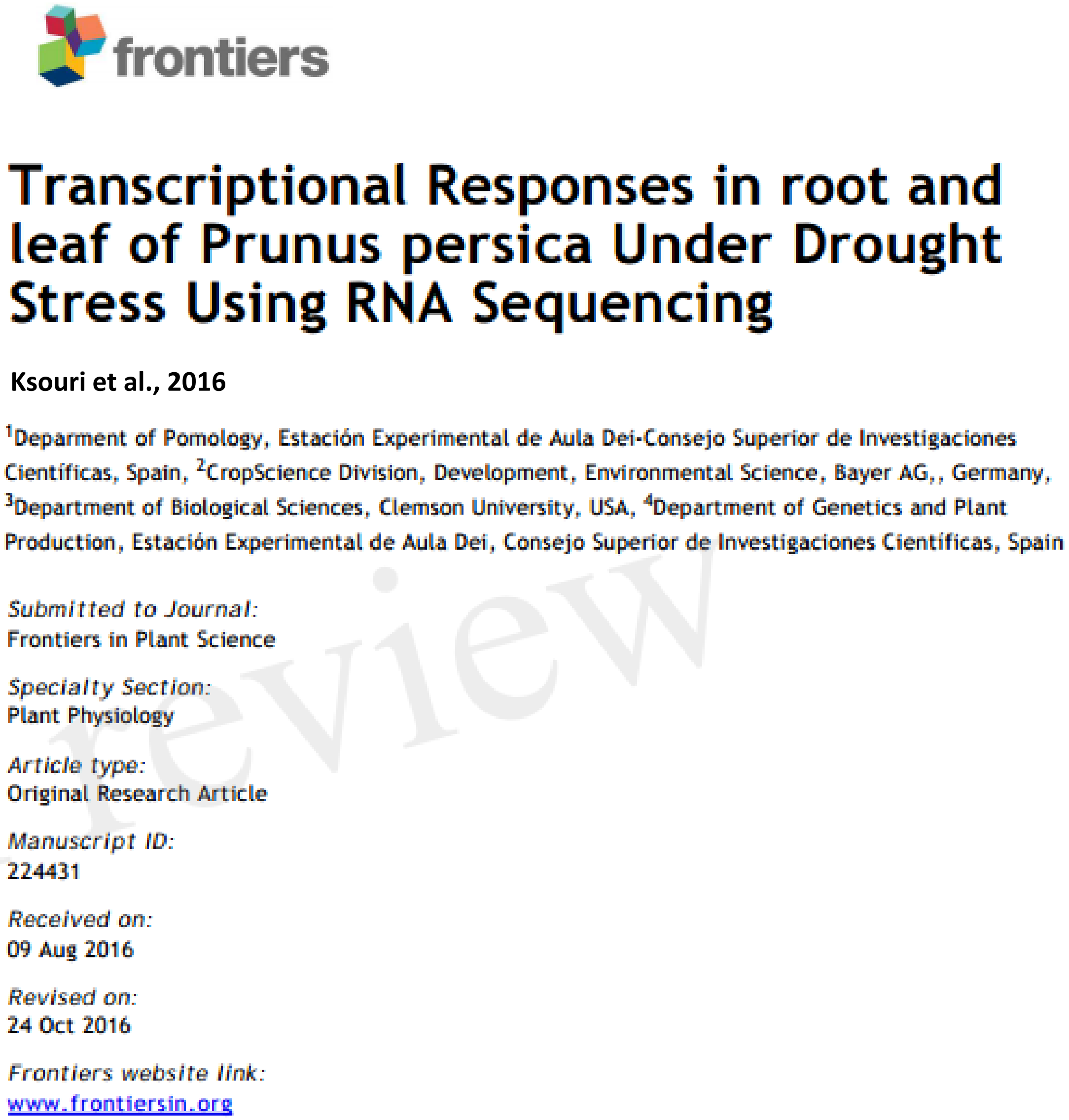
3. The differentially expressed genes (DEGs) in roots are approximately twice the number of DEGs found in leaves, suggesting thereby that roots undergo more complex gene regulation, highlighting the role of rootstock as stress buffer during water deprivation.

5- Conclusions (II)

4. Functional annotation allows dissecting molecular aspects of drought responses, selection of candidate genes, and the formulation of hypotheses that can be validated experimentally.

5. Abscisic acid (ABA) is the only inducible hormone under drought stress conditions, while other hormones, and their associated metabolic pathways, are repressed.

6. GRF5 and AtHB12 are candidate genes potentially involved in drought stress adaptation providing thus a good starting point for investigation in *P. persica*.



Transcriptional Responses in root and leaf of Prunus persica Under Drought Stress Using RNA Sequencing

Ksouri et al., 2016

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Submitted to Journal:
Frontiers in Plant Science

Specialty Section:
Plant Physiology

Article type:
Original Research Article

Manuscript ID:
224431

Received on:
09 Aug 2016

Revised on:
24 Oct 2016

Frontiers website link:
www.frontiersin.org

In review

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