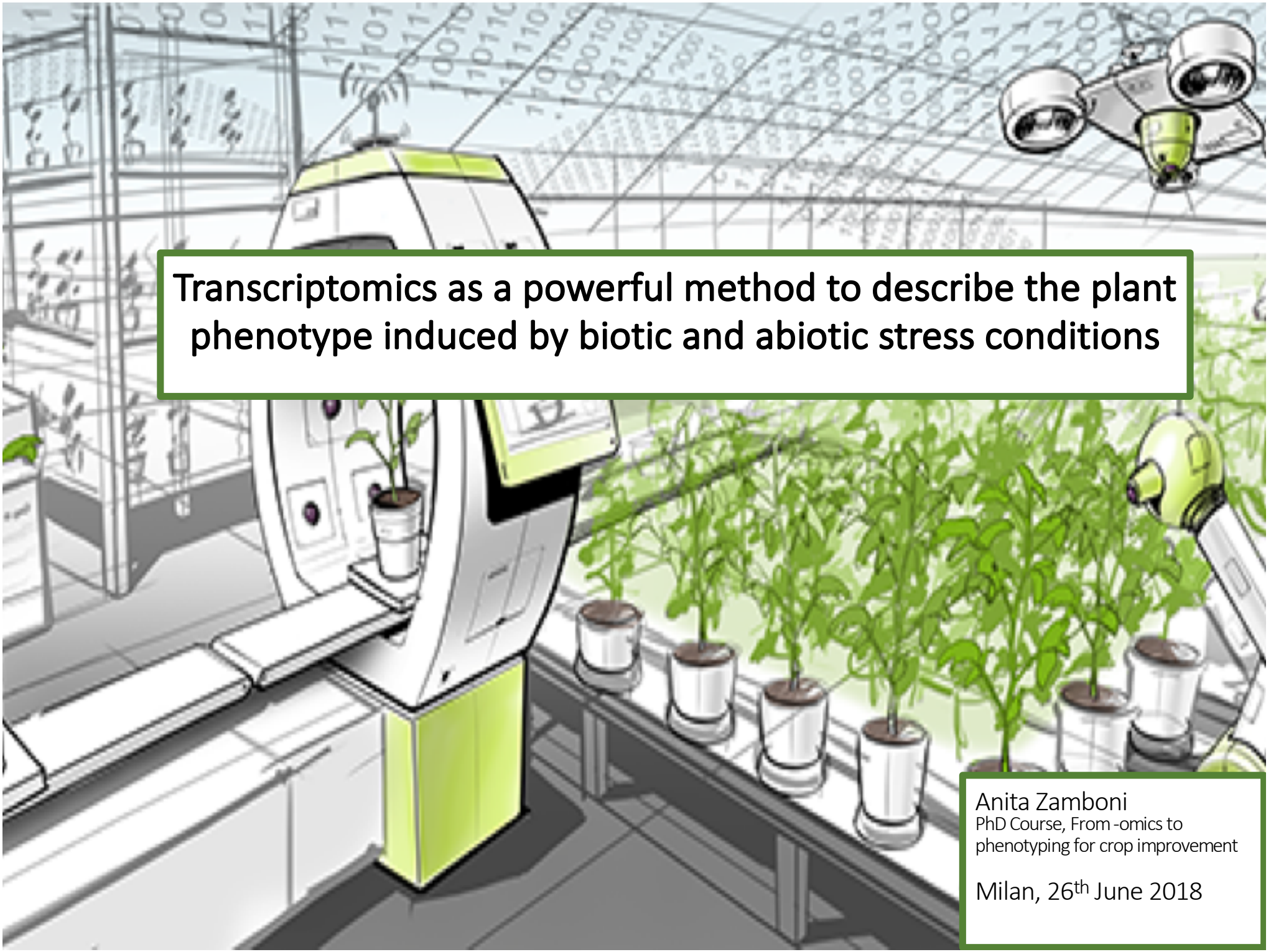


Transcriptomics as a powerful method to describe the plant phenotype induced by biotic and abiotic stress conditions

Anita Zamboni

PhD Course, From -omics to phenotyping for crop improvement

Milan, 26th June 2018

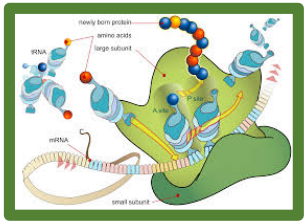
Transcriptome:

is the **complete set of RNA molecules** produced by a cell, tissue or organism. It includes mRNA, rRNA, tRNA and other non-coding RNAs, although in many cases the mRNA profile is the most sought after because it corresponds to the expression of protein-encoding genes. The transcriptome depends on gene expression and therefore changes qualitatively and quantitatively according to cell type, developmental stage and in response to external conditions or physiological states. The **dynamic** nature of the transcriptome is **highly informative**.

- ✓ **direct analysis:** procedures involving nucleotide sequencing and fragment sizing (EST sequencing, SAGE, MPSS, SSH and cDNA-AFLP)

- ✓ **indirect analysis:** cDNA microarray and oligo-chip (Affymetrix, Combimatrix, NimbleGen, Agilent…)

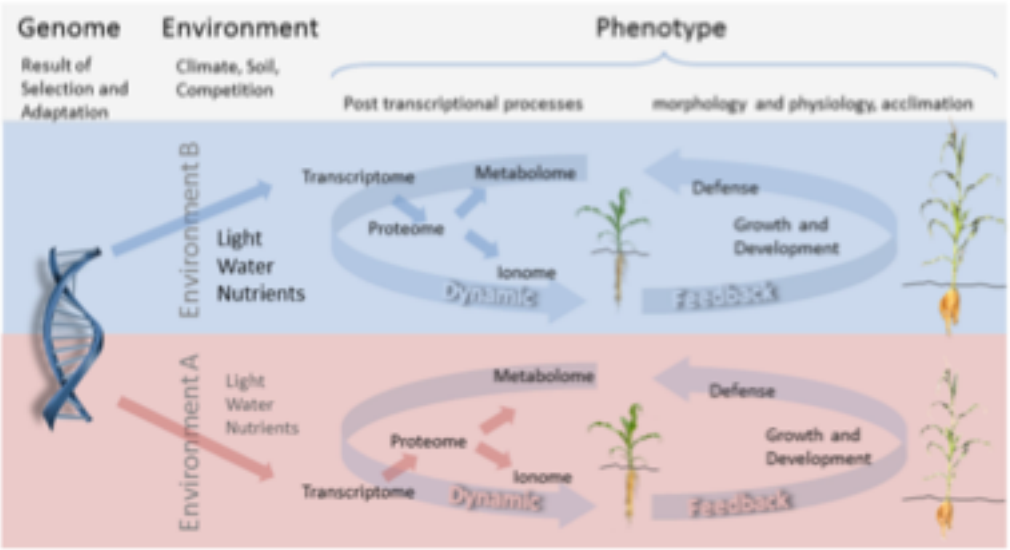
- ✓ the word **transcriptome** was used for the first time in the 1990s (Velculescu *et al*., 1997; Piétu *et al*., 1999)



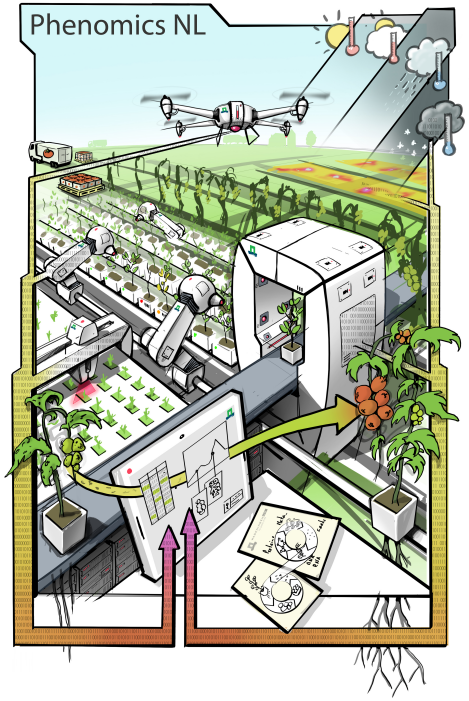
Plant phenotyping:

is the quantitative appraisal of traits from a given plant genotype in a give environment and experiment, which range from scalar (e.g. plant height), multi-value (chemical and transcriptional) to image-based (pictures) and includes both direct measured attributes and those derived from analysis (*e.g*. leaf area from shoot images; Bolger *et al*., 2017).

Heterogeneous data: are a problem for the analysis but also for a long-term access



Walter et al., 2015



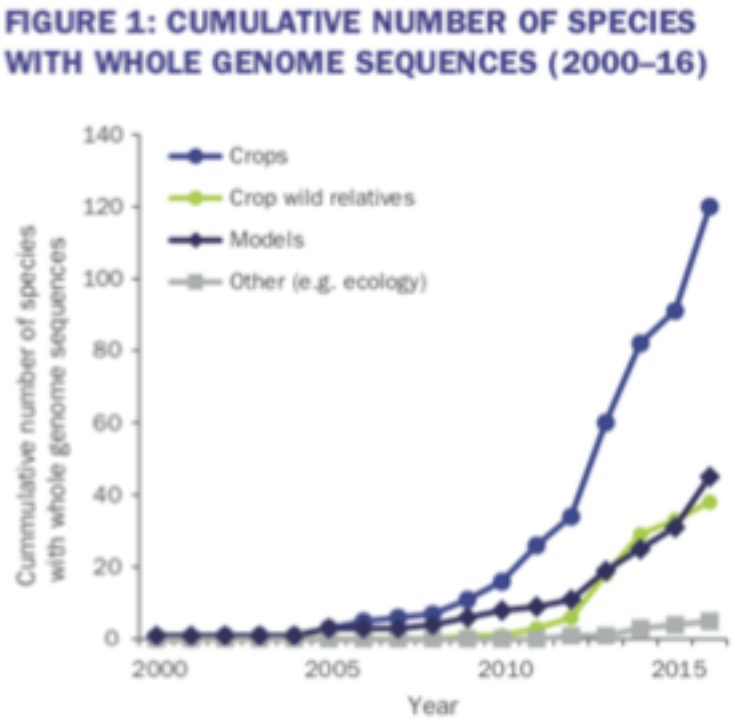
Wageningen University and Research

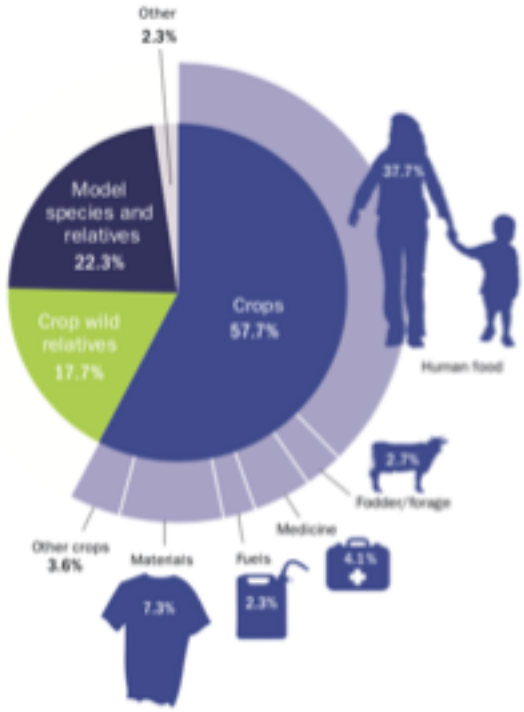
Arabidopsis thaliana

✓ sequence of the entire plant genome: 2000 (Nature **48:** 796-815)

✓ develop of microarray platform: 2003 (Yamada *et al.,* 2003)

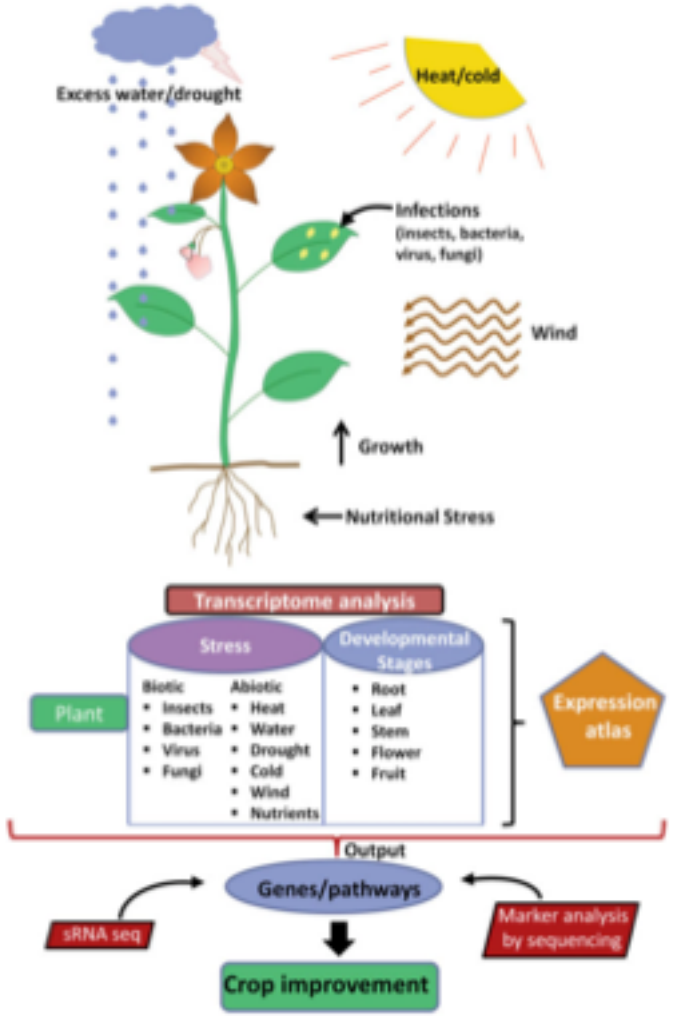
✓ first transcriptome report by next generation sequencing (NGS): 2007 (Weber *et a*l., 2007)





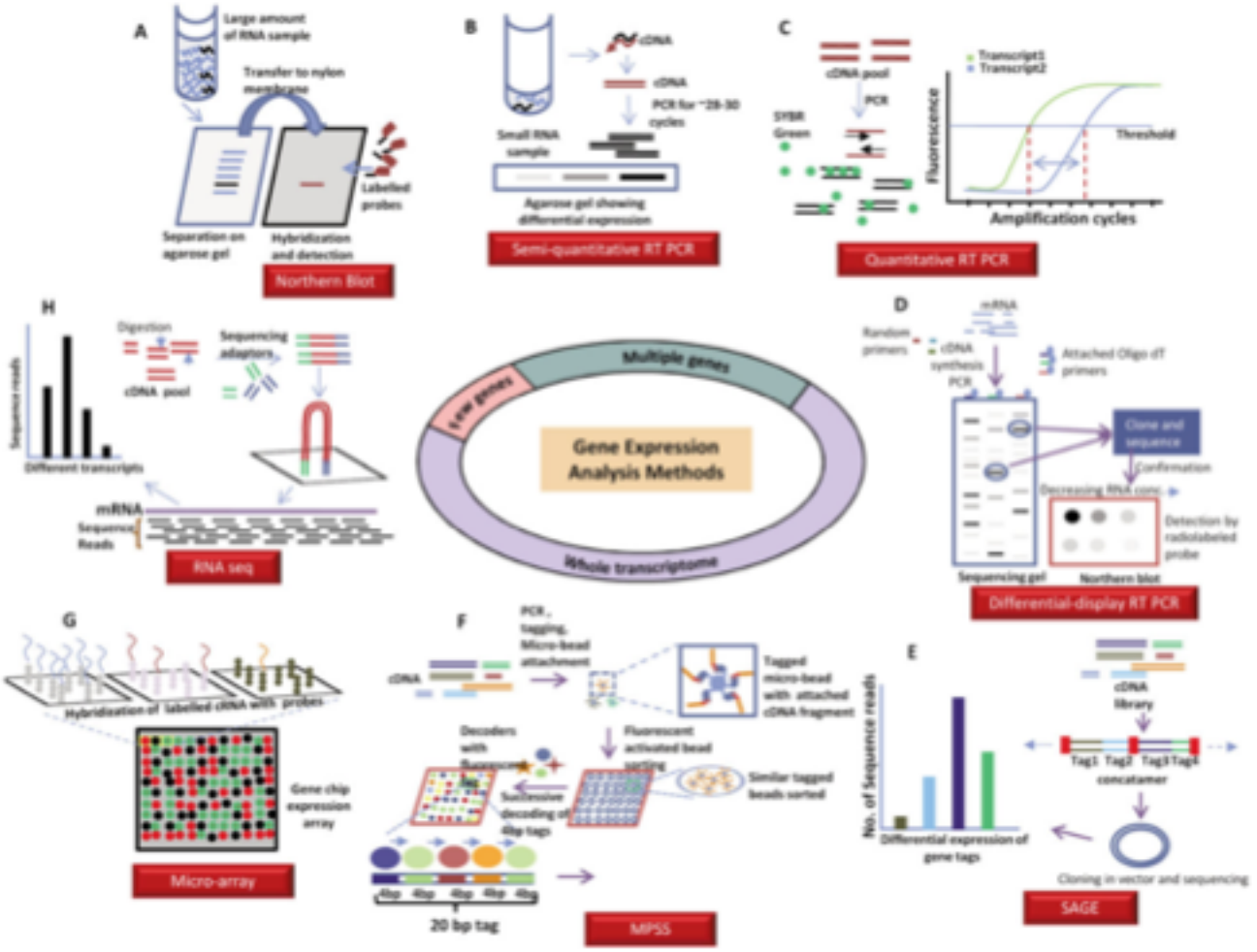
Applications of transcriptomics:

- ✓ identification of development and stressed-associated genes and pathways
- ✓ development of molecular markers
- ✓ insight about downstream genes (plants with altered expression of genes of interest)
- ✓ expression atlas: it spans the complete range of tissues and developmental stages; it presents the snapshot of the mRNA profile of the entire life-cycle of plants
- ✓ transcriptome analysis is an essential component of functional genomics



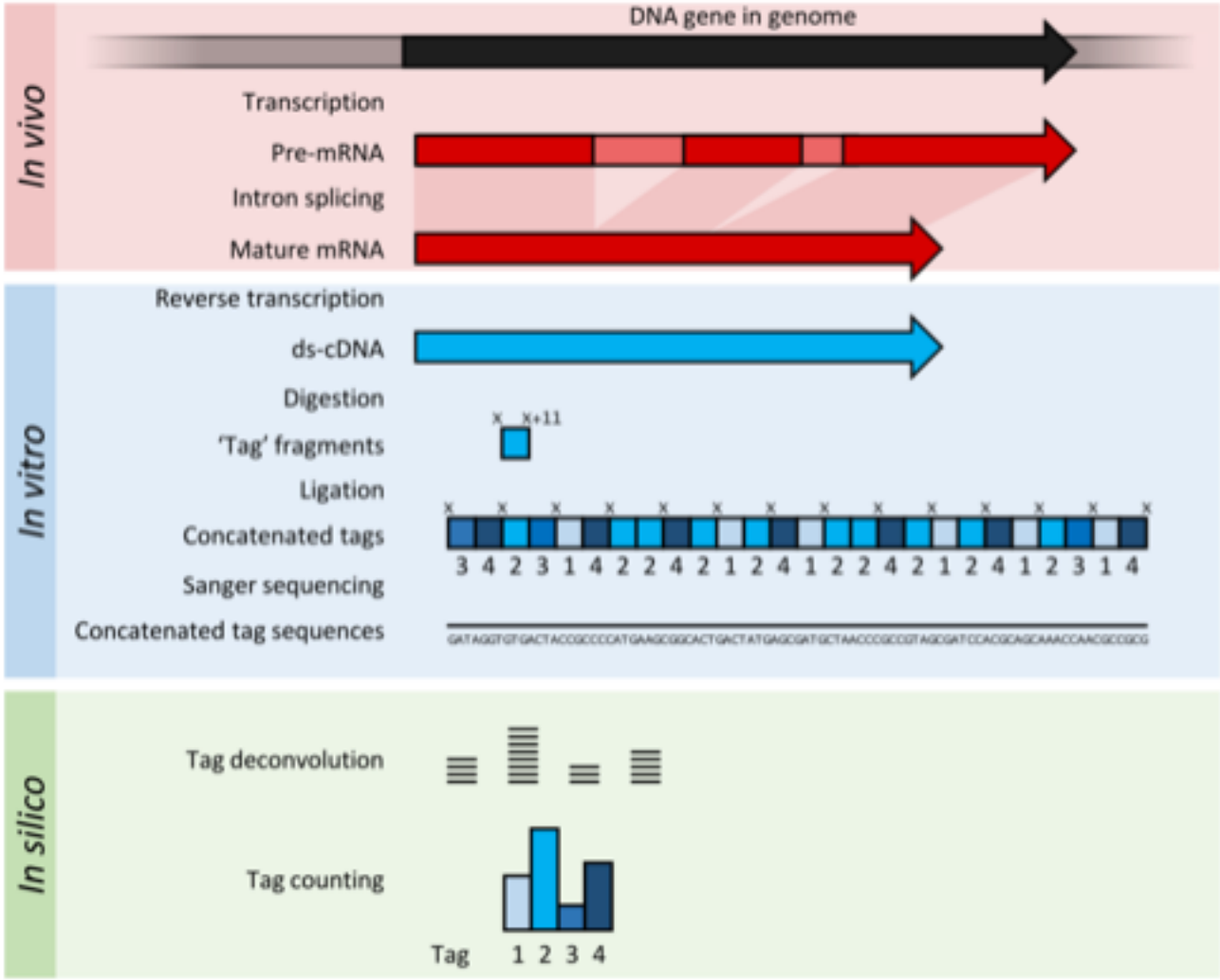
Agarwal et al., 2014

Progress in methods for gene expression analysis:



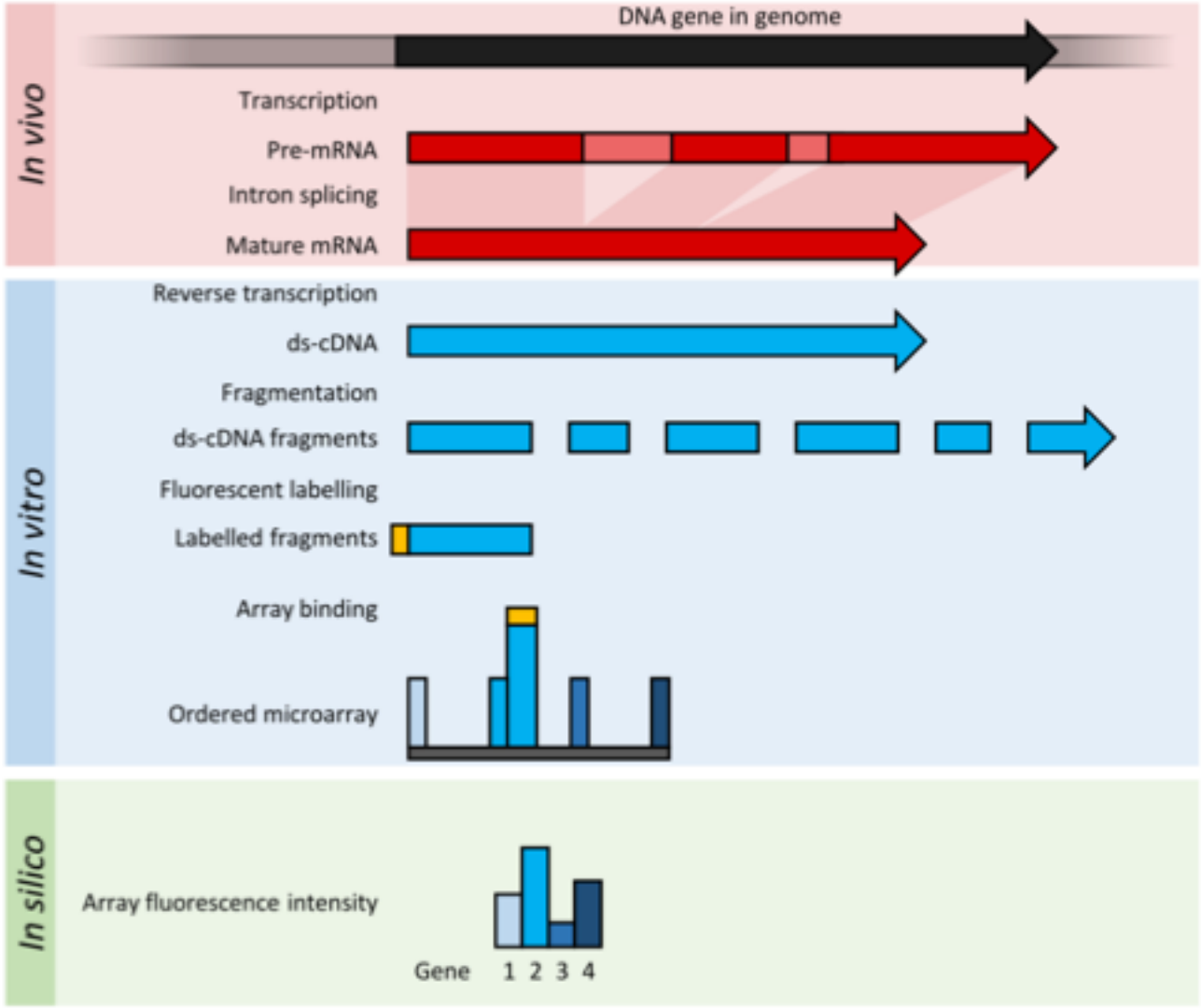
Agarwal et al., 2014

Serial analysis of gene expression (SAGE)



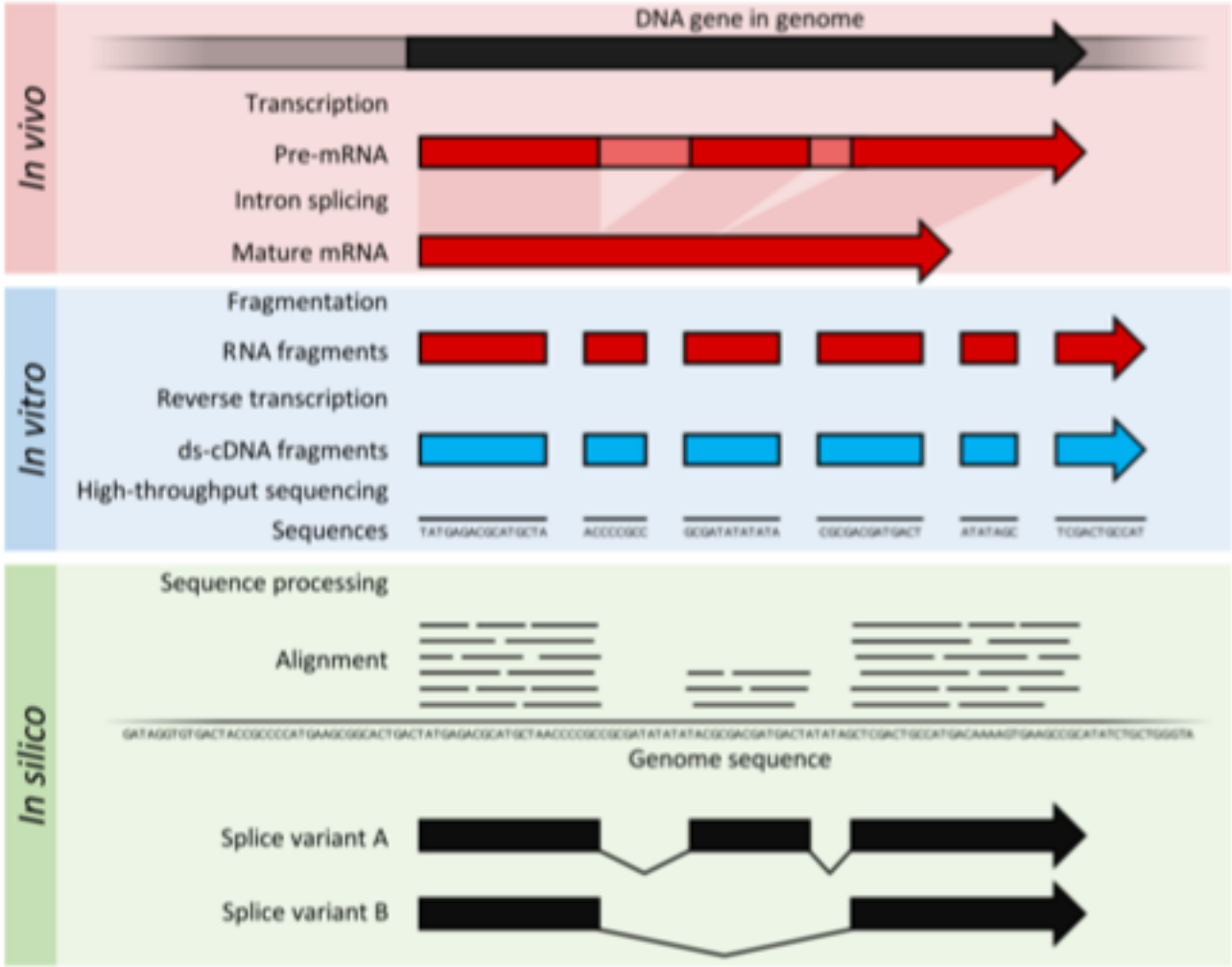
Lowe et al., 2017

DNA microarray

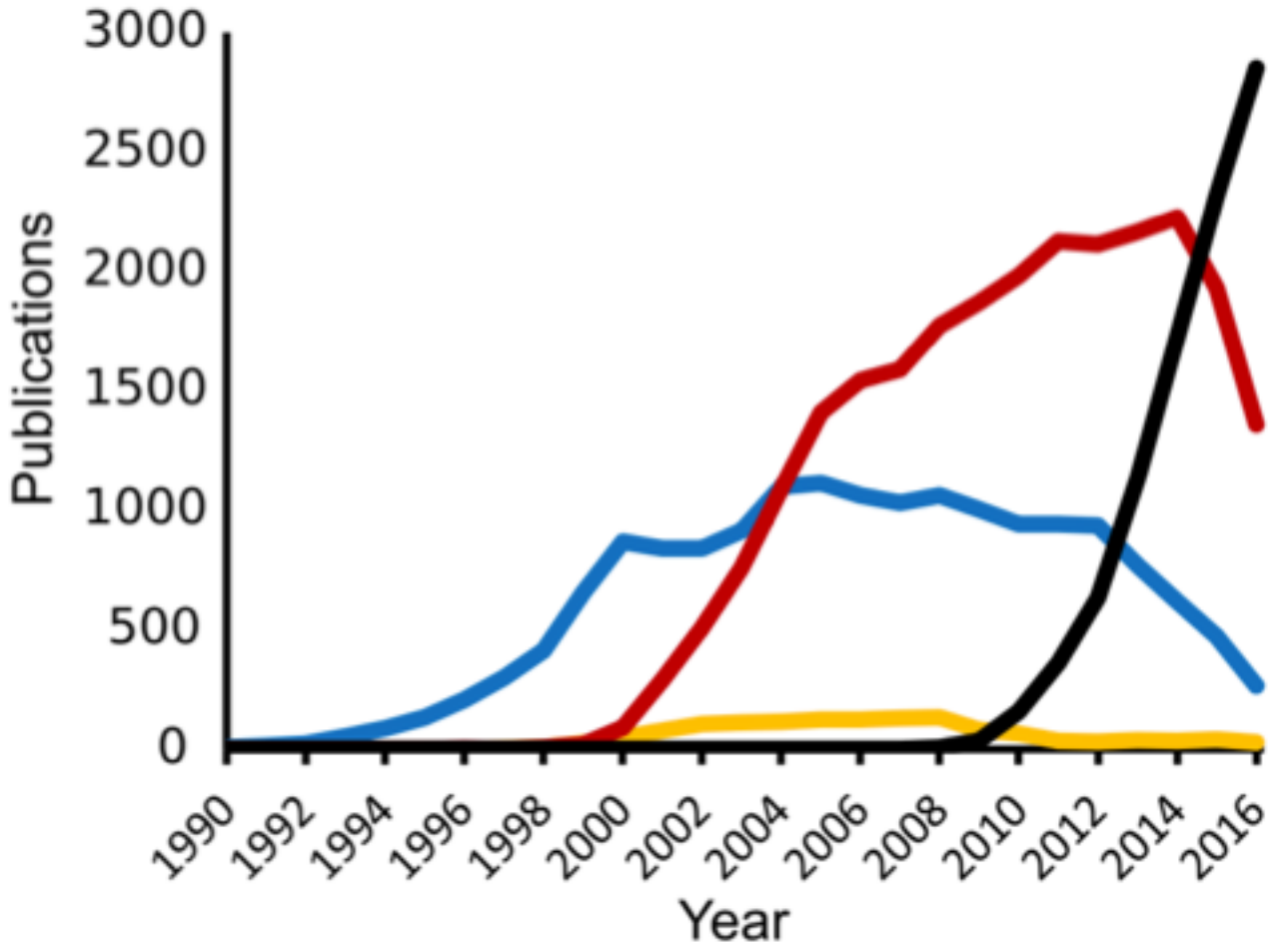


Lowe et al., 2017

RNA sequencing



Lowe et al., 2017



Lowe et al., 2017

DNA Microarray: an orderly arrangement of DNA sequences on a small solid support, usually a membrane or glass slide, used to quickly survey the simultaneous expression of many genes. A sample containing DNA or RNA is placed in contact with the gene chip. Complementary base pairing between the sample and the gene sequences on the chip produces light that is measured.

✓ **glass DNA microarray:** micro-spotting of pre-fabricated cDNA fragments on a glass slide

✓ **high-density oligonucleotide microarray ("chip"):** *in situ* oligonucleotide synthesis

Different technologies: Affymetrix, Combimatrix, NimbleGen, Agilent, OpArray

Microarray: transcriptional profiling, copy-number variation, SNP genotyping and DNA-protein interaction

RNAseq: deep sequencing of RNA (RNASeq) reverse-transcribed to complementary DNA for measuring RNA expression and detecting changes in RNA structure (Goldman and Domschke, 2014).

RNAseq: measuring gene expression

differential expression

novel transcripts

splicing junction analysis

de novo assembly

SNP analysis

allele specific expression

RNA editing

small/microRNAs

Table 2. Sequencing technology platforms commonly used for RNA-Seq [72][73].

Platform (Manufacturer)	Commercial release	Typical read length	Maximum throughput per run	Single read accuracy	RNA-Seq runs deposited in the NCBI SRA (Oct 2016) [74]
454 (Roche, Basel, Switzerland)	2005	700 bp	0.7 Gbp	99.9%	3548
Illumina (Illumina, San Diego, CA, USA)	2006	50–300 bp	900 Gbp	99.9%	362903
SOLiD (Thermo Fisher Scientific, Waltham, MA, USA)	2008	50 bp	320 Gbp	99.9%	7032
Ion Torrent (Thermo Fisher Scientific, Waltham, MA, USA)	2010	400 bp	30 Gbp	98%	1953
PacBio (Pacbio, Menlo Park, CA, USA)	2011	10,000 bp	2 Gbp	87%	160

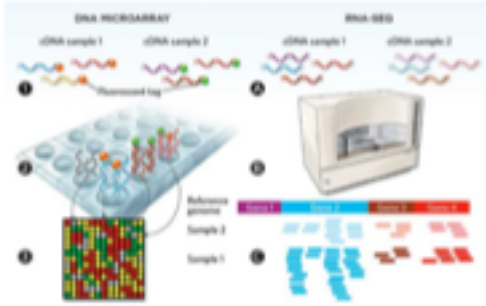
NCBI, National Center for Biotechnology Information; SRA, Sequence Read Archive; RNA-Seq, RNA sequencing.

https://doi.org/10.1371/journal.pcbi.1005457.t002

Lowe et al., 2017

Microarray *vs* RNAseq

2014: 54,000 samples analyzed through microarray were deposited into the Gene Expression Omnibus (GEO) database, compared to data from just around 9,000 samples analyzed using RNA-seq (Su *et al*., 2014).

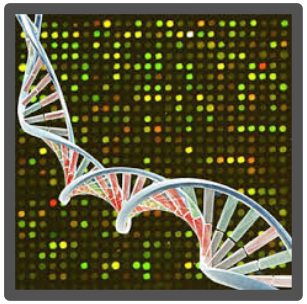


RNAseq advantages:

- ✓ very low background signal
- ✓ higher dynamic range of expression level
- ✓ more accurate in term of fold change values
- ✓ can reveal previously uncharacterized transcripts,
- ✓ gene fusions and genetic polymorphisms

but….

Microrrays allow analyzing large numbers of samples rapidily and methods for data analysis are fully mature straightforward (Su *et al*., 2014).



Method	RNA-Seq	Microarray
Throughput	High [10]	Higher [10]
Input RNA amount	Low ~ 1 ng total RNA [25]	High ~ 1 µg mRNA [26]
Labour intensity	High (sample preparation and data analysis) [10][23]	Low [10][23]
Prior knowledge	None required, though genome sequence useful [23]	Reference transcripts required for probes [23]
Quantitation accuracy	~90% (limited by sequence coverage) [27]	>90% (limited by fluorescence detection accuracy) [27]
Sequence resolution	Can detect SNPs and splice variants (limited by sequencing accuracy of ~99%) [27]	Dedicated arrays can detect splice variants (limited by probe design and cross-hybridisation) [27]
Sensitivity	10^{-6} (limited by sequence coverage) [27]	10^{-3} (limited by fluorescence detection) [27]
Dynamic range	$>10^{5}$ (limited by sequence coverage) [28]	10^{3}–10^{4} (limited by fluorescence saturation) [28]
Technical reproducibility	>99% [29][30]	>99% [31][32]

Lowe et al., 2017

Microarray *vs* RNAseq

Which technology?

- ✓ number of gene evaluated
- ✓ accuracy
- ✓ sensitivity
- ✓ data interpretation
- ✓ cost

RESEARCH Open Access

An investigation of biomarkers derived from legacy microarray data for their utility in the RNA-seq era

Nucleic Acids Research Advance Access published June 30, 2015

RESEARCH ARTICLE

RNA-seq and microarray in transcriptome profilir

A nested parallel experiment demonstrates differences in intensity-dependence between RNA-seq and microarrays

David G. Robinson, Jean Y. Wang and John D. Storey

nature biotechnology

Comparative RNA-Seq and Microarray Analysis of Gene Expression Changes in B-Cell Lymphomas of *Canis*

Review Cell

The concor data depen abundance

PLOS

Comparison of RNA-Seq and Microarray in Transcriptome Profiling of Activated T Cells

Shanrong Zhao, Wai-Ping Fung-Leung, Anton Bittner, Karen Ngo, Xuejun Liu

iparing microarrays and next-eration sequencing technologies nicrobial ecology research

oon Roh, Guy C.J. Abell, Kyoung-Ho Kim, Young-Do Nam and Bae

Microarray *vs* RNAseq

…a more comprehensive picture of a transcriptome applying multiple profiling methods…..

The choice of technology depends on the aim

Public databases

Four main categories

✓ primary archives: data obtained from different high-throughput technologies (ArrayExpress and GEO)

✓ added-val… n user-based inte…

✓ topical da…

✓ integrativ… otation, pathways …

Table 5. Transcriptomic databases.

Name	Host	Data	Description
Gene Expression Omnibus [142]	NCBI	Microarray RNA-Seq	First transcriptomics database to accept data from any source. Introduced MIAME and MINSEQE community standards that define necessary experiment metadata to ensure effective interpretation and repeatability [143][144].
ArrayExpress [145]	ENA	Microarray	Imports datasets from the Gene Expression Omnibus and accepts direct submissions. Processed data and experiment metadata are stored at ArrayExpress, while the raw sequence reads are held at the ENA. Complies with MIAME and MINSEQE standards [144] [145].
Expression Atlas [146]	EBI	Microarray RNA-Seq	Tissue-specific gene expression database for animals and plants. Displays secondary analyses and visualisation, such as functional enrichment of Gene Ontology terms, InterPro domains, or pathways. Links to protein abundance data where available.
Genevestigator [147]	Privately curated	Microarray RNA-Seq	Contains manual curations of public transcriptome datasets, focusing on medical and plant biology data. Individual experiments are normalised across the full database, to allow comparison of gene expression across diverse experiments. Full functionality requires licence purchase, with free access to a limited functionality.
RefEx [148]	DDBJ	All	Human, mouse, and rat transcriptomes from 40 different organs. Gene expression visualised as heatmaps projected onto 3D representations of anatomical structures.
NONCODE [149]	noncode.org	RNA-Seq	ncRNAs excluding tRNA and rRNA.

DDBJ, DNA Data Bank of Japan; EBI, European Bioinformatics Institute; ENA, European Nucleotide Archive; MIAME, Minimum Information About a Microarray Experiment; MINSEQE, Minimum Information about a high-throughput nucleotide SEQuencing Experiment; NCBI, National Center for Biotechnology Information; ncRNAs, noncoding RNAs; RNA-Seq, RNA sequencing.

Data analys…

✓ data analy… ntial to provide th…

✓ the quality of data influences the quality of the outcome but it also true that the best quality of data in unlikely surrender insights without appropriate data analysis

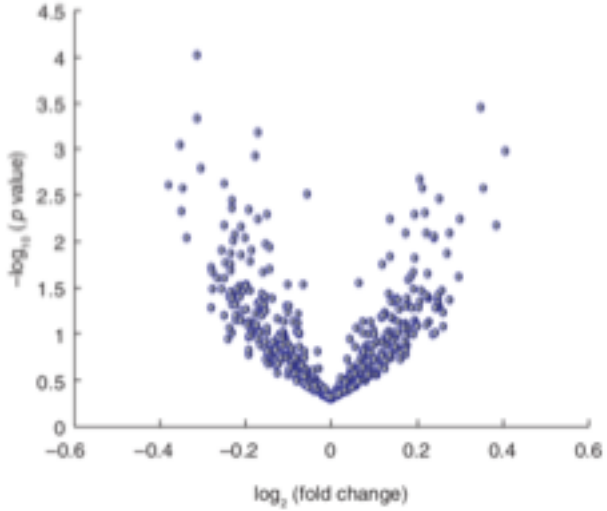
Data analysis

To assess the statistical significance of the results, multiple biological replicates for each cell type or treatment are needed.

$m_a >> m_b$ (transcript up-regulated) or $m_a << m_b$ (down-regulated)

The pertinent question: "how confidently can this transcript be called differentially expressed?"

- ✓ Student's t-test (normal distribution)
- ✓ Wilcoxon rank sum test or the SAM (Significance Analysis of Microarrays; Tusher *et al.*, 2001)



Hung and Weng, 2017

Data analysis

✓ multiple testing correction: statistical methods for correcting statistical confidence estimates based on the number of tests performed

✓ these test control the false discovery rate (FDR): the percentage of prediction that are false positive

✓ multivariate analyses: principal component analysis (PCA) and Orthogonal Projections to Latent Structures Discriminant Analysis (OPLS-DA)

Data analysis

✓ RNA-sequencing data take the form of counts, so model based on the Gaussian distribution are unsuitable

✓ normalization is challenging because different sequencing experiments may generate quite different total number of reads

✓ quality control (*e.g.* analysis of sequence quality, GC content, the presence of adaptors, overrepresented *k*-mers and duplicated reads in order to detect sequencing errors, PCR artefacts or contaminations)

✓ read alignment (mapping quality parameters: percentage of mapped reads and uniformity of read coverage on exons and the mapped strand)

✓ metrics for gene and transcript expression: RPKM (reads per kilobase of exon model per million reads); is a within-sample normalization method removing the effects of feature-length and library size

✓ identification of differentially expressed transcripts: Poisson distribution and negative binomial distribution (discrete probability distributions)

Gene coexpression networks

- ✓ the correlation in expression pattern between pairs of genes is measured, and those exhibiting strong correlations are “joined” in a graphical representation to create a network, which can be visualized with graph network viewers

- ✓ cross-level correlation is an area that will become more important as genome wide association studies (GWAS) could be used to link genotype to environmental factors or perturbations through changes in the transcriptome, epigenome, or other ‘omes of a plant

- ✓ 4 components are necessary for co-expression analysis: collection of gene expression profile from different samples and/or different perturbation, a method for computing expression pattern similarity, a way for assessing the degree of significance of expression pattern similarity and a tool to visualize and analyze statistically significant coexpression patterns

- ✓ metrics: Pearson’s correlation coefficient (PCC) and Spearman’s (Rank) correlation coefficient

Gene coexpression networks

	URL and comments
Correlation network DB	
ATTED-II (Obayashi et al., 2009)	http://atted.jp/: explore condition-independent coexpression networks for up to 100 genes in Arabidopsis using the NetworkDrawer tool . Coexpression analyses may also be performed for rice.
CressExpress (Srinivasasainagendra et al., 2008)	http://cressexpress.org: generate condition-independent coexpression analyses or custom condition-dependent coexpression analyses for Arabidopsis with up to 30 genes. Results are easily imported into Cytoscape for visualization.
PlaNet (Mutwil et al., 2011)	http://aranet.mpimp-golm.mpg.de/: use this tool to explore condition-independent coexpression networks in seven plant species. Networks are displayed as static SVG images, but networks may also be downloaded for easy viewing and further manipulation in Cytoscape or Pajek.
CSB.DB (Steinhauser et al., 2004)	http://csbdb.mpimp-golm.mpg.de/: use this tool to explore both condition-independent or condition-dependent coexpression networks in Arabidopsis for up to 60 genes. Networks may be immediately viewed as images, or downloaded for further manipulation into the visualization tools below.
GeneMANIA (Mostafavi et al., 2008)	http://genemania.org/: this tool allows functional network generation in Arabidopsis based on user-selected or default expression data sets, protein–protein interactions, subcellular localization, shared protein domains, etc. Results are easily visualized via an embedded Cytoscape Web (Lopes et al., 2010) application.
SeedNet (Bassel et al., 2011)	http://bnw.cs.nott.ac.uk/arabidopsis/: explore condition specific (i.e., seed-expressed) gene networks from Arabidopsis in a custom network explorer.
AraNet (Lee et al., 2010)	http://www.functionalnet.org/aranet/: like GeneMANIA this tool allows functional network generation in Arabidopsis. Results may be visualized via activation of Cytoscape Web.
Network visualization tools	
Cytoscape (Shannon et al., 2003; Kohl et al., 2011)	http://www.cytoscape.org/: use this powerful open-source desktop tool to visualize coexpression and other networks, such as those generated by protein–protein interaction studies. Nodes and edges may be appended with additional, user-defined information.
Biolayout (Theocharidis et al., 2009)	http://biolayout.org/: the current iteration of this desktop tool, Biolayout *Express*[3D], permits visualization of coexpression and other networks in three-dimensional space. Cytoscape "sif" files may be imported into the tool.
Pajek (Batagelj and Mrvar, 1998)	http://pajek.imfm.si/doku.php

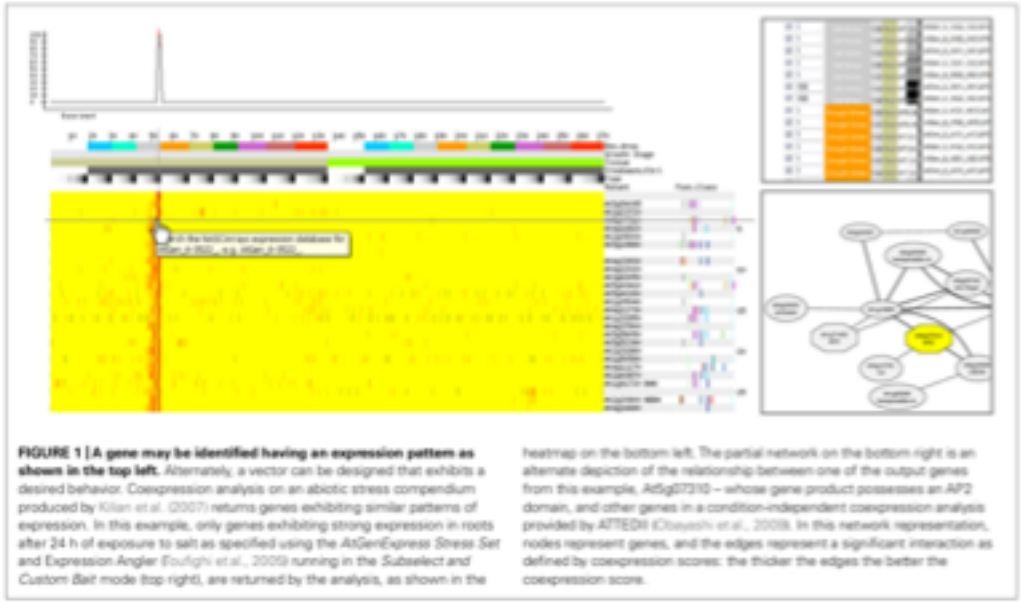
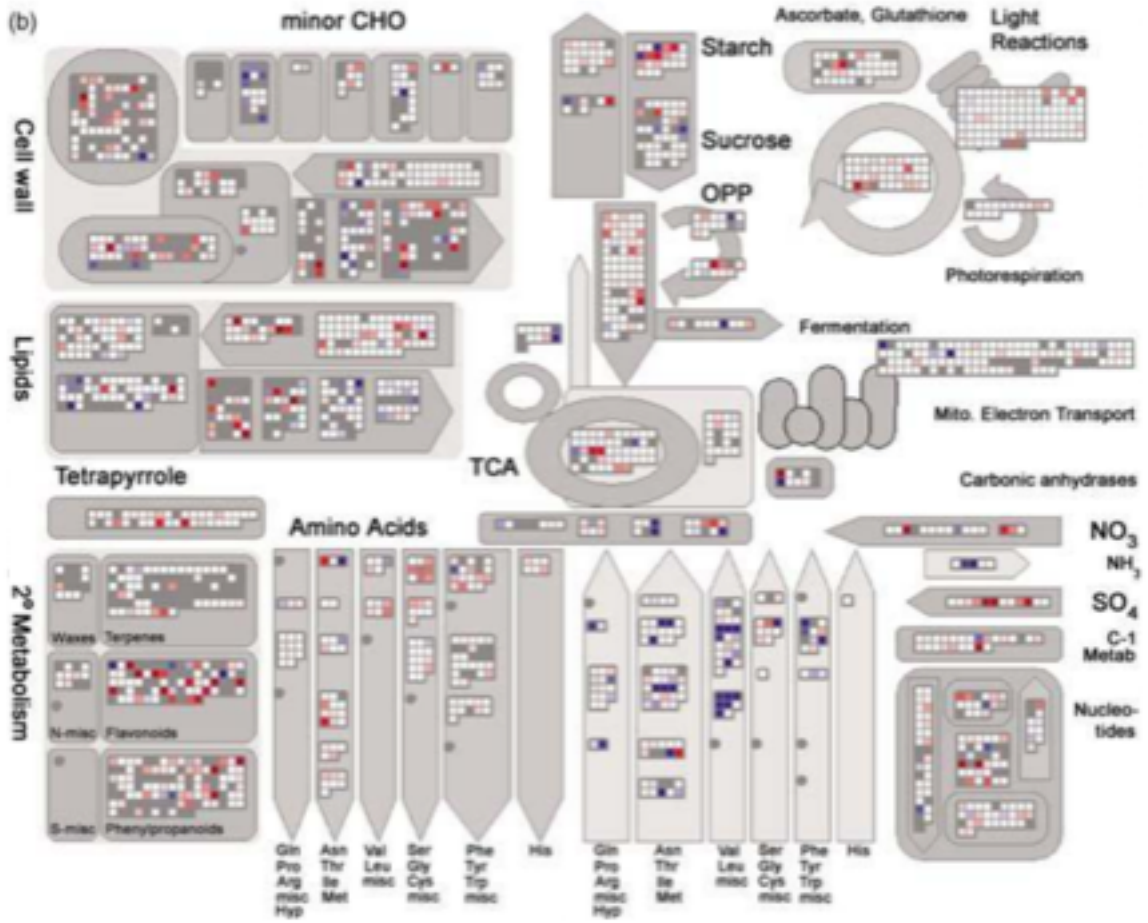


FIGURE 1 | A gene may be identified having an expression pattern as shown in the top left. Alternately, a vector can be designed that exhibits a desired behavior. Coexpression analysis on an abiotic stress compendium produced by Kilian et al. (2007) returns genes exhibiting similar patterns of expression. In this example, only genes exhibiting strong expression in roots after 24 h of exposure to salt as specified using the *AtGenExpress Stress Set* and Expression Angler (Toufighi et al., 2005) running in the *Subselect and Custom Bait* mode (top right), are returned by the analysis, as shown in the heatmap on the bottom left. The partial network on the bottom right is an alternate depiction of the relationship between one of the output genes from this example, At5g07310 – whose gene product possesses an AP2 domain, and other genes in a condition-independent coexpression analysis provided by ATTED-II (Obayashi et al., 2009). In this network representation, nodes represent genes, and the edges represent a significant interaction as defined by coexpression scores: the thicker the edges the better the coexpression score.

***Provat** et al., 2012*

Data visualization

✓ MapMan: for displaying omics data onto diagrams of metabolic pathways or other processes.



Thimm et al., 2004

Gene Ontology (GO)

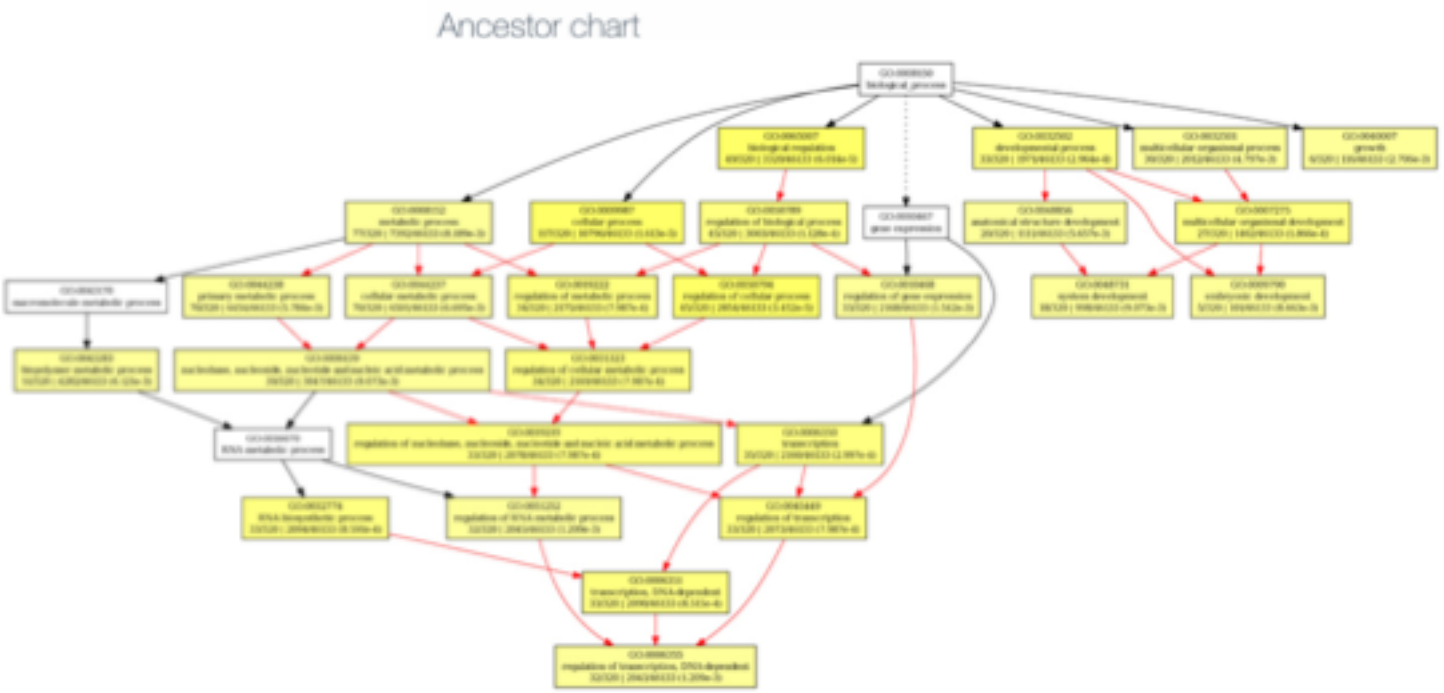
✓ GO project provides the most comprehensive resource currently available for computable knowledge regarding the functions of genes and gene products

✓ **Gene Ontology**: provides the logical structure of the biological functions ('terms') and their relationships to one another, manifested as a directed acyclic graph

✓ the corpus of **GO annotations**, evidence-based statements relating a specific gene product to a specific ontology term

✓ GO: classifies functions along three aspects a) **molecular function** (molecular activities of gene products) b) **cellular component** (where gene products are active) c) **biological process** (pathways and larger processes made up of the activities of multiple gene products)

Gene Ontology (GO)



GO enrichment analysis: analysis performed using a gene set; this analysis allow to identify GO terms over-represented or under-represented using the annotation for that gene set (p-value)

http://www.geneontology.org/

Responses to biotic stresses

Polesani et al. BMC Genomics 2010, 11:117
http://www.biomedcentral.com/1471-2164/11/117

BMC Genomics

RESEARCH ARTICLE Open Access

General and species-specific transcriptional responses to downy mildew infection in a susceptible (*Vitis vinifera*) and a resistant (*V. riparia*) grapevine species

Marianna Polesani[1], Luisa Bortesi[1], Alberto Ferrarini[2], Anita Zamboni[2], Marianna Fasoli[2], Claudia Zadra[2], Arianna Lovato[1], Mario Pezzotti[1], Massimo Delledonne[2], Annalisa Polverari[1*]

Combimatrix chip
24,571 transcripts, *Vitis vinifera* Gene Index release 5.0 (19062 probes) + genomic sequences produced by the International Grape Genome Project that were not already represented by the tentative consensus
Probe: 35-40 nt (3 probe for each transcript)

- ✓ downy mildew is a destructive grapevine disease caused by *Plasmopara viticola* (Berk. and Curt.) Berl. and de Toni, which can only be controlled by intensive fungicide treatments
- ✓ *Plasmopara viticola* is an obligate pathogen that obtains nutrients from infected plant cells through specialized structures known as haustoria
- ✓ signals and effectors involved in resistance in this important crop species were not well understood
- ✓ early transcriptional changes associated with *Plasmopara viticola* infection in susceptible *Vitis vinifera* and resistant *Vitis riparia* plants were analyzed

Responses to biotic stresses

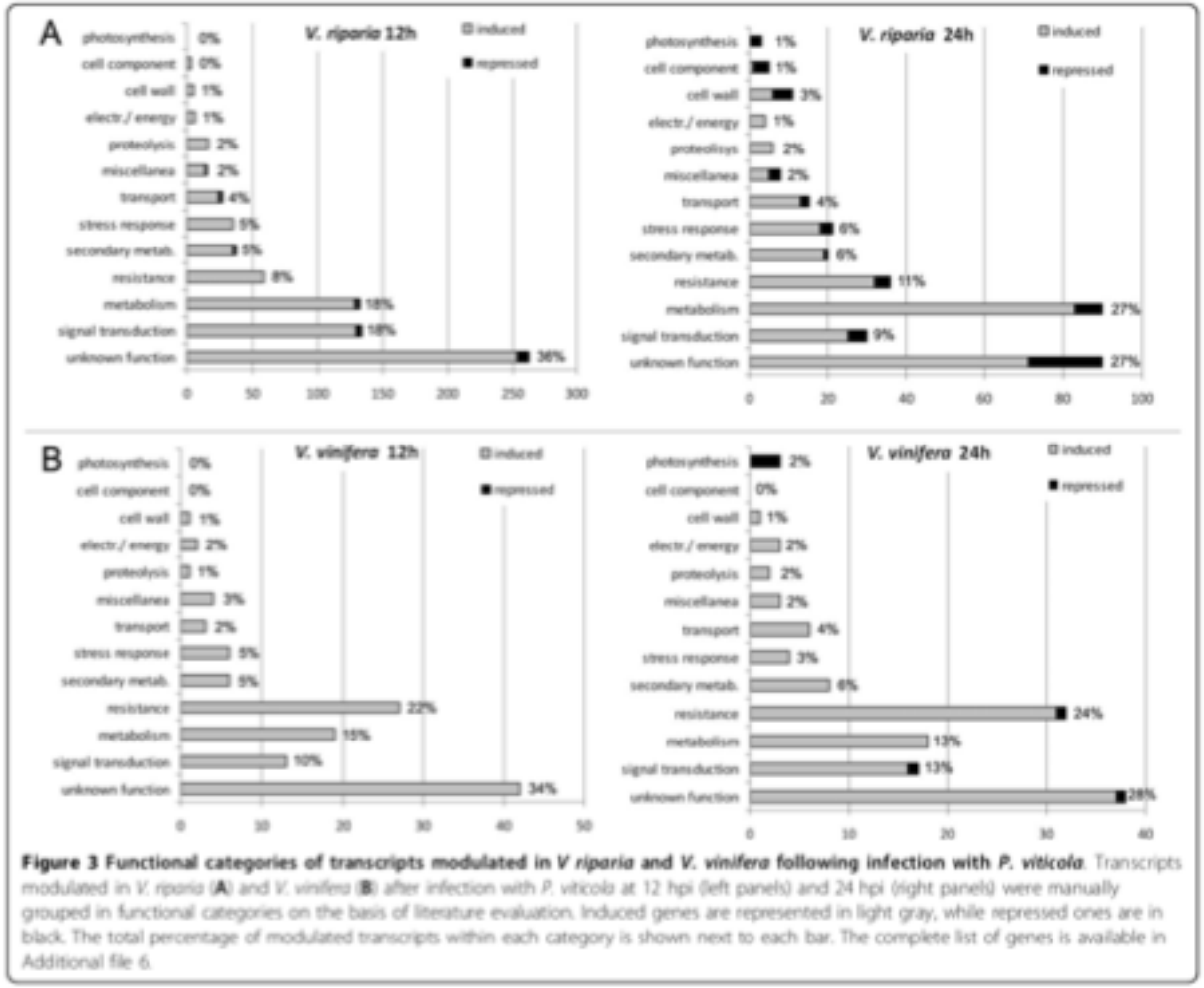


Figure 3 Functional categories of transcripts modulated in *V riparia* and *V. vinifera* following infection with *P. viticola*. Transcripts modulated in *V. riparia* (**A**) and *V. vinifera* (**B**) after infection with *P. viticola* at 12 hpi (left panels) and 24 hpi (right panels) were manually grouped in functional categories on the basis of literature evaluation. Induced genes are represented in light gray, while repressed ones are in black. The total percentage of modulated transcripts within each category is shown next to each bar. The complete list of genes is available in Additional file 6.

Responses to biotic stresses

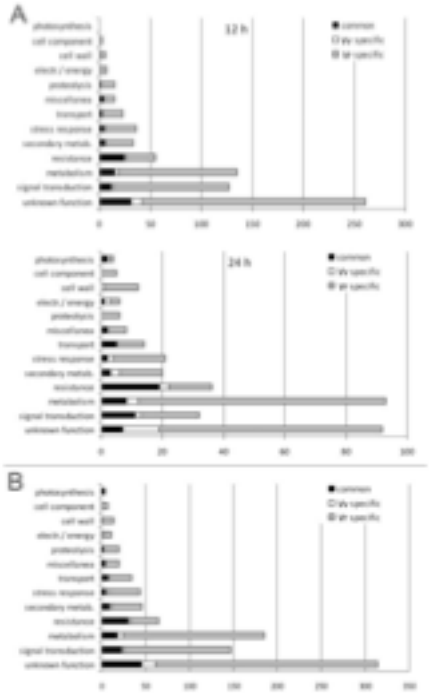


Figure 4 Specificity of transcriptional changes in infected *V. vinifera* and *V. riparia* within selected functional categories. A. Proportion of transcripts modulated in *V. vinifera* (Vv) or *V. riparia* (Vr) or in both species at either 12 (upper panel) or 24 hpi (lower panel). **B.** Proportion of transcripts modulated in *V. vinifera* (Vv) or *V. riparia* (Vr) or in both species considering either time point collectively.

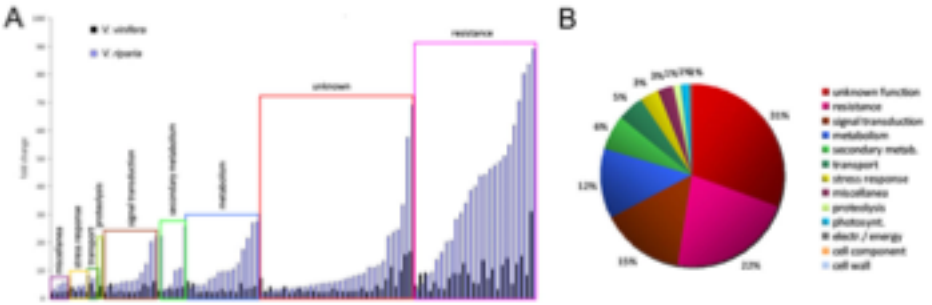


Figure 5 Common transcriptional changes in *V. vinifera* and *V. riparia* following infection with *P. viticola*. A. Intensity of the transcriptional changes of 'common' genes in *V. riparia* and *V. vinifera* at 12 hpi. Each functional category is shown in a different color. **B.** Distribution of the 147 'common' genes, modulated in both species at one or both time points, into functional categories.

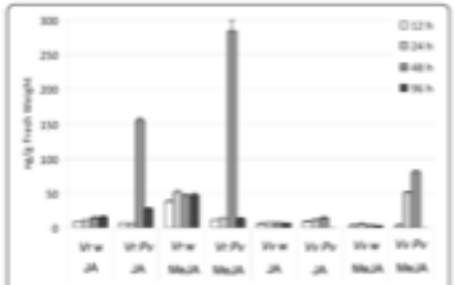


Figure 6 Endogenous levels of jasmonic acid and MeJA in *V. riparia* (Vr) and *V. vinifera* (Vv). Measurements were taken using leaf samples collected at 12, 24, 28 and 96 hpi with *P. viticola* (Pv) or on the mock-inoculated control samples (w) at the corresponding time-points. Values are the average of three measurements, with standard errors.

Responses to biotic stresses

- ✓ data strongly support the view that resistance in *Vitis riparia* is a post-infection phenomenon, characterized by a rapid wave of signal transduction (12 hpi) followed by a shift in primary and secondary metabolism (24 hpi) to implement a defense mode

- ✓ early transcriptional changes in *Vitis vinifera* indicate a weak and abortive defense response and do not provide information about the possible downregulation of resistance mechanisms by pathogen effectors, which might occur later on

- ✓ basal levels of defense gene expression in the two species do not seem to be responsible for the different infection outcomes

- ✓ the upregulation of genes involved in jasmonic acid biosynthesis and the increase in jasmonate levels indicate that this hormone may play a role in *Vitis riparia* resistance against *Plasmopara viticola*

Response to environmental stress: nutrient availability

Essential elements for plant growth: for higher plants, the essentiality of 14 elements is now well established, although the requirement for the micronutrients Cl and Ni is as yet restricted to a limited number of plant species

three criteria to be an essential elements:

a) given plant must be unable to complete its lifecycle in the absence of the element

b) the function of the element must not be replaceable by another element

c) the element must be directly involved in plant metabolism – for example, as a component of an essential plant constituent such as an enzyme – or it must be required for a distinct metabolic step such as an enzyme reaction

Classification in 4 group

TABLE 1.2 Classification of plant nutrients

Nutrient	Uptake	Biochemical functions
Group 1		
C, H, O, N, S	as CO_2, HCO_3^-, H_2O, O_2, NO_3^-, NH_4^+, N_2, SO_4^{2-}, SO_2 ions from the soil solution, gases from the atmosphere	Major constituents of organic material. Essential elements of atomic groups involved in enzymatic processes. Assimilation by oxidation-reduction reactions.
Group 2		
P, B, Si	as phosphates, boric acid or borate, silic acid from the soil solution	Esterification with alcohol groups. Phosphate esters involved in energy transfer reactions.
Group 3		
K, Na, Ca, Mg, Mn, Cl	as ions from the soil solution	Non-specific functions establishing osmotic potential. More specific functions for optimal confirmation of enzymes (enzyme activation). Bridging of reaction partners. Balancing anions. Controlling membrane permeability and electrochemical potentials.
Group 4		
Fe, Cu, Zn, Mo	as ions or chelates from the soil solution	In chelated form in prosthetic groups of enzymes. Enable electron transport by valency change.

From Mengel and Kirkby (2001) with kind permission from Springer Science Business Media.

TABLE 1.3 Average concentrations of mineral elements in plant shoot dry matter sufficient for adequate growth

Element	Chemical symbol	µmol g^{-1} dw	mg kg^{-1}
Molybdenum	Mo	0.001	0.1
Nickel	Ni	0.001	0.1
Copper	Cu	0.1	6
Zinc	Zn	0.3	20
Manganese	Mn	1.0	50
Iron	Fe	2.0	100
Boron	B	2.0	20
Chlorine	Cl	3.0	100
Sulphur	S	30	1,000
Phosphorus	P	60	2,000
Magnesium	Mg	80	2,000
Calcium	Ca	125	5,000
Potassium	K	250	10,000
Nitrogen	N	1,000	15,000

From Epstein (1965), Epstein and Bloom (2005), Brown *et al.* (1987b).

Normally, these minerals are taken up by plant roots from the soil solution in ionic form with the metal Ca^{2+}, Mg^{2+} and K^+ present as free cations, P and S as $H_2PO_4^-/HPO_4^{2-}$ and sulfate (SO_4^{2-}) and N as anionic nitrate (NO_3^-) or cation ammonium (NH_4^+).

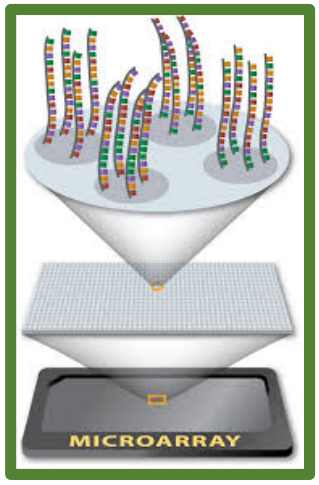
Microarray and plant nutrition

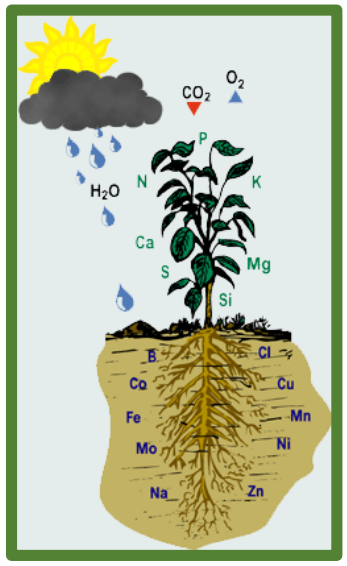

NEWS FEATURE FOOD

AN UNDERGROUND REVOLUTION

Plant breeders are turning their attention to roots to increase yields without causing environmental damage. **Virginia Gewin** unearths some promising subterranean strategies.

In the last ten years high-throughput gene expression analyses (in particular microarray) have been applied in order to have a picture of molecular changes in response to a nutritional condition.



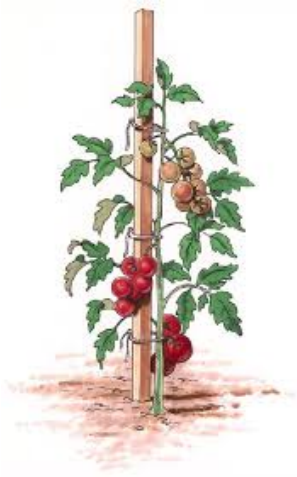


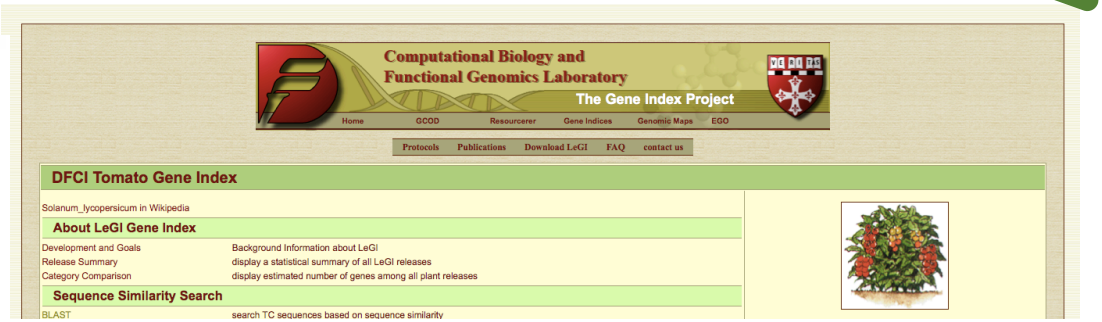
Fe

Tomato Array 2.0

(25,789 transcripts, DFCI Tomato Gene Index, Release 12.0)

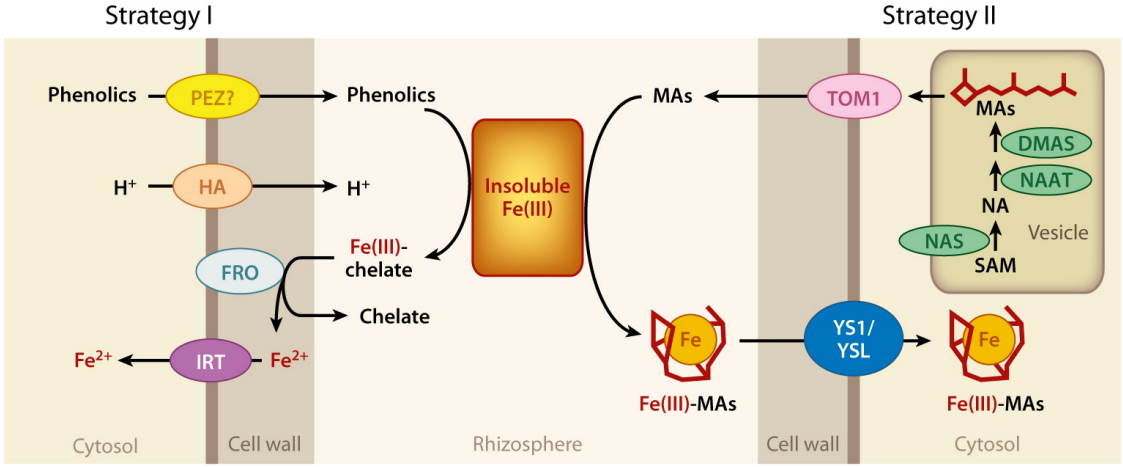
Probe: 35-40 nt (3 probes for each transcript)





Fe and plants

- ✓ Fe is an essential nutrient for plants, which catalyzes crucial cellular functions such as chlorophyll synthesis, chloroplast development, and antioxidative cell protection
- ✓ despite being abundant in soils, Fe mainly exists as the insoluble, not available to plants, ferric Fe(III) form; solubility of Fe is, however, extremely low, especially in aerated alkaline soils. In aerated systems in the physiological pH range, the concentrations of ionic Fe(III) and Fe(II) are below 10^{-15} M due to formation of Fe hydroxides, oxyhydroxides and oxides
- ✓ plants have developed two separate strategies to acquire Fe(III) from soils



Kobayashi T, Nishizawa NK. 2012.
Annu. Rev. Plant Biol. 63:131–52

Zamboni et al. BMC Genomics 2012, 13:101
http://www.biomedcentral.com/1471-2164/13/101

BMC Genomics

RESEARCH ARTICLE Open Access

Genome-wide microarray analysis of tomato roots showed defined responses to iron deficiency

Anita Zamboni[1*], Laura Zanin[2†], Nicola Tomasi[2], Mario Pezzotti[1], Roberto Pinton[2], Zeno Varanini[2*] and Stefano Cesco[3]

Table 1 Leaf SPAD index values and root Fe^{III}-chelate reductase activity

Sample	SPAD index[a]	Fe^{III}-chelate reductase (mol g^{-1} root FW h^{-1})[b]
Fe-sufficient	29.5 ± 0.3	0.37 ± 0.04
Fe-deficient	16.8 ± 0.6	1.41 ± 0.06

[a]SPAD index value of fully expanded young leaves was determined using a SPAD-502 meter (Minolta, Osaka, Japan); mean and SD using data of the three biological replicates.
[b]Mean and SD of three biological replicates.

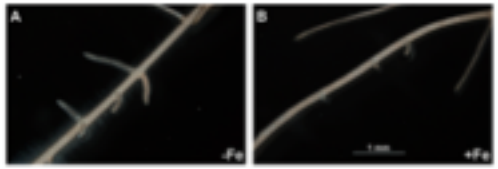


Figure 2 Root apparatus of tomato plants grown under different Fe-supply condition. Detail of root apparatus of **A**) Fe-deficient and **B**) Fe-sufficient plants.

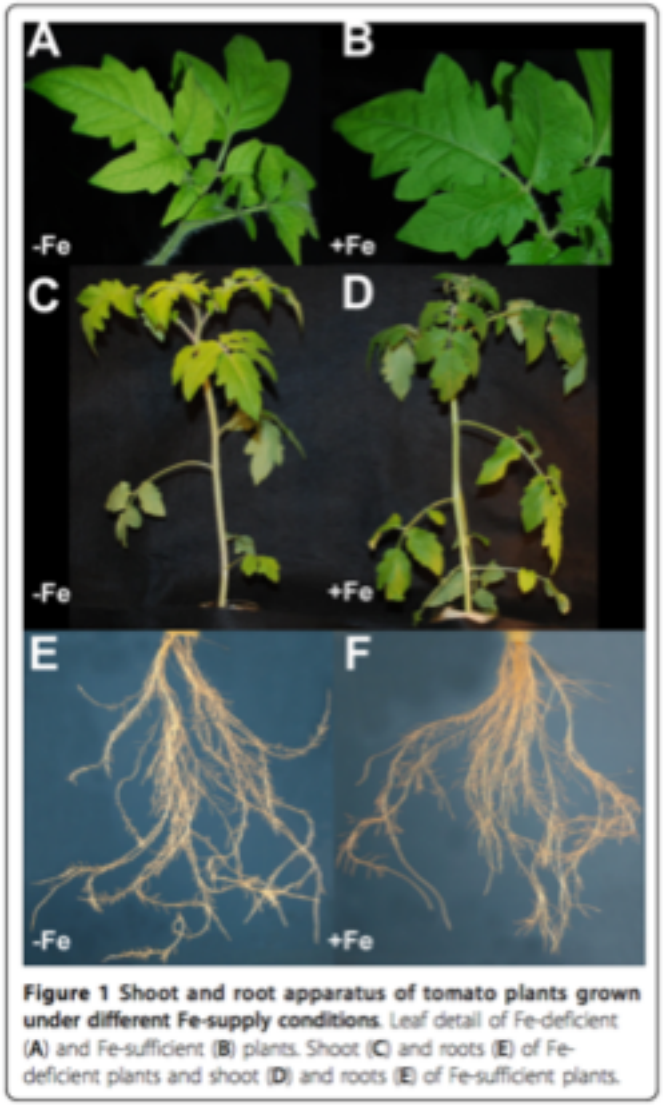


Figure 1 Shoot and root apparatus of tomato plants grown under different Fe-supply conditions. Leaf detail of Fe-deficient (**A**) and Fe-sufficient (**B**) plants. Shoot (**C**) and roots (**E**) of Fe-deficient plants and shoot (**D**) and roots (**E**) of Fe-sufficient plants.

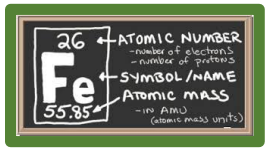
Fe-deficient *vs* Fe-sufficient

75 up- (↑) and **22** (↓) down-regulated transcripts in roots (LIMMA, adjusted p-value ≤ 0.05; |FC| ≥ 2)

Our transcriptional results suggested that….

- ✓ tomato roots respond to Fe deficiency by modulating the expression of a number of transcripts similar to the model plant *Arabidopsis thaliana*
- ✓ tomato roots modulate transcripts involved in homeostasis of Fe and heavy metal cations (*e.g.* IRT, NRAMP, MTP, ferritin) and others cation (*e.g*. AMT)
- ✓ tomato, as *Arabidopsis*, requires the up-regulation of transcripts related to glycolysis (*e.g.* PFK) and methionine cycle (*e.g.* MTK), the latter pathway being putatively linked to NA biosynthesis in response to Fe deficiency
- ✓ tomato roots seem to be more characterized by root morphological adaptation, mainly linked to hair root production, as suggested by the strong up-regulation of extensin transcripts
- ✓ flavonoid biosynthesis and root morphological changes are revealed as specific tomato responses to Fe shortage

Response to supply with different natural Fe-chelates



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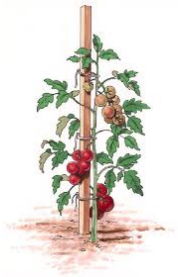
DOI 10.1186/s12864-015-2331-5

BMC Genomics

RESEARCH ARTICLE Open Access

Early transcriptomic response to Fe supply in Fe-deficient tomato plants is strongly influenced by the nature of the chelating agent

Anita Zamboni[1], Laura Zanin[2], Nicola Tomasi[2], Linda Avesani[1], Roberto Pinton[2], Zeno Varanini[1*] and Stefano Cesco[1]



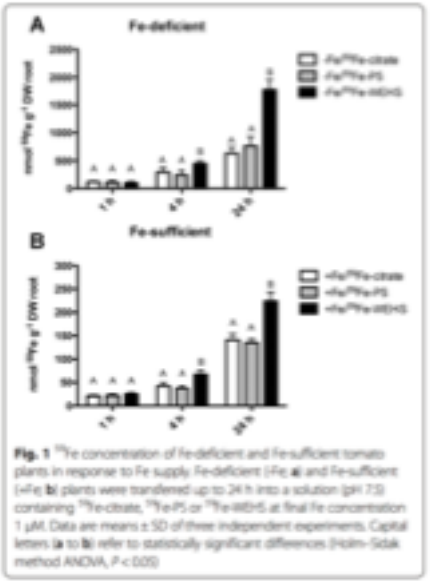


Fig. 1 ^{59}Fe concentration of Fe-deficient and Fe-sufficient tomato plants in response to Fe supply. Fe-deficient (-Fe; **a**) and Fe-sufficient (+Fe; **b**) plants were transferred up to 24 h into a solution (pH 7.5) containing ^{59}Fe-citrate, ^{59}Fe-PS or ^{59}Fe-WEHS at final Fe concentration 1 μM. Data are means ± SD of three independent experiments. Capital letters (**a** to **b**) refer to statistically significant differences (Holm–Sidak method ANOVA, $P < 0.05$)

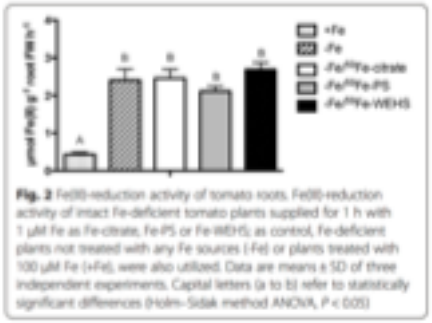


Fig. 2 Fe(III)-reduction activity of tomato roots. Fe(III)-reduction activity of intact Fe-deficient tomato plants supplied for 1 h with 1 μM Fe as Fe-citrate, Fe-PS or Fe-WEHS; as control, Fe-deficient plants not treated with any Fe sources (-Fe) or plants treated with 100 μM Fe (+Fe), were also utilized. Data are means ± SD of three independent experiments. Capital letters (a to b) refer to statistically significant differences (Holm–Sidak method ANOVA, $P < 0.05$)

Table 1 Number of differentially expressed transcripts resulted by transcriptional profile comparisons of Fe-deficient plants supplied with the three natural sources of Fe and Fe-deficient plants

Comparison	Upregulated transcript	Downregulated transcripts
-Fe/Fe-citrate *vs* -Fe	260	468
-Fe/Fe-PS *vs* -Fe	91	317
-Fe/Fe-WEHS *vs* -Fe	1	1

Differentially expressed transcripts were identified by each transcriptional profile comparison through LIMMA analysis (adjusted p-value ≤ 0.05; $|Log_2(R)| \geq 1$); -Fe: Fe-deficient; -Fe/Fe-citrate, -Fe/Fe-PS or -Fe/Fe-WEHS: Fe-deficient plants supplied for 1 h with Fe citrate, Fe-PS or Fe-WEHS, respectively

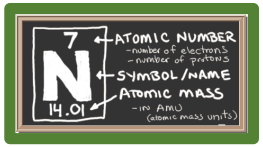
Fig. 3 Shared transcripts modulated in response to supply with the three natural Fe sources relative to Fe-deficient plants. Fe-deficient plants were supplied for 1 h with Fe-WEHS (-Fe/Fe-WEHS) or with Fe-PS (-Fe/Fe-PS) or with Fe-citrate (-Fe/Fe-citrate). As control, Fe-deficient plants were used (-Fe)

Response to supply with different natural Fe-chelates

Differentially expressed transcripts suggest:

- ✓ the root transcriptional response to Fe supply depends on the nature of the ligand (WEHS, citrate and PS)
- ✓ Fe-WEHS did not cause relevant changes in the root transcriptome with respect to the Fe-deficient plants, indicating that roots did not sense the restored cellular Fe accumulation
- ✓ the responses to supply with Fe-citrate and Fe-PS are fast and based on a back regulation of molecular mechanisms modulated under Fe deficiency
- ✓ citrate is also adsorbed by roots causing a negative regulation of the TCA cycle and influencing mainly cell wall metabolism and the response to stress
- ✓ Fe-PS specific responses seem to be mainly based on a negative regulation of lipid metabolism and phospholipid-based signal that control ROS responses in the presence of heavy metals

N (NO_3^-)

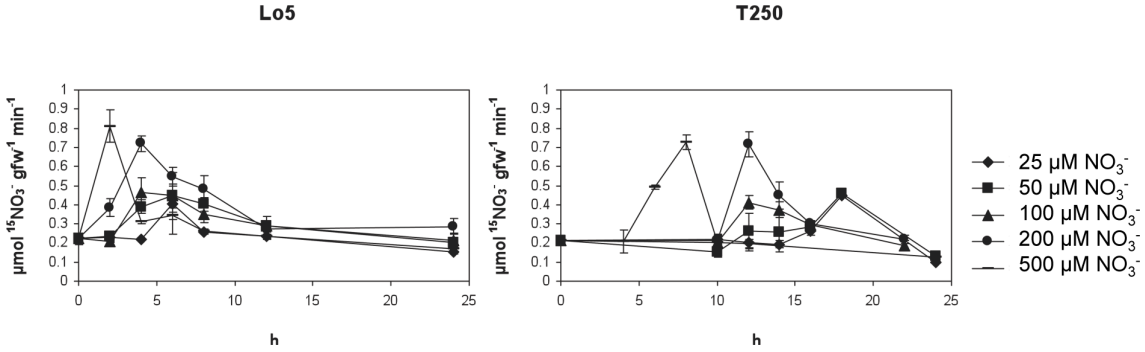


- ✓ N is the element required in largest amounts by plants; the major sources of N taken up by the roots of higher plants are NO_3^- and NH_4^+

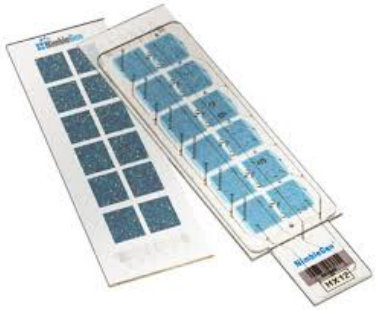
- ✓ plants cope with this rapid changes of NO_3^- concentration in soil solution increasing its uptake rate following the exposure of the roots to this anion; this type of response is known as “induction”

- ✓ roots possess at least three, kinetically distinct, NO_3^- transport systems (cHATS, iHATS and LATS); iHATS appears to play the key role in induction and NO_3^- uptake rates.

Two maize inbreed line with different nitrogen use efficiency (NUE)



N (NO_3^-)



59,756 transcripts predicted from the ZmB73 reference genome (Release 5b)

Probe: 60 nt

JIPB Journal of Integrative Plant Biology

Nitrate induction triggers different transcriptional changes in a high and a low nitrogen use efficiency maize inbred line

Anita Zamboni[1], Stefania Astolfi[2], Sabrina Zuchi[2], Youry Pii[3], Katia Guardini[1†], Paola Tononi[1] and Zeno Varanini[1*]

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Article

Differentially expressed transcripts

Parametric Analyis of Gene Set Enrichment (PAGE): AgriGO

GO Information					CM			1h			2h			4h		
No	GO Term	Onto	Number	Description	1	2	3	Z-score	Mean	FDRnbsp;	Z-score	Mean	FDRnbsp;	Z-score	Mean	FDRnbsp;
1	GO:0044267	P	496	cellular protein metabolic process				6.7	0.34	7.3e-10	-8.4	-0.4	2.9e-16	9.3	0.28	0
2	GO:0019538	P	564	protein metabolic process				6.5	0.32	1.5e-09	-8.4	-0.37	4.5e-16	8.7	0.24	0
3	GO:0009987	P	1443	cellular process				6	0.23	3e-08	-9.9	-0.26	1.3e-21	7.9	0.13	3.3e-14
4	GO:0044237	P	1140	cellular metabolic process				5.5	0.23	4.2e-07	-8.6	-0.25	1.8e-16	7.3	0.14	1.6e-12

GO Information					CM			5h			11h			12h		
No	GO Term	Onto	Number	Description	1	2	3	Z-score	Mean	FDRnbsp;	Z-score	Mean	FDRnbsp;	Z-score	Mean	FDRnbsp;
1	GO:0006807	P	206	nitrogen compound metabolic process				3.4	0.029	0.029	2.7	0.038	0.048	1.7	-0.09	0.27
2	GO:0044249	P	200	cellular biosynthetic process				3	0.012	0.029	2.1	-0.017	0.17	1	-0.15	0.6
3	GO:0006519	P	48	cellular amino acid and derivative metabolic process				2.9	0.15	0.029	3	0.37	0.022	2.3	0.2	0.074
4	GO:0006091	P	11	generation of precursor metabolites and energy				2.8	0.43	0.029	4.5	1.6	0.00025	4.2	1.5	0.00071
5	GO:0019538	P	160	protein metabolic process				-2.8	-0.27	0.029	-3.1	-0.54	0.022	-3.6	-0.64	0.0032
6	GO:0006464	P	111	protein modification process				-2.9	-0.3	0.029	-3.3	-0.64	0.016	-4.2	-0.79	0.00071
7	GO:0043412	P	112	macromolecule modification				-2.9	-0.31	0.029	-3.3	-0.63	0.016	-4	-0.77	0.00082
8	GO:0044238	P	452	primary metabolic process				-0.34	-0.14	0.79	-1.3	-0.3	0.49	-2.7	-0.42	0.036
9	GO:0044260	P	278	cellular macromolecule metabolic process				-0.18	-0.13	0.87	-1.7	-0.35	0.28	-2.8	-0.48	0.033
10	GO:0044267	P	129	cellular protein metabolic process				-2.2	-0.26	0.099	-2.4	-0.5	0.1	-3.1	-0.62	0.012
11	GO:0008152	P	661	metabolic process				-0.63	-0.14	0.64	-2	-0.32	0.17	-3.3	-0.43	0.0065
12	GO:0043170	P	319	macromolecule metabolic process				-0.85	-0.16	0.64	-2.6	-0.41	0.064	-3.5	-0.52	0.0047

23	GO:0006810	P	260	transport				-0.36	0.097	0.88	-4.4	-0.27	2.8e-05	0.94	0.028	0.5
24	GO:0051179	P	268	localization				-0.091	0.11	0.97	-4.5	-0.27	2.2e-05	0.92	0.027	0.5
25	GO:0010467	P	573	gene expression				1.3	0.15	0.54	-4.7	-0.17	9.9e-06	7.8	0.21	7.3e-14
26	GO:0006464	P	214	protein modification process				0.5	0.14	0.88	-5	-0.35	2.5e-06	1.4	0.054	0.29
27	GO:0043412	P	222	macromolecule modification				0.83	0.15	0.74	-5.2	-0.36	7.3e-07	1.8	0.071	0.17
28	GO:0044249	P	678	cellular biosynthetic process				2.1	0.17	0.12	-5.5	-0.19	2e-07	7.8	0.19	5.9e-14
29	GO:0009058	P	704	biosynthetic process				2.1	0.17	0.12	-5.6	-0.19	8.5e-08	7.6	0.19	1.7e-13
30	GO:0006259	P	36	DNA metabolic process				0.55	0.18	0.86	-2.1	-0.37	0.058	3.5	0.39	0.0014

Differentially expressed transcripts

Log_2(+N/-N) values of transcripts differentially expressed at all three sampling time points in T250 line

#	Probe_ID	UniProtID	Description	Log_2(+N/-N) 5 h	Log_2(+N/-N) 11 h	Log_2(+N/-N) 12 h
15	AC191113_2_FGT002	I1J2F1	Uncharacterized protein	1.45	2.07	1.72
16	AC198414_2_FGT001		no hits found	1.25	2.11	1.70
17	AC210731_3_FGT002	C5YVI5	Putative uncharacterized protein Sb09g028370	1.27	1.62	2.42
18	GRMZM2G000739_T01	B4F7W3	Putative uncharacterized protein	1.16	2.34	1.75
19	GRMZM2G000739_T02	B4F7W3	Putative uncharacterized protein	1.29	3.25	1.73
20	GRMZM2G001205_T01	B6TTL8	ZFP16-1			
21	GRMZM2G002498_T01		no hits found			
22	GRMZM2G004161_T03	B4F8C0	Putative uncharacterized protein			
23	GRMZM2G004161_T05	B4F8C0	Putative uncharacterized protein			
24	GRMZM2G007546_T02	B4FZR3	Putative uncharacterized protein			
25	GRMZM2G009223_T01	B6SRN7	Glucose-6-phosphate/phosphate translocator 2			
26	GRMZM2G016462_T01	B6STD0	Putative uncharacterized protei			
27	GRMZM2G017319_T01	Q75HN5	LOB domain protein 40 putative, expressed			
28	GRMZM2G020423_T02	B6SJJ4	Jasmonate-indu			
29	GRMZM2G020508_T01	B8A130	Putative			
30	GRMZM2G022538_T04	B6THF0	Putat			
31	GRMZM2G022538_T05	B6THF0	Putati			
32	GRMZM2G026532_T01	C5XBH7	Putative Sb02g03			
33	GRMZM2G035370_T04	C5Y9Z6	Putative u Sb06g0319			
34	GRMZM2G041980_T01	Q9ATN4	Aquaporin N			
35	GRMZM2G041980_T02	Q9ATN4	Aquaporin NI			
36	GRMZM2G041980_T03	Q9ATN4	Aquaporin NI			
37	GRMZM2G041980_T04	Q9ATN4	Aquaporin N			
38	GRMZM2G041980_T05	Q9ATN4	Aquaporin			
39	GRMZM2G041980_T06	Q9ATN4	Aquapori			
40	GRMZM2G046601_T01	B6UDS5	Glutami isozym			
41	GRMZM2G046601_T02	B6UDS5	Gluta isozy			
42	GRMZM2G046601_T03	B6UDS5	Glutamin			
43	GRMZM2G046601_T04	B6UDS5	Glutamine synt isozyme 5			
44	GRMZM2G047835_T01	Q2QM58	Cation/hydrogen exchan putative, expressed			
45	GRMZM2G056975_T02	Q9FXZ7	1-deoxy-D-xylulose 5-phospha reductoisomerase			
46	GRMZM2G065655_T04	B4FZ50	Serine/threonine protein kinase			
47	GRMZM2G067402_T01	Q9M593	Non-symbiotic hemoglobin			
48	GRMZM2G071704_T01	C5Y9Q9	Putative uncharacterized protein Sb06g018650			
49	GRMZM2G076075_T01	C0PAU7	Glucose-6-phosphate isomerase			
50	GRMZM2G076075_T02	C0PAU7	Glucose-6-phosphate isomerase			
51	GRMZM2G079381_T01	B6SY01	Ferredoxin--nitrite reductase	2.15	4.11	3.96
52	GRMZM2G079381_T02	B6SY01	Ferredoxin--nitrite reductase	2.20	3.78	3.96
53	GRMZM2G079381_T03	B6SY01	Ferredoxin--nitrite reductase	1.99	4.11	3.91
54	GRMZM2G079381_T04	B6SY01	Ferredoxin--nitrite reductase	2.29	4.53	3.82
55	GRMZM2G079381_T05	B6SY01	Ferredoxin--nitrite reductase	1.88	3.53	3.34
56	GRMZM2G080871_T02	C5XG72	Putative uncharacterized protein Sb03g043700	-1.36	-1.13	-1.98
57	GRMZM2G081554_T01	Q41771	Kaurene synthase A	-1.98	-1.32	-1.36
58	GRMZM2G081554_T06	Q41771	Kaurene synthase A	-2.54	-1.72	-1.90
59	GRMZM2G090114_T01	B6U0J5	Putative uncharacterized protein	2.17	1.80	2.57
60	GRMZM2G091656_T01	C5Y560	Putative uncharacterized protein Sb05g003820	-1.98	-1.58	-2.05
61	GRMZM2G093705_T01	C5X9H9	Putative uncharacterized protein Sb02g002880	-1.03	-1.23	-1.12
62	GRMZM2G098290_T03	B4FMX4	Glutamine synthetase. chloroplastic	1.67	3.74	3.19
63	GRMZM2G098925_T01	C5XZ31	Putative uncharacterized protein Sb09g023310	-1.32	-2.06	-2.33
64	GRMZM2G102959_T01	P17847	Ferredoxin--nitrite reductase. chloroplastic	1.57	3.55	3.28
	GRMZM2G105604_T01	P93628	Uroporphyrinogen III methyltransferase	2.19	5.30	4.79
	ZM2G105604_T02	P93628	Uroporphyrinogen III methyltransferase	1.20	1.92	2.10
			Ferredoxin-6. chloroplastic	1.38	2.42	2.38
			Putative uncharacterized protein Sb09g023310	-1.13	-1.45	-1.14
			Putative uncharacterized protein Sb02g025240	-1.11	-1.02	-1.05
			tative uncharacterized protein	-1.84	-2.19	-1.05
			ochrome P450 CYP71Y10	-1.20	-1.13	-1.19
			Factor	1.38	2.13	2.00
			protein	1.29	1.38	1.22
			erized protein	-1.06	-1.47	-1.11
				1.51	3.42	3.51
				-1.21	-1.27	-1.16
				-1.07	-1.90	-1.22
				1.59	4.18	3.43
			hosphate	-2.43	-2.48	-1.90
				1.72	3.72	3.58
			P72A124	-1.44	-2.02	-2.30
				-1.84	-1.71	-1.06
			SIZ1	-1.26	-1.15	-1.18
				-1.38	-1.79	-1.34
				1.13	3.32	3.32
				1.07	1.47	1.31
				1.32	1.63	1.50
				1.50	2.65	2.56
			e hydroxylase	1.07	3.00	2.22
			oid cleavage ygenase	-1.89	-2.38	-2.08
			tative uncharacterized protein 06g030720	1.71	1.00	1.14
			tative uncharacterized protein Sb02g022270	-1.17	-1.43	-1.21
			1-deoxy-D-xylulose 5-phosphate reductoisomerase	-1.98	-1.78	-1.63
			Putative uncharacterized protein	1.19	1.63	1.22
			Putative uncharacterized protein	1.13	1.71	1.14
	GRMZM5G828229_T01	B4FQK0	Putative uncharacterized protein	1.27	3.38	2.41
97	GRMZM5G830545_T03	C5XRD6	Putative uncharacterized protein Sb03g041580	1.10	1.73	1.78
98	GRMZM5G856297_T01	B4FEU2	Putative uncharacterized protein	1.73	2.61	1.97
99	GRMZM5G856297_T02	B4FEU2	Putative uncharacterized protein	1.62	2.78	2.17
100	GRMZM5G878558_T01	C5XTG6	Nitrate reductase	1.31	3.52	2.77

Log_2(+N/-N) values (5, 11 e 12 h) between treated (+N; $+NO_3^-$) and control (-N; $-NO_3^-$) samples in T250 inbred line (adjusted p-value ≤0.05; $|Log_2(+N/-N)| \geq 1$).

Transcripts modulated in T250 line in response to NO_3^- are in line with responses previously identified in other plant species (*e.g.* NO_3^- uptake and assimilation)

Transcripts involved in NO_3^- uptake and assimilation

Log_2(+N/-N) values at each sampling time points for transcripts involved in NO_3^- uptake and first steps of its assimilation recorded for Lo5 and T250 inbred lines

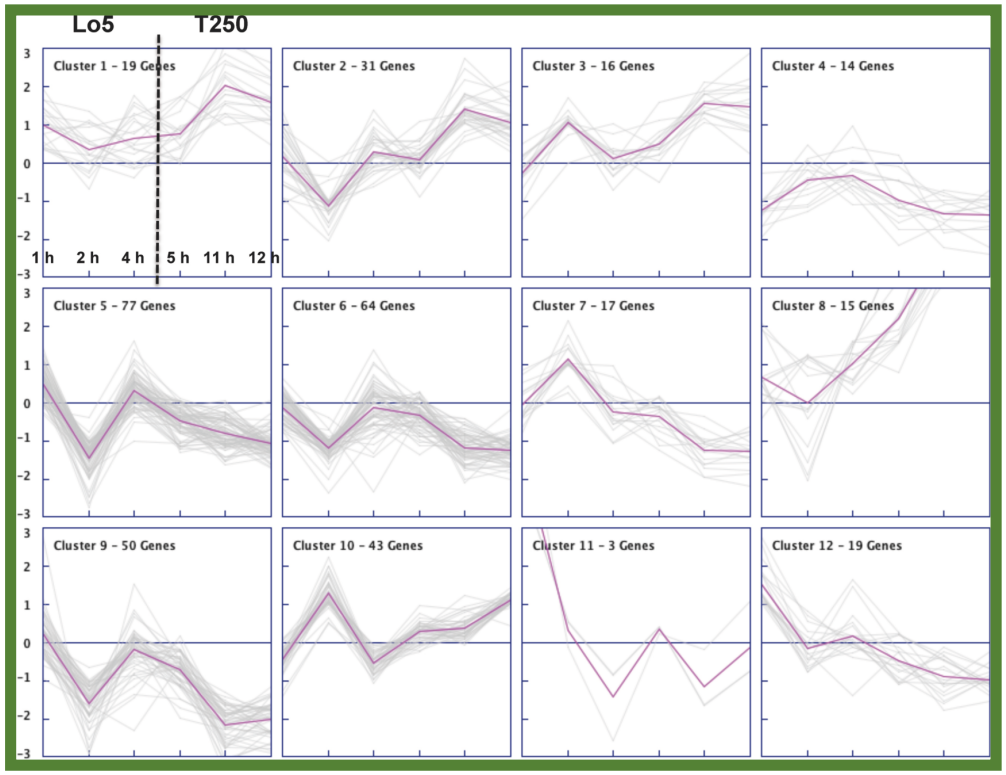
		Lo5		
Probe ID	**Description**	**Log_2(+N/-N) 1 h**	**Log_2(+N/-N) 2 h**	**Log_2(+N/-N) 4 h**
GRMZM2G010280_T01	ZmNRT2.1	0.23	0.30	-0.03
GRMZM2G010251_T01	ZmNRT2.2	0.55	0.41	0.05
GRMZM2G163866_T01	ZmNRT2.3	1.17	0.45	1.24
GRMZM2G455124_T01	ZmNRT2.5	-0.55	**1.06**	-0.64
GRMZM2G179294_T01	ZmNRT3.1A	0.13	0.60	0.03
GRMZM2G163494_T01	ZmNRT3.1B	-0.19	-0.55	-0.21
GRMZM2G568636_T01	nitrate reductase1	0.20	0.27	0.10
GRMZM5G878558_T01	nitrate reductase	0.56	0.37	0.53
GRMZM2G079381_T01	nitrite reductase	0.70	**1.23**	0.61
GRMZM2G098290_T01	glutamine synthetase1	0.52	0.30	0.20
GRMZM5G872068_T01	glutamine synthetase4	-0.30	-0.49	-0.27
GRMZM2G036464_T01	glutamine synthetase5	-0.19	-0.49	-0.33
GRMZM2G046601_T01	glutamine synthetase	0.39	-0.02	0.58
GRMZM2G050514_T03	glutamine synthetase	0.05	**1.44**	-0.20
GRMZM2G024104_T01	glutamine synthetase2	0.02	0.79	-0.23
GRMZM2G036609_T02	Ferredoxin-dependent glutamate synthase, chloroplastic	0.32	-0.26	0.04
		T250		
Probe ID	**Description**	**Log_2(+N/-N) 5 h**	**Log_2(+N/-N) 11 h**	**Log_2(+N/-N) 12 h**
GRMZM2G010280_T01	ZmNRT2.1	-0.35	**2.35**	**1.70**
GRMZM2G010251_T01	ZmNRT2.2	0.32	**3.38**	**3.01**
GRMZM2G163866_T01	ZmNRT2.3	0.58	0.67	**1.46**
GRMZM2G455124_T01	ZmNRT2.5	-0.61	0.37	0.18
GRMZM2G179294_T01	ZmNRT3.1A	-0.06	**1.84**	**1.56**
GRMZM2G163494_T01	ZmNRT3.1B	0.00	-0.43	**-1.17**
GRMZM2G568636_T01	nitrate reductase1	0.67	**1.99**	**1.89**
GRMZM5G878558_T01	nitrate reductase	**1.31**	**3.52**	**2.77**
GRMZM2G079381_T01	nitrite reductase	**2.15**	**4.11**	**3.96**
GRMZM2G098290_T01	glutamine synthetase1	0.52	**1.44**	**1.21**
GRMZM5G872068_T01	glutamine synthetase4	-0.61	-0.92	-0.79
GRMZM2G036464_T01	glutamine synthetase5	-0.47	-0.36	-0.38
GRMZM2G046601_T01	glutamine synthetase	**2.09**	**5.36**	**3.94**
GRMZM2G050514_T03	glutamine synthetase	0.85	**1.22**	**1.74**
GRMZM2G024104_T01	glutamine synthetase2	0.62	0.94	0.61
GRMZM2G036609_T02	Ferredoxin-dependent glutamate synthase. chloroplastic	0.48	**1.67**	**1.17**

The differentially transcripts (adjusted p-value ≤0.05; $|Log_2(+N/-N)| \geq 1$) between treated (+N; $+NO_3^-$) and control (-N; $-NO_3^-$) samples were in bold for each sampling time point.

Results were confirmed by Real-time RT-PCR experiments

Transcripts related to NO_3^- uptake and assimilation are more greatly expressed and modulated in response to treatment in T250 than Lo5.

Common NO_3^--modulated transcripts



- ✓ NO_3^- has an opposite effect on the modulation of some transcripts in two inbred lines (clusters 2,4,7,8,11 and 12)

- ✓ clusters 1,3,10 and 5,6,9 grouped transcripts from both inbred lines, respectively, positively and negatively affected by NO_3^- in at least one sampling-point

Transcripts with a different transcriptional behaviour between the two inbred lines:

- ✓ transcripts encoding transcription factors
- ✓ transcripts encoding protein kinases and phosphatases
- ✓ transcript encoding CBL protein positively modulated at 1h in Lo5 and down-regulated at 5h in T250 that can interact with CIPK Ser/Thr kinases; CIPKs are involved in the signalling system associated with NRT1.1 in *Arabidopsis thaliana* (Ho *et al*., 2009; Hu *et al*., 2009)
- ✓ transcripts related to ethylene synthesis down-regulated in T250 and up-regulated in Lo5 could be involved into the positive modulation of NRT2 transcripts only in T250
- ✓ transcripts encoding heat-shock proteins and heat-shock factors are strongly up-regulated at 1h in Lo5
- ✓ transcripts encoding aquaporin (*ZmTIP4.1* and *ZmNIP1.1*) were positively affected by NO_3^- in T250

Transcripts with a similar transcriptional behaviour in both inbred lines:

confirm the involvement of metabolic pathways previously described in *Arabidopsis* (Wang *et al*., 2000; Wang *et al*., 2003; Scheible *et al*., 2004), tomato (Wang *et al*., 2001) and maize (Liu *et al*., 2008) in response to NO_3^- supply, such as trehalose metabolism, NO_3^- assimilation, phenylpropanoid metabolism, cytokinin homeostasis and cell expansion.

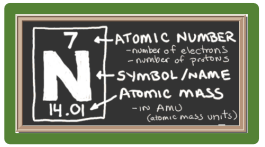
Results suggest that:

- ✓ different timing in the response to the changes in the solution bathing the roots (*e.g.* contact with NO_3^- and subsequent increase in its uptake rates) are mirrored by a different transcriptional behaviour during the induction stage (0-4 h and 0-12 h in Lo5 and T250, respectively)

- ✓ the two inbred lines differed extensively both in the number of modulated transcript during the NO_3^- induction (10% *vs* 3.5% of the maize transcriptome)

- ✓ our results suggested that the two maize inbred lines seem to have developed different strategies to respond to NO_3^- changes in the environment

N (NO_3^-) and water-extractable humic substances (WEHS)



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Water-extractable humic substances speed up transcriptional response of maize roots to nitrate

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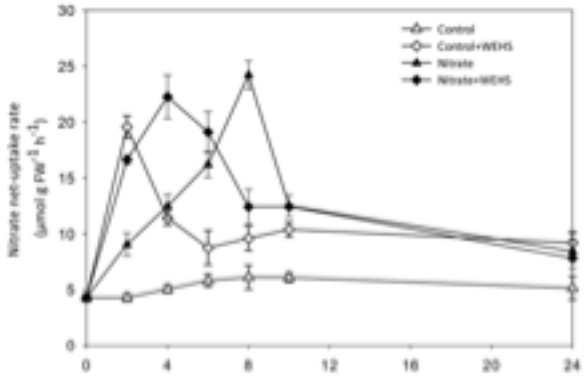
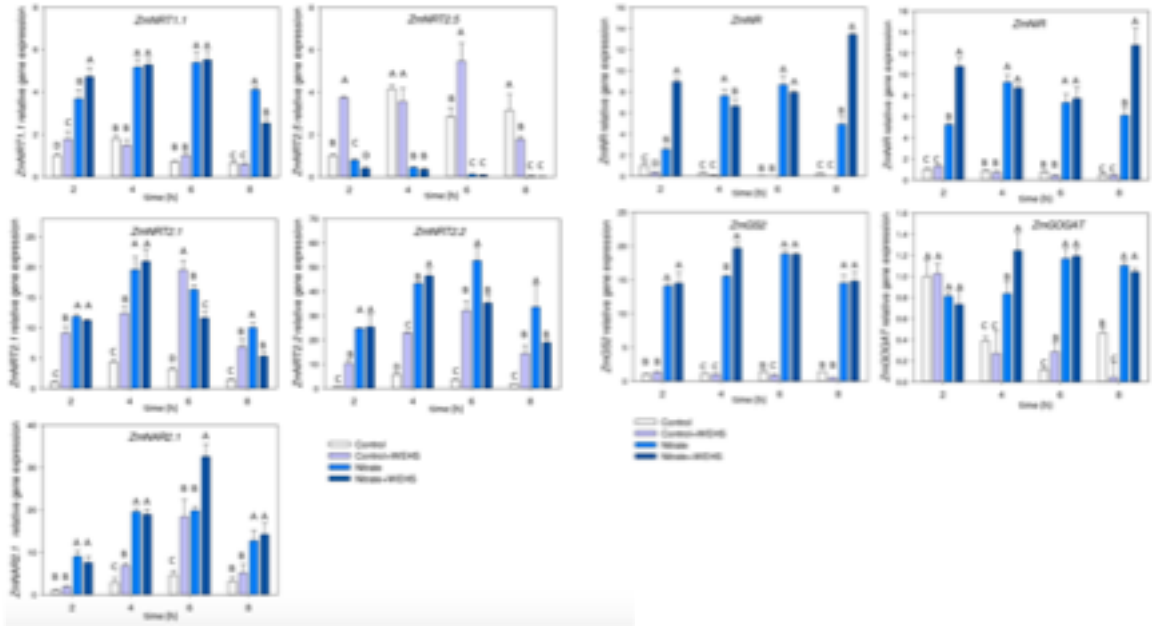




Treatment	Length (cm)	Surface (cm²)	Avg Diam (mm)	Root volume (cm³)	Tips	Primary root length (cm)
Control	208.51 ± 19.29	36.37 ± 2.28	0.56 ± 0.03	0.51 ± 0.03	468 ± 79	23.80 ± 0.98
Control+WEHS	231.10 ± 16.02	40.68 ± 4.16	0.56 ± 0.02	0.57 ± 0.08	545 ± 63	28.47 ± 1.81
Nitrate	281.18 ± 21.72	44.87 ± 0.84	0.51 ± 0.03	0.57 ± 0.03	567 ± 87	25.63 ± 1.19
Nitrate+WEHS	250.95 ± 26.72	39.35 ± 3.71	0.50 ± 0.01	0.49 ± 0.04	673 ± 26	28.36 ± 1.60

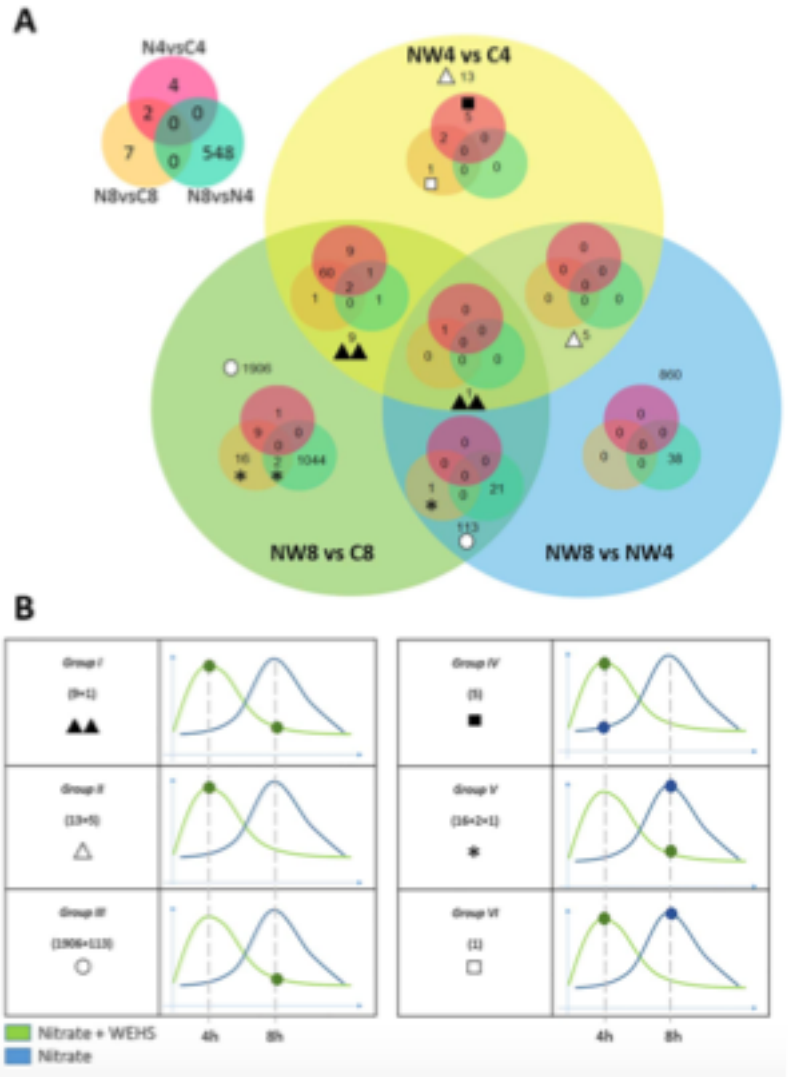
ANOVA (Student-Newman-Keuls Method, P<0.05)

Control	C	B	A	ns	B	B
Control+WEHS	BC	AB	A	ns	B	A
Nitrate	A	A	B	ns	B	B
Nitrate+WEHS	AB	AB	B	ns	A	A

N (NO_3^-) and water-extractable humic substances (WEHS)

Table 1
Number of differentially expressed transcripts comparing the following transcriptomic profiles of maize roots: C4, *Control* roots at 4 h; C8, *Control* roots at 8 h; N4, *Nitrate* roots at 4 h; N8, *Nitrate* roots at 8 h; NW4, *Nitrate + WEHS* roots at 4 h; NW8, *Nitrate + WEHS* roots at 8 h (FC≥ |2.00|, n = 3, adjusted P-value ≤ 0.05).

Comparison	*Total transcript*	*Up-regulated*	*Down-regulated*
N4 *vs* C4	96	+93	−3
N8 *vs* C8	104	+95	−9
N8 *vs* N4	1657	+1061	−596
NW4 *vs* C4	111	+98	−13
NW8 *vs* C8	3198	+1354	−1844
NW8 *vs* NW4	1040	+836	−204



Venn diagram: **6** clusters of transcripts correlating to physiological changes in plants

- ✓ *I* (9+1): modulated only by NW at 4 and 8h
- ✓ *II* (13+5): modulated only by NW at 4h
- ✓ *III* (1906+113): modulated only by NW at 8h

- ✓ *IV* (5): modulated by NW and N only at 4h
- ✓ *V* (16+2+1): modulated by NW and N only at 8h
- ✓ *VI* (1): modulated by NW and N at 4 and 8h

N (NO_3^-) and water-extractable humic substances (WEHS)

- ✓ NW affects transcripts involved into hormonal metabolism (Groups *I* and *II*)

- ✓ NW affects transcripts involved into N-metabolism (Group *III*); *ZmNR* and *ZmNiR* are strongly up-regulated in N+WEHS *vs* N explaining the observed physiological pattern; at 8h are strongly up-regulated the transcripts involved into the following step of N assimilation (e.g. *ZmGS*1, *ZmGS2*, *ZmGOGAT*, *ZmCNX* and *ZmASN*) promoting the de-induction of HATS

- ✓ NW and N upregulate at 8h (Group *V)* the *ZmNRT1.1* that could play a role in NO_3^- uptake, N translocation and in the expression of NO_3^--responsive genes (Hu et al., 2015)

- ✓ NW and N downregulate at 8h (Group *V)* the *ZmNRT2.5* suggesting an adequate availability of N; this pattern of the expression could be explained by the upregulation of the transcript encoding the LBD37 transcription factors which functions as repressor of some NO_3^- transporter including NRT2.5 (Kiba and Krapp, 2016; Rubin et al., 2009; Konishi and Yanagisawa, 2013; Sawaki et al., 2013)

Biostimulants (protein hydrolysates e and free amino acids)

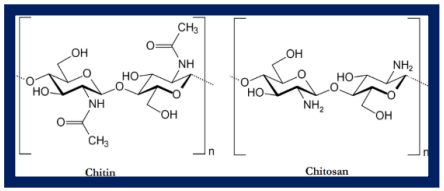
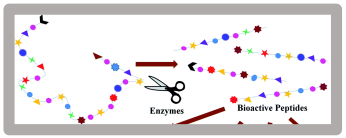
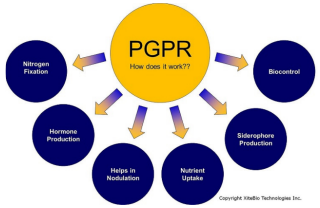


TABLE 2| Proposed Biostimulant Categories.

	Filatov, 1985'a	Arbna and Kolbin, 2004	Kauffman et al., 2007	Du Jardin, 2012	Calvo et al., 2014	Halpern et al., 2015	Du Jardin, 2015	Torre et al., 2016
1	Carboxylic fatty acids (oxalic acid and succinic acid)	Microorganisms (bacteria, fungi)	Humic substances	Humic substances	Microbial inoculants	Humic substances	Humic and fulvic acids	Humic substances
2	Carboxylic fatty hydroxy acids (malic and tartaric acids)	Plant materials (land, freshwater and marine)	Hormone containing products (seaweed extracts)	Complex organic materials	Humic acids	Protein hydrolysate and amino acid formulations	Protein hydrolysates and other N-containing compounds	Seaweed extracts
3	Unsaturated fatty acids, aromatic and phenolic acids (cinnamic and hydroxycinnamic acids, coumarin)	Sea shellfish, animals, bees	Amino acid containing products	Beneficial chemical elements	Fulvic acids	Seaweed extract	Seaweed extracts and botanicals	Hydrolyzed proteins and amino acids
4	Phenolic aromatic acids containing several benzene rings linked via carbon atoms (humic acids)	Humate- and humus-containing substances		Inorganic salts (such as phosphite)	Protein hydrolysates and amino acids	Plant growth-promoting microorganisms (including mycorrhizal fungi)	Chitosan and other biopolymers	Inorganic salts
5		Vegetable oils		Seaweed extracts	Seaweed extracts		Inorganic compounds	Microorganisms
6		Natural minerals		Chitin and chitosan derivatives			Beneficial fungi	
7		Water (activated, degassed, thermal)		Antitranspirants			Beneficial bacteria	
8		Resins		Free amino acids and other N-containing substances				
9		Other raw materials (oil and petroleum fractions, shale substance)						

Yakhin et al., 2017





- ✓ free amino acid mixtures or protein hydrolysates constituted by short peptides and free amino acids in different proportions are marketed as crop biostimulant
- ✓ these products are obtained by the hydrolysis of proteins from plant, animal, and microbial sources, but also from industrial and agricultural residues

Biostimulants (protein hydrolysates e and free amino acids)

The protein hydrolysates used in this project are a mixture of amino acids and peptides of different length, that derive from animal origin by-products (collagen) (SICIT 2000).

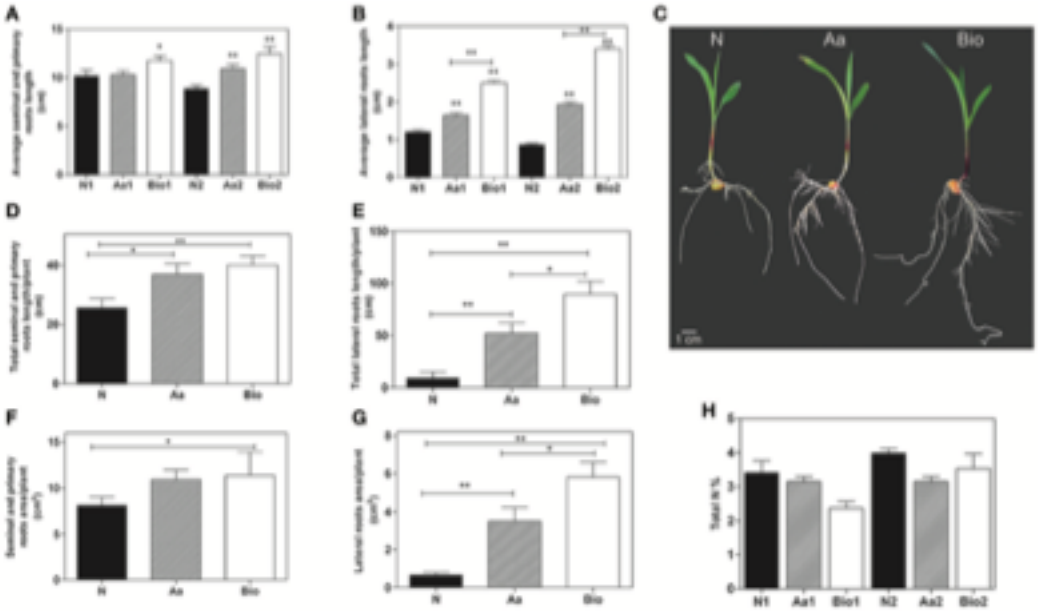
PRODUCT	organic N %	organic C %	amino acids % total	free	formulation
PROTIFERT LMW 10	10.0	30	62	10	Liquid (SC)

Maize seedlings grown in nutrient solution supplemented with:

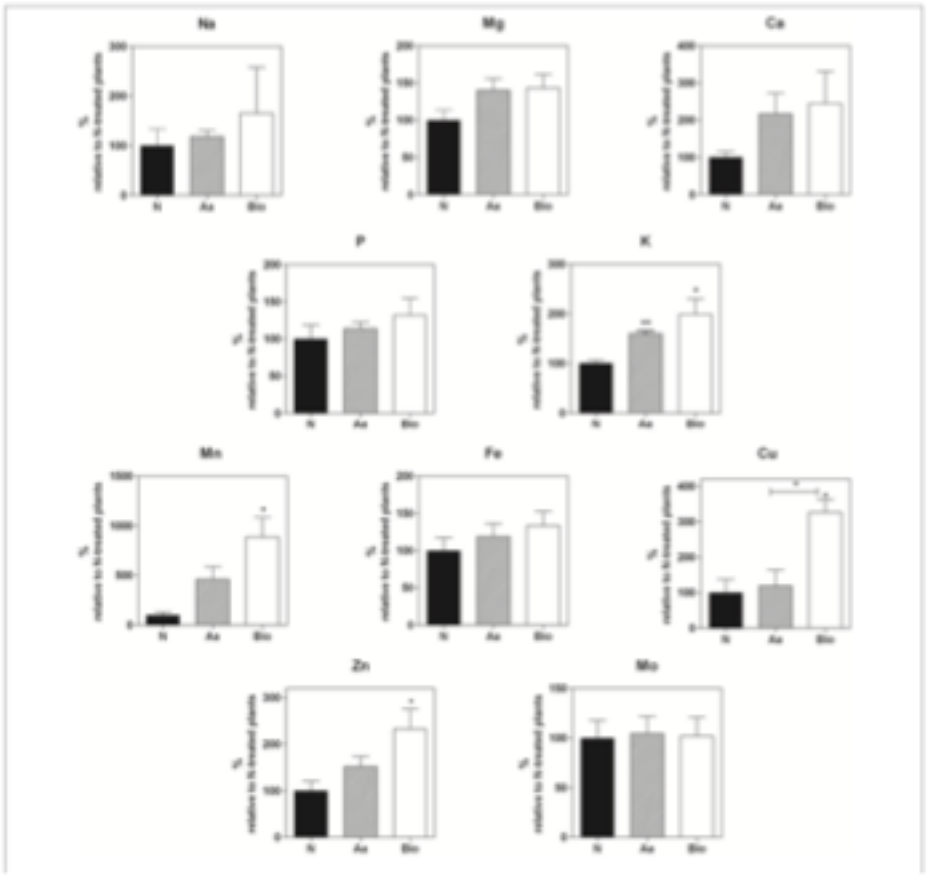
Equal total N content

- Biostimulant containing 10% free aminoacids — ***Bio***
- Aminoacid mixture (same aa composition of the biostimulant) — ***Aa***
- Inorganic N ($NH_4H_2PO_4$) — ***N***

0.05 and 0.1 ml L^{-1} ➡ 5.65 and 11.3 mg L^{-1} total N



Biostimulants (protein hydrolysates e and free amino acids)



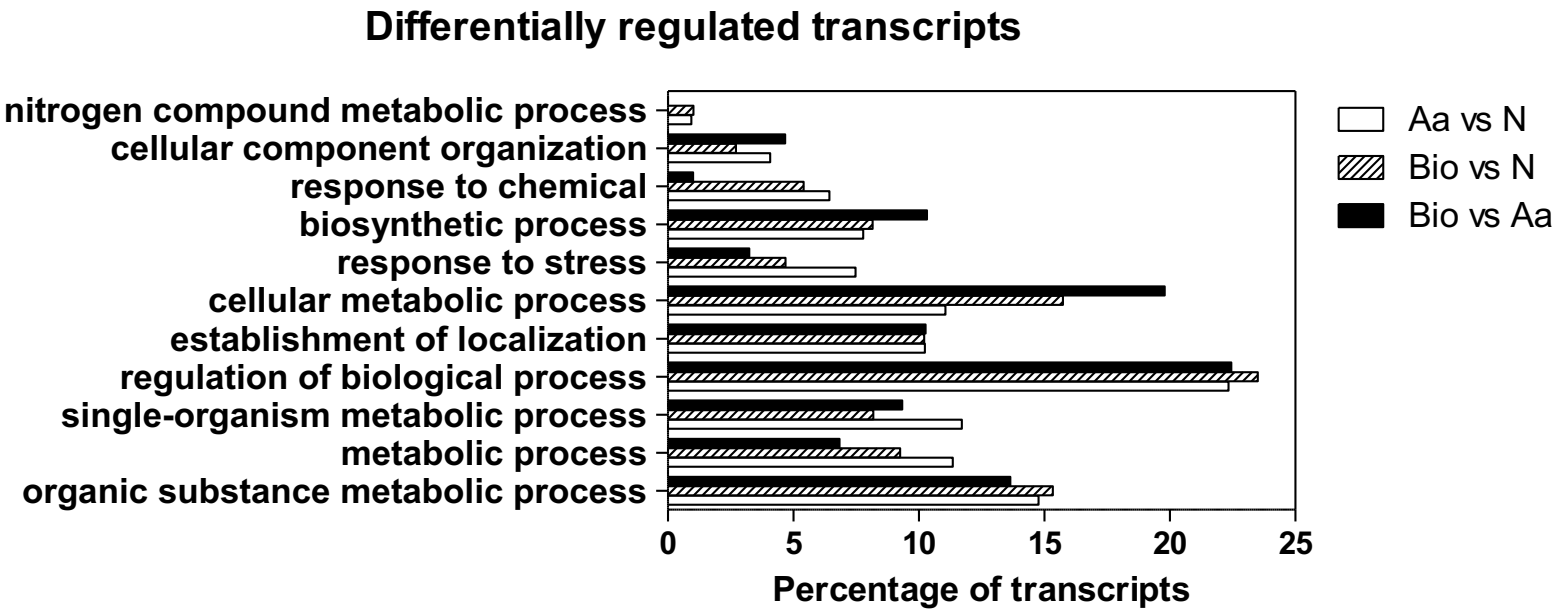
Agilent chip

39,372 of transcripts predicted from the ZmB73 reference genome (Release 5b)

Probe: 60 nt

Differentially expressed transcripts Fold-change ≥ 2.0 or ≤ 2.0		
BIO vs N	**BIO vs Aa**	**Aa vs N**
282 ↑ 305 ↓	333 ↑ 98 ↓	385 ↑ 610 ↓
587 tot	431 tot	995 tot

Functional categories



Transport

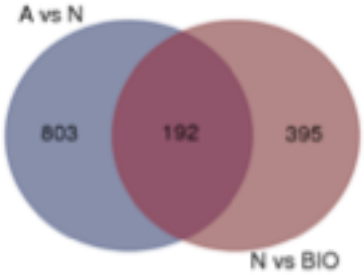
Stress response

Hormonal metabolism

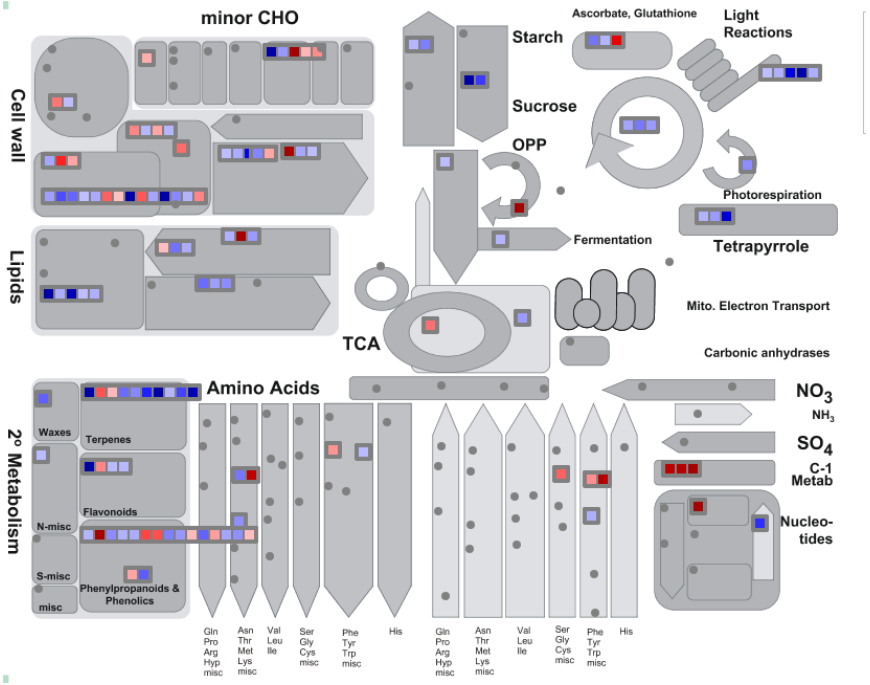
Cell wall

Transcription factors

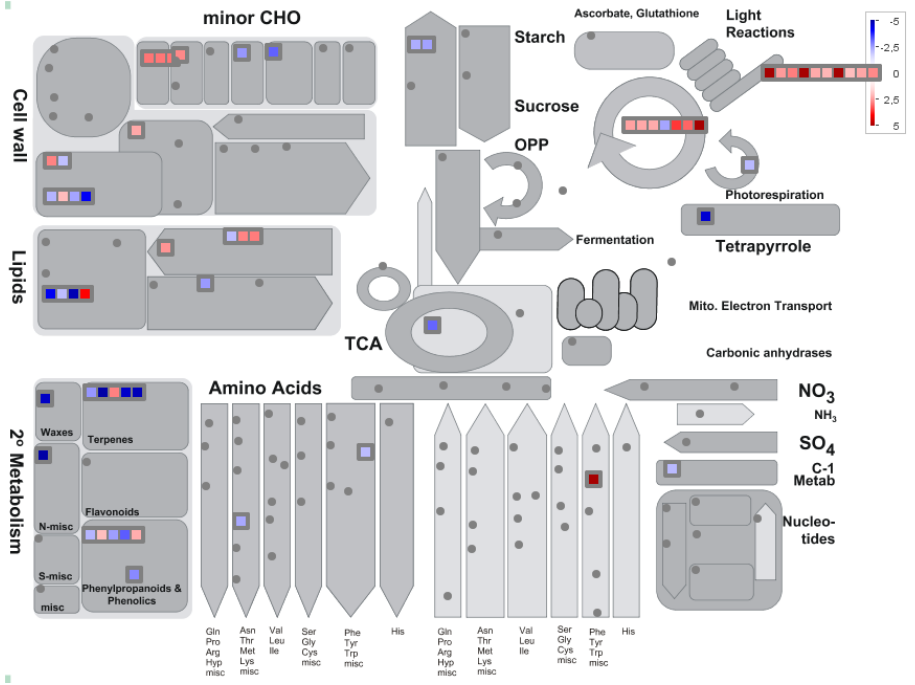
Aa *vs* N and Bio *vs* N



Aa



Bio



Common responses of maize roots to free amino acids and protein hydrolysate

- ✓ modulation of transcripts encoding transcription factors related to nutrient stress and involved in root growth and metabolism (NAC, MYB, WRKY, bHLH, AP2-EREB)
- ✓ modulation of genes involved in cell wall remodeling that can regulate root growth and lateral root formation
- ✓ modulation of transcripts involved into transport processes (transport of Fe chelates and other divalent cations, peptides and amino acids, nitrate and ammonium)
- ✓ transcripts involved into gibberellin metabolism and auxin signalling and transport are induced by both biostimulants
- ✓ higher potassium accumulation

Aa-specific responses

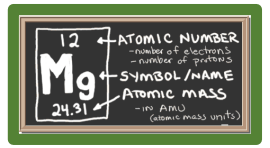
- ✓ stress-related transcripts modulation (mostly peroxidases)
- ✓ active uptake of amino acids (induction of amino acids permease transcripts)
- ✓ positive modulation of transcripts involved in the synthesis of metal chelators

Bio-specific responses

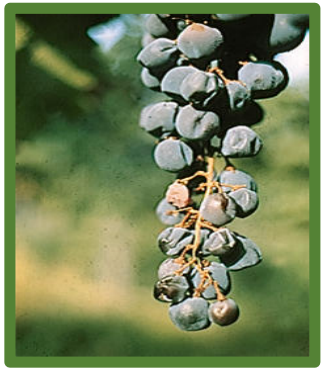
- ✓ modulation of transcripts encoding specific peptide transporters
- ✓ modulation of transcripts involved into cytokinin and jasmonate metabolism
- ✓ higher micro-nutrients accumulation

Transcriptomics coupled with phenotypic and ionomic analyses are useful tools to highlight the mechanisms of action of biostimulants

Mg deficiency in grapevine

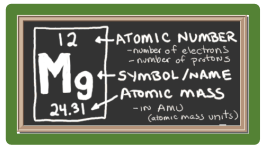


- ✓ only field observations
- ✓ no physiological and molecular characterization of grapevine responses to Mg deficiency

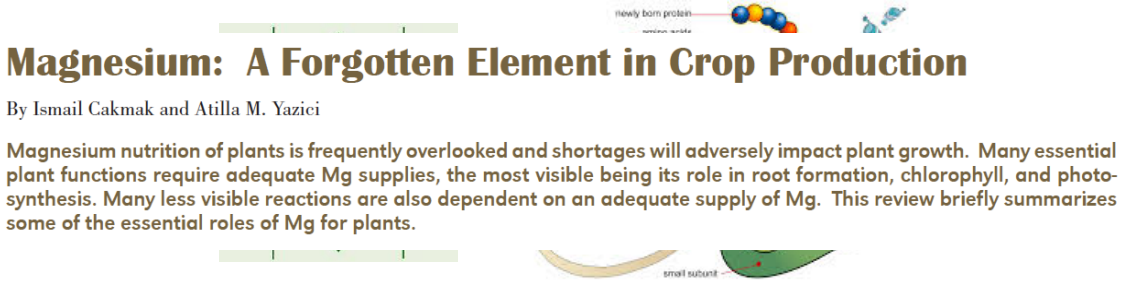


- ✓ interveinal chlorosis and necrosis (old leaves)
- ✓ early leaf fall
- ✓ bunch stem necrosis

Mg



- ✓ Mg is the 4th element mainly adsorbed by plants
- ✓ Mg^{2+} is the most abundant free divalent cation in the plant cytosol
- ✓ Mg concentration in soil solution is commonly quite high (3-4 mM) and its uptake by plant roots is negatively influenced by competition with other cations (K^+, NH_4^+, Ca^{2+}, Mn^{2+}, H^+ e Al^{3+})



Magnesium: A Forgotten Element in Crop Production

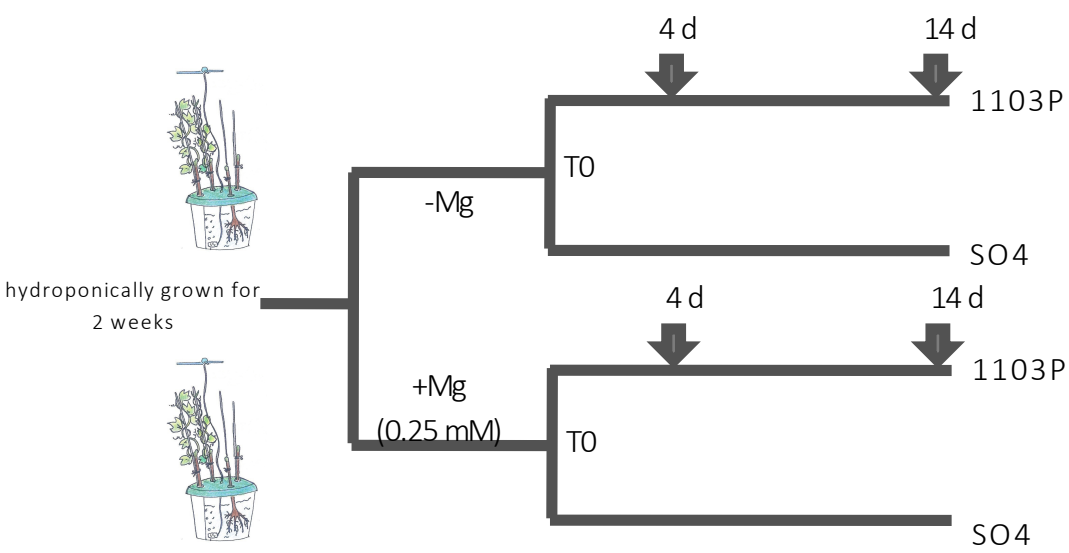
By Ismail Cakmak and Atilla M. Yazici

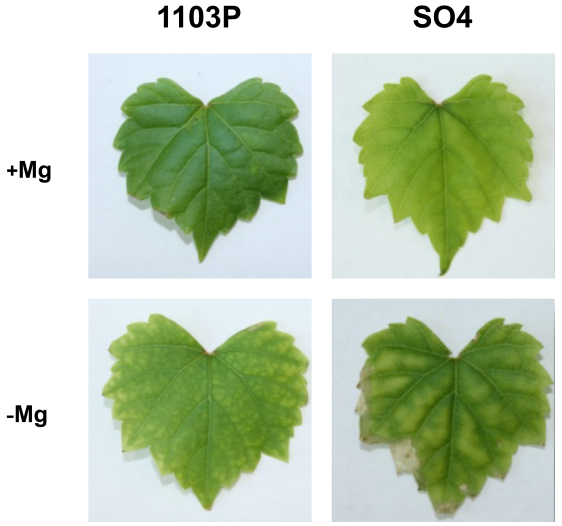
Magnesium nutrition of plants is frequently overlooked and shortages will adversely impact plant growth. Many essential plant functions require adequate Mg supplies, the most visible being its role in root formation, chlorophyll, and photosynthesis. Many less visible reactions are also dependent on an adequate supply of Mg. This review briefly summarizes some of the essential roles of Mg for plants.

Physiological and molecular characterization of rootstocks responses to Mg deficiency

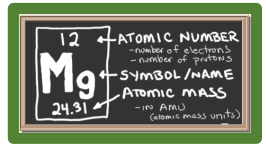
1103P: tolerant

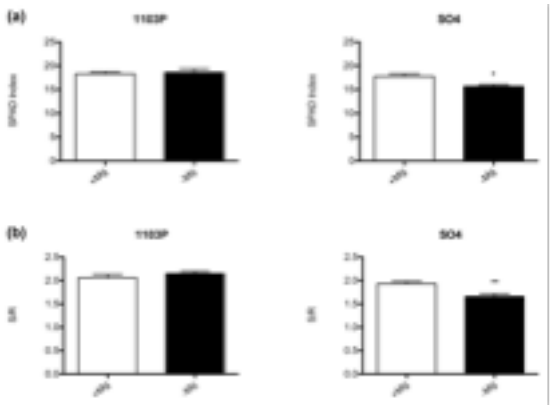
SO4: susceptible

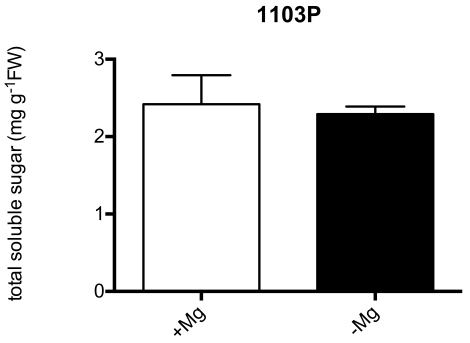


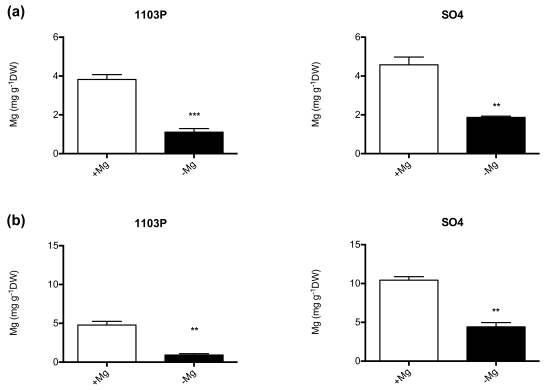


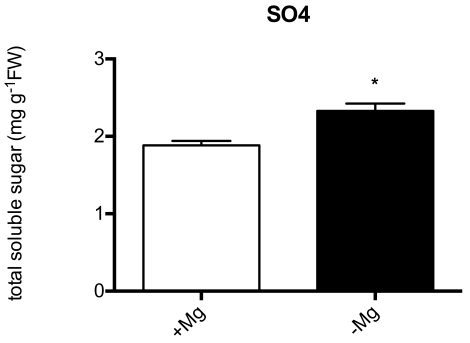
Physiological and molecular characterization of rootstocks responses to Mg deficiency





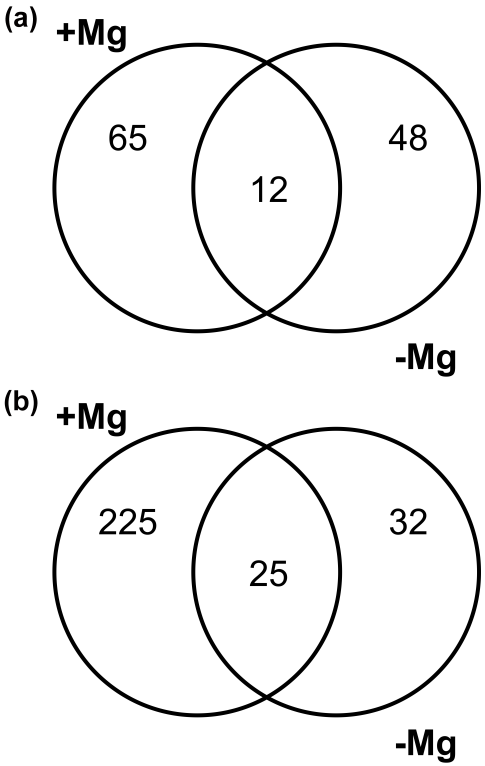






Physiological and molecular characterization of rootstocks responses to Mg deficiency

Root metabolite analysis (UPLC-MS and GC-MS)



4 days

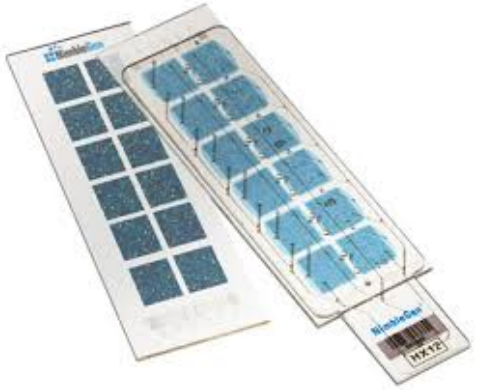
✓ higher levels of carotenoids in 1103P roots

✓ monosaccharides (*e.g.* $\boldsymbol{\alpha}$-L-arabinopyranosio, $\boldsymbol{\alpha}$ -L-arabinofuranose, $\boldsymbol{\alpha}$-D-xylose, $\boldsymbol{\beta}$-D-xylose) involved into the synthesis of constituents of the cell wall xyloglucans and pectins (Harris and Stone, 2008)

14 days

✓ higher levels of metabolites involved into responses to biotic and abiotc stresses in the susceptible rootstocks

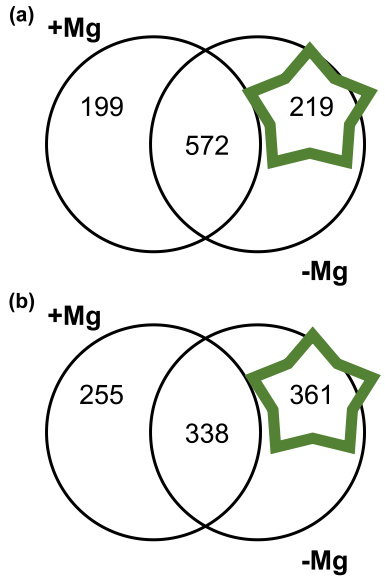
Microarray analysis



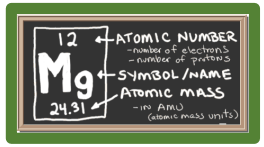
NimbleGen chip:

29,549 predicted grapevine transcripts (98.6% of the genes predicted from the V1 annotation of the 12x grapevine genome)
Probe: 60 nt (4 probes for each transcripts)

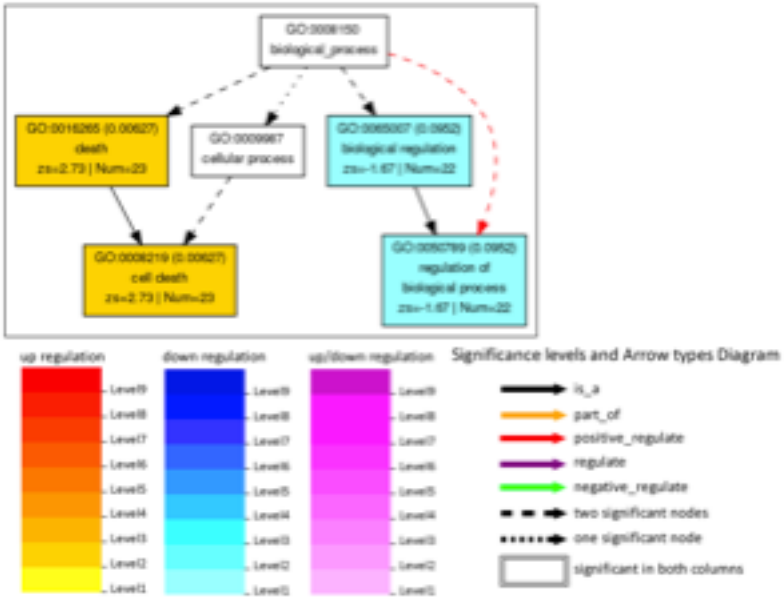
	4 days	
	1103P +Mg *vs* SO4 +Mg	1103P -Mg *vs* SO4 -Mg
up-regulated	412	421
down-regulated	359	370
	14 days	
	1103P +Mg *vs* SO4 +Mg	1103P -Mg *vs* SO4 -Mg
up-regulated	183	247
down-regulated	410	452



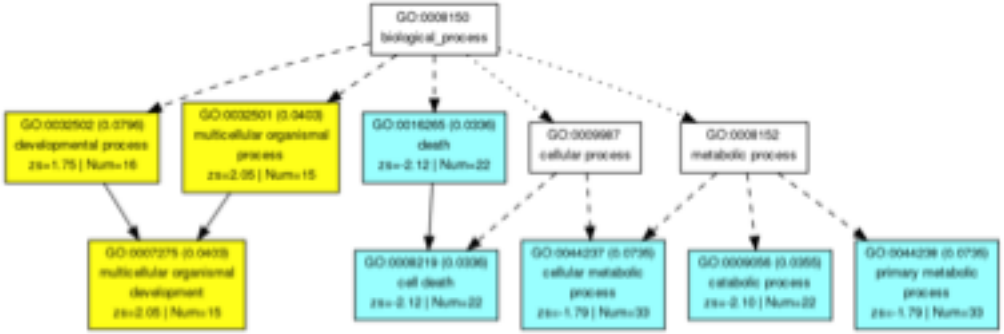
1103P *vs* SO4 (-Mg)



4 days



14 days



Singular Enrichment Analysis (SEA); AgriGO

1103P *vs* SO4 (-Mg)

- ✓ the tolerant rootstock exhibits a lower oxidative stress in the first phase of the response to Mg deficiency (*e.g.* 4 days; down-regulation of transcripts encoding Respiratory burst oxidase protein D and a peroxisomal biogenesis factor 11)

- ✓ the tolerant rootstock produce lower levels of phenolic compounds (transcripts involved in this process are down-regulated)

- ✓ the tolerant rootstock respond to Mg deficiency changing cell wall structure through a decrease in a cellulose content and its stiffness through an increase in pectin with a lower level of methyesterification and a higher quantity of arabinose-containing polysaccharides putatively tightly linked to the cell wall (up-regulation of cellulose synthase, endo-1,3;1,4-beta-D-glucanase, pectinesterese and polygalacturonase)

- ✓ the higher level of a HKT2 transcript in roots of 1103P relative to SO4 under 4-day Mg deficiency suggests that this transporter could be involved into Mg uptake

In conclusion

- ✓ microarray analyses give a genome-wide picture of transcript levels in relationship to different biotic and abiotic stresses

- ✓ microarray analysis allow us comparing the transcriptional profiles of different genotypes in response to a treatment (e.g. root NO_3^- exposure) or stress (*e.g.* Mg deficiency)

- ✓ we can correlate the physiological responses to a nutrient deficiency with changes in transcriptome

- ✓ we can obtain a list of transcripts for further functional analyses

- ✓ we can obtain a list of putative biomarkers for genotype selection

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Thank you for attention