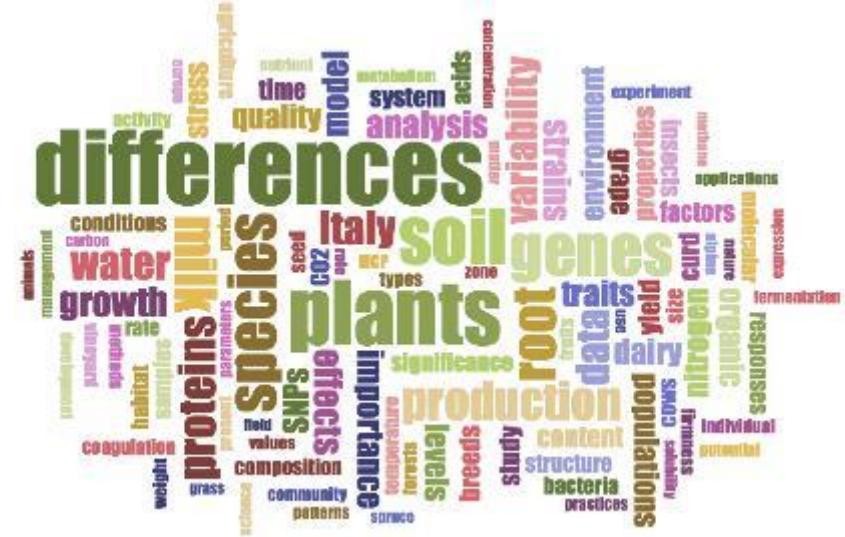


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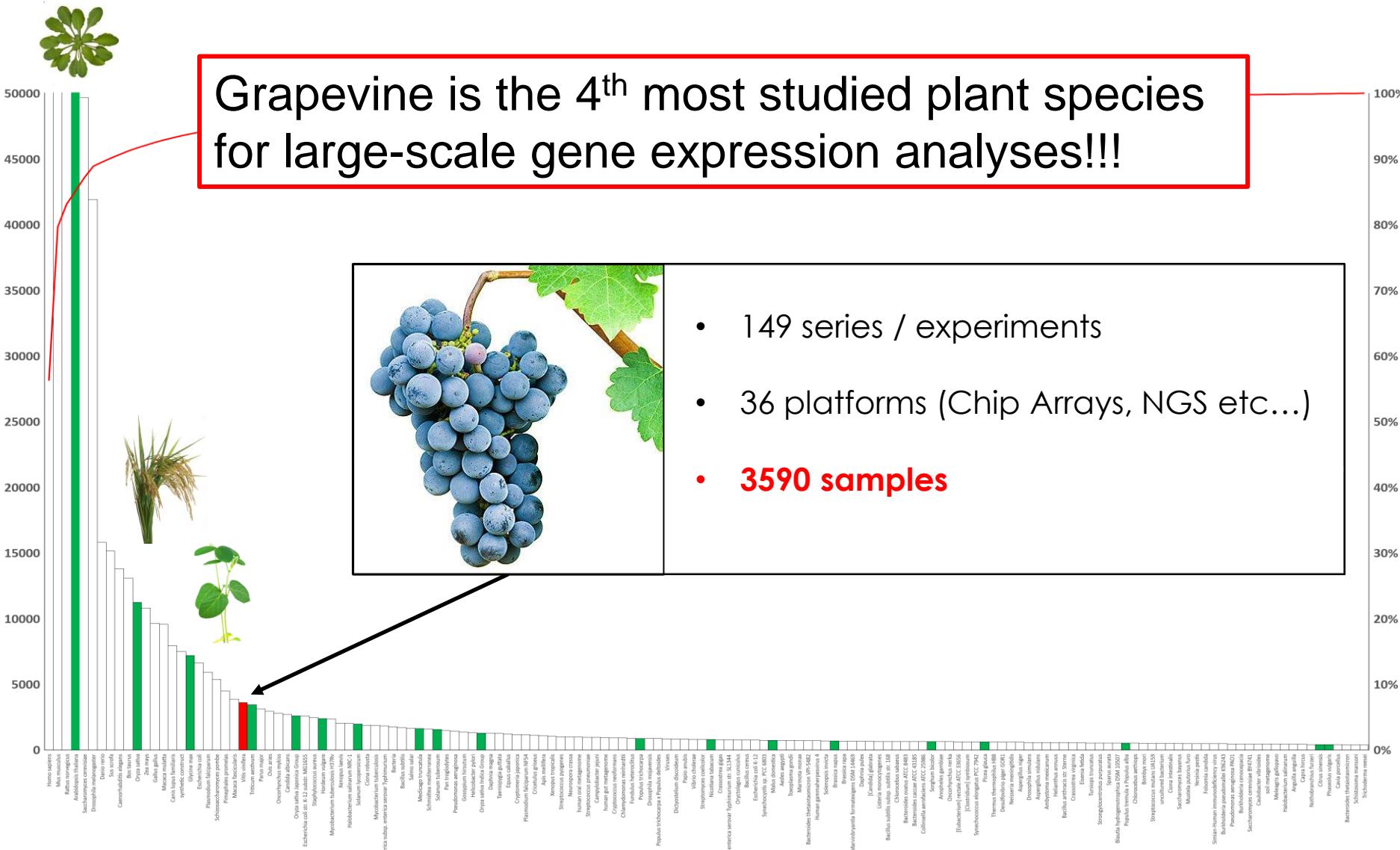
# The combined role of WRKY and MYB TFs in the regulation of stilbene synthase genes in grapevine

Alessandro Vannozzi

**Big data is like teenage sex:**  
everyone talks about it, nobody  
really knows how to do it,  
everyone thinks everyone else  
is doing it, so everyone claims  
they are doing it...

(Dan Ariely)

# Top 150 organisms in Gene Expression Omnibus database



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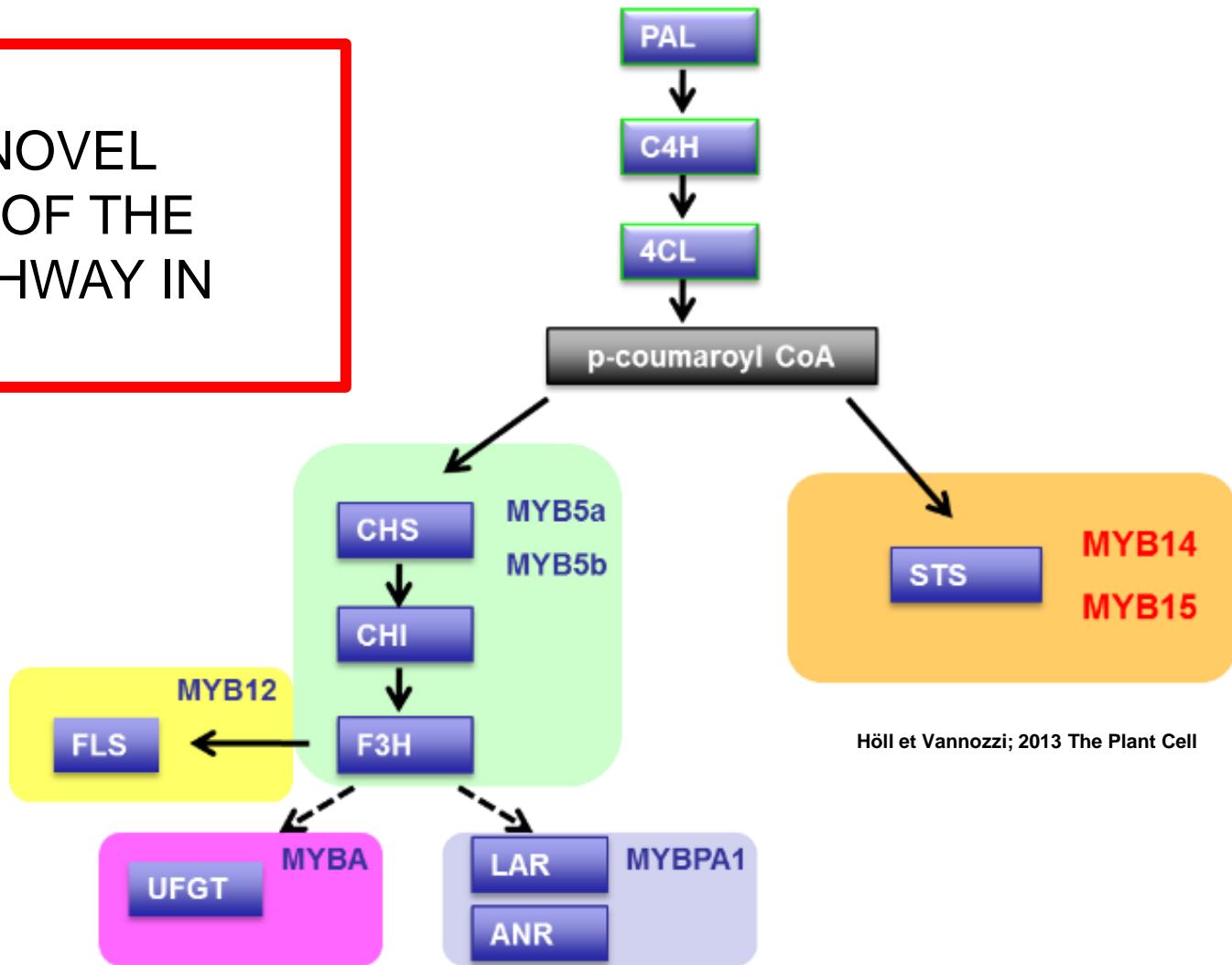
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How can we use  
all this  
information?

# The regulation of the stilbene biosynthetic pathway

## A case study:

IDENTIFYING NOVEL  
REGULATORS OF THE  
STILBENE PATHWAY IN  
GRAPEVINE

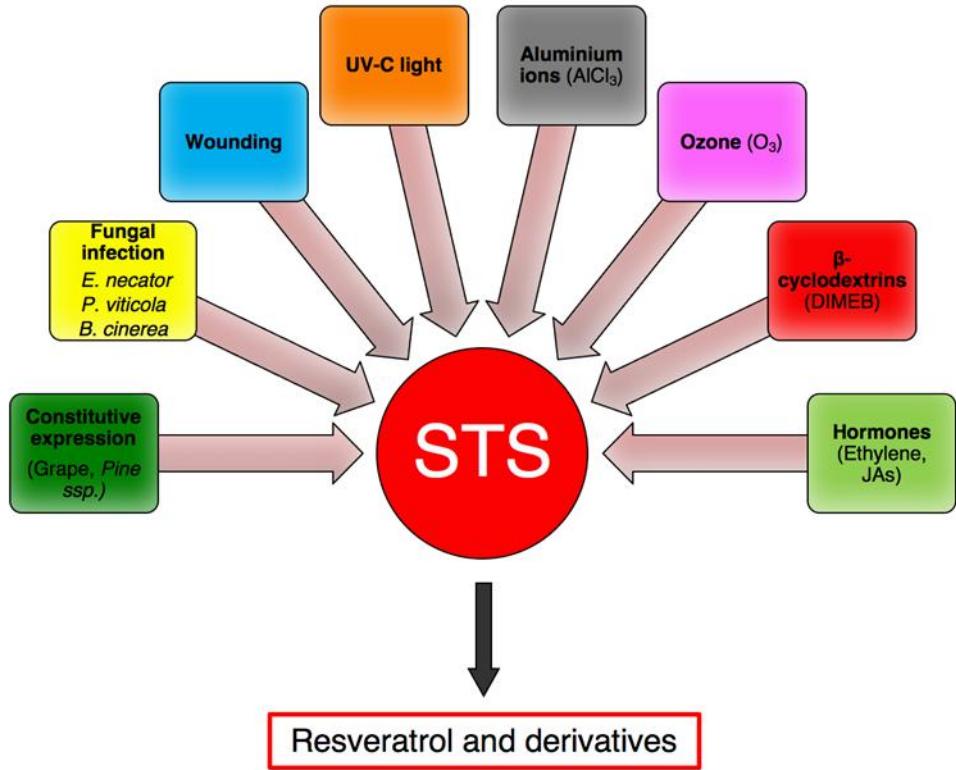
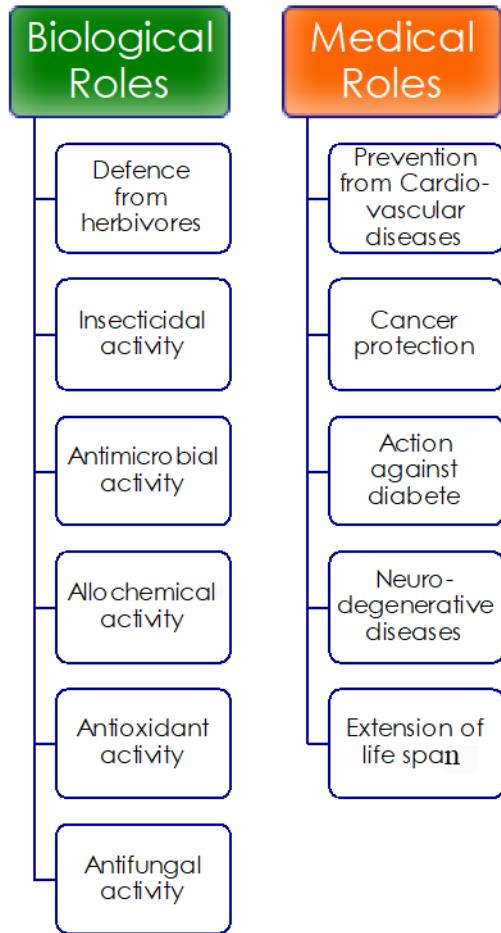


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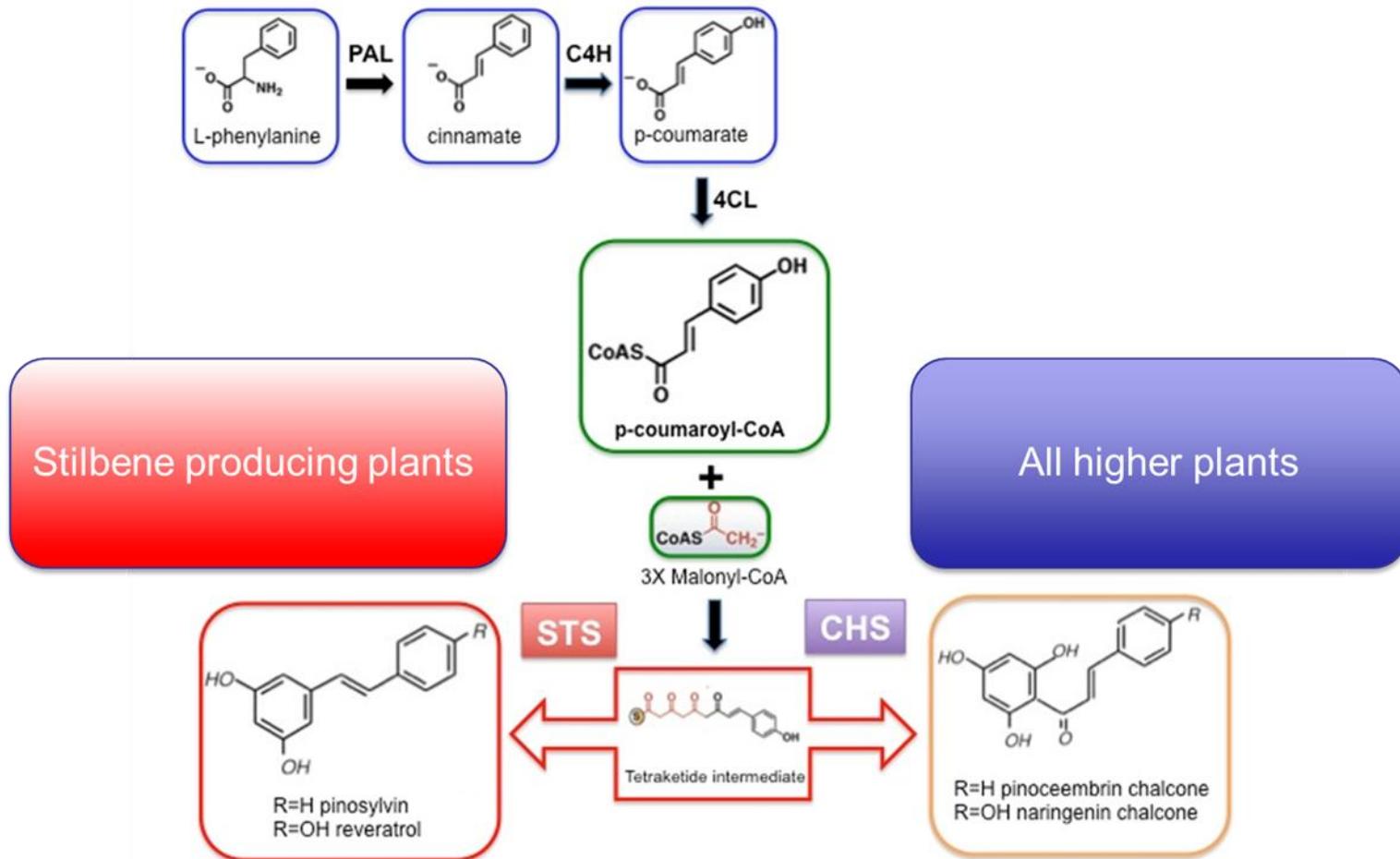
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# Roles and regulation of stilbene biosynthesis



# Stilbene biosynthesis



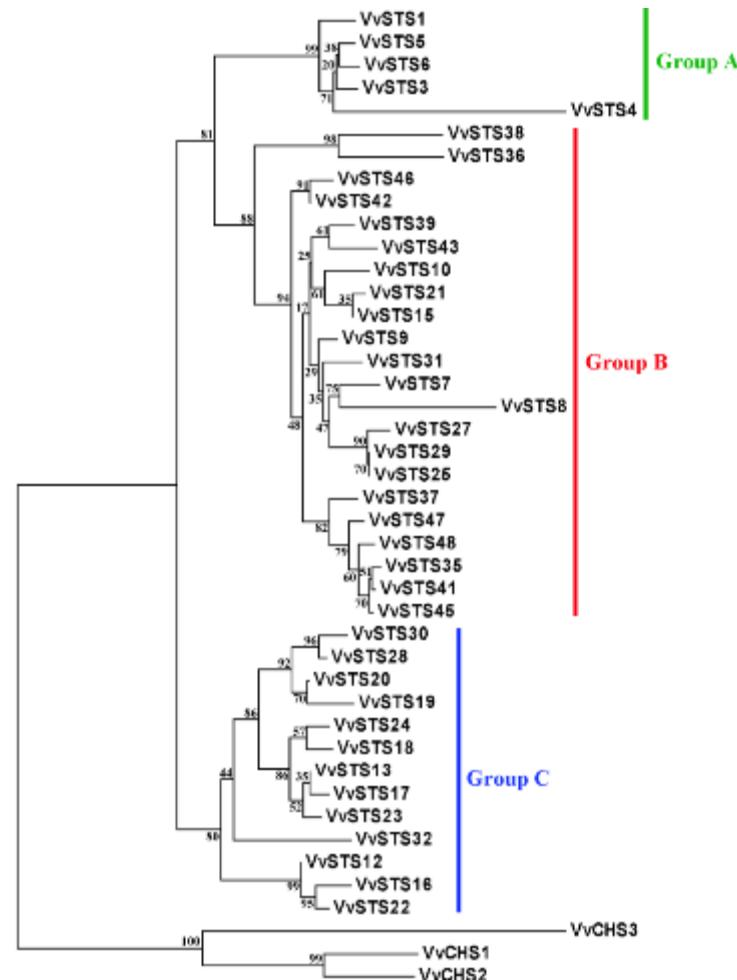
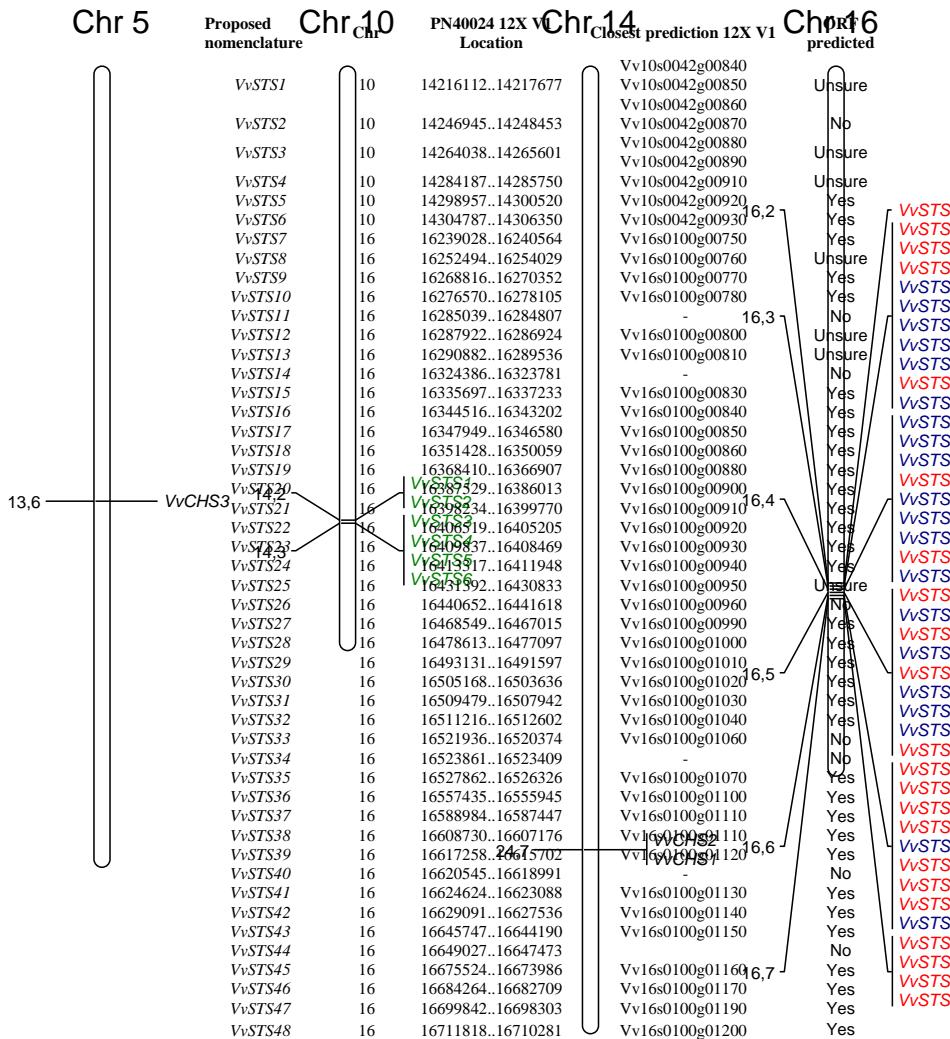
Vannozzi et al. (2012). BMC Plant Biology



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# The grapevine *STS* gene family



Vannozzi et al. 2012 BMC Plant Biol



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# The principle

**'guilt-by-association'**  
genes involved in biologically related  
pathways or processes exhibit  
comparable expression dynamics  
across a wide range of experimental  
conditions.



# Construction of a mutual-rank Gene Co-expression Network

## 29K NimbleGen

23 experiments, 359 conditions  
averaged from 914 NimbleGen arrays

RMA-normalization of row intensity data

**mutual rank (MR) gene co-expression analysis**

VvSTS top 200 MR-ranked genes

TF filtering based on Plant TFdb

GCN network

## RNA-seq

21 experiments, 236 conditions  
averaged from 1353 replicates

Row single/paired-end reads trimming/filtering

Alignment vs **12X v1 PN40024** reference genome

Estimation for **FPKM** transcript abundance

**mutual rank (MR) gene co-expression analysis**

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Merged GCN



# Mutual Rank VS Pearson Correlation Coefficient

Top 5 co-expressed list for **gene A**

Rank	PCC	Locus
1	1.0	Gene A
2	<b>0.93</b>	Gene B
3	0.94	Gene C
4	0.90	Gene D
5	0.87	Gene E

Top 5 co-expressed list for **gene B**

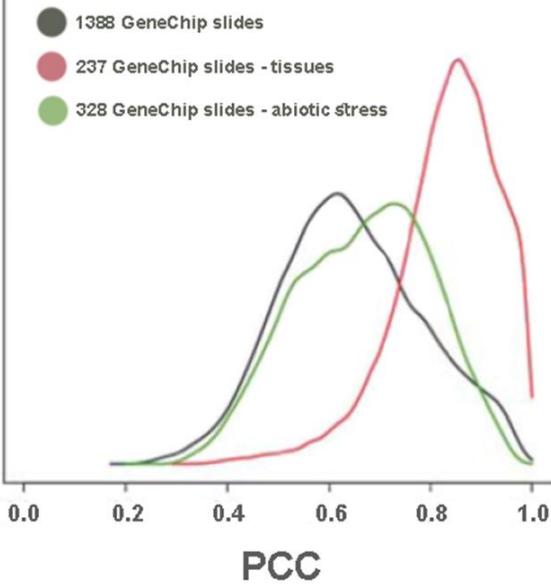
Rank	PCC	Locus
1	1.0	Gene B
2	0.99	Gene C
3	0.97	Gene D
4	<b>0.93</b>	Gene A
5	0.87	Gene E

**Correlation rank is asymmetric:** the rank of gene B from gene A is not the same as the rank of gene A from gene B!!!

$$MR_{(AB)} = \sqrt{(Rank_{(A \rightarrow B)} \times Rank_{(B \rightarrow A)})}$$

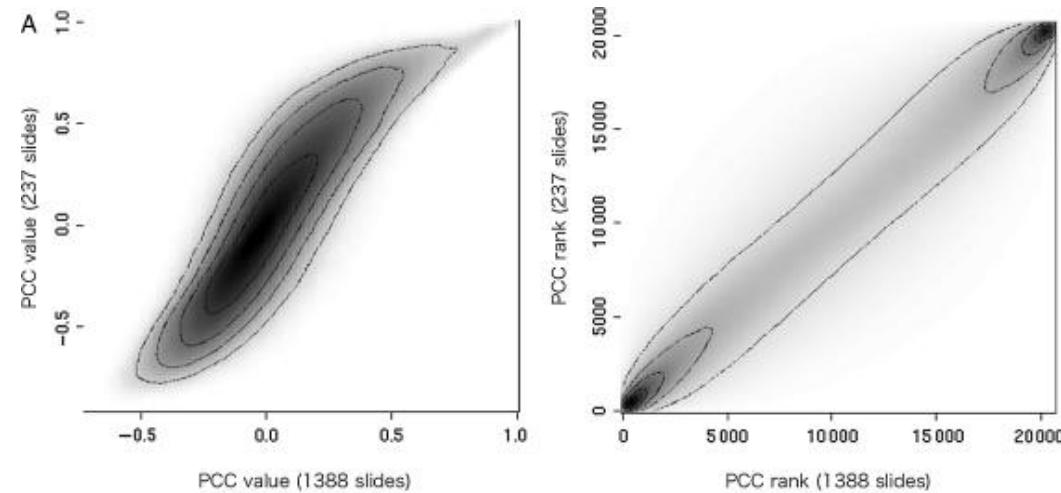


# Why mutual ranking instead of PCC?



- 1) **Mathematical:** smaller samples produce larger amplitude of correlation between any two genes
- 2) **Biological:** gene expression changes amongst developmental samples are far larger than those induced by abiotic stresses. **Changes in gene expression amplitude decrease experimental noises that decrease any gene correlation.**

PCC rank distribution aligns in the diagonal indicating linear correspondence between the PCC ranks between different samples



Obayashi & Kinoshita, DNA RESEARCH 2009, 16:249-260



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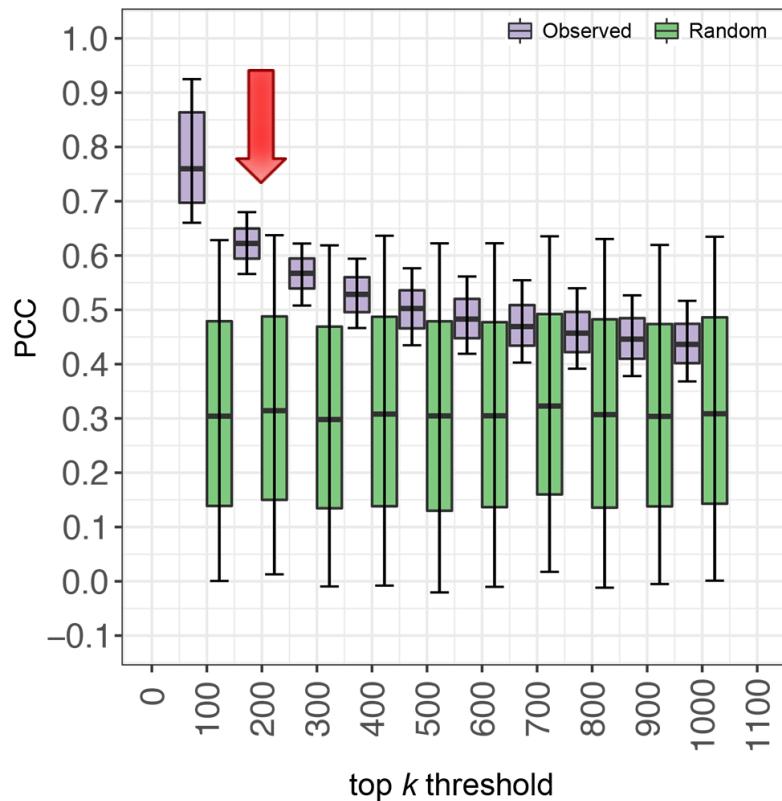
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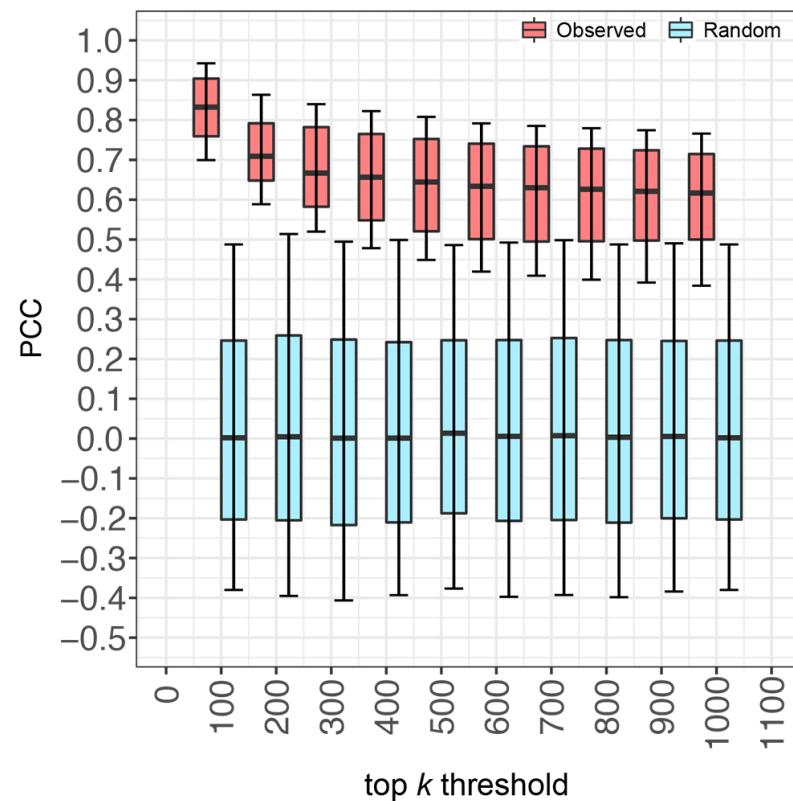
# Why the top 200 ranking genes?

1) Based on the relationship of different  $k$  thresholds (100–1,000) to the distribution of PCC values for each member of the grapevine STS family in observed and random GCN

Array



NGS



# Construction of a mutual-rank Gene Co-expression Network

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## RNA-seq

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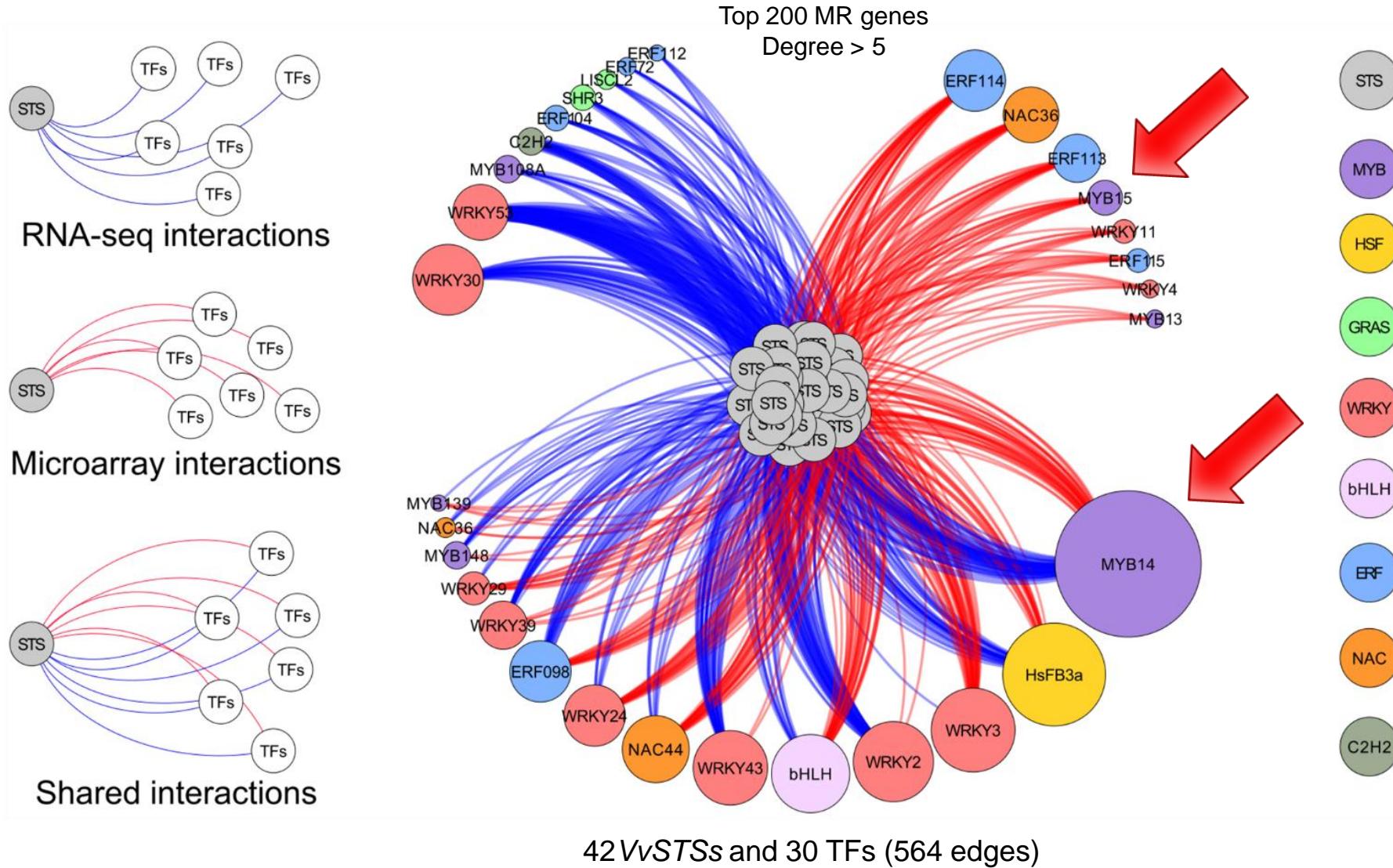


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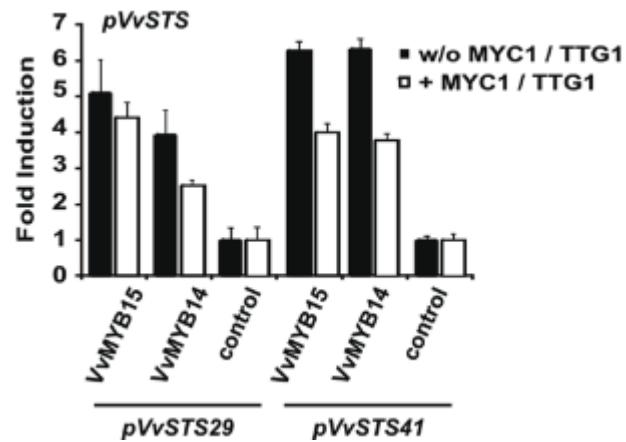
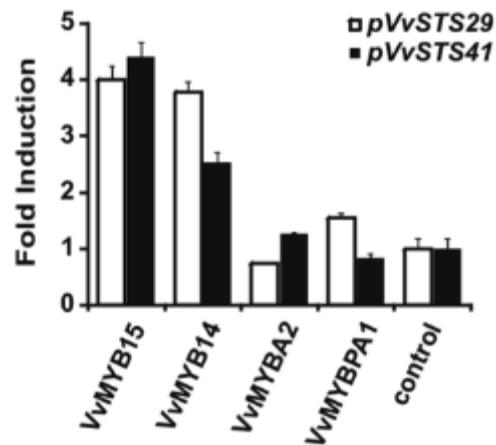
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# Condition-independent STS-TF Gene Co-expression Network

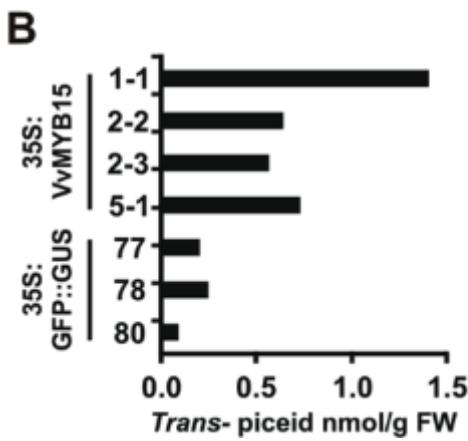
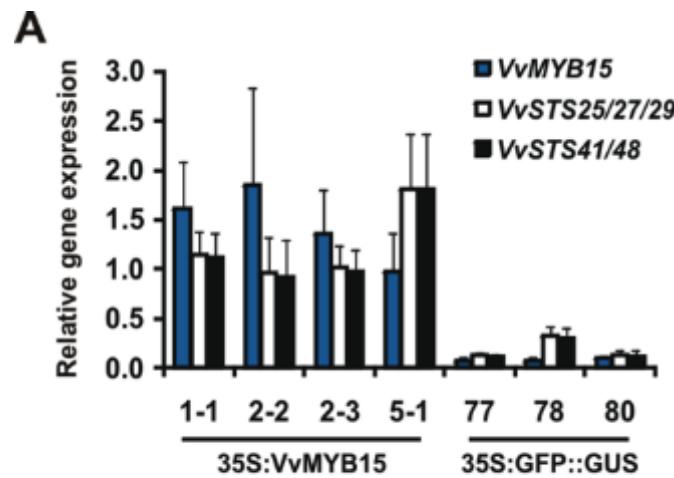


# VviMYB14 and VviMYB15 regulate two VviSTS genes

## Dual reporter luciferase assay



## VviMYB15 overexpression in hairy roots



Höll et Vannozzi; 2013 The Plant Cell

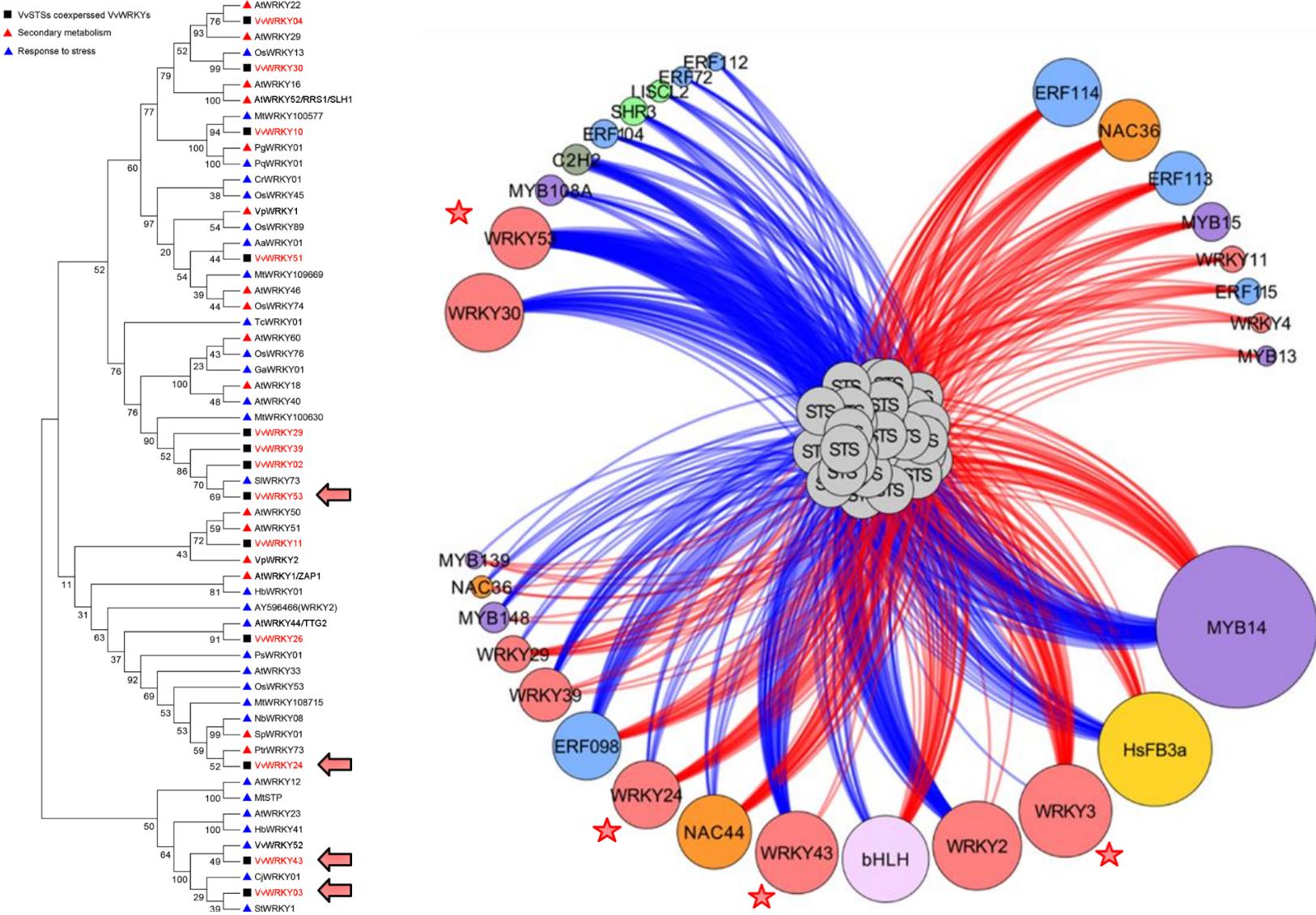


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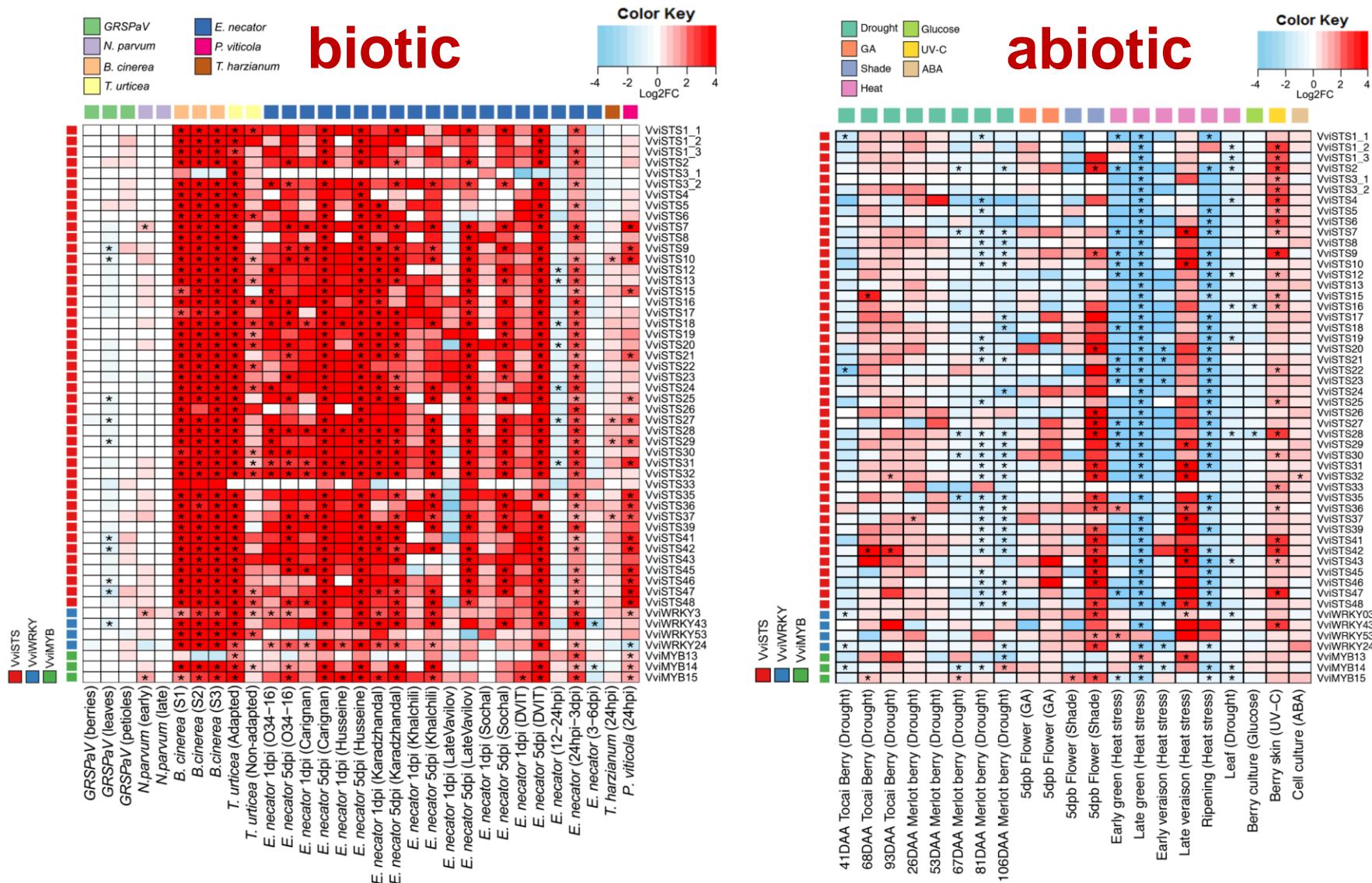
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# Condition-independent STS-TF Gene Co-expression Network

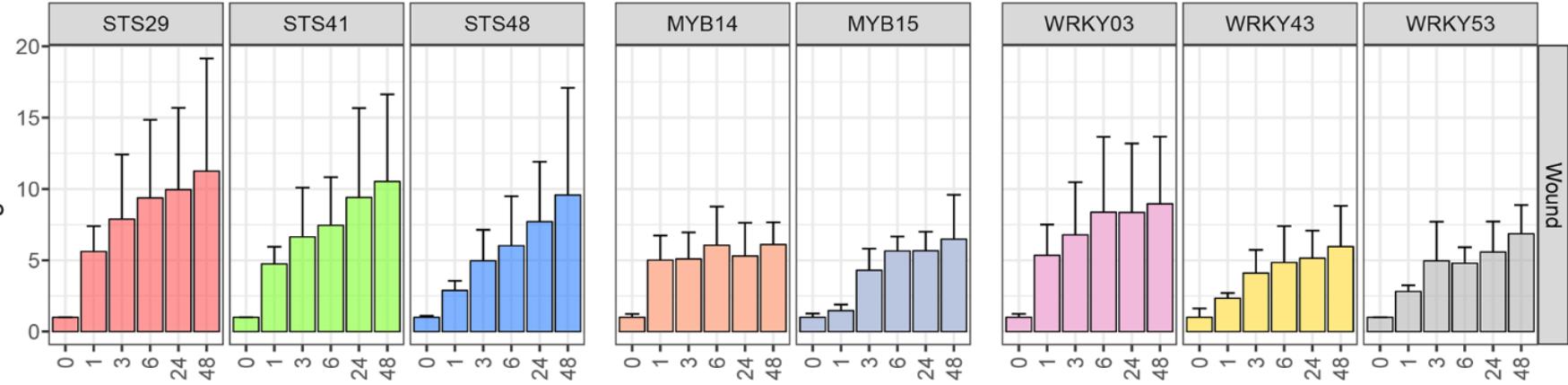


## Heat-map of *VvSTSs* and candidate TFs under stress conditions

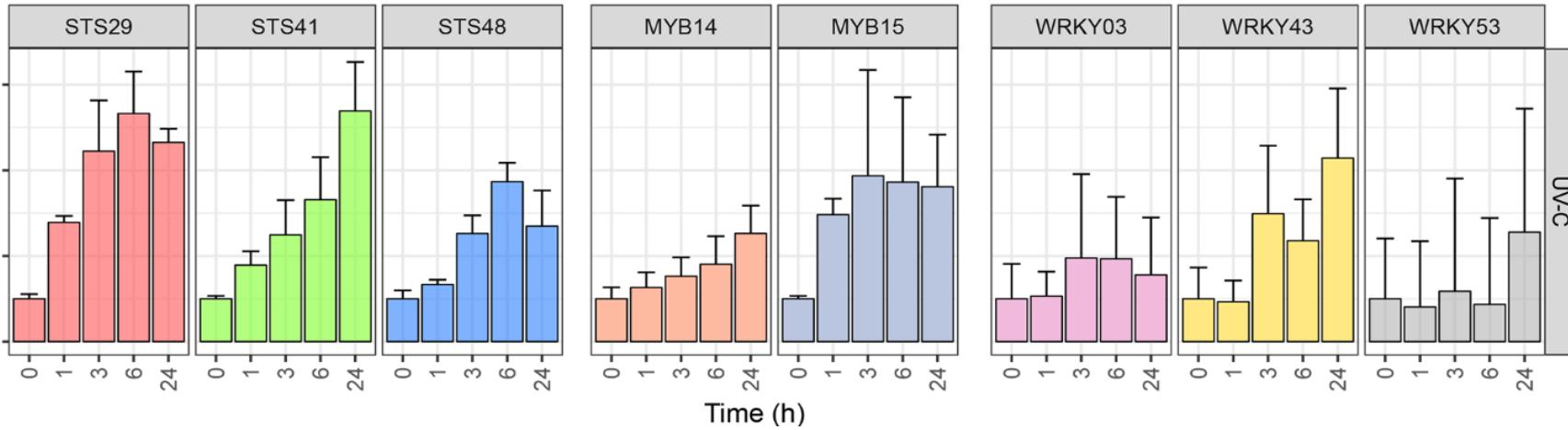


# *VvSTS* and TFs expression under abiotic stresses

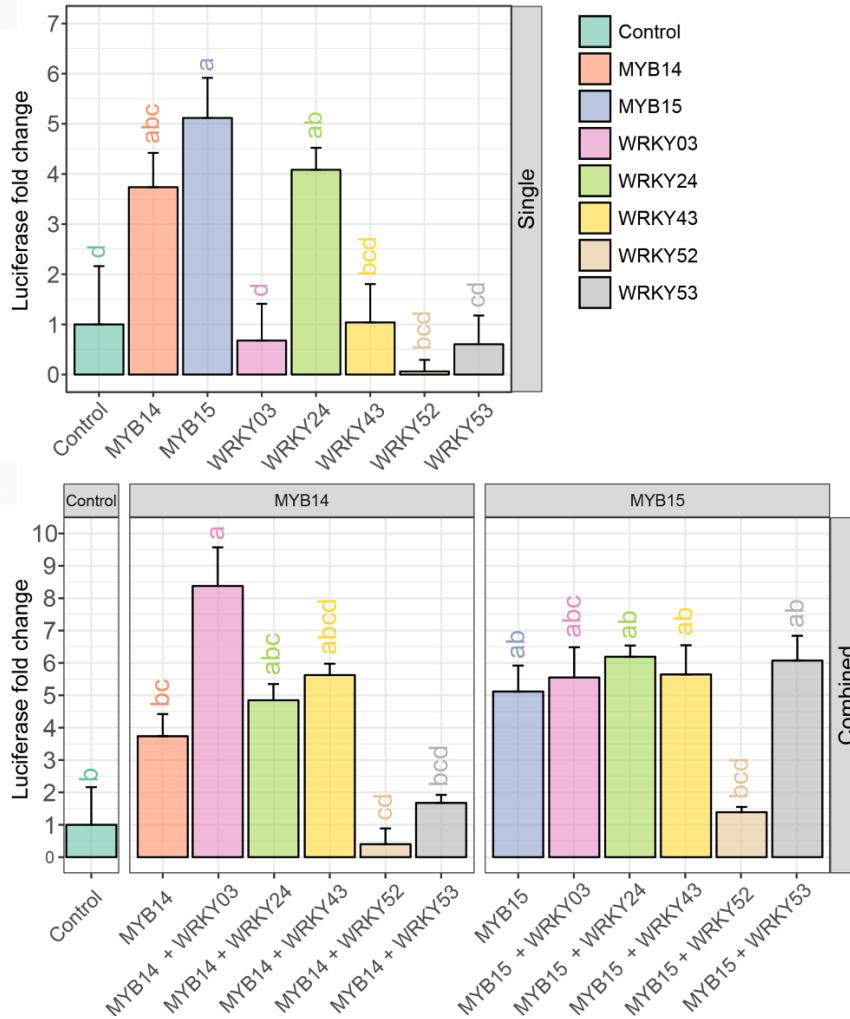
wound



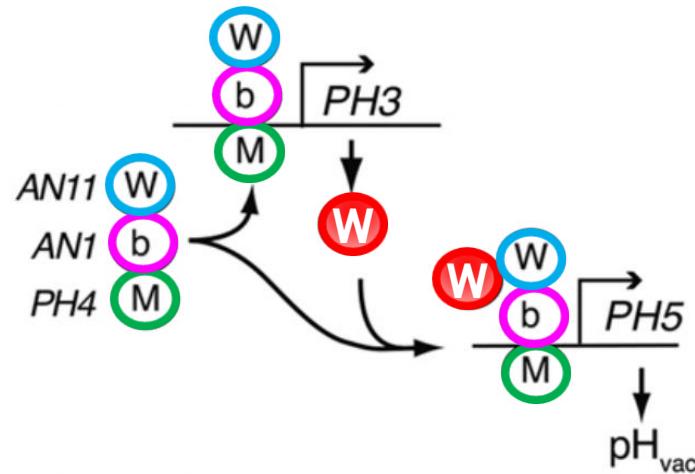
UV-C



# Single and combined effector Dual reporter luciferase assay



- **VvWRKY24** effector increases the luciferase activity as much as VviMYB14 and VvMYB15
- **VvWRKY3** shows a **combinatorial** effect in association with VviMYB14 leading to a promoter induction higher than MYB14 alone. **TF-TF interaction?**

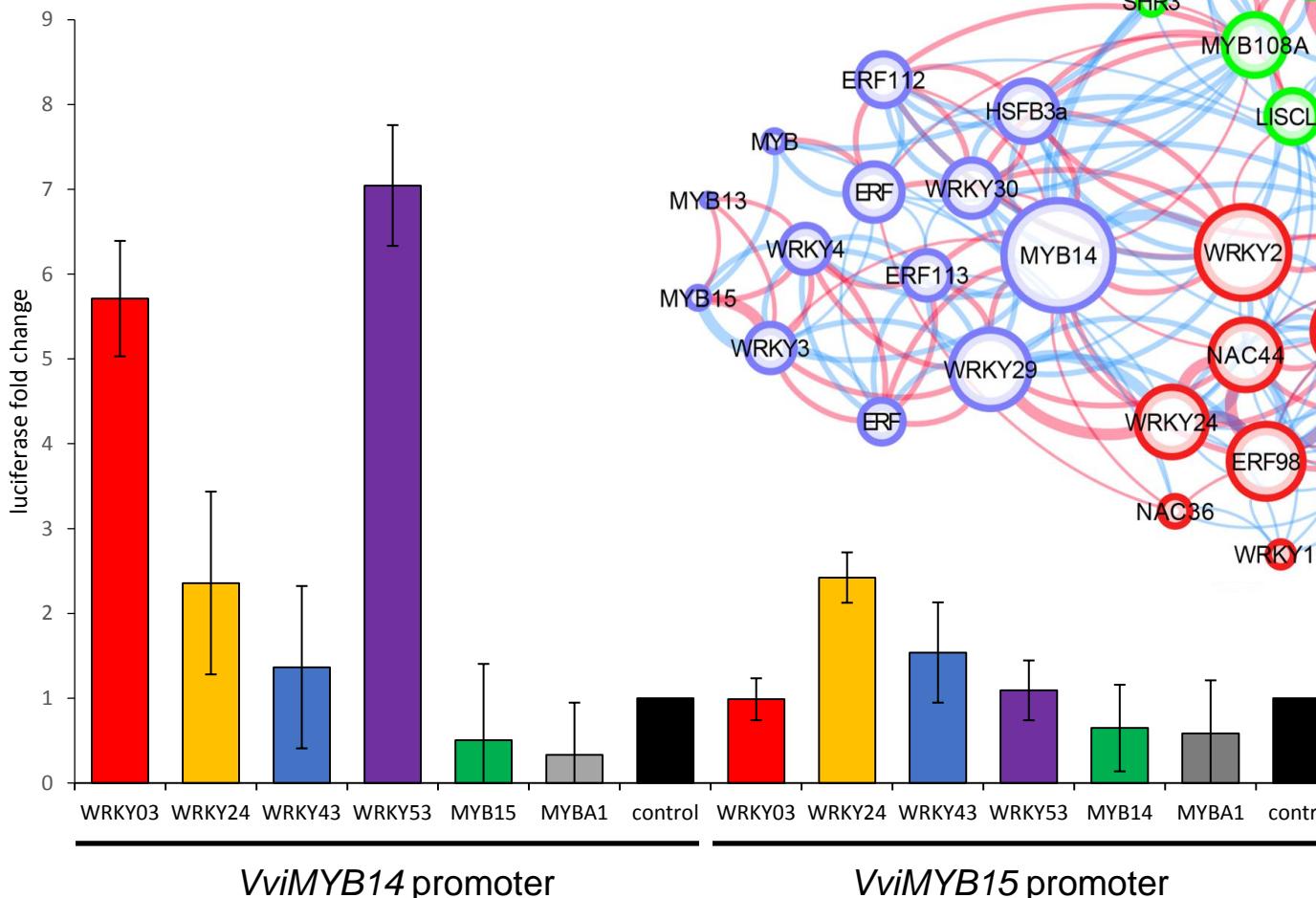


Modified from Verweij et al. 2016 The Plant Cell



# TF-TF CGN

**VvWRKY3 and VvWRKY53 regulate the *VviMYB14* expression**



# Conclusions

- We identify a number of TFs representing good candidates for further studies on the regulation of the stilbene pathway in grapevine including **R2R3-MYBs**, **WRKYs**, ERF and NAC genes and a **bHLH** gene.
- We confirmed the validity of the GCN “guilt by association” approach for inferring the biological function of genes (confirmed the role of VviMYB14 and VviMYB15 TFs).
- We identified a novel regulator, namely **VvWRKY24** which appears to induce the promoter activity of at least one *VvSTS* gene in transfected grapevine cells.
- We described a combinatorial effect of **VvWRKY3** and **VviMYB14** in the regulation of the promoter of a *VvSTS* gene.
- Preliminary results suggest that **VvWRKY3** (and **VvWRKY53**) could act upper level (regulatory circuitry?) in the STS regulation controlling the transcription of *VviMYB14* TF.
- To test the interaction between VvWRKY3 (VvWRKY53) and VviMYB14 (and VviMYB15) proteins by Yeast-2-hybrid and BiFC assays.
- Confirm the role of WRKY3, VvWRKY24 and VvWRKY53 by functional assays *in planta*



# Thanks to



## DAFNAE

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**Adelaide SA**

Dr Ian Dry

Dr Paul Boss

Dr Angelica Jermakow

**CSIC-IRTA-UAB-UB**

**Barcelona**

Dr Tomàs J. Matùs

**Australian National University**

**Acton**

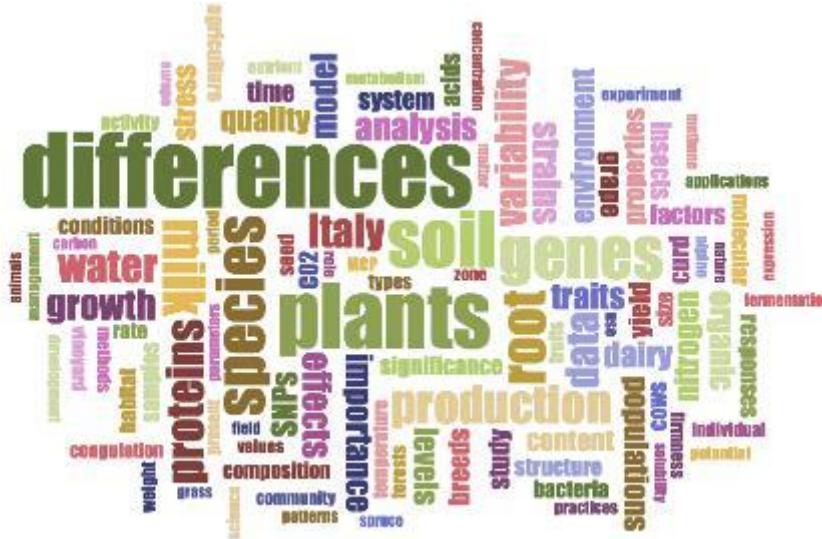
Dr Darren C.J. Wong



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# Thank you



# Roles and regulation of stilbene biosynthesis

