Crops coping with water scarcity

CHIARA TONELLI

Università degli Studi di Milano

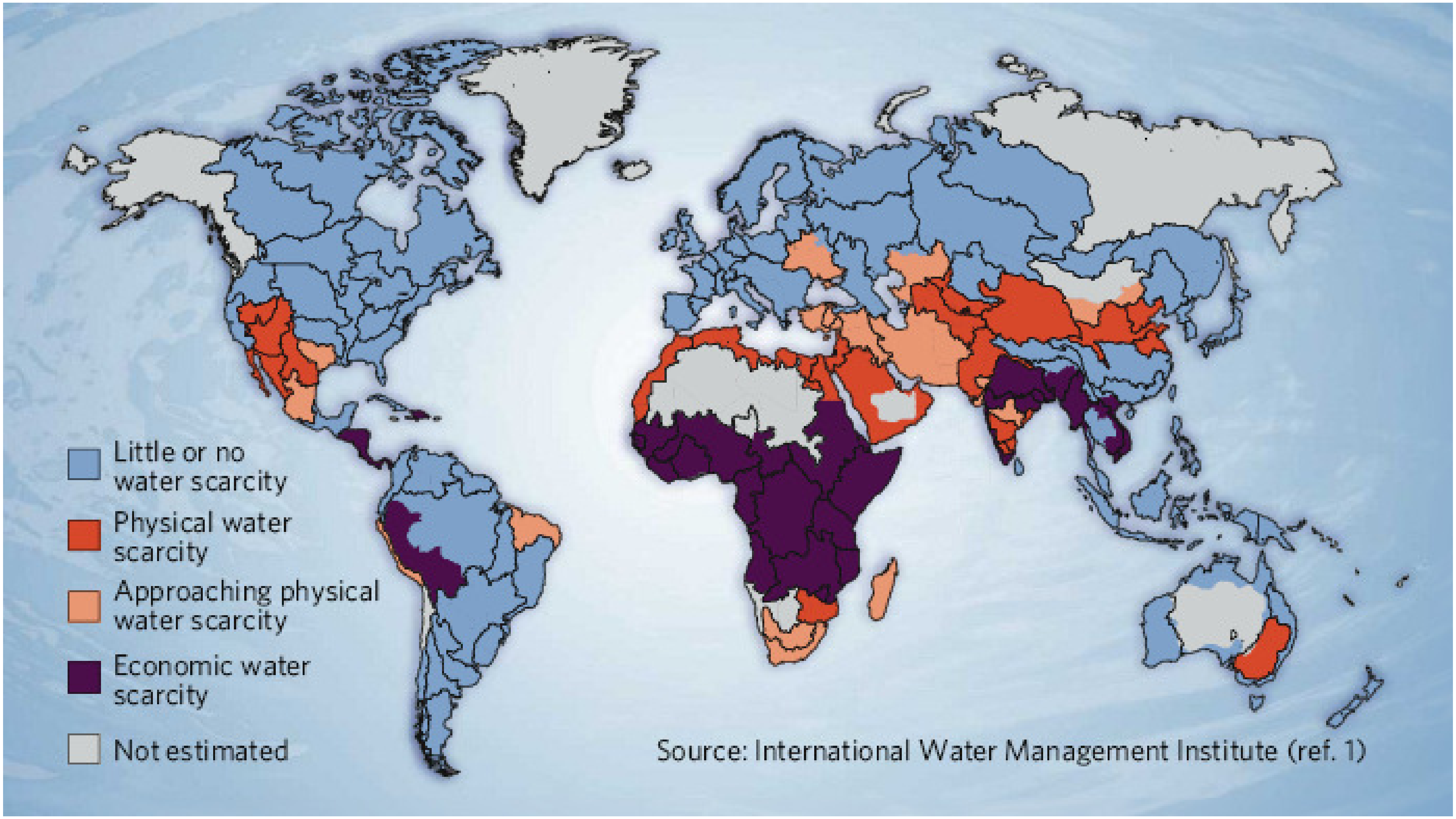
Dipartimento di Scienze Biomolecolari e Biotecnologie

World water distribution

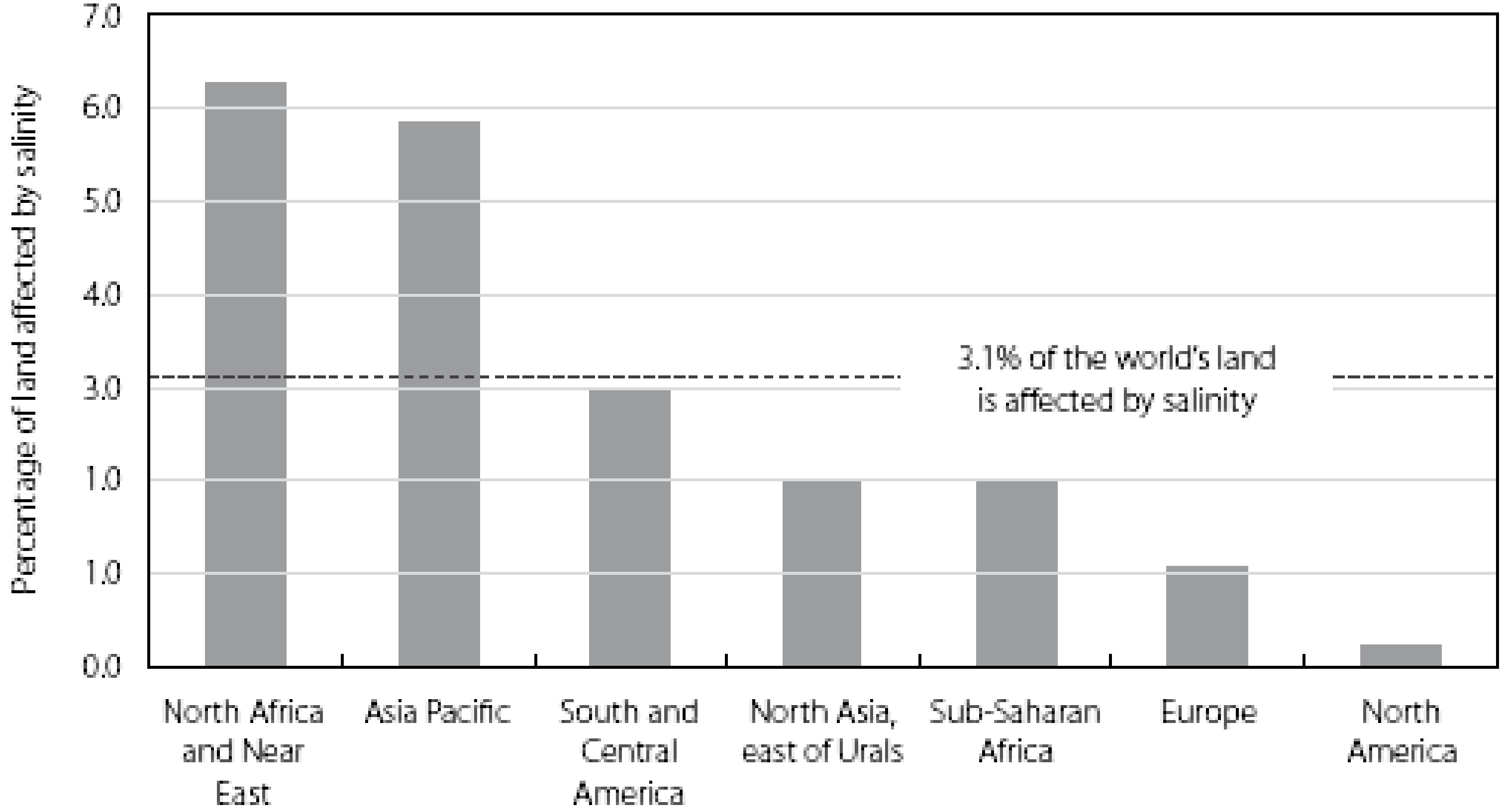
	Water volume (million km³)	Percent of freshwater	Percent of total water
Total water	**1 386**		**100.00**
Freshwater	**35**	**100.0**	**2.53**
Glaciers and ice caps	24.4	69.7	1.76
Groundwater	10.5	30.0	0.76
Lakes, rivers, atmosphere	0.1	0.3	0.01
Saline water	**1 351**		**97.47**

Source: FAO 2002; Crops and drops: make the best use of water for agriculture

Areas of physical and economic water scarcity

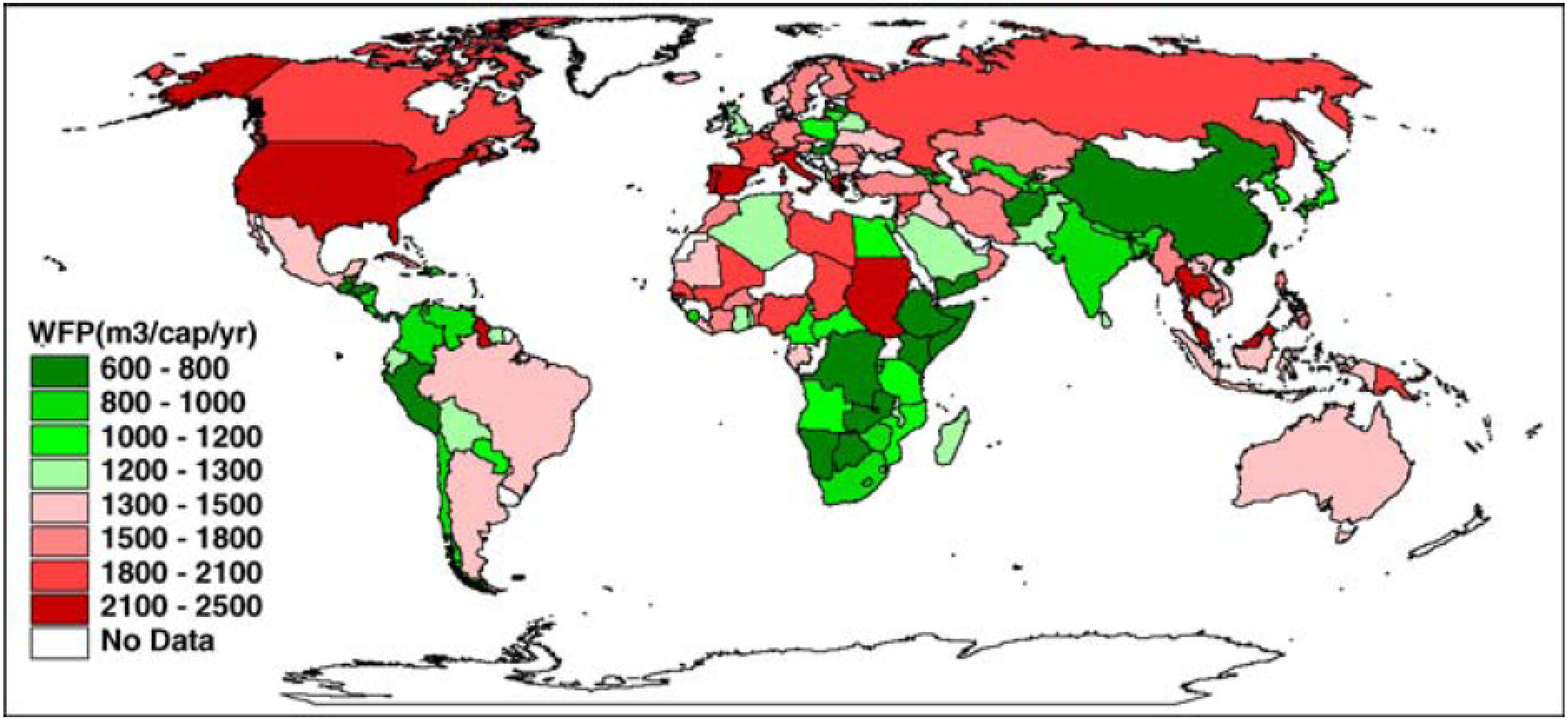


Increase in soil salinity is another big problem associated to water scarcity



Source: FAO 2006

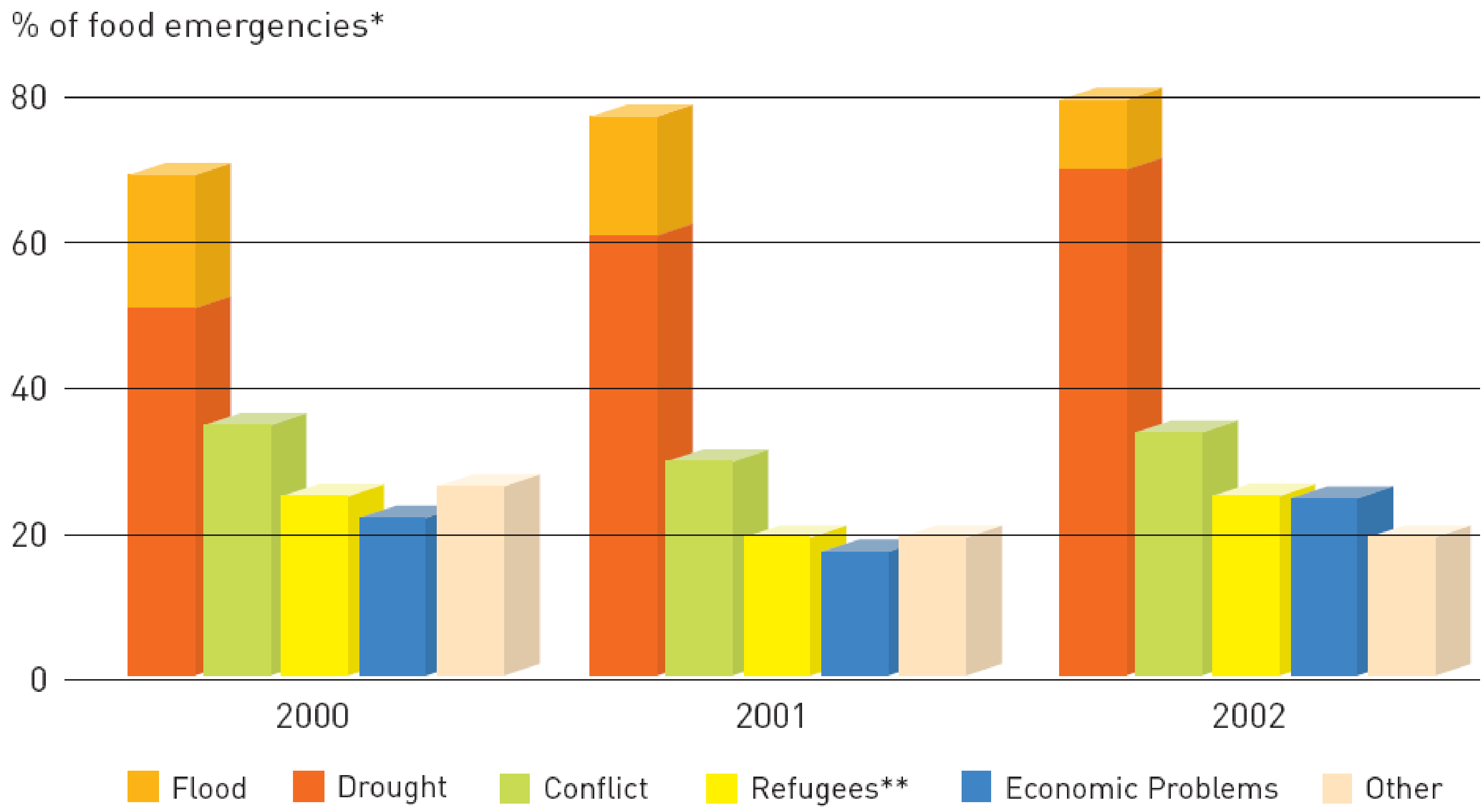
The **water footprint** of a person, company or nation is defined as the total volume of freshwater that is used to produce the commodities, goods and services consumed by the person, company or nation.



Average national water footprint per capita (m3/capita/yr).

A. Y. Hoekstra · A. K. Chapagain, Water Resource Management (2007) 21:35–48

Drought ranks as the single most common cause of severe food shortages in developing countries

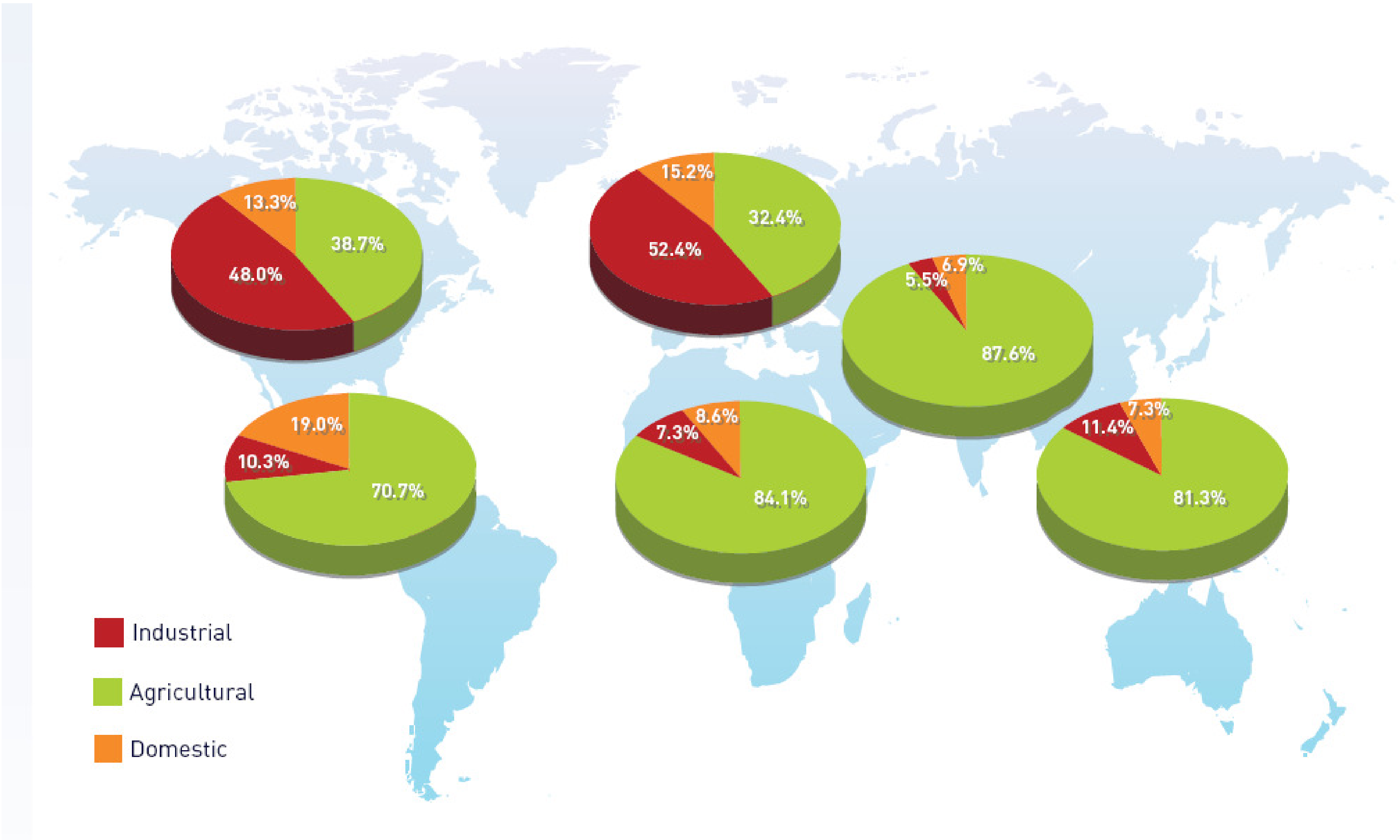


* total exceeds 100% because of multiple causes and cited for many emergencies

** includes internally displaced people

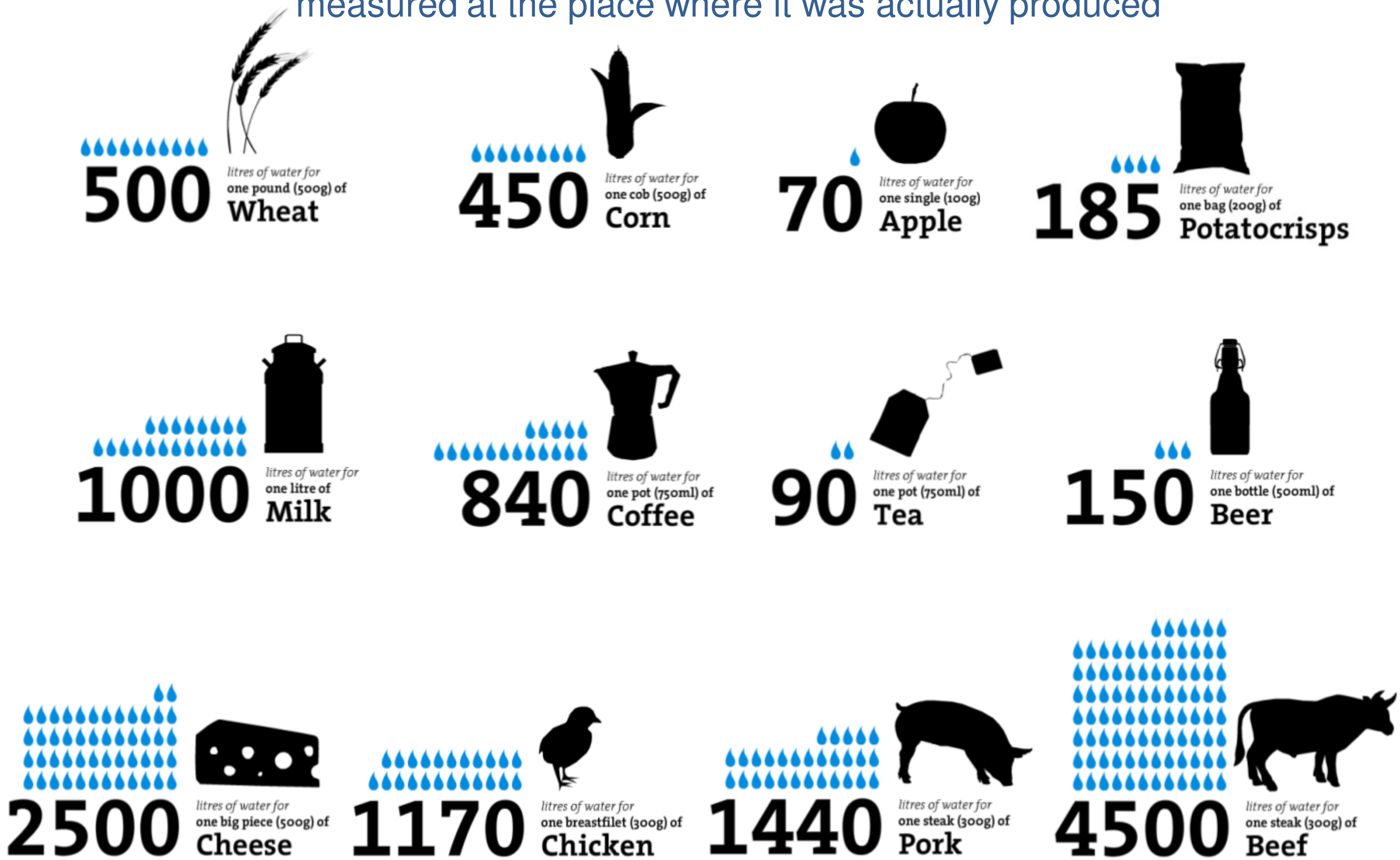
http://www.fao.org/nr/water/

Agriculture makes the biggest demands on fresh water, followed by industry and household use



http://www.fao.org/nr/water/

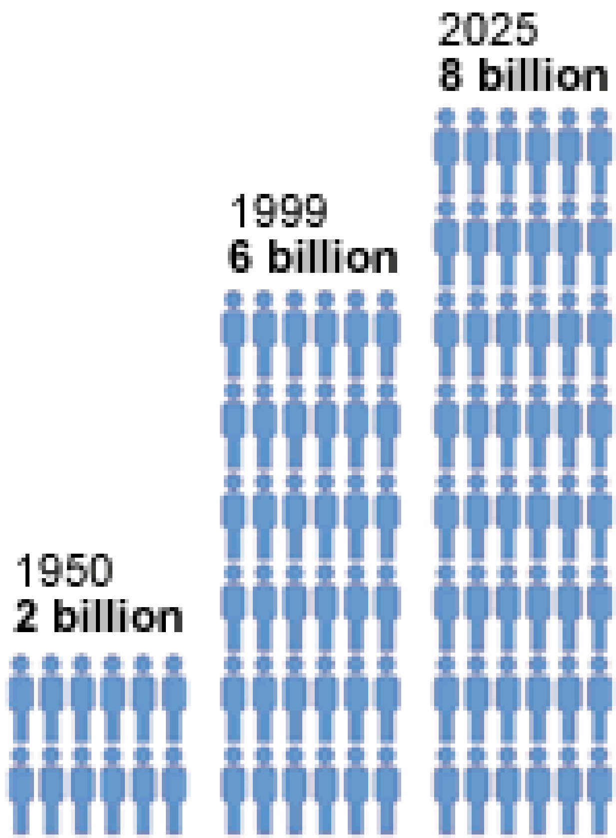
VIRTUAL-WATER CONTENT: volume of freshwater used to obtain a product, measured at the place where it was actually produced



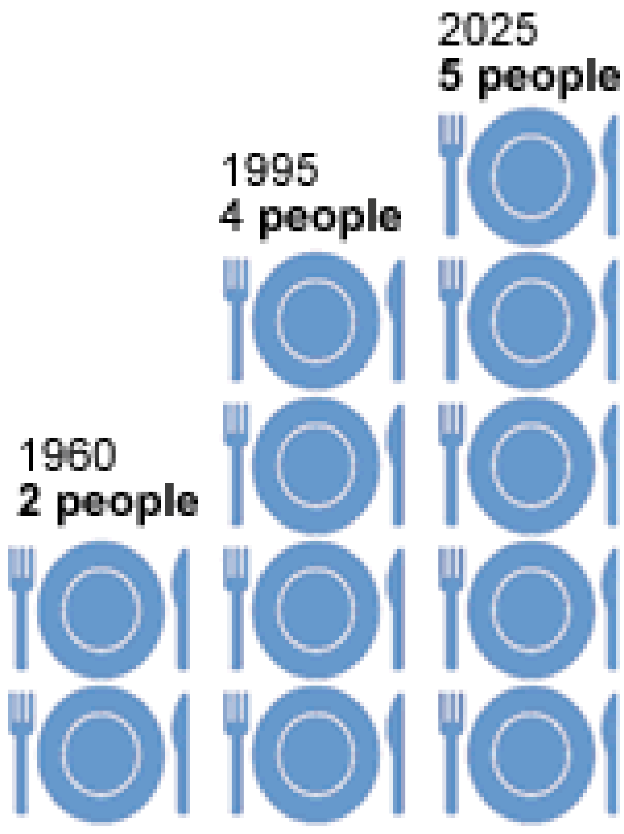
Designed byTimm Kekeritz from the study: Chapagain, A.K. and Hoekstra, A.Y. (2004), Water footprints of nations, Value of Water Research Report Series No. 16, UNESCO-IHE, Delft, the Netherlands.

Demand for drought-tolerant crops driven by population growth and land scarcity

World population



People fed per hectare



Source: FAO, World Bank statistics

How increasing in demand can be met

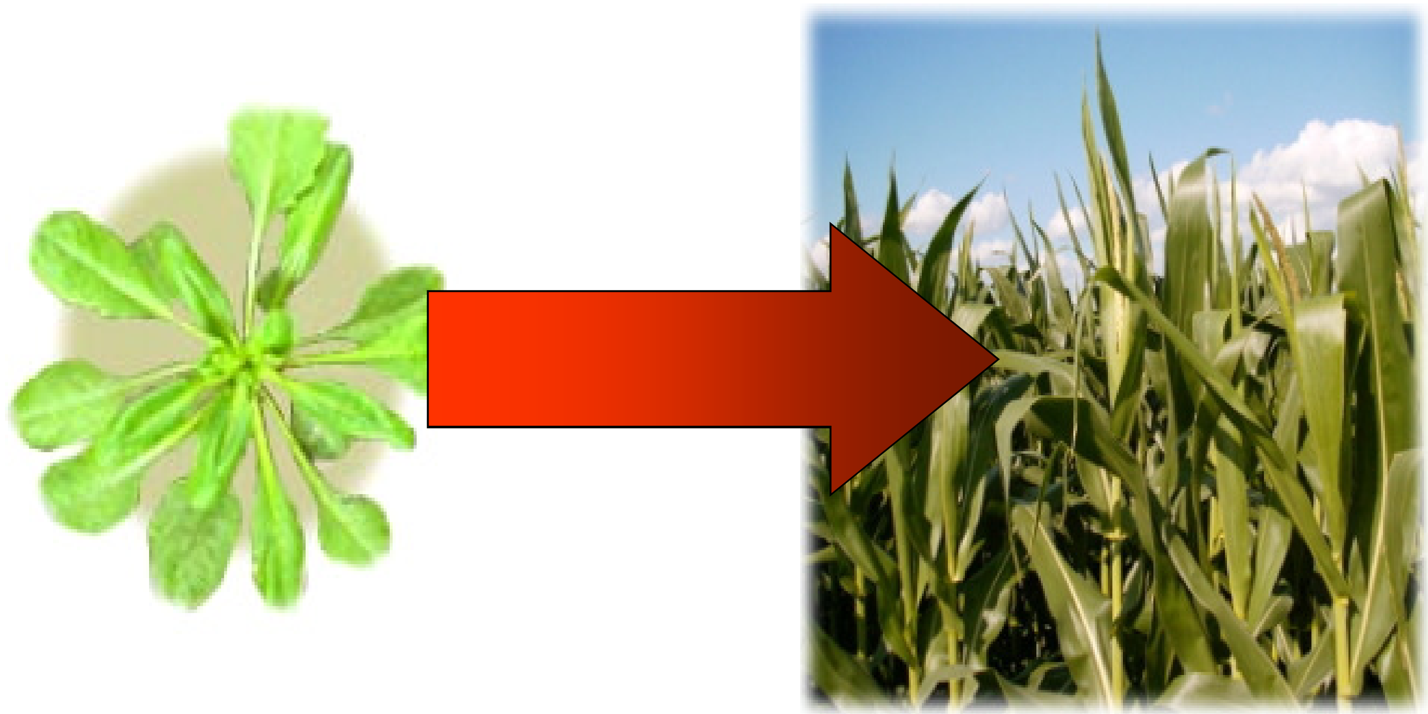
- ❖ increasing the area of arable land
- ❖increasing agricultural yield
- ❖ increasing cropping intensity (number of crops per year)

through

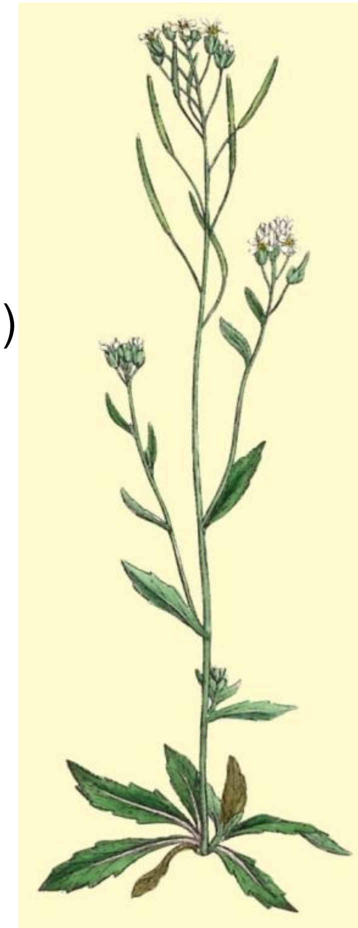
- ❖ improvement in irrigation efficiency
- ❖ desalination of sea-water
- ❖ cultivation of species with relatively low water requirements
- ❖ improvement of plant "water use efficiency" through selection, breeding or **genetic engineering approaches**
- ❖ improvement of plant performance and yield under drought conditions through selection, breeding or **genetic engineering approaches**

Technology transfer

from model plants to crops



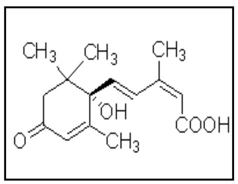
Arabidopsis thaliana as a model plant



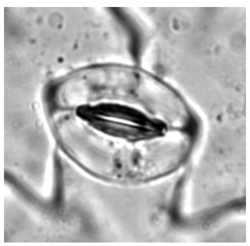
- ❖ small genome (about 130 Mbp, 5 chromosome, 27,000 genes)
- ❖ the first plant genome to be sequenced (completed in 2000)
- ❖ rapid life cycle (2 months from germination to mature seed)
- ❖ small size convenient for cultivation in small spaces
- ❖ many seeds (several thousand)
- ❖ high transformation efficiency
- ❖ genomics resources (insertion mutants collections, genome arrays etc.)

Physiological responses of plants to drought stress

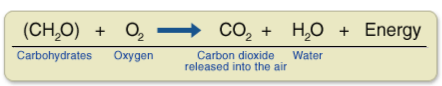
❖ inhibiton of cell growth and photosynthesis



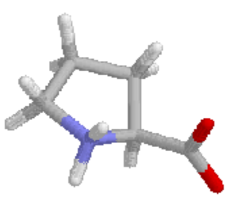
❖ biosynthesis of abscisic acid



❖ stomata closure



❖ increase of respiration



❖ production of osmolytes and stress proteins

Improvement of plant drought tolerance through biotechnology

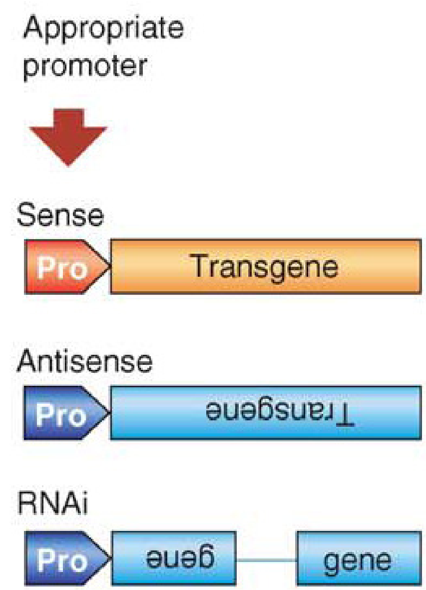
GENE DISCOVERY IN STRESS RESPONSE

↓

GENES USED FOR ENGINEERING

↓

CONSTRUCTION OF TRANSGENES →

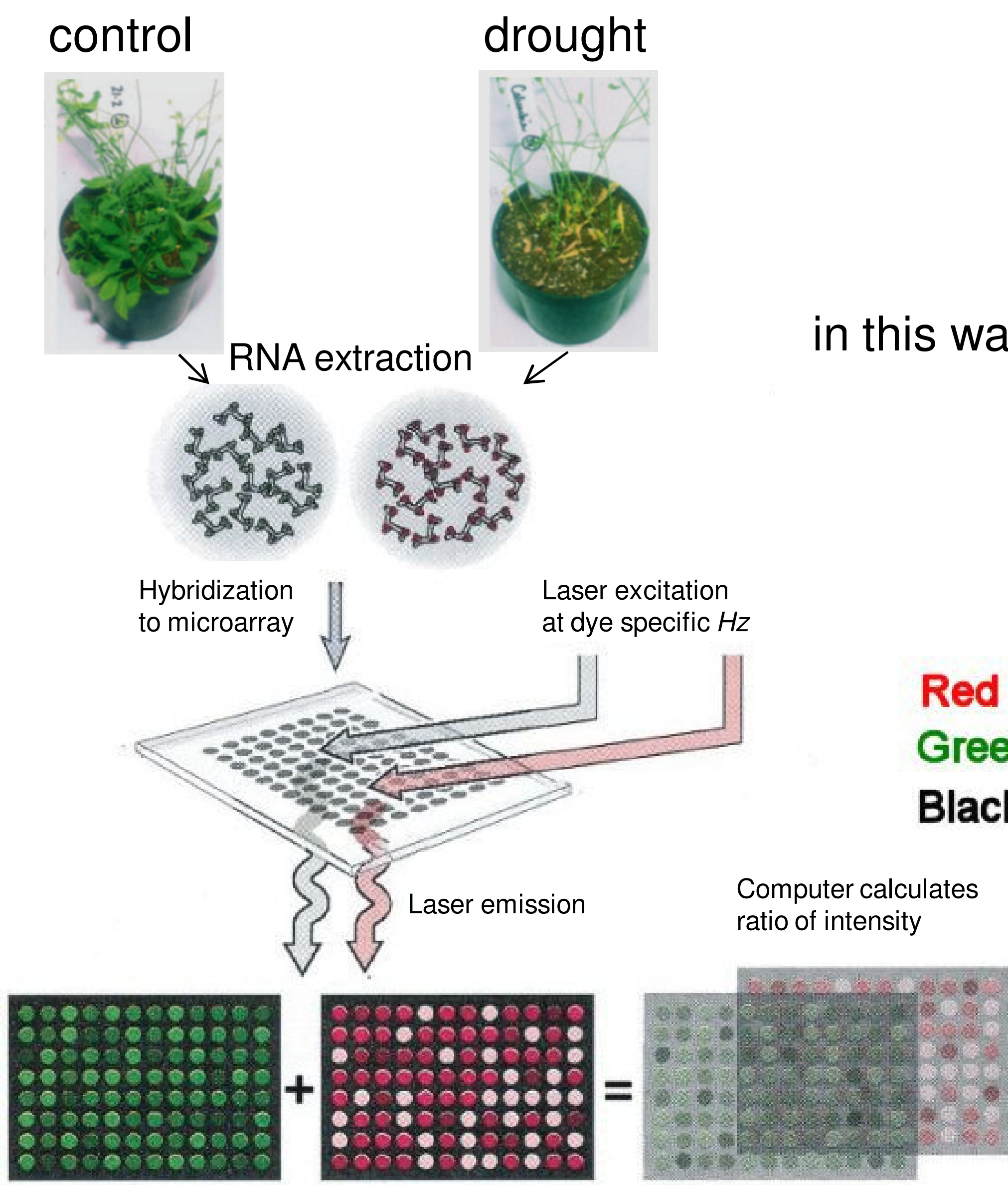


↓

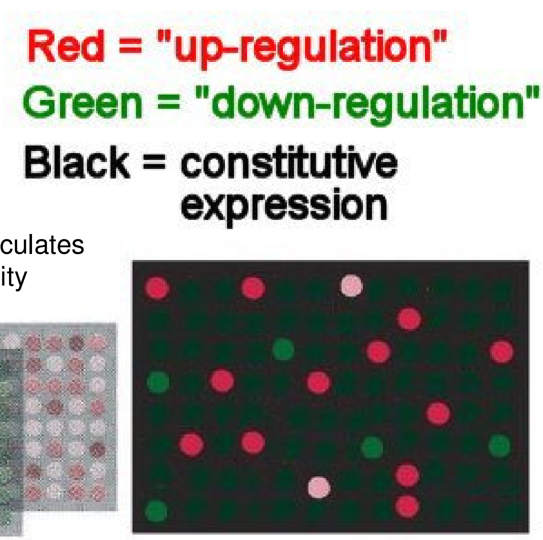
GENETIC ENGINEERING

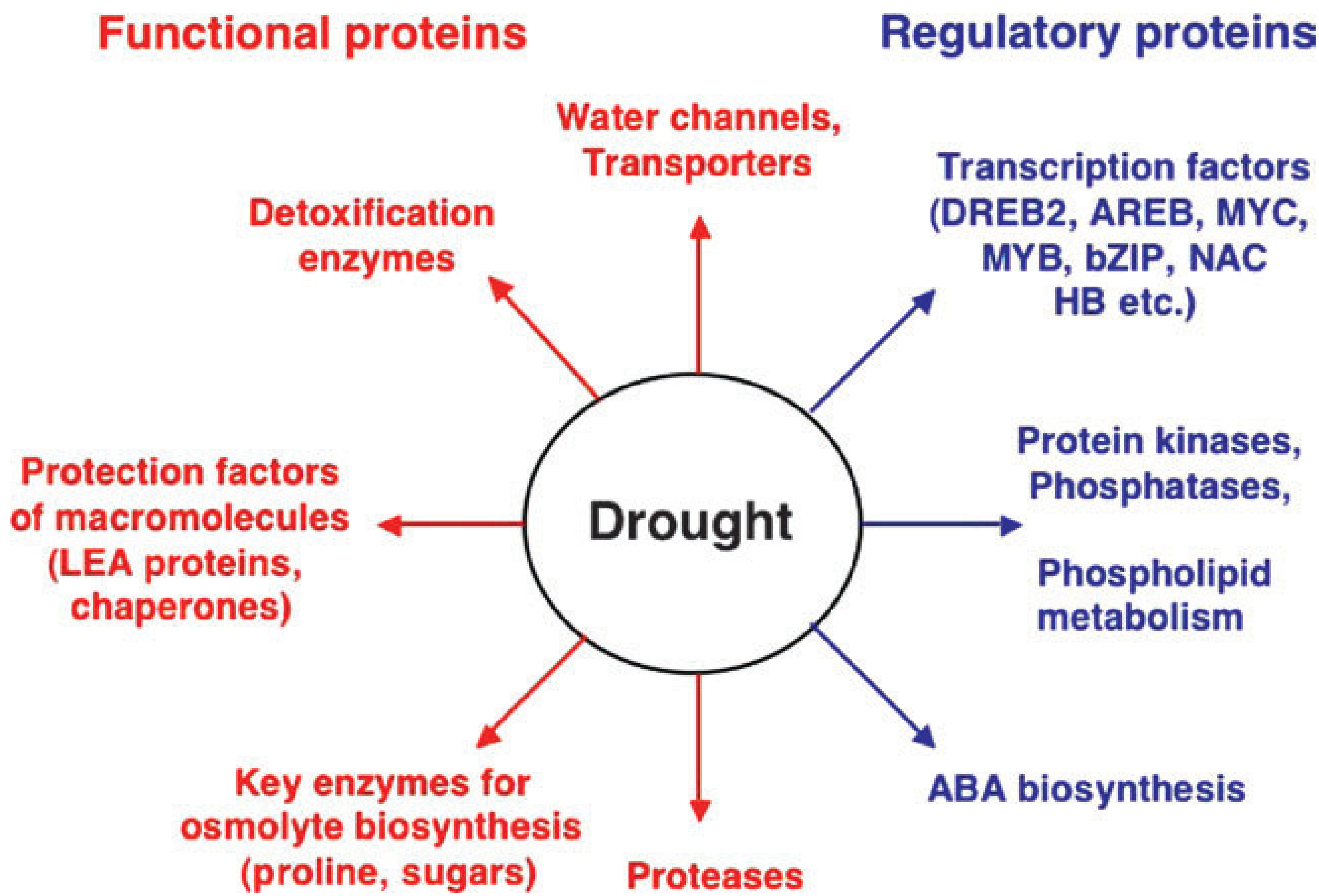
modified from Umezawa et al., 2006

Identification of genes involved in drought stress response

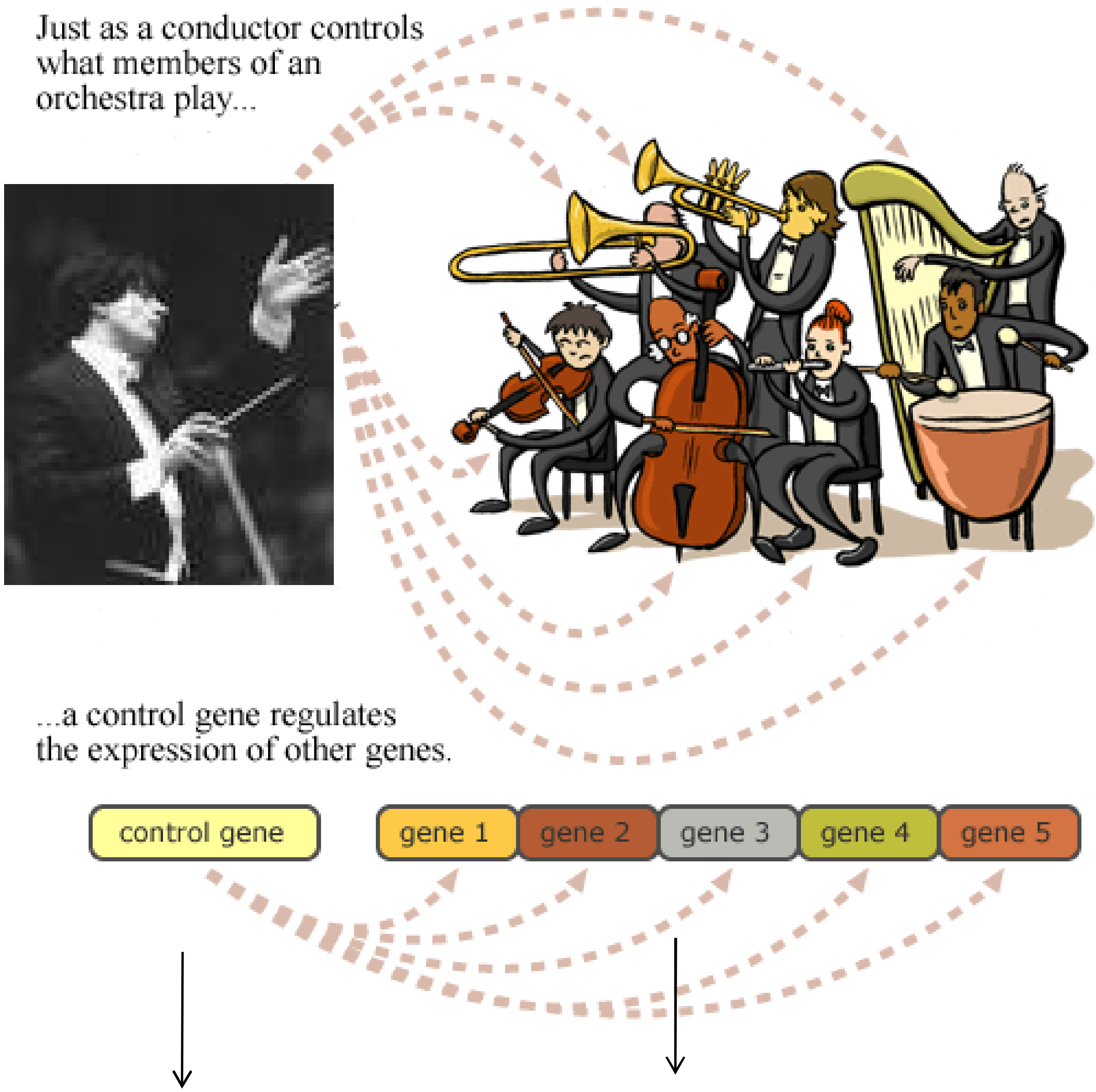


in this way in Arabidopsis ~1000 genes identified!!!





Shinozaki and Yamaguchi-Shinozaki, 2007

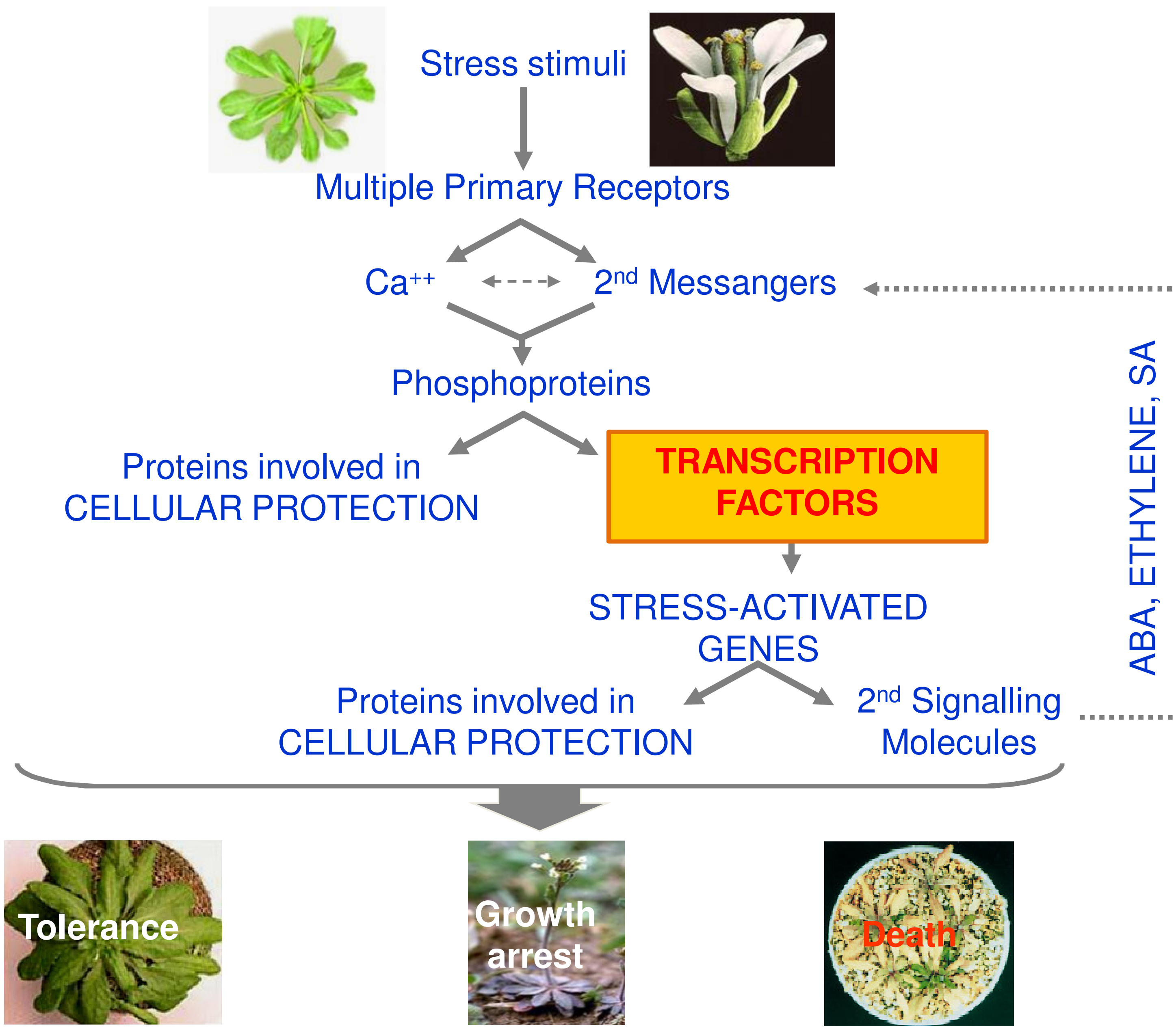


only 1 regulatory protein

many functional proteins

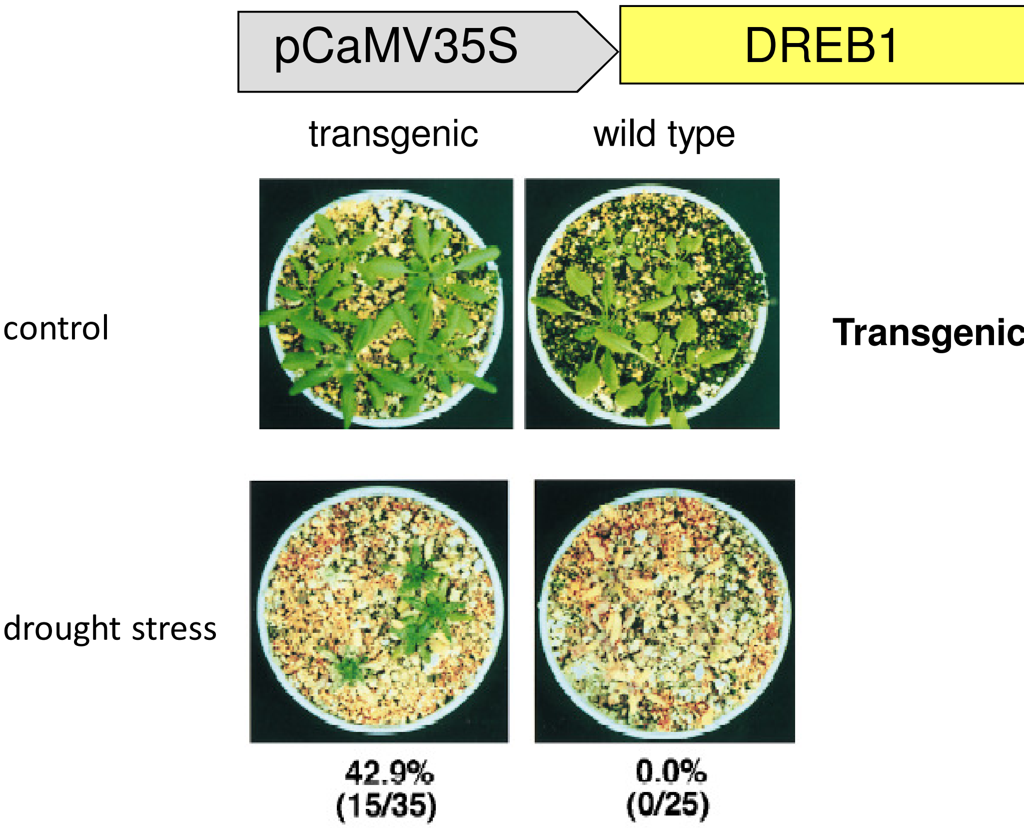
http://evolution.berkeley.edu

Strategy: *Arabidopsis* & Transcription Factors



Constitutive expression of DREB1 in *Arabidopsis*

under the control of a promoter that confers expression in any organs in any growth conditions



Transgenics are more drought tolerant than wild type

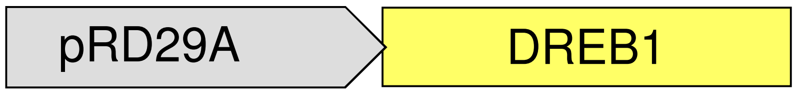
BUT

- **dwarf phenotype**
- **growth retardation**

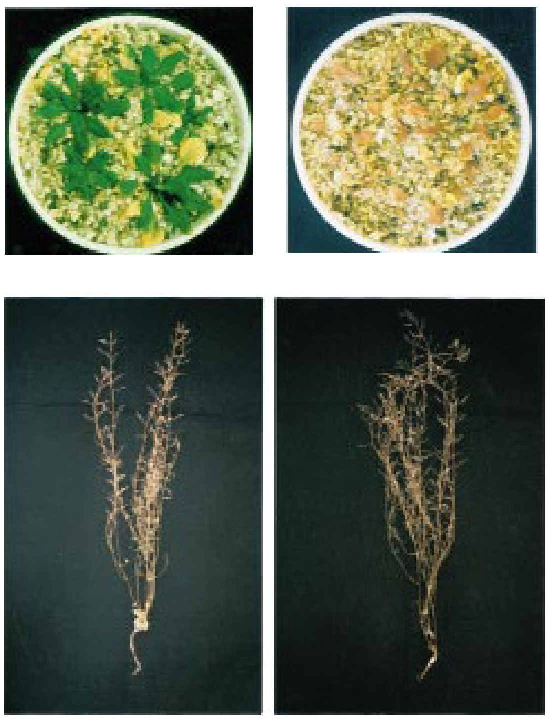
Liu et al. (1998) Plant Cell 19:1391-1406

Inducible expression of DREB1 in *Arabidopsis*

under the control of a promoter that confers **high expression only in response to abiotic stress**



transgenic wild type



40.8±5.5 cm 42.2±5.4 cm

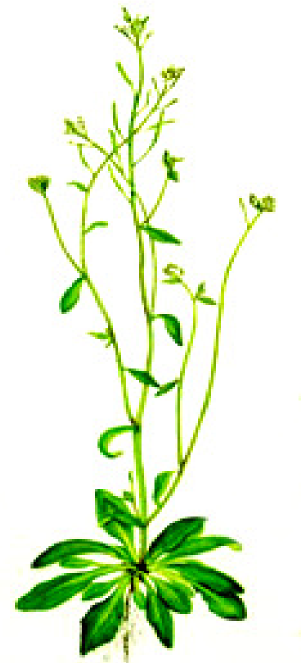
transgenics are more drought tolerant than wild type

AND

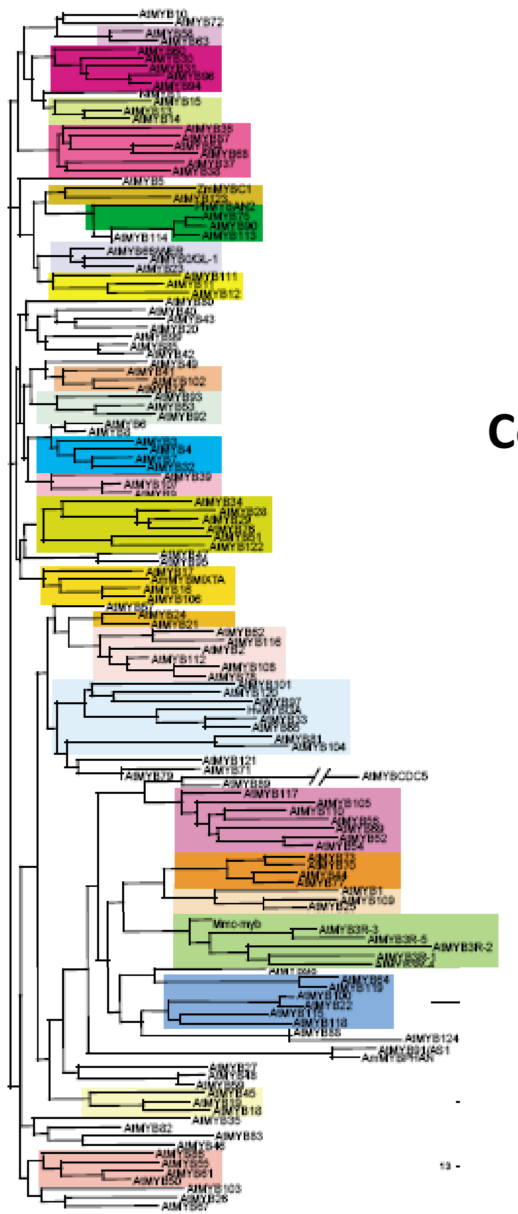
their growth is NOT affected

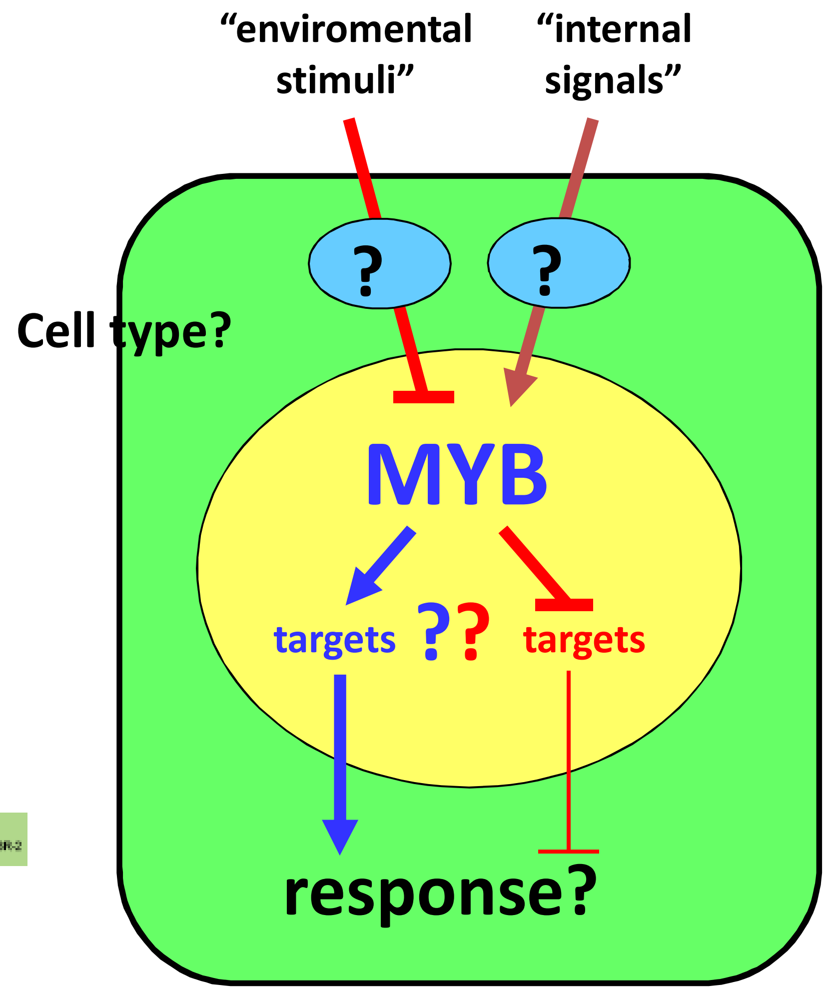
Kasuga et al. (1999) Nature Biotech. 17:287-291

“Functional genomics” of the *Arabidopsis* R2R3-MYB transcription factors family



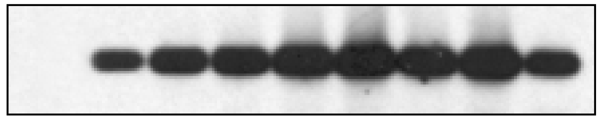
REGIA project



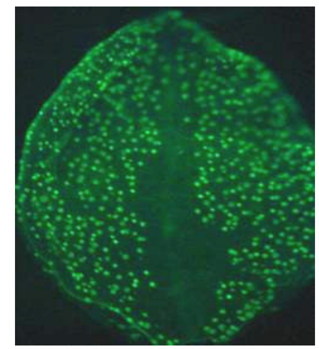


Approaches used to determine the function of a gene involved in stress response

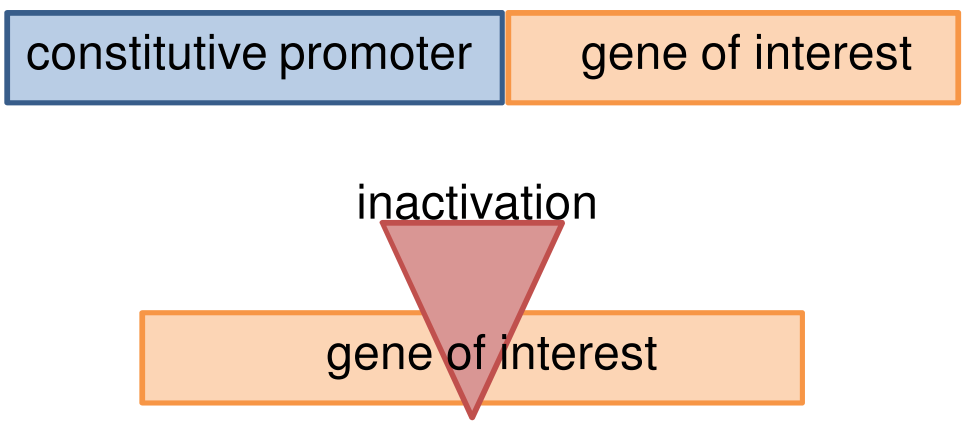
❖ Analyisis of gene expression in response to different stresses



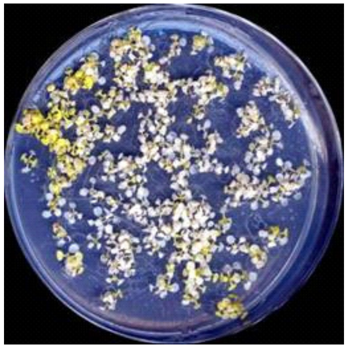
❖ Analysis of expression pattern through promoter fusion to reporter genes

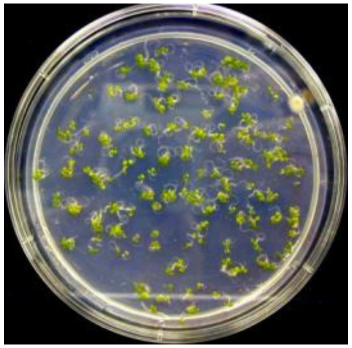


❖ Analysis of phenotype of overexpression and mutant lines in stress conditions



First example: **overexpression** of **AtMYB90** transcription factor confers salt tolerance in *Arabidopsis*

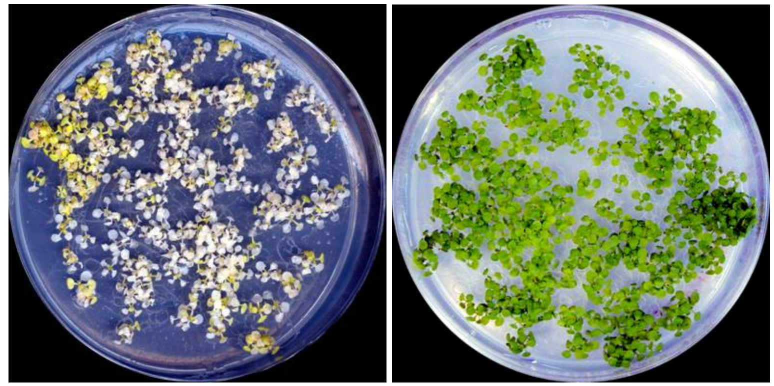




Second example: **silencing** of **AtMYB60** transcription factor confers drought tolerance in *Arabidopsis*

Overexpression of **AtMYB90** transcription factor confers salt tolerance in *Arabidopsis*

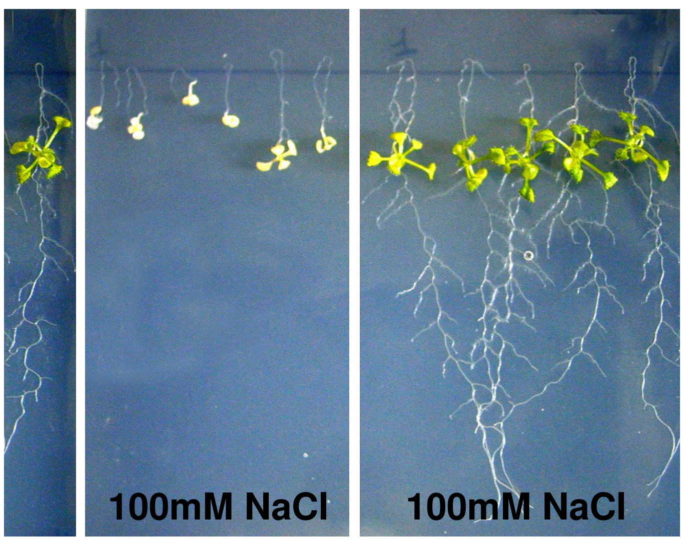
1. Growth on 100mM NaCl:

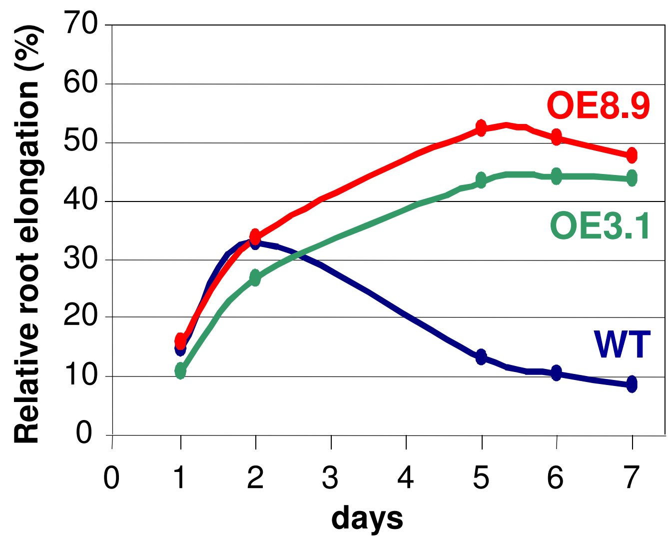


wild type OE3.1

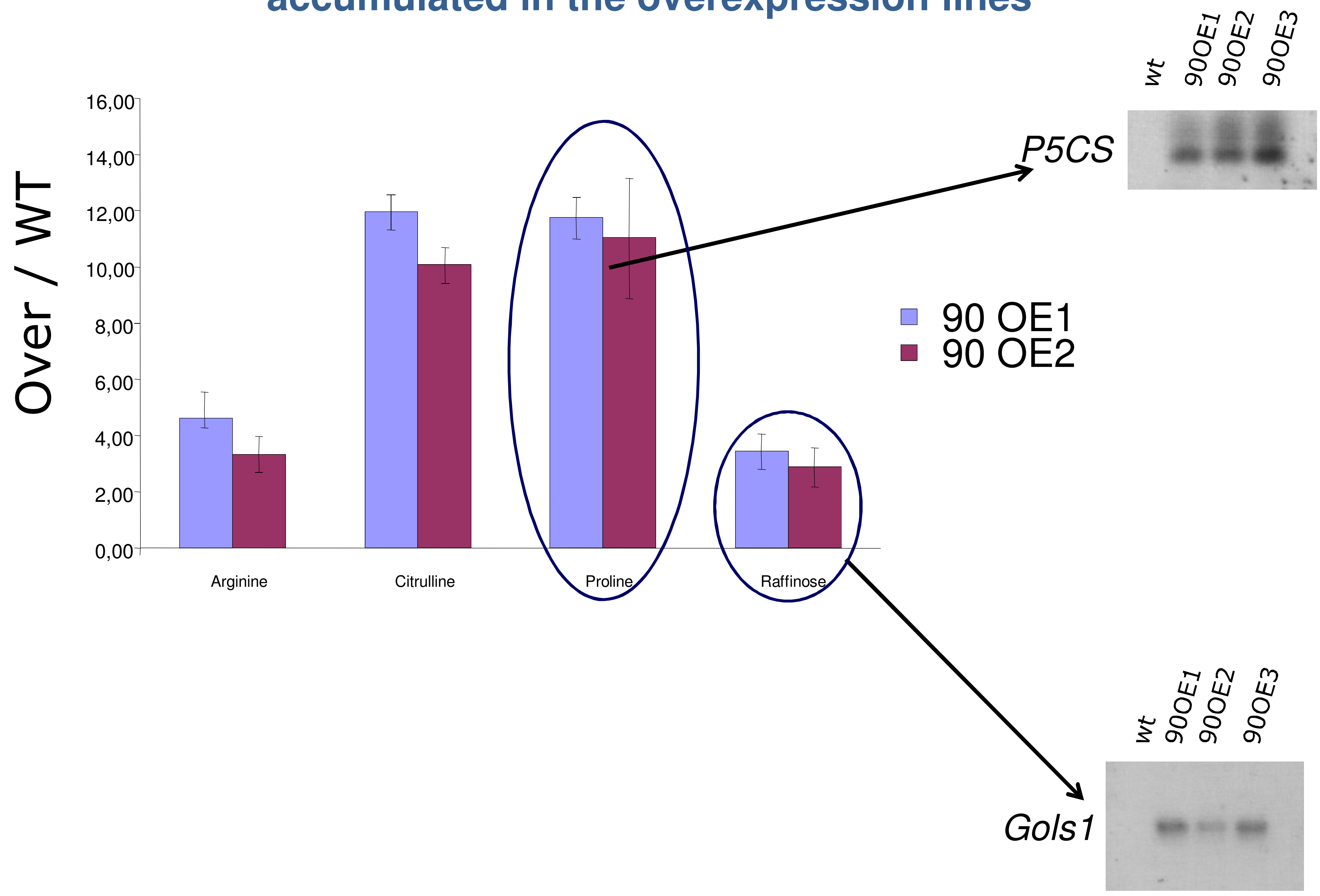
2. Root bending assay:

wild type OE3.1

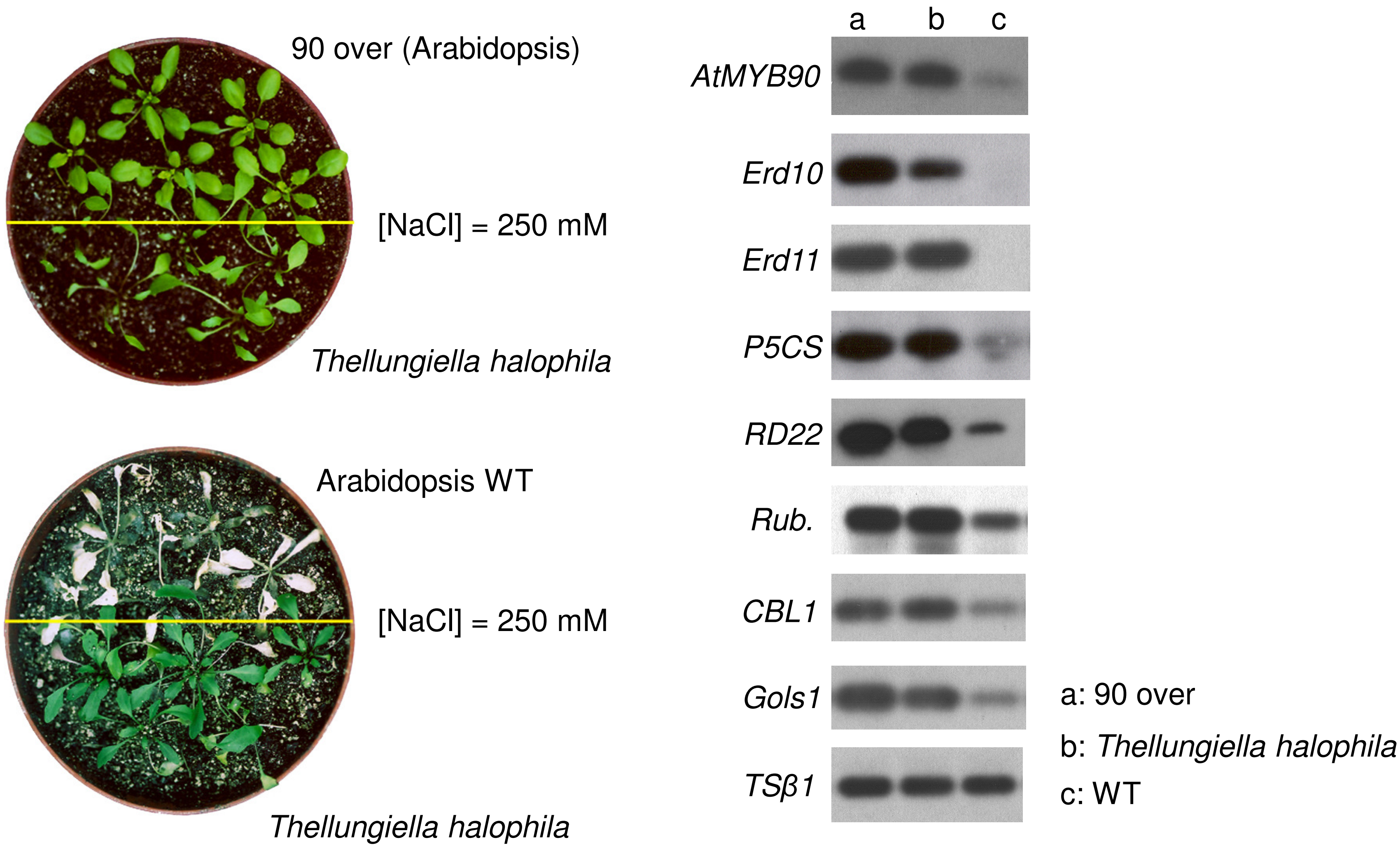




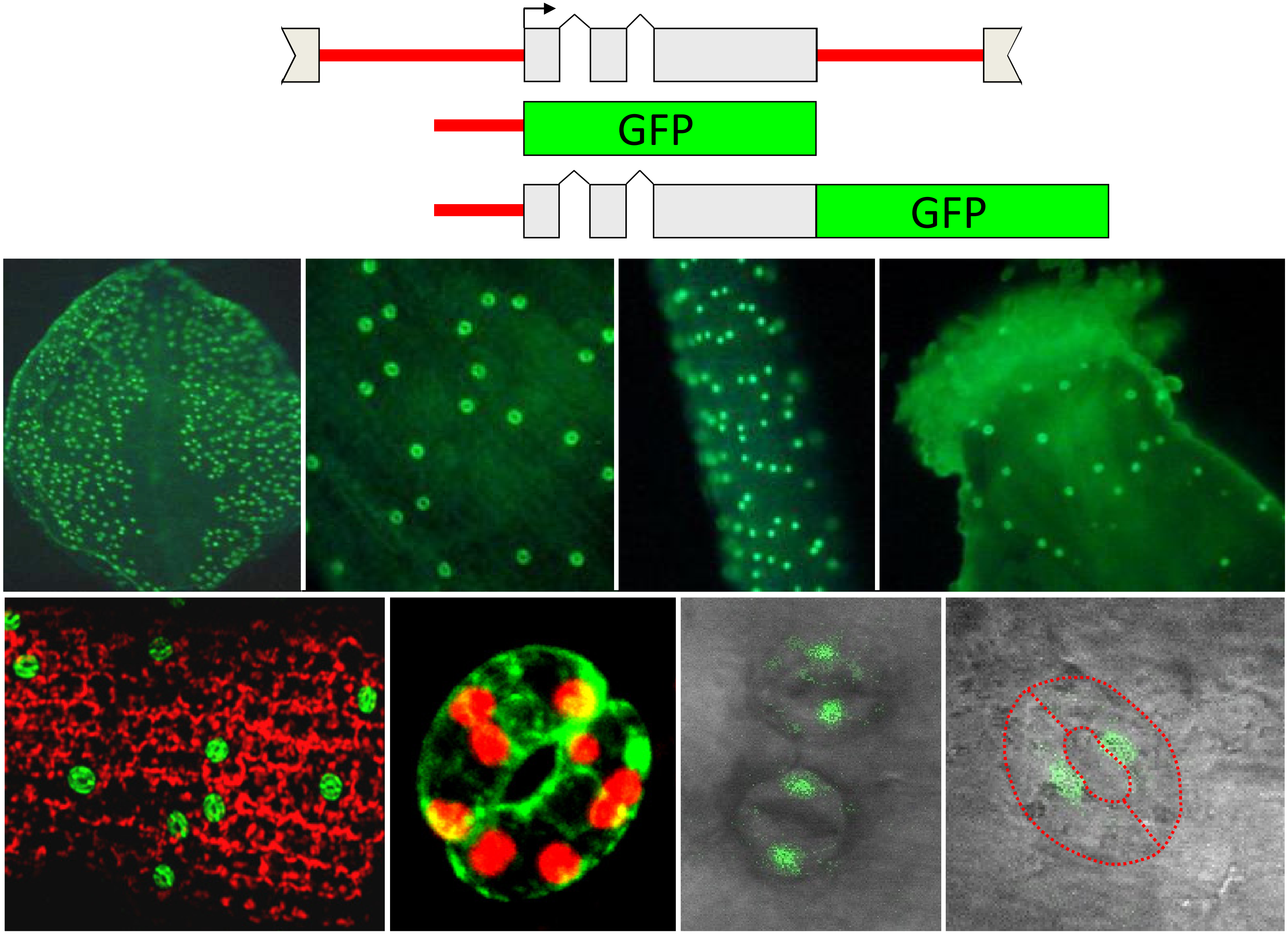
Metabolic profiling analysis reveals that osmoprotectants are highly accumulated in the overexpression lines



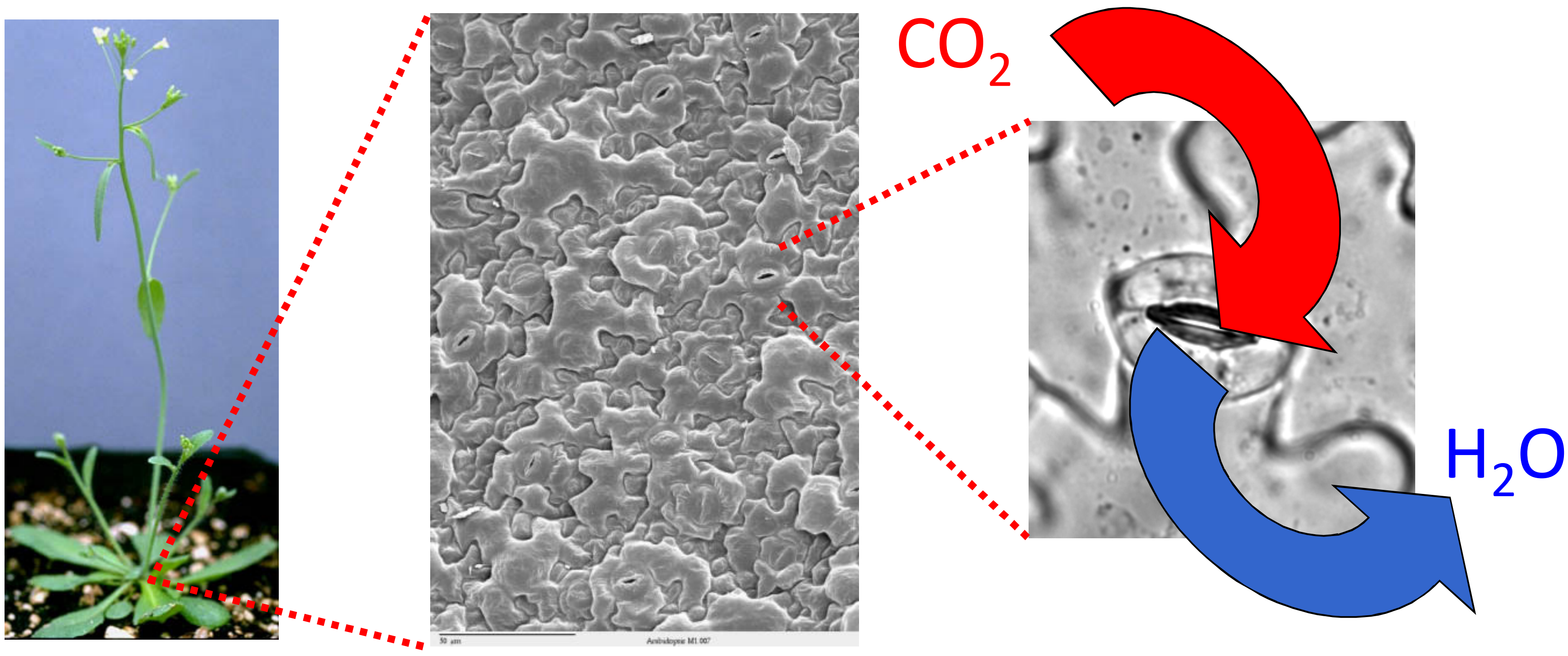
The ortholog of *AtMYB90* in *Thellungiella halophila*, a salt tolerant plant, is highly expressed



AtMYB60 is expressed in guard cells



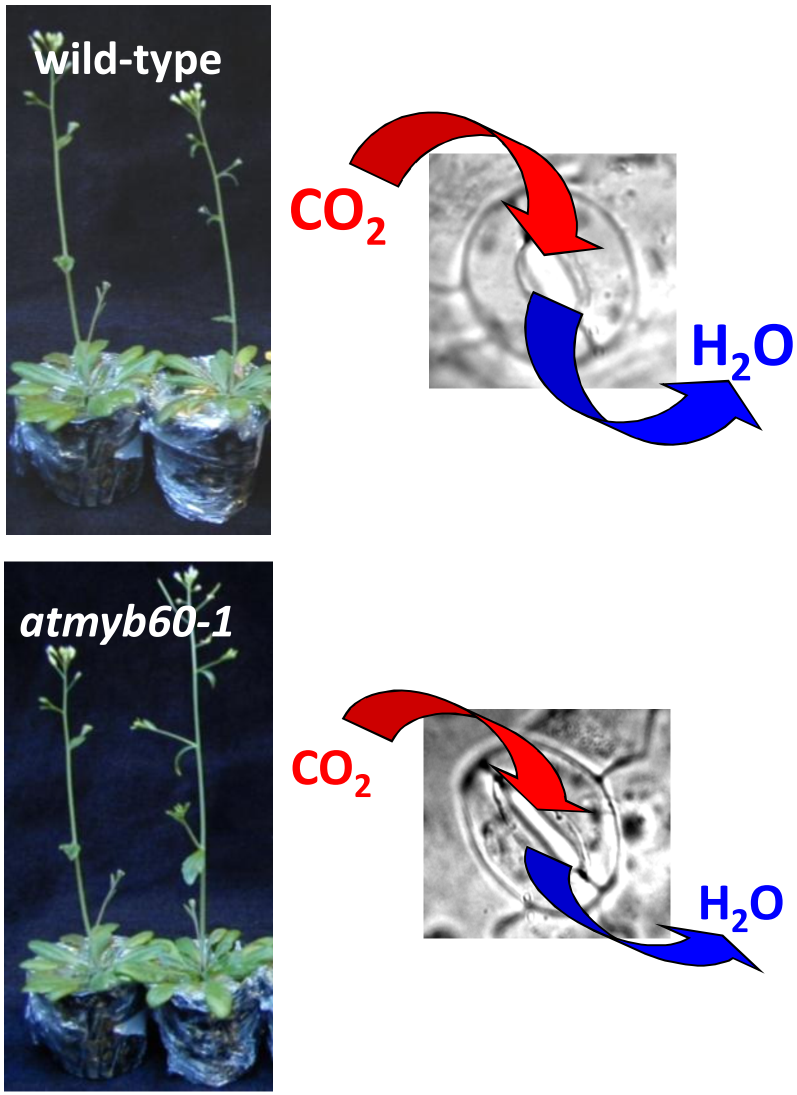
Stomatal pores: a lot more than simply holes!

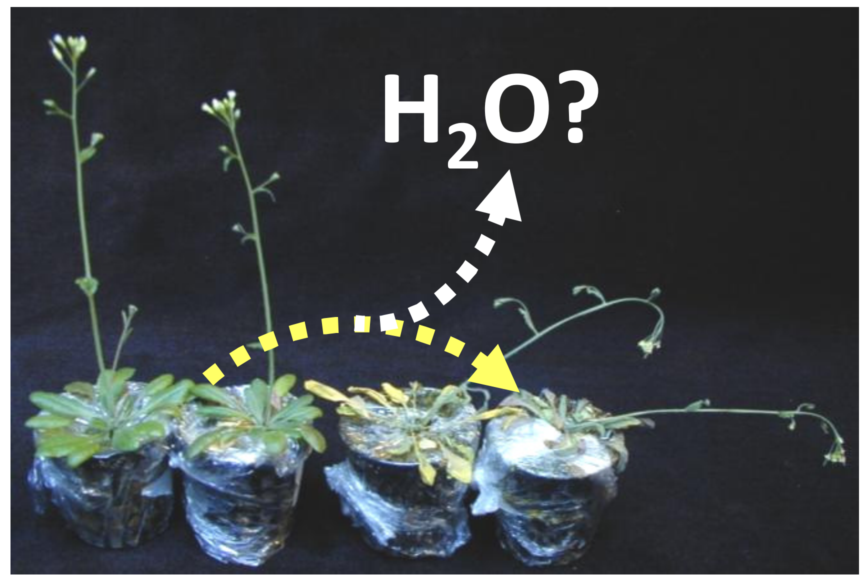


Guard cells integrate environmental stimuli to modulate stomatal aperture

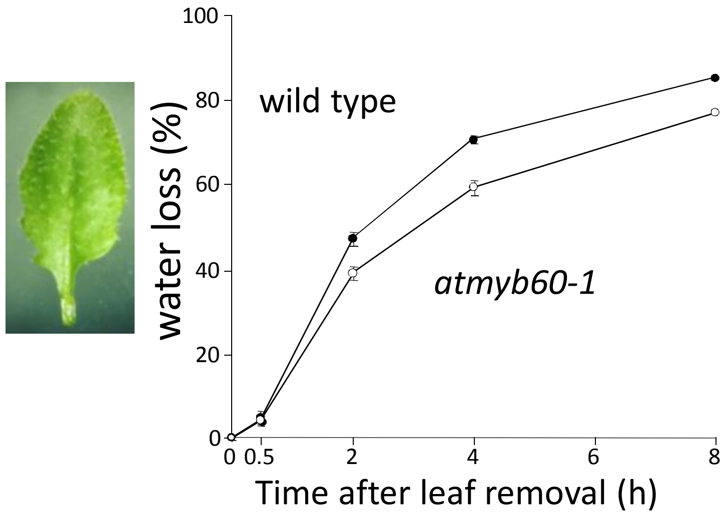
opening **closure**

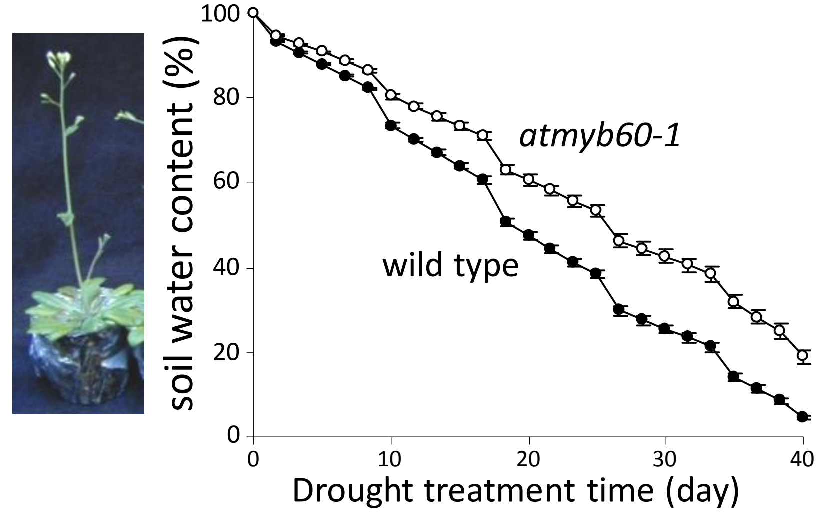
“Whole-plant” effects of the *atmyb60-1* mutation

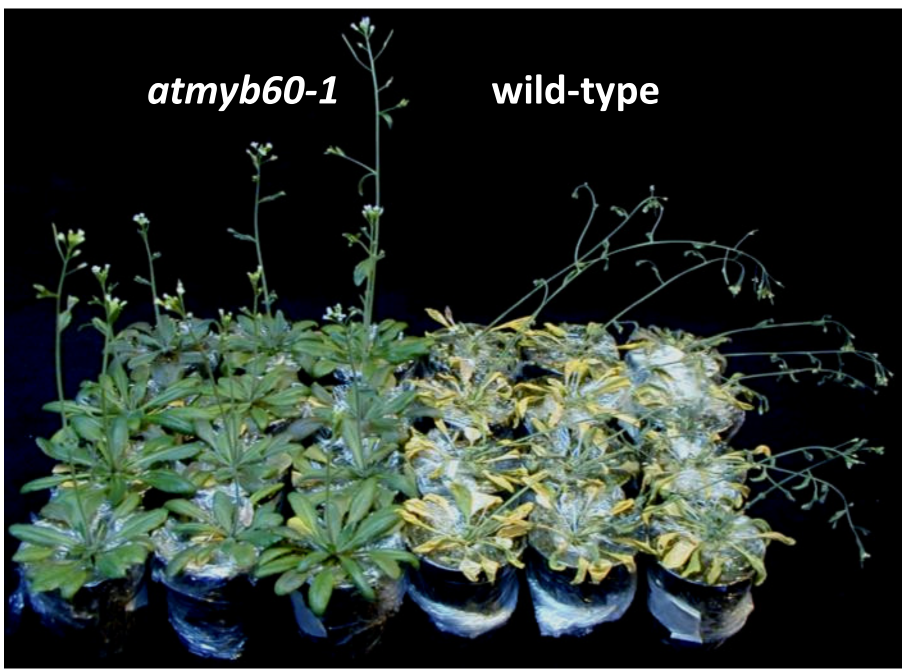


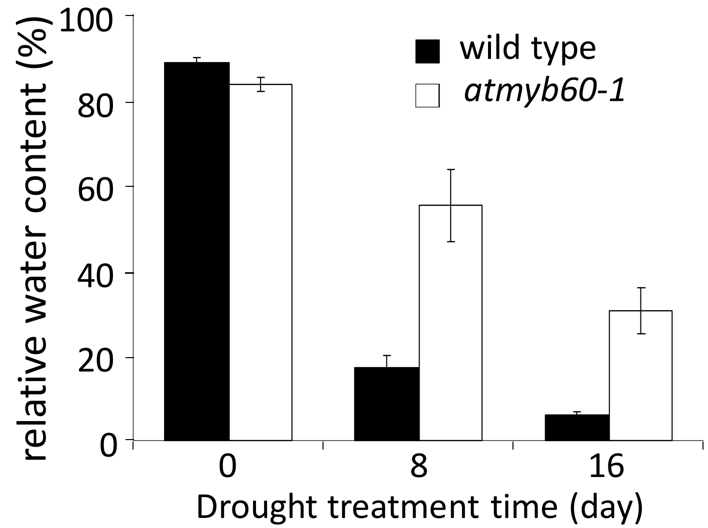


atmyb60-1 plants are more tolerant to desiccation than wild-type

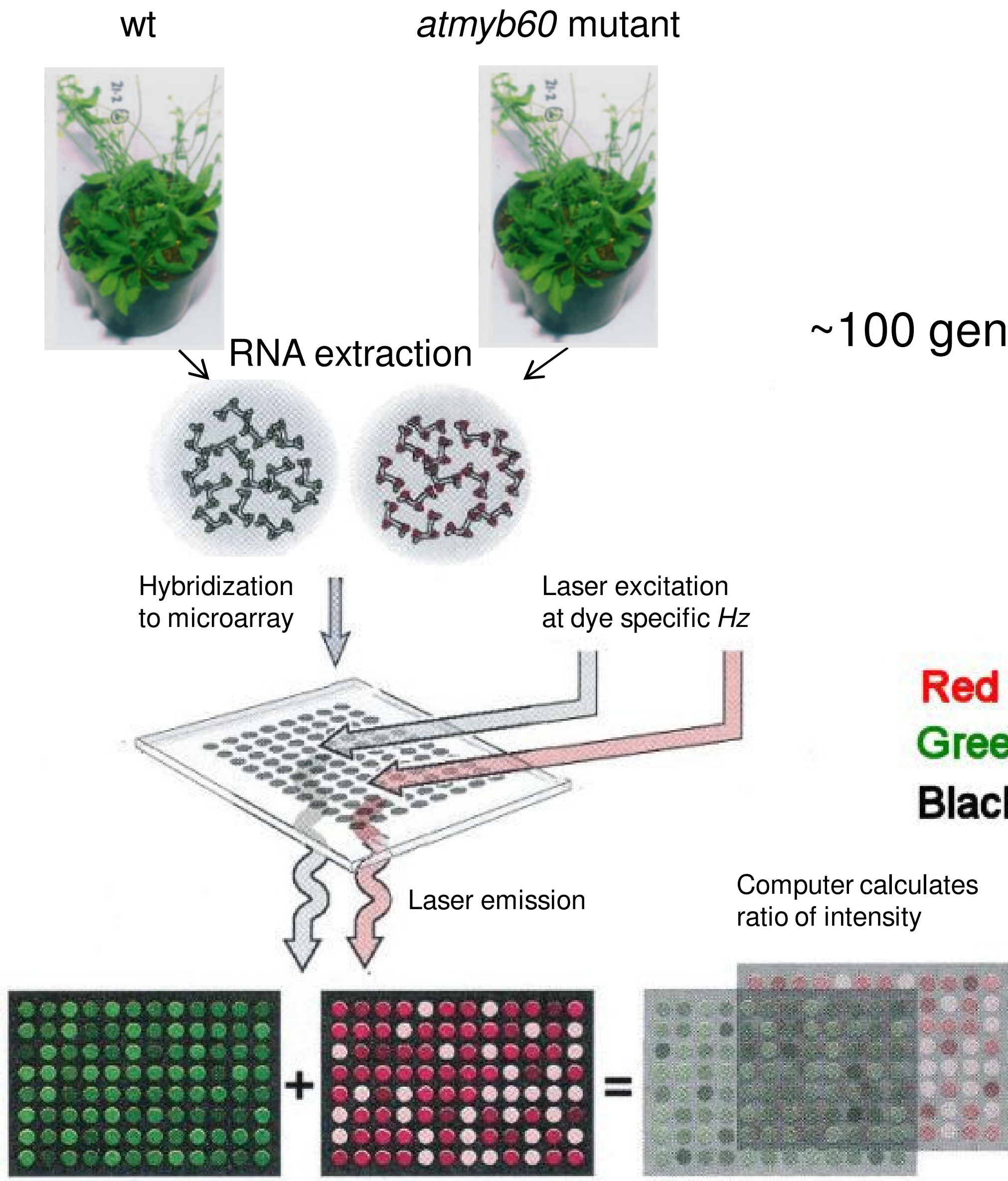




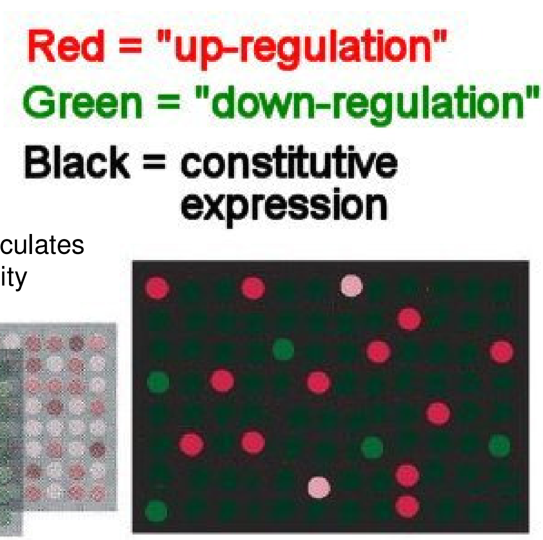




Identification of genes modulated by AtMYB60 in standard growth conditions



~100 genes are differentially expressed
in mutant than in wt



Genes differentially modulated in *atmyb60* mutant and in wild type plants

Gene ID	Description	Fold change
At4g22710	Cytochrome P450-like protein (CYP706A2)	- 2.03
At4g32020	Expressed protein, unknown function	- 2.04
At1g20450	Dehydrin ERD10	- 2.12*
At1g73600	Phosphoethanolamine N-methyltransferase -related	- 2.14
At4g38550	Putative phospholipase	- 2.15
At5g61600	AP2 domain transcription factor, similar to AtERF5	- 2.16
At5g59820	Putative zinc finger protein (C2H2 type) ZAT12	- 2.18*
At1g14880	Expressed protein, unknown function	- 2.21
At4g31500	Cytochrome P450 83B1 (CYP83B1)	- 2.33
At1g21130	O-methyltransferase 1 putative	- 2.33
At2g30870	Glutathione S-transferase (ERD13)	- 2.39
At5g06320	NDR1/HIN1-like protein 3 (NHL3)	- 2.41
At1g27020	Expressed protein, unknown function	- 2.43
At1g73500	Mitogen-activated protein kinase kinase, putative MKK9	- 2.46
At2g26560	Similar to patatin-like latex allergen	- 2.62
At2g40100	Lhcb4:3 protein (light-harvesting chlorophyll-binding)	- 2.68
At3g46620	Similar to RING-H2 finger protein RHC2a	- 2.68
At3g15210	Ethylene-responsive element-binding factor 4 (AtERF-4)	- 2.72
At2g40000	Expressed protein, unknown function	- 2.83
At4g27280	Calcium-binding EF hand family protein	- 2.83*
At1g57990	Purine permease-related	- 2.86*
At4g17490	Ethylene-responsive element-binding factor 6 (AtERF-6)	- 2.94*
At4g02380	Late embryogenesis abundant protein homolog (SAG21)	- 2.98*
At4g24570	Mitochondrial substrate carrier family protein	- 3.00*
At4g32940	Putative vacuolar processing enzyme gamma-VPE	- 3.21
At5g47220	Ethylene-responsive element-binding factor 2 (AtERF-2)	- 4.02*
At3g52400	Syntaxin, putative (SYP122)	- 4.29*
At1g07135	Glycine-rich protein, unknown function	- 4.47*
At1g27730	TFIIIA-type zinc finger protein (ZAT10)	- 5.10*
At4g29780	Expressed protein, unknown function	- 5.28*

Plant responses:

dehydration

pathogen attack

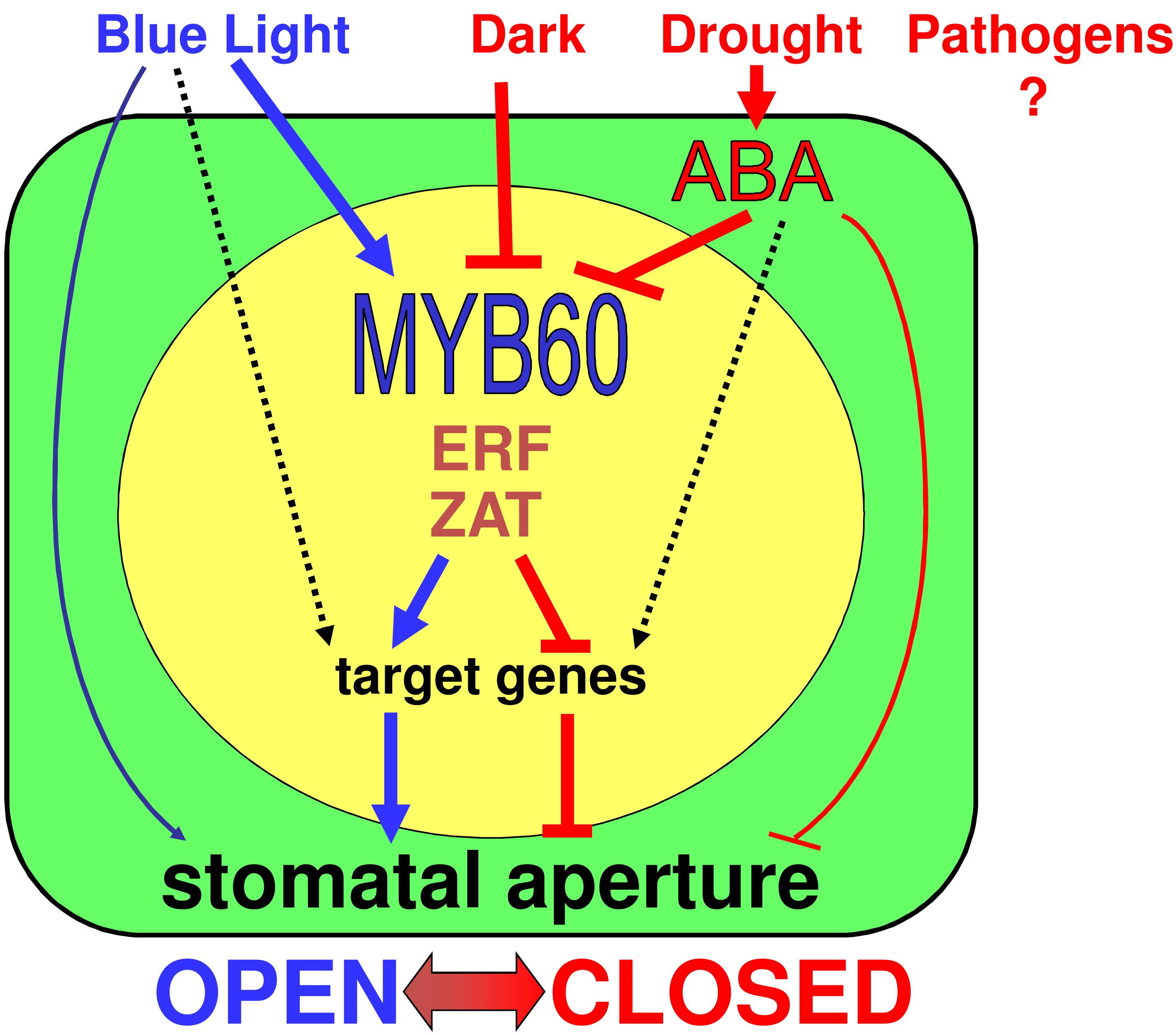
abiotic stress

biotic stress

Transcription Factors

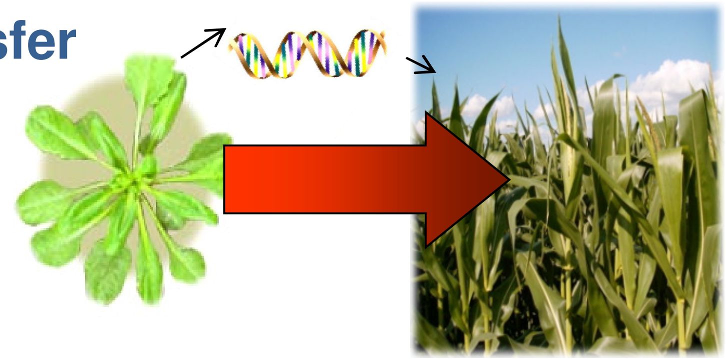
(ERF, ZAT)

Transcriptional integration of guard cell-signalling pathways

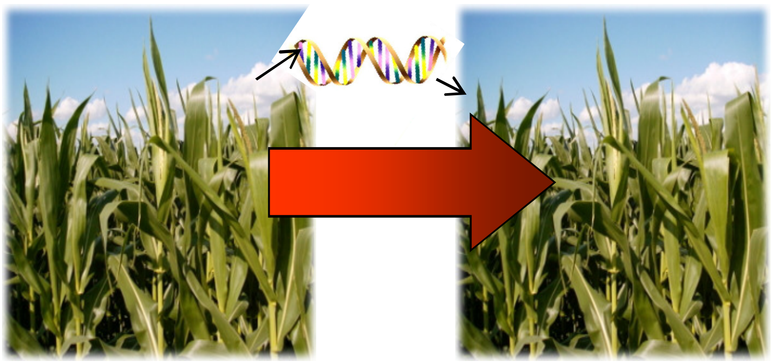


Different strategies of technology transfer

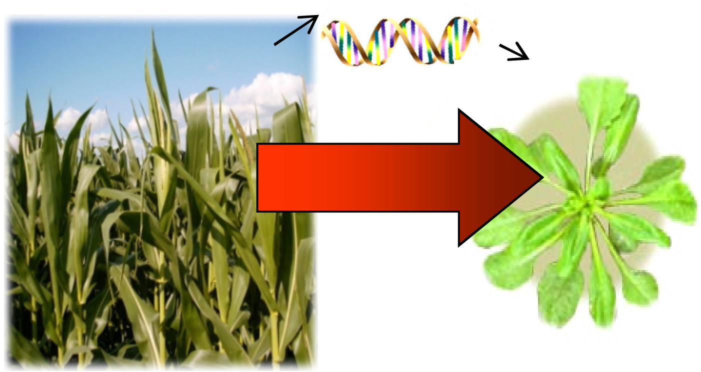
Transfer of Arabidopsis genes and their regulatory sequences to crops



Identification of othologs in crops through bioinformatic search and modulation of their expression



Preliminary study of function of crop orthologs and their regulatory sequences in Arabidopsis



Drought tolerance of DREB1 transgenic wheat

pRD29A:DREB1 wt pRD29A:DREB1 wt

Pellegreneschi *et al*., Genome 2004

Field trials indicate that leaf temperature in DREB transgenic wheat is lower than in wild type, suggesting that DREB plants need less water than wild type for their growth

Shinozaki *et al*., unpublished

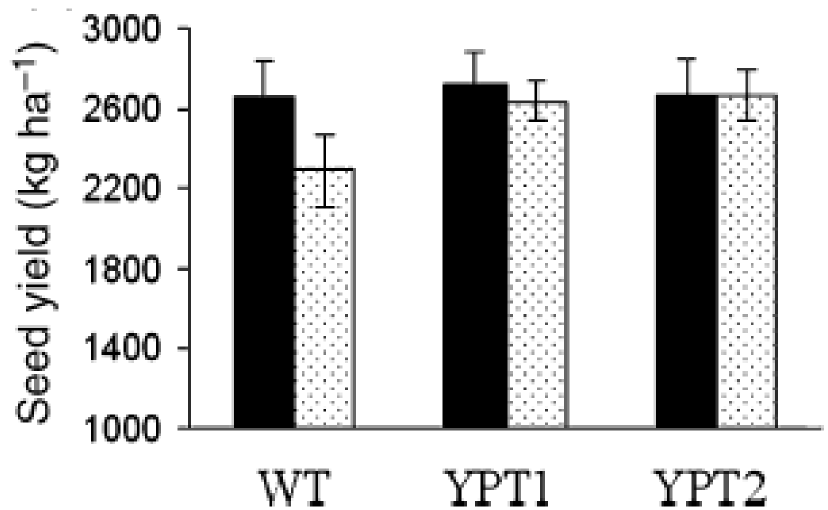
Downregulation of *ERA1* gene improves drought tolerance in *Arabidopsis* and canola

wt transgenic

Arabidopsis

canola

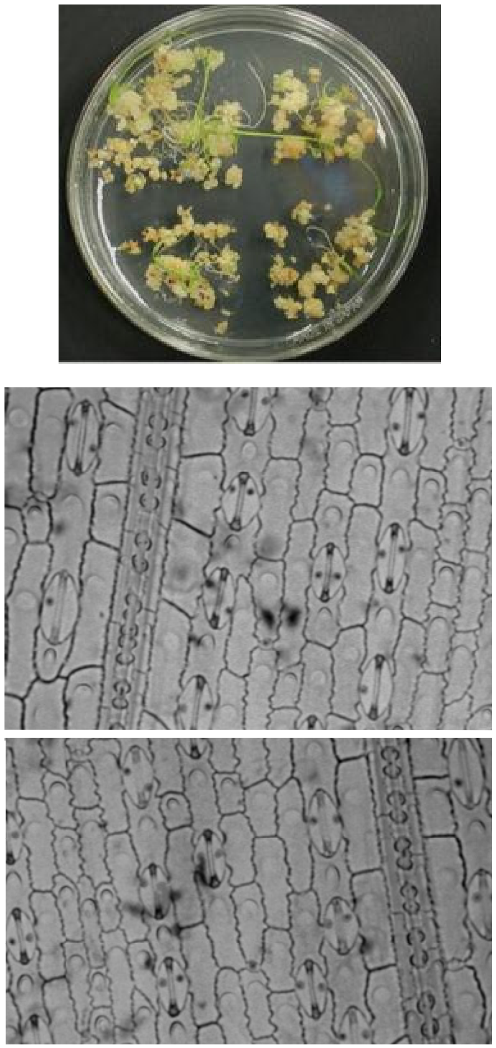
seed yield of transgenic canola is significantly higher than the control under drought stress



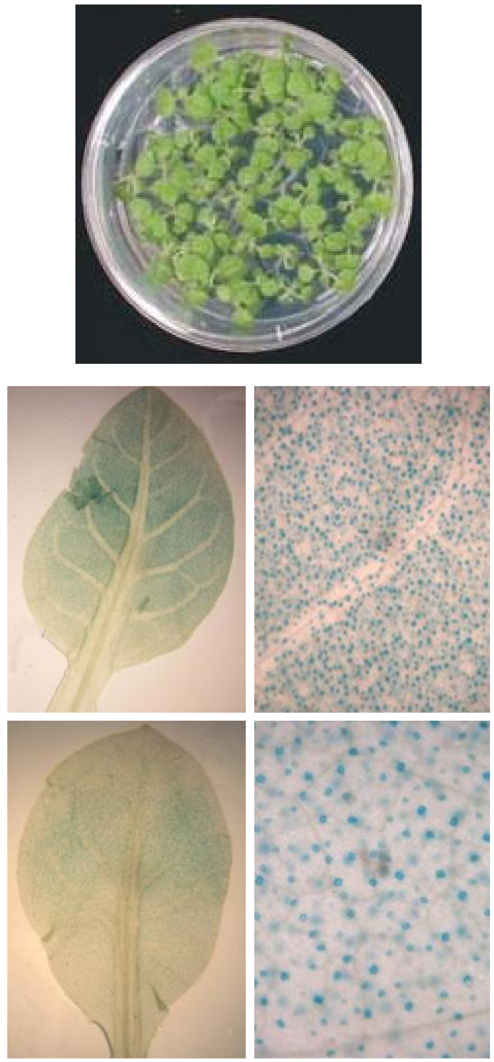
Wang et al., Plant Journal 2005

AtMYB60 promoter activity in crops

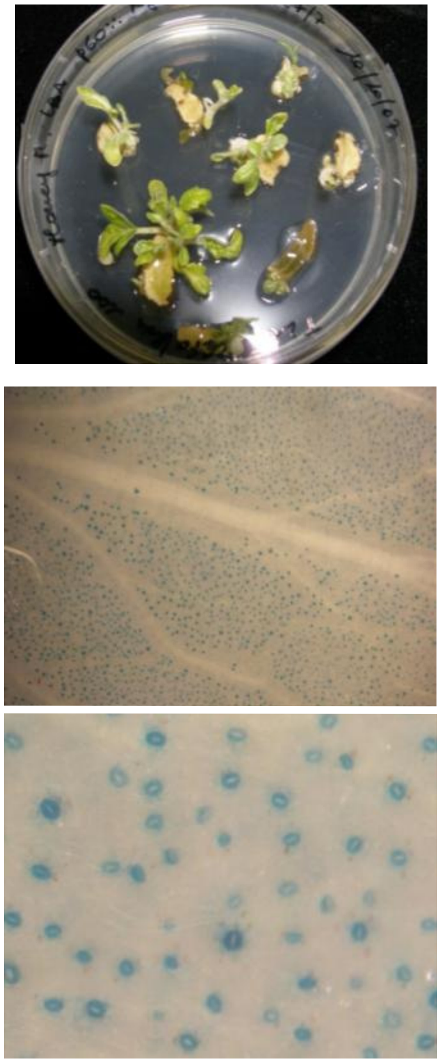
rice



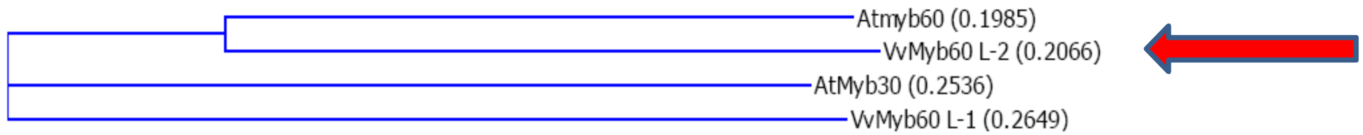
tobacco



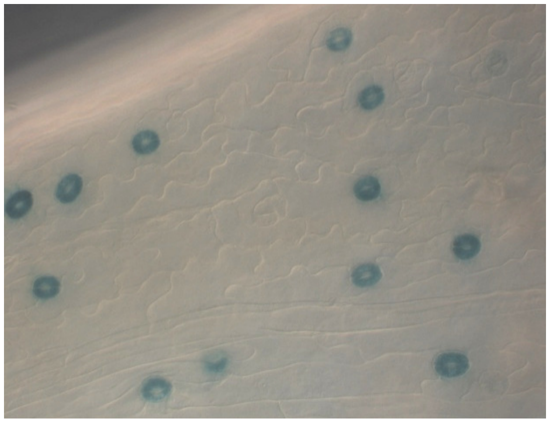
tomato



VvMYB60L-2 identified in grape as putative ortholog of *AtMYB60*



promoter of *VvMYB60* fused to GUS reporter and transferred in Arabidopsis

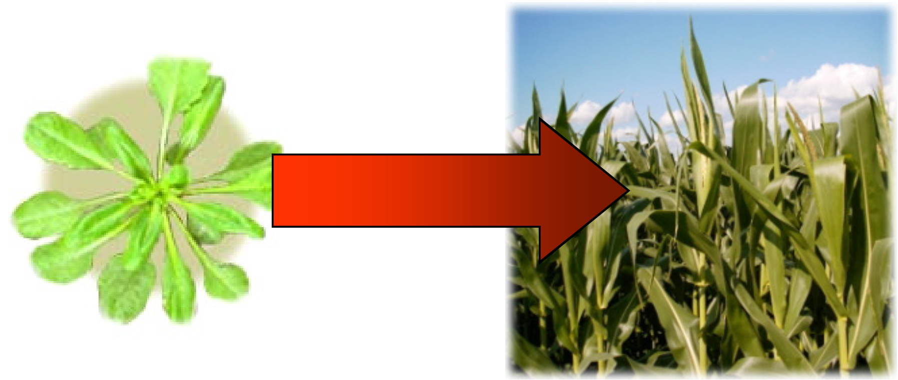


The same expression pattern as *AtMYB60*!!!

Future Perspectives

1. Technology transfer:

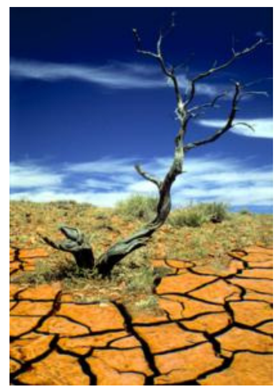
- from *Arabidopsis* to crops



2. Long term goal:

- growing corn in the desert?

+ reducing water requirement
+ increasing tolerance to “moderate stress”
+ cultivation of marginal areas



Grants

European projects: REGIA, EXOTIC, FLORA

Italian projects: FIRB 2002, FIRB 2007

Collaborators

• Alain Vavasseur
• Nathalie Leonhardt
Commissariat à l'Energie Atomique
Laboratoire des Echanges Membranaires et Signalisation, St Paul lez Durance, France

• Marnik Vuylsteke
Department of Plant Systems Biology, Ghent University, Ghent, Belgium

• Stephen Dellaporta
Department of Molecular Cellular and Developmental Biology Yale University, New Haven CT, USA

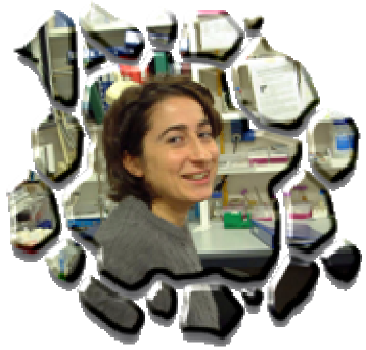
• Cathie Martin
• Jonathan Jones
John Innes Centre, Norwich, UK

• Jose Tomas Matus
Departamento de Fruticultura y Enología, Pontificia Universidad Católica de Chile. Santiago, Chile

Group Leader
Chiara Tonelli

Assistant Professor
Katia Petroni

Research Associates
Massimo Galbiati **Michela Landoni**



Postdoctoral Fellows

Domenico Allegra

Valentina Calvenzani

Eleonora Cominelli

Laura Simoni

PhD Students

Monica Fornari **Priscilla Francia**

Graduate Student
Alessandra Albertini

Students: **Sara Amodeo, Silvia Bellatti, Foteini Bounartzidi, Silvia Galimberti, Matteo Riboni**