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BIOTECHNOLOGY

...seeding a sustainable future

New transcription factor based technology & tools for future crop improvement

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Mendel Biotechnology, Inc.

New Phytologist Tansley Symposium
18 September, 2008
Timberline Lodge, Mt. Hood, Oregon USA

The challenge

John Stringer & Linda Horton

we need to improve crop productivity!

Ag. Biotechnology Product Timelines

First generation (mid 1990s)

- single gene traits
  - herbicide and insect resistance

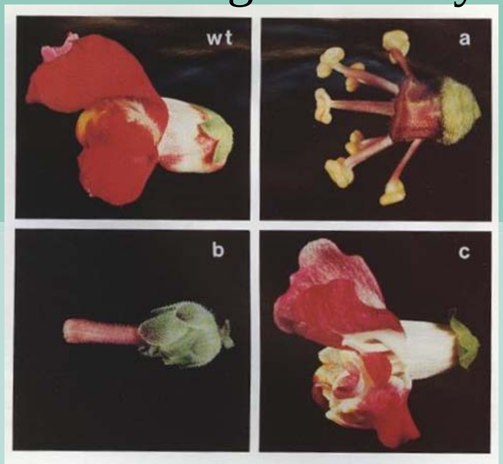
Next generation (early 2010s)

- complex polygenic traits
  - e.g. yield and stress
- Based on whole pathway regulation

Body plan patterning

e.g. Homeodomain proteins
McGinnis et al., 1984, Nature 308

Floral organ identity



e.g. MADS domain proteins
Coen & Meyerowitz, 1991, Nature 353

Floral symmetry

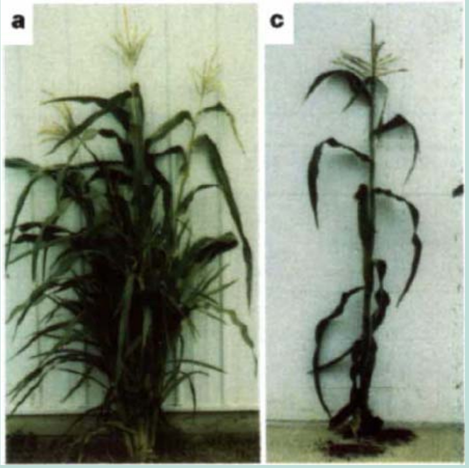
e.g. CYCLOIDEA-related proteins
Luo et al., 1996, Nature 383

Transcription factors: master regulators of genetic pathways

Flowering time

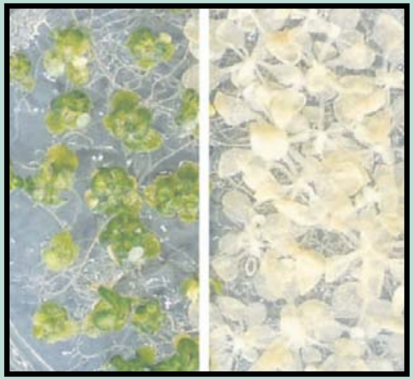
e.g. CONSTANS
Putterill et al., 1995, Cell 80

Branching pattern



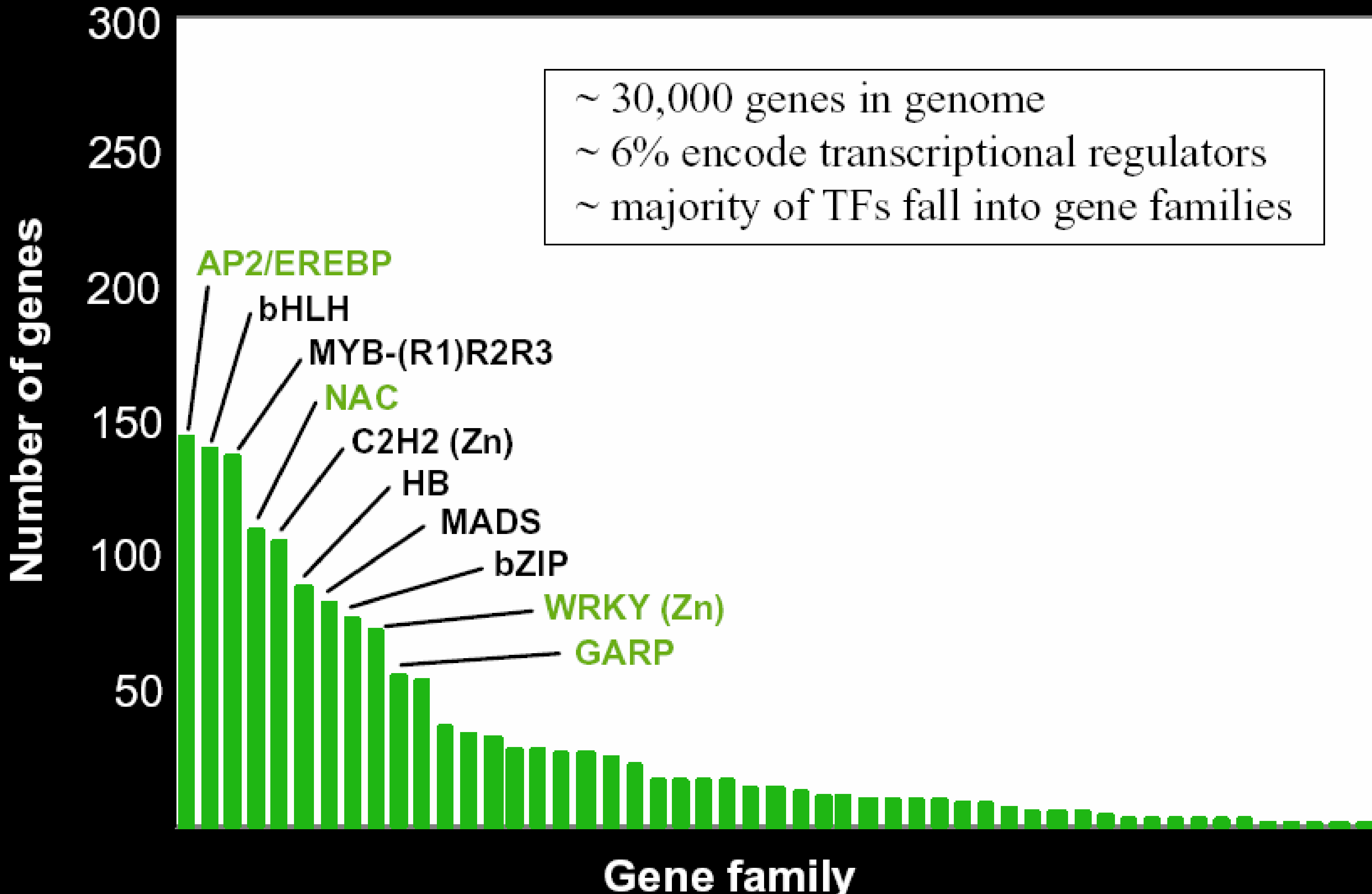
e.g. teosinte branched (CYC-family)
Doebley et al., 1997, Nature 386

Stress tolerance



e.g. CBF genes (AP2 family)
Stockinger et al., 1997, PNAS, 94

Arabidopsis thaliana transcription factor complement



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Riechmann et al., 2000, Science 290

Screens on transcription factors have produced hundreds of leads

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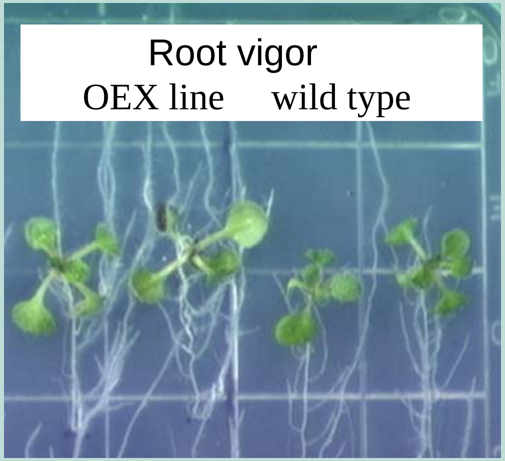
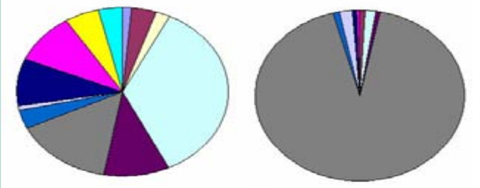
Developmental Traits...

seed size



Biochemical Traits...

Glucosinolate composition

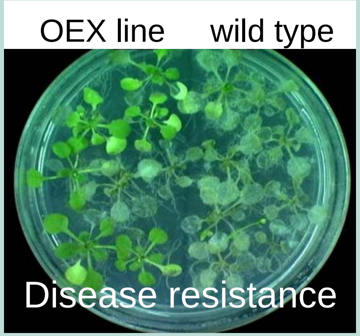


wild type OEX line

Flavonoid production

Physiological Traits...

Drought tolerance



N-use

Typical Ag Biotech Product Development Process

Typical development timeline & costs:
- 10 -15 years
- $50M – $100M

Discovery -> Validation	Phase I – Crop proof of concept	Phase II – Early Product Development	Phase III – Advanced Development	Phase IV – Regulatory Approvals	Market Launch
High throughput screening; lead validation in model crops.	**Test gene leads in crop plants; select best candidates.**	**Field testing in crop Plants. Regulatory assessment started.**	**Field testing in elite germplasm. Develop Regulatory data**	**Field testing in elite germplasm. Develop Regulatory data**	

Adapted from: Monsanto 2003 Annual report

MOA work supports
early-mid stage development

An understanding of MOA is important for successful commercialization

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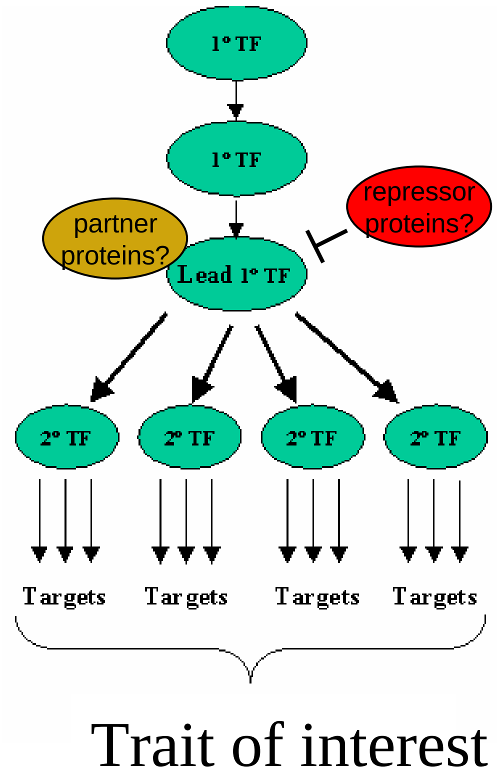
Mode of Action analysis:

- define phenotypic basis of the trait
- define pathways through which the leads act
  => helps prioritize among leads
  => support the future regulatory process/acceptance
  => identify optimization strategies
- the genetic tools in Arabidopsis make it a valuable model



Two general trait categories are of high priority

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Intrinsic yield e.g.

- photosynthetic performance
- enhanced growth and vigor
- modification of architecture

Yield stability (stress tolerance)

- drought tolerance/water use efficiency
- cold/freezing tolerance
- N utilization
- fungal disease tolerance

For recent review see:
Century et al., 2008
Plant Physiology 147, 20-29

We need a “Blue Revolution” (Kofi Annan, 2000)

- **Drought is the primary limitation to agriculture**

- **Many TFs have been implicated in drought responses**

- Mostly from large TF families:
  AP2/EREBPs, bZIP, NAC, MYB, MYC, C2H2, WRKY

- Recent comprehensive reviews in:
  Umezawa et al., 2006 and Bhatnagar-Mathur et al., 2008

Ref: Pennisi, 2008. The Blue Revolution, Drop by Drop, Gene by Gene. Science **320**: pp. 171 - 173

NF-YB1 – a promising drought tolerance technology

Nuclear Factor Y (NF-Y) transcription factor family

• binds CCAAT box elements

• single copy TFs in yeast & mammals with roles in:
- energy metabolism
- cell cycle

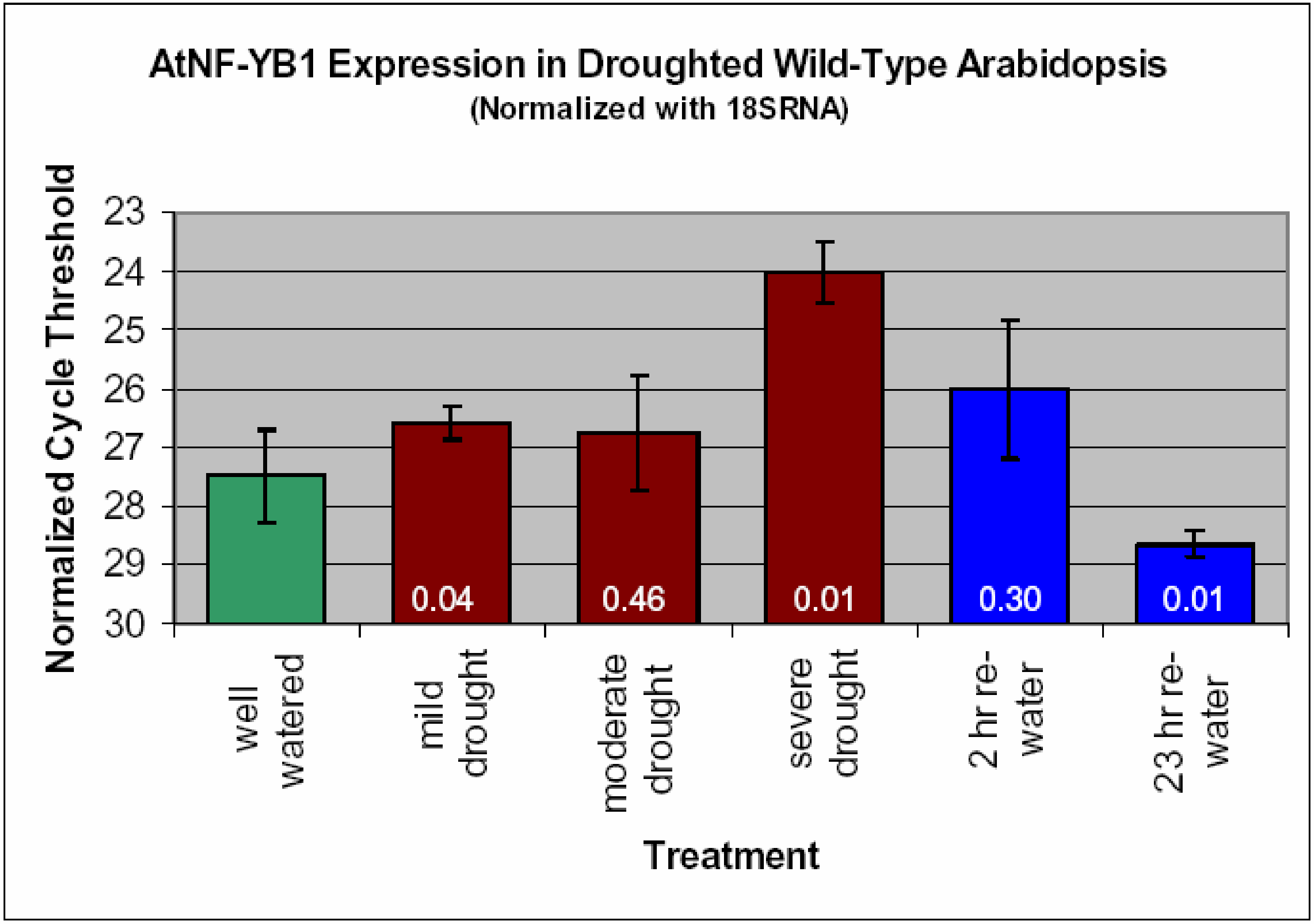
• family greatly expanded in plants emerging roles in plant in:
- flowering time
- photosynthesis biology
- drought responses

35S::*NF-YB1* **wild type**

8 days drought

After Re-water

NF-YB1 is part of a native drought response



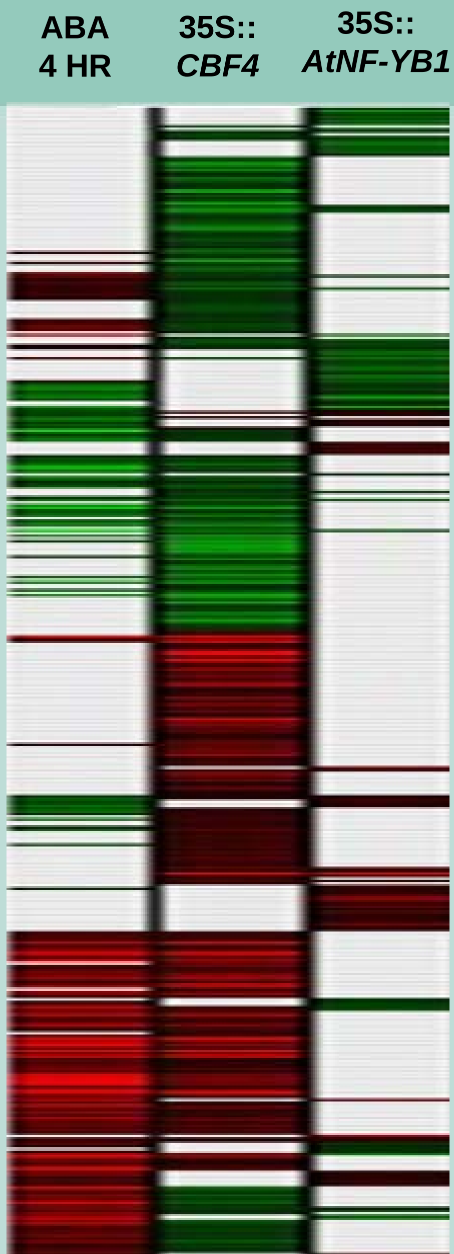
***NF-YB1* is upregulated ~12 fold by severe drought**

NF-YB1 regulates a novel genetic pathway for control of drought responses

- Microarray fingerprint shows little overlap with well characterized drought response pathways (ABA and CBF pathway).

- Key targets observed in 35S::*NF-YB1* profiles
• photosynthesis components
• pathways for synthesis of protective pigments
• components of energy metabolism

- Key components of phenotype
• increased WUE
• increased chlorophyll levels
• maintenance of turgor during stress
• higher rate of photosynthesis during stress



red bar: up-regulated gene

green bar: down-regulated gene

Both The Genes and Control Pathways Are Conserved Across Arabidopsis....

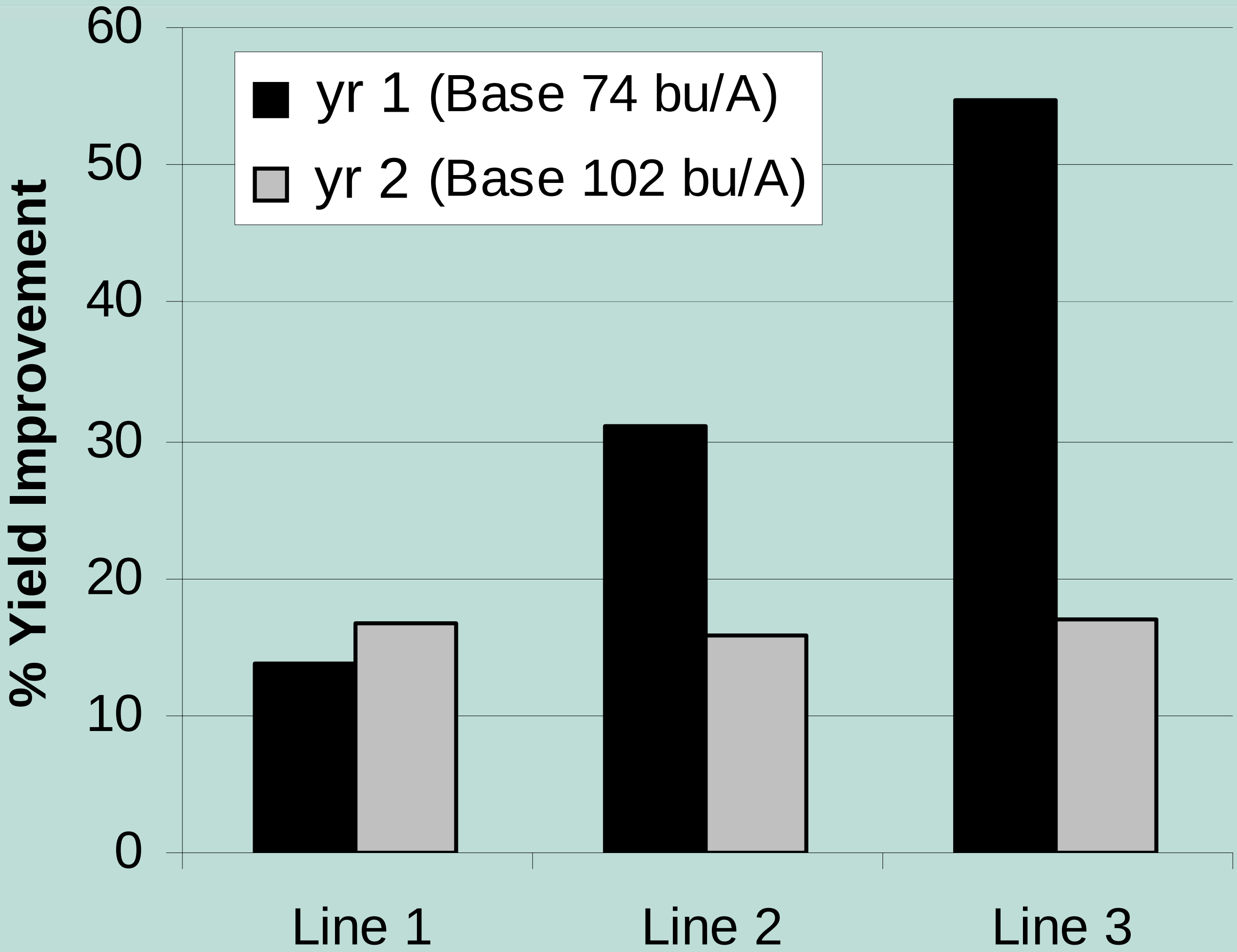
Non-transgenic control | AtNF-YB1 Transgenic | Non-transgenic control | AtNF-YB1 Transgenic | Non-transgenic control | ZmNF-YB1 Transgenic

....soybean, cotton... ...and corn

Courtesy of Monsanto Company

Field trial results in corn

Significant Yield Advantage obtained on dry acres



Courtesy of Monsanto Company

year 1: single, 6-replication drought efficacy trial using a Split-Split-Split block design in Kansas
year 2: multi-location study using group block design with 3 replicates within each location

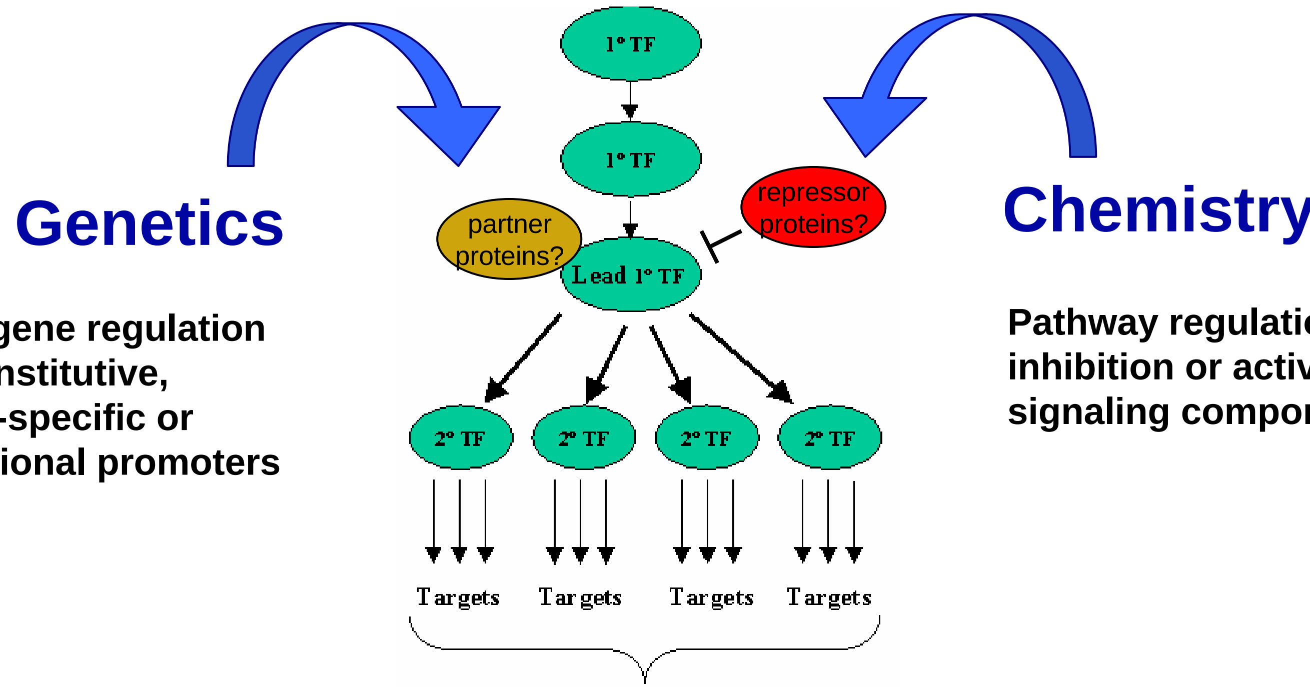
Nelson et al., 2007, PNAS 104: 16451

New tools to meet the future goals of plant biotech

Excerpt from Monsanto press release from June 04, 2008

“ ... Monsanto will double yield in its three core crops of corn, soybeans and cotton by 2030, compared to a base year of 2000...”

Chemical approaches:
complementary to genetics for modulating transcription factor (TF) regulated pathways



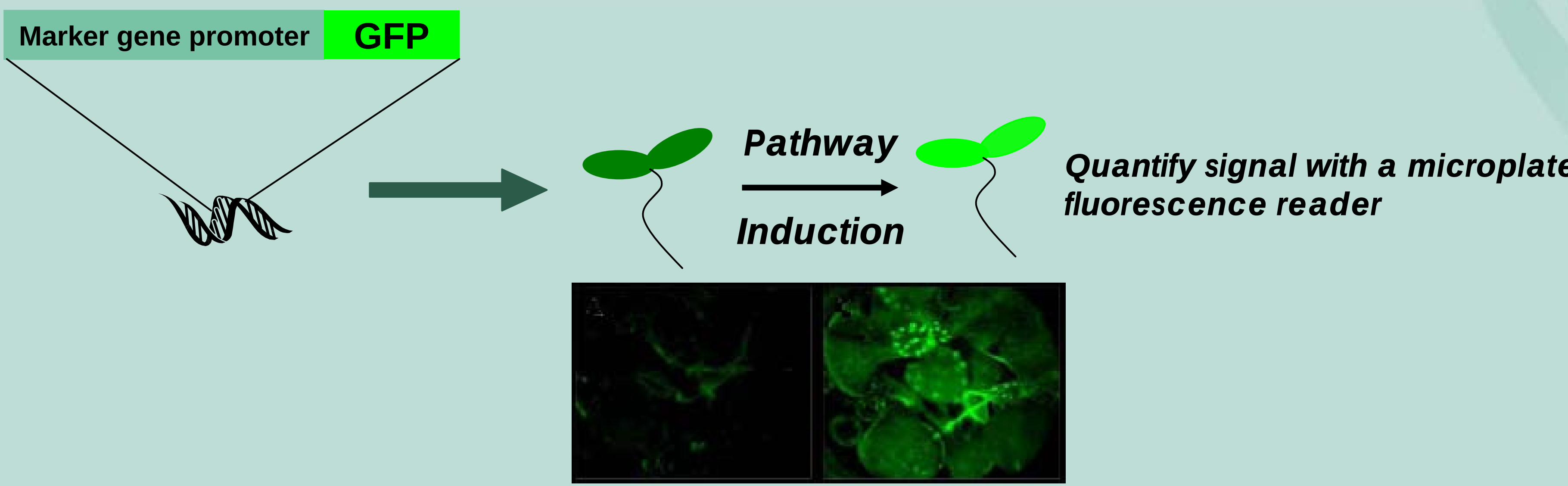
Transgene regulation via constitutive, tissue-specific or conditional promoters

Pathway regulation via inhibition or activation of signaling components

Trait of interest

(e.g. abiotic stress tolerance, disease resistance, enhanced growth)

Reporter lines enable quantification of pathway induction



- Promoters are selected using
  - Public data (genetic pathways)
  - Proprietary data (transcriptional profiling (TxP) analysis)
- An ideal promoter
  - Conserved across species
  - Induced specifically in the pathway of interest
  - Expressed in leaf/cotyledon tissue
  - Downstream of multiple regulatory pathways

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Compound imparts drought tolerance on soil-grown plants

Arabidopsis

DMSO

Compound (350μM)

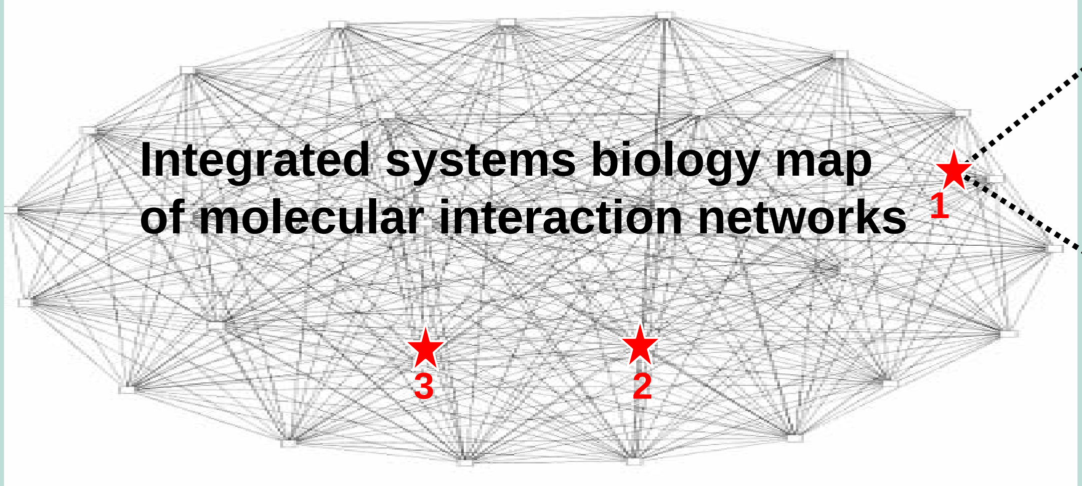
Survival following drought treatment and rewater

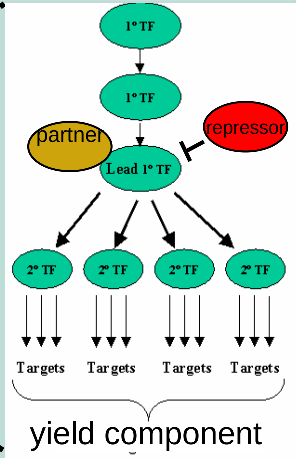
Barley

CONTROL | H144217 (500g/ha) | H144217 (1000g/ha)

Courtesy Bayer CropScience AG

Systems biology will be important in delivering future technology

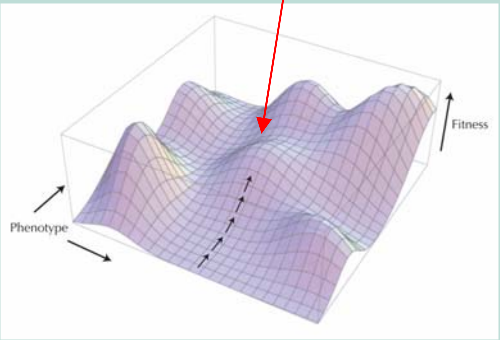




TFs form network hubs

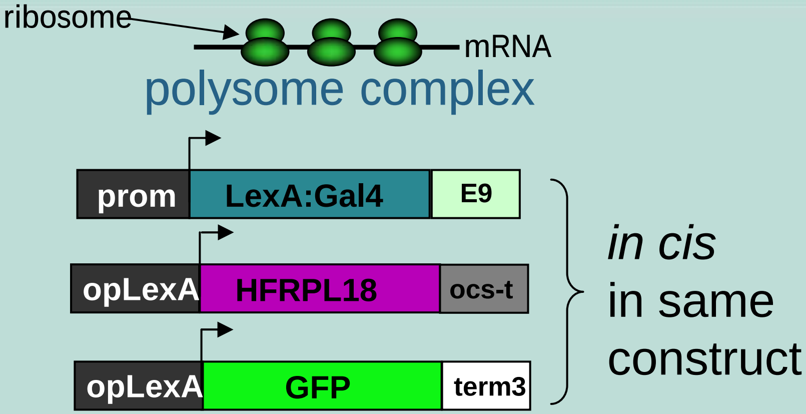
Network knowledge directs future rounds of discovery work

e.g. combination of 1 + 2 + 3 gives fitness peak

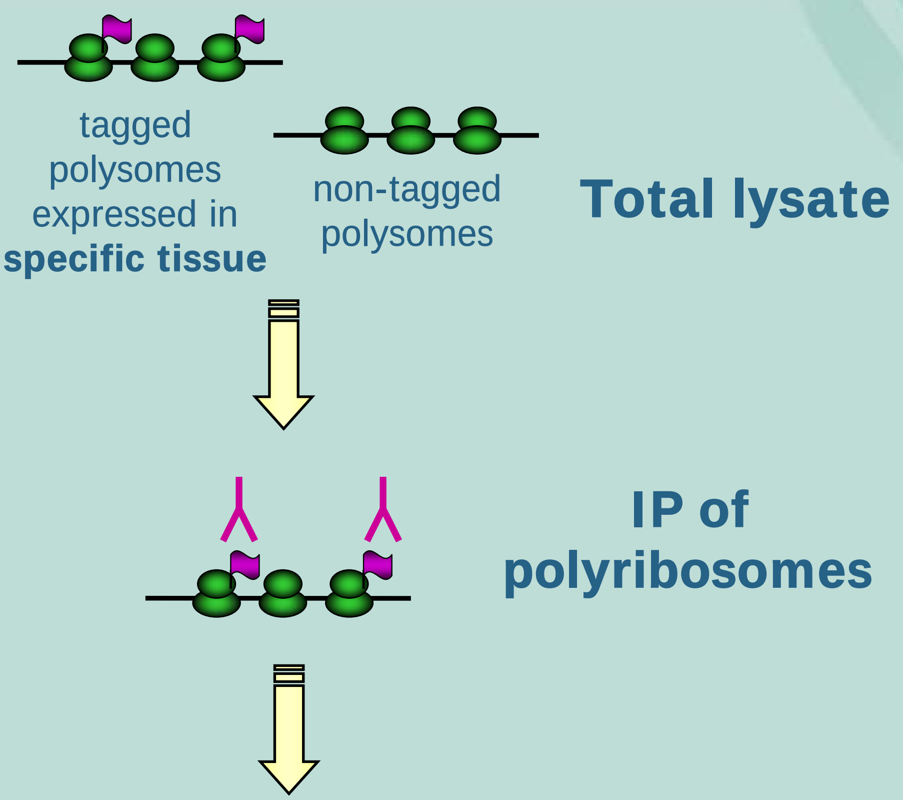


High resolution maps depend on high quality *in-vivo* TF x DNA and TF x protein interaction data at the ***level of the individual cell-type***

Ribotag: an emerging tool for cell-type specific expression studies



Stable transformation of Arabidopsis



RNA isolation and expression analysis

Reference: profiling of RNA associated with polysomes via tagging of RPL18
Zanetti et al. (2005). Plant Physiol 138, 624-635

Validation: dramatic enrichment of specific markers in the phloem

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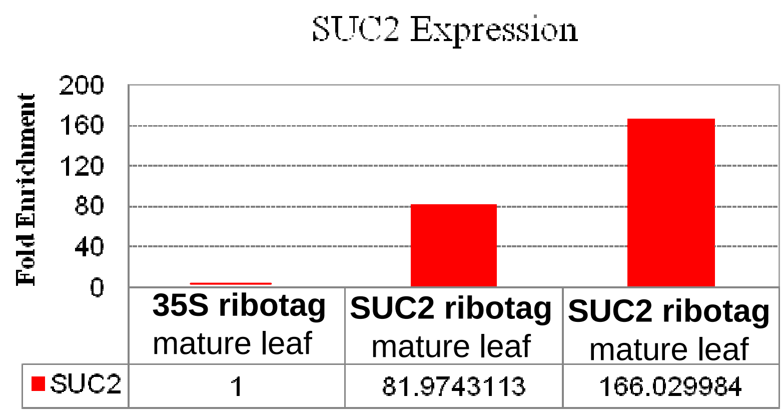

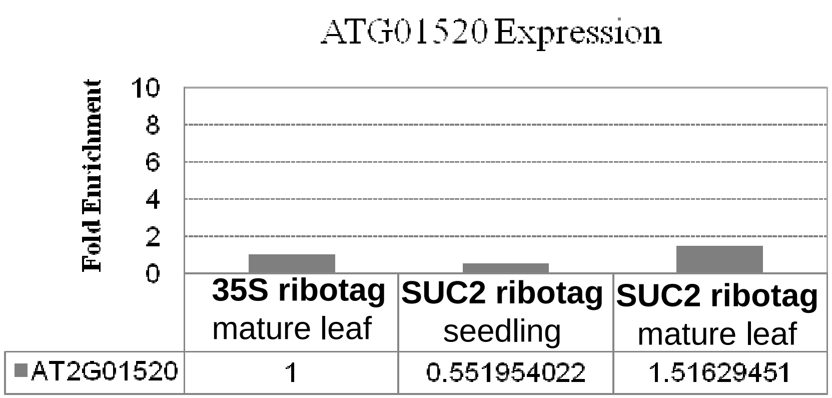

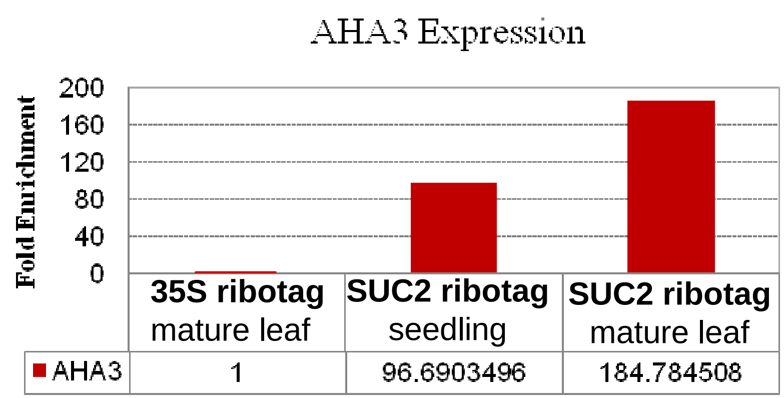

Projected timelines for biotechnology products

Impact of molecular biology

first generation (mid 1990s)

- **single gene traits – herbicide and insect resistance**

Impact of Genomics

next generation (early 2010s)

- **complex traits – yield and stress**
- initial lead technologies identified from screens

Impact of Systems Biology & Chemistry

later generations (> 10 years hence)

- **super-enhanced complex traits – yield and stress**
- optimal intervention points in regulatory pathways

- rationalized from "holistic" knowledge of entirety of pathways

Concluding remarks

- TF-based technologies are excellent candidates for enhancement of crop yield and stress tolerance
- Genetics and chemistry offer complementary approaches to pathway regulation
- Systems biology will enable future generations of super-enhanced products
- **Plant biologists have a critical role to play in ensuring a sustainable future**