Improving water use efficiency and water capture in *Solanum* and *Brassica*, transgenic and QTL approaches

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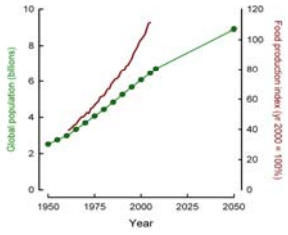
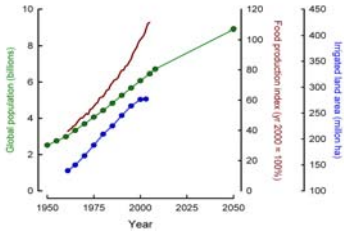
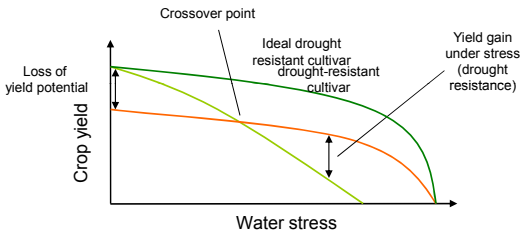
Growth in food production has exceeded population growth (so far!), but production must double by 2050



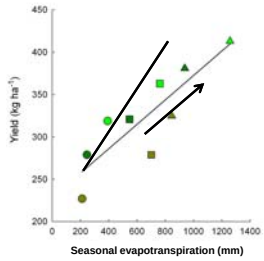
Scope for further growth in food production by increasing irrigation is severely limited



What is drought resistance?



Crop yield is closely related to seasonal water use



Grain yield as a function of seasonal water use, oilseed crops, Eastern India

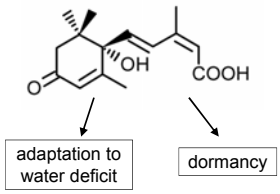
Yield in water limited environment can be improved by:

- Increasing the slope of the line - increased water use efficiency (ton ha^{-1} mm^{-1})
- Increased use of available water - increased water capture, e.g. deeper roots (but may reduce river flows/aquifer recharge)

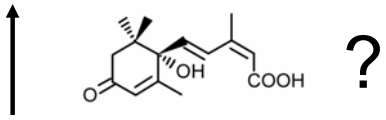
From: Kar et al 2007 Ag. Wat. Manag. 87:73-82

Topics

1. Improving water use efficiency by increasing the production of the plant hormone abscisic acid (tomato, *B. napus*). *GM and natural variation*
2. Quantitative trait loci (QTL) for water use efficiency (vegetable brassicas)
3. QTL for water capture (tomato)



1. Abscisic acid

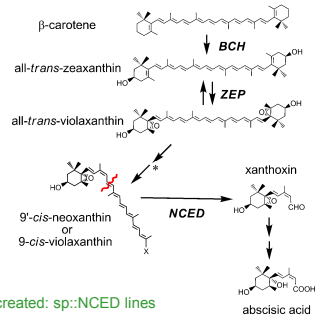


- Demonstrate that we can engineer plants with higher ABA content
- Describe the impact of long-term high ABA on plant growth and development, and water use
- To explore how we should optimise ABA biosynthesis to maximise crop productivity when water is limiting

Abscisic acid synthesis

Key regulatory step:

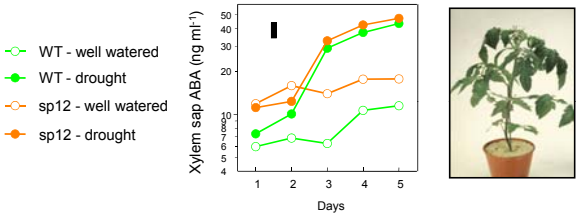
9-*cis*-epoxycarotenoid dioxygenase (NCED)



"High ABA" tomato plants created: sp::NCED lines

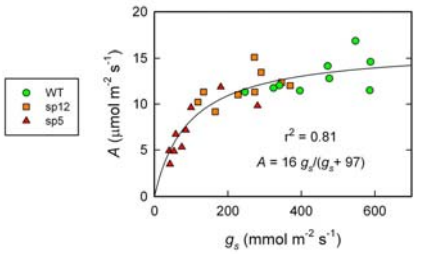
Thompson et al 2000, Plant J

Transgenic tomato lines: **sp::NCED** plants have higher abscisic acid in well-watered conditions



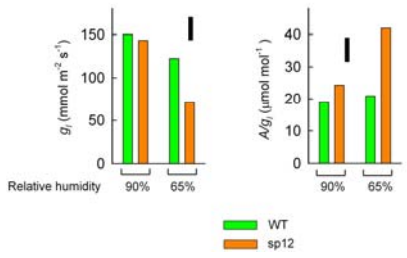
- sp::NCED plants become equivalent to WT plants once stress is imposed
- Physiology under well-watered conditions is different to WT

Closure of stomata in **sp::NCED** "high ABA" tomato plants has a bigger impact on water loss than on photosynthesis, so improves intrinsic water use efficiency of leaves



- Stomatal conductance (g_s) is "capped" in sp lines
- Reducing stomatal conductance from 600 to 300 has little impact on rate of photosynthesis (A)

"High ABA" sp::NCED plants only close stomata in dry air when transpiration is high (maximising photosynthesis when the risk of water loss is low)



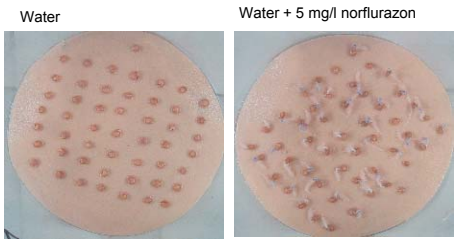
sp::NCED "high ABA" lines have up to 80% increase in water use efficiency at the whole plant level

	Genotype		
	WT	sp12	sp5
Initial biomass (g DW)	3.31	3.50^{NS}	3.29^{NS}
Biomass gain over 25 days (g DW)	37.96	31.37^{NS}	34.91^{NS}
Transpiration (kg H_20 plant^{-1})	8.5	5.5*	4.4*
WUE_p (g DW kg^{-1} H_2O)	4.5	5.7**	8.0**

Thompson et al 2007, Plant Physiol.

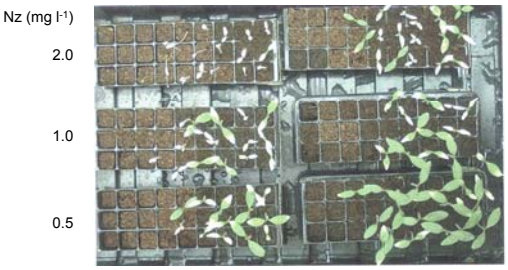
Growth of "high ABA" plants

In sp::NCED plants germination is delayed or prevented - this is reversed by norflurazon



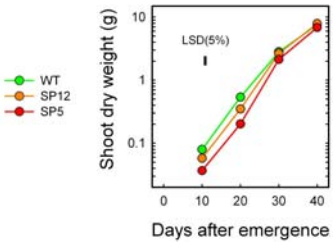
6 days from imbibition

Healthy seedlings can be produced using low concentrations of the herbicide norflurazon



sp::NCED plants have delayed early growth

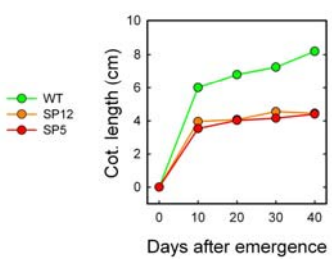
- Shoot dry weight at 10 DAE was greatly reduced (sp12 -28%, sp5 - 54%)
- By 40 DAE, dry weight differences were non-significant (sp12 +4%, sp5 -10%)



n = 10

sp::NCED plants have delayed early growth

40-50% reduction in cotyledon size:



n = 10

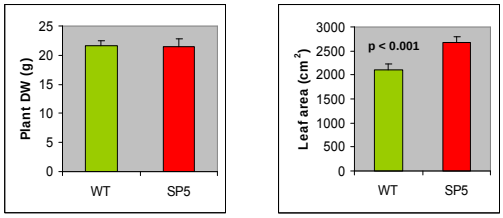
Cotyledon expansion and early growth may be inhibited by ABA signalling pathways: e.g. post-germination growth arrest (*Arabidopsis*)

"…ABA…can also reversibly block growth during a narrow developmental time interval following germination and before the onset of vegetative growth".

Luis Lopez-Molina et al 2001 PNAS **98**:4782-4787

Long-term biomass accumulation of sp::NCED plants is normal, leaf area is increased (glasshouse-grown plants in pots)

n = 9



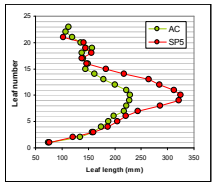
Sp5 has higher specific leaf area ($cm^2\ g^{-1}$)

Increased leaf expansion in sp::NCED plants

Petiole length and epinasty

WT SP5

n = 9



Conclusions from sp::NCED construct

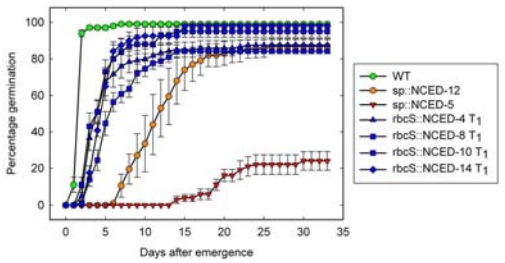
Moderate increases in ABA:

Positive effects
- dramatically increase water use efficiency by limiting g_s at high VPDs
- little or no impact on long-term biomass (well watered CE/glass)
- enhances expansion of leaves (turgor/ethylene?)
- enhances biomass under water limited conditions (drought resistant)
- increases root hydraulic conductivity

Negative effects
- inhibition of germination and seedling establishment (increased soil evap.)

Further optimization of ABA biosynthesis for productivity – can we separate the good from the bad?

NCED over-expression in tomato using a light-regulated gene promoter (rbcS::NCED) to avoid germination problems



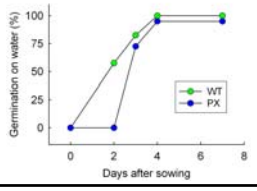
Tung et al 2008, PCE

rbcS::NCED lines: too much ABA severely inhibits growth

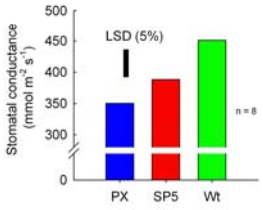
+ T - T

High ABA Wild-type

PX::NCED1 (het) SP::NCED (SP5) WT

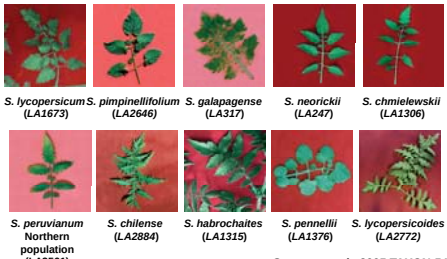


Optimising ABA content with different gene promoters: promoter X



Avoiding GM: exploiting natural variation in NCED activity

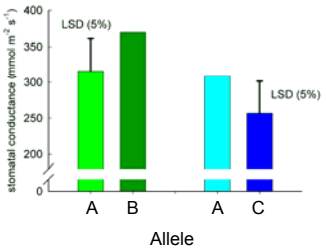
- NCED alleles from ten wild species are being introgressed into tomato c.v. Ailsa Craig



Spooner et al., 2005 TAXON 54 (1)

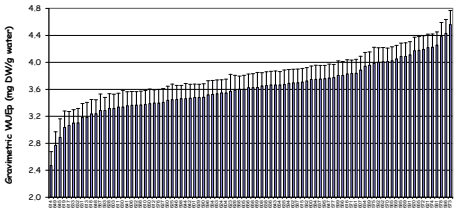
Avoiding GM: exploiting natural variation in NCED activity (preliminary data)

- comparing the effect of three NCED alleles on stomatal conductance in near-isogenic lines



"High ABA" *Arabidopsis thaliana* transgenics: how do they match up to natural variation in WUE?

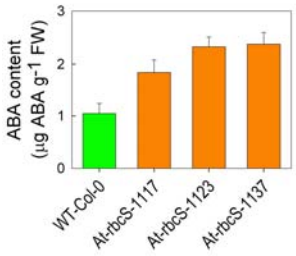
Genetic variation in water use efficiency in 96 Arabidopsis ecotypes



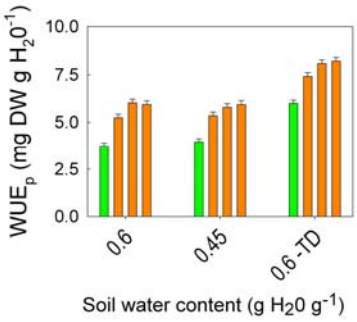
Very significant genotype effect on WUE, but also on anatomical traits (Chlorophyll content, Leaf Thickness, water content, etc)

"High ABA" transgenic *Arabidopsis thaliana*

ABA content of rosette leaves



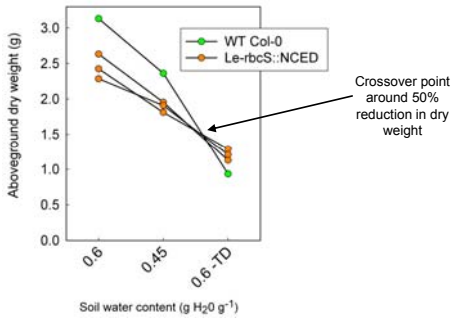
"High ABA" transgenic *Arabidopsis thaliana* always have higher water use efficiency



30-50% increase in WUE under all conditions

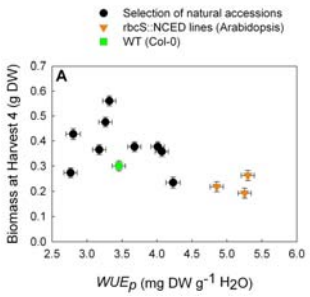
"High ABA" *Arabidopsis* plants grow better under terminal drought

15-26% penalty in yield potential



Crossover point around 50% reduction in dry weight

"High ABA" *Arabidopsis* transgenic plants have much greater WUE than is found in nature

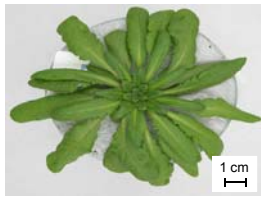


- Ecotypes may have been under selective pressure to use soil water rather than leave it in the soil for their competitors to use

2. Quantitative trait loci for water use efficiency

Brassica oleracea (vegetable brassicas)

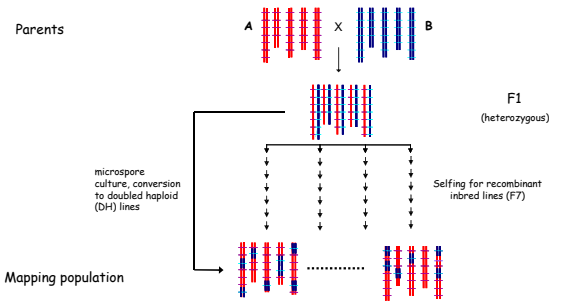
Arabidopsis



Field crop ⟷ Model plant

Comparative genetics/genomics

QTL analysis in two-parent mapping populations



A12 x GD33 DH population – QTL trials

(A12 = Chinese kale, GD33 = broccoli/calabrese)

2 x sites
2 x treatments (well-watered & drought)
2 x transplantings
2 x replicates per transplanting
8 plants per plot

Automatic rain shelters – Gleadthorpe (sandy soil)

Spanish tunnels – Kirton (heavy soil)

Trait assays to asses both WUE and indicators of yield potential

- WUE is a ratio, selection on this alone could drive g_s down too far, leading to loss of yield (very low g_s will limit A)
- Other trait data is needed to assess components of the ratio

leaf chl (SPAD), leaf thickness (MLA)

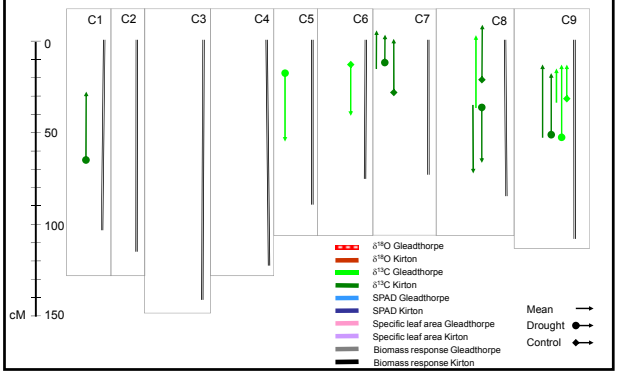
$$WUE_{Intrinsic} = \frac{A}{g_s} \approx \frac{A}{T} \times VPD$$

biomass

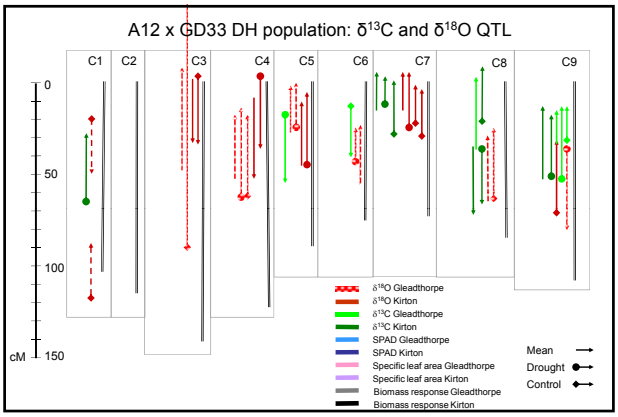
$\delta^{13}C$ $\delta^{18}O$

A = CO_2 assimilation rate
g_s = stomatal conductance
T = transpiration
VPD = vapour pressure deficit

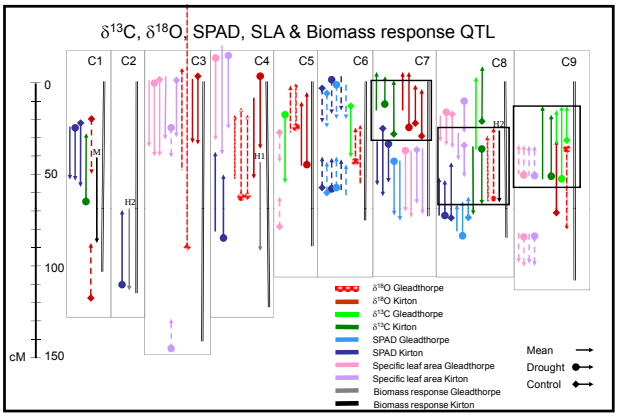
A12 x GD33 DH population: $\delta^{13}C$ QTL



A12 x GD33 DH population: $\delta^{13}C$ and $\delta^{18}O$ QTL

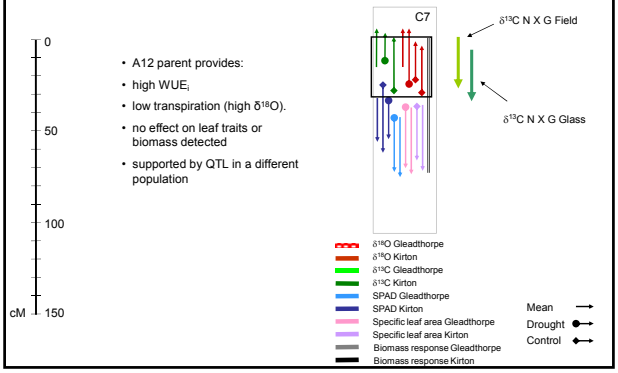


$\delta^{13}C$, $\delta^{18}O$, SPAD, SLA & Biomass response QTL

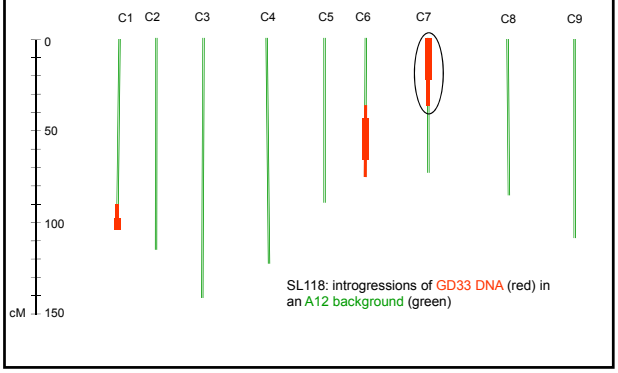


Co-localisation of traits on C7

- A12 parent provides:
- high WUE_i
- low transpiration (high $\delta^{18}O$).
- no effect on leaf traits or biomass detected
- supported by QTL in a different population



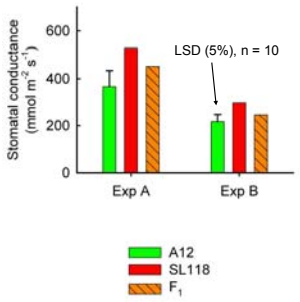
Investigation of top of C7 with substitution line SL118



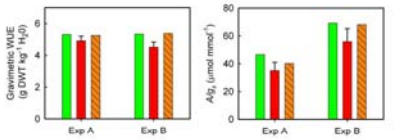
SL118: introgressions of GD33 DNA (red) in an A12 background (green)

Line SL118 (with a substitution on chr. 7):

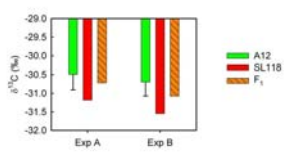
- A12 alleles causes 20-25% lower stomatal conductance relative to GD33



Line SL118 (with a substitution on chr. 7):



- A12 alleles causes 15-20% higher WUE relative to GD33



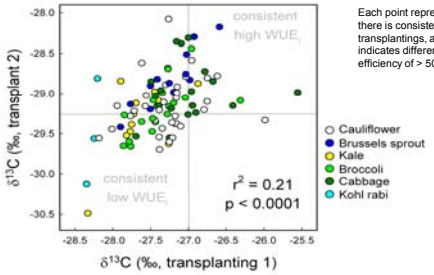
Association mapping in *B. oleracea*?

BoDFS: *Brassica oleracea* diversity foundation set

- Collect trait data from BoDFS and genotype within regions of interest
- Search for association between trait values and specific haplotypes

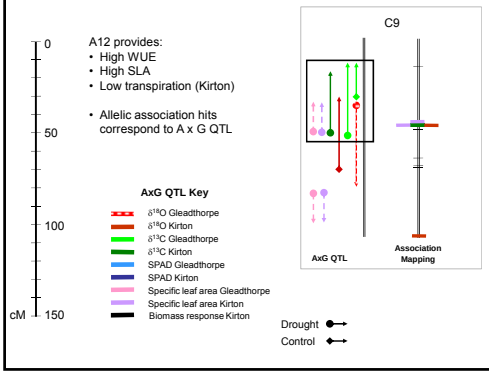
Genetic variation in intrinsic water use efficiency in vegetable brassicas

WUE was assessed with carbon isotope composition (δ^{13}C) in two field transplantings at Kirton (107 accessions selected from the vegetable gene bank at Wellesbourne)



Each point represents a single genotype: there is consistency between the two transplantings, and the range of value indicates differences in actual water use efficiency of > 50%

Comparison of two-parent QTL mapping and allelic association mapping on C9



3. Traits for water capture

- Root depth and root length density at depth
- Ability to penetrate sub-soil
- Ability to conduct water to the shoot

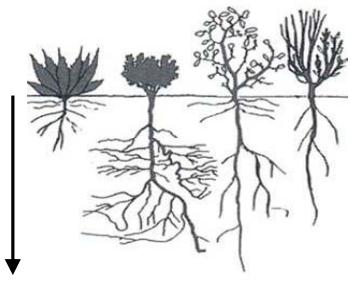


Image from Larcher 4th ed

S. pennellii x *S. lycopersicum* (M82) introgressed line (IL) population
Dani Zamir, The Hebrew University of Jerusalem

S. pennellii growing on dry rocky slopes in central Peru

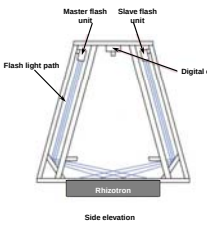
S. lycopersicum (LA3475, cv M82) Determinate (bush) cultivated field tomato

Images courtesy of the TGRC, UC Davis

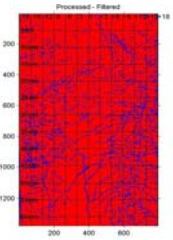
50 introgressed lines screened in 64 rhizotrons
6 reps per line, 2 chromosomes per run

Image analysis of root images
using a custom-designed MATLAB based program
(*Nick Parsons, University of Warwick*)

Captured root image

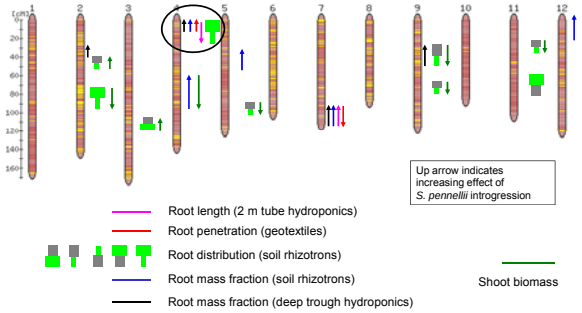


Photographic hood

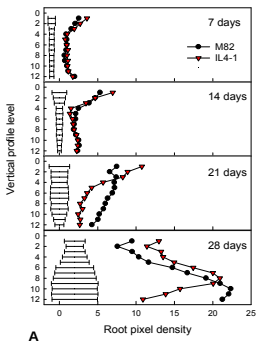


Processed root image (pixel density at 12 depths)

QTL for root traits
12 chromosomes of *Solanum lycopersicum*

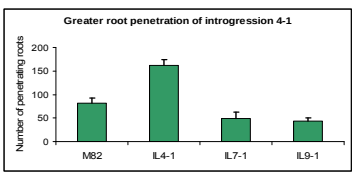


A QTL for the vertical distribution of the tomato root system

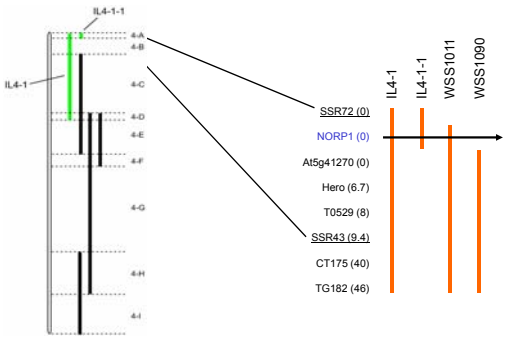


Data from imaging of soil-grown plants in rhizotrons

Identification of QTL for penetration of geotextile membranes

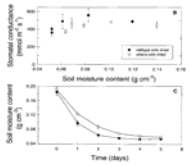


Fine mapping of root penetration QTL (NORP1) on chr 4

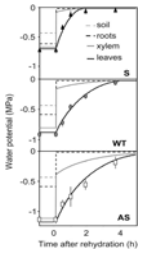


- Root hydraulic conductivity is important for water capture and for regulating shoot water status under variable transpiration rates
- Root hydraulic conductivity is strongly regulated by abscisic acid

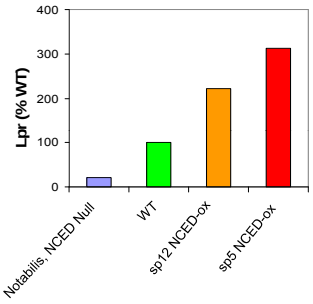
ABA deficient roots are worse at extracting water from drying soil in 3-way grafts (Holbrook et al 2002)



High ABA maize lines recover leaf water potential faster when rehydrated (Parent et al 2009)



14-fold range of root hydraulic conductivity (Lpr) from ABA deficient or ABA over-producing tomato lines

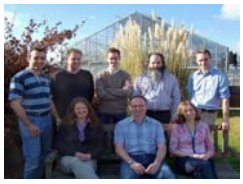


Data from:
Tal and Nevo (1973) (notabilis)
Thompson et al (2007) (NCED overexpression)

Conclusions

- Much of agriculture uses water in an **unsustainable** way, breeding for WUE is one approach to address this issue
- **Transgenics**: over-expression of NCED doubles WUE
  - a strong effect of a single gene! Beyond natural variation.
  - but need to understand the pleiotropic effects and impact on productivity
  - more subtle engineering to enhance benefits and limit negative effects
- **Marker assisted selection** for WUE will be difficult, but rewards are large
  - there is a wealth of natural genetic variation in WUE
  - many genes with small effects
  - different genes will be important in each environment – which trait to measure?

Acknowledgements



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