Development of informative markers through association mapping in maize to improve drought tolerance in cereals

GCP Project Number 13

CIMMYT MR

Collborators

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Collaborators

Link to other subprograms

- SP1 – Genetic diversity – alleles
- SP3 – Trait capture for crop improvement – MAS
- SP5 – Capacity building - workshops

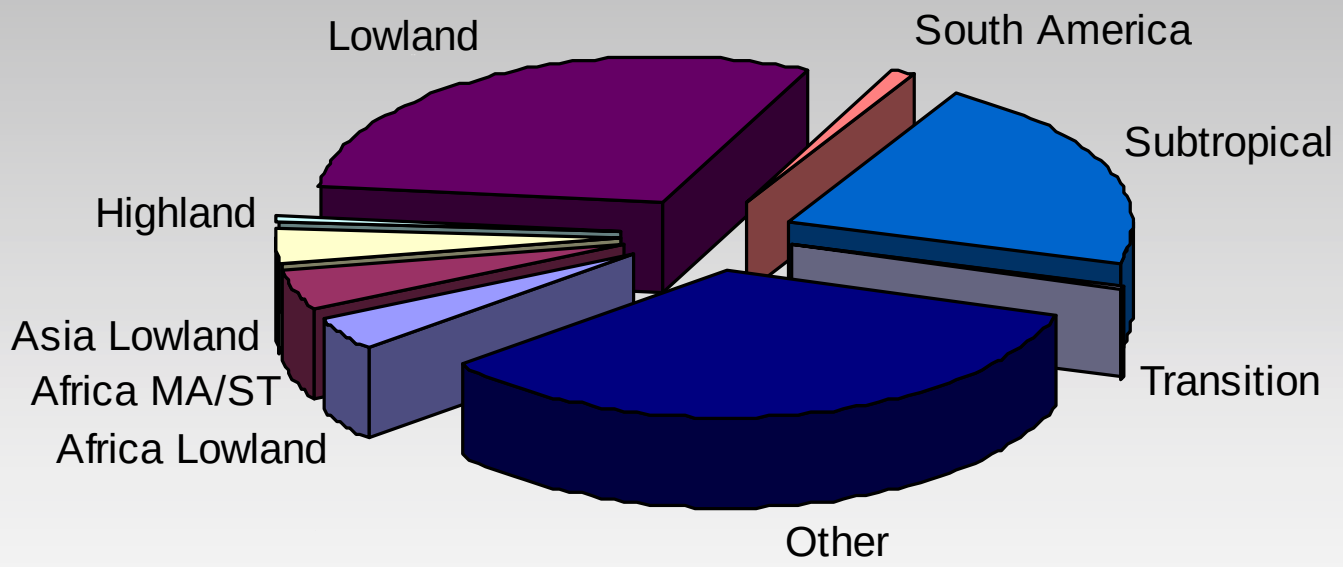
Main activities 2005

- Germplasm selection
- Field trials (Phenotying and tissue collection)
- Metabolite analysis
- Genotying and candidate gene selection

Germplasm selection

- Initial selection of 600 diverse maize lines selected
  - Material from partners, Ed Buckler, pedigree information, lines used at CIMMYT to develop segregating pops for QTL detection
- Planted in Mexico (2004) to check adaptation to local conditions
  - Flowering time
  - Verify inbreeding level of lines. Homogeneity within plots. 5 plants selfed to provide adequate seed for distribution. If homogeneity visually OK seed from different ears bulked. If not, then line discarded.
- **460** subsequently selected for further evaluation

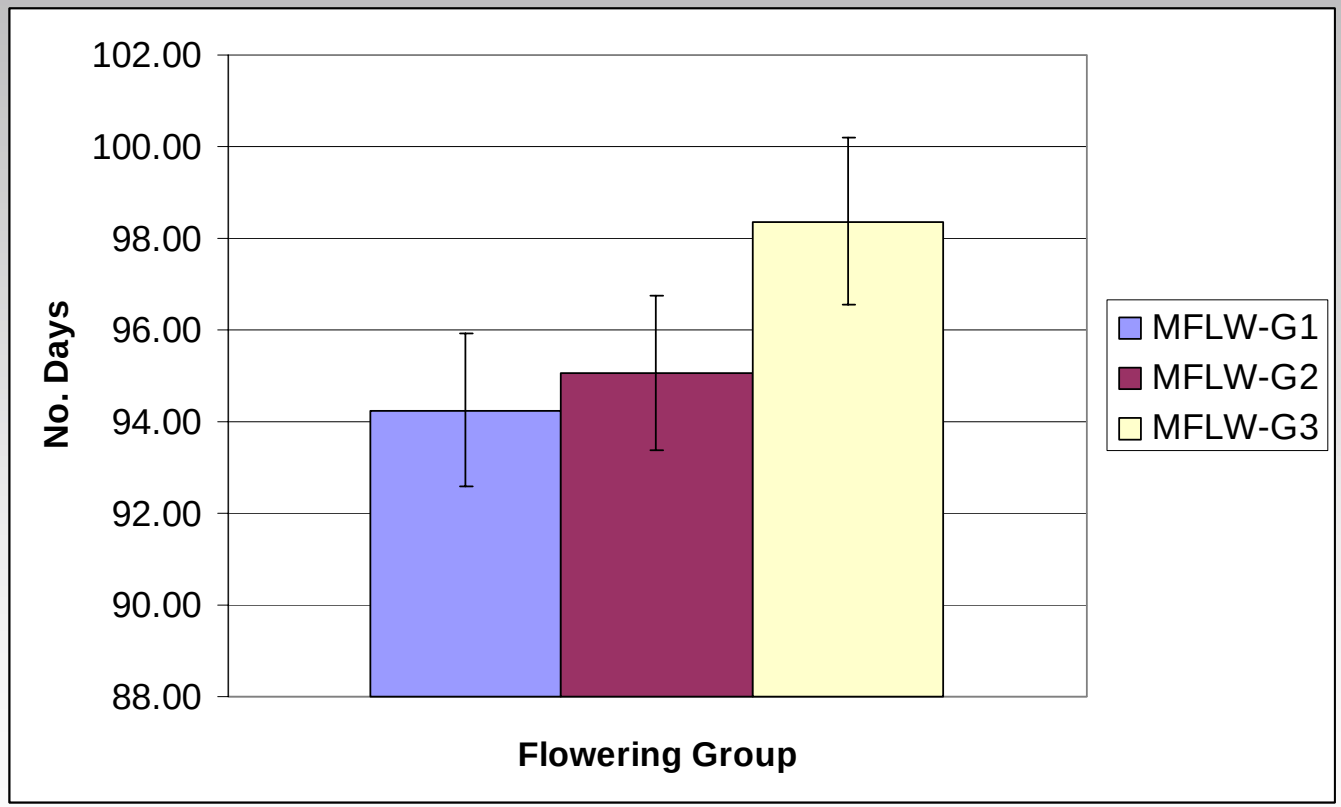
Germplasm


Lowland
South America
Subtropical
Highland
Asia Lowland
Africa MA/ST
Africa Lowland
Transition
Other

Germplasm

- Interested at drought at flowering
- Design field trials where stress occurs at flowering
- Diverse germplasm – divergent selection for flowering time
- Required classification into different groups based on precocity data

Germplasm – Precocity groups



Field trials

- Seed increase and hybrid seed production with CIMMYT tester CML312
- Parallel trials to evaluate lines under drought
- Seed distribution to partners in Thailand, China, Kenya and Zimbabwe
- Sample collection for DNA (10 leaf discs pooled)

Drought trials

- Three randomized trials – each flowering group
- Two replications per trial
- Data collected for standard phenotypic data
  - Flowering
  - Plant size
  - Chlorophyll content
  - Root conductivity

Drought trials

- Seed distribution
  - Subset of 60 genotypes (20x3) and the corresponding hybrids sent to collaborators. Being evaluated in Kenya and China under optimal conditions for flowering and adaptation
  - Kenya - full set of hybrids (n=430) planted in mid July. Currently being evaluated under stress and normal conditions

Metabolite analysis

- 5520 samples harvested from the field under stress
  - Three tissues – ear tips, silks & leaves
  - Two timepoints – 0 days & 7 days
  - Two stress replicates
- Samples placed in 80% methanol in the field
- Stored for 1 month in cold storage for exodiffusion

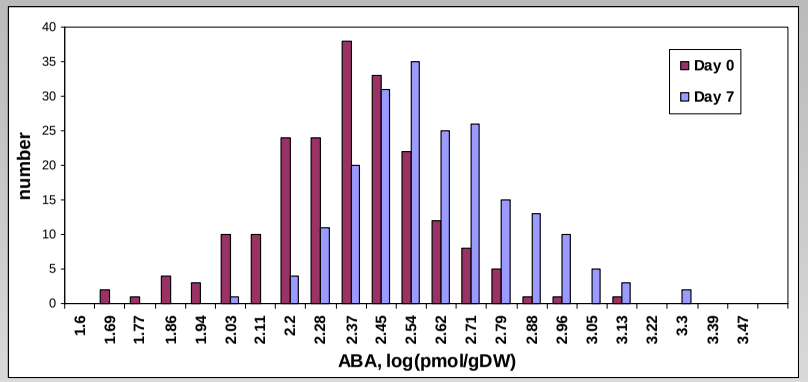
Metabolite analysis - Cornell

- Dried aliquots sent in 96 well plates to Cornell
  - Rapid processing of samples
- Dry weights of all tissue taken to normalize for differences in amounts of tissue harvested between lines
- Dried tissue also being ground for analysis of starch and cellulose

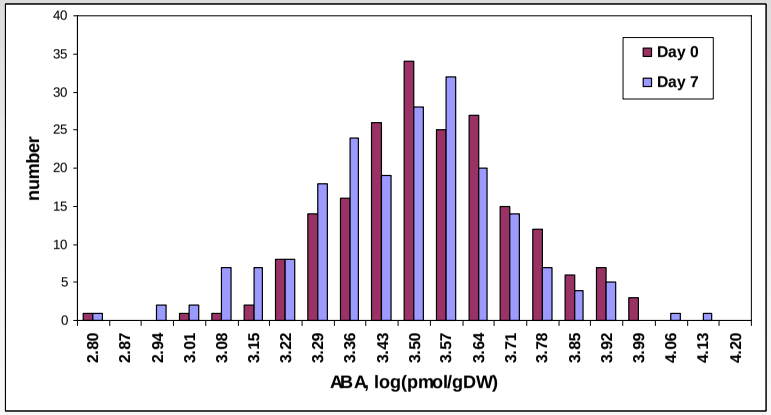
Metabolites assayed

- Sucrose
- Glucose
- Abscisic acid
  - analysis of sugars and ABA run twice/run until one obtains satisfactory results and average taken
- ABA glucose ester (ABA-GE)
- Phaseic acid (PA) being run

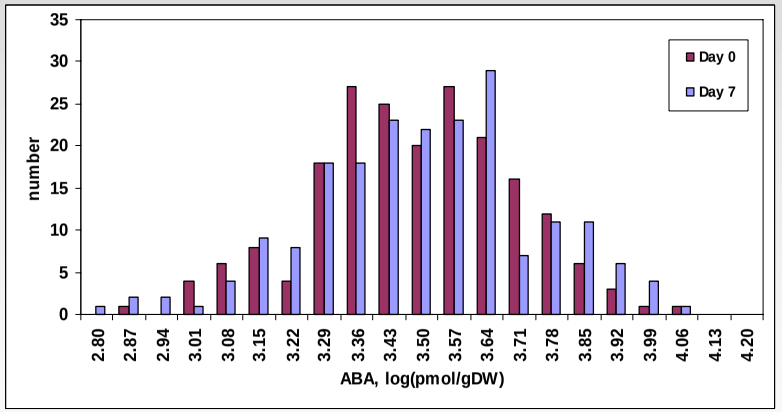
Preliminary results - ABA



Leaves

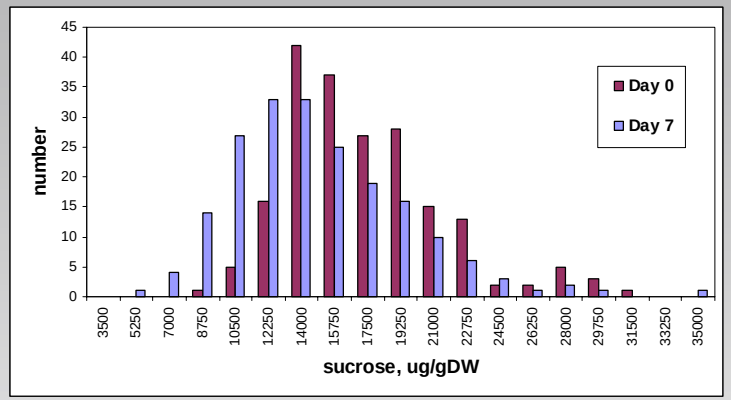


Silks

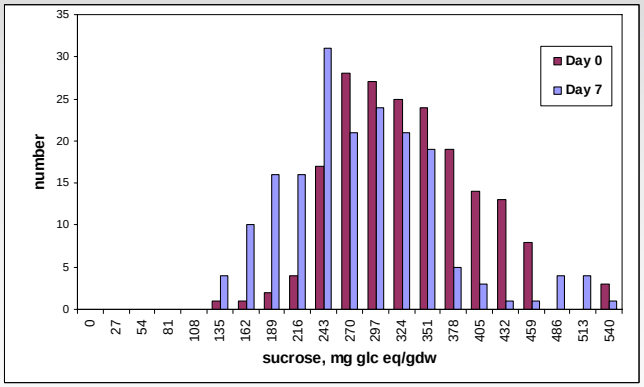


Ear tips

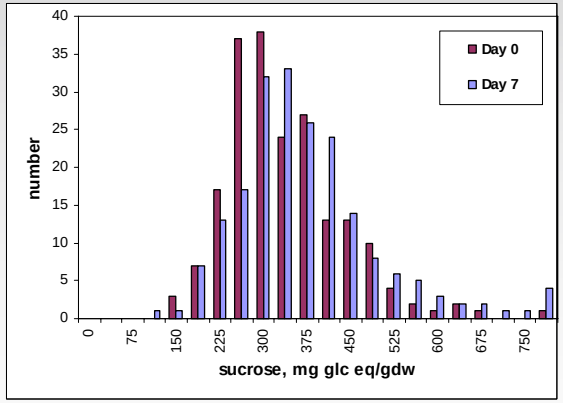
Preliminary results - Sucrose



Leaves

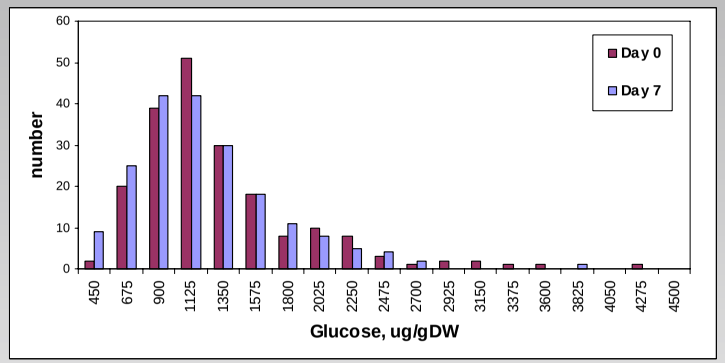


Silks

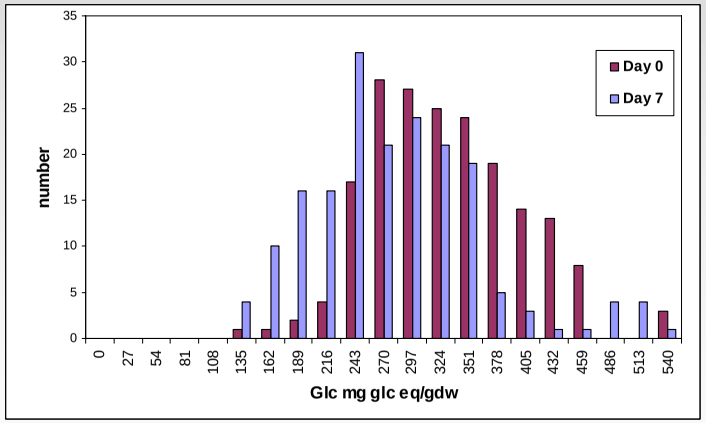


Ear tips

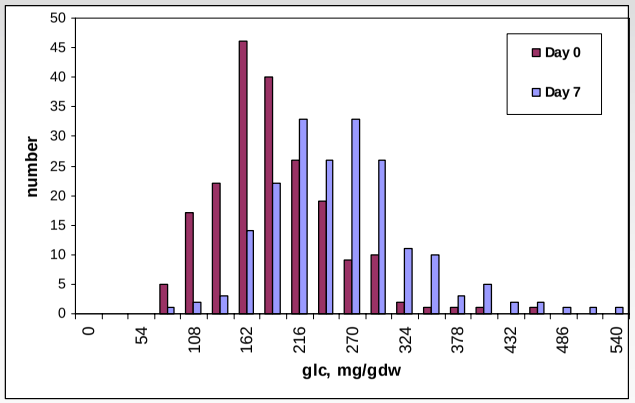
Preliminary results – Glucose



Leaves



Silks



Ear tips

Metabolite results

- Leaves - ABA increases from the first to second sampling. In contrast, sucrose decreases. Some increase in glucose
- Ear tips - Glucose concentration per dry weight increases (0-7d)
- Silks – Sucrose decrease (0-7d). Possibly some decrease in glucose
- **TREAT WITH CAUTION**
  - Error checking; repeat assays

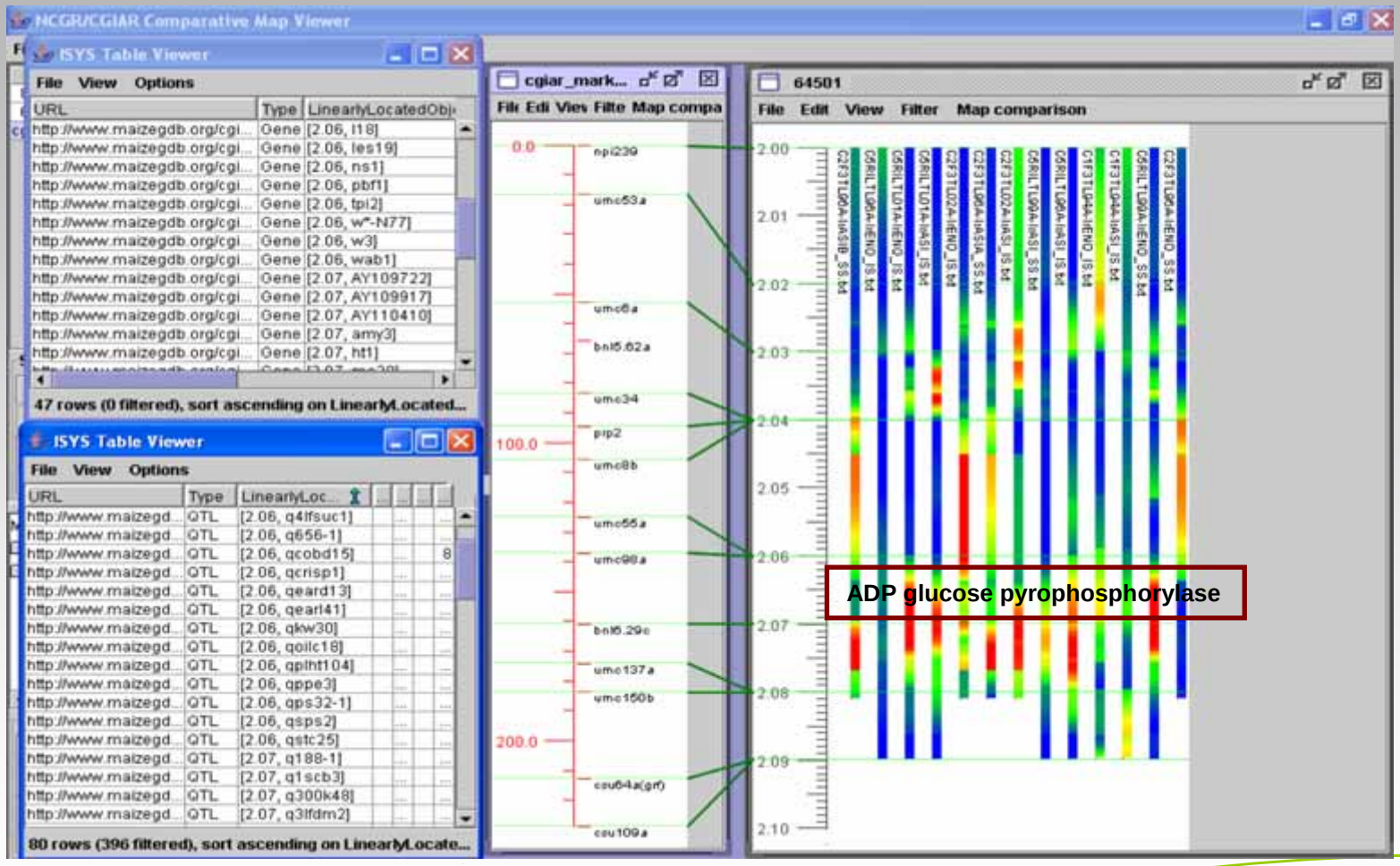
Genotyping and candidate gene selection

- Diverse set of inbred lines selected for study
- What is the genetic relationship among lines?
  - Genotyping for population structure using an unbiased set of SNPs
  - Ca. 1000 EST SNPs available from www.panzea.org.

Candidate gene selection – Criteria for selection

- Genes that have established functional roles in carbohydrate and ABA pathways
- Genes whose expression in transcript profiling differed under drought in contrasting genotypes
- Positional candidates (QTL location)

Positional candidates



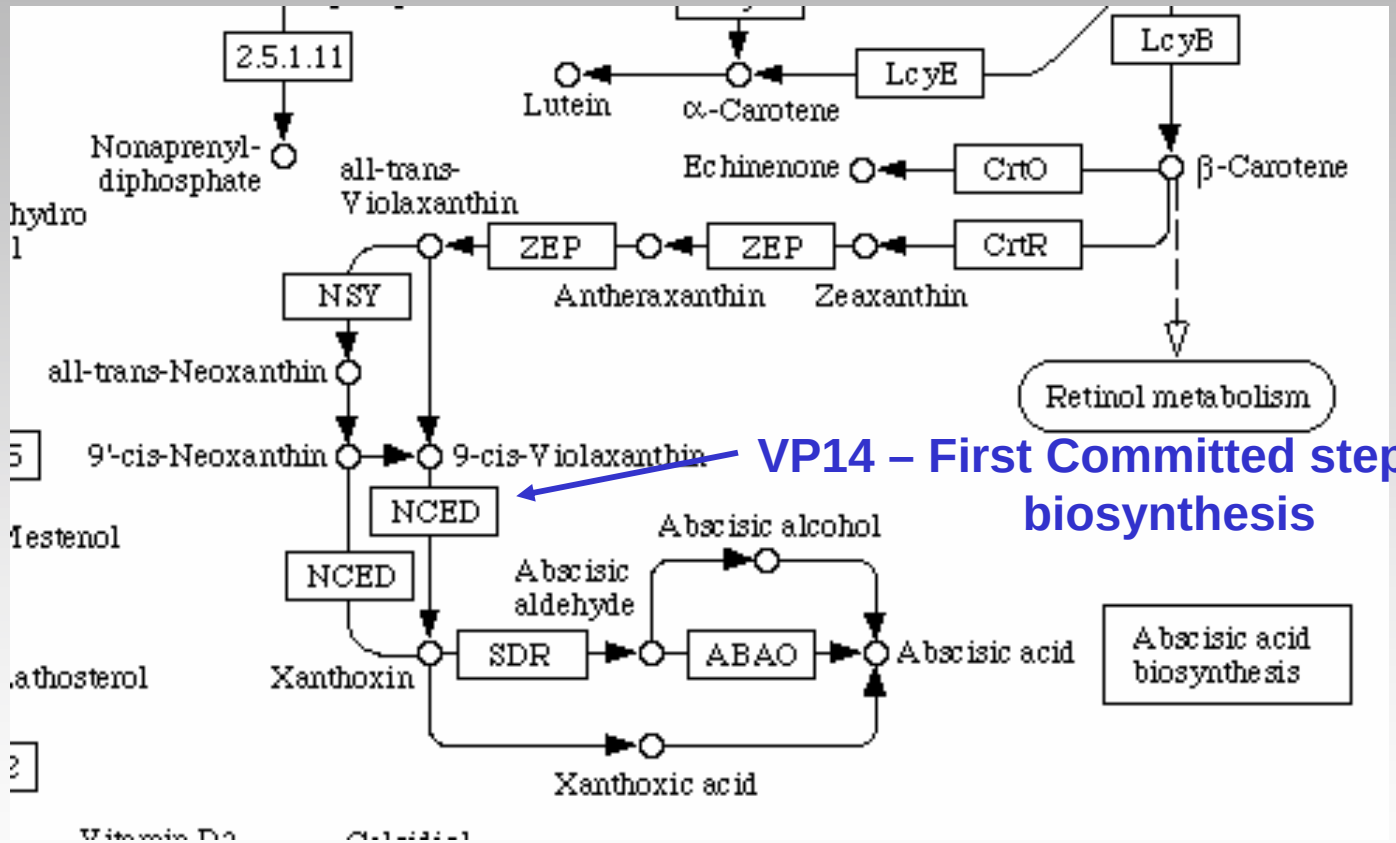
First set of candidates

Gene		Bin
ae1	starch branching enzyme IIB	5.04
sh1	shrunken 1 - Sucrose synthase sus2	9.01
su1	debranching enzyme	4.05
wx1	granule bound starch synthase	9.03
Agp1	ADP-glucose-pyrophosporylase - small subunit	6.07
Agp2	ADP-glucose-pyrophosporylase - large subunit	2.06
sh2	ADP-glucose-pyrophosporylase - large subunit	3.09
bt1	ADP-glucose-pyrophosporylase - small subunit	5.04
beta amylase		
maltose transporter		
Sus1	Sucrose synthase 1	9.04
Sps1	Sucrose phosphate synthase	8.06
Sps2	sucrose phospate synthase	3.05
Ivr1	invertase 1	2.02
Ivr2	invertase 2	5.03
spp1	sucrose phosphatase	8.05

First set of candidates

- ABA anabolism
  - ZEP (zeaxanthin epoxidase; *aba*1/*npq*2/*los*6 in *Arabidopsis*)
  - SDR (short-chain alcohol dehydrogenase/reductase; *aba*2/*gin*1/*isi*4/*sis*4 in *Arabidopsis*)
  - NCED (9-cis-epoxycarotenoid dioxygenase; *vp*14 in maize)
  - MoCo Sulfurase (molybdenum cofactor sulfurase; *aba*3/*los*5/*gin*5 in *Arabidopsis*)

First set of candidates



VP14 – First Committed step in ABA biosynthesis

First set of candidates

- ABA catabolism – storage/inactivation
  - ABA 8‘ hydroxylase (ABA → Phaseic acid (PA) → DPA - Oxidation)
    - Bin 4.06 – QTL ABA-Ear (CIMMYT)

Projected activities

- For many of the carbohydrate genes polymorphisms can already be screened on our lines
- What about new alleles in tropical maize?
  - Ed Buckler – core set 304 lines. 41 CMLs
  - This study – 460 lines containing 305 CMLs (32 same as core set)
- New candidates – isolated in maize and screened across a subset of lines

Conclusions – Year 1

- Field evaluation and phenotyping – Done
- Genotyping – Underway (4-6 weeks turnaround)
- Candidate genes – screening of polymorphisms
  - Carbohydrate metabolism – OK
  - ABA genes – some further work