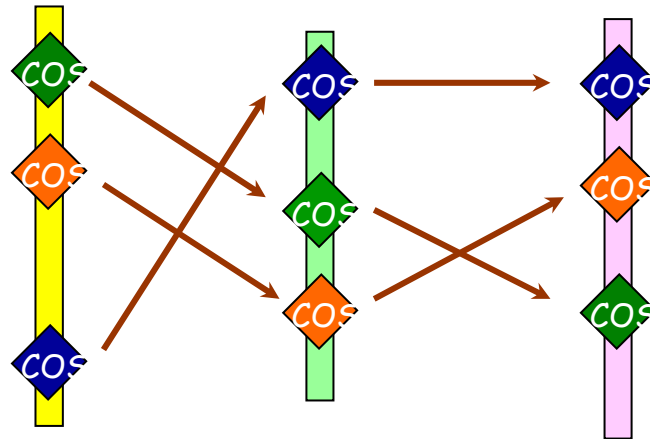


SP2-7

Validation of orthologous gene markers



CIP, ICARDA, CAAS
INIBAP, U Leicester

Overall Objective

- To develop and validate universal primers for orthologous genes for use in comparative mapping and phylogenetic studies in a broad range of species using convenient PCR
 - Monocots
 - Dicots
- "Proof of Concept" following Fulton et al. (2002, Plant Cell 14:1457) in 2004...2005

Specific objectives 2004

- 1: Develop, test and validate universal markers for functional genes
2. Identify/ tag putative orthologous candidate genes for drought resistance
- 3: Enhance comparative genetic maps



COS
(conserved orthologous set)



Markers
for
transcribed
sequences

- Targeting exonic sequences flanking introns in putative orthologues
- EST-SSRs

End of Year 1: Jury still out

Too few PCRable COS mapped & validated

- Unexpected multiple-copy
- Difficulty in discerning among copies
- Non-homologous products

Proposal: More intensive computational algorithms/ stringency to identify putative orthologues

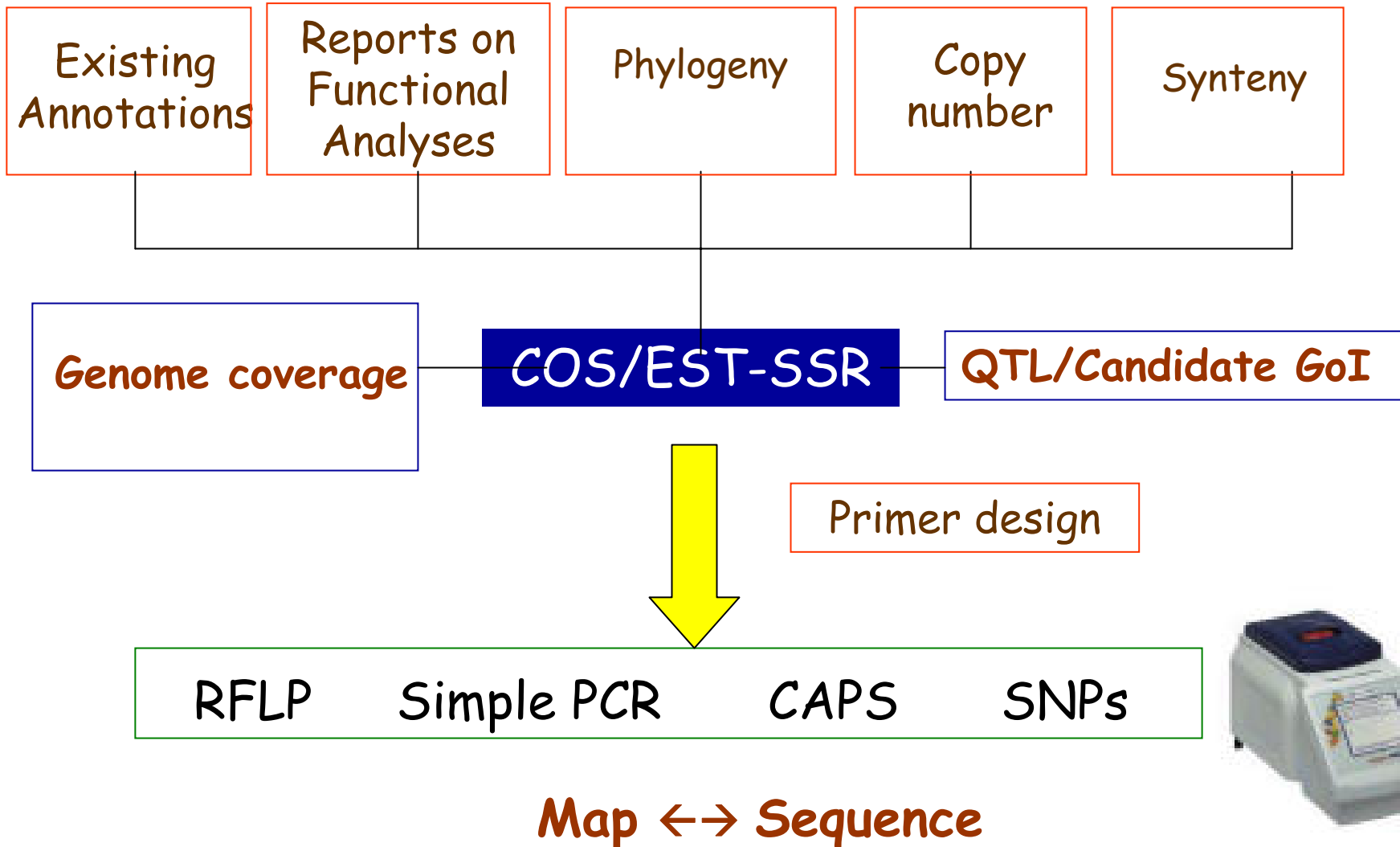
- Rapid evolution of EST databases
- RBM >no. of species (*A.t.*, tomato, pepper, potato, eggplant, petunia, coffee; Wheat, rice, maize barley; Rice, Musa);
- Multiple seq. alignment tool, manual editing; phylogeny

Objectives (2005):

- Consolidate and expand the available set of orthologous genes by revisiting bioinformatics approaches for identifying COS
- Test and validate universal markers developed in Year 1 related to drought tolerance, disease resistance and other agronomically important traits to enhance comparative maps within and across crop families
- Develop gene-based markers universally or separately for monocots and dicots based on conserved regions of orthologous genes to enhance gene discovery.

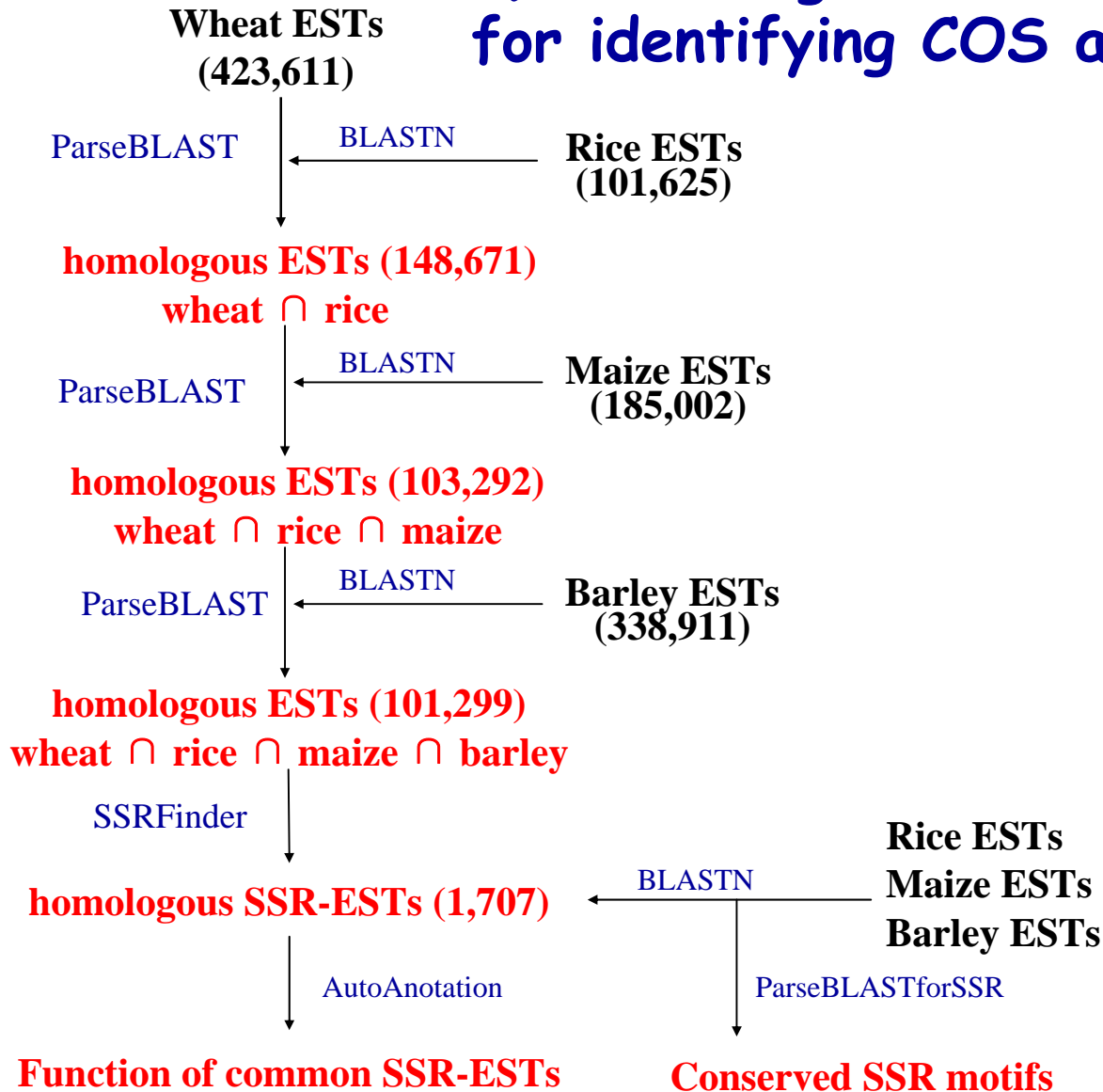


Approaches



Monocots: Grasses

1) Revisiting bioinformatics approaches for identifying COS at CAAS

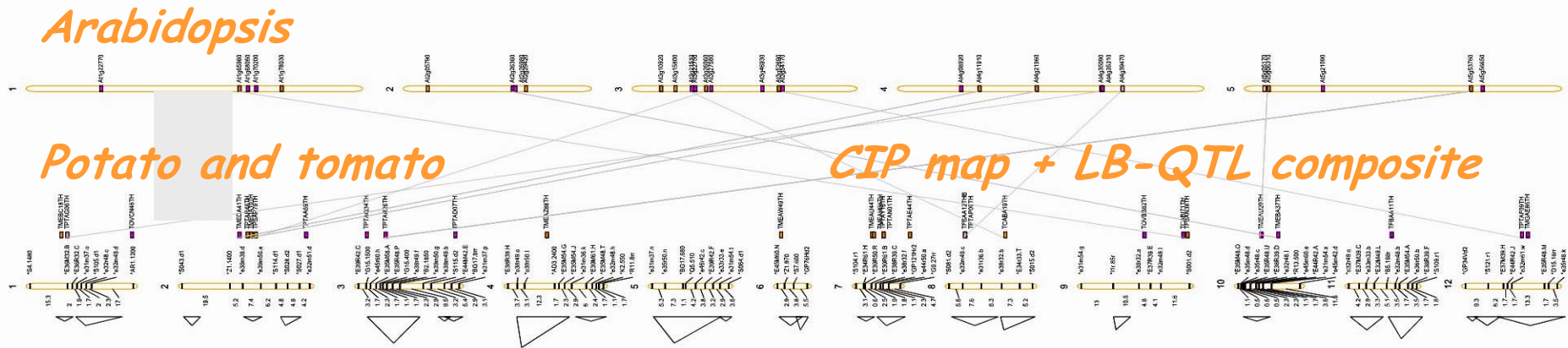


2) Disease resistance universal markers among monocots

- 51 SSR-containing RGA were discovered
- 63 EST-SSR primers for 65 SSRs were designed based on wheat ESTs.
- Of which, 25 ESTs contain LRR or NBS-LRR domain, 22 contain PK or LRR-PK domain, the other 4 ESTs were related to disease resistance.

Selection of COS for potato

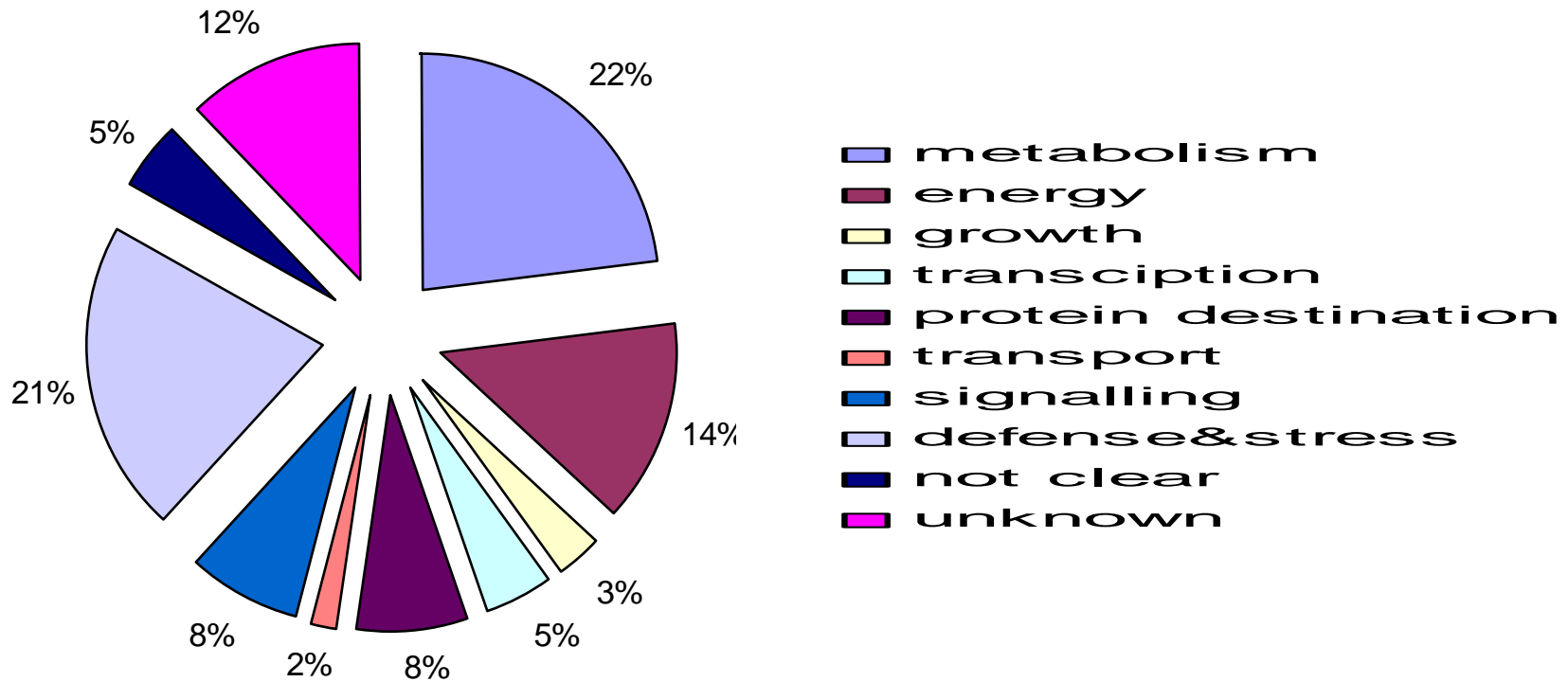
1) Position and Function



- 400 COS mapped in tomato, defense related genes selected: e.g. biogenesis, signal transduction, cell rescue related (Fulton et. al.)
- Locations in potato predicted from tomato
- Connecting lines represent similarities by BLAST of potato ESTs (StGI) against AtGI. Triangles represent intervals identifying QTL & strength of evidence
- Primer design and testing in 2 MapPop samples

CIP

2) Distribution of genes per high level category (MIPS/SGN) selected from COSI & COSII collections (SGN)



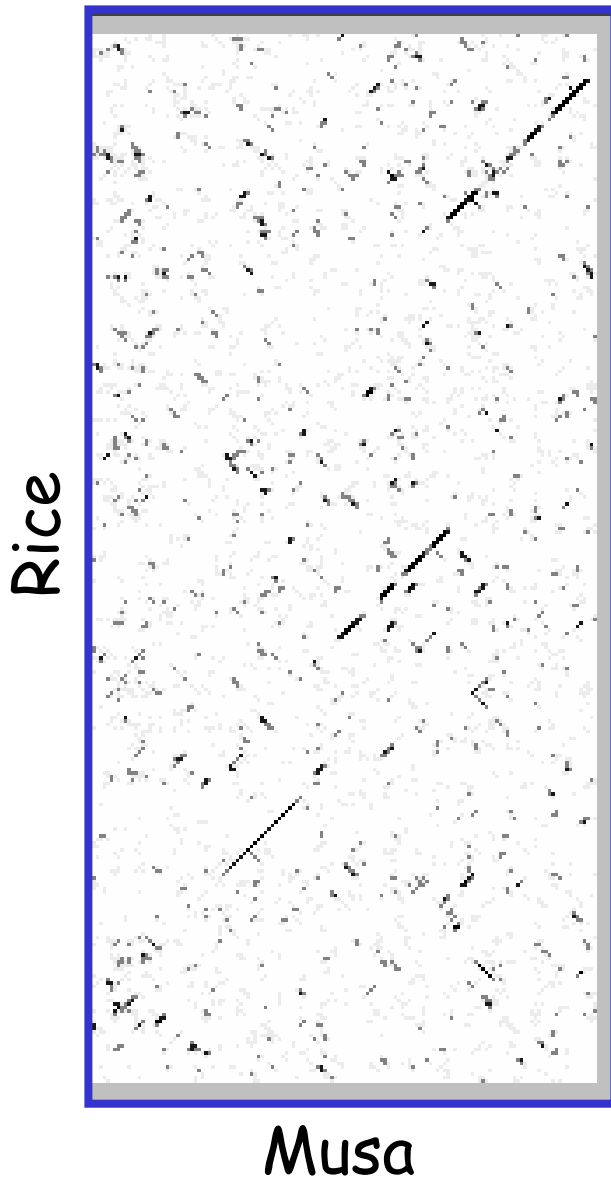
CIP

Monocot: Rice and Musa

1) Targeted gene and informatic approach

- Genes in the flavonol pathway and genes related to abiotic stress responses.
 - EST sequences from *Musa* were included where available
 - Both predicted protein and DNA sequences were used for alignment
- Primer design was more difficult for proteins rich in amino acids encoded by 6 codons (eg leucine, serine) or with AT-rich codons (eg lysine, tyrosine).

2) Targeting exonic sequences flanking introns



- Dotplot of Musa EST (x) vs Rice BAC (y) showing two exons and three introns.
- Comparison of ESTs and heterologous BACs shows locations of exons and introns permitting design of intron-spanning primers which are often polymorphic.

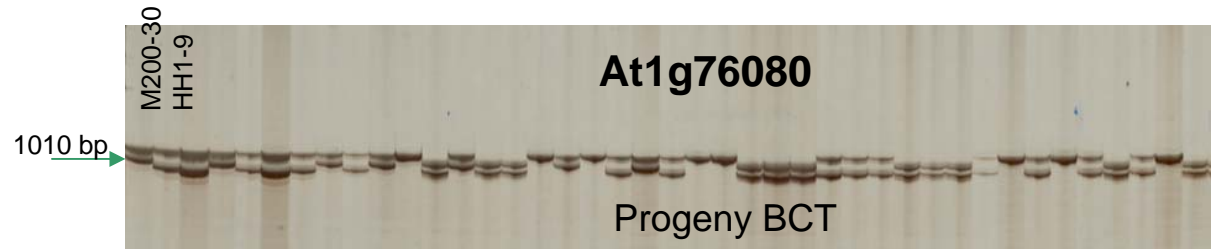
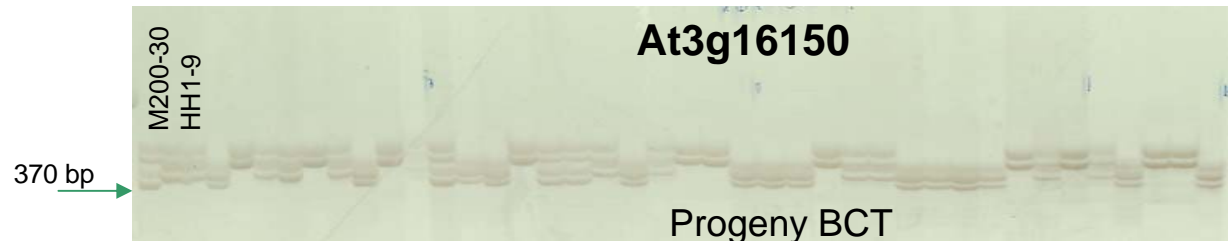
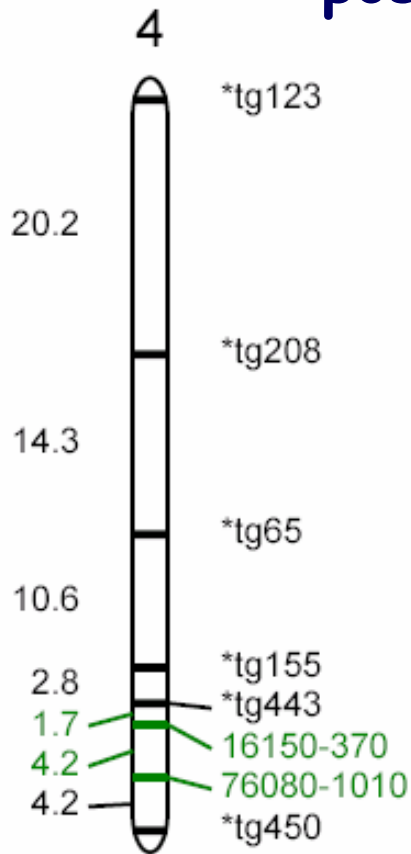
Musa Consortium

Cereal & legumes:

- Select a Mapped Conserved Ortholog Set Markers available on SGN / Arabidopsis full length cDNA clone involved in stress
- BLAST Search for TIGR Unique Gene Indices
- Tentative Ortholog Group was identified as referred by TIGR (EGO)
- Identified conserved regions across legume species
- Primers were designed and used for amplification (4 legume species)

Progress

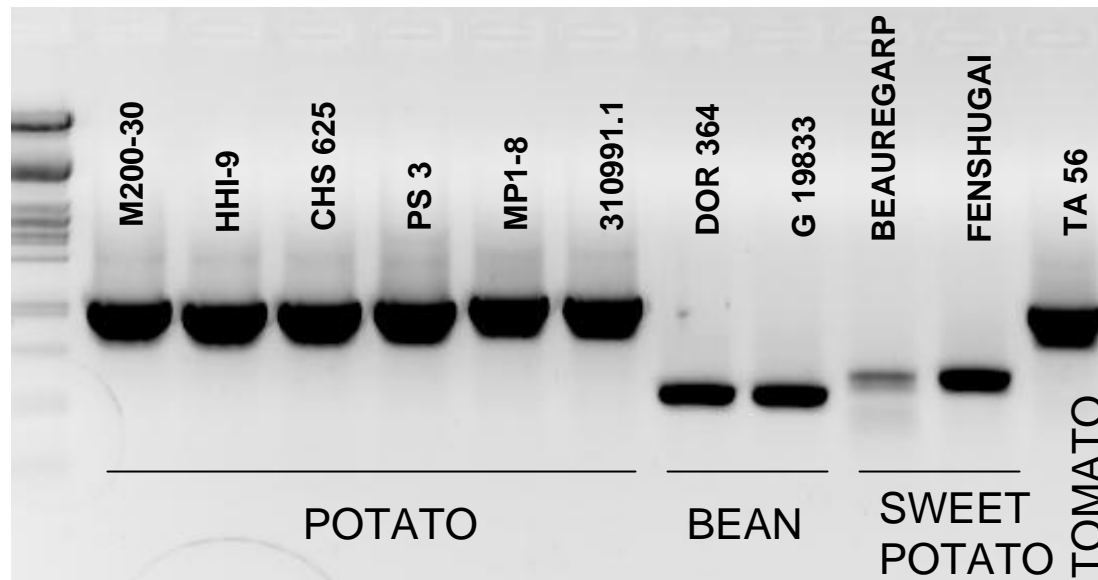
COS II markers mapped to homeologous positions on Potato/tomato chr. 4



CIP

Amplification of COS II marker in 4 dicot species

At5g62390

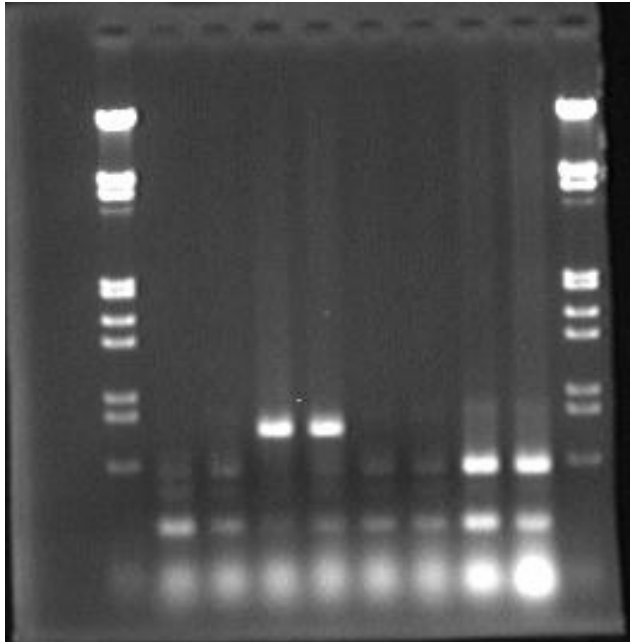


1% Agarose gel

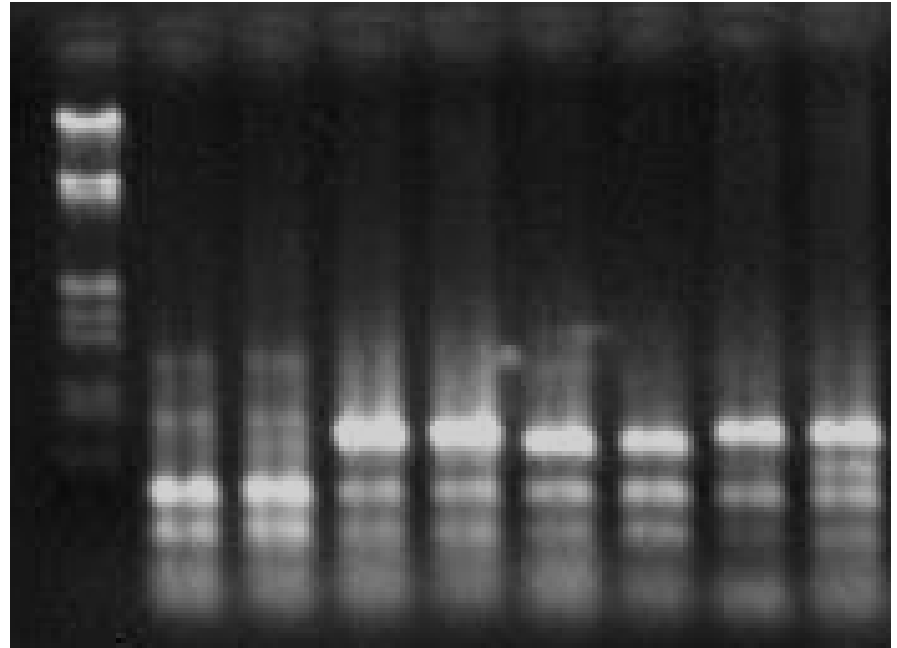
CIP

COS Amplification in 3 Legumes

RAFL04-12-K17 -2



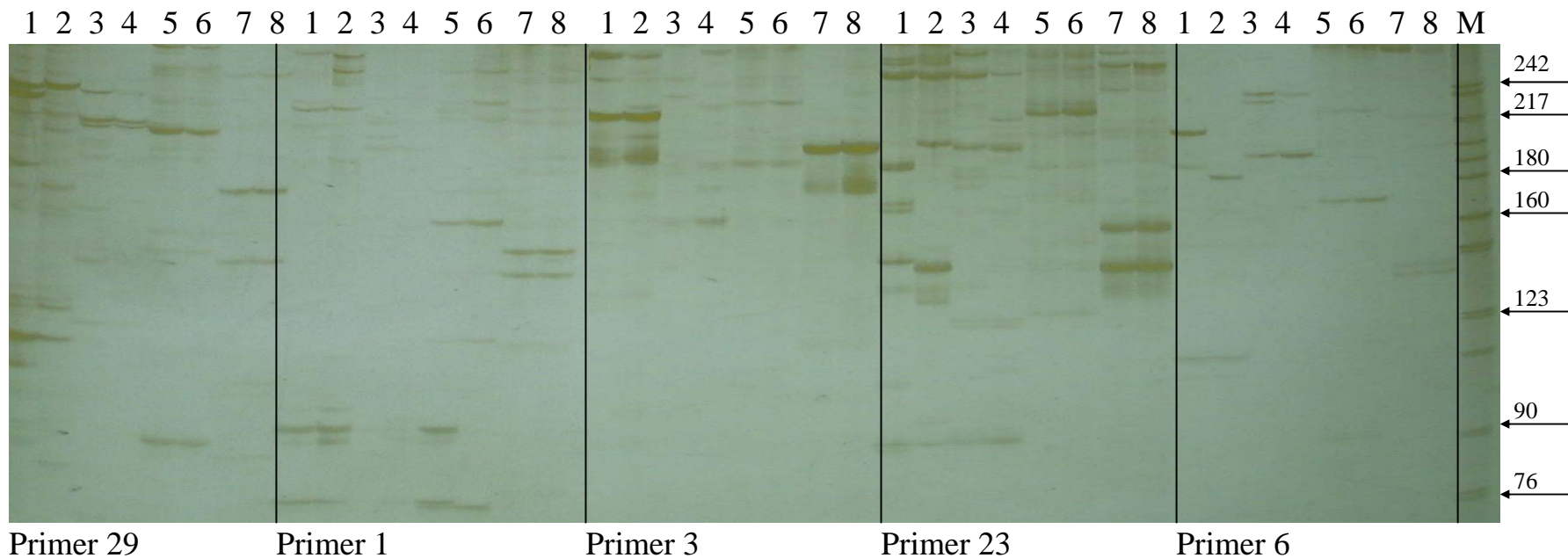
TO51



Lanes: 1: Mol. Wt. Marker, 2-3: Faba bean, 4-5:
Lentil, 6-7: Chickpea, 8-9: Grasspea

ICARDA

Screening RGA-SSR primers across monocots



1,2: rice varieties; 3,4: maize varieties; 5,6: barley varieties; 7,8: wheat varieties

CAAS

Mapping RGA-SSRs onto wheat chromosomes

Mapped RGA-SSR 5 and 6 to Chr7B of
Yanzhan1 × Neixiang188 population

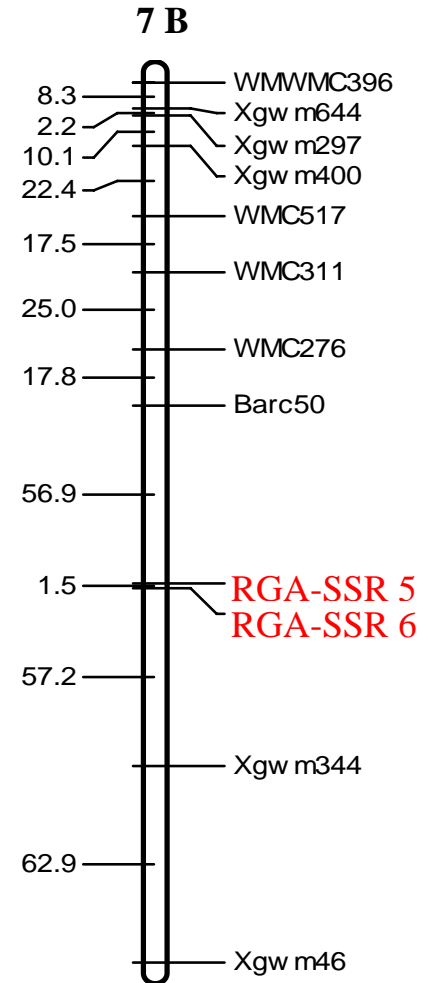
Progeny 1-51



Progeny 53-102



P1: W7984; P2: Opata; M: pBR322 DNA/Mspl DNA marker



CAAS

Diversity analysis using RGA-SSR in wheat

Primer 1



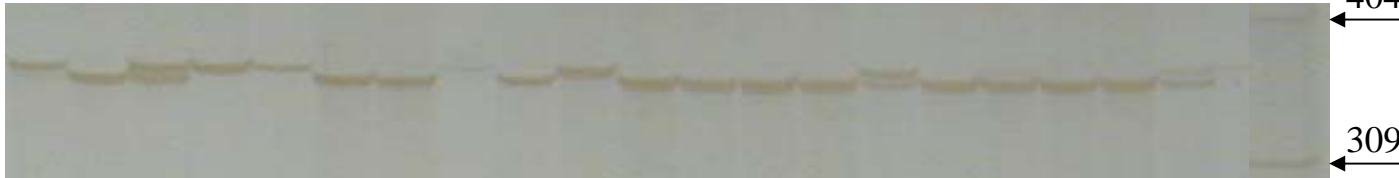
Primer 5



Primer 6



Primer 14



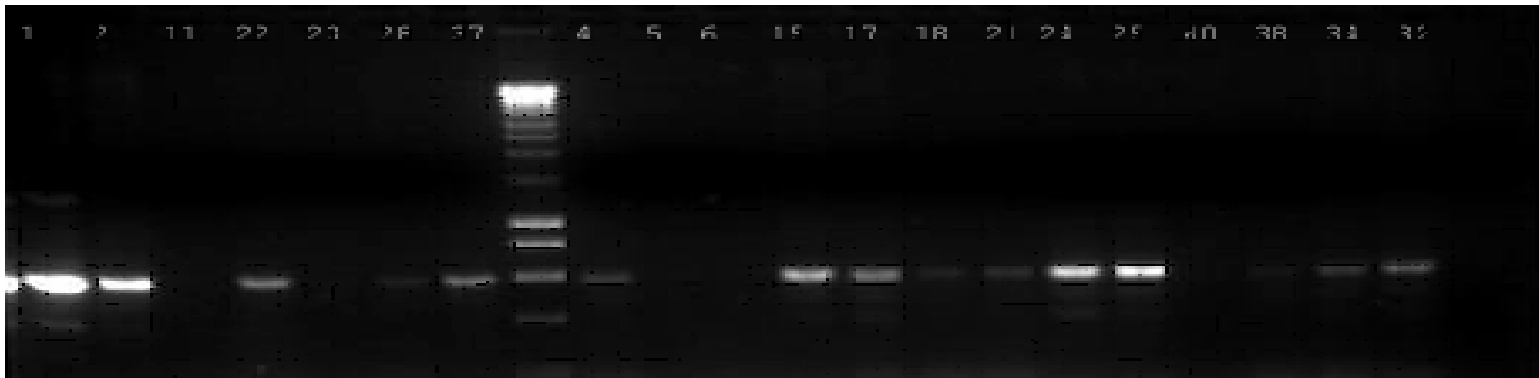
Primer 15



CAAS

COS1263

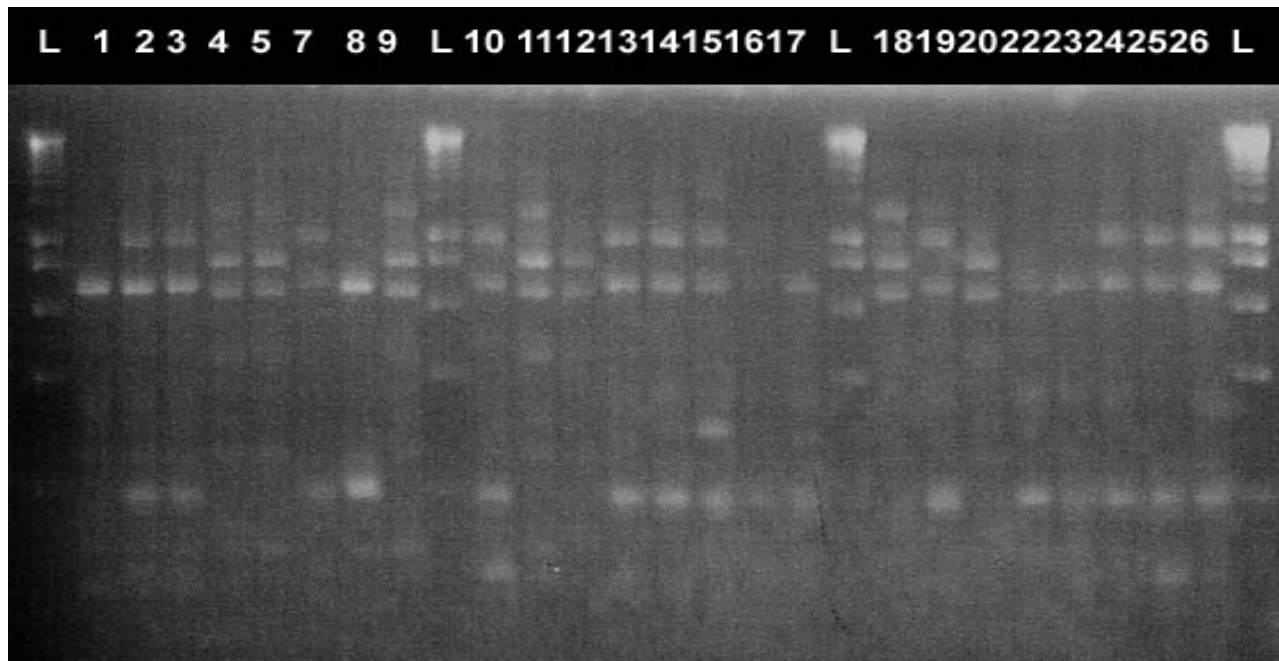
Amplification in 13/20 *Musa* accessions



Even across large taxonomic distances and using non-optimized primers, about a third of genes are amplified.

Musa Consortium

Musa EST - rice - wheat - Arabidopsis comparison and primer design.



- DREBP

INIBAP/ U Leicester

Tangible outputs midyear

- 100 COS II primers (SGN) synthesized
- 45 COS II (SGN) amplified, surveyed for polymorphism and/or mapped in potato
- 46 EST-SSR primers synthesized
- 24 EST-SSR primers amplified, surveyed for informativeness and/or mapped in wheat
- 12 EST-SSR primers amplified across 4 monocots, and 3 more produced amplicons in wheat, rice and barley.
- Abiotic stress genes identified *in silico* and aligned for COS marker development
- Primer designed and amplified on a panel of four legumes
- Sequence analysis of PCR products initiated
- Utility and transferability of wheat SSR-EST markers across barley, rice and maize (submitted to Molecular Breeding; CAAS)

Follow-up



2005: Complete Commissioned research

- Complete sequencing
- Exchange validated primers
- Extend garden panels → additional spp. Exchange mapping parents
- Database/emphasize drought candidates
- SNP identification in validated COS

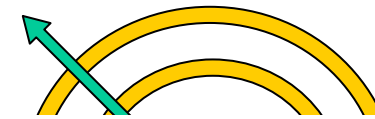
Beyond 2005

SP1 links: Functional markers for SP1: Association studies

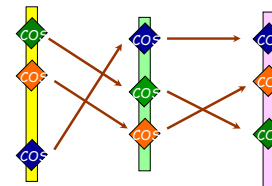
SP2 links: Expression experiments to implicate COS in trait variation

- Continue the EST-SSR validation, mapping and function assessment
- Development of new orthologous functional markers related to stress tolerance and disease resistance (SNP, Indel)

- Jizeng Jia, CAAS
- Lifeng Gao,
- Qingming Sun,
- Nicolas Roux, INIBAP
- Mathieu Rouard,
- Pat Heslop-Harrison, U. Leicester
- Suresh Kumar
- Merideth Bonierbale, CIP
- Reinhard Simon
- Leticia Portal
- Michael Baum, ICARDA
- Sripada M. Udupa



SP2-7
Validation of
orthologous gene
markers



CIP, ICARDA, CAAS
INIBAP, U Leicester