

Validation of Diversity Arrays Technology (DArT) as a Platform for Whole-Genome Profiling in Orphan Crops

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GCP meeting
Rome, September 2005

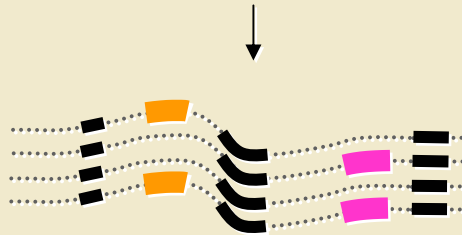
Why DArT?

- Gel-based marker assays: Serial analysis
 - Costly
 - Limited throughput
 - Susceptible to human error
- DArT genome scans: Parallel analysis of several hundred markers
 - Approximately 10-fold cost reduction
 - Amenable to full automation → data integrity
 - Quantitative evaluation of marker quality and individual score confidence
 - User defined „certified” quality - tailored to application
 - Fast and inexpensive technology development (no sequence information necessary)
 - FTO through non-exclusive licenses

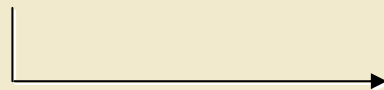
How it works

Array development

"Genepool"



Clone and
microarray
fragments



Routine assays

Sample 1

Genomic DNA

Sample 2 ...



Genomic
representation

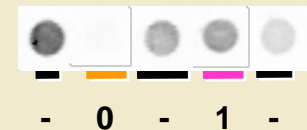


Hybridisation



Pattern 1

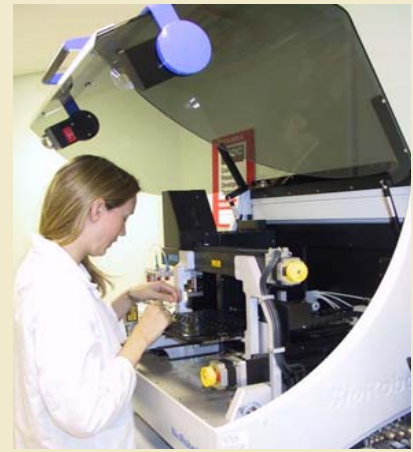
DArT
chip



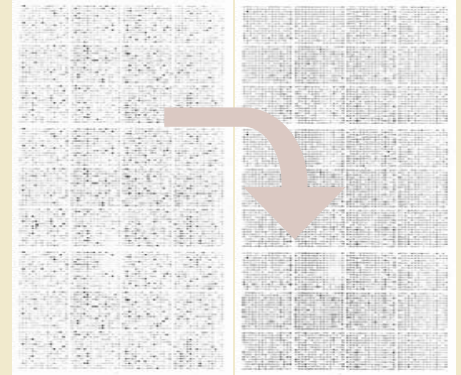
Pattern 2

Workflow

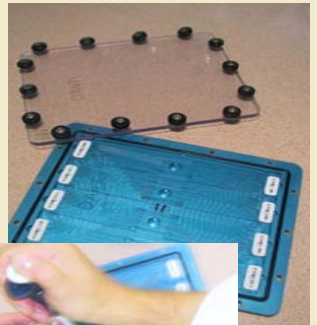
library and target preparation



slide spotting



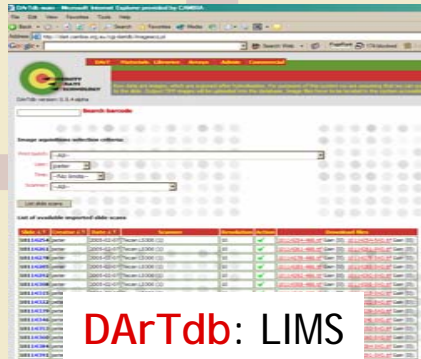
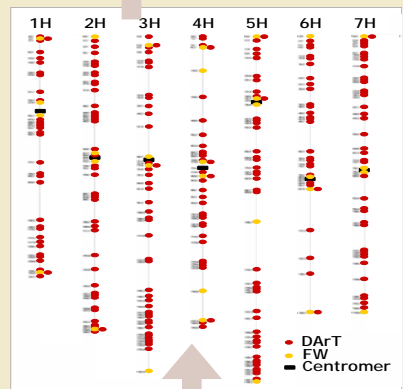
hybridization with targets



slide scanning



results



DArTdb: LIMS

DArTsoft: data analysis



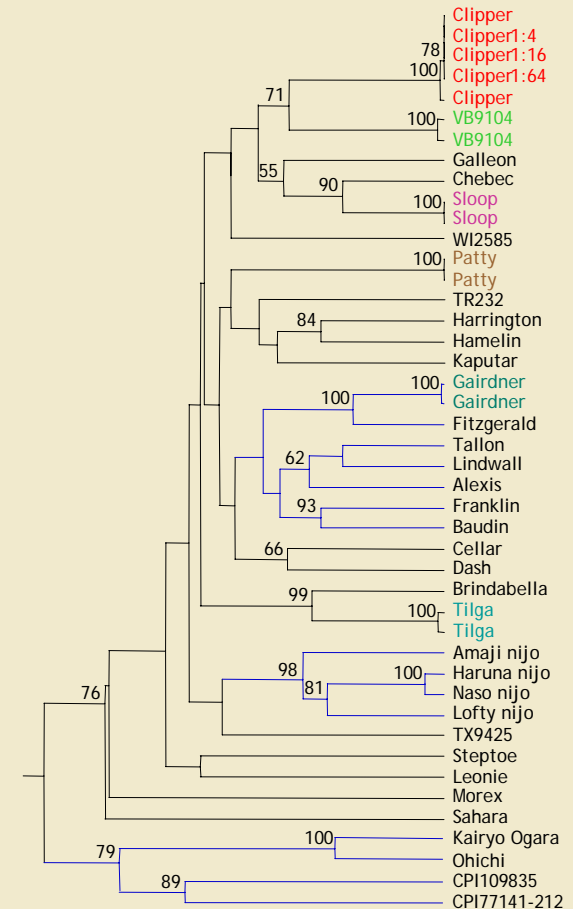
Project Objectives

- Validate existing DArT arrays for **barley**, **sorghum** and **rice**
- Develop and validate a high-density array for **cassava**
- Develop and validate medium-density arrays for **coconut** and **banana**
- Compare genetic-diversity patterns revealed with **DArT** and **SSR**
- Train GCP scientists in DArT to facilitate technology transfer



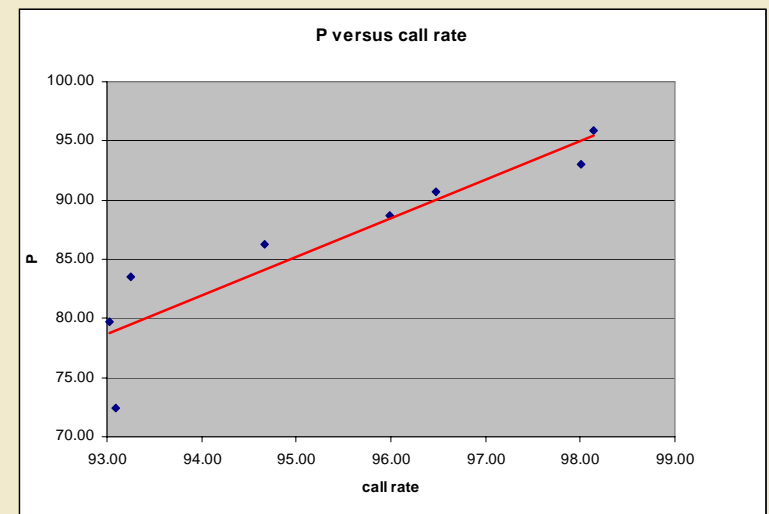
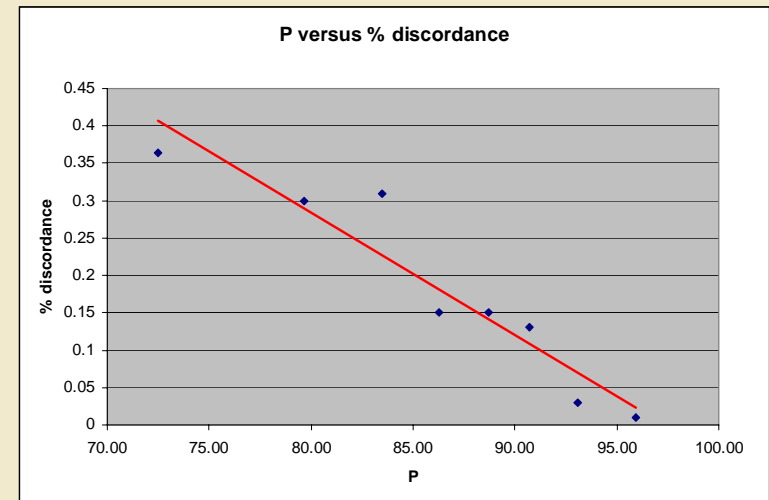
Barley/wheat: Triticarte service

- Triticarte: 50-50 partnership between Value Added Wheat CRC and DArT P/L
- DArT service based at DArT P/L
- 2,571 barley and 2,609 wheat lines analysed from 10/04 - 7/05
- Approximately 2.5 million data points at average cost of 9 cents
- Allele-calling consistency:
 - 99.75 ± 0.29 % for barley
 - 99.92 ± 0.06 % for wheat
- Call rate:
 - 94.9 ± 2.0 % for barley
 - 92.2 ± 2.0 % for wheat
- Approximately 20 barley maps and 15 for wheat
- Numerous diversity studies including several studies with 400+ lines

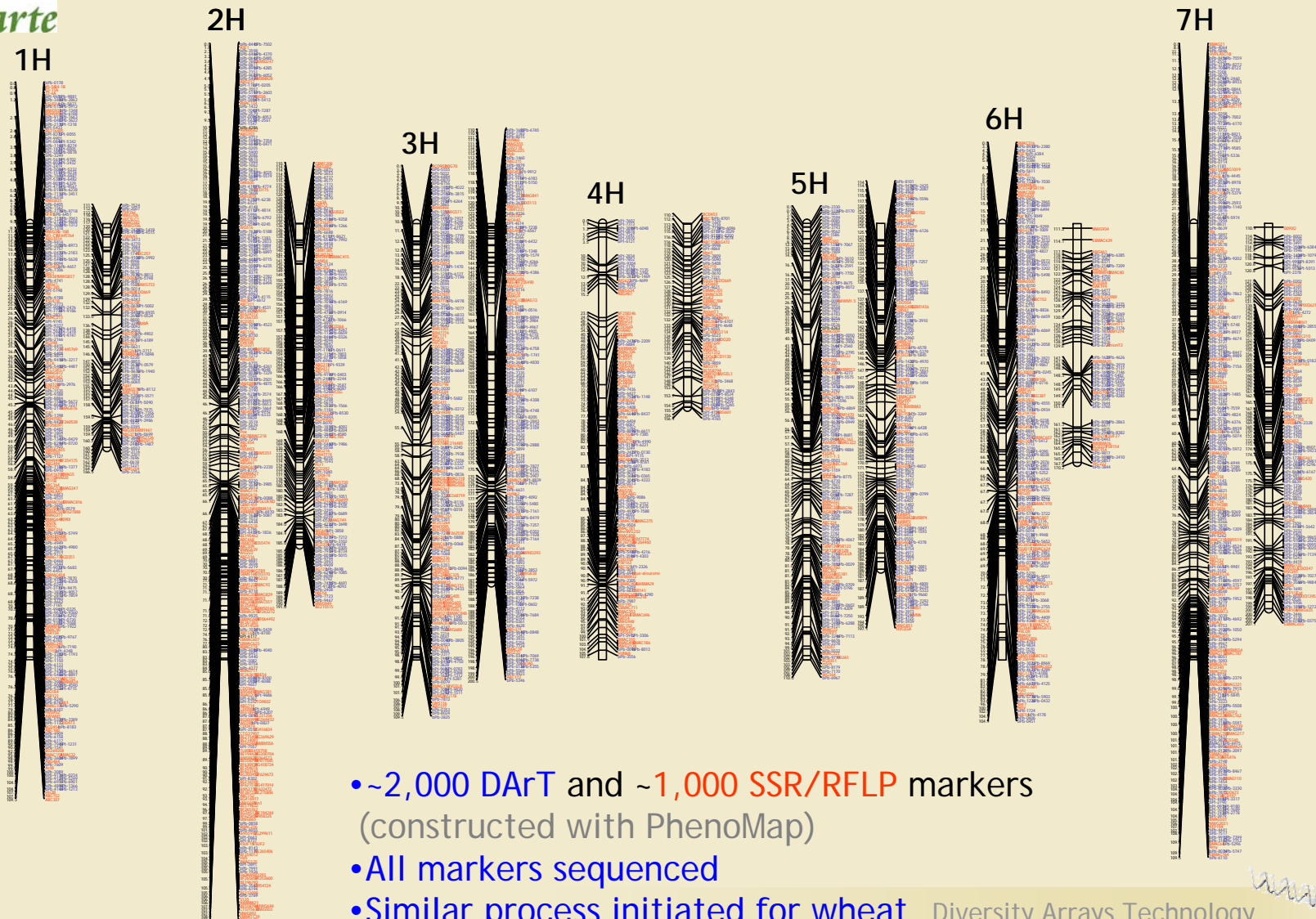


Marker reproducibility test - wheat

- 13 wheat cultivars analysed with triplicate arrays in three experiments
 - 9 arrays per genotype
 - 117 assays per DArT marker
 - 2 different fluorescent dyes
- Over 1000 markers discovered
- Marker quality evaluated by a number of statistics
 - P (percentage of bimodality)
 - Call rate (data completeness)
 - Discordance (reproducibility of scores)



Barley integrated map: 10 maps draft

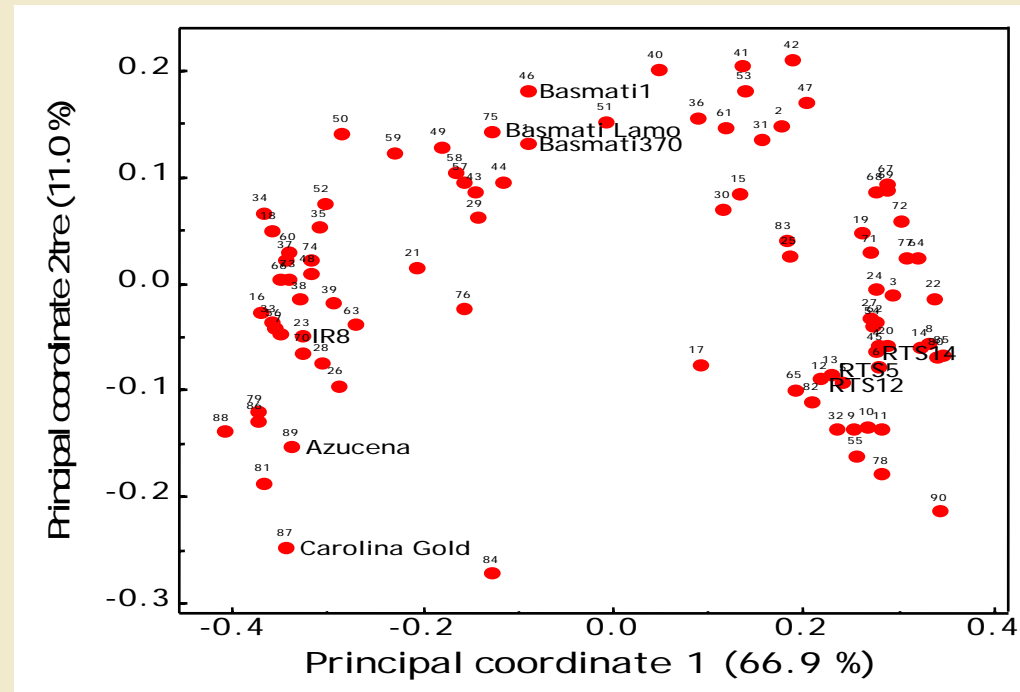


- ~2,000 DArT and ~1,000 SSR/RFLP markers (constructed with PhenoMap)
- All markers sequenced
- Similar process initiated for wheat

Rice: DArT PL

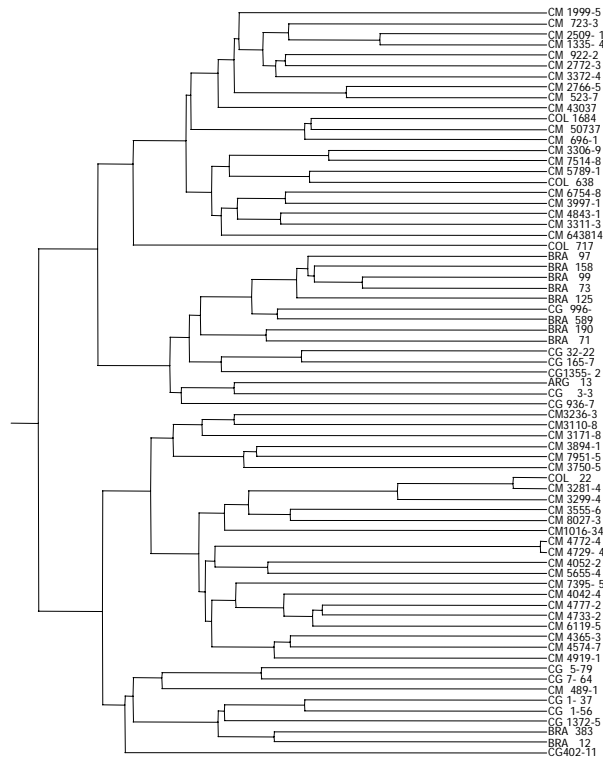
- Rice array developed by DArT PL with >6000 clones
- MITE-DArT complexity reduction method
- 519 markers discovered with the GCP materials selected by CIRAD scientists
- 3 day experiment to generate data
- Data available for comparison with SSR

PCO for 90 lines x 519 markers



Cassava: Prapit Wongtiem (Thailand)

Cassava: Diversity analysis (620 DArT markers)



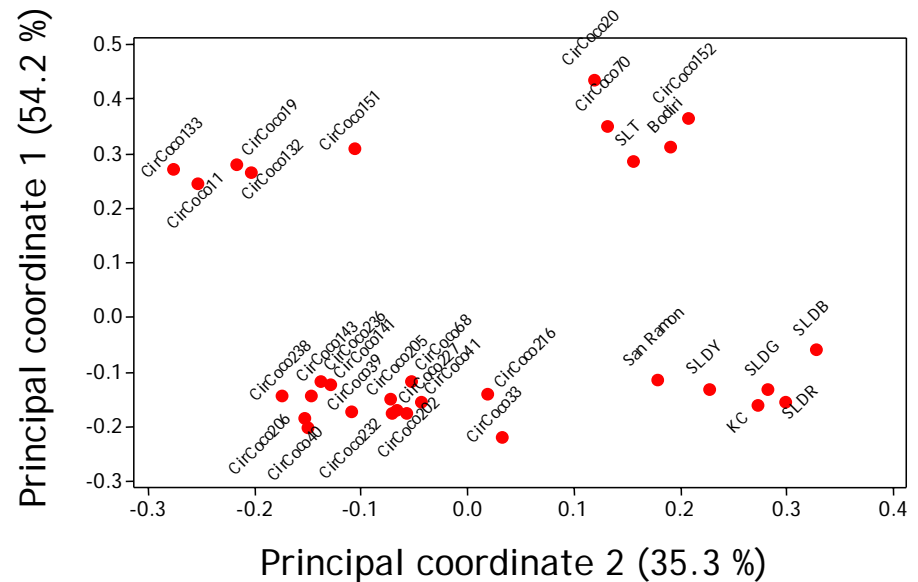
(UPGMA clustering)

- 3 months visit (second visit to DArT PL)
- Rearranged markers polymorphic identified in the previous visit
- Created new library with 5,376 new clones from materials selected by CIAT team
- Genotyped 120 cultivated lines varying in dry matter content + 20 wild accessions
- Average number of markers per line >800
- New complexity reduction method in preparation

Coconut: Chandrika Perera (Sri Lanka)

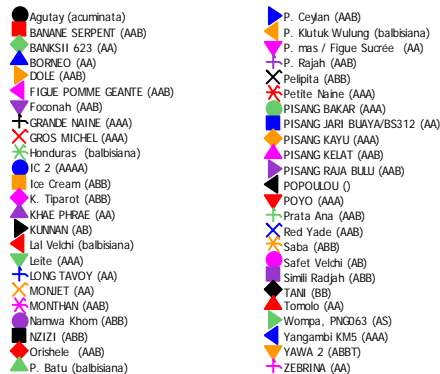
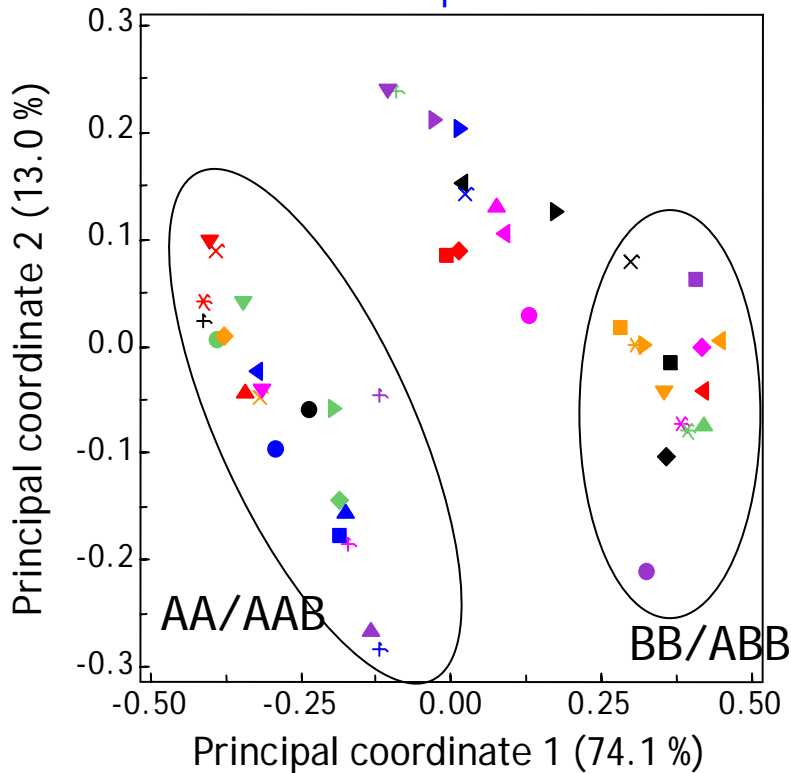
- Trainee visiting for 2 months
- 128 markers discovered in the initial experiment (3000 clones, 31 genotypes)
- First array (PstI/BstNI) fully developed (>6000 clones)
- > 350 markers discovered among 120 coconut lines from CIRAD and Sri Lanka

Coconut: PCO analysis for 31 lines × 128 markers



Banana: Ange-Marie Risterucci (CIRAD)

332 PstI/TaqI markers



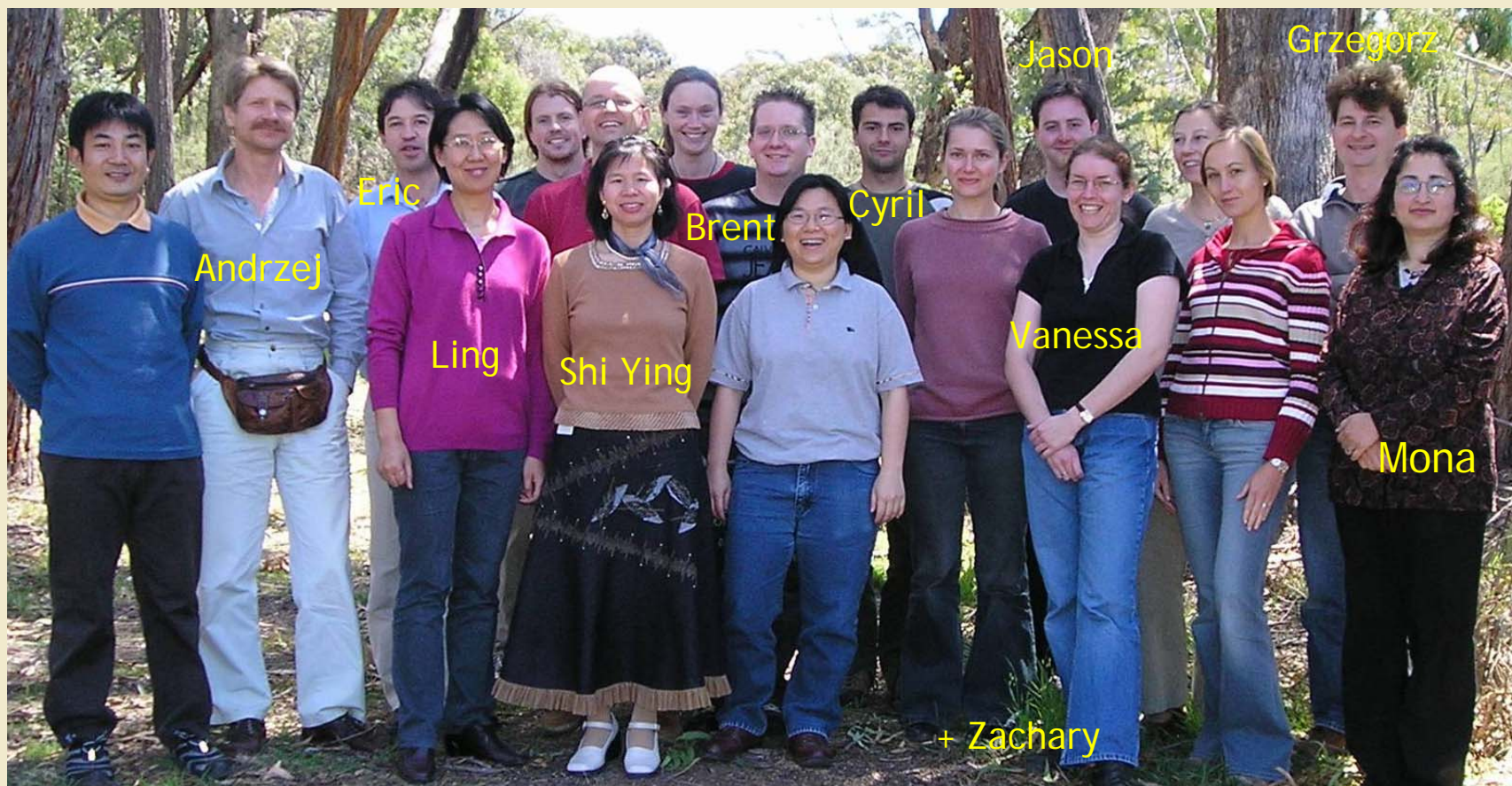
- Arival of the trainee: late August
- Banana DNA samples selected by CIRAD scientists
- 48 samples representing wide diversity for array construction
- 4 complexity reduction methods tested
- Initial results available for PstI/TaqI and PstI/MseI representations
- PCO plot groups samples with similar genome compositions
- >1000 markers available in 2 months

	# of markers (HQ)	P Marker Quality	% of polymorphic markers	Call Rate	PIC Average
<i>PstI / MseI</i>	279 (123)	83.2	18.2	90.7	0.43
<i>PstI / TaqI</i>	332 (172)	83.7	21.3	91.9	0.43

Summary

- ~100,000 data points generated using sorghum and rice arrays developed by DArT PL (1 week, \$7K)
- Wheat and barley genotyping service fully operational with growing interest among breeders
- 7 DArT arrays developed by GCP trainees to DArT PL within approximately 2 months
- Most of the time spent on training (primarily IT)
- ~200,000 data points generated by the trainees
- >350 new markers identified for coconut and cassava and >700 markers for banana
- Project on track to deliver contracted outcomes in substantially shorter timeframe

Triticarte / DArT team



www.Triticarte.com / www.DiversityArrays.com

Diversity Arrays Technology

