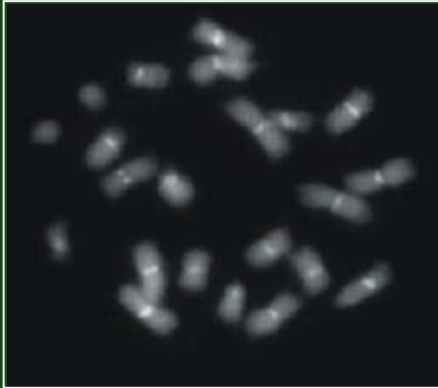


# Unlocking the genetic diversity in groundnut's wild relatives



Diploid species with  
"AA" genome

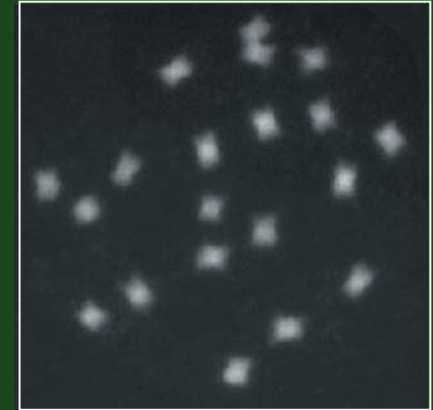


$$n = 10 \times n = 10$$



Sterile AB hybrid  $2n = 20$

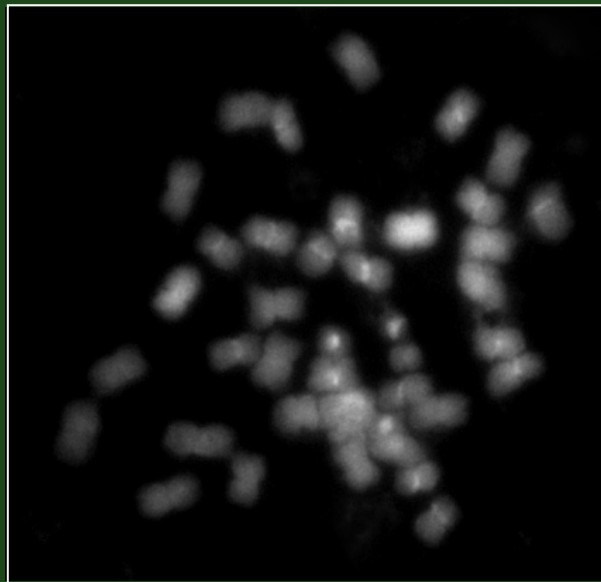
Diploid species with  
"BB" genome



Genetic bottle-neck !



Spontaneous duplication



*Arachis hypogaea*

**AABB**

# Project Rationale

*A. hypogaea*

Few alleles

Tetraploid

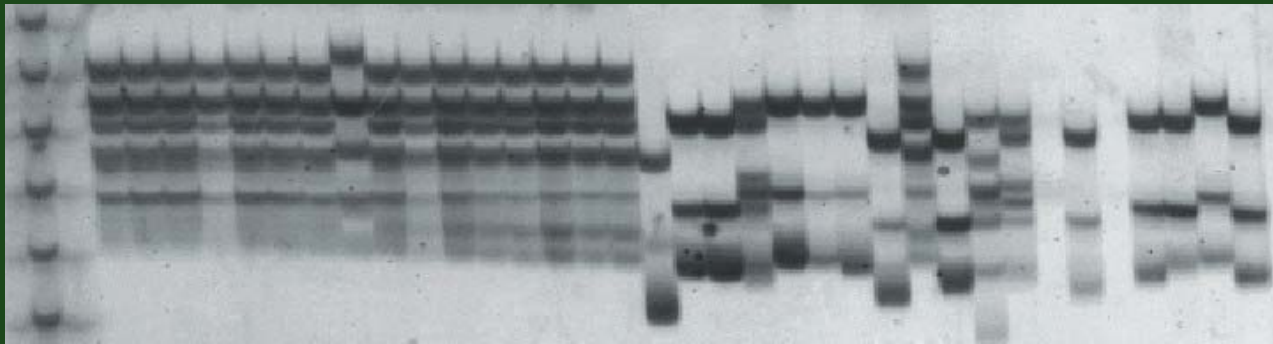


Wild alleles bring many new traits  
and provide polymorphism for genetics

Wild species:

Many alleles

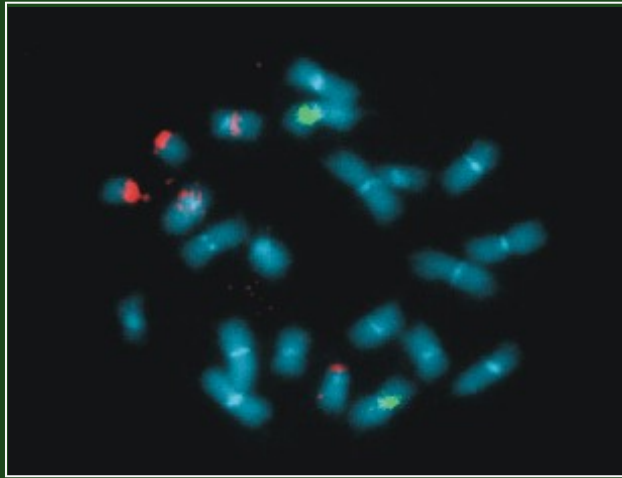
Diploid



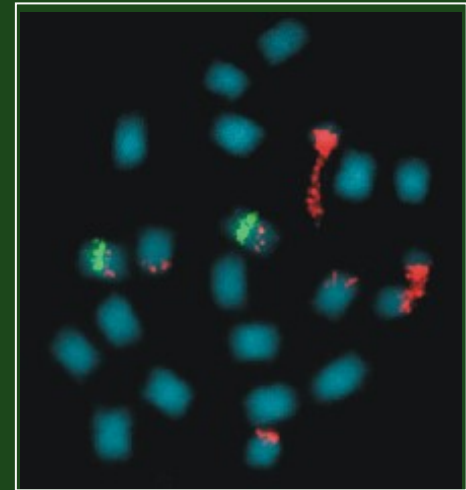
## Project Rationale

Resynthesis is a way to introduce variability to cultivated peanut

"A" genome  
*A. duranensis*



"B" genome  
*A. ipaensis*



×



Sterile **AB** F1 hybrid

colchicine



**AABB** Tetraploid (Synthetic amphidiplo)

crossing/backcrossing with *A. hypogaea*



# Project Rationale

## At Project start

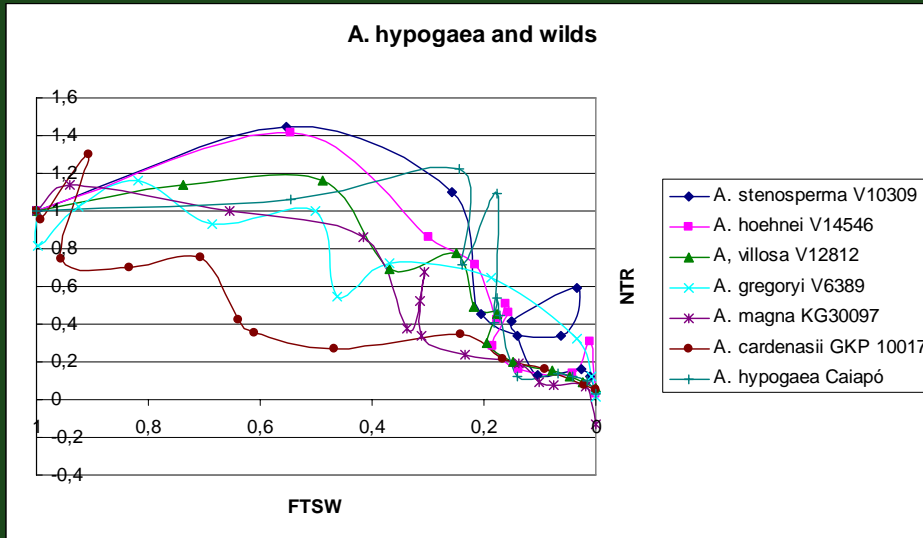
- Only two RFLP maps
- 6 SSR markers published
- Only one case of wild introgression - Charles Simpson

## Project aimed for

- Characterization of wild genomes and traits
- Genetic maps and Genomic resources for Molecular breeding
- Introduction of wild genes into breeding programs

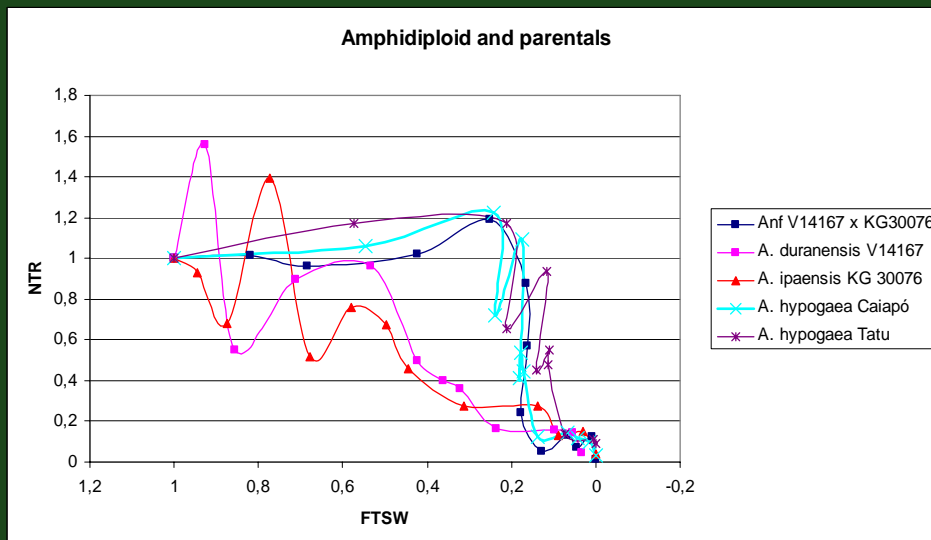
# Activities and Results

## Wild, synthetic and cultivated drought responses better characterized



Range of transpiration responses

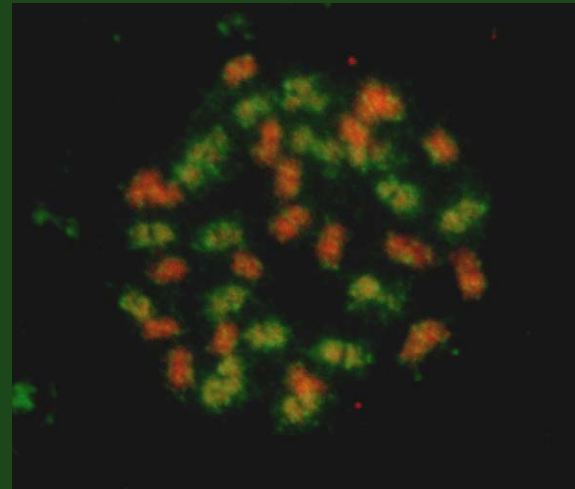
Very high - wilds  
Surprisingly high - cultivated



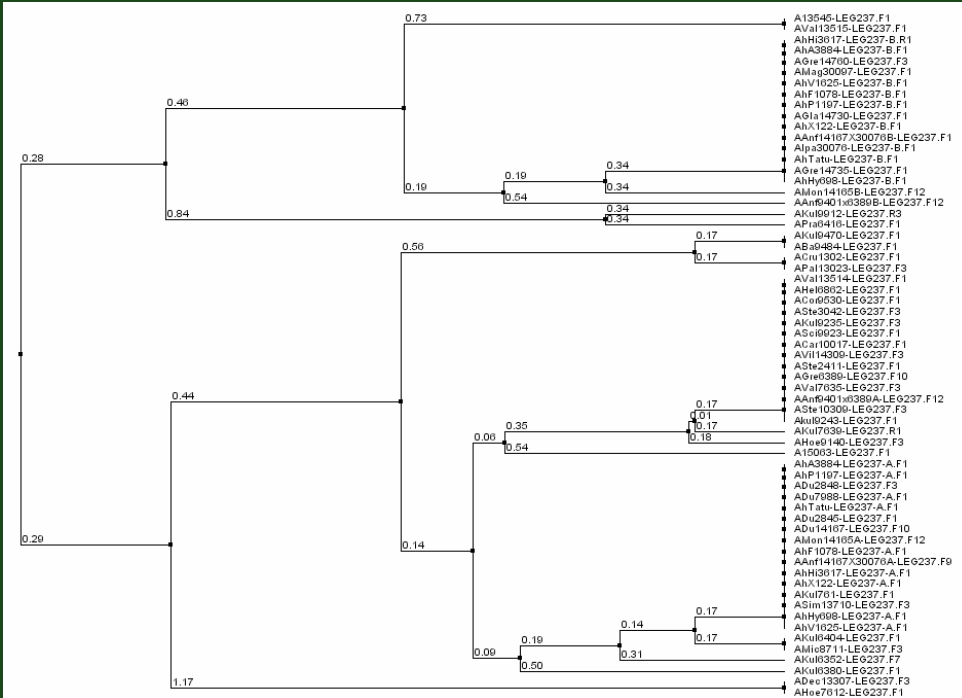
Sythetic amphiploids  
are completely different from  
parental species

- large effect of ploidy

# Activities and Results Characterization of wild genomes to guide resynthesis



## Cytogenetics - FISH and GISH



Wild genomes better characterised

eg.  
New potential BB donors  
*A. williamsii*  
*A. gregoryi*  
*A. valida*

New species being used for amphidiploid production

Single copy gene intron phylogenies

## To make maps - we needed markers:

- **Microsatellites - SSRs**

Co-dominant, transferable between populations

Useful for plant breeders

Software and more than 350 SSRs developed ←

- **Anchor markers:**

For comparison of genomes - legume synteny

Software - 450 universal legume anchor primer pairs ←

- **Candidate genes:**

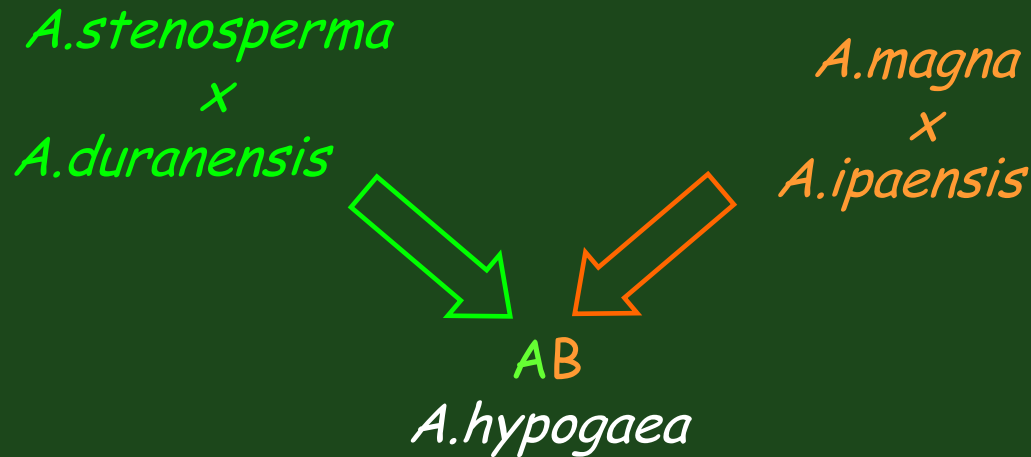
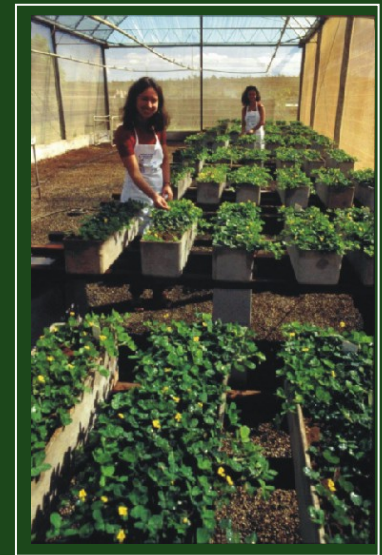
More likely to be linked to traits of interest.

markers for RGAs and drought response genes ←

# Activities and Results

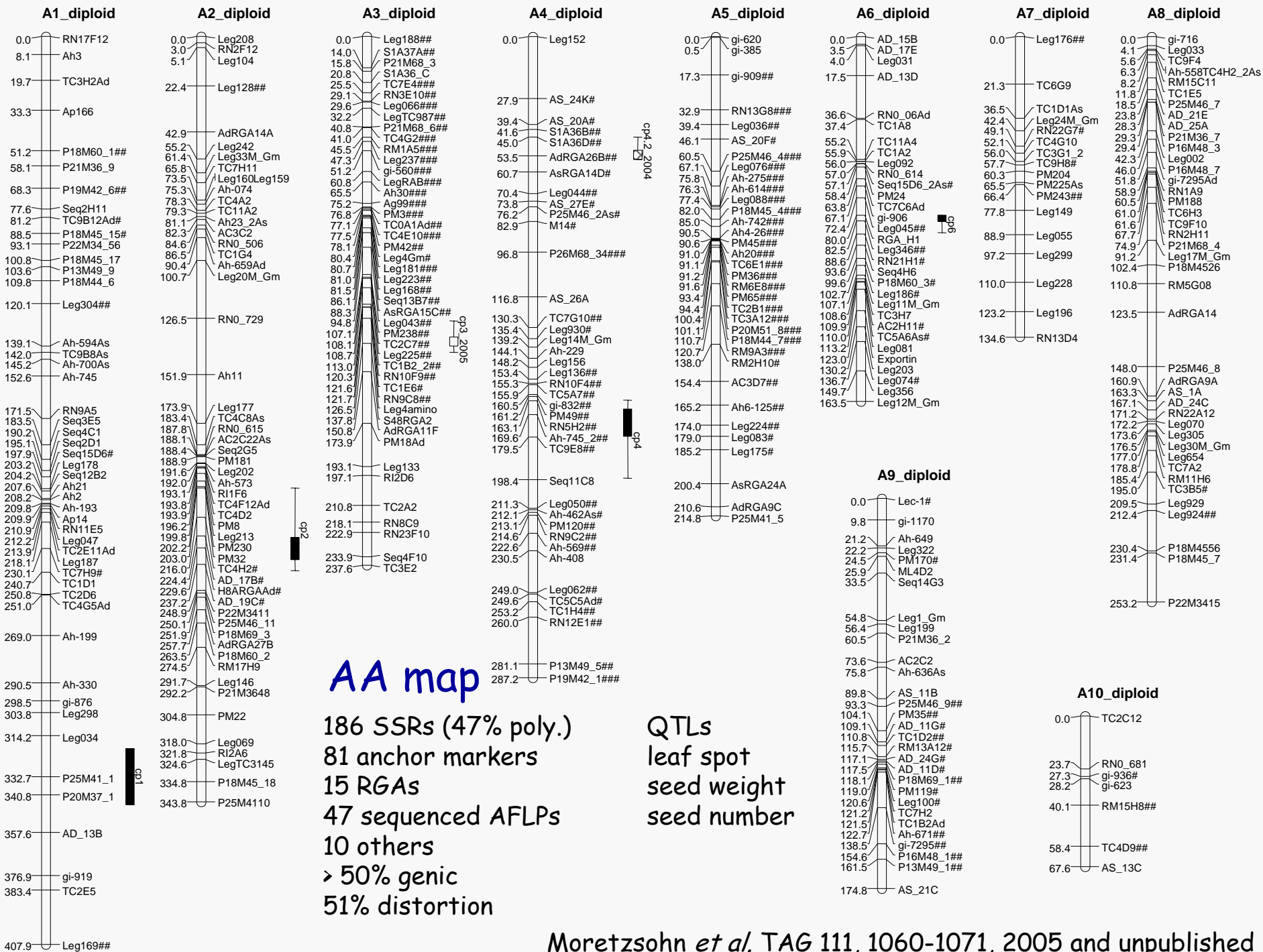
## To make maps - we needed populations

- With wild polymorphism
- To dissect the tetraploid problem
- 2 diploid and one tetraploid population



x  
(*A.duranensis* x *A.ipaensis*)<sup>c</sup>

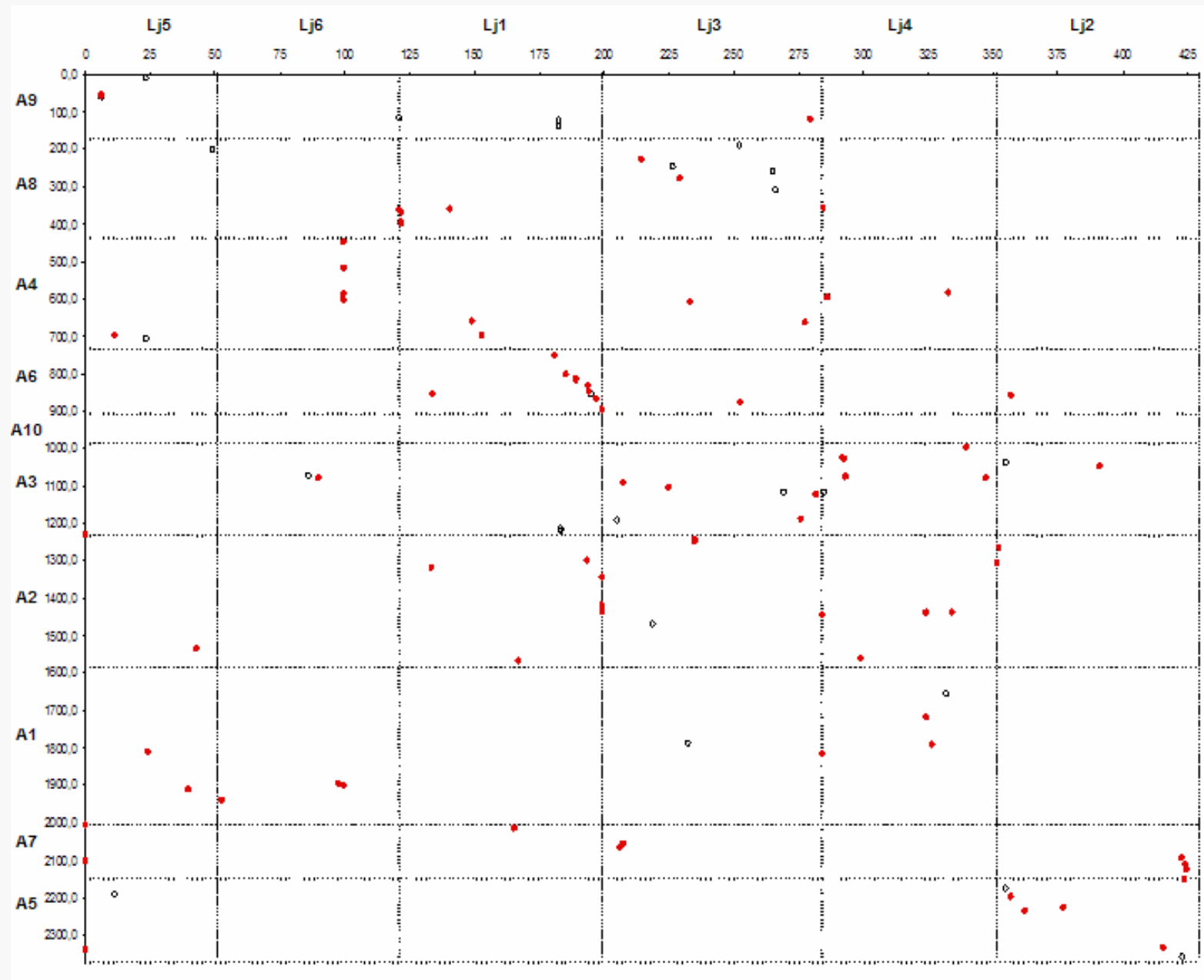
*A. hypogaea*  
x  
*A. hypogaea*





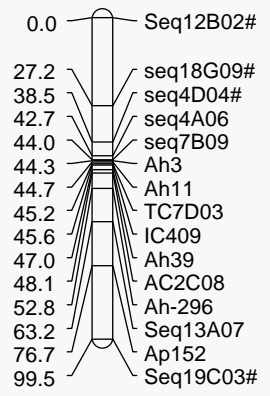
# Activities and Results

# Arachis AA vs Lotus

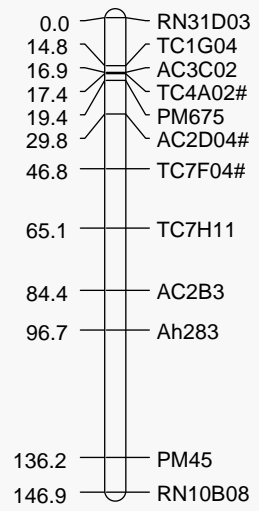


# Activities and Results

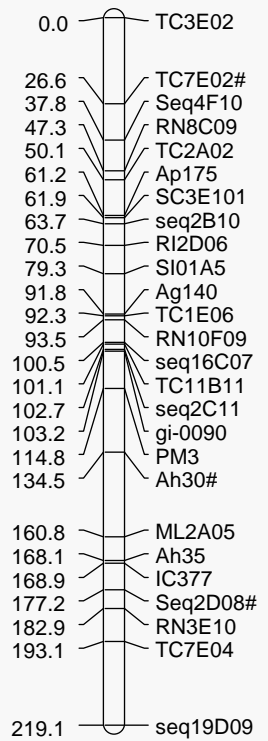
**B1\_diploid**



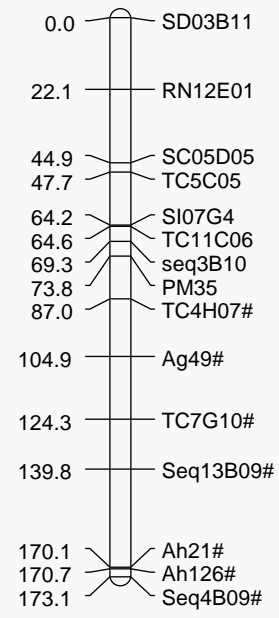
**B2\_diploid**



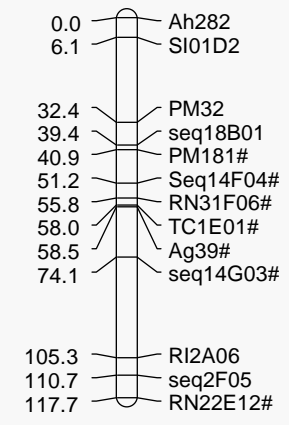
**B3\_diploid**



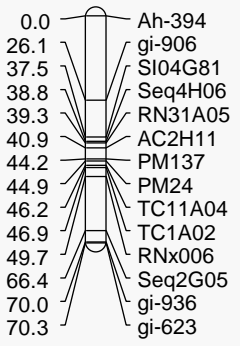
**B4\_diploid**



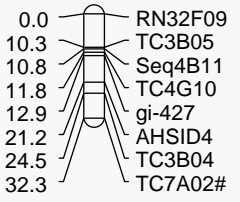
**B5\_diploid**



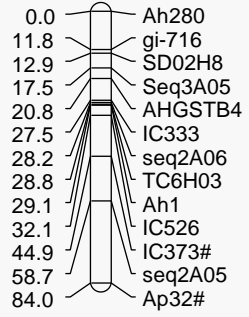
**B6\_diploid**



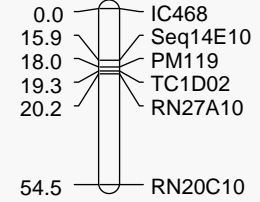
**B7\_diploid**



**B8\_diploid**



**B9\_diploid**



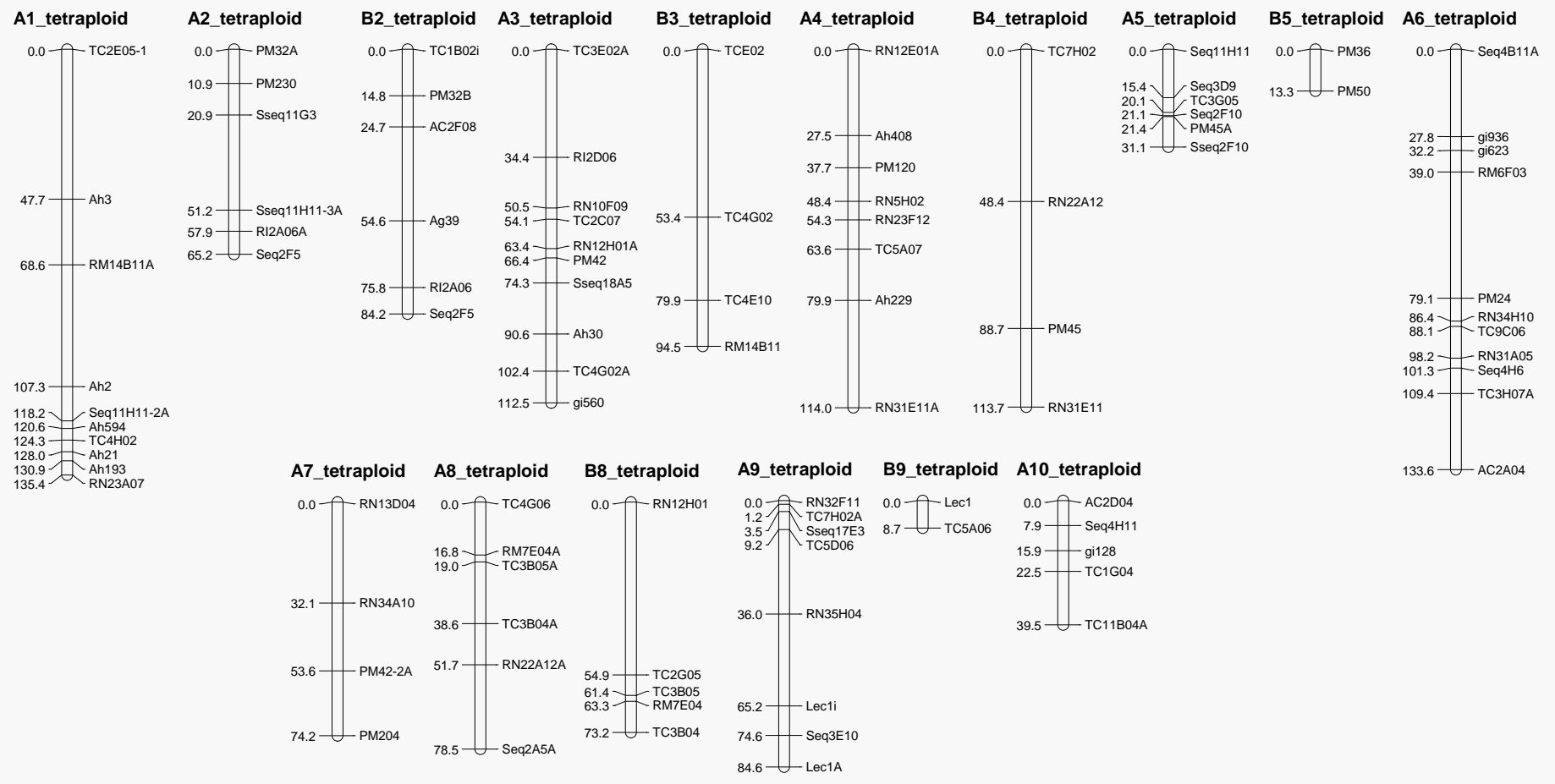
**B10\_diploid**



**BB map**

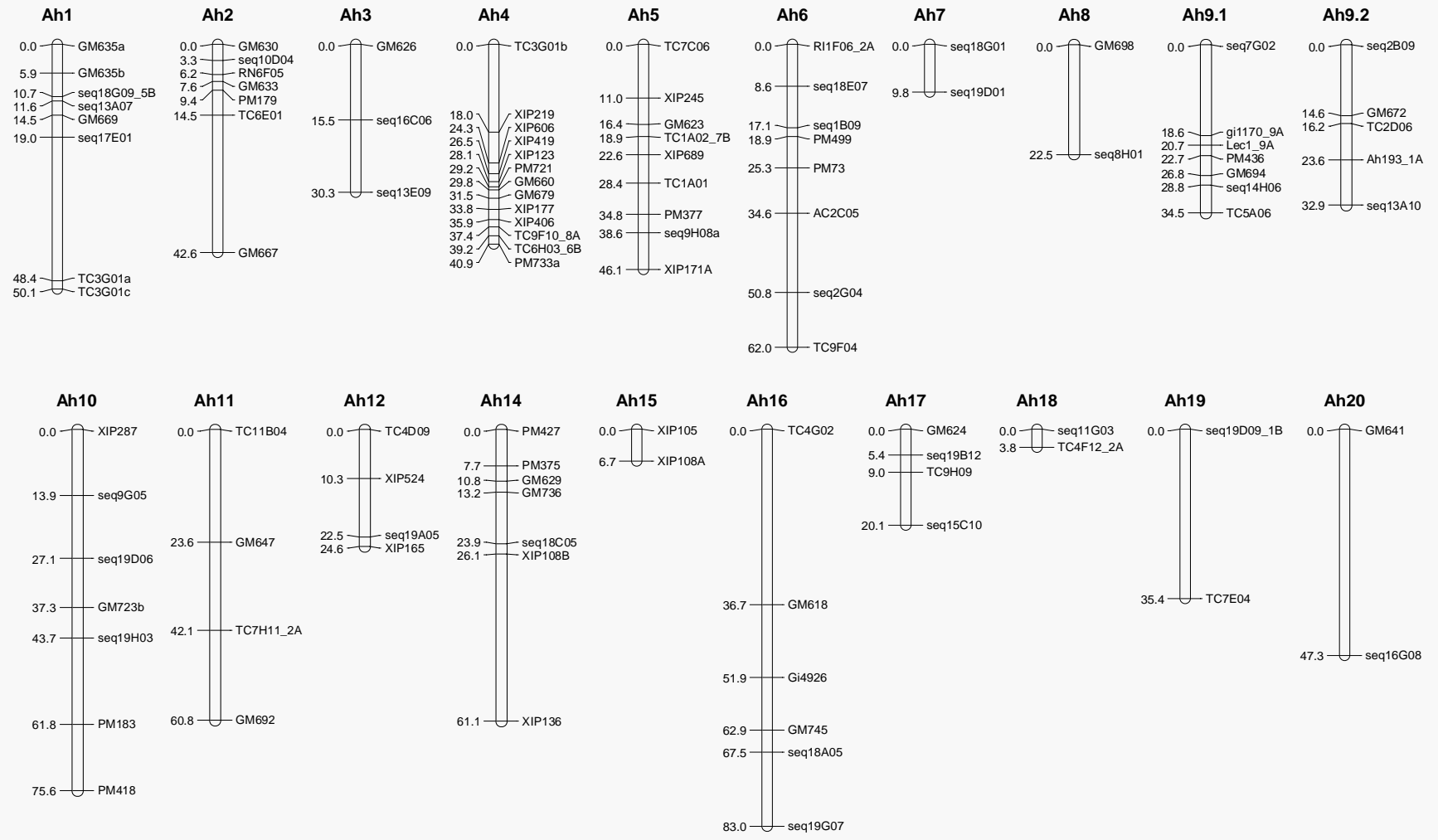
**130 SSRs (24% poly.)**  
**LOD > 4**  
**Distortion 23.8%**  
**1234.6 cM**

# Activities and Results



Synthetic x Cultivated

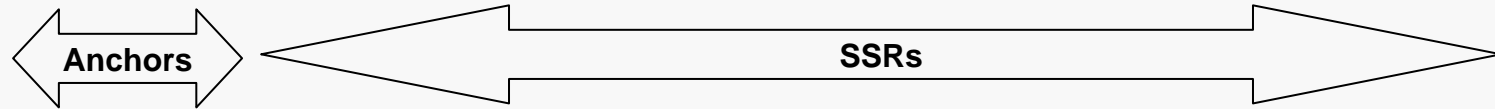
# Activities and Results



Cultivated x Cultivated

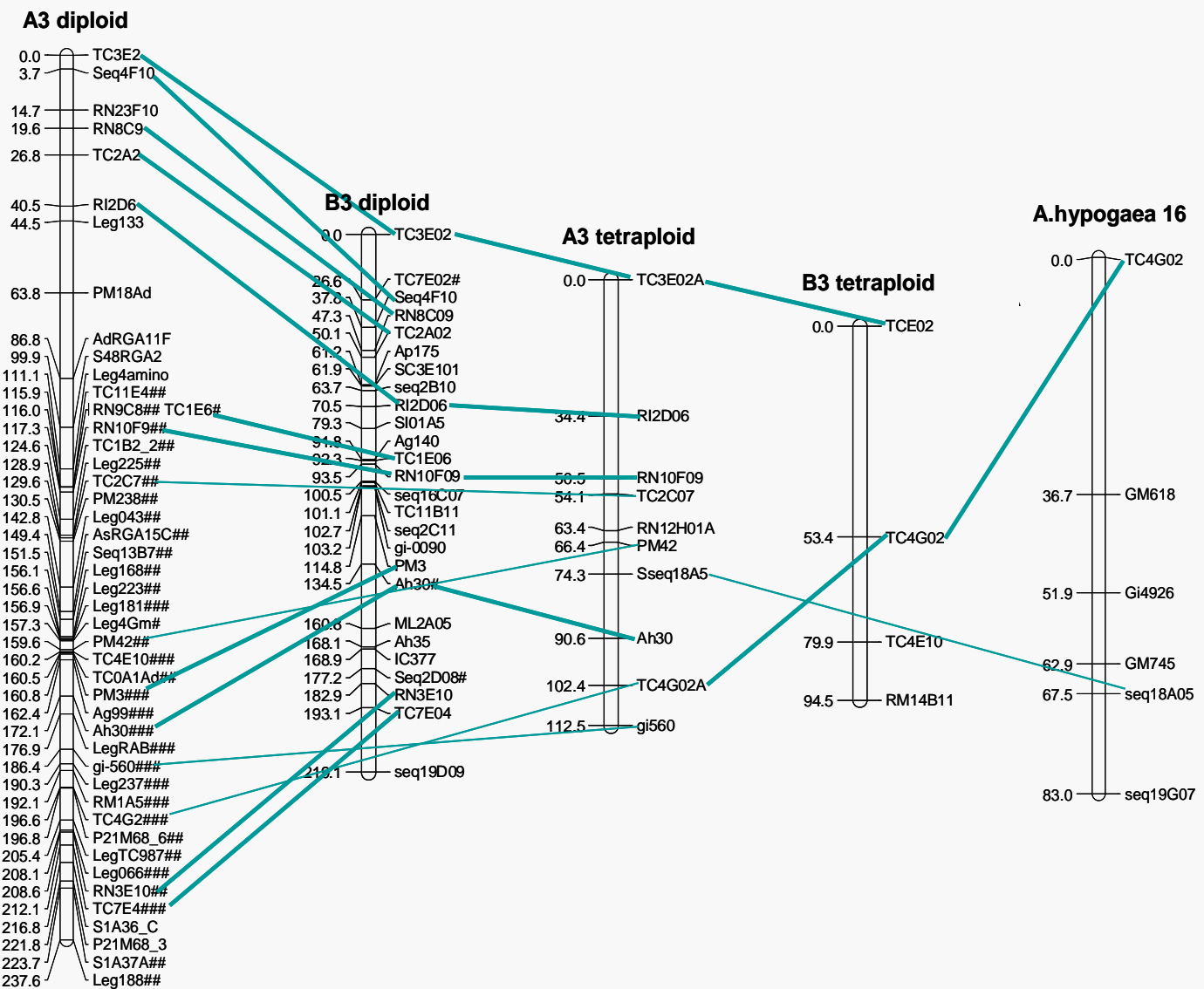
# Activities and Results

Models → Reference diploids → Cultivated



**Mt Lj**

- 
- 
- 
- 
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- 
- 
- 
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- 
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- 



# Activities and Results

## New Genomic Resources

6,264 ESTs

*A. stenosperma*

x

*A. duranensis*

BAC (*Hind*III)  
c. 80,000 clones  
c. 6.5x

*A. magna*

x

*A. ipaensis*

BAC (*Hind*III)  
c. 80,000 clones  
c. 6.5x

AB

*A. hypogaea*

x

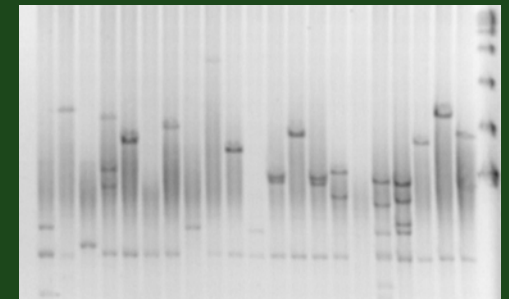
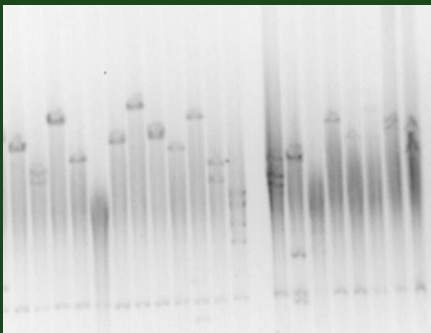
(*A. duranensis* x *A. ipaensis*)<sup>c</sup>

*A. hypogaea*

x

*A. hypogaea*

subtractive library TAG24 and ICGV86031  
water stressed -FTSW 0.11  
500 non-redundant candidate sequences



Now's the time to move to the field !



# Activities and Results

## Incorporation of amphiploids to breeding programs

*(A. ipaensis x A. duranensis)*<sup>c</sup> - EMBRAPA

*(A. gregoryii x A. linearifolia)*<sup>c</sup> - EMBRAPA

*[(A. cardenasii x A. diogoi) x A. batizocoi]*<sup>c</sup> - Prof. C. Simpson



# Activities and Results

## Incorporation of amphiploids to breeding programs

Female parent	Male parent	Generation	Number of seed
Fleur11	AixAd	BC1	336
55-437	AixAd	BC1	333
GC8-35	AixAd	BC1	383
GC8-35	TxAG6	F1	65
73-30	TxAG6	F1	24
IAC-Runner	AixAd	BC1	150
Caiapo	AixAd	BC1	120

➔ ILs



# Data Format and Release

ESTs from a wild *Arachis* species for gene discovery and marker development 2007 BMC Plant Biology, 7:7 doi:10.1186/1471-2229-7-7 \*Open Access Journal\*

All marker information at:



<http://www.biomedcentral.com/content/supplementary/1471-2229-7-7-S1.xls>

EST sequences have been submitted to Genbank. (numbers EH041934- EH048197)



A general pipeline for the development of anchor markers for comparative genomics in plants 2007 BMC Genomics 2006, 7:207 \*Open Access Journal\*

Table of anchor markers available at:



<http://cgi-www.daimi.au.dk/cgi-chili/GeneticMarkers/table>

GeMprospector - Online Design of Cross-Species Genetic Marker Candidates in Legumes and Grasses. Nucleic Acids Research 34

(Web Server issue): W670-W675 \*Open Access Journal\*

The web program GeMprospector available at:



<http://cgi-www.daimi.au.dk/cgi-chili/GeMprospector/main>

Microsatellite based, gene-rich linkage map for the AA genome of *Arachis* (Fabaceae). 2005 Theoretical and Applied Genetics. 111:1060-1071.

Genetic map info in paper.

All marker information at:



<http://dx.doi.org/10.1007/s00122-005-0028-x>

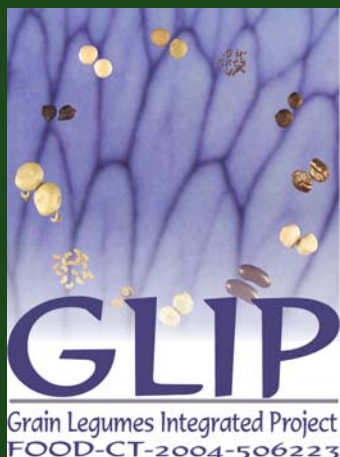
New softwares for automated microsatellite marker development. 2006 Nucleic Acids Research, 34 (Web Server issue): E31. \*Open Access Journal\*

Software available at:



<http://finder.sourceforge.net/>

## Links with other projects



### **GCP - TLI**

**Improving tropical legume productivity  
for marginal environments  
in sub-Saharan Africa**

### **GCP – Capacity building Grant**

**Application of molecular tools for  
controlled wild introgression into Peanut  
cultivated germplasm in Senegal**

### ICRISAT

India

Vincent Vadez  
Rajeev Varshney  
Aruna Rupakula  
David Hoisington

### UCB, Brazil

David Bertioli  
Ana Carolina José  
Dione Mendes  
Rinaldo Perreira

### UCG, Brazil

Wellington Martins

### University of Aarhus, Denmark

Jens Stougaard  
Leif Schauser  
Birgit Hougaard  
Anna Marie Nielsen  
Jakob Fredslund  
Lene Madsen  
Niels Sandal

### Kazusa DNA Research Institute

Satoshi Tabata

### EMBRAPA, Brazil

Soraya Bertioli  
Marcio Moretzsohn  
Patricia Messenberg  
José Valls  
Karina Proite  
Stephan Nielen  
Ana Cristina Brasileira  
Simone Ribeiro  
Marcos Gimenes  
Alessandra Favero  
Fernando Campos

### CIRAD

France

Angelique d'Hont  
Olivier Garsmeur  
Jean-Francois Rami

### IBONE, Argentina

Guillermo Seijo

### CERAAS/ISRA- Senegal

Ousmane Ndoye  
Issa Faye



# Summary

