Conserving and mining plant genetic resources using knowledge of the genome

Theo van Hintum & Rob van Treuren

Centre for Genetic Resources, The Netherlands (CGN)

WAGENINGEN UR

For quality of life

Conserving and mining PGR

- introduction
  - PGR management
  - new approaches and tools
- molecular marker applications in PGR management
  - detailed case study (apple)
  - brief snapshots of selected CGN research
- Generation Challenge Programme
  - genomic approaches to mining PGR
- conclusions

Conserving and mining PGR

- plant genetic resources (PGR) conservation evolved from classical taxonomy: qualitative approach
  - number of accessions rather than diversity captured
  - ‘having it’ more important than ‘knowing it’

- State of the World (FAO 1996)
  - 6.1 million accessions in 1308 collections
  - major crops

wheat	774 500 accessions
barley	485 000 accessions
rice	408 500 accessions
maize	277 000 accessions

Conserving and mining PGR

- current trend *ex situ* PGR management
  - from qualitative
    - collecting, documenting, evaluating individual accessions
  - to quantitative
    - optimising genebank management strategies by approaching the collection as a whole
    - screening large parts of the collections with genomics based techniques

Conserving and mining PGR

- new approaches
  - improving genebank efficiency and quality
    - genebank economy
    - genebank quality management
    - accession management
  - stimulating use
    - web interfaces
    - search strategies
    - identifying new alleles

Conserving and mining PGR

- new tools
  - molecular genetics
    - marker technology
    - allele mining techniques
  - information and communication technology
    - Internet
    - bioinformatics

Conserving and mining PGR

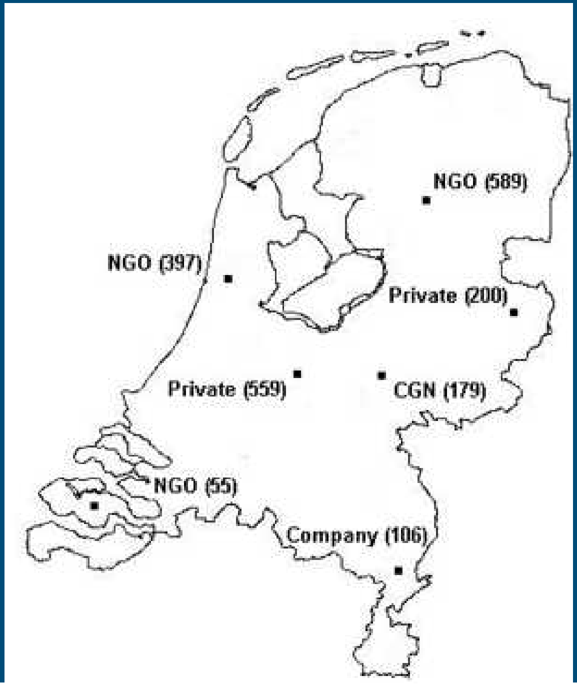
- application molecular markers in PGR management
  - acquisition
    - improving sampling strategies
    - identifying gaps and improving composition
  - maintenance
    - measuring and reducing genetic drift/shift
    - observing contamination
  - characterization
    - fingerprinting / diversity studies
  - utilization
    - creation of core collections
    - allele mining

Conserving and mining PGR

- examples use molecular markers at CGN
  - support Dutch apple NGO’s
    - detailed description
  - various other examples CGN studies using molecular markers to improve PGR management
    - lettuce
    - potato
    - white cabbage
    - barley
    - ryegrass
    - flax
    - Dutch grasslands
    - enset

Conserving and mining PGR

- molecular study of Dutch apple collections
  - total ~2000 accessions



Conserving and mining PGR

- molecular study of Dutch apple collections
  - interest CGN
    - to improve knowledge of diversity in genetic resources of apple in the Netherlands
    - to determine the degree of representation of CGNs collection
    - to improve efficiency of conservation by supporting actors

Conserving and mining PGR

- molecular study of Dutch apple collections
  - study material for microsatellite analysis
    - entire collection of CGN
    - selection from 8 other collections
      - proper documentation
      - Dutch origin
      - foreign material that has been important for cultivation in the Netherlands
    - total sample size: ~700 trees

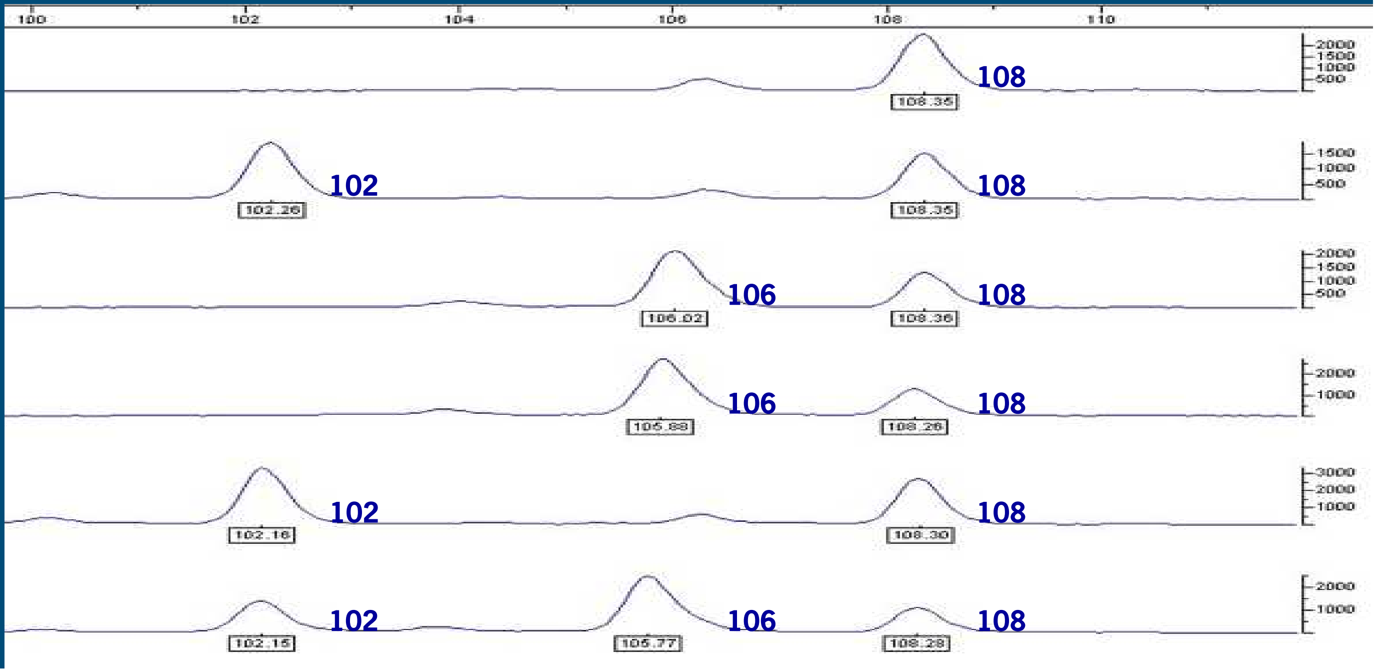
Conserving and mining PGR

- molecular study of Dutch apple collections
  - selection of apple microsatellite markers
    - literature: 150-200 microsatellites
    - selection criteria
      - technical qualities
      - degree of variability
      - genome coverage
      - proximity to important genes
      - linkage of data to other studies
    - 16 markers selected
      - 11 of the 17 chromosomes covered
      - traits: scab resistance, canker resistance, aphid resistance, firmness, malic acid, allergy genes

Photo [illegible] A. L. Jones and T. B. Sutton

Conserving and mining PGR

- molecular study of Dutch apple collections
  - identification of microsatellite variation



Conserving and mining PGR

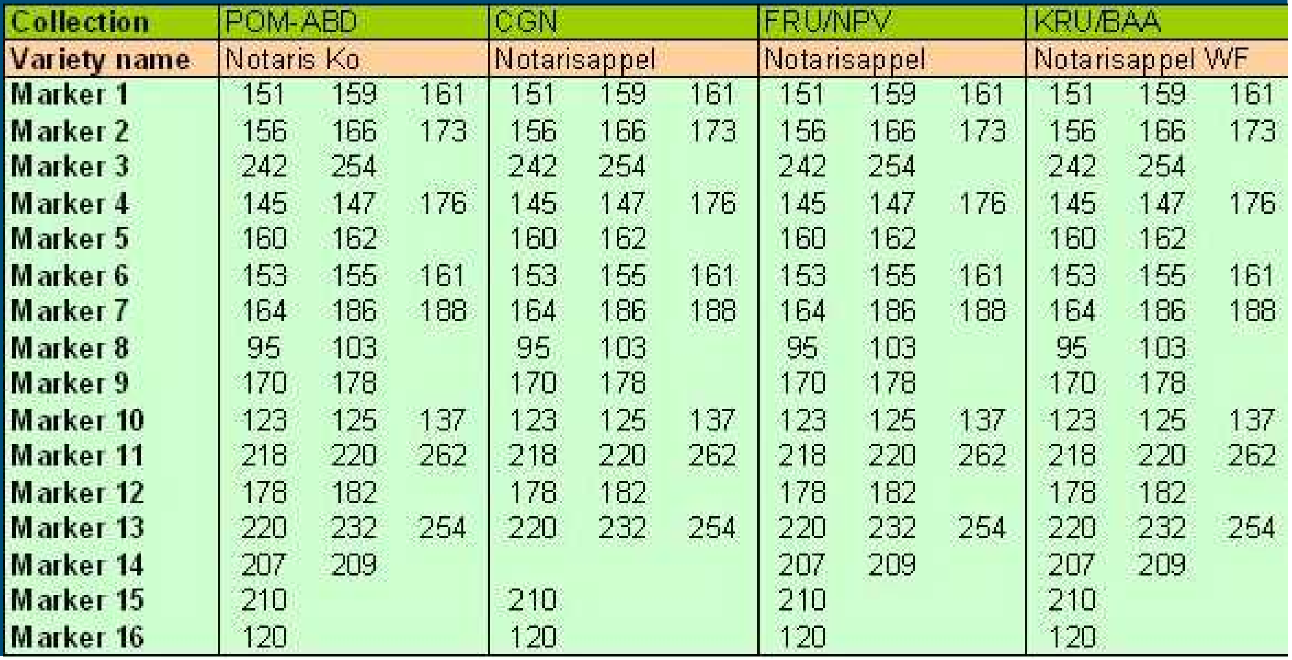
- molecular study of Dutch apple collections
  - resolution of the microsatellite markers
    - observed number of alleles
      - 3 - 37 alleles per microsatellite
      - 296 alleles for the total set of 16 microsatellites
      - 18.5 alleles on average per microsatellite
  - probability to find identical genotypes by chance
    - depending on frequencies of the alleles in total sample
    - estimation: $6.4 \times 10^{-20} = 0.0000000000000000000064$

Conserving and mining PGR

- molecular study of Dutch apple collections
  - potential duplicates
    - duplication groups based on passport data
      - number of groups: 138
      - total number of trees: 355
    - verification with microsatellite data
      - duplicates confirmed: 147 (41%)
      - duplicates rejected: 202 (57%)
      - to be determined: 6
    - majority of potential duplicates are different despite similarity in accession name

Conserving and mining PGR

- molecular study of Dutch apple collections
  - example of confirmed duplicates



Collection	POM-ABD			CGN			FRU/NPV			KRU/BAA		
Variety name	Notaris Ko			Notarisappel			Notarisappel			Notarisappel WF		
Marker 1	151	159	161	151	159	161	151	159	161	151	159	161
Marker 2	156	166	173	156	166	173	156	166	173	156	166	173
Marker 3	242	254		242	254		242	254		242	254	
Marker 4	145	147	176	145	147	176	145	147	176	145	147	176
Marker 5	160	162		160	162		160	162		160	162	
Marker 6	153	155	161	153	155	161	153	155	161	153	155	161
Marker 7	164	186	188	164	186	188	164	186	188	164	186	188
Marker 8	95	103		95	103		95	103		95	103	
Marker 9	170	178		170	178		170	178		170	178	
Marker 10	123	125	137	123	125	137	123	125	137	123	125	137
Marker 11	218	220	262	218	220	262	218	220	262	218	220	262
Marker 12	178	182		178	182		178	182		178	182	
Marker 13	220	232	254	220	232	254	220	232	254	220	232	254
Marker 14	207	209					207	209		207	209	
Marker 15	210			210			210			210		
Marker 16	120			120			120			120		

Conserving and mining PGR

- molecular study of Dutch apple collections
  - potential duplicates based on marker data
    - total sample: 695 trees
      - duplicate groups: 119
      - number of trees involved: 339
      - redundancy: 220 (32%)
      - observed genotypes: 475 (68%)
    - unexpected duplicates
      - 66% of the observed groups
    - majority of identical microsatellite genotypes are not in line with the passport data

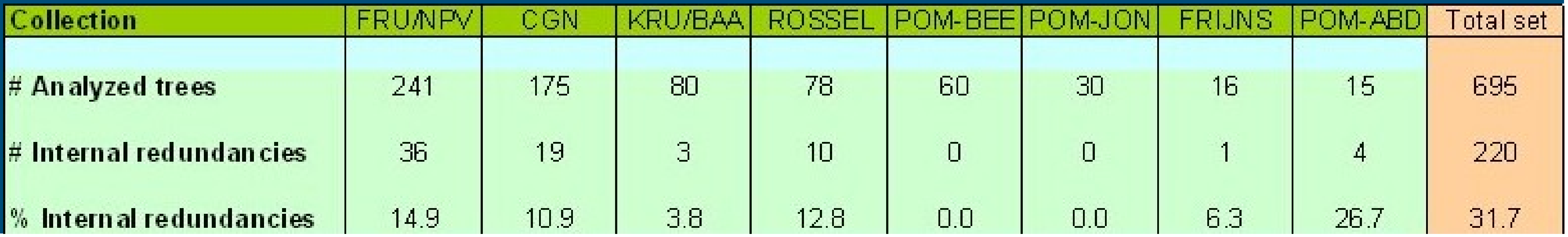
Conserving and mining PGR

- molecular study of Dutch apple collections
  - example unexpected duplication group

CGN	Reinette Rouge Etoilée
ROSSEL	Sterappel
POM-JON	Paradijszoet
KRU/BAA	Dubbele ster
FRU/NPV	Duchesse's Favourite

Conserving and mining PGR

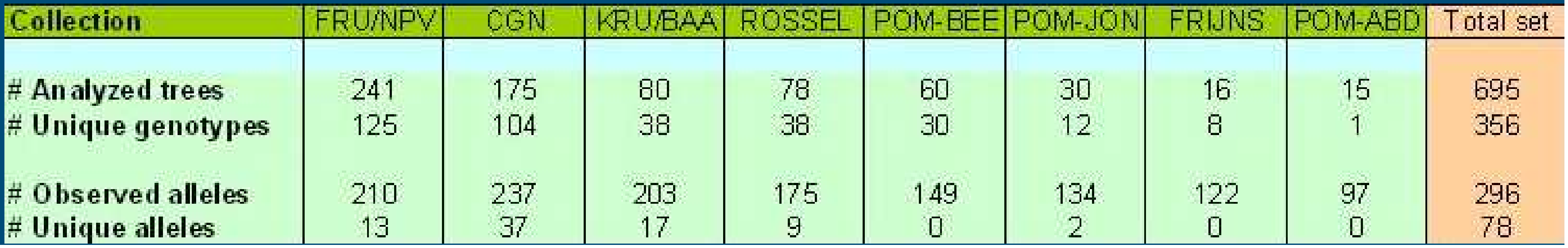
- molecular study of Dutch apple collections
  - potential redundancy per collection
    - potential redundancies (220)
      - within collections 73 (33%)
      - between collections 147 (67%)
    - majority of redundancies are between collections



Collection	FRU/NPV	CGN	KRU/BAA	ROSSEL	POM-BEE	POM-JON	FRIJNS	POM-ABD	Total set
# Analyzed trees	241	175	80	78	60	30	16	15	695
# Internal redundancies	36	19	3	10	0	0	1	4	220
% Internal redundancies	14.9	10.9	3.8	12.8	0.0	0.0	6.3	26.7	31.7

Conserving and mining PGR

- molecular study of Dutch apple collections
  - unique diversity within collections
    - each collection consists for about half of unique genotypes
    - majority of the alleles occurs in multiple collections



Collection	FRU/NPV	CGN	KRU/BAA	ROSSEL	POM-BEE	POM-JON	FRIJNS	POM-ABD	Total set
# Analyzed trees	241	175	80	78	60	30	16	15	695
# Unique genotypes	125	104	38	38	30	12	8	1	356
# Observed alleles	210	237	203	175	149	134	122	97	296
# Unique alleles	13	37	17	9	0	2	0	0	78

Conserving and mining PGR

- molecular study of Dutch apple collections
  - management implications apple study
    - improvement of documentation
    - improvement of variety determination
    - identification of redundancies and removal of internal duplicates
    - identification of unique material (guidelines for safety duplication)
    - formation of a network of co-operating collections

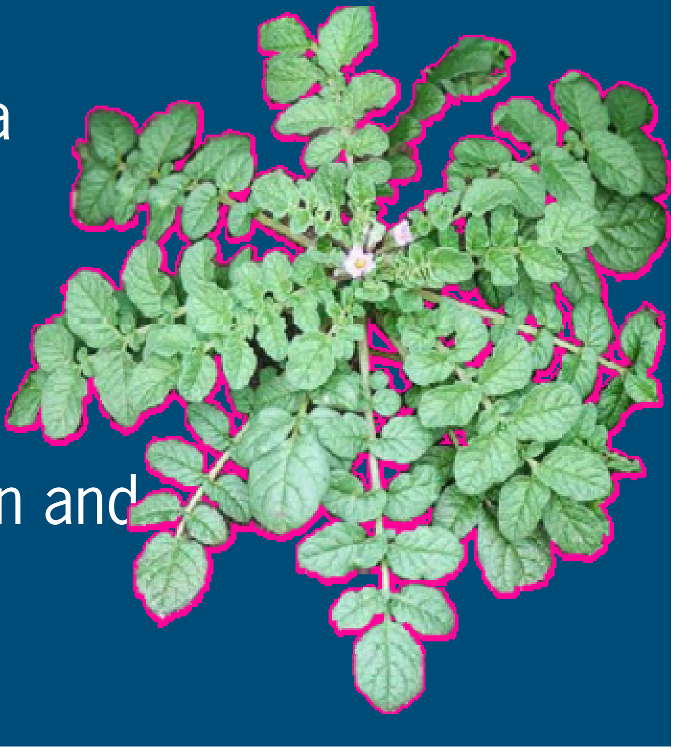
Conserving and mining PGR

- optimization of protocol for adding modern lettuce varieties to CGN collection
  - comparison new varieties with CGN collection
  - information from breeders
  - microsatellite study
  - outcome: improved protocol to select varieties with highest added value

Treuren et al. 2008, GRACE 55: 319-330.

Conserving and mining PGR

- reduction size CGN's potato collection
  - about 2000 accessions
    - 110 wild species
  - 314 accessions of the series Acaulia
  - AFLP study
  - taxonomic validation
  - sampling strategies
  - outcome: considerable rationalization and optimisation collection strategy



McGregor et al. 2002, TAG 104: 146-156.

Conserving and mining PGR

- study of diversity and validation regeneration protocol in CGN cabbage collection
  - comparison drift/shift during regenerations with distances between accessions
  - sampling after ‘real’ regeneration
  - AFLP study
  - outcome: need to improve regeneration protocol or collection composition

Hintum et al. 2007, TAG 114: 777-786.

Conserving and mining PGR

- intra-accession variation in barley
  - improvement of regeneration methods
    - avoidance of genetic drift
    - avoidance of selection
  - case study in barley
    - cultivars, landraces and wild material
  - AFLP study
  - outcome: methodological knowledge and recommendations for splitting accessions

Treuren & Hintum 2001, GRACE 48: 287-295.

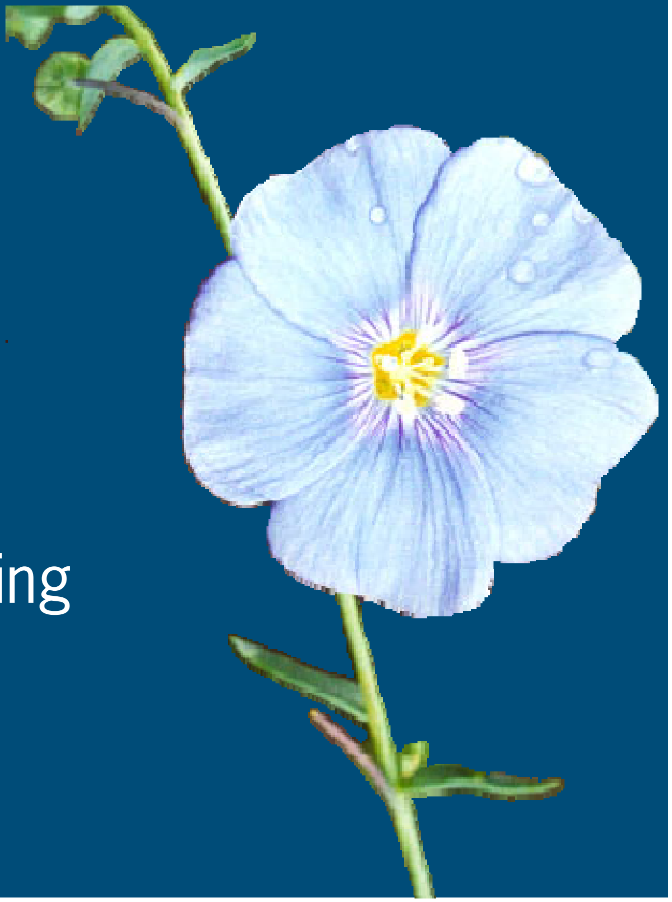
Conserving and mining PGR

- regeneration of perennial ryegrass
  - variation in pollination
  - loss of genetic integrity
  - paternity analysis
  - microsatellite study
  - outcome: methodological knowledge and recommendations for regeneration protocol improvement

Treuren et al. 2006, Euphytica 147: 367-382.

Conserving and mining PGR

- redundancy study in flax
  - CGN collection: ~1000 accessions
    - 317 accessions of breeder's lines
    - many similar accession names
    - poorly documented
    - hardly requested for utilisation
  - AFLP study
  - outcome: recommendations for bulking accessions



Treuren et al. 2001, TAG 103: 144-152.

Conserving and mining PGR

- development *in situ* conservation strategy for old Dutch grasslands
  - perennial ryegrass, white clover and Kentucky bluegrass
  - comparison of old grasslands to nature reserves and cultivars
  - AFLPs and morphological variation
  - outcome: support to policy decisions for *in situ* conservation

Treuren et al. 2005, Mol Ecol 14: 39-52.

WAGENINGEN UR
For quality of life

Conserving and mining PGR

- study clonal variation in Ethiopian enset
  - on farm conservation in Ethiopia
  - many synonyms
  - AFLP and morphological study
  - identification of duplicates
  - outcome: rationalisation conservation program

Negash et al. 2002, Crop Science 42: 1105-1111.

WAGENINGEN UR For quality of life

Conserving and mining PGR

- Generation Challenge Programme
  - an international initiative to use genomic science to mine PGR collections of major crops and use it in poor framers crops
  - initiative of the CGIAR

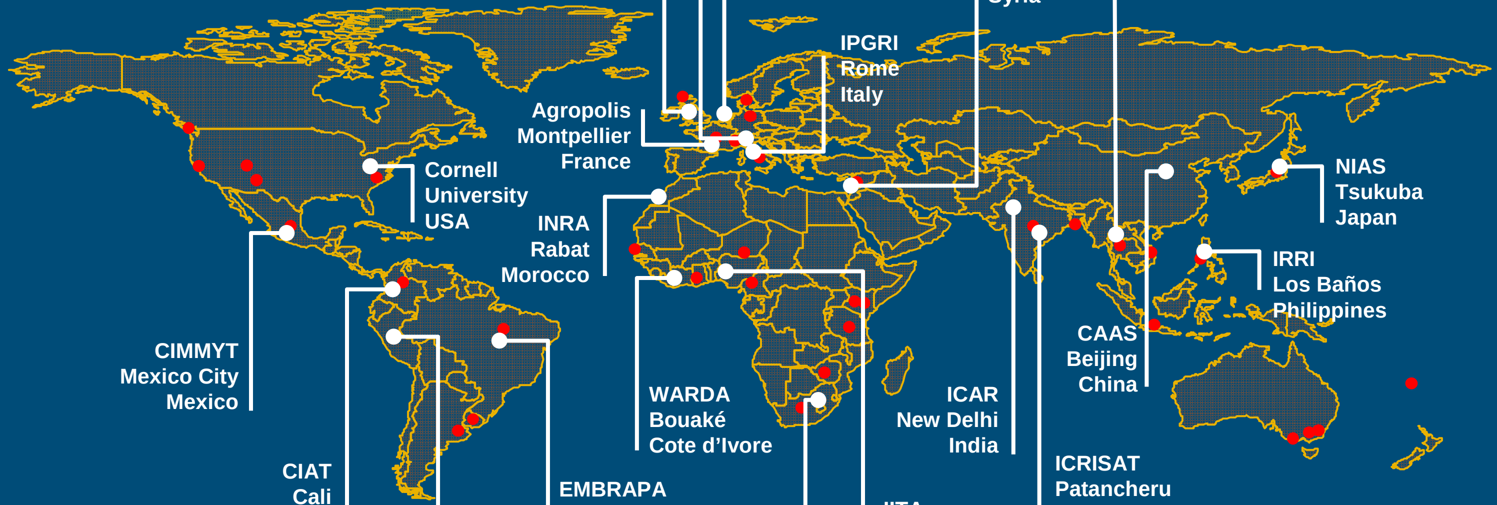
Generation

Conserving and mining PGR

- Generation Challenge Programme
  - objectives
    - utilize plant genetic diversity for crop improvement
      - develop low cost technologies for germplasm genotyping, allele mining and molecular breeding
      - integrate genomics with plant physiology and/or biology
    - capacity building and delivery of products
      - create and enable adoption of mechanisms for products to reach end-users

Conserving and mining PGR

- Generation Challenge Programme
  - consortium



Instituto Agronomico per l'Oltremare
Florence
Italy

Wageningen University
Netherlands

BIOTEC
Bangkok
Thailand

John Innes Centre
Norwich
UK

ICARDA
Aleppo
Syria

IPGRI
Rome
Italy

Agropolis
Montpellier
France

Cornell
University
USA

NIAS
Tsukuba
Japan

INRA
Rabat
Morocco

IRRI
Los Baños
Philippines

CAAS
Beijing
China

CIMMYT
Mexico City
Mexico

WARDA
Bouaké
Cote d'Ivore

ICAR
New Delhi
India

ICRISAT
Patancheru
India

CIAT
Cali
Colombia

EMBRAPA
Brasilia
Brazil

IITA
Ibadan
Nigeria

ACGT
Pretoria
South Africa

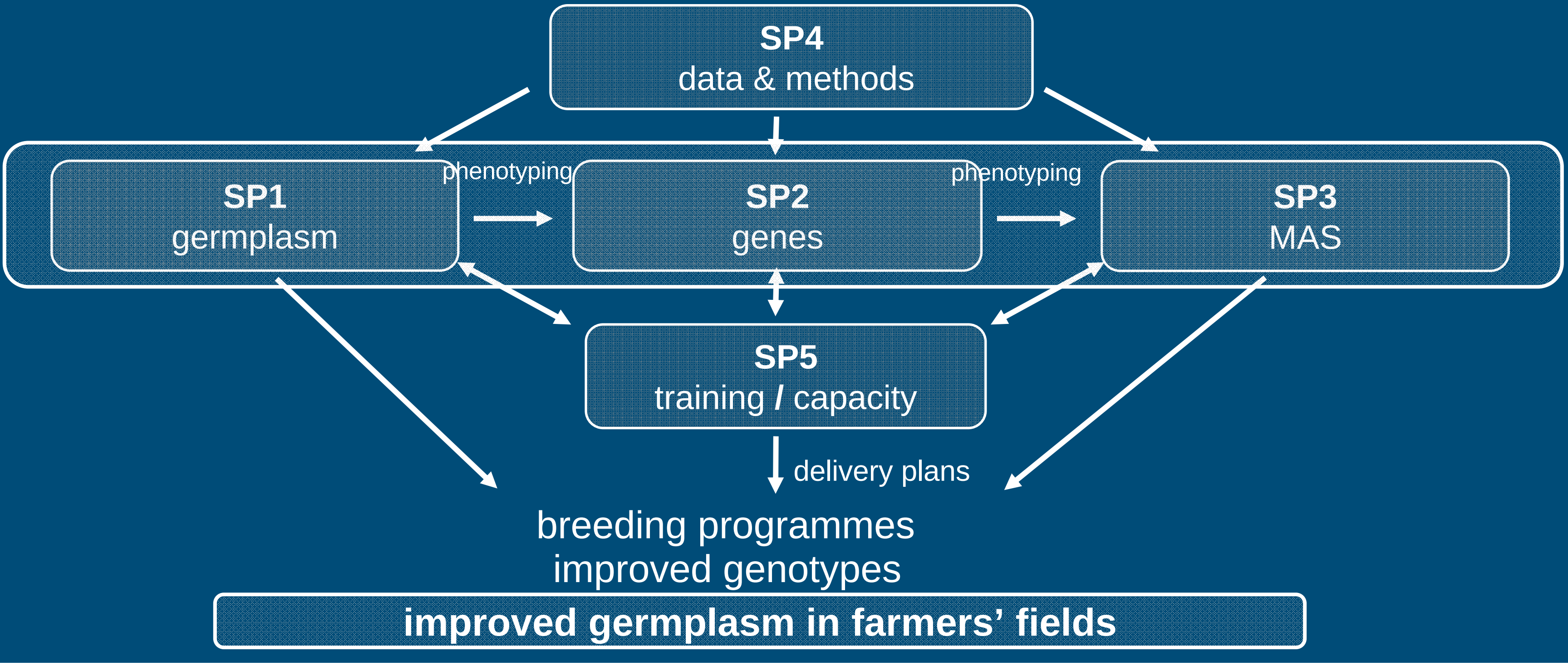
CIP
Lima
Peru

Conserving and mining PGR

- Generation Challenge Programme
  - some facts
    - launched in August 2003 (10 year framework two 5 year periods)
    - target areas: marginal environments in Sub-Sahara Africa, Latin America and South East Asia
    - mandate crops: all CGIAR crops
    - mandate traits: drought tolerance and associated traits
    - budget c. $16M / year of which 70% for research
    - major donors
      - European Union
      - UK Department for International Development
      - Bill and Melinda Gates Foundation

Conserving and mining PGR

- Generation Challenge Programme
  - research structure



SP4
data & methods

SP1
germplasm

phenotyping

SP2
genes

phenotyping

SP3
MAS

SP5
training / capacity

delivery plans

breeding programmes
improved genotypes

improved germplasm in farmers' fields

Conserving and mining PGR

- Generation Challenge Programme
  - genomics: central component
    - large scale fingerprinting of all crops
      - up to 50 microsatellites x 3000 accessions
      - development hi-throughput technologies / pipelines
    - candidate gene identification
      - forward and reverse genetics
      - QTL analysis
    - allele mining
      - EcoTILLING
      - ‘mining strategies’
    - study of orthologous candidate genes

Conserving and mining PGR

- Generation Challenge Programme
  - expected outputs relevant to PGR management
    - knowledge about the diversity in germplasm collections (and methodologies to study this)
    - methods and tools for identifying allelic diversity in germplasm collections based on DNA sequences

Conserving and mining PGR

- conclusions
  - molecular marker technology has great potential to improve PGR management
    - but little impact so far
  - applications are aimed at specific research questions, not at routine operations
  - costs of molecular analysis are still limiting factor in most cases
    - high throughput technology is required