CPVO co-funded R&D project CPV.8648

"MANAGEMENT OF PEACH TREE REFERENCE COLLECTIONS"

May 5, 2008 – May 4, 2011

FINAL REPORT

Creation of a common database through a collaboration

between the examination offices in France, Hungary, Italy and Spain.

Participants
GEVES – coordinator (Cavaillon and Le Magneraud, France)
INRA (Avignon, France)
MgSzH (Budapest, Tordas, Helvécia Hungary)
CRA-FRU (Roma, Italy)
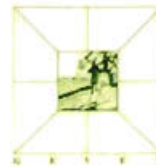IVIA (Valencia, Spain)
CITA (Zaragoza, Spain)

Table of content

Introduction

The Grant Agreement of the CPV.8648 R&D project « Management of Peach tree reference collections » was signed in December 13, 2007. The project effectively began after the approval of the Inception report, the May 5, 2008. It finished the August 4, 2011. The request the benefit of the 2 additional months to provide the Final report was validated the July18, 2011.

The project involved all the peach tree examination offices indentified by the CPVO in 2007.
The respective involvement of each partner was validated as follows:

	France		Hungary	Italy	Spain	
	GEVES	INRA	MgSzH	CRA-FRU	IVIA	CITA
Thematic 1 Compilation of **morphological data**		X	X	X	X	X
Thematic2 Creation of standardised **digital data**		X	X	-	X	X
Thematic3 Generation of **molecular marker data**	X		X BioGEVES	X	X	X
Thematic 4 Creation of a phenotypic and molecular variety **database**	X		X	X	X	X

The aim of the project was to produce a database compiling both phenotypic data, including standardised morphological descriptions, digital pictures, and a large data set of DNA profiles (microsatellites) in order to optimize the management of variety reference collections in peach tree, *Prunus persica* L.

Seven axes of works were approved:

1. Definition of a list of varieties included in the project
2. A set of General data for each variety (T01_varieties table)
3. The Applicant phenotypical description (part of T04_Phenotypical_Description table)
4. The Complete phenotypical description
5. A set of Digital data
6. Biomolecular data
7. A Database to store and manage all the previous data

I) Program results

1. Varieties included in the project

GEVES Cavaillon unit, as coordinator of the project, proposed to the partners the following rules to select the varieties to include in this project, as a representative sample of the Peach tree reference collection:

1- Variety officially listed and / or protected (UE / national level)
2- Variety effectively available in an orchard of at least one partner
3- Priority was done to the latest varieties.

On the basis of the proposal of each partner and the CPVO global grant (149 990€), 510 varieties were initially chosen:

- 12 varieties, effectively available for each partner (base of the phenotypical ring test to set up)
- 498 varieties fulfilling the imposed choice criteria.
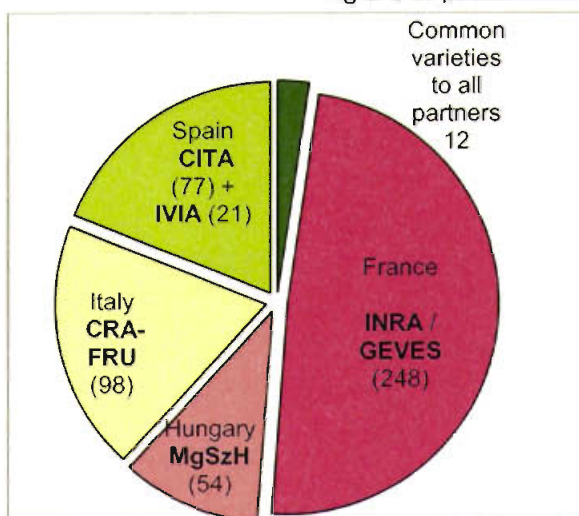
Due to some agronomical unpredictable troubles, some varieties were not available anymore. The investment of each partner was exposed as following in the latest interim report.

	France		Hungary	Italy	Spain	
	GEVES	INRA	MgSzH	CRA-FRU	IVIA	CITA
Common varieties (phenotypic harmonization purposes)	11		12	12	12	10
Specific varieties studied by office (without common varieties)	247		54	97	21	75
TOTAL	258		66	109	33	85

2. General data (T01 Variety table)

Variety identification data necessary to fulfill T01 table, the main entrance into the data base, were defined as below:

T01 VARIETIES   **Field Description**

o Variety_Id	numerical identifier used in the country	
o Country_Code	country code ISO3166	
o Species_Id	Identifier of a species (as SpecieId in CPVO)	
o **Denomination**	the denomination or the breeder's reference	(**)
o **Location_of_the_orchard**	orchard identifier used in the country	(*)
o **Number_Available_Trees**	number of tree per location	(*)
o **Tree_year_of_plantation**	Year of plantation in the orchard	(*)
o **Year_Of_Application**	Year of first Application in the country	(**)
o **Year_Of_End_Protection**	Year of end of Protection	(**)
o **Year_Of_Listing**	Year of listing of the variety	(**)
o **Mutant_variety_of**	denomination of the original variety	(**)
o Breeder_Reference	mentioned in the application form	
o **Trade_Mark**	Trade mark of the variety	(**)
o **Breeder**	Name of the breeder	(**)
o Present_Status		
o Comments_On_Variety	comments concerning the variety	
o **Date_Last_Modification**	Date of last modification of any information concerning the cultivar	(**)
o Phenotypical_Remark	Phenotypical remark for the Variety	
o **Unique_Identifier**	filled only when agreed by crop experts	(**)
o Virus_Tested	1 for YES or 0 for NO	

They correspond to administrative data (**) and collection data (*), which need to be up dated (frequency to define).The suppression of a variety in this table will destroy all data associated, whatever the subject (quantitative data, biomolecular data...).

In the case of varieties withdrawn (or rejected) during the study, the content of the T01 table has to be conserved, with a remark, but the other associated data are deleted.
In all cases, the date of last modification must be specified for this strategic table.

3. Applicant phenotypical description

Applicant phenotypical description corresponds to the Technical Questionnaire (TQ), fulfilled by the applicant.

It is not a central aim of this project, but the management of the TQ has to be included in the future use of the database :

- In application of CPVO protocol, the TQs of new applications have to be exchanged between Examination Offices.
- These data are identified as grouping characteristics.

A brainstorming between the partners (Budapest, September 2010) on :

- the current CPVO TQ
- results of the phenotypical ring test (shown in part 4)

allowed the definition of a <u>list of characteristics</u> to revise the current TQ.

This result was finally very positively crossed with the TQ of the new UPOV Draft Guideline TG/53/7(proj.2) – 2010-02-10

French point of view		Spanish point of view		UPOV draft Guideline TG/53/7(proj.2) – 2010-02-10			
Curent TQ of CPVO TP/53/1 – 27/03/2003							
1	Tree: size	1	Tree: size				
6	Flowering shoot: anthocyanin coloration	6	Flowering shoot: anthocyanin coloration				
10	Flower: type	10	Flower: type	9	Flower: type		
14	Petal: size	14	Petal: size				
31	Petiole: nectaries	31	Petiole: nectaries				
32	Petiole: shape of nectaries	32	Petiole: shape of nectaries	31	Petiole: shape of nectaries		

French point of view		Spanish point of view		UPOV draft Guideline TG/53/7(proj.2) – 2010-02-10			
Curent TQ of CPVO TP/53/1 – 27/03/2003							
46	Fruit: pubescence	46	Fruit: pubescence	45	Fruit: pubescence of skin		
50	Fruit: firmness of flesh	50	Fruit: firmness of flesh			-	Fruit: flesh type "NEW"
51	Fruit: ground color of flesh	51	Fruit: ground color of flesh			52	Fruit: carotenoid coloration of flesh "NEW"

57	Fruit: acidity	57	Fruit: acidity	58	Fruit: acidity (Acidity titrable) in meq 1000/ml	
63	~~Stone: adherence of flesh~~	63	Stone: adherence of flesh	65	Stone: adherence to flesh	
66	~~Time of beginning of flowering~~	66	Time of beginning of flowering	68	Time of beginning of flowering	
68	Time of maturity for consumption	68	Time of maturity for consumption	69	Time of maturity for consumption	

Some characteristics are very rarely relevant to drive the choice of the examination office in the selection of example varieties. In these very specific cases, some relevant pictures could be very useful.

This discussion allowed drawing the basis of:
- the improvement of the applicant's appropriation of this form to reduce the misunderstanding of the usefulness of the characteristics included in the TQ.
- the improvement of the efficiency of the Applicant form, which is the first step of each new variety, to allow an Examination Office to identify the closest example varieties
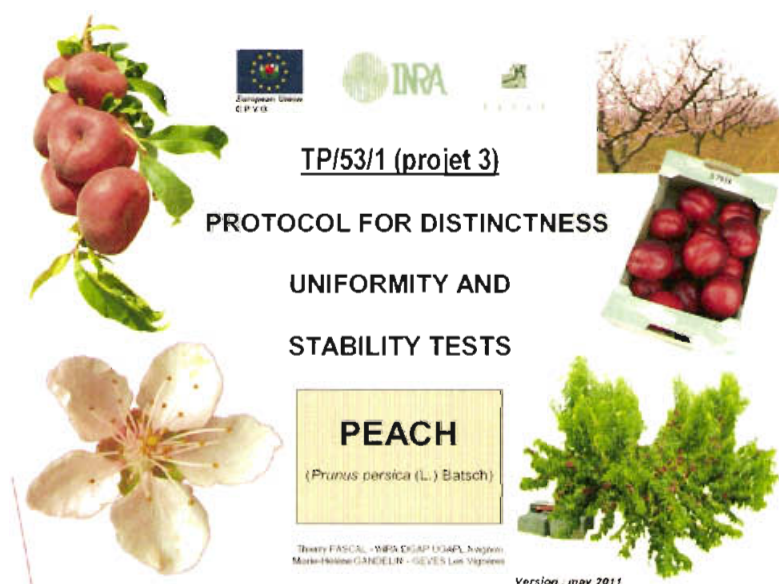
Eventually, 6 characteristics were chosen to be taken into account for phenotypical description:
10-Flower type, 32- Petiole : shape of nectarines, 46-Fruit pubescence, 51-Fruit colour of flesh, 57-Fruit acidity and 68-Time of maturity for consumption.

4. Complete phenotypical description

The phenotypical description is based on the use of the current CPVO TP 53/1 as a central reference, for the present time and to update old descriptions.

The INRA colleagues finalized a new version of the illustrated protocol with details of the scales used for the transformation of quantitative data in qualitative data in France.

TP/53/1 (projet 3)

PROTOCOL FOR DISTINCTNESS

UNIFORMITY AND

STABILITY TESTS

PEACH

(Prunus persica (L.) Batsch)

Thierry FASCAL - INRA DGAP UGAPL Avignon
Marie-Hélène GANDELIN - GEVES Les Vignères

Version : may 2011

4.1 Phenotypical ring test

Twelve varieties common to all partners were described by each partner to assess the level of discrepancies between partners for varieties theoretically redundant (same denomination).

1	Babygold 5
2	Babygold 6
3	Dixired
4	Early Redhaven
5	Fantasia

6	Flavortop
7	Loadel
8	Redhaven
9	Snow Queen
10	Springcrest

11	Springtime
12	Suncrest

The aims followed in this work was :

1. to secure the use of reliable characteristics(*) which can be used in sorts.
2. to identify the variability between the descriptions (which can be explained and tolerated).
3. to reduce the mistyping as far as possible.

➢ Results of the phenotypical ring tests leaded to select 16characteristics (*) to enter into the database, in order to complete the **Applicant phenotypical description**.

3	Tree: habit
6	Flowering shoot: anthocyanin coloration (excluding brindilles, side away from sun)
10	**Flower: type**
15	Petale: number
18	Anthers: pollen
19	Ovary: pubescence
28	Leaf blade : colour
29	Leaf : red mid-vein on the lower side
31	Petiole : nectaries
32	**Petiole : shape of nectaries**
41	Fruit : ground colour
42	Fruit : over colour
46	**Fruit : pubescence**
51	**Fruit : ground colour of flesh**
57	**Fruit : acidity**
68	**Time of maturity for consumption**

Moreover, it could be useful to add :

1	Tree : size	to sort easily dwarf varieties
35	Fruit: shape in ventral view	to sort easily flat fruits

For some characteristics, the partners were able to define groups of values which can be considered equivalent when sorting:

50	Fruit: firmness of flesh	1= very soft 2 = 3 = soft	4 = 5= medium 6=	7=firm 8= 9 = very firm
51	Fruit: ground color of flesh	1 = greenish white 2= white 3= cream white	4= light yellow 5= yellow 6= orange yellow 7= orange	8 = red
57	Fruit: acidity	1= 2= 3= low	4= 5= medium 6= 7=high 8 = 9=	
66	Time of beginning of flowering	1= very early	3 = early 5= medium 7= late	9= very late
68	Time of maturity for consumption	1= very early	3 = early 5= medium 7= late	9= very late

4.2 Quantitative data

16 quantitative data are included in the database *(except for old trees)*

4q	Flowering shoot: thickness (excluding brindilles)
5q	Flowering shoot: length of internodes (excluding brindilles)
8q	Flowering shoot: density of the flower buds (excluding brindilles)
14q	Petale: size
20q	Young shoot: length of stipule
21q	Leaf blade: length
22q	Leaf blade: width
23q	Leaf blade: ratio length / width
26q	Leaf blade: angle at base
27q	Leaf blade: angle at apex
30q	Petiole : length
34q	Fruit: size
45q	Fruit: extent of over color
56q	Fruit: sweetness
57q	Fruit: acidity
68q	Time of maturity for consumption

Each quantitative data is associated to a qualitative characteristic.

It is important to notice that

- Sorts *are only available with qualitative data.*
- Only numerical field can be used in the database for the expression of the date
  - a calendar (ie: 3-July = 184) was proposed to illustrate more precisely the qualitative data associated. Excel formulas can perform conversions (remark: a small gap can appear, depending of the year (leap year))

Precautions to take before the integration of measured data

- define all the Excel file column as **numerical** and check for **eventual text conversion**
- use always the same **numerical separator** (comma)
- use an unique value (an average, no interval, no symbol like >....)
- check aberrant values

check the consistency of measured data with associated qualitative score.

5. A set of digital data

A protocol to create <u>standardized digital pictures</u> related to the CPVO peach tree project was defined in August 2008.

Pomological view	Mass effect view
HU-0004-2009-A	HU-0004-2009-B
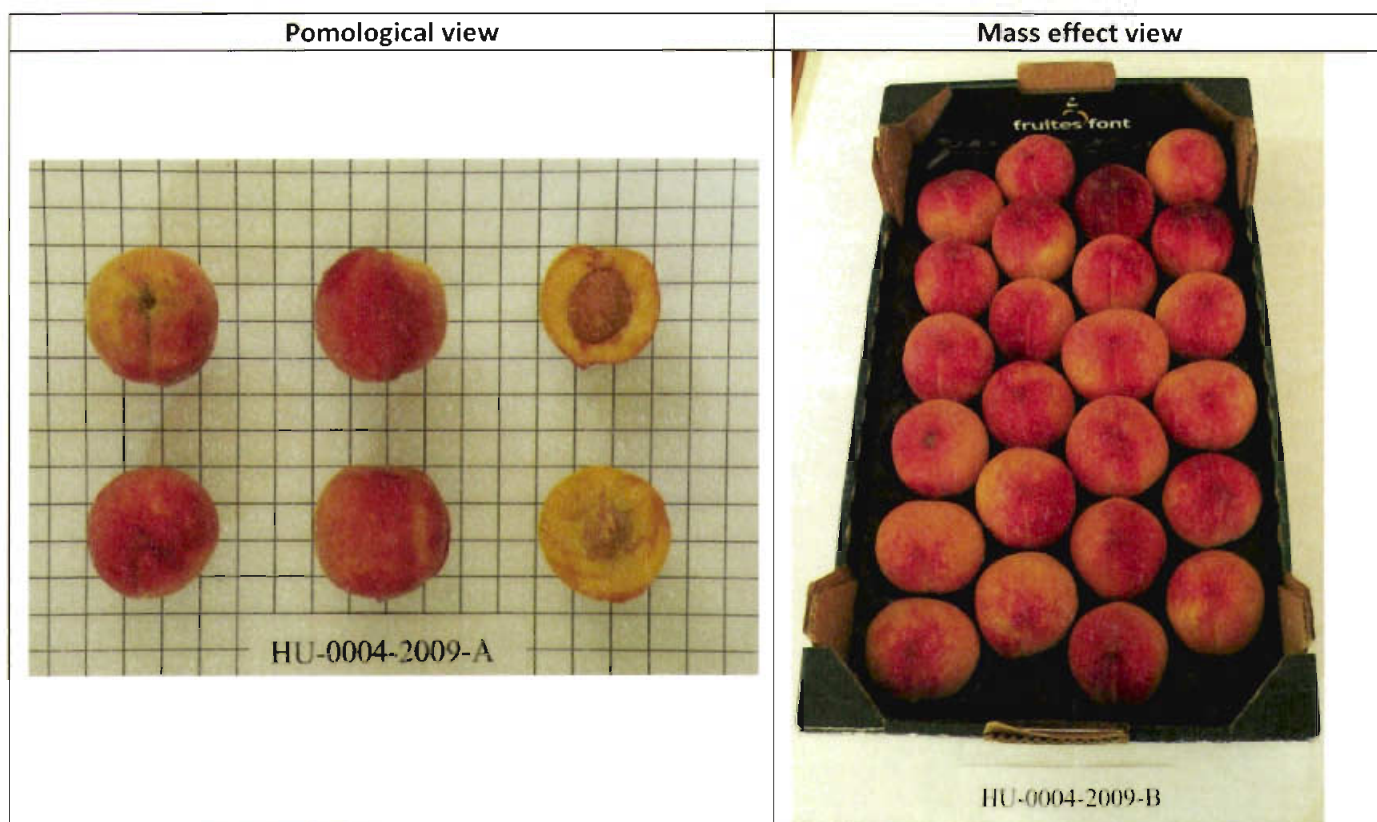
After three seasons of harvest, in different places, the standardized digital protocol was updated in October 2010 (Annex 1).

Additional precisions are included:
- The mass effect picture has to show the fruit pistil view (rather than the peduncle view)
- Whatever the picture, some elements which can influence the picture has to be taken into consideration:
  - Place and year of harvest
  - Type and setting of the camera.

This information cannot be a substitute for conventional phenotypic characterization but may summarize a few characteristics. The use of such document is restricted to advanced users only.

6. Biomolecular data : generation of molecular fingerprints

6.1 - Ring tests

The first step of this work was the harmonization of the protocols between 4 biomolecular laboratories (BioGEVES, CRA-FRU, IVIA, CITA), to allow the choice of SSR primers for the collections characterization. The first ring test allowed the identification of 16 SSR primers (amongst 21 proposed primers).

Linking group	Microsatellite	
G1	UDP96-005	UDP98-022
G2	CPPCT044	BPPCT001
G3	BPPCT007	UDP96-008
G4	CPDCT045	BPPCT015
G5	BPPCT017	BPPCT038
G6	BPPCT025	UDP98-412
G7	CPPCT022	EPPCU5176
G8	CPPCT006	UDP98-409

The harmonization of the allele scoring was the purpose of the second ring test. The NIAB's «unified allele score» system was used by all the partners. Between the first and the second ring test, 164 alleles were scored.

6.2 - Characterization of the collections

This project allowed the characterization of 535 genotypes, included in the varietal list of the CPV.8648 project and around one hundred additional varieties, for which DUS tests were running between 2008 and 2011.

The collaborative work between the partners allowed to start the writing of a scientific paper. It is to finalize.

This work was compared to the similar works in 2006 (Yoon *et al.*, 2006) and 2010 (Aranzana *et al.*, 2010).

6.3 - Genetic structure of the collection

An interpretation work on the genetic structure of the reference collections was barely discussed during the project. Preliminary observations were drawn, but have to be completed.

The interpretation of the pool of data (SSR profiles AND phenotypical characterizations) should be continued.

A synthesis of the biomolecular work done during the CPV.8648 project was the subject of a paper for the next **UPOV** working group on Biochemical and Molecular Techniques and DNA- Profiling in Particular (**BMT**) in Brasilia (November 22-24, 2011) and is annexed to this report (Annex 2).

7.   A Database to store and manage all the previous data

The storage and the management of all the data produced during this project was this opportunity to build a phenotypic and molecular variety **database,** and to go further with the **GEMMA framework.**

7.1 - The data model
The database structure developed for maize by Spain, Germany and France, in the frame of the programme CPV4961 supported by CPVO (2003-2005) was a good starting point for the GEVES IT.

The data structure was adapted and optimized:
- To integrate different type of data (Molecular data + Digital pictures) whatever the couple Species/Country
- To support multi language
- To define access rights
- To manage different species.

The relational model of Peach tree reference collection is shown in Annex 3.
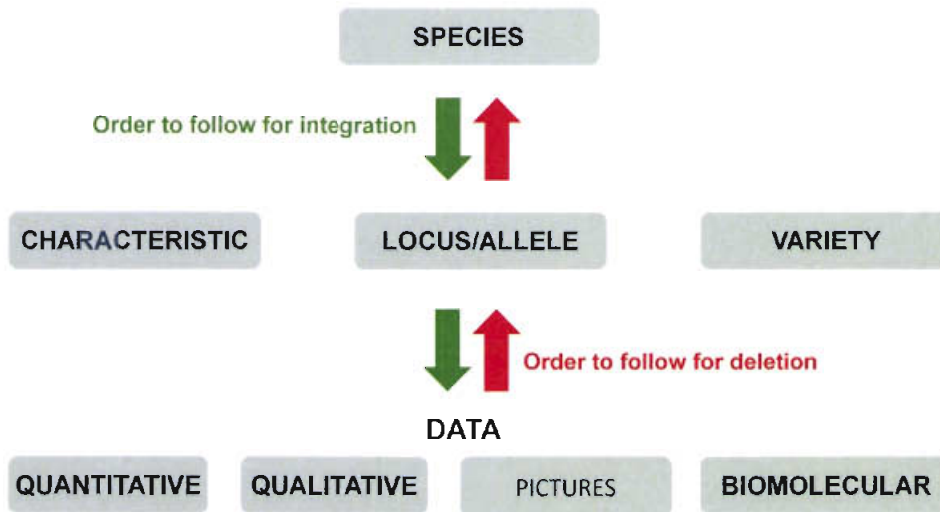
7.2 - Web site approach
The web site approach allows to provide:
- an easy way to share data
- a tool able to correct problems quickly
- an easy system to manage data whatever the type of data (digital pictures, quantitative data, qualitative data, bio molecular profils...)
- some useful tools (ie: sorting, ...) automatically available for all users.

An Excel format to import and export data from GEMMA was favored, because:
- Excel is user friendly and known by a wide community of users
- People don't need to know the structure of database to Import/Export
- People who use a central database to manage and store their DUS data can develop procedures to generate automatically the creation of Excel files for GEMMA
- It allows more flexibility when modification of database model when needed.

The integrity of the database GEMMA is guaranteed thanks to the following cascade of information:

SPECIES

Order to follow for integration

CHARACTERISTIC   **LOCUS/ALLELE**   **VARIETY**

Order to follow for deletion

DATA

QUANTITATIVE   **QUALITATIVE**   PICTURES   **BIOMOLECULAR**

The access rights to the database are defined as follows:

- The system of rights access is based on 3 different levels
  - Administrator:
    - one data administrator per country and species with a specific Account/Password
    - management of his own data
    - display data from other country (same species) only with authorization
  - User:
    - restricted access that require a specific Account/Password
    - each user is created by an administrator
  - Public:
    - access that not require a specific Account/Password
- The access level is defined by the data administrator (country/species) for each variety
  - 1 = Public access level: everyone can see the variety
  - 2 = Restricted access level: only user and administrators accounts defined by the data administrator of the couple Species/Country, will see the variety
  - 3 = Administrator access level: only administrator accounts defined by the data administrator of the couple Species/Country, will see the variety.

http://gem.geves.info

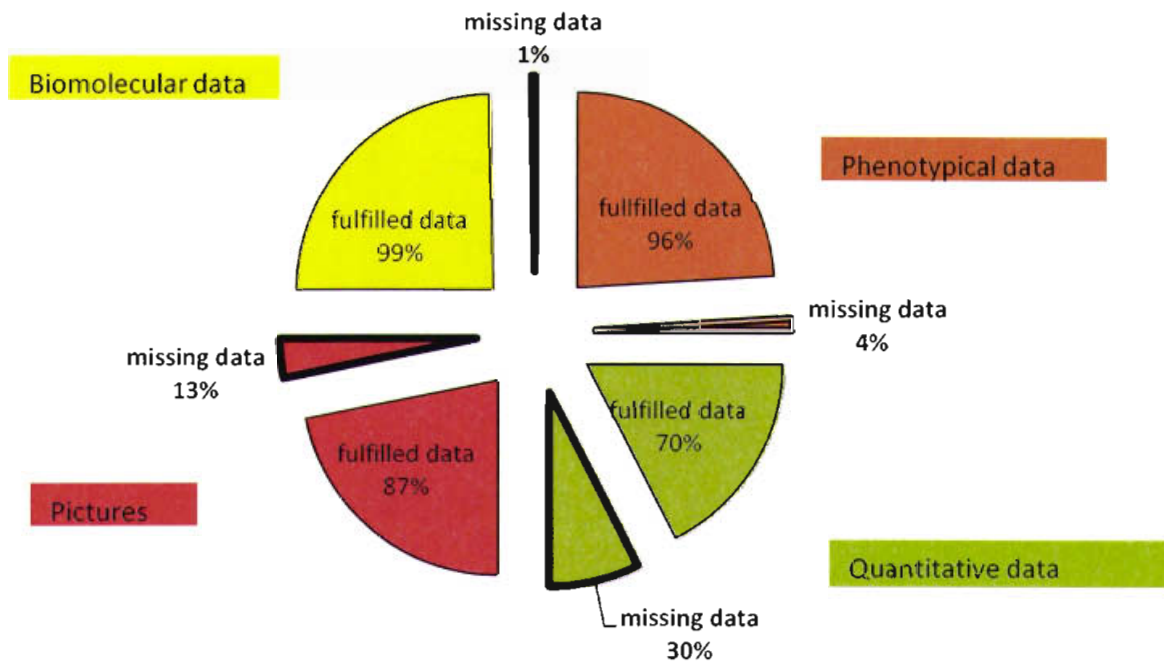## 7.3 – Synthesis of technical results included in GEMMA database

All the partners included as far as possible their respective contributions in regards to the inception report (April 2008).
The following pie charts allow a quick appreciation of the whole work done per partner.
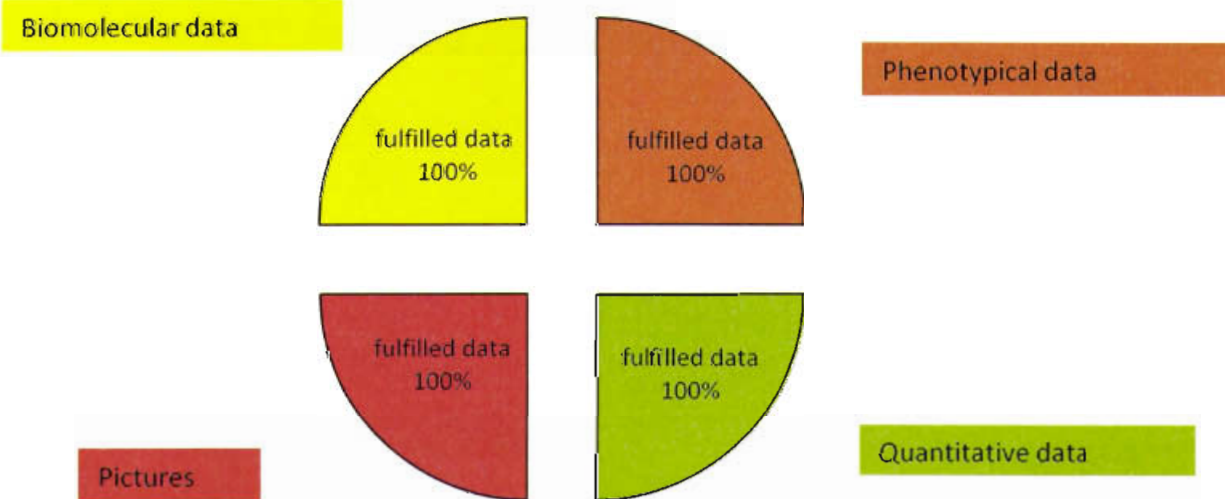The missing data were most of the time announced in the annual technical reports. They are generally linked to unexpected difficulties (dead trees, trees to early grafted, diseases that prevented from carrying out some notations (*Monilia*...)). From the beginning of the program, CRA-FRU (Italy) stated that they won't perform the digital data acquisition.

Some quantitative data couldn't be recorded because they referred to trees too old or too young to be representative.
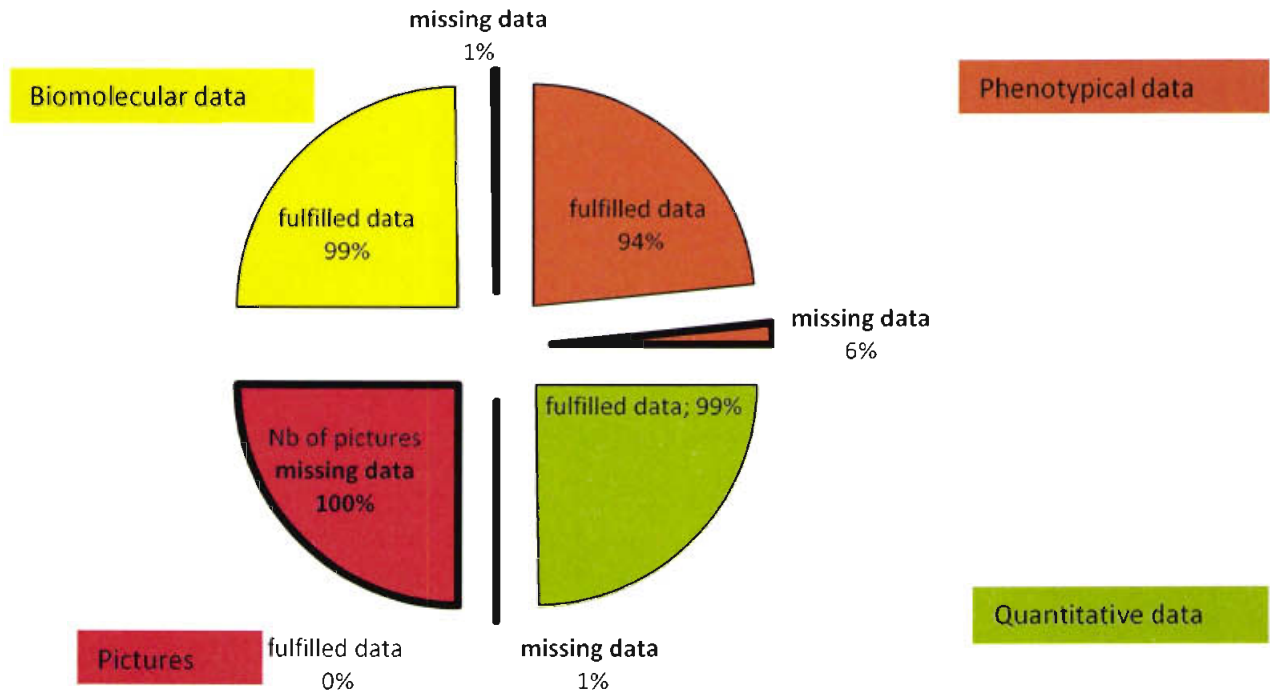
Synthesis of the FR partner results - **260 varieties**

missing data
1%

Biomolecular data

Phenotypical data

fulfilled data
99%

fullfilled data
96%

missing data
4%

missing data
13%

fulfilled data
70%

fulfilled data
87%

Pictures

Quantitative data

missing data
30%

Synthesis of the HU partner results - **66 varieties**

Biomolecular data

Phenotypical data

fulfilled data
100%

fulfilled data
100%

fulfilled data
100%

fulfilled data
100%

Pictures

Quantitative data

Synthesis of the IT partner results
110 varieties

Biomolecular data
Phenotypical data
Quantitative data
Pictures

missing data 1%
fulfilled data 99%
fulfilled data 94%
missing data 6%
Nb of pictures missing data 100%
fulfilled data; 99%
fulfilled data 0%
missing data 1%

Synthesis of the ES partner results
110 varieties

Biomolecular data
Phenotypical data
Quantitative data
Pictures

missing data 6%
fulfilled data 94%
fulfilled data 73%
missing data 27%
missing data 22%
fulfilled data 78%
fulfilled data 72%
missing data 28%
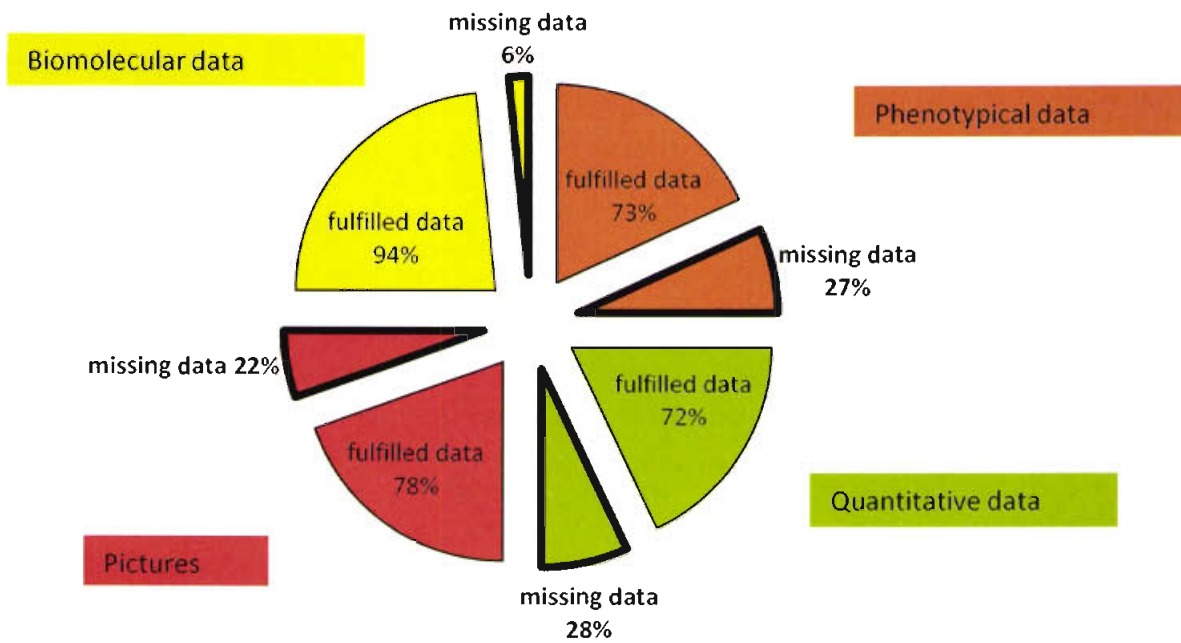
II) Program prospects

1 - Scientific and technical topics

1 - On the basis of the data produced during this project

Further interpretation of results (cross-interpretation of phenotypic and bio molecular data) has to be performed. Some axes of interpretation were only sketched to identify elements of structuration of the genetic diversity, elements to follow the evolution of the breeding objective...

The added scientific value of this project would be substantially increased by a detailed interpretation of the results. This could be the basis for future collaborations.

2 - To continue the technical work initiated during this project

- on all the common knowledge varieties, with a priority done to the varieties which were declared DUS since the beginning of the CPV.8648 project

> *INRA – GAFL Avignon produced additional data during the project (statement – end 2010)*
> - *phenotypical characterization : 82 varieties*
> - *SSR fingerprint: 471 varieties*
> - *pictures: 208 varieties (pomological view + mass effect)*

3 - To open the GEMMA framework on other species

The IT department of GEVES had clearly integrated this need before developing the scope of the GEMMA database.

- Hungarian colleagues expressed their interest on Pear (Pyrus communis) (Sept 2010).
- Such a work is in progress on Apricot *(Prunus armenica)* by the INRA GAFL Avignon colleagues.

2 - Political and strategic topics – Access to database

Access rights to database remain to be defined, as well as the way to go on completing the database.
Proposals and points of view of the different partners lie below.

1- Access to database

- Data produced during the CPV.8648 program, IN the convention
  The program's partners benefit of an exclusive and whole access to all the data produced.
- Data produced OUT of the convention
  Two alternative accesses can be considered for an Examination Office:
  - A whole access (phenotypic data /digital data/ biomolecular data from all the partners), if the partner is an actor which contributes to all the chapters.
  - A restricted access to its own contribution only, if the contribution does not cover all the chapters of the base.

  Cooperations between partners have to be considered to allow the production of the data (ie: biolomolecular data for HU partner for the CPV.8648 program).
  In case of an Examination Office (previous partner or not) would join the program, it must undergo the obligations that has to be finalized.

2- Contribution to the database management

- The level of the compulsory data to provide has to be defined.

GEVES and INRA proposed a **reduced compulsory phenotypical data list**:

CPVO n°	CPVO characteristic
1	Tree: size
3	Tree: habit
6	Flowering shoot: anthocyanin coloration (excluding brindilles, side away from sun)
10	Flower: type
15	Petale: number
18	Anthers: pollen
19	Ovary: pubescence
28	Leaf blade : colour
29	Leaf : red mid-vein on the lower side
31	Petiole : nectaries
32	Petiole : shape of nectaries
35	Fruit: shape in ventral view
41	Fruit : ground colour
42	Fruit : over colour
46	Fruit : pubescence
51	Fruit : ground colour of flesh
57	Fruit : acidity
68	Time of maturity for consumption

Legend

PNT ring test – robust characteristics
CPVO TQ
Additional characteristic

The **pictures** and the **biomolecular data** are considered as **compulsory**.

- A committee is required to lead the annual exchanges of information:
  - o The monitoring of the integration in GEMMA database (by each contributor) of
    - The new applications.
    - The common knowledge varieties, (prior to the CPV.8648 program or not).
  - o The timing and rhythm of integration has to be defined.
  - o The data validation (no aberrant data, error correction, manage unique Ids).
- When a problem is identified, CPVO and partners review the arrangements.
- A written commitment of each partner has to be formulated to respect the predefined rules.

CRA-FRU point of view

De : **Ignazio Verde [mailto:ignazio.verde@entecra.it]**
Envoyé : **mercredi 1 juin 2011** 11:01

"The involvement of CRA-FRU in the CPVO project "Management of peach tree reference collection" has been of great value both at the scientific and practical level. The CRA-FRU group has had the opportunity to collaborate with valuable scientific partners around Europe and establish durable collaborations with colleagues. The set of primers chosen during the project has greatly improved the quality of fingerprinting in our Centre having this set a high power of discrimination among accessions minimizing the number of primers that need to be analyzed for these purposes. The individuation of a set of Reference accession would greatly help in the comparison and the harmonization of our data with those of colleagues around the world.

The availability of a database containing phenotypic and genotypic information would greatly help in the evaluation of the new cultivars. Updating the database is an action that need to be coordinated among the various national institutions participating. And this is, in our opinion, an action that must be taken by CPVO. Of course the morphological descriptors will be done in any case and adding to the database should not be a big problem; but about the molecular data, an extra expensive work needs to be done. We think the molecular database would be useful but this is an action that must be taken in all countries. The CPVO in its guideline should suggest or enforce this action.

The choice of descriptors is somewhat strange for peach, as important agronomical monogenic and polygenic characters are missing. For example:
- the melting-non melting character, which is not too difficult to score, and could be taken together
- the freestone-clingstone, being controlled by the same gene.
Another important missing character is the consistency of the pulp, but we understand it is much more difficult to score."

De : **Maria Teresa Dettori [mailto:mariateresa.dettori@entecra.it]**
Envoyé : jeudi **15 septembre 2011** 12:40

"Me and Ignazio talked again with our Director, DrGuido Cipriani and with the new CPVO Responsible, Dr Roberto De Salvador.
They both agreed that CRA-FRU would have no problem in keeping the database up-to-date, the only problem would be with the molecular data, that are costly and time consuming. The costs are increased by the knowledge that, if it is expected to cover all the alleles now known, perhaps 25-30 reference peach cultivar should be added to each experiment (I did not had an exact count , but the number of reference cultivars should not be far). For this reason we think it could be better to choose a single institution for each species, otherwise it could happen that the number of reference cultivars is higher than the number of cultivars in examination.
We could obviously do the work, provided the costs would be covered by CPVO.
That is all we have to add to the observations Ignazio sent you with the final report."

MgSzH point of view

Szani Zsolt [mailto:SzaniZs@mgszh.gov.hu]
Envoyé : **mercredi 14 septembre 2011** 16:41

"We would like to express our opinion on the future of the peach project. We regard the establishment of the peach database as a significant improvement in the Community plant variety system. Moreover it is more than a special project for peach and nectarine varieties. This is a perfect model for other fruit species also. It would be very useful to cover other species by the GEMMA also.

We definitely support to go ahead with the database after the official close of the project. We are ready to feed more data on other peach varieties continuously.

However our point of view differs firmly in one topic from the French implosion. The representative of the GEVES proposed to restrict partners within the databases in the future based on their special expertise. In fact the GEMMA includes more fields, like morphological data, standardized digital data and molecular data. Many Institutes take part in this project still only one of us supplied all kind of data. In this way almost all the partners could use only partly the database. We expressed the above mentioned doubt immediately in the Fruit Open Day 15th June 2011. Moreover we can not acquiesce that the 'closed Institutes' would be absolved from this rules as the verbal explanation proposed it. It could result different rules based on the country and based on the nationality.
We can support an approximation that keeps the main aim of this project: to integrate the European knowledge on the peach varieties and improve it in the coming years.

We hope that we can make an agreement in this kind of mentality."

CITA point of view

De : manualonseg@gmail.com [mailto:manualonseg@gmail.com] De la part de **JOSE MANUEL ALONSO SEGURA**
Envoyé : **jeudi 28 juillet 2011** 14:36

" Regarding the Spanish position to the future cooperation to feed the Gemma database with data of new varieties, I am waiting for a meeting in Madrid (Spanish Plant Variety Office – CITA – IVIA). I hope this meeting will celebrate before September 16th, and hope you will have an official answer to the French proposal.

I only can express my personal position. For me this project was very positive, and it allowed me to interchange different points of view with other peach experts, and I think I learn from them. For me it would be wonderful to continue the work started in this project and to feed the GEMMA every year with phenotypical, quantitative and molecular data, and pictures. There is only one problem. We will need to receive more financing from the Spanish Plant Variety Office to do this work, especially for the molecular analysis. I wish the Spanish Office think that it is interesting to continue the work of this project too."

Acknowledgments

List of Annexes

1- Protocol of the digital pictures (October 2010)
2- BMT2011 Collective paper
3- GEMMA relational model
4- Partners' contacts

Protocol to
Create standardized digital pictures related to
## the CPVO peach tree project

1 – Pomological view: picture focused on 6 fruits views

Background

a. To use a white squared sheet of 40cm long x 28cm wide

b. Used grid: 2cm x 2cm

c. To use the total area to take the picture even if the fruits are small. It is a way to work with a constant scale.

Subject of the picture

d. position in the picture

i.	upper left corner:	top view (peduncle)
ii.	lower left corner:	bottom view (pistil scar)
iii.	upper middle position:	ventral view (with the peduncle attach to the top)
iv.	lower middle position:	lateral view (with the peduncle attach to the top)
v.	upper right corner:	longitudinal cross section (cut along the suture line and put the peduncle attach to the top)
vi.	lower right corner:	transversal cross section

e. to try to lay out the top and left side of each fruit on the grid

Good   Wrong
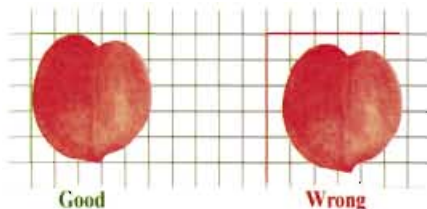
f. to space the different pieces enough to avoid shadows

g. to adjust the position of fruit in vertical plane, you can use small supports (always the same) or sticky paper or small pieces of modelling clay.

Label of the picture

- to precise:
    1. the country code (two capital letters) : *FR* / *HU / IT / SP*
    2. the numerical identifier used in the country : a number with four positions (0001 for example)
    3. the year of production of the picture with four positions : 2008 for example
    4. the type of picture (one position) : the pomological view (6 views of the fruit) - A

- to put the label below the middle views
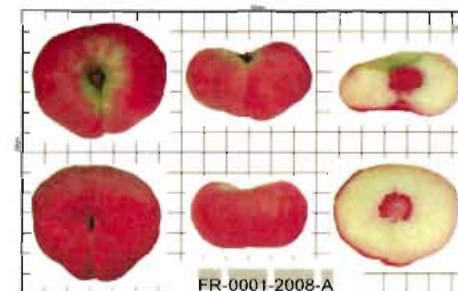
The denomination is deliberately forgotten at this stage to avoid typing mistakes, and too long file name.

FR-0001-2008-A

Size of the picture

- necessity to adjust your camera resolution to obtain a maximum picture size of 500Ko
- to adjust the camera zoom to see all the squared sheet (40cm long x 28cm wide)

File name : like the label of the picture with hyphen between each element.
For example : FR-0001-2008-A.jpg

2 – Mass effect view: picture focused on an harvest box (fruit pistil view)

- Illustration of the variability of the variety
- Fully complementary to the previous picture.

Label of the picture

- to precise:
    1. the country code (two capital letters) : *FR* / *HU / IT / SP*
    2. the numerical identifier used in the country : a number with four positions (0001 for example)
    3. the year of production of the picture with four positions : 2008 for example
    4. the type of picture (one position) : the mass effect view (in an harvest box): B

- to put the label below the box

The denomination is deliberately forgotten at this stage to avoid typing mistakes, and too long name of file.

FR-0001-2008-B

Size of the pictures

A picture should be less than 500 Ko in **JPEG format.**

File name : like the label of the picture with hyphen between each element.
For example : FR-0001-2008-B.jpg

Management of Peach Tree Reference Collections
CPV.8648 Research & Development Project
Part 1: Bio molecular results

C Jouy[1-1], MH Gandelin[1-1], C Guitouni[1-3], D Zhang[1-4], J Lallemand [1-4], C Colonnier[1-2], A Luciani[1-2], T Pascal[2], C Tuero[2], Zs Füstös[3-1], Zs Szani[3-1], P Chomé[4], MT Badenes[5], JM Alonso[6], I Verde[7], MT Dettori[7], S Semon[8]

[1-1]GEVES Cavaillon,4790 route des Vignères, 84250 Le Thor, France
[1-2]GEVES Beaucouzé, rue George Morel, BP 90024, 49071 Beaucouzé Cedex, France
[1-3]GEVES Le Magneraud, Saint-Pierre-d'Amilly, BP52, 17700 Surgères, France
[1-4]BioGEVES, Saint-Pierre-d'Amilly, BP52, 17700 Surgères, France
[2]Institut National de la Recherche Agronomique (INRA), UGAFL, BP 94-84143 Avignon-Montfavet, France
[3-1]MgSzH, Központ Keleti Károly u. 24, Budapest, H-1024, Hungary
[4]Oficina Española de Variedades Vegetales (OEVV), Calle Alfonso XII, 62, 28014 Madrid, Spain
[5]Instituto Valenciano de Investigaciones Agrarias (IVIA), Carr. Moncada-Náquera, 46113 Valencia, Spain
[6]Centro de Investigación y Tecnología Agroalimentaria (CITA), Av. Montañana 930, 50059 Zaragoza, Spain
[7]Centro di Ricerca per la Frutticoltura (CRA-FRU) Via di Fioranello 52, 00134 Rome, Italy
[8]Community Plant Variety Office (CPVO), 3 Bvd. Maréchal Foch, 49101 Angers Cedex 02, France

Background

Peach/nectarine is an important fruit tree crop in Europe and indeed world-wide. The registration of new varieties, either for Plant Breeders' Rights or National Listing (PBR/NL) purposes in the European Union (EU), requires the completion of a distinctness, uniformity and stability (DUS) test in one of the EU Member States.

The number of candidate varieties and potentially required example varieties entered for DUS testing is steadily increasing annually. Moreover, DUS testing of this type of tree crops requires the maintenance of large orchards, particularly with the aim of having a complete reference collection. In France, Hungary, Spain and Italy, peach tree breeding activity is characterized by an important number of varieties available and a short turn-over of the varieties. This situation will extend to new EU members states in the coming years, with the development of the European trade.

However, although the diversification increases the number of segments, for each segment, new released varieties are genetically closer and closer. The consequence is that it is now difficult in some cases to distinguish between varieties.

the development of means of "managing" reference collections is highly desirable in order to be able to compare candidate varieties with the closest varieties of common knowledge in the reference collections prior to planting them, and so reduce the number of varieties that need to be grown side by side, without eroding the strength of PBR and the relevance of DUS tests. Effective means of such a management include the definition of a standardised way to compare phenotypic data and to use molecular markers to remove the reference varieties which do not need to be compared to the candidate varieties. In peach tree, various molecular markers such as DNA microsatellites (SSRs) have been developed and evaluated, but they have not yet been used for the characterization of large collections.

Introduction

The current Peach tree examination offices, GEVES and INRA for France, MgSzH for Hungary, CRA-FRU for Italy, and OEVV, represented by IVIA and CITA, for Spain joined their forces in a R & D Project supported by CPVO (CPV.8648, 2008-2011), to construct an integrated microsatellite and phenotypical (68 characteristics, two digital pictures) database.

The aim of the project was to produce a database dealing with all the information needed in order to optimize the management of variety reference collections in peach tree, *Prunus persica* L.

The project generated a database compiling both phenotypic data, including standardised morphological descriptions, digital pictures, and a large data set of DNA profiles for more than 500 peach tree varieties selected among the EU granted and listed varieties.

The steps of the project were:
1. compilation and production of a representative varieties set of standardized phenotypic data, including morphological descriptions and digital pictures
2. selection and test a set of SSR markers,
3. characterisation of the varieties with these SSR markers,
4. creation and implementation of a database with these data.

This paper focuses on the results on Bio molecular data obtained in CPV.8648 project.

Aspects concerning the compilation of morphological data (on the base of the 68 characteristics included in the CPV TP/53/1- protocol, with a phenotypical ring test between the official examiners), standardized digital data (definition of an harmonized protocol to produce a pomological view and a mass effect view per variety), and the creation and use of a phenotypic and molecular variety database will be developed in further communications.

Materials and methods

- **List of varieties included in the project**

The number of varieties included in this project was sized to represent a realistic sample of the *Prunus persica* L. diversity. All these varieties are –at least:
- o Registered in a National List (trading authorization), and / or protected at a national or European level (Plant Breeder Rights)
- o Physically available in at least one of the partners' orchards.

Amongst the pool of varieties identified, the priority was given to the latest material.

Figure 1: Repartition between partners of the number of varieties included in the CPV.8648 project
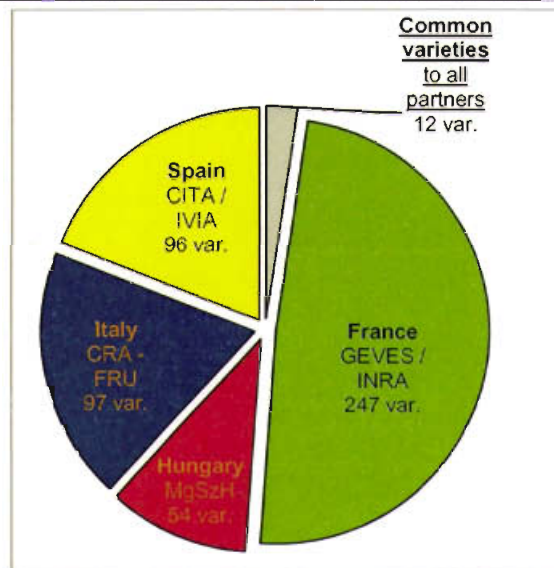
The final list of this project covers 506 varieties: 494 original varieties and 12 common varieties to all partners.

- **Set of varieties for the ring test**

A molecular ring test was performed by the participating laboratories. The purposes of the molecular ring test were (i) to harmonise the protocols and the allelic rating of the three countries involved, and (ii) to assess the capacity of the set of markers tested to be used for the evaluation of the allelic richness and the level of heterogeneity of the varieties studied in the programme.

In order to reveal the maximum number of different alleles at each tested locus, the set of varieties to be used in the ring test has to be chosen to represent as much variability as possible. For that matter, the set of 12 example varieties common to all countries and identified for phenotypic purposes did not appear to be perfectly adapted (varieties genetically to close to each other).

The partners validated another list of varieties widely distributed on a dendrogram published in a study on *Prunus* ssp. phylogeny (Aranzana *et al.*, 2003). In total, **12 varieties** were used for the molecular ring test: REDHAVEN, MAYCREST, YUMYEONG, GIALLA DI VERONA, DUCHESSA D'ESTE, ZAIFRANC (ROYAL MOON), BABYGOLD 8, ZAINARA (ALEXANDRA), CASAROB, BINACED, CATHERINA, and FANTASIA. Presenting a large number of alleles at the loci tested in this study, these varieties should cover a large diversity inside *Prunus persica* varieties. The annex 1 shows the location of the example varieties for the biomolecular ring test included in the CPV.8648 project , on the dendrogram produced by Aranzana (2003).

- **Bio molecular equipment**

All partners involved in this task (GEVES, CRA-FRU, IVIA, CITA) worked with several PCR machines and a capillary sequencer, which was validated to be a very favorable point for the scoring of the molecular data.

- **Keys to choose the SSR primers**

All the partners shared their experiences in the matter and their bibliographic references. To characterize the reference collections, suitable markers should respect the following criteria:

a. Primer sequences should be publicly available;
b. Markers should be highly polymorphic (i.e. high power of discrimination and high number of alleles);
c. Markers should be mapped on *Prunus* maps; markers should be in linkage equilibrium (two markers per linkage group with a recombination frequency of about 50%) as well as Hardy-Weinberg equilibrium;
d. Single locus markers with no null alleles are desirable;
e. Strong amplification pattern should be easily noted.

- **Protocols used for SSR analysis**

After tests, comparisons and exchanges of technical information among the participating laboratories (BioGEVES, CRA-FRU, IVIA and CITA), the SSR analysis protocol supplied by CRA-FRU showed appear to be the better adapted and was therefore accepted by all participating labs to use for the ring test as well as for the future genotyping of the peach collections The chosen protocol is describes in annex 2.

Results and discussions

1. Ring tests

- **First ring test**

A first ring test was performed on a set of 21 SSRs primers from Wunsch (2006) and Dirlewanger E. (1997) and carried out on a set of 12 varieties. The aim was to select 16 SSRs with two SSRs per linkage group. To optimize the work, only two labs, CRA-FRU (IT) and IVIA (SP), performed DNA extraction and managed the samples distribution to the involved partners. Two criteria were used for the selection of SSRs for the future genotyping of the collections: quality of SSR and genomic coverage. The final list of SSR markers to use in the characterization of collections (510 varieties) is the following:

Tab. 1: 16 SSR primers selected during the first bio molecular ring test

Linkage group	SSR primers	
1	UDP96-005	UDP98-022
2	CPPCT044	BPPCT001
3	BPPCT007	UDP96-008;
4	CPDCT045	BPPCT015
5	BPPCT017	BPPCT038
6	BPPCT025	UDP98-412
7	CPPCT022	EPPCU5176 (IRTA primer set)
8	CPPCT006	UDP98-409

The annex 3 shows the location of the 16 SSR primers used in the CPV.8648 project on the TxE SSR map from Dirlewanger et al., 2004.

- **Second ring test**

After this first ring test the bio molecular experts exchanged their experience in the case of appearance of new alleles, which can cause the disappearance of other ones. These preliminary results were validated by all the labs involved, on the base of a larger number of varieties.

Meanwhile, each lab had conducted analyses on their own varieties and identified 89 new alleles (i.e. not present in the first 12 genotypes). This leaded to a second ring test during which, the DNAs of the varieties exhibiting the new alleles were shared within the laboratories. Putting all the data together and comparing the results obtained on doubloons performed a final check.

The second ring test was the opportunity to exchange the DNA for the samples with new alleles and to send the obtained profiles. Some close varieties pairs have been identified to assess the powerfulness of the method. The results are encouraging.

2.   Characterization of the collection : 506 genotypes (included in the CPV.8648 project)

In total, more than 600 varieties were genotyped with less than 1% missing data. The first results revealed a few mislabeling cases in the collection as well as picking errors. This enabled the partners to clean up their collections and to construct procedures to detect picking errors.

Tab. 2: Summarized molecular statistics for the 16 SSR markers

	Marker	Major.AlleleFrequency	AlleleNo	Availability	PIC
1	UDP005	0.65	15	1.00	0.50
2	UDP022	0.53	9	0.96	0.61
3	CPPCT044	0.43	15	0.99	0.69
4	BPPCT001	0.43	13	0.99	0.73
5	UDP008	0.67	9	0.95	0.38
6	BPPCT007	0.44	13	0.99	0.55
7	CPDCT045	0.44	8	0.99	0.53
8	BPPCT015	0.57	19	0.99	0.59
9	BPPCT017	0.56	14	1.00	0.49
10	BPPCT038	0.62	12	1.00	0.51
11	UDP412	0.43	13	1.00	0.66
12	BPPCT025	0.61	17	0.99	0.58
13	CPPCT022	0.27	20	0.99	0.79
14	EPPCU5176	0.53	12	0.99	0.58
15	CPPCT006	0.58	8	0.97	0.52
16	UDP409	0.74	11	1.00	0.40
	Mean	**0.53**	**13**	**0.99**	**0.57**

- **Comparison with other works**

A preliminary processing of the data showed that the "CPVO program" collection offered a good coverage of the genetic variability encountered in peach. A higher number of alleles was encountered in this collection as compared with other works described in the scientific literature. We observed 33 supplementary alleles on a common set of 12 markers as compared with Aranzana (2010). To a lesser extent, we found more alleles than Yoon et al., (2006).

Tab. 3: Number of alleles found in CPV Project for each marker compared with two other works

	SSR	Number of Alleles			SSR	Number of Alleles		
		CPVO	Aranzana (2010)	Difference		CPVO	Yoon, (2006)	Difference
1	UDP 005	9	7	2	UDP 005	9	12	-3
3	CPPCT044	12	10	2				
4	BPPCT001	11	9	2				
5	UDP 008	7	5	2	UDP 008	7	6	1
6	BPPCT007	8	7	1				
8	BPPCT015	17	15	2				
9	BPPCT017	15	9	6	BPPCT017	15	12	3
10	BPPCT038	10	9	1	BPPCT038	10	12	-2
12	BPPCT025	12	10	2	BPPCT025	12	5	7
13	CPPCT022	16	10	6				
15	CPPCT006	6	3	3	CPPCT006	6	5	1
16	UDP 409	9	5	4	UDP 409	9	11	-2
	Total alleles	132	99	33	**Total alleles**	68	63	5

- **Genetic structure of the collection**

The varieties proposed by the 4 countries cover different ranges of genetic diversity:

Spain was mostly represented by low chilling, yellow, non-melting varieties (4 o'clock part of the tree). Although France and Italy display the largest variability, there are regions in the tree where the varieties proposed by both counties do not overlap.

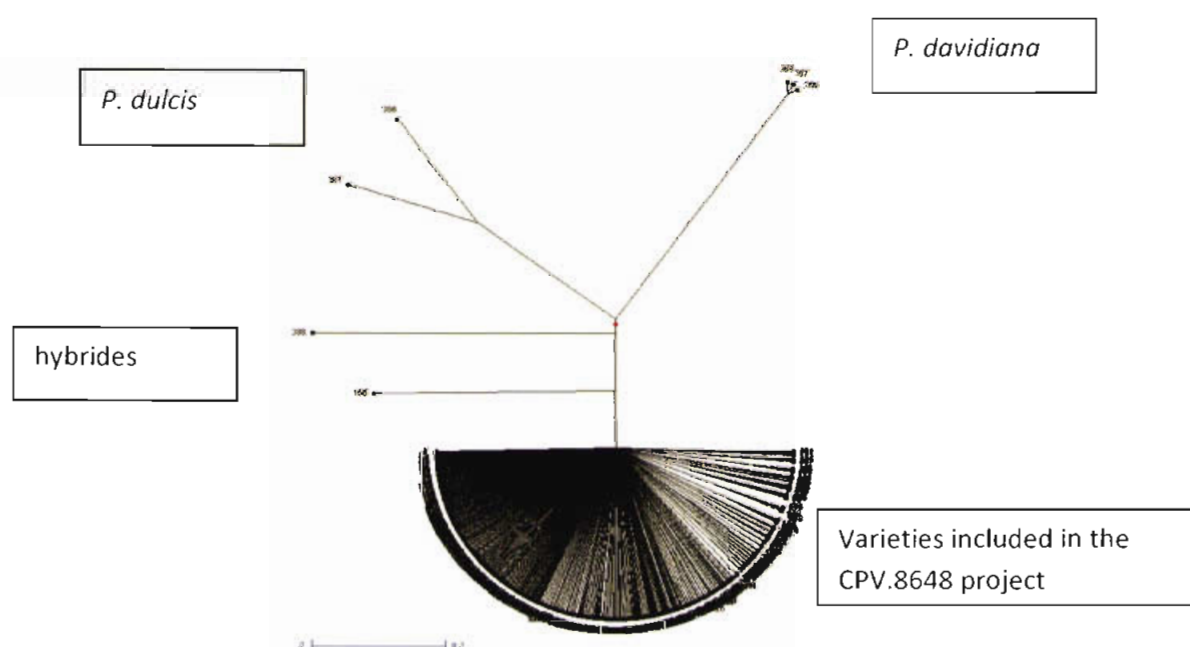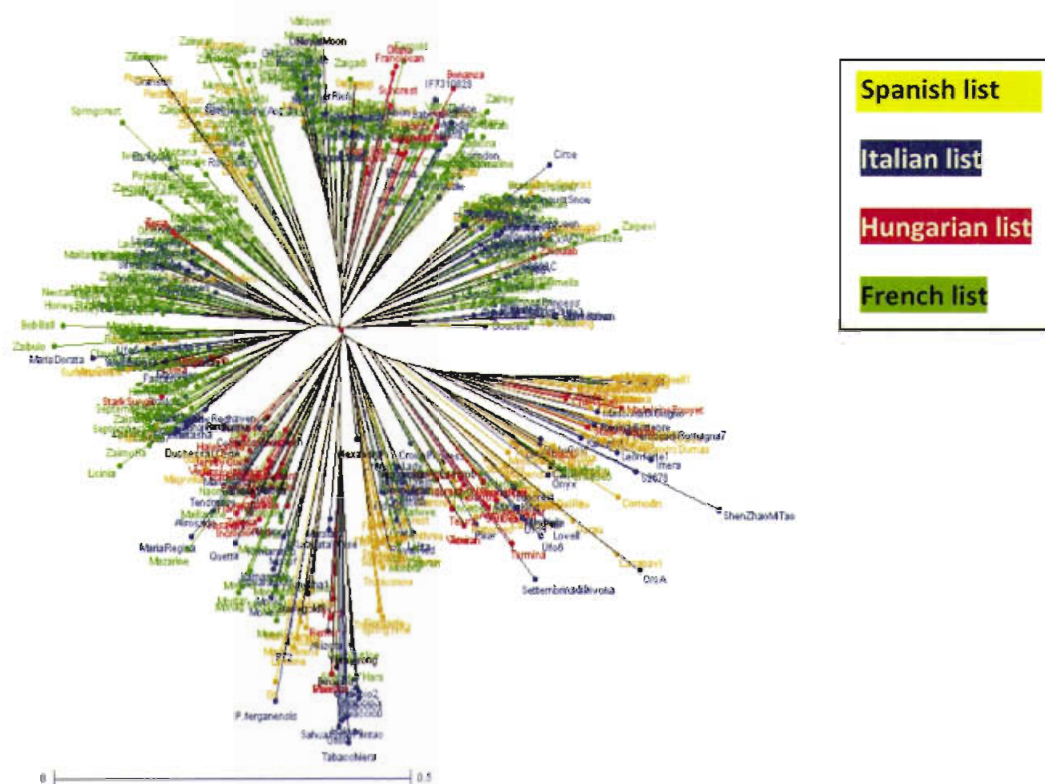**Figure 2: Phylogenetic tree presenting the CPV project collection varieties, hybrids, P.dulcis and P.davidiana**

P. davidiana

P. dulcis

hybrides

Varieties included in the CPV.8648 project

Figure 3: Tree representation of the CPV.8648 collection.

Conclusion

The CPV.8648 project allowed the establishment of a common data base to characterize routinely the peach tree reference collection. Its content will be enhanced annually, through a collaborative work of the examination office involved in DUS trials.

The fingerprint (with 16 SSR primers in this project) of the example varieties and new applications (whatever the purpose) provides a tool for topics such as:

- Maintenance of a living reference collection, through the verification of the bio molecular identity after a new supplying
- Use of a discriminatory and reproducible bio molecular pattern, obtained in different laboratories (interest in case of infringement for example)
- Screening - in association with the phenotypical characterization - of the reference collection to identify the possible examples varieties for a new application.

The use of this combined tool, could allow

- a better characterization of the candidate varieties by the examination offices (harmonized phenotypic and bio molecular descriptors)
- an earlier and more efficient pre-selection of the standard varieties to compare with the candidate variety (i) to provide an assistance in the case of very close varieties in order to assess of the minimal genetic distance, (ii) to limit as far as possible the use of additional cycles to finalize the DUS test, in the aim of maintaining the strength of the Plant Breeders Rights system.

A large data set was produces in CPV.8648 project, with more than 500 varieties during the program, and several partners followed their individual involvement with new candidate varieties in study.

The analysis of the dataset and the relationship between the bio molecular and phenotypical data is at its beginning. This very rich prospect (interpretation of raw data (biomolecular / phenotypical data) should be pursued further.

7

References

Aranzana M.J., Pineda A., Cosson P., Dirlewanger E., Ascasibar J., Cipriani G., Ryder C.D., Testolin R., Abbott A., King G.J., Iezzoni A.F., Arus P. (2003) A set of simple-sequence repeat (SSR) markers covering the Prunus genome. Theor. Appl. Genet. 106: 849-825.

Aranzana *et al.* (2010) Genetic variation, population structure and linkage disequilibrium in peach commercial varieties. BMC Genetics

Dirlewanger E. (1997) Recherche de marqueurs moléculaires pour la distinction de variétés de pêchers. Comité Technique Permanent de la Sélection des Plantes Cultivées (FRA). Marquage moléculaire appliqué à la distinction variétale. Estimation d'indices de distance entre variétés Séminaire, Paris (FRA) 1997/10/30:69-83.

Dirlewanger E, Graziano E, Joobeur T, Garriga-Calderé F, Cosson P, Howad W, Arús P (2004) Comparative mapping and marker-assisted selection in Rosaceae fruit crop. Proc Natl Acad Sci U S A 101: 9891-9896

Wunsch A, Carrera M, Hormaza JI. (2006) Molecular characterization of local Spanish peach [Prunus persica (L.) Batsch] germplasm. Genet. Res. Crop Evol. 53 (5): 925-932.

Yoon JH, Liu DC, Song WS, Liu WS, Zhang AM, Li SH (2006) Genetic diversity and ecogeographical phylogenetic relationships among peach and nectarine cultivars based on simple sequence repeat (SSR) markers. *J Amer Soc Hort Sci*, 131:513-521.

Annex 1 – BMT 2011:

Location of the example varieties for the biomolecular ring test included in the CPV.8648 project

On the dendrogram produced by Aranzana (2003)

Aranzana et al 2003
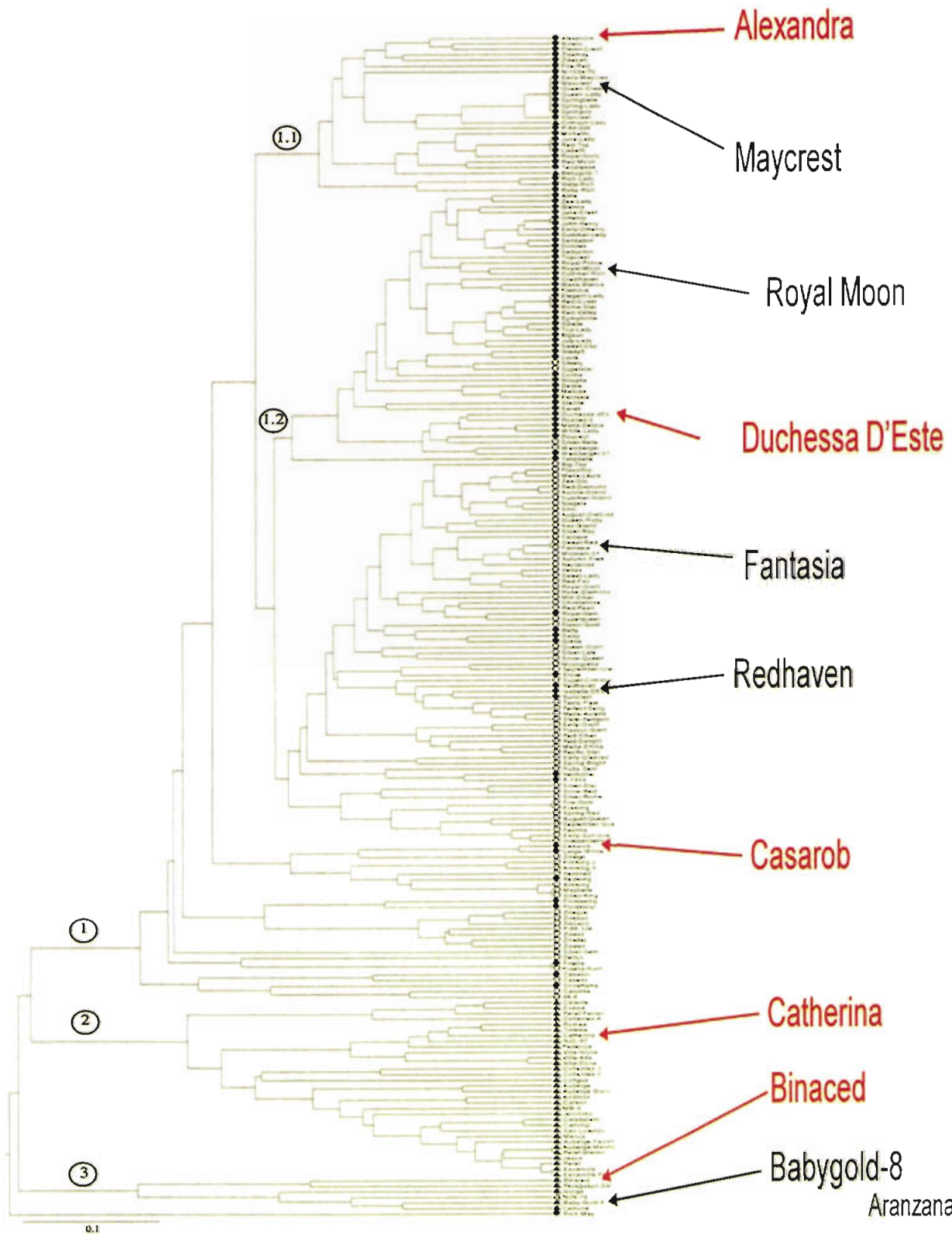
Annex 2 – BMT 2011 :

Protocol for SSR analysis validated for the CPV.8648 project

Amplification Mix

(CRA – FRU)

Buffer	1X
MgCl$_2$	1.5 mM
dNTPs	0.2 μM
primer F	0.1 μM
primer R	0.1 μM
Platinun Taq (Invitrogen)	0.5 U
DNA	da 2,5ng a 20ng/μl

PCR amplification

	10 cycles			25 cycles				
94°C	94°C			94°C				
10 :00	0 :30	*	72°C	0 :30		72°C	72°C	
		62°C	1 :00		50°C	1 :00	30 :00	
		0 :45			0 :45			4°C

*The annealing temperature is decreased 0.5°C per cycle from 62°C to reach 55°C.

TxE SSR Map (Dirlewanger et al 2004)

EPPCU5176: BIN 7:56 (interval position 49-56 cM via BIN mapping)

SSRs mapped in maps other than TxE

Annex 3 : Peach tree reference collection database : relational model
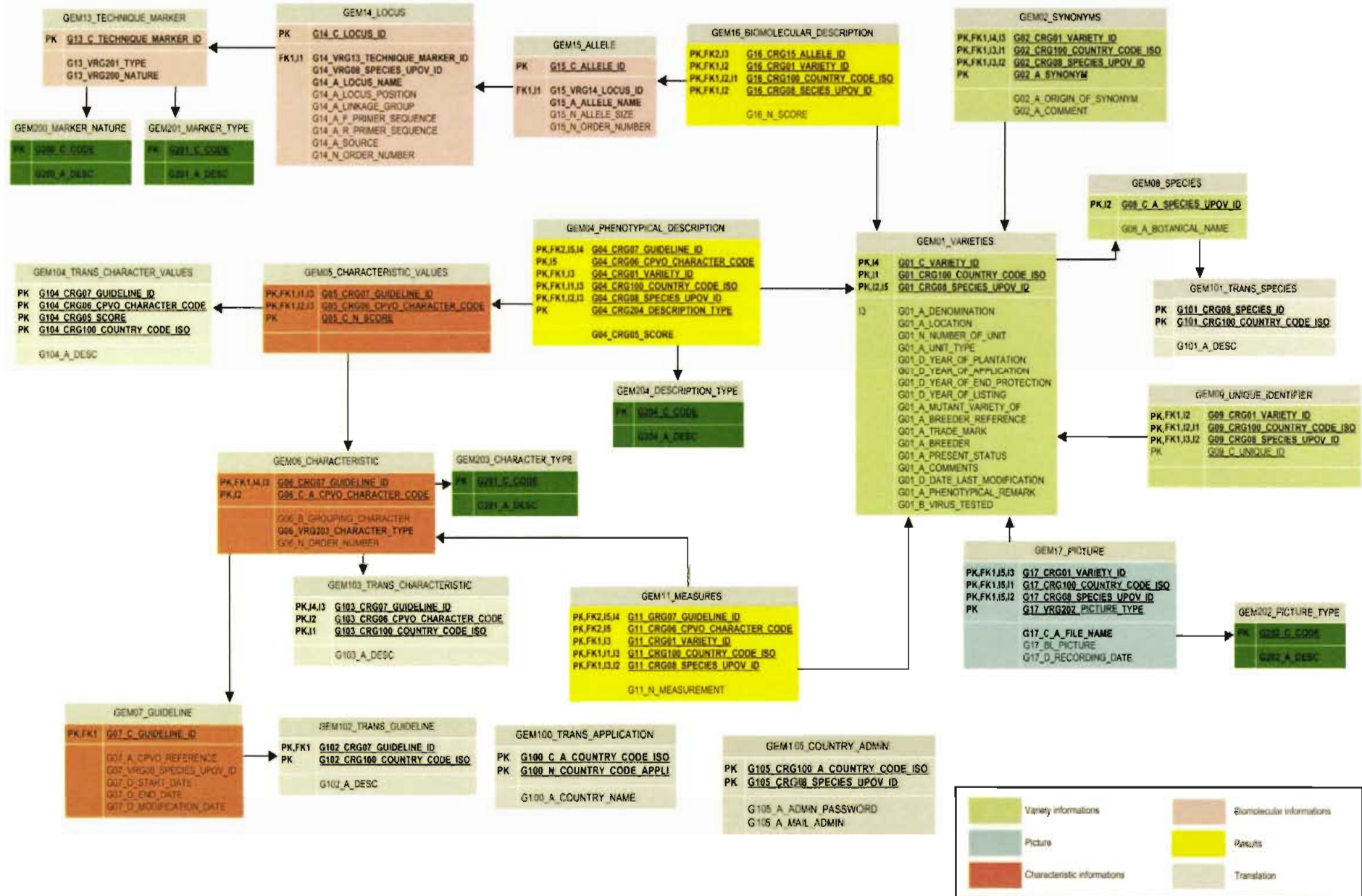
France (Coordinator):

Central contact: **Chrystelle JOUY: GEVES Cavaillon, 4790 route des Vignères, F-84250 Le Thor. Tel: 33 (0)4 90 78 66 64 - Fax: 33 (0)4 90 78 01 61 - e-mail: chrystelle.jouy@geves.fr**

Administrative and financial aspects: René PANN (till 31/12/2010)- Marie-Noëlle RACAT (since 01/01/2011) ; Filomena JUIGNET: GEVES Beaucouzé, Rue Georges Morel, F-49071 Beaucouzé CEDEX. Tel: 33 (0)2 41 22 86 10 - Fax: 02 41 22 86 02 e-mail: filomena.juignet@geves.fr

Scientific coordinator: Aurélia LUCIANI: Beaucouzé, Rue Georges Morel, F- 49071 Beaucouzé CEDEX. Tel: 33 (0)2 41 22 86 46 - Fax: 02 41 22 86 01- e-mail: aurelia.luciani@geves.fr

Database: Christelle GUITOUNI: GEVES Le Magneraud, Saint Pierre d'Amilly - BP52, F-17700 SURGERE. Tel: 33 (0)5 46 68 30 27 – Fax: 33 (0)5 46 68 30 24 – e-mail: christelle.guitouni@geves.fr

Digital Pictures, DUS tests: Marie-Hélène GANDELIN: GEVES Cavaillon, 4790 route des Vignères, F-84250 Le Thor. Tel: 33 (0)4 90 78 66 77 - Fax: 33 (0)4 90 78 01 61 - e-mail: marie-helene.gandelin@geves.fr

Christophe TUERO, Thierry PASCAL: INRA GAFL, Domaine St Maurice, F-84143 MONTFAVET - Tel: 33 (0)4 32 72 27 64 - Fax: 33 (0)4 32 72 27 02 - e-mail: christophe.tuero@avignon.inra.fr, tpascal@avignon.inra.fr

Molecular data: David ZHANG, Joëlle LALLEMAND

Anne BERNOLE: BIOGEVES, Domaine du Magneraud, Saint Pierre d'Amilly - BP52, F-17700 SURGERES. Tel: 33 (0)5 46 68 30 31– Fax: 33 (0)5 46 68 31 00– e-mail: anne.bernole@geves.fr

Hungaria:

Central contact: **ZS.FÜSTÖS: MgSzH Központ, BUDAPEST, Keleti Károly u.24. H-1024. Tel: 36 1 336 91 60 – Fax: 36 1 336 90 97 – e-mail: fustoszs@ommi.hu**

DUS tests and digital pictures: ZS. SZANI: MgSzH Központ, BUDAPEST, Keleti Károly u.24., H-1024 – Tel: 36 1 336 93 07 – Fax: 36 1 336 90 97 – email: szanizs@ommi.hu

Italy:

Central contact: **Ignazio Verde: CRA Centro di Ricerca per la Frutticoltura via di Fioranello, 52 00134 Roma – Tel: +390679348183 – Fax: +390679340158 – e-mail : ignazio.verde@entecra.it, ignazio.verde@mclink.it**

Database: Maria Teresa Dettori: CRA Centro di Ricerca per la Frutticoltura via di Fioranello, 52 00134 Roma – Tel: +390679348144 – Fax: +390679340158 – e-mail : mariateresa.dettori@entecra.it

Spain :

Central contact: PEDRO M . CHOMÉ: OEVV C/ Alfonso XII 62, 28014, Madrid – Tel: 34-913476913 – Fax: 34-913476703 – e-mail: pchomefu@mapya.es

Administrative and financial aspects and Database: VICENTE CEBOLLA: IVIA, Carretera Moncada-Náquera s/n, 46113 Moncada, Valencia Spain – Tel: 34-963424060 – Fax: 34-963424001 – e-mail: vcebolla@ivia.es

DUS tests, Molecular data, Digital pictures, Database and Administrative and financial aspects: J. M. ALONSO: CITA Montañana 176, Apartado de correos 617, 50008, Zaragoza, Spain – Tel: 34-976716310 – e-mail: jmalonsos@aragon.es

Mª LUISA BADENES: IVIA Carretera Moncada-Náquera s/n, 46113 Moncada, Valencia Spain – Tel : 34-963424049 - Fax: 34-963424106 – e-mail : mbadenes@ivia.es