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MANAGEMENT OF PEACH TREE REFERENCE COLLECTIONS

Document prepared by experts from France, Hungary, Spain and Italy

BACKGROUND

1. Peach/nectarine is an important fruit tree crop in Europe and indeed world-wide. The registration of new varieties, either for Plant Breeders' Rights (PBR) or National Listing (NLI) 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 relevant varieties of common knowledge (reference collection) to be included in the DUS test is steadily increasing annually. Moreover, DUS testing of this type of tree crop 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 a large number of varieties 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 standardized 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

2. The current Peach tree examination offices, GEVES (*Le Groupe d'Etude et de contrôle des Variétés et des Semences*) and INRA (*Institut National de la Recherche Agronomique*) for France, MgSzH (Mezőgazdasági Szakigazgatási Hivatal) for Hungary, CRA-FRU (*Centro di Ricerca per la Frutticoltura*) for Italy, and OEVV (*Oficina Española de Variedades Vegetales*), represented by IVIA (*Instituto Valenciano de Investigaciones Agrarias*) and CITA (*Centro de Investigación y Tecnología Agroalimentaria*), 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.

3. 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 standardized morphological descriptions, digital pictures, and a large data set of DNA-profiles for more than 500 peach tree varieties selected among the EU PBR granted and NLI varieties.

4. The steps of the project were:

a. for representative varieties, compilation and production of a set of standardized phenotypic data, including morphological descriptions and digital pictures
b. selection and test a set of SSR markers,
c. characterization of the varieties with these SSR markers,
d. creation and implementation of a database with these data.

5. This paper focuses on the results on biomolecular 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.

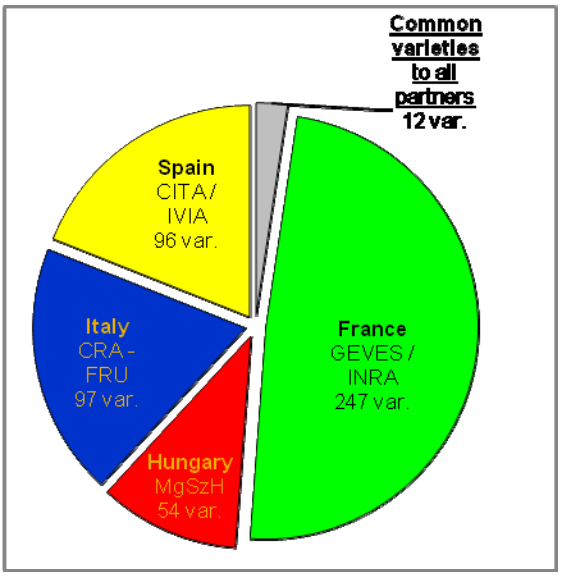
MATERIALS AND METHODS

- List of varieties included in the project

6. The number of varieties included in this project was selected to represent a realistic sample of the *Prunus persica* L. diversity. All these varieties are –at least:
    - Eegistered in a National List (trading authorization), and / or protected at a national or European level (Plant Breeder Rights)
    - 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

7. A molecular ring test was performed by the participating laboratories. The purpose of the molecular ring test was (i) to harmonize 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 program.

8. 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).

9. 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. 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

10. All partner 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

11. 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

12. 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 appeared to be the best adapted and was therefore accepted by all participating laboratories to use for the ring test as well as for the future genotyping of the peach collections The chosen protocol is described in Annex 2.

RESULTS AND DISCUSSIONS

Ring tests

First ring test

13. 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 laboratories, 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

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

14. After this first ring test the biomolecular 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 laboratories involved, on the base of a larger number of varieties.

15. Meanwhile, each laboratory had conducted analyses on their own varieties and identified 89 new alleles (i.e. not present in the first 12 genotypes). This led 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.

16. 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.

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

17. 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

18. 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

		Number of Alleles				Number of Alleles		
	SSR	CPVO	**Aranzana (2010)**	Difference	**SSR**	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

19. 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

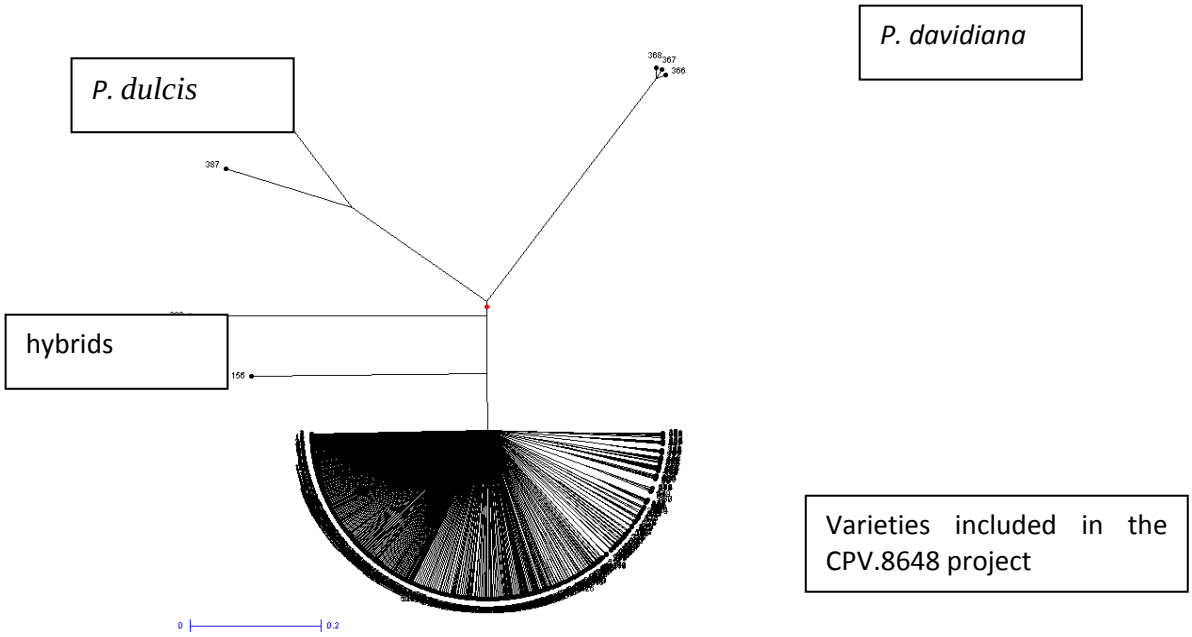
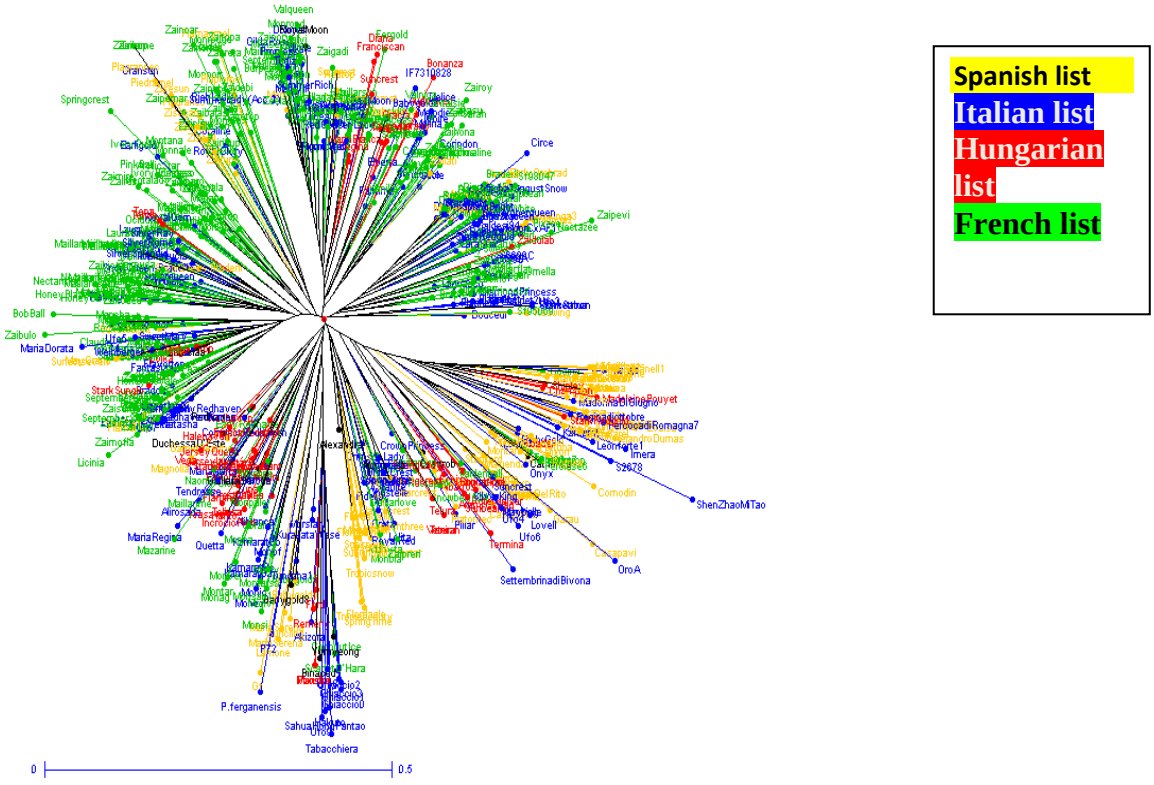


Figure 3: Tree representation of the CPV.8648 collection.



CONCLUSION

20. 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.

21. 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 biomolecular identity after a new supplying
- Use of a discriminatory and reproducible biomolecular 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.

22. 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 varieties of common knowledge 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.

23. 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.

24. 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.

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