

1. Final publishable summary report

1.1 Executive summary

The FP7 European research project FruitBreedomics has come to an end. It has been 54 months of intense research activity by many researchers from 28 research institutes and private companies to fulfil the goal of bridging the gap between genomics and breeding in fruit trees. The approach taken to close this gap has been comprehensive. It has encompassed the generation of new tools, methods and plant material that commercial breeders of fruit varieties can implement and use in their day to day effort to create new cultivars better suited to growers, shippers, retailers and consumers. These are the highlights of what the project has achieved during this endeavour:

1. New plant material of apple and peach that can be used as parent lines on breeding programs with new resistances to apple scab, powdery mildew and fire blight (apple) and green aphid and brown rot (peach) thanks to the use of genomics tools.
2. Fast breeding on apple. First case of fast introduction of fire-blight resistance to parent lines. A total of 39 BC'4 plants carrying the early flowering gene and the fire blight resistance from 'Evereste' or M. ×robusta 5 were generated in less than 7 years.
3. New genomic tools that enable the discovery of new associations of markers and traits of economic interest: two new apple array chips with 20000 SNP and 487000 SNP markers. These chips are the foundations of genetic and genomic studies to find robust markers for traits of economic interest like disease resistances, fruit quality, and biotic stress resistances among others.
4. New molecular markers closely linked to important traits such as fruit quality and biotic and abiotic resistances to be used for marker assisted selection and genetic studies. These new SNP markers have been found by using two different approaches: Pedigree Based Analysis (PBA) and Genome Wide Association studies (GWAS).
5. New knowledge about the European apple and peach germplasm structure. This knowledge will help breeders to choose for new parent material to grasp and introduce new variability into their breeding programs.
6. New methods to assess new traits of interest for apple and peach. In particular, methods to assess fruit texture in both crops, resistance to *Monilinia* in peach and drought tolerance in apple have been developed.
7. A complete and thorough assessment of a pipeline for Marker Assisted Breeding on apple and peach. Pilot studies on apple and peach have shown fully the applicability and robustness of Marker Assisted Breeding on these species.
8. First attempts to further increase the efficiency of breeding programs by using Genome Wide Selection on apple and peach.
9. A platform, FruitBreedomics Marker Assisted Breeding Services, to transfer results to fruit breeding companies.

Thanks to the effort and the reactivity of the whole consortium, the project has achieved much beyond the objectives established at the beginning of the project and has contributed to a higher efficiency in commercial breeding programs by generating new knowledge and putting it into work. For the longer term, new, better suited cultivars will be available to all actors as a result of the project impacts

1.2 Summary description of the project context and objectives

FruitBreedomics main aim was to fill in the gap between research and fruit breeding. It was structured in 7 scientific WPs, one dissemination WP and one management WP. WP1 and WP2 were dealing with breeding and prebreeding respectively; WP3 and WP4 aimed at deciphering genetic bases of traits taking into account allelic diversity; WP5 was dedicated to improve

methodologies to assess for fruit quality, biotic and abiotic stresses. WP6 developed the genotyping tools necessary for the previous WPs; WP7 created and managed the database that stored the data of the project and develop tools for the breeders and scientists to make the best use of these data.

The general objective of WP1 was to develop more efficient breeding programmes applying markers assisted breeding. To reach this goal, WP1 aimed at designing and developing i) breeding strategies and schemes to be tested in pilot studies; ii) molecular markers (SNPs) tightly linked to specific major horticultural traits; iii) cost-efficient marker-assisted breeding (MAB) pipeline(s); and iv) Genome Wide Selection first tests.

WP2 was aimed at the establishment of pre-breeding material in apple and peach in a more efficient way and the improvement and application of a fast-breeding system in apple. Pre-breeding material can be defined as material carrying new traits or trait combinations which can be used as donors to improve cultivar breeding by the introduction of the traits. The development of pre-breeding material is necessary if desired traits or combinations are not present in existing advanced breeding material and when the horticultural characteristics of these donors are far from advanced breeding material and/or when the conventional introgression process is too time consuming or expensive. Availability of new resistance genes, new combinations of resistance and/or quality traits and material homozygous for some important genes are useful for breeders to improve breeding of apple and peach cultivars more suited for sustainable production. The efficiency of pre-breeding has been increased by the choice of donors, the selection of progeny with less genetic drag (apple and peach) and the development of strategies to shorten the generation cycle in apple.

The overall aim of WP3 was to increase knowledge on the inheritance of important traits and to provide DNA diagnostic tools which would support breeders in the breeding of new cultivars with excellent fruit characteristics, resistance to some major diseases, and, for regions with warm winters, adaptive traits to global warming. WP3 also aimed at providing geneticists with new tools and approaches for the performance evaluation of DNA based inheritance studies. The following sub-aims were considered: i) making available new approaches and software for the discovery of marker-trait correlations, ii) increasing knowledge on the number of genes that underlie observed phenotypic variation of major traits, iii) pinpointing the position of these genes to a narrow region of the relative chromosomes, iv) identifying various sources for the desired genes (alleles), v) identifying predictive SNP markers for most of these genes and sources, and vi) increasing knowledge on the genetic diversity that is currently used in European breeding programs.

WP4 objectives were to: i) improve the knowledge of genetic variability in European apple and peach collections by exploring the phenotypic and allelic diversity available in European apple and peach germplasm collections (variability, population structure, LD) and defining potential national and European Core collections (CC); ii) identify genomic regions contributing to the genetic control of agronomic traits through GWAS by discovering new and by confirming previously identified QTLs, exploring QTL-allele diversity and reducing confidence interval (CI) around QTLs; and iii) in combination with WP3, supply WP1 with tightly linked molecular markers for implementation into the MAB pipeline.

The objectives of WP5 were to develop tools for novel trait determination and make them high throughput and applicable for the research community and the breeding industry. To breed innovative fruit varieties it is necessary to characterize thousands of individuals to select only a few with the best interesting profiles. Some traits like resistances to diseases of certain fruit attributes are either difficult to assess or time consuming. Taking into account new consumer trends, global climate change, environmental concerns and consultation with stakeholders, FruitBreedomics decided to develop new phenotyping tools for attributes considered to be of key importance for the fruit industry, i.e. fruit texture and resistance for some biotic and abiotic stresses for which no simple, reliable and high throughput assessment methods were previously available.

The objective of WP6 was to develop advanced technological tools to allow genome-wide genotyping of the apple and peach genomes at high marker density and with molecular markers well distributed along the chromosomes. These tools allowed: (a) to assess LD decay in the apple and peach genomes; (b) to efficiently construct high density genetic maps in apple and peach; (c) to

perform Pedigree Based Analyses (PBA) and genome wide association (GWA) analysis on apple and peach. To reach these goals, WP6 technical objectives were:

- To develop a low-density genotyping array for efficient gene/QTL mapping in apple.
- To develop species-specific SNP arrays suitable for pedigree-based analyses and for GWA mapping in apple and peach
- To test new low-expensive high-throughput technologies for marker-assisted breeding.

The FruitBreed database planned by WP7 was intended to provide a unique tool specifically adapted for geneticists and breeders who aim to combine phenotypic and molecular marker data. This database will be useful in intercontinental collaboration on marker-trait associations, validation of candidate genes and functional allelic diversity. Its structure forms a firm foundation on which other users can build their own applications. Several software tools, explicitly designed to leverage the wide range of stored data, will help both breeders and scientist to design marker-assisted breeding programs.

The objectives of WP8 were to: i) make the best possible use of the project results by the stakeholders of the fruit industry, notably but not exclusively breeders; ii) ensure fruitful exchange with the scientific community, including individuals and collaborative initiatives, senior and early scientists; and iii) inform European citizens and policy makers about the stakes of the project, the challenges encountered by the fruit industry, the impact on citizens' well-being, and the contributions brought by the project to respond to stakeholders' needs and meet consumer demands.

1.3 Description of the main Scientific and Technical results/foregrounds

FruitBreedomics has achieved all the goals which were planned at the beginning; the consortium has been very active and reactive to take into account the last methodologies and technologies and make them available for the fruit scientists, germplasm curators and breeders.

WP1:

During the first year of the project WP1 performed two surveys, one for the apple and one for the peach. Stakeholders as well as partners of the project were asked to describe their breeding programs and breeding aims. The surveys enabled us to identify the most important traits for apple and peach breeders. For the apple the most important five traits in breeding are: resistance to apple scab, storability, juiciness, crispness and firmness. For the peach the most important traits refer to fruit quality (considering both appearance and sensorial aspects) and resistance: fruit size, homogeneity and type, juiciness, crispness and sweetness, and finally brown rot, mites and green peach aphid resistance. The traits that have been prioritized by the stakeholders are in good agreement with the traits that have been selected for the genetic studies of FruitBreedomics. The same survey also allowed having a first insight on how apple and peach are currently breed and selected.

At the same time the identification of SNP markers associated to specific apple and peach traits/loci started. The work led to the identification of several SNP markers. For the apple SNP markers have been found for the apple scab resistance genes *Rvi2*, *Rvi4*, *Rvi5*, *Rvi6*, *Rvi11*, *Rvi12*, *Rvi13*, *Rvi15*, and resistance QTLs on LG17 of Fiesta and Discovery, the fire blight resistance genes *FB-MR5*, *FB-E* and the QTL *FB-F7*, the powdery mildew resistance gene *PI2* and two QTLs on LG 2 and 13 of TN10-8, and the rosy apple aphid resistance gene *Dp-FI*. For the peach SNP markers have been found for fruit low acidity (locus *D*); fruit shape (flat/round, locus *S*) glabrous fruit epidermis (peach/nectarine locus *G*); fruit flesh colour (white/yellow locus *Y*), non-melting (locus *F*) and for the green peach aphid resistance gene *Rm2* and two QTLs on LG3 and LG5, and finally for the powdery mildew resistance gene *Vr2* and two QTLs on LG6 and LG8.

Another aim of this workpackage was to develop a protocol in which all the steps necessary to perform MAB have been optimized. Such a protocol has been developed. Firstly, a very efficient

system for the tracking of seedlings has been proposed which avoids the time consuming single labelling of each seedling. Secondly, in collaboration with WP6 a genotyping platform has been identified and tested in the apple and peach pilot studies. This platform can be used also by breeders without an own DNA lab. The apple and peach pilot studies had two aims: the first was to validate the DNA based predictions using the SNP markers; the second was to test the optimized MAB protocol and the selected genotyping platform. For the loci that could be validated, the predictions done with the SNP markers were correct in close or above 90% of the cases. The selection strategy tested in an apple pilot study allowed identifying 89 seedlings among over 5000 which combined pyramided resistance genes with best growth characteristics and potentially best fruit quality traits (i.e. low ethylene production and best firmness).

Beside the “regular” ways of making these scientific results publically available through peer reviewed publications, efforts were made to encounter breeders. Stakeholder days dedicated to the breeders as well as a workshop dedicated to MAB have been organized, and a booklet on MAB has been written and made available through the homepage of the project.

In the course of the project we realized that the development of cost-effective markers (SNP), the optimization of a MAB protocol and identification of an efficient genotyping platform was not sufficient to facilitate the access of MAB of breeders that are willing to start applying molecular markers in their program. Coaching of such “beginners” showed to be necessary. To cope with this problem FruitBreedomics MAB Services have been launched. FruitBreedomics MAB Services is a system devised to efficiently transfer Marker Assisted Breeding related information to public and commercial Rosaceae breeding programs. It is a service offered on a commercial basis targeting FruitBreedomics stakeholder breeding programs. Initially it has been provided by a reduced number of service providers related to project partners (INRA, FEM and IRTA). The service has been provided based on information gathered within FruitBreedomics and other relevant external sources.

Another task performed in the frame of WP1 was the preparation of the list of specifications of what we called the “breeder-interface”. The breeder interface is a tool to interrogate the FruitBreedomics database in order to allow breeders to retrieve the information necessary to select the best combinations of parents for the development of a specific ideotype and to interpret MAB results. Such an interface has been produced by WP7 and tested by WP1 breeders that proposed tools/queries to be implemented in the interface.

Finally in WP1 a Genome Wide Selection (GWS) experiment in apple was successfully performed. A GWS prediction model from the marker and phenotypic data on the training families, mostly originating from WP3 has been constructed and the Genomic Breeding Values (GBV) for the application families has been predicted. The accuracy of estimated genomic breeding values was estimated as the correlation between these predicted GBV's and observed phenotypes. It varied largely among traits and among applications progenies and ranged between -0.31 and 0.68. Genomic relatedness between test populations and training population did not explain well differences in accuracy between the test progenies.

WP2:

Detailed descriptions of available donors were established. A total of 42 donors have been listed for apple. Among these were six with the *Rvi11* scab resistance gene originating from *M. baccata* jackii, seven with the markers surrounding the fire blight QTL from *M. x robusta* 5 and the marker for *PI1*, three with the pyramided powdery mildew resistance genes *PI1* and *PI2*, seven with *PI1*, *PI2* and *Plm*, six with different combinations of *Rvi2*, *Rvi4* and *Rvi6*. Some of these later possess additionally one or two powdery mildew resistance genes and/or the fire blight QTL of Fiesta on linkage group 7 (*FB-F7*). Furthermore donors with single or two resistances as well as donors for fruit quality and for low chilling have been described and the data transferred in to the data base. For peach a total of 124 donors have been described. This includes 8 individuals with strong, 8 individuals with medium and 17 with low resistance to brown rot. Some of them carry additionally powdery mildew, green peach aphid or leaf curl resistances. Powdery mildew resistance genes are present in 42

individuals, the majority carrying *Vr1*, some *Vr2*, two both *Vr1* and *Vr2*, and some additionally a powdery mildew QTL. One plant carrying a combination of the two green peach aphid resistance genes *Rm1* and *Rm2* is described as well as combinations of powdery mildew and green peach aphid resistance genes. Resistance to leaf curl is present in six peach individuals and resistance to plum pox virus in one plant. Among these 124 donors both nectarine and peach types are present as well as melting and non-melting and acidic and sub acidic types.

A total of 36 crosses were done in apple for the following purposes: introduction of scab resistance genes originating from *M. baccata* jackii (*Rvi11*) and Hansen's *baccata* No. 2 (*Rvi12*), pyramiding of scab and powdery mildew resistance genes, improving of quality and the introduction of low chilling, specific shape, rich flavour and low allergenicity. Since Hansen's *baccata* No. 2 carries two resistance genes the mapping and development of markers for *Rvi12* (*Vbsc*) was performed.

Using phenotypic and molecular tools genotypes with *Rvi11*, *Rvi12*, individuals with three scab resistance genes, two powdery mildew resistance genes and the fire blight QTL from Fiesta (*FB-F7*) as well as individuals showing the markers for two scab resistance genes, three powdery mildew resistance genes and *FB-F7*, too, could be selected. The first pseudo-backcrosses to improve quality could be performed with progenies carrying pyramided powdery mildew resistance genes and seedlings with *Rvi11*. Further selection for fruit quality is required.

In peach 83 crosses were aimed to combine resistances to powdery mildew, green peach aphid, and brown rot. An interspecific cross of peach x almond was performed to introduce the strong resistances to plum pox virus (PPV), green peach aphid, and powdery mildew observed in the almond cultivar Del Cid into peach. Crosses were done regarding the traits peach/nectarine, flat shape (*S**), glabrous skin (*g*), non-acid flesh (*D*) and bloody flesh (*bf*), too. Selection was done using phenotypic screenings, like the artificial inoculation of brown rot, and/or molecular markers. Up to now two peach/almond hybrids resistant to the PPV, green peach aphid and powdery mildew as well as 52 seedlings with pyramided green peach aphid resistances, three individuals tolerant to brown rot and two nectarine types with good resistance to powdery mildew could be selected. Further selection work is in progress.

The early flowering experiments performed in the frame of FruitBreedomics clearly confirmed that this approach allows a turnaround of one generation per year. Individuals with the *FB_E* fire blight resistance locus from 'Evereste' have been developed from five successive crosses (one cross pollination and 4 backcrosses) in less than 7 years (note that one year was "lost" due to technical problems) for. Similarly for *FB_MR5*, 4 successive crosses were made in only four years. By classical breeding the production of the same generation would have taken at least 25 years. Performing artificial inoculations with *E. amylovora* demonstrated that the final products of the early flowering approach possess a high level of fire blight resistance. As a result, very valuable pre-breeding material became available for the selection of new fire blight resistant cultivars by classical breeding. As start for conventional breeding, pre-breeding material (at least BC₄) without the early flowering gene but carrying the fire blight resistance gene will be selected. As in the USA such pre-breeding genotypes are not considered as genetically modified, US apple breeders can readily start using them. This chance will be precluded to European apple breeders until these genotypes will not be deregulated.

The improvement of the fast breeding approach by using a heat shock promoter to induce flowering by the repression of *TFL1* (terminate flower 1 gene) giving a heat shock failed. Nevertheless the development of transgenic lines carrying the early flowering gene (*BpMADS4*) on different linkage groups was successful. *BpMADS4* lines carrying the flowering gene on linkage groups 2, 4, 5, 7, 8, 9, 10, 14, 15, 16 are available now.

WP3:

Multiple segregating families from known parents were examined. Each individual of these families was characterised for various traits, including phenology, fruit quality, productivity and resistance to pathogens, and by its DNA profile obtained with thousands of SNP markers. Next the data were

surveyed for the presence of strong co-inheritance patterns between traits and markers, in which case one or more predictive DNA markers had been found for each trait. The traced markers revealed the position of the involved genes on the apple or peach chromosomes and also provided information on the number of genes involved. This information can be used by breeders, amongst others, to predict which combination of parental cultivars have the best chance to give outstanding progeny.

For the inheritance studies, FruitBreedomics decided to analyse multiple segregating families simultaneously as this would increase statistical power compared to current approaches on single families. FruitBreedomics also headed for the use of breeding germplasm as this would increase applicability of results compared to 'scientific' crosses.

New software

Developed software packages provide an efficient workflow for genetic studies using SNP markers, starting from the filtering and calling of markers through the building of haploblocks and haplotypes to PBA based QTL analyses. Firstly, the ASSIsT software allows a smooth and fast filtering of those SNP markers that can be called in a reliable way. This software is dedicated to SNP that are generated through the Infinium platform.

FlexQTL™ was developed in the previous EU-HiDRAS project to enable QTL analyses on multi-family and was ready for the use of SSR markers. FruitBreedomics has successfully extended the software FlexQTL™ for use on SNP markers. As tens of thousands SNP markers can now be tested for less than the price of a single SSR marker, sizes of datasets increased dramatically. On the other hand, single SNP markers are more difficult in use on complex germplasm because biallelic markers are less informative. Thanks to FruitBreedomics, FlexQTL™ was successfully adapted for application to high density SNP- genotyping.

To further enhance the applicability of SNP data in genetic analyses, the PediHaplotyper software has been developed. It compresses the information from sets of SNP markers into a single, virtual haploblock marker, thus providing a much smaller data set without losing in information. These compressed data require less computation time and computer memory and are also more amendable for visual inspection.

Marker-trait associations & predictive markers

Numerous marker trait associations have been discovered for traits related to taste (e.g. texture, sourness, sweetness, juiciness), appearance (skin colour, size), disease resistance (*Neonectria* canker, *Monilinia* rot), production (crop load, pre-harvest drop), storability, maturity and flowering date, and for traits for adaptation to global warming (chilling & heat requirement). Many of these associations are new. In cases where these were known, current results often allowed to precise the position of the related genes and to identify a range of cultivars that have these desired genes. The discovered associations also provided the first sets of predictive markers for these traits. The first three papers have been submitted. Nevertheless, most of the outcomes have still to be documented through papers, which are on their way.

Location of genes: genetic linkage maps & new SNP markers

The accuracy by which gene positions can be localised is affected by many factors, including the order of the markers used in the analyses. Ideally, this order is identical to their true order on the chromosomes. For peach, this order can be adequately deduced from the known genome sequence, due to the high quality of this sequence. For apple, this approach could not be followed as use of this sequence resulted in too many mismatches. Therefore, the order of markers was determined by means of new co-inheritance studies: the more nearby, the stronger the co-inheritance of markers from parents to offspring. For this work, 21 families were used. New innovative approaches were used in the analyses, which led to a high quality genetic map that includes over 14 thousand SNP markers. Next, this map was used in another FruitBreedomics work package (WP6) to improve the apple reference genome sequence.

In peach, the ability to precise gene positions to a very confined region is regularly limited due to shortage of informative markers. Here, informative means that a marker reveals a difference

(polymorphism) between two homologous chromosomes. In FruitBreedomics over 1.3 million new polymorphisms have been identified by examining the genome sequence of four important parents through a re-sequencing experiment. Next, some new SNP markers were designed and applied on some specific families, which effort narrowed down the potential position of a Maturity Date gene by 27% to only 159Kb. The new polymorphism will also be used for the design of an updated Illumina peach SNP chip in the context of an international collaboration involving FruitBreedomics and RosBREED partners.

Genetic Sources

Knowledge on the presence of specific alleles in the parents and ancestors of the above mentioned families was extrapolated to other cultivars through the use of pedigree information and marker data. Hereto, over 800 apple cultivars and breeding selections were SNP genotyped. The data allowed validation of known pedigree records and also to trace parentages of over 50 individuals for which pedigree records were lacking. Thanks to this effort, breeders can now for the first time know which cultivars share desired alleles for a large set of cultivars and genes. FruitBreedomics thus generated unique information that can be used in choosing breeding parents.

Explored genetic diversity

In Europe, apple production has a narrow genetic base: half of the production comes from just four cultivars. Moreover, the 20 most produced cultivars show close genetic relationships: 75% of their ancestry is based on four dominant founding clones. This tendency towards mono-culture could be 'vulnerable to a catastrophe', e.g. in case a new disease would show up. In 1991 Way et al. already alerted that 'careful consideration of pedigrees and increased size of the genetic base are needed in future apple breeding strategies'. FruitBreedomics surveyed the genetic diversity that is currently used in apple breeding. Seventeen European programs participated, as well as a program from New Zealand and the USA. Over 3200 crosses and over 3000 ancestors were examined for their founder composition. Usually, programs build on a genetically narrow framework of a few founders. However, overall up to 40% of the genetic composition of modern germplasm is of highly diverse origin coming from up to 90 founders within a single breeding program. The data document the huge efforts of breeders to include new traits from very distinct genetic resources, including wild species. These results will be detailed in a coming paper, together with some recommendations to come to an even better use of the available genetic diversity.

WP4:

Considering that a large number of apple and peach accessions had been maintained and evaluated for several traits and years in European repositories, we first surveyed and made available a list of the accessions existing within a number of key European genebanks (5,477 apples and 2,885 peaches), together with a short description of the traits and phenotypic records available, with their respective protocols. After several meetings and debates, common protocols and descriptors were defined for both apple and peach. Afterwards subsets of accessions were selected for each collection (1,560 apples and 1,296 peaches) and their phenotypic records transferred to the FruitBreedomics database following the scales agreed. The peach collections considered were those maintained by the UMIL, CRA, INRA and IRTA plus a valuable Chinese collection representing a large portion of peach variability absent in the European germplasm collections (ZJU leader group of Chinese partners). The apple collections were those maintained by CRA-W, VNIISP, NCRRIH&V, SLU, University of Bologna, RBIPH, University of Reading and INRA-Angers. Phenotypic records for tree architecture, phenology, fruit quality and some disease resistances records were uploaded to the FruitBreedomics database. Thereafter subsets of accessions were selected as representative of the collections based on curators knowledge and/or available genotypic (SSR) data. These subsets of accessions have been phenotyped for key selected traits for at least two years in their respective location. As a result, thousands of data entries are now available to all FruitBreedomics partners through the FruitBreedomics database. This database will be made available to the public 2 years after the end of the project. This

information stands as a valuable tool for breeders, for diversity analysis and for genome-wide association studies (among others).

Genotypic diversity was evaluated in both species. In several apple collections (those from INRA, UNIBO and READING) a large fraction of the accessions had been previously genotyped with SSR markers. Eight of these markers were commonly used in the three collections. A large number of additional apple accessions were genotyped for diversity analysis with these 8 SSRs and complemented with 8 more. The initial sample selected for genotyping contained 1,000 accessions. However the high interest shown by apple curators from additional collections raised the number of accessions to 2,700. The final sample included accessions from germplasm collections located in Belgium, France, Italy, United Kingdom, Czech Republic, Sweden, Switzerland, Spain, Russia and Kyrgyzstan. SSR data were used to investigate the genetic structure of these 2,700 accessions, detecting differentiation according to the geographic origin (West, South, North-East). Overall, the genetic diversity was very high, but with a weak structure, confirming large gene flow across Europe. Genetic diversity of more than 1,500 peach accessions was evaluated with the Illumina 9K peach SNP array. Data allowed the detection of synonyms. Peach population structure correlated with breeding strategies (breeding and local landraces) and also with geographic origin (Occidental and Oriental (Asia)). Such data have been extremely useful to identify clones and synonyms within and between collections, which will aid in a more efficient management of the collections. This combined and co-ordinated dataset represents a significant development in the co-ordination of European genetic resource collections, and will offer valuable base for continued efforts in bringing further germplasm collections together in the future.

Genotypic data together with phenotypic information obtained before and during FruitBreedomics allowed the definition of core collections (CCs) representative for partner's repositories as well as a European CC per species intended for future research studies. This European CC is planned to be grown and evaluated in various locations over Europe (subject to further funding and/or initiatives): for apple, experimental plots will be located in Belgium, France, Italy, Poland, Spain, and Switzerland. For peach, plots will be located in Italy (2 sites), Spain, and Greece. This collection should allow evaluating the behaviour of the same genotypes under different climatic conditions, deciphering interaction between genotype and environment. Such analyses will be critical to addressing the new challenges of breeding fruit suited to changing climates.

Peach SNP data have been used to study the pattern of linkage disequilibrium (LD) in peach confirming its slow decay with distance, especially in the Chinese collection where loci separated by 1.8 Mbp can still pass to the offspring simultaneously. Despite these large DNA regions inherited as blocks, a GWAS analysis on monogenic traits validated the use of the SNP array for LD mapping. A detailed analysis of the most frequent SNP haplotypes in European breeding programmes for the loci *G* (peach/nectarine), *Y* (white/yellow flesh), *S* (flat/round fruit shape) and *D* (acid/subacid fruits) allowed markers to be designed for marker assisted selection (MAS). Such markers were transferred to WP1 and their use was demonstrated in pilot studies. In apple, the performance of the previously available 20k SNP chip was evaluated on a subset of 48 most diverse cultivars from the INRA collection. Analysis confirmed a fast decay of LD, indicating that markers at distance higher than 55 kb were already inherited independently (i.e. a SNP marker 55 Kb from a causal allele could not predict the trait); consequently a SNP array with higher density was required for GWAS analysis. Coordinated efforts between FruitBreedomics WP4, WP3 and WP6 partners succeeded in the design of an Axiom array with 487k-SNPs. This array has been used to genotype a collection of 1,100 accessions and two small mapping populations, and represents a significant step forward in genotyping tools for the European (and global) research community.

In summary, a large collection of apple and peach genotypes has been characterized with state-of-the-art SNP chips; robust data have been deposited in the FruitBreedomics database. For both crops, genotypic data and phenotypic information for agronomic key traits (tree architecture, phenology, fruit quality, disease resistances) acquired before and during FruitBreedomics have been put together to carry out Genome-wide association studies. Such analyses have been performed per National collection as on the entire SNP genotyped collection for traits with high

heritability. In general the local analysis detected already known but also some new QTLs, while the integrated analysis reduced the confidence interval of the QTLs.

These results are the successful outcome of a strong and fluent coordinated effort of curators, breeders and geneticists, generating new tools which are ready to be applied by curators and breeders and providing valuable knowledge which will underpin future success in fruit tree genetic research and breeding.

WP5:

Fruit texture

On apple, fruit texture, is a complex trait of several components. Classical approaches to assess fruit texture include mechanical assessments through a penetrometer and sensorial assessments. In FruitBreedomics a new approach was examined that also includes the acoustic properties of fruit. A novel device was used which simultaneously acquires the two texture components represented by mechanical and acoustic profiles. Results showed that crispness is only partially related to the mechanical texture behaviour which means that current methods fail to capture this component of texture. With this new instrument it is now possible to dissect the texture more precisely and to perform a more detailed investigation of each single component.

In peaches, texture is generally classified into melting flesh (MF), slow melting flesh (SMF), non-melting flesh (NMF) and stony-hard (SH) based on the different fruit softening patterns. While the assessment of NMF or SH traits are relatively easy and clear, the trait of SMF, which is characterised by a slower firmness loss than MF fruit after harvest, is quite difficult to phenotype and may yield different assessments depending on who actually carries out the evaluation. On the other hand, SMF varieties, like 'BIG TOP®' have become the 'facto' standard in the industry because of their high fruit quality, better handling after harvest resulting in increasing demand by breeders, peach industry and consumers. Among methodologies developed, two were promising and further tested on progenies: i) time-course postharvest evaluation with a hand-held penetrometer ii) rheograms acquired from digital penetrometer (Fruit Texture Analyzer, FTA, RG strumenti) to calculate three parameters representing: elasticity, firmness and fracturability of the peach fruit flesh. They both discriminated between melting and slow softening flesh phenotypes. Both of them are now recommended to be used by breeders that develop this type of cultivars.

Resistances to biotic stresses

There are no widely adopted peach varieties resistant to diseases. Some of these diseases are quite disruptive like the case of *Monilinia* not only due the economic damage it might cause in the orchard but also because it can develop during fruit transport breaking the confidence between shippers and retailers and making very difficult the control of the disease. Results obtained during the life of the project have related *Monilinia* resistance to biochemical characteristics, explicitly surface compounds, waxes and epidermis phenolics may contribute to the resistance of the fruits in the middle stage of growth (pit hardening). In addition, phenolic compounds may also play a role in the defence reaction of infected cells of the pulp. A fast method to screen brown rot impact on tens of seedlings per day directly on the field was developed and used to screen multiple progenies. Though environmental control may be important, the variability of susceptibility to *M. laxa* and fruit characteristics observed within three progenies indicated a quantitative inheritance of these traits, further studied via QTL analyses. The genotypes which displayed infection probability equal to zero for different years, sites or tests are of great interest for the development of peach cultivars resistant to brown rot.

Resistances to abiotic stresses

Global climate change predictions indicate shorter water availability in many important fruit growing areas. Selection of apple and peach cultivars that endure water stress is going to be in the 'to do list' of fruit breeding programs as it is in other plant species. However the assessment of water stress resistance is neither simple nor fast. FruitBreedomics has explored two methods to assess

tolerance/resistance to water scarcity. First, a multivariate statistical approach to assess leaf photo-assimilation performance using a portable photosynthesis system was developed. Water stress tolerance of genotypes could be discriminated by the IPL index (Impulse Photography of Luminescence), correlated with photosynthetic activity and variables representing their morphological responses. Second, genetic diversity of water use efficiency (WUE) was explored in a large apple germplasm collection using high-throughput phenotyping technologies (HTPT; PhenoArch platform) for the first time in fruit trees. A group of genotypes have been identified with high WUE under both well-watered (WW) and water-stress (WS) treatments, with the capability to maintain a relatively high biomass production under WS and use less water for transpiration under WW. This result may encourage apple breeders to improve apple material for the use of water.

Besides water availability concerns, erratic phenology may also hamper production in some regions in the future. Despite time consuming, forcing tests proved relevant to select cultivars with desired chilling and heating requirements. Objectives may focus on the selection of cultivars with either lower chilling requirements or higher heat requirements of the floral buds to prevent from spring frost risk or excessive flowering advances.

WP6:

For the purpose of fast genotyping of apple germplasm and mapping populations, three successive single nucleotide polymorphism (SNP) arrays have been developed of increasing size. SNP selection was based on transferability across cultivars (Micheletti et al. 2011) and even distribution across the apple genome (Velasco et al. 2010). At the beginning of the project a low-density array of 384 highly informative SNPs was designed. This low-density array has been tested and validated using an Illumina BeadXpress® Reader with VeraCode® Technology and building of various genetic linkage maps. This array became a valuable resource for the fast genotyping of germplasm to build framework linkage maps.

This array was intended to be utilized for an apple germplasm survey in WP3 and WP4. At the time of the project submission, the gap in terms of cost between a 384 SNP array and a high density array such as a 9-20K chip was enormous. Nowadays this gap has been considerably reduced and the cost of medium/high density arrays has become more affordable. As a result, a

8K Infinium SNP array became available through the International RosBREED Single nucleotide polymorphism (SNP) Consortium at an early stage of the project. This array was evaluated and showed to not yet meet WP3 requirements, Next, FruitBreedomics build the new 20K Infinium array, which included 3.7K robust markers from the 8K array and additional 16.3K SNPs which were predicted from re-sequencing data derived from 14 apple genotypes and two double-haploids. A haplotype-targeting strategy similar to that adopted to design the IRSC array was implemented for the design of the array, in order to combine the information from individual SNPs into haploblocks and provide fully informative multi-allelic markers. An ad-hoc pipeline for SNP selection has been devised to reach the target of 20K SNPs, while at the same time avoiding the pitfalls presented by paralogous sequence variants. The performance of the SNP array has been assessed, using data from more than 2,000 seedlings and more than 500 progenitors of WP3 and a number of diverse accessions of the FB collection. Evaluation of the chip in segregating populations and germplasm accessions revealed that more than 75% of the SNP assays are polymorphic and segregate in a Mendelian fashion, therefore providing high-quality markers for pedigree-based QTL mapping. Its excellent performance was demonstrated by its use on the FruitBreedomics full-sib families, since the genetic linkage maps of the individual families had an average of 6.8K SNPs uniformly distributed along the genome.

The genetic position of ca. 16K SNPs, and the assembled genome of two doubled haploids have been used to improve the current version of the apple genome (v3.0) now available at the GDR database (www.rosaceae.org). Using the information obtained from the 20K array it was also possible to study the decay of linkage disequilibrium (LD) for each apple chromosome. As expected,

LD decays quite rapidly, at an average distance of 55 kb. A much higher density array was therefore necessary to perform Genome Wide Association (GWA) studies in apple.

A 487K SNP array, the largest ever produced for a fruit plant and among the largest for higher plants, was developed for the Linkage Disequilibrium studies in WP4. It is based on Affymetrix technology. For SNP discovery a high coverage whole-genome re-sequencing strategy across a panel of 63 highly diverse apple cultivars and two double haploids was adopted to call individual genotypes and reduce the number of false positive SNPs included in the array design. This also allowed the selection of sets of both more widely and more narrowly polymorphic markers that together represented the diversity of the discovery panel, and resulted in a final set of 465K. Next, all the SNP markers from the previous 20K Infinium array were included together with 1.5K SNPs derived from a Genotyping By Sequencing experiment. This Axiom Apple480K Chip has been successfully produced and used to genotype 1324 apple accessions for GWA studies and to construct four high-density parental maps. Using a quality prediction model based on logistic regression (developed within Task 4.4), a total of 275,223 highly informative and robust SNPs (56% of the 487K) have been identified.

This newly developed Axiom array with its high density of SNPs is expected to be of great utility for GWA studies. It will also be a valuable tool to help dissect the genetic mechanisms controlling important traits, to aid the identification of marker-trait associations suitable for the application of Marker Assisted Selection (MAS) in apple breeding programs, and to clarify pedigrees for individuals for which parentages are still unknown.

For peach, the IPSC (the International Peach SNP Consortium) 9K SNP array has been successfully used for genotyping both segregating populations and germplasm collections WP3 and WP4. Since LD in peach was estimated at about 13-15 cM, the density of the array is sufficient to perform optimal GWAS in peach with the goal of obtaining SNP closely linked to agronomic interesting traits. An average of 2,300 validated SNPs were obtained for each mapping population. Therefore, no new arrays had to be developed. The IPSC 9K SNP array was used to genotype the full-sib families of WP3 and a large collection of peach accessions including an ample set of Occidental and Oriental materials within WP4.

Different methods have been evaluated to perform high-throughput genotyping for the pilot studies of WP1 in which a relative low number of SNP markers (10-25) had to be tested on few thousands of samples. Costs and laboratory protocols of five different technologies (KASP™-SNP Genotyping technology, Illumina BeadXpress®, Sequenom MassARRAY Analyzer, Fluidigm SNPtype™ Assays and Applied Biosystems TaqMan® OpenArray® Genotyping) were compared for both apple and peach. Illumina BeadXpress and Applied Biosystems TaqMan OpenArray Genotyping are fixed arrays technologies and were not considered for MAS because of several disadvantages. Fluidigm SNPtype Assay and Sequenom MassARRAY Analyzer would have been a valid alternative to the KASP-SNP Genotyping technology since they meet the required throughput for a MAS application (i.e. multiplex up to 36 SNPs in the case of Sequenom) but the cost of outsourcing to service providers was too high compared to the cost of KASP™ genotyping offered by LGC Genomics. So for the FruitBreedomics pilot studies, it was decided to choose the KASP™-SNP Genotyping technology. Considering the needs of the pilot studies this technology looked to be the most cost-effective and robust but also ideal for breeding companies not having a DNA laboratory that want to start applying MAS.

WP7:

WP7 designed and deployed the bioinformatics infrastructures necessary to provide efficient storage and access of phenotypic data and genome variation information. We developed and managed web-based relational data banks specifically created for apple and peach data, and provided specific bioinformatics tools for data integration and visualization. All data are accessible through a stable and well-maintained application programming interface and browser.

Two main results are produced. The first one is the capability for the user to explore the corpus of stored data through a seamless approach that highlights the interconnections present in the originally separated datasets. This result has been achieved crafting several data exploration tools exposing a homogeneous approach over search forms, graphical representation, and capability to download the required data. In this way, phenotype data, genome variation data, information on traits of interest and linked markers, as well as a collection of publications are available, both online and through download links.

The second result is represented by two analytical tools called “The Breeder's Interface” and “LD explorer”. These tools have been designed in collaboration with other partners of the project and respond to the explicit requirements of the scientists/breeders community. The Breeder's Interface is intended to help breeders to plan a cross that will guarantee the best chances of success. To achieve this it leverages the stored information relative to a number of monogenic traits. After asking the breeder to define the target combination of phenotypes, the tool examines the chances of success of all possible couples of varieties among the stored ones, and proposes a ranking of crosses, from the most advantageous to the least one. The tool allows also fixing one of the parents, so that the breeder can plan crosses to improve his/her existing selection.

The second tool, called LDexplorer, is intended to help scientists explore linkage disequilibrium across single chromosomes or on the entire genome of the studied species. It uses the stored SNP chip information, and allows the user to select the subset of samples on which to operate. Several parameters of the analysis can be decided before submitting the request. The tool offers results in the form of linkage disequilibrium decaying (R^2) charts available both online and through download links. Several analyses are also available for download. The tool contains a queue mechanism that stores the user requests and process them sequentially, so to not overload the server capacity and thus disrupting the other services. The user is given a special link to be used to check the status of his requests. Analysis results are stored on the server for at least two weeks after completion.

WP8:

A total of 23 papers have been published since the beginning of the project. More are currently being written and in the process of being submitted to review journals. A series of contributions (orals and posters) to congresses and meetings have been produced.

An active and dynamic internet oriented system has been established to disseminate scientific and technical results from the project. This system is composed by a set of coordinated tools: project web page, FruitBreedomics Slideshare portal and FruitBreedomics Vimeo Channel. Up to date, the web site has served 48854 pages by more than 9916 visitors. FruitBreedomics videos (215) in Vimeo have been loaded more than 59901 times and presentations (163) in Slideshare have been viewed more than 49964 times.

A stakeholder network has been created with 367 individuals representing 61 commercial breeding programs encompassing different *Rosaceae* species. As part of the interaction with the stakeholders, 9 online surveys have been organized to get information on breeders' practices and expectations. Since the initiation of the project, 5 stakeholder meetings (including the kick-off meeting and an International Conference) have been held in different parts of Europe.

A total of 6 training sessions covering different outputs of the project from all workpackages have been organized. These training sessions were targeted to the different types of stakeholders, mainly breeders and germplasm curators.

A series of 6 FruitBreedomics booklets have been produced highlighting the main practical results of the project. These booklets are targeted to the stakeholders of the project. Their content is related to the training sessions.

An international conference has been organized as part of the official program of the XIV Eucarpia Fruit Breeding and Genetics Symposium in June 2015 in Bologna (Italy). 19 selected results disseminated in 5 sessions were presented. 214 people attended this event

Finally, a system to ensure effective Marker Assisted Breeding technology transfer to commercial breeding programs has been put in place by the creation of FruitBreedomics MAB Services.

1.4 Potential impact and main dissemination activities and exploitation of results

A constant goal all along the duration of FruitBreedomics has been to make its results easily available for the partners of the project and, at longer term, to all the stakeholders. This has been a general effort from all the WP to keep this trend and to make this project as useful as possible to the whole fruit community.

Thanks to the new genotyping tools developed in the frame of FruitBreedomics and the upmost scientific results obtained, impacts of FruitBreedomics will cover wide areas from genetics, genomics and bioinformatics. The most practical impacts will be for the scientists, for the germplasm curators and the breeders.

After the end of the project, we are working in some areas to further enhance the use of FruitBreedomics output; for example:

- The setting up of reference population networks on both apple and peach which are directly built and designed thanks to the results of FruitBreedomics and which will help to better take into account GxExP interactions in European genetic studies and breeding programs.
- Improvement of the tools available for further genetic studies such as the development of new genotyping SNP chips.
- Preliminary studies to develop a common Fruit European Breeding Platform which could provide common tools and material for the fruit breeders.

WP1:

Thanks to the activities performed in WP1, the way to a larger application of molecular markers in apple and peach breeding has been paved.

SNP markers for important apple (primarily resistance genes against apple scab, powdery mildew, rosy apple aphid and fire blight) and peach traits (like: low acidity, fruit shape, glabrous fruit epidermis, fruit flesh, non-melting and for the green peach aphid and powdery mildew resistance) have been developed and validated. The availability of SNP markers for such traits together with the genotyping pipeline tested in the pilot studies will allow on the short term more apple and peach breeders to start applying MAB in their programs as the genotyping costs with such type of markers are continuously decreasing.

A larger application of MAB and/or GWS will make breeding more efficient and cost-effective. New cultivars with innovative characteristics, like for instance pyramided resistances with outstanding fruit and agronomical traits will become faster available to farmers and will permit at mid-term a more ecological and economic production of fruits.

To foster the application of MAB, FruitBreedomics MAB Services have been launched. These companies will offer more than the mere genotyping service. The FruitBreedomics MAB Services will include onsite visits to breeding programs, explanation on the possibilities of using markers in breeding programs, detailed planning for using Marker Assisted Breeding in the breeding programs, on site assistance for application of protocol for collecting samples, genotyping service, on site discussion of the genotyping results and assistance in the selection phase based on genotyping results. This MAB service will be the basement of the European Fruit Breeding Platform we are working on right now.

WP2:

WP2 was aimed at the establishment of pre-breeding material. The main focus of WP2 was the improvement of resistance to important diseases affecting apple and peach. Considered were single resistances and QTLs as well as combinations of resistances. The overall aim of resistant material is the possibility to produce fruits in a more sustainable, resilient way. This will have an impact on growers, distributors and consumers. The input of pesticides could be reduced to minimize the exposure of nature with chemicals and to produce fruits without chemical residues which will benefit the health of consumers. Furthermore the reduction of chemical pesticides will reduce costs for growing making fruit production more competitive. In such way growers as well as consumers will benefit from resistant cultivars.

Besides resistance, new traits like low allergenicity, specific fruit shape and low chilling in apple as bloody flesh in peach have been introduced by crosses. These traits can open new markets by offering more variability. Low chilling varieties could ensure apple production in regions suffering from climate changes and make apple production possible in new.

The successful testing and improvement of the fast breeding approach in apple will allow the establishment of new pre-breeding material with traits introduced from wild *Malus* species in an extremely reduced time. In general one generation is possible in one year thus reducing the time of necessary backcrosses to eliminate genetic drag from decades to less than one decade. The gimmick is the segregation of the early flowering gene which reduces juvenility to few weeks. After a series of pseudo-backcrosses genotypes can be selected carrying the introduced trait but not the early flowering gene. The selected plant will be not transgenic. But still the question remains how these plants will be regarded by the authorities. In the US such pre-breeding genotypes are not considered as genetically modified. How these plants will be regarded in Europe is unclear.

The exchange of material developed by WP2 in consideration of bilateral material transfer agreements will allow apple and peach breeders to introduce new resistances in their breeding program not using the original wild donors but advanced pre-breeding material. The knowledge on and the use of that material opens the door for a better cooperation in breeding which could succeed in the development of better, resistant varieties.

WP3:

Thanks to the activities performed in WP3 prospects have increased to soon widen Marker Assisted Breeding to a series of new traits and genes as through the use of knowledge on the genetic composition of potential parents in terms of founder contributions. This leads to the further increases in breeding efficiency, especially for some genetically complex traits. At the end this will help to develop new cultivars that will contribute to a financially and environmentally sustainable production of delicious fruit, and which finally increase the competitiveness of the European breeding industry, and, even more importantly, the European fruit production sector

WP3 provided also results that increase the power and prospects of genetic research on QTL discovery and characterization, thus supporting the coming of diagnostic/predictive markers for new traits or for new genetic sources. It hereby strengthened the competitiveness of European research. Several projects world-wide showed interest in the Pedigree Based Analyses approach. It has been adapted by research projects on grape, strawberry and wheat, and by the multi-state and multi-crop USDA-SCRI RosBREED project and fostered new interactions with Japan. The competitiveness is illustrated by the interest of USA groups to employ previous WP3 collaborators.

The gained knowledge on the genetic diversity that is currently explored in European breeding programs and the increased understanding on some of the drivers and limitations for increased use of genetically distinct germplasm may be useful to policy makers and breeders in efforts to widen the genetic bases in apple production, and hereby re-direct the current tendency towards mono-culture, thus reducing the current vulnerability of current European apple production to a catastrophe, e.g. for the showing up of a new disease.

The PBA approach and WP3 results have been disseminated through presentations during the FruitBreedomics Stakeholders Platform days, a full day training workshop for stakeholders on the use of software, release of software (3 public available), scientific publications (10 published and 3 submitted and 9 more planned), two FruitBreedomics newsletters, international symposia (more than 9 international meetings, 14 oral presentations and 4 posters), 4 webpages and providing content for video's (17x) that are available at FruitBreedomics Vimeo Channel

Moreover, several research organisations which are not partners of the project showed interest in taking part in the Pedigree Based Analyses approach, the core element of WP3. As a result, during the project added two full partners KULeuven and RCL and the two invited partners SLU and VNIISPK joined WP3 even not receiving any fund from the EC. They have been trained in the performance of PBA Analyses,

Finally, WP3 software was readily used by partners of the SCRI-USDA RosBREED project for their analyses on Apple, Peach, Cherry and Strawberry.

Activities performed in WP3 resulted in a solid base established for facilitating DNA informed parent selections by breeders, for the development of diagnostic marker tests for new traits/genes, and for increased efficiency in the performance of QTL discovery and QTL characterization studies by geneticists.

Hundreds of apple cultivars and their progenitors are characterized for their favourable and unfavourable genes as well as profiled for their genome-wide genetic constitution. Access to this information would give breeders completely new type of information for the selection of parents that well complement each other, thereby increasing chances for the raise of new top cultivars. Hereto, database infrastructure needs to be developed and training programs for breeders in the understanding and use of the data are to be implemented.

Corrections to pedigree records have been made and this information is valuable for breeders securing assignment of favourable and unfavourable alleles to correct sources and plan more performant crosses.

The genetic diversity explored in European apple breeding has been inventoried. Results are of use to breeders and policy makers to take measures that allow for a better use of the already available diversity. On the long term, this widens the genetic base in apple production thereby reducing its current vulnerability to a catastrophe like the appearance of a new disease or a pest and strengthening the resilience in the view of climate change.

For a series of traits, the genetic base has been further clarified and predictive SNP markers have been identified. On the short term scientists and companies may use this to develop diagnostic assays for use in Marker Assisted Breeding approaches. On the midterm this enlarges the set of diagnostic DNA tests available to breeders. The traits relate in both crops to acidity, sugar, skin colour, and size. Apple-specific traits are firmness, pre-harvest drop, and adaptation to global warming (chilling and heat requirement). Peach-specific traits are firm/soft melting, maturity date, flowering period, fruit development period and resistance to brown rot (*Monilinia fructigena*).

New software has been developed that facilitates the discovery of new marker-trait relations. On the short term this supports scientists in their efforts to find new predictive markers and on the midterm leads to an enlarged set of diagnostic DNA tests.

Scientific results have been made public through publications. Software has been made available for free through public accessible websites, and a workshop for breeders was organized dedicated to the use of the Pedimap software, which is a useful tool allowing to visualize the pedigree structure as well as get a breeding program-specific overview of the transmission of phenotype and marker-based genotypes. The content of this workshop is available on the website of the project in the form of videos of the presentations and a dedicated booklet.

WP4:

Diversity is crucial for fruit innovation; consequently a good knowledge of the diversity (phenotypic and genotypic) underlying the peach and apple germplasm available is of extreme importance for breeding. Apple and peach germplasms are preserved in collections maintained and evaluated at some level during years. Such accessions represent a repository of variability that can be introduced as desired in breeding programs. FruitBreedomics has characterized a large collection of apple and peach accessions, allowing the identification of clones, synonyms, validation of the identity of the trees and discovery of triploid accessions. This information will allow a more efficient management of the collections, allowing to discard replicates or to better preserve diversity and will provide a base for the further co-ordination of germplasm collections around Europe. Additionally a good knowledge of the genetic and phenotypic diversity is now available for breeders in an organized and homogeneous way in a database. Breeders will be able to investigate the behaviour of the accessions in a given location as well as select those with more potential to introduce variability in their breeding programs, which will aid in fruit improvement and innovation.

Phenotypic and genotypic data generated by FruitBreedomics have allowed also the selection of core European germplasm collections for further research. Indeed efforts have been initiated to establish germplasm collections (one per species) that will be grown in different locations, which will allow researchers to explore the interaction of genes and environment as part of a collaborative research network. Results will allow the selection of the best genetic pool for a given environment to improve fruit quality.

Thousands of apple and peach accessions have been fingerprinted and assigned to subpopulations based on allelic frequencies. This information will be public and maintained in database repositories for the scientific community once the papers describing the work will be accepted for publication, and this resource will also be relevant to a range of further scientific studies.

GWAS analysis allowed to generate markers for MAB based on SNP haplotypes, whose efficiency has been proved in WP1 pilot studies. This tool generated in FruitBreedomics may have a big impact in breeding efficiency, allowing for early seedling selection. Moreover GWAS proved to more accurately focus on small genomic regions containing QTLs, which will help in more quickly identifying the underlying genes and understanding the involved mechanisms.

Results have been presented in scientific publications (3 published, 2 submitted and at least 2 more planned), two FruitBreedomics newsletters, international symposiums (more than 10 international meetings, eight oral presentations and two posters) and disseminated to Stakeholders (EUFRIN working group, breeders, etc.).

WP5:

The availability of innovative assessment methods for traits difficult to assess and with significant interest for the fruit industry should help the adoption of breeding for these objectives in commercial breeding programs. The new tools and methods developed can already be used by variety testers and germplasm curators to better describe genetic variability. In addition, project outcomes should facilitate the development of new cultivars with improved texture, *Monilinia* resistant peach cultivars or fruit varieties with better tolerance to water scarcity and with adapted phenology to future climate.

If selection on texture is already a reality, progress can still be made by targeting different components of this complex trait. Concerning the other traits, they are still in the research pipeline towards commercial breeders. The results presented here on large number of individuals confirm their feasibility for large scale phenotyping and their accuracy to describe phenotypic variations among genotypes. However, the transition to the industry has not been tested.

In contrast, the evaluation of thousands of individuals for traits like fruit texture, *Monilinia* resistance or water scarcity resistance at a fraction of the cost of what was in the past will facilitate a better understanding of the genetics of these traits. In this way, part of the studies has already enabled quantitative genetics analyses and detection of QTL.

These stimulating results have been spread on the one hand towards the scientific community and on the other hand to stakeholders, through international meetings and workshops. In addition, a huge effort has been targeted to the writing and delivery of the standardized tools and protocols set up. Finally, partners are already engaged in a process of publication to ensure a large dissemination of the results.

WP6:

High throughput SNP array-based genotyping has revolutionized the study of genome-wide genetic variation, reducing costs and increasing the reliability and efficiency of data produced, as well as significantly reducing the time spent on genotyping. The newly developed SNP arrays will greatly increase the opportunities for apple and peach researchers to dissect the genetic mechanisms controlling important traits, and to identify marker-trait associations suitable for the application of Marker Assisted Breeding (MAB) in apple and peach breeding programs. Thanks to the genotyping tools developed, MAS is more and more applied than at the beginning of the project but it is still mainly focus on traits controlled by major genes. The new dense SNP chips will allow locating complex traits on the genetic maps and so broadening the field of use of molecular markers for the breeders. To advance the accuracy of prediction, including the contributions from small to intermediate effect QTL, genomic selection (GS) has been tested as an extension of MAB. Looking at the first results obtained in the project, this could be an efficient way for breeding in apple and peach.

A very good representation of the apple and peach genome diversity in the newly developed SNP arrays has been achieved thanks to a large SNP discovery panel which has been re-sequenced at high coverage. In particular, for the apple Illumina 20K array, SNPs were predicted from re-sequencing data derived from the genomes of 13 *Malus x domestica* apple cultivars and one accession belonging to a crab apple species (*M. micromalus*). The array has been successfully used to genotype over 26 FruitBreedomics mapping populations and to perform a pedigree-based analysis within WP3 as to identify the parents for numerous individuals for which pedigree records were lacking or incorrect. The array had an innovative design in that SNPs were centred to well-spaced Focal Points. This allowed new approaches in the generation of genetic linkage maps and increased statistical and computational power in PBA analyses.

For the development of the Axiom Apple480K array a panel of 63 more diverse cultivars has been used. The array is used to finely dissect the genetic basis of important agronomical traits such as phenology, fruit quality, disease resistance, and drought tolerance which have been assessed for several years over hundreds of apple cultivars in the frame of the European project FruitBreedomics (Laurens et al., 2012). It will be a reference tool for further Genome Wide Association (GWA) studies to be performed on other apple collections worldwide and will help in the construction of high-resolution linkage maps and in the improvement of the current reference genome sequence. The *Malus x domestica* genome is an improved high quality draft genome (<http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3854948>) and continuous work is put in to improve it. The Axiom Apple480K array provides a wealth of marker information which may be a useful resource for further improvement of the current version of the *Malus x domestica* genome assembly v3 (<https://www.rosaceae.org/>).

For peach, the International Peach SNP Consortium (IPSC), which includes institutions from the U.S., Italy, and Spain, has worked together to identify genome-wide sequence variation and to develop a moderate-density peach SNP array relevant for worldwide peach breeding germplasm. SNPs were discovered from the whole genome resequencing of 56 peach breeding accessions. The array has become a fundamental genotyping tool for the peach community for high-throughput linkage and QTL mapping and GWA studies.

WP7:

The FruitBreedomics database and breeding interface offer scientists and breeders direct access to data and tools developed by the project to apply them in their own research and breeding programmes. As explained in the previous WP parts, the biggest impacts of WP7 will be for the breeders thanks to the Breeding interface (WP1) and with LDexplorer to help scientists explore linkage disequilibrium. This will result in faster progress in genetic dissection of important traits, gene and marker discovery, and more efficient breeding of novel peach and apple cultivars with positive impacts on the European fruit sector.

WP8:

A relevant impact of the activity of WP8 is the creation of an active stakeholder network of public and private commercial breeders. More than 350 stakeholders are part of the network. This network is an asset for future activities in the field of research an innovation on fruit breeding and genetics.

Although the project aimed mainly at breeders and germplasm curators, efforts have been put also to extend the network to variety testers, growers, shippers and other relevant actors of the fruit chain.

The project focused on apple and peach as model species. However, to extend the significance of the project to other species, during the life of the project, connections have been established with other relevant projects like EUBerry dealing with berries or the organization of workshops with researchers of other rosacea species.

Another significant result that will have strong impact in the future is the creation of the FruitBreedomics MAB Services, a set of 3 companies facilitating as a service the application of the project results in commercial breeding programs. This approach should ensure an efficient technology transfer to the majority of fruit breeding programs which are characterized to be small and medium private companies lacking of trained personnel to apply MAB. These services together with the stakeholder network can be the foundation of sustainable public-private cooperation at European level in the area of fruit breeding and cultivar development.

FruitBreedomics has maintained links with other European projects related to genetics, genomics and breeding in fruits, like EUBerry but most notably has established strong connections with other international projects outside Europe, like RosBreed in the USA and other research teams in the same field like in China, New Zealand or Russia.

Main dissemination activities:

1. An active and dynamic internet oriented system to disseminate scientific and technical results from the project. This system is composed by a set of coordinated tools: project web page, FruitBreedomics Slideshare portal and FruitBreedomics Vimeo Channel. Up to date, the web site has served 48854 pages by more than 9916 visitors. FruitBreedomics videos (215) in Vimeo have been loaded more than 59901 times and presentations (163) in Slideshare have been viewed more than 49964 times.
2. Five stakeholder meetings, including an International FruitBreedomics conference held in 5 different countries.
3. Six training sessions targeted to project stakeholders
4. Six FruitBreedomics booklets
5. International FruitBreedomics Conference within the framework of the Eucarpia Symposium on Fruit Breeding and Genetics.
6. FruitBreedomics Marker Assisted Breeding Services. Three companies providing on a commercial basis the practical application of MAB in private or public commercial breeding programs

Exploitation of results:

FruitBreedomics MAB Services are companies operating on a commercial basis transferring results obtained in the project to small and medium fruit breeding companies. This approach, which was not originally planned in the project, will facilitate implementation of MAB in the majority of the commercial breeding programs independently of the size of the programs.

1.5 Project public website

The website address is the following: <http://www.fruitbreedomics.com/>

1.6 Relevant contact details

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1.7 Project logo