

EUBerry – WP1

Improving berry varieties through the identification and utilisation of the best genetic resources

Leader – Rex Brennan (James Hutton Institute, Scotland, UK)



Objectives and aims of WP1

- Evaluating, selecting and obtaining new genetic material of *Fragaria*, *Rubus*, *Ribes* and *Vaccinium* with improved adaptability to cultivation conditions and systems
- Identification of the best materials exhibiting enhanced fruit quality, incl. nutritional quality and nutraceutical value



Partners in WP1

- **P1** - Marche Polytechnic University, Italy (Bruno Mezzetti)
 - Phenotyping (F, V), Functional genomics (F)
- **P2** – Research Institute of Horticulture, Poland (Edward Zurawicz)
 - Phenotyping (F, R, Ri), Genotyping (Ri)
- **P3** – James Hutton Institute, UK (Rex Brennan)
 - Phenotyping (R, Ri, V), Genotyping (R, Ri)
 - Subcontractor: East Malling Research (David Simpson)
 - Genotyping (F)
- **P4** – INRB, Portugal (Pedro Oliveira)
 - Phenotyping (R)
- **P5** – IFAPA, Spain (José Sánchez Sevilla)
 - Phenotyping (F), Genotyping (F), Functional genomics (F)



Projected outputs from WP1

- Integrated sets of characterised germplasm, both cultivars and advanced breeding lines, for *Fragaria*, *Rubus*, *Ribes* and *Vaccinium* across the (3 main) climatic zones within the EU
- Full development of phenotypic databases beyond the information collected in existing GENBERRY and RIBESCO databases, to incorporate elite pre-commercial germplasm from EU-based breeding programs.
- Genotyping protocols for strawberry, raspberry and currant based partly on genome-wide sequencing approaches.
- Identification of QTLs and some key genes underlying important developmental and quality traits in strawberry, raspberry and currant
- Markers linked to key traits in strawberry, raspberry and currant for subsequent deployment by breeders in downstream breeding programs.
- Validation of functionality of genes for flowering and fruit quality in strawberry

Partners in WP1

- **P6 – INRA, France** (Beatrice Denoyes)
 - Phenotyping (F, R), Genotyping (F), Functional genomics (F)
 - Subcontractor 1 – CIREF (Phillipe Chartier)
 - Subcontractor 2 - Invenio (Marie-Noëlle Demene, Jean-Jacques Pommier)
- **P7 – MTT, Finland** (Saila Karhu)
 - Phenotyping (Ri)
- **P8 – Bioforsk, Norway** (Rolf Nestby)
 - Genotyping (F)
- **P10 – Geisenheim Research Centre, Germ**
Krüger-Steden)
 - Phenotyping (F, R, Ri)
- **P12 - JKI Quedlingburg** (Detlef Ulrich)
 - Phenotyping (F, R, Ri)




Task 1.1 Phenotyping and characterisation of pre-commercial germplasm

- Sub-tasks
 - 1.1.1 – Data mining of existing characterisation data
 - Partners 6 (INRA) and 7 (MTT)
 - Based on EU-generated Genberry and Ribesco databases
 - Strawberry (INRA) and Blackcurrant (MTT)
 - 1.1.2 – Assessment of pre-commercial material
- Outputs
 - 1.1 Fully integrated databases for each genus
 - 1.2 Integrated sets of characterised germplasm for each genus
 - Define new varieties for cultivation in specific areas



Sub-task 1.1.1 - progress

- *Fragaria* (P6 – INRA) 
 - GenBerry database is being utilised for the description of pre-commercial germplasm
 - Focused on hierarchical structures between accession groups, accessions from different locations and also from different agronomic regimes (eg. field vs. tunnel production).
- *Ribes* (P7 – MTT)
 - Identifying subsets of accessions with valuable characteristic combinations, both for breeding and commercial use
 - Different methods to manage the large amount of phenotypic data have been tested, and correlations between different traits have been assessed
 - Work with SAS/STAT® 9.2 (SAS Institute Inc. 2008) has been recently started with statements created to identify accessions that contain different combinations of selected valuable traits
 - The work is ongoing and will be finished during year 2.

EUBERRY ... Identification of the best genetic resources ...
Sub-Task 1.1.1 Data mining of existing characterisation data

Aim: To identify subsets of accessions with valuable characteristic combinations, both for breeding and commercial use

- The work of Partner 7 (MTT, Finland) is based on the information collected in the EU AGRI GEN RES project RIBESCO (2007– 2011)



- Quality information (more than 18 000 observations) of over 600 Northern and Central European accessions of blackcurrant has been collected



The ECP/GR Ribes and Rubus Database
Vilnius University Botanical Garden, Kašėnai 43, LT-2040 Vilnius, Lithuania
Tel: +370 2 317944 Fax: +370 2 317429
Webmaster: [D. Danys](mailto:D.Danys@botanika.lt)
Database manager: [D. Danys](mailto:D. Danys@botanika.lt)

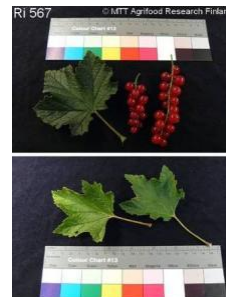


The Ribes database contains 2624 records of Ribes accessions held in 11 countries, namely Czech, Denmark, Estonia, Finland, Germany, Latvia, Lithuania, Poland, Romania, Sweden and U.K. in the meantime.

The Rubus database contains 965 records of Rubus accessions held in 6 countries, namely Poland, Czech, Germany, Lithuania, Romania and Sweden (Nordic Gene Bank) in the meantime.

[Download Ribes](#)
[Download Rubus](#)

Page last updated 10 May 2010



- The traits recorded include :
 - Key agronomic characters
 - yield, pathogen resistance, climatic adaptation, etc.
 - Fruit quality parameters
 - soluble solids, vitamin C content, phenolic content
- An “elite collection” for central and Northern European blackcurrant breeding will be identified



Subtask 1.1.2 - Phenotypic data collation for pre-commercial germplasm

- Phenotypic data is being collected at all relevant partners as follows
 - *Fragaria* – 6 sites
 - *Rubus* – 4 sites
 - *Ribes* – 4 sites
 - *Vaccinium* – 2 sites
- Covers agronomic and fruiting traits (incl. compositional analysis)
- Breeding lines as well as control varieties
- Collation done by P2 (Poland)
- Repeated throughout project's duration



Sub-task 1.1.2

- Set of elite pre-commercial genotypes have been selected for each crop (strawberry, raspberry, blueberry, blackcurrant), with up to 12 key genotypes for each species selected at each partner.
- Selections represent main varieties used in each partner country and region, new seedlings and newly released materials.
- Common genotypes have been included across partners, for benchmarking of results
- Monitoring has begun
 - Some data for 2011, full recording in 2012



WP 1. ACTIVITIES OF PARTNER 2 (IO – POLAND)

Task.1.1 Phenotyping and Characterisation of pre-commercial berry Germplasm; Task Leader Edward Zurawicz, P2

Sub-Task 1.1.2 Assessment of pre-commercial material

Strawberry (*Fragaria*) - MASNY A.


1. 302 genotypes in the cultivar collection and 563 genotypes in the breeding clones collection were evaluated.
2. 10 genotypes selected for detailed assessment.
3. In 2011 plant vigour, fruit ripening time, yield, fruit weight, firmness, soluble solid content and ascorbic acid content were assessed.

Blackcurrant (*Ribes*) – PLUTA S.


1. 121 genotypes maintained in the clone collection and 609 interspecific hybrid seedlings were evaluated.
2. 5 genotypes were selected for the detailed assessment.
3. In 2011 fruit ripening time, yield, fruit weight, soluble solid content and ascorbic acid content were assessed.

Raspberry (*Rubus*) – LEWANDOWSKI M.


1. 199 genotypes in the clone collection were evaluated.
2. 10 genotypes were selected for the detailed assessment.



Standard
'Elsanta'



new
'Romina'



campusgeisenheim
Forschung.
Bildung.
Praxis.
ZUKUNFT WÄCHST.

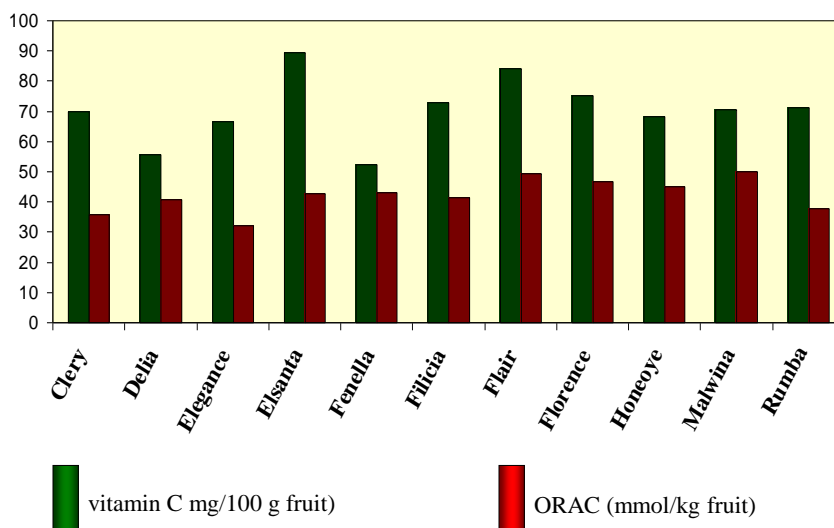
WP 1 - Task 1.1. –
Sub-task 1.1.2 Assessment of pre-commercial and commercial material
Strawberry - Activities of P 10 (Geisenheim Research Center)

- Testing of 13 varieties in 2011
- Testing of 9 varieties in 2012 and 2013, varieties are also grown at other research places within the EuBerry-Project

Evaluated traits

- Sensorial assessment according a common protocol developed by P15 (JKI Germany) due to the tasks of WP 3
- Yield parameters, fruit weight
- Fruit colour L*, chroma (Tristimulus method)
- Chemical analyses (Soluble solids content, Titratable acidity, Total Antioxidant Capacity (ORAC) Vitamin C content

Strawberry Varieties 2011: Content of Vitamin C and Antioxidant Activity – GRC (P10)



Work package number 1

Task 1.1 Phenotyping and Characterisation of pre-commercial Berry Germplasm

Sub-task 1.1.2 Assessment of pre-commercial material

P1 Strawberry: evaluation of genotypes included in its germplasm collection and new genotypes generated from inter-specific back cross combinations of *Fragaria virginiana* spp. *glauca* X *Fragaria x ananassa* and intra-specific cross combination of *Fragaria x ananassa*.

In the specific the parameters evaluated were:

- **Phenotyping characterization:** germplasm collection genotypes
- **Productive parameters:** total plant production, commercial plant production, average fruit weight, undersized and misshapen fruit production;
- **Sensorial parameters:** fruit color, fruit firmness, soluble solids content and total acidity;
- **Nutritional parameters:** total antioxidant capacity, total phenol content and anthocyanin content.

Genotypes	Precocity index	Commercial Production (g)	Total Production (g)	Fruit weight (g)	Soluble Solids (*brix)	Titrateable Acidity (meqNaOH)	Firmnes (g)	Chroma index	Total Antioxidant Capacity	Total Phenol Content	Anthocyanin Content
ADRIA	149	1232	1677	31,2	6,1	10,8	461	41,5	13,9	1426	205
ALBA	135	1088	1351	24,0	6,7	13,1	480	47,3	10,4	1463	275
ALBION	137	398	543	20,3	8,1	12,9	596	39,9	20,4	1869	340
AMIGA	140	1017	1185	21,4	7,3	9,8	769	48,0	15,5	1555	437
ANTEA	140	747	956	21,8	8,5	9,6	496	47,5	16,0	1438	219
CLERY	137	894	1165	20,5	7,9	10,1	394	46,7	13,4	1498	315
CRISTAL	139	622	797	16,5	8,1	11,7	616	45,3	19,9	2069	402
CRISTINA	152	1219	1407	30,8	7,3	10,6	334	48,7	10,0	1176	344
FIGARO	145	486	738	18,6	7,5	9,7	405	47,3	16,8	1615	202
JOLY	142	918	1124	26,1	7,9	10,5	320	42,5	15,4	1403	370
JUDIBELL	163	347	719	18,6	7,2	15,6	264	52,1	19,1	1617	242
KAMILA	140	784	1031	23,0	6,4	10,1	537	45,6	17,8	1542	355
MALWINA	164	366	490	23,8	9,1	17,7	318	42,0	22,1	2099	551
PRIMORIS	136	391	606	16,1	7,9	9,5	671	41,1	21,4	2021	308
ROMINA	135	810	1043	18,8	7,5	10,2	520	45,1	13,2	1396	274
SAN ANDREAS	139	450	722	15,0	7,9	12,0	651	45,8	14,3	1685	250
SONATA	147	810	1118	18,0	8,5	9,3	288	44,6	12,7	1236	233
SVEVA	151	601	957	24,9	6,6	12,6	391	44,1	19,3	1969	522
SWEET EVE	142	197	320	14,0	11,9	11,5	592	45,9	18,9	1909	256
AN00,239,52	143	365	458	17,0	8,2	13,4	425	41,9	13,1	1450	401
AN00,239,55	146	274	400	13,1	10,3	15,9	396	43,7	20,6	1937	600
AN01,184,55	138	838	969	27,6	7,1	12,3	396	37,4	17,3	1465	283
AN01,243,54	130	545	706	22,7	8,1	12,6	464	46,1	12,5	1183	273
AN01,246,55	138	616	732	21,9	7,3	8,8	453	40,7	15,0	1397	287
AN02,119,53	141	412	563	19,9	8,8	11,2	507	41,6	14,0	1293	210

P1 Blueberry: development of experimental blueberry field located by the UPM agronomic experimental farm P. Rosati.

The plant material was purchased by Plant and Food Research and was composed by *Vaccinium corymbosum* varieties and elite selections of Northern highbush, Southern highbush and Rabbiteye genotypes developed outside the EU. Till now it was not possible to evaluate the yield and adaptation parameters to the recent development of the trial.

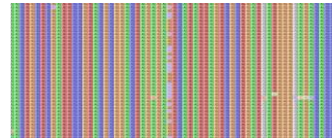
V. corymbosum L. Varieties n° 13: BLUE RAY; BLUECROP; COVILLE; DUKE; EARLY BLUE; ELLIOT; HORTBLUE PETITE; LATE BLUE; NUI; PATRIOT; POPPINS; REKA. New selections n°10;

V. virgatum Aiton ; n°1 new selection.



T1.2 Development of molecular tools

- Sub-tasks
 - 1.2.1 – *Fragaria*
 - 1.2.2 – *Rubus*
 - 1.2.3 – *Ribes*
 - 1.2.4 – *Vaccinium*
 - 1.2.5 - Strategy for MAB
- Deliverables
 - Development of marker-assisted breeding strategies
 - Berry size, anthocyanin content, water use efficiency, *Verticillium* resistance
 - Identification of markers linked to key traits
 - Marker-assisted tools for breeding



Sub-task 1.2.1 - *Fragaria*



- Flowering traits (P1, P6, P8)
- Major QTL linked to continuous flowering and date of flowering in the octoploid strawberry was identified using two segregating populations (Capitola x CF1116 and Mara des Bois x Pajaro).
- Molecular markers have now been added to identify those co-locating in the linkage map with the continuous flowering QTL
- 5 putatively linked SSR markers have been identified.
- Markers linked to everbearing will be available in end of 2012 – beginning of 2013
- In parallel, the segregating population has been enlarged in order to have more crossing over in the region of the major QTL.
- Further new segregating populations will be genotyped in the coming year to confirm QTL and markers for this trait.
- Later in the project, interspecific populations of *Fragaria virginiana* ssp. *glauca* X *Fragaria x ananassa* will be analysed for QTL using NGS-derived data.



Sub-task 1.2.1 - *Fragaria*



- *Disease resistance (P1, P2, P3*, P6)*
- Populations segregating for *Verticillium* wilt resistance (Redgauntlet x Hapil, Elsanta x Dukat) have been propagated for use to validate markers linked to resistance.
- Phenotyping of Elsanta x Dukat population has enabled the identification of 2 putative QTL regions correlated with resistance.
- Simultaneously, differentially-expressed candidate genes have been identified and markers will be generated in years 2-3. The candidates include ascorbic peroxidase, PGIP and 1-3 β glucanase.
- *Water use (P1, P3*, P6)*
- A population of *F. ananassa* x *F. chiloensis* segregating for water use has been subject to preliminary QTL analysis prior to the EUBerry project
- Markers linked to water use efficiency markers will be tested in germplasm from other partners.

Task 1.2 Development of molecular tools for support and enhancement of berry fruit breeding

Sub-task 1.2.1 Strawberry (*Fragaria*) (P1, P2, P3, P5, P6, P7, P8)

P1 have planned inter-specific cross combination of *Fragaria virginiana* ssp. *glauca* X *Fragaria* x *ananassa* to generate new populations that will be submitted to P6 for QTL identification, Association mapping, and Next generation sequencing.

Cross Combination Planned:

Male Parental		Female Parental
FRA 982.000 (<i>F. virginiana</i> ssp. <i>glauca</i>)	X	Romina (<i>F. x ananassa</i>)
FRA 982.000 (<i>F. virginiana</i> ssp. <i>glauca</i>)	X	Cristina (<i>F. x ananassa</i>)

Sub-task 1.2.4 Blueberry (*Vaccinium*)

Design of primers for sequences of MYB17, MYB10, bHLH & F3'S'H involved in anthocyanin production and regulation. Four sets of these primers have been found to be polymorphic in a diploid blueberry population. Work is in progress to test these markers in two tetraploid mapping populations to examine the extent of co-segregation with anthocyanin quantity.

Work in this crop is planned for Yrs. 2 and 3. Using Plant & Food Research *Vaccinium* EST library

Sub-task 1.2.5 Elaboration of a strategy to develop marker assisted breeding (MAB) for strawberry, raspberry, blackcurrant and blueberry and for important traits with breeders and associated SMEs (P1, P2, P3, P5, P6, P7, P8, P10, P13)

P1 have planned the cross combination program to develop new breeding population generated by inter-specific cross combination of *Fragaria* x *ananassa* X *Fragaria virginiana* ssp. *glauca* using varieties with high sensorial and nutritional quality.

Male Parental		Female Parental
FRA 982.000 (<i>F. virginiana</i> ssp. <i>glauca</i>)	X	Romina (<i>F. x ananassa</i>)
FRA 982.000 (<i>F. virginiana</i> ssp. <i>glauca</i>)	X	Cristina (<i>F. x ananassa</i>)

WP 1. ACTIVITIES OF PARTNER 2 (IO – POLAND)

Task.1.2 Development of molecular tools for support and enhancement of berry fruit breeding through the use of marker assisted breeding; Task Leader Beatrice Denoyes-Rothan, P6

Sub-Task 1.2.1 Strawberry (*Fragaria*) – KORBIN M.

1. Searching of molecular tools useful for selection in breeding for resistance to *Verticillium* wilt disease.
2. Study on 'Elsanta' x 'Dukat' breeding population - 112 individuals were molecularly evaluated for their origin, two putative QTL regions correlated with plant tolerance to wilt disease were located on strawberry map, single candidate genes were selected from HTS data-base obtained in IO (over 6 million tags).

Sub-Task 1.2.3 Blackcurrant (*Ribes*) – KORBIN M.

1. Extracted genetic material is ready for verification of new markers (Scottish partner – P3) for blackcurrant selection in regard to different trait characterized fruit quality (200 accession form IO collection, parental forms and 152 individuals from three breeding populations):
 PC-7/13 (small fruits, high content of polyphenol and vit. C) x 'Big Ben' (large fruits, high content of polyphenol and vit. C)
 'Titania' (medium fruits, medium content of polyphenol and vit. C) x 'Big Ben' (large fruits, high content of polyphenol and vit. C)
 'Foxendown' (small fruits, high content of polyphenol and vit. C) x PC-7/13 (small fruits, high content of polyphenol and vit. C).

Sub-task 1.2.4 - *Vaccinium*

- Plant & Food Research (NZ) *Vaccinium* EST library used to design primers for sequences of MYB17, MYB10, bHLH & F3'5'H involved in anthocyanin production and regulation (P1).
- Four sets of primers found to be polymorphic in diploid blueberry population (obtained through a US collaborative project)
- Work is in progress to test markers in two tetraploid mapping populations to examine the extent of co-segregation with anthocyanin quantity (P1, P3).



T1.3 Validation of key genes in strawberry

- Sub-task 1.3.1 Validation of nutritional quality genes (P1, P6)
- Sub-task 1.3.2 Validation of flowering genes (P1 – MPU, P5 - IFAPA, P6 – INRA)



- Outputs
 - Validation of genes controlling
 - fruit nutritional quality
 - Flowering traits



Sub-task 1.3.1 - Validation of nutritional quality genes

- Transformation of varieties of Sveva and Calypso with ANS (anthocyanidin synthase) and DFR (dihydroflavonol) constructs
- Transformants awaiting analysis
- Further transformation experiment on Sveva with the same ANS gene is planned



Subtask 1.3.2 – Validation of flowering genes

- Genes used:
 - KSN -flowering repressor from *Fragaria vesca*
 - homologue of TFL1 gene for flowering repression from *Arabidopsis*
 - FvFTx – floral activator from *F. vesca*
- Transformation of varieties Sveva and Calypso with KSN from P6
- Further transformation planned using FTx



WP1 – P06



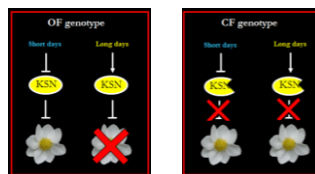
WP1 - Improving berry varieties through the identification and utilisation of the best genetic resources

• Task 1.3 Validation of the role of key genes in strawberry traits

Functional validation

Flowering habit – seasonal vs. recurrent,
KSN, an orthologue of TFL1 is responsible of recurrent flowering in *F. vesca*.

Model for continuous flowering in diploid *Fragaria*

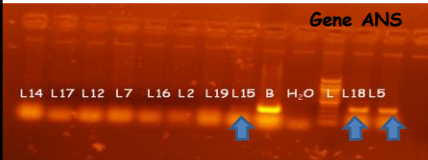


Constructions with KSN were sent to P1
New constructions will be obtained.

Task 1.3 Validation of the role of key genes in strawberry traits

Sub-task 1.3.1 Validation of nutritional quality-related genes

Transformation of varieties of Sveva and Calypso with 35S-driven ANS and DFR constructs mediated by *Agrobacterium tumefaciens* using strawberry leaves propagated in vitro. Strawberry transformed leaf are in selection phases on regeneration and selection substrate added with Kanamycin. Plants were propagated and grown in green house following the rules of GM restricted conditions evaluation.



PCR analysis of ANS gene has been done on propagated "Calypso" plants generated from transformation. Three lines, L15, L18 and L5 of gm plants resulted positive to ANS gene.



DFR plants has been analyzed by PCR with DFR gene and NPTII gene for Kanamycin resistance. DFR plants resulted negative to PCR analysis of DFR gene, while resulted positive to NPTII gene.

Investigation research are now mainly focused on ANS GM plants L5, L15 and L18 from which have been harvested fruits that has to be sent to P5 for RNA extraction.

Sub-task 1.3.2 Validation of flowering-related genes

Transformation of varieties Sveva and Calypso has been done by P1 with constructs CaMV35S FvKSN from P6, mediated by *Agrobacterium tumefaciens*, were performed using strawberry leaves propagated in vitro. Strawberry transformed leaf are in selection phases on regeneration and selection substrate added with Hygromycin.

