Breeding sweet cherries at INRA Bordeaux: from conventional techniques to marker-assisted selection
José Quero-Garcia, José Antonio Campoy, Sophie Castede, Teresa Barreneche, Loick Le Dantec, Bénédicte Wenden, Jacques Joly, Lydie Fouilhaux, Elisabeth Dirlewanger

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Breeding sweet cherries at INRA-Bordeaux: from conventional techniques to marker-assisted selection

José Quero García (INRA – Bordeaux)
Plan

- First breeding programmes (1968 – 2005) (Raymond Saunier-Jacques Claverie)

- Transition (2000-2005) (Jacques Claverie-Elisabeth Dirlewanger)

- Present (2007- today) (UREF-A3C)
Second breeding programme (1980-2005)

New orientations:
- Introduction of numerous (400) foreign cultivars
- Establishment of experimental networks (A-B) via a collaboration between INRA and CTIFL

New breeding objectives:
- Big fruits (= or > 10 g) and firm fruits (Durofel > 60-65)
- Enlargement of the maturity range
- Yield precocity (3rd year)
- High production potential
Second breeding programme (1980-2005)

STRUCTURE OF THE PROGRAMME

- Year 0: Hybrid production
- Year 1: Nursery
- Year 2: Field planting on own roots
- Years 5-7: Hybrid evaluation
- Years 8-11: Level 1 evaluation: 3 sites/ 2 cl
- Years 12-15: Level 2 evaluation: 10 sites/ 10 cl
Second breeding programme (1980-2005)

HYBRID PRODUCTION TECHNIQUES

- Hand-pollination for controlled crosses, open pollinations
- In-vitro culture for early-maturing hybrids
- Classical stratification
Second breeding programme (1980-2005)

INRA FERCER

INRA FERPRIME

INRA FOLFER

INRA FERTARD
Second breeding programme (1980-2005)

Poster ‘The partnership between INRA and CEP INNOVATION’- Quero-Garcia et al.
<table>
<thead>
<tr>
<th>Variety</th>
<th>Maturity period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primulat®Ferprime</td>
<td>Burlat</td>
</tr>
<tr>
<td>Folfer\textsuperscript{(COV)}</td>
<td>Between Burlat and Summit</td>
</tr>
<tr>
<td>Ferdouce\textsuperscript{(COV)}</td>
<td>Summit</td>
</tr>
<tr>
<td>Fertille\textsuperscript{(COV)}</td>
<td></td>
</tr>
<tr>
<td>Fermina\textsuperscript{(COV)}</td>
<td>Belge</td>
</tr>
<tr>
<td>V3467</td>
<td></td>
</tr>
<tr>
<td>Ferdiva\textsuperscript{(COV)}</td>
<td>Late</td>
</tr>
<tr>
<td>Fertard\textsuperscript{(COV)}</td>
<td></td>
</tr>
</tbody>
</table>
New research programme on rain-induced fruit cracking:

- **Crosses between tolerant** (Regina, Fermina, Ferobri) and **susceptible** varieties (Lapins, Garnet, Brooks...)

- Establishment of a **first genetic map** : Regina x Lapins (125 hybrids) with Prunus SSR markers (Dirlewanger et al., 2004, PNAS 101: 9891-9896)

- Establishment of an original system to study cracking under tunnels
Diversity studies conducted on sweet cherry (Tavaud et al, Heredity 2004) with AFLP and 6 SSR markers:

- Large sample of mazzards and sweet cherry varieties
- Important genetic differentiation between wild materials from West Europe, Romania and Georgia
- Insufficient sample to determine the centers of domestication of sweet cherry. Proximity between wild and cultivated materials due to either gene flow or to domestication and breeding
Present (2007-today)

- 2007-2008: new breeding programme and new scientific project: ‘Adaptation of sweet cherry to climate change’. Two main targets: **phenology-related traits** and **tolerance** to rain-induced fruit **cracking**

- Close relationship with **UEA (Unité Expérimentale Arboricole)**: two sites: Toulenne and Bourran

- **A3C**: 5 researchers, 5 technicians, 1 PhD student, 1 post-doc
Scientific project

**GENOMICS**
- Development of genomic resources: Unigene - RNAseq
  - Identification of CG: Fonctionnal - Expressionnal
    - Mapping of CG

**PHENOMICS**
- Search for accurate phenotypic criteria
  - Phenotyping
    - QTL detection: Multi-site trials, Interaction G x E
    - Structure, LD: Association, Genetics
      - Phenotyping/Collections

**MODELLING**
- Genotyping
  - Climatic data
    - Co-localisation CG-QTL
      - GC Studies
        - Functional analyses
          - qRT PCR-epigenetic variation -transformation

**MAS**
- Genotype
- Phenotype
  - Ideotypes
Breeding programme

- **Selection criteria:**
  - Fruit weight and fruit firmness
  - Phenology-related traits: chilling requirements, flowering and maturity dates
  - Cracking tolerance
  - Self-fertility
  - Organoleptic and nutritional quality
  - Tolerance to diseases (monilia, aphids)

- **Marker-assisted selection (MAS) strategy**
- **Diversification of genetic resources used**
Marker-assisted selection

- **Plant materials for QTL detection studies:**
  - Regina x Lapins (RxL): 125 inds. (enlarged to 200). Planted in the field on own roots and grafted on Tabel in pots (tunnel system)
  - Regina x Garnet (RxG): 120 inds. on own roots (enlarged to 1300)
  - Fercer x Burlat (FxB): 115 inds. On own roots

- **Genetic maps:**
  - RxL and RxG with the **6000 SNP chip** (RosBREED project). FxB in progress

- **Phenotypic data:**
  - Phenology traits: **6-7 years** for flowering and maturity dates (all progenies); **3 years** for CR on RxG
  - Fruit quality traits (weight, firmness, cracking): **5-7 years** for field data (all progenies) and **3 years** for tunnel data (RxL)
Gb of markers = 728
Coverage = 624cM
1 marker every 0.86 cM

Campoy et al. (2013): PlosONE
QTL detection analyses - Phenology traits

Dirlewanger et al. (2012), Heredity 109: 280-292

R×L
1  2  3  4  5  6  7  8
R1 R2 R3 R4 R5 R6 R7 R8
L1 L2 L3 L4 L5 L6 L7 L8

R×G
R1 G1  R2 G2  R3 G3  R4 G4  R5 G5  R6 G6  R7 G7  R8 G8

Chill requirement  Flowering date
Heat requirement  Maturity

Poster P23 – Castède et al.
Cracking tolerance evaluation

- **Field**: 100 fruits at maturity → percentage

- **Tunnel**: 100 fruits, counting during 5 days → percentage

4 types analyzed
QTL detection analyses - Fruit quality traits

Dirlewanger et al. (2012), RGC6
**Candidate Genes (CGs)**

- **Flowering**

  - Poster P18 – Dirlewanger *et al.*

- **Fruit weight:** Collaboration with Amy Iezzoni’s group concerning the **CNR genes** (see next presentation; De Franceschi *et al.*, Molecular Breeding, 2013)

- **Cracking tolerance:** Collaboration to initiate with Herman Silva’s group to map CGs of the biosynthesis pathway of the **cuticle**
Multi-parental analyses (new QTLs; QTL stability; no need to produce large progenies)

- Collaboration with Amy Iezzoni’s group for fruit weight, use of pedigree-based mapping with FlexQTL software (Rosyara et al., 2013, Mol Breed, accepted with revisions): identification of 6 QTLs (3 on LG2, one on LGs 1, 3 and 6)
- Integration of different crosses, including FxB

Fine mapping: development of a large RxG population with 1300 hybrids. Planted in the field in 2013. Used for the validation of a MAS strategy

Environmental stability (QTLxE) studies: multi-site trials implemented within the COST Action: RxL population planted in France, Slovenia, Spain and England. Other populations.
Development of a fine mapping progeny
Establishment of multi-site trials

Bordeaux, France (2001-2002)

Murcia, Spain (2012-2013)

Bordeaux, France (2010-2011)

Maribor, Slovenia (2009-2010 and 2011-2012)
Phenotypic decomposition of complex traits – fruit cracking:

- New studies under tunnel with RxL progeny grafted on MM14
- Analyses of **cell wall composition** of the RxL progeny on-going (collaboration with Dr. Marc Lahaye, INRA Nantes)
- Analyses of the main **metabolites (sugars, acids)** initiated in 2013 in collaboration with Dr. Yves Gibon. Fruits collected from the progenies RxL and FxB. Three replicates per genotype, with three fruits per replicate and three technical replicates.
- Tri-lateral KBBE project (Spain, Germany and France) submitted in 2012 (waiting for answer). Objective: develop new protocols for assessing cracking tolerance taking into account the composite nature of cracking (water transport characteristics and mechanical constitution of exocarp) (Prof. Moritz Knoche)
QTL/CG analyses - Perspectives

- **QTL/CG validation by GWA/association genetics analyses:**
  - First ‘Structure’ studies conducted with 26 SSR markers on 207 varieties (141 landraces and 66 modern varieties) (Mariette et al., 2010, BMC Genetics)
  - Study of DL: rapid decay, in particular in wild accessions (Arumyawat et al. 2012, TGG), promising for future association genetics studies

- Second ‘Structure’ analyses on-going with the 6K SNP chip on 140 varieties (92 landraces, 55 modern varieties). DL analyses not still initiated (Sandra Robert, MSc).

- Genetic resources collection genotyped with a subset of 41 CGs SNPs
QTL/CG analyses - Perspectives

Phenotypic variability of collections:

- Based on 20 phenotypic traits (3 years data): flowering date, maturity date, fruit weight, sugar content, etc.
- 380 accessions, among which 160 belong to the French National Collection.

Flowering and maturity range of the collection:
2 years 380 accessions

Large variability for flowering and maturity dates
Discovery and validation of CGs:

- Transcriptomics: expressional CGs (DGE-RNAseq): ongoing for flowering-related genes
  - 18 cDNA banks: 2 genotypes (Regina and Garnet), 3 stages (endo-dormancy, dormancy-release, eco-dormancy)
  - 12-50 millions read/ 100 bases

- Expressional validation: qRT-PCR (PhD S. Castède, 12 flowering CGs)
Modelling: from phenology data to MAS

Data
- Phenology data
  - Flowering
  - Maturity
- Climatic data
  - Temperature
- QTLs
  - Flowering date
  - Chill requirement
  - Heat requirement
- Molecular data
  - Candidate genes
  - Signaling pathways

Models
1. Flowering and maturity date response to temperature
   - 1 cultivar – 1 site
   - + various sites
2. Flowering and maturity date
   - Wide range of climatic conditions
   - + cultivars and segregating populations
3. Flowering and maturity date
   - Model based on mechanisms
   - Genetic parameters

Outputs
- Analyses of past trends
  - Temperature / Flowering
- Predictions
  - Flowering and Maturity dates
- Predictions
  - Effect of genotype on flowering and maturity dates
  - G*E interaction
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Wide range of climatic conditions

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Genetic parameters

Outputs

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  Effect of genotype on flowering and maturity dates
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Ideotypes construction

Prediction tests

Allele combinations
Climatic scenarios

Predicted phenotype

Ideotypes
Ideal alleles combination for future climatic conditions

MAS
MAS strategy - Conclusions

- Promising for traits with stable and high-effect QTL: flowering and maturity dates, fruit weight and firmness (problem of negative correlations). Still early for very complex traits such as cracking tolerance.

- Trial to validate a multi-trait strategy: select two sets of 150 inds with best and worst allelic combinations and phenotype them (RxG pop).

- Hybrid production challenge: medium to large sized families, incorporation of genetic diversity.

- Logistic challenge: implement a pipeline. Learn from other experiences (WSU in cherry; other crops, apple, peach...). Which genotyping strategies?
Main objective:

Develop innovative strategies to safeguard European cherry production through active networking by:

- The adaptation of cherry cultivation to climate change
- The implementation of new cultivation practices aimed at promoting sustainable agriculture
- The promotion of high-quality fruits
Acknowledgements

A3C TEAM
VERY LONG LIST OF NATIONAL AND INTERNATIONAL COLLABORATORS!!!!!
THANKS FOR YOUR ATTENTION !!!!!

TIME FOR QUESTIONS!!