III) Construction of a new genetic map

- Discovery of SNPs
  - First Wonder / Chilli de Morelos 334

- Selection of SNPs in Candidate Genes

- Mapping

Plan of action

To deliver new markers:
- Expected to be functional markers,
- Optimize the estimation of allele values for the genotype-specific models.

Validation by a genetic association study between the traits and sequence polymorphisms in a pepper core-collection

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Plan

I) Selection of 384 SNPs

- After eQTL and bulk analysis, a list of 1139 sequences was established.
- Among these sequences, 351 presented a mappable SNP:
  - 33 in isoflavin singleton sequences,
  - 18 in sequences that were absent in the transcriptome sequencing.
- 33 SNP found in a prior candidate genes, were added.
- Genotyping using the VeraCode technology.

II) Genotyping of complete F5YC population

- Among these 230 SNPs:
  - 215 in candidate genes from Bulk QTL or eQTL analyses,
  - 14 in a prior candidate genes with 2 cyclines, 2 CDM, Fas, sun, ...
- These data were transmitted to Belgian and Dutch colleagues for further analysis.

III) Construction of a new genetic map

- Candidate genes
- Plant traits
- eQTL
- Trait
- Gene

- SNPs

- Validation by a genetic association study between the traits and sequence polymorphisms in a pepper core-collection

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III) Construction of a new genetic map

Distribution on the 12 pepper chromosomes

We will further look for Mark and Nurudeen responses

III) Genetic association study - MLM analysis

- Significant marker-trait associations were identified using the mixed linear model (Tassel software):
  - It takes account of population structure and kinship.
  - It reduces error due to relatedness and population structure.

- Construction of 4 data files (input):
  - Core collection was genotyped
  - 362 accessions/362 SNPs

- Genotype
- Phenotype
- Structure
- Kinship

Calculation of pairwise kinship coefficients
- Procedure of Loiselle et al. (1995)

Compared with pQTLs from Nurudeen: 2 associations were promising.

13 sequences of isolate blasted to the sequences of ESTs (array)

Pepper array ESTs originated from 3 databases:
- NCBI (USA),
- Dena-Farber Cancer Institute-The gene index project (USA),
- KRBB.

4 sequences presented a significant homology to only 1 EST,
9 sequences to several ESTs,
2 sequences no homology.

5 are located in the same chromosomal region as an eQTL.

IV) Genetic association study - Construction of core collection

- Structure of pepper collection: 6 clusters
- Capsicum annuum: 3 clusters
- 3 clusters of C. annuum: 3 varieties

Core collection groups:
- 3 separate core collections from each of the 3 C. annuum clusters.
- (the size of each of the 3 core collections being proportional to the genetic diversity within each cluster)
- Associations from clusters 4, 5, and 6 were added to be used as an out-group.

Total of 391 accessions

III) Genetic association study - Results

<table>
<thead>
<tr>
<th>Trait</th>
<th>pQTLs 1</th>
<th>pQTLs 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PVT resistance</td>
<td>0.0004</td>
<td>0.0005</td>
</tr>
<tr>
<td>TMV resistance</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Apical fruit diameter</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fruit length</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fruit shape</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>(fruit diameter: apical fruit diameter)</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Pericarp thickness</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Fruit softness</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Pompey</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Flowering earliness</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Plant habit</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Asin length</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
<tr>
<td>Leaf size</td>
<td>0.0001</td>
<td>0.0002</td>
</tr>
</tbody>
</table>

Seventeen significant marker-trait associations exceeding Bonferroni household (P value ≤ 10⁻4)

- Control
- pQTLs
- TMV resistance
- CDKA and L gene: same region
- Linkage disequilibrium

Several important associations with different fruit traits and plant traits

III) Genetic association study - Analysis of results

Compared with eQTLs from Nurudeen: 2 associations were promising.

1. SNP 15785: leaf size
2. SNP 22599: leaf length

1- SNP 15785: leaf size
2- SNP 22599: leaf length

Different QTLs related to the same traits

QTLs on different chromosomes (78) and (12): leaf length decrease in 508, leaf size increase in 518.
Conclusion

- 230 new SNP markers were mapped in the RIL YC progeny, generating a new genetic map.
- This saturated map was constructed with 460 markers distributed in the 12 pepper chromosomes.
- The previous results of genetic association study highlights 17 significant marker-trait associations.
- In comparison with the position of eQTLs and the phenotypic QTLs, 2 of these associations seem to be promising.
- We expect further colocalizations from the analysis on the relative position of these SNPs with the eQTL and the pQTL positions.

Yesterday, last information

<table>
<thead>
<tr>
<th>SNP in candidate genes obtained after eQTL and Bulk QTL analysis</th>
<th>a priori candidate genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mapped SNPs</td>
<td>351</td>
</tr>
<tr>
<td>Seq homologue to one EST</td>
<td>216</td>
</tr>
<tr>
<td>Seq homologue to different ESTs</td>
<td>117</td>
</tr>
<tr>
<td>Seq re-eQTL</td>
<td>147</td>
</tr>
<tr>
<td>Cis</td>
<td>130</td>
</tr>
<tr>
<td>Trans</td>
<td>17</td>
</tr>
</tbody>
</table>

Co-localization with pQTL

Project participants

Alain Pallot
Véronique Lelebrore
Anne-Marie Sage-Pallot : person in charge of pepper collection
Ghislaine Nemouchi
Bruno Savio
Alexandre Bachelez
Melissa Cantet

Thank you for your attention!