Association and Linkage Mapping of Walnut (Juglans regia L.) Phenological Traits
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To cite this version:
Anthony Bernard, Annarita Marrano, Armel Donkpegan, Patrick J Brown, Charles A Leslie, et al.. Association and Linkage Mapping of Walnut (Juglans regia L.) Phenological Traits. International Plant & Animal Genome XXVIII, Jan 2020, San Diego, United States. hal-03358866

HAL Id: hal-03358866
https://hal.inrae.fr/hal-03358866
Submitted on 29 Sep 2021

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Association and Linkage Mapping of Walnut
(*Juglans regia* L.)
Phenological Traits
The world in-shell walnut production is increasing.
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- China > California > Iran
- France: 7th with 40,000 tons
- Area of orchards: 2nd French fruit crop
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Walnut breeding goals in France:
increased yield, larger nut size, ease of cracking, adaptation to climatic conditions (late spring frosts) → phenology
1. The world in-shell walnut production is increasing.

2. China > California > Iran
   France: 7th with 40,000 tons
   Area of orchards: 2nd French fruit crop

3. Walnut breeding goals in France:
   increased yield, larger nut size, ease of cracking, adaptation to climatic conditions (late spring frosts) → phenology

4. Effect of climate change, but breeding possible since phenology-related traits are also controlled by genetic background.
The world in-shell walnut production is increasing.

China > California > Iran
France: 7\textsuperscript{th} with 40,000 tons
Area of orchards: 2\textsuperscript{nd} French fruit crop

Walnut breeding goals in France:
- increased yield
- larger nut size
- ease of cracking
- adaptation to climatic conditions (late spring frosts) → phenology

Axiom™ \textit{J. regia} 700K SNP array (Marrano \textit{et al.}, 2019)
GWAS on walnut: in-shell walnut and kernel traits (Arab \textit{et al.}, 2019), water use efficiency (Famula \textit{et al.}, 2019), and yield, lateral bearing, pellicle color, leafing date and harvest date (Marrano \textit{et al.}, 2019)
Goals: study of *Juglans regia* genetic resources for the implementation of a marker-assisted selection

→ basic research: genetic diversity and structure evaluation of INRA germplasm repository (Bernard *et al.*, 2018), and genetic architecture deciphering of main traits of interest

→ applied research: establishment of necessary tools for marker-assisted selection
1

Goals: study of *Juglans regia* genetic resources for the implementation of a marker-assisted selection
→ basic research: genetic diversity and structure evaluation of INRA germplasm repository (Bernard et al., 2018), and genetic architecture deciphering of main traits of interest
→ applied research: establishment of necessary tools for marker-assisted selection

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Action plan:
→ phenotyping: 2017, 2018, 2019, 2020 for many traits related to phenology, nut in-shell and kernel
→ genotyping: using SSRs and Axiom™ *J. regia* 700K SNP array
→ plant material: F₁ progeny (78 individuals) segregating for phenology + unique genetic resources core-collection (170 accessions)
→ GWAS, combined with QTLs detection for phenology
→ marker validation on other plant material
→ choice of genitors
Phenotypic evaluation of phenology and data analysis

Association and Linkage Mapping of Walnut (*Juglans regia* L.) Phenological Traits – Fruits/Nuts Workshop
Phenotypic evaluation of phenology and data analysis

1. **Budbreak date** (leaves and female flowering)
   - Beginning of female flowering date
   - Full female flowering date
   - End of female flowering date
   - Beginning of male flowering date
   - Full male flowering date
   - End of male flowering date

2. **Best Linear Unbiased Predictions (BLUPs)**
   - $P_{ik} = \mu + Y_i + g_k + e_{ik}$
   - $P_{ik}$ - observed phenotype of the $k^{th}$ accession in the $i^{th}$ year;
   - $\mu$ - mean value of the trait; $Y_i$ - fixed effect of the $i^{th}$ year;
   - $g_k$ - random effect of the $k$ genotype; and $e_{i(j)k}$ - residuals of the model

→ 2018 data
→ 2019 data

**lme4**
By calculating the BLUPs, budbreak date within the GWAS panel is considered as normally distributed.
Descriptive statistics and heritability, a case study with budbreak date

Association and Linkage Mapping of Walnut (*Juglans regia* L.) Phenological Traits – Fruits/Nuts Workshop
1. By calculating the BLUPs, budbreak date within the GWAS panel is considered as normally distributed.

2. Broad-sense heritability \( H^2 = \sigma^2_G / [(\sigma^2_G + (\sigma^2_\varepsilon / n_{\text{obs/g}}))] \)

where \( \sigma^2_G \) - genotypic effect variance;
\( \sigma^2_\varepsilon \) - variance of residuals;
and \( n_{\text{obs/g}} \) - number of observations by genotype

\( \Rightarrow 0.93 \)
Step 1. To keep SNPs of high resolution from Axiom® Analysis Suite

<table>
<thead>
<tr>
<th>Conversion Type</th>
<th>Number of markers</th>
<th>Percentage of markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>PolyHighResolution</td>
<td>397,921</td>
<td>65.27</td>
</tr>
<tr>
<td>NoMinorHom</td>
<td>75,564</td>
<td>12.39</td>
</tr>
<tr>
<td>MonoHighResolution</td>
<td>36,684</td>
<td>6.02</td>
</tr>
<tr>
<td>CallRateBelowThreshold</td>
<td>27,761</td>
<td>4.55</td>
</tr>
<tr>
<td>OffTargetVariant</td>
<td>4,787</td>
<td>0.79</td>
</tr>
<tr>
<td>Other</td>
<td>66,941</td>
<td>10.98</td>
</tr>
<tr>
<td><strong>Total of retained SNPs</strong></td>
<td><strong>510,169</strong></td>
<td></td>
</tr>
</tbody>
</table>

Step 2. To keep SNPs with mendelian inheritance using \( F_1 \) progeny

SNPs having no mendelian inheritance 661

Total of retained SNPs 509,508

Step 3. To keep SNPs having genotyping rate >90%

SNPs having genotyping rate <90% 13,993

Total of retained SNPs 495,515

Step 4. To keep SNPs having minor allele frequency >5%

SNPs having minor allele frequency <5% 123,751

Total of retained SNPs 371,764

Step 5. To delete duplicated SNPs

Duplicated SNPs 7,489

Total of retained SNPs 364,275

\[ 609,658 \rightarrow 364,275 = 59.8\% \]
Structure analysis shows clustering according to geographical origin

K2: one group with accessions from Western Europe and America, other with accessions from Eastern Europe and Asia

K3: highlights hybrids
Confounding effects for GWAS

Structure analysis shows clustering according to geographical origin

- **K2**: one group with accessions from Western Europe and America, other with accessions from Eastern Europe and Asia

- **K3**: highlights hybrids

Structure also investigated with PCA

Cryptic relatedness calculated using kinship matrix may account for structure

→ Best number of PCs to include = 0 (Bayesian Information Criterion)
GWAS results for budbreak date

Other work on leafing date: 3 SNPs on chr 1, between 3,187,214 and 4,805,396 bp (Marrano et al., 2019)
Intraspecific F₁ mapping progeny of 78 individuals

Female: ‘Franquette’ (late budbreak date)
849 SNPs

Male: ‘UK6.2’ (intermediate to early)
1,088 SNPs

Pseudo-testcross strategy:
JoinMap4.0
LOD 16.0 for mapping
Kosambi’s function
MultiQTL 2.6
Multiple Interval Mapping
Female flowering dates

Beginning

Full

End
Other phenology-related traits

**1. Female flowering dates**
- **Beginning**
- **Full**
- **End**

**2. Male flowering dates**
- **Beginning**
- **Full**
- **End**
Female flowering dates
Chr 1 – 9,298,520 bp

*Associated SNP
LD blocks and candidate genes

**1**

**Female flowering dates**

Chr 1 – 9,298,520 bp

*Associated SNP

**2**

**Male flowering dates**

Chr 4 – 5,966,307 bp

*Associated SNP

**Chr 11 – 31,874,617 bp**

*Associated SNP

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**Association and Linkage Mapping of Walnut (Juglans regia L.) Phenological Traits – Fruits/Nuts Workshop**
96 unreleased breeding line accessions from WIP, University of California, Davis
KASP marker development and validation for budbreak date

1. 96 unreleased breeding line accessions from WIP, University of California, Davis

2. Kruskal-Wallis test, p-value = $6.88 \times 10^{-13}$
   Budbreak date vs. leafing date
Thank you for your attention!
Any questions?