Development of highly-multiplexed SNP arrays in Maritime pine for multi-objective genetic applications

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Introduction / Objectives

➢ SNPs are the most abundant class of polymorphisms in the genome. They are frequent in coding regions providing gene-based markers for different genetic and genomic applications.

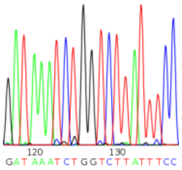
➢ The reduction in cost and the increased throughput of SNP assays have made these markers very attractive especially in conifers.

➢ 6 highly multiplexed customized SNP genotyping assays have been constructed so far in Maritime pine (*Pinus pinaster* Ait.), the first species used for reforestation in Southwestern Europe.

➢Here we report on three different applications related to linkage analysis, genetic diversity and association mapping.

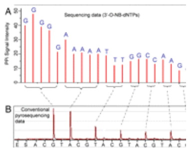
Methods / Applications

Maritime pine transcriptome



Targeted amplicon resequencing (Sanger)

Next Generation Sequencing Technology (454 Roche)



In vitro SNPs ⇔ *In silico* SNPs

Pipeline for SNP detection and Goldengate assay (Illumina Inc.) preparation

Genetic linkage mapping

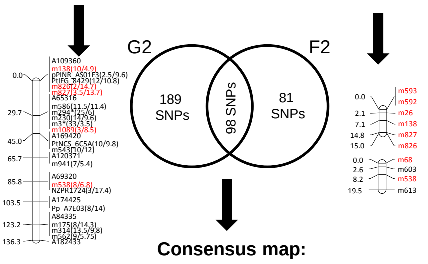
1,536 SNPs in 885 contigs

⇩

1056 SNPs correctly genotyped

Outbred pedigree

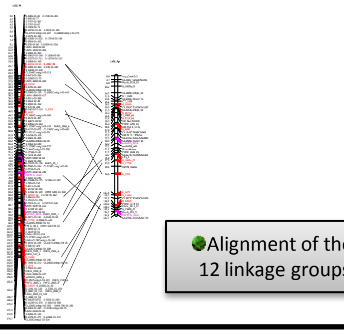
Inbred pedigree



Consensus map:

357 SNPs mapped corresponding to <u>292 different loci</u>

⇨ Search for orthologous markers between maritime and loblolly pine



Alignment of the 12 linkage groups

Population structure analysis

384 SNPs in 240 contigs

⇩

260 SNPs correctly genotyped

36 populations in 8 ecotypes → 672 genets

⇩

Clear population genetic structure identified

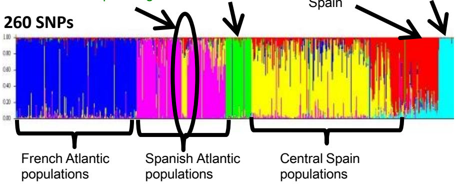
A plantation with Central Spain origin!

Corsica

Southern Spain

Morocco

260 SNPs



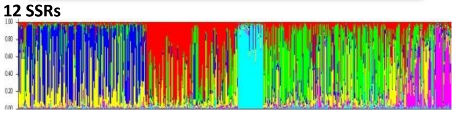
French Atlantic populations

Spanish Atlantic populations

Central Spain populations

Higher discrimination power with SNPs than with SSRs

12 SSRs



Association study

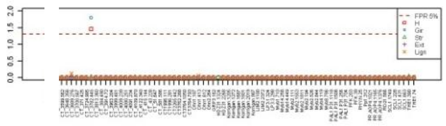
384 SNPs in 240 contigs

⇩

257 SNPs correctly genotyped

➢160 1st generation plus-trees (progeny tested)

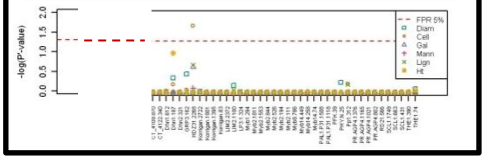
141 non redundant SNPs, 1 with significant association with <u>growth</u>: CT_3782.445, <u>fasciclin-like arabinogalactan protein</u>



- Total height
- Diameter at 1.3m
- Stem straightness
- Lignin and cellulose content

➢162 2nd generation plus-trees (clonaly tested)

121 non redundant SNPs, 1 with significant association with <u>cellulose content</u>: HDZ31.2268, <u>class III homeodomain-leucine zipper</u> transcription factor



Conclusion / Prospects

GoldenGate assays can be used successfully for high-throughput SNP genotyping in maritime pine. A 12k Infinium-array has been constructed and is being used for high density linkage mapping (7 pedigrees) and association studies (breeding population).

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