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Resources and potential for candidate-gene association studies in European black poplar for leaf rust resistance

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High levels of variability for partial resistance to *Melampsora larici-populina* leaf rust in black poplar (*Populus nigra* L.) across Europe offers unique opportunity to identify favourable alleles through association studies.

Nucleotide polymorphism (SNP and indels) within candidate genes has been explored in a discovery panel of 21 French *P.nigra* genotypes.

Phenotyping for field and laboratory resistance components revealed significant diversity at both the intra and inter-metapopulation levels, with a suspected North vs. South geographical contrast.



Studied material: origins and neutral diversity

1,141 *P.nigra* trees were sampled from 14 natural metapopulations in Germany, Netherlands, Italy and France. Altitudinal range goes from 10 m to 1,630 m. All sampled genotypes were cloned and evaluated for rust resistance in a replicated field experiment in Orléans (France).



Figure 1. Geographical distribution of the studied *P.nigra* metapopulations and respective sample sizes.

>60% of the French metapopulation was genotyped for 10 SSR markers covering 9 linkage groups to test for population structure at neutral loci.

Global $F_{st} = 0.0391$ (highly sign.)
 Mean $H_0 = 0.647$, mean $A_e = 4.79$

Although significant, the level of metapopulation differentiation at neutral loci was low and mostly driven by the outlying Adour meta-population. No clear geographical gradient was found.

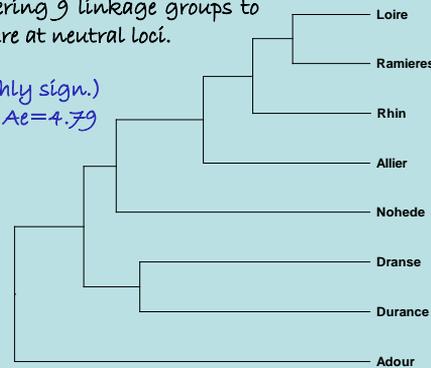


Figure 2. Dendrogram based on Nei's (1972) genetic distances between the 8 French metapopulations (subset of 499 individuals for 10 SSR markers) (Method: UPGMA).

Nucleotide diversity in candidate genes

Table 1. Summary of SNP and diversity indices for 5 of the 24 genes re-sequenced in 21 French *P. nigra* individuals. L: fragment length (bp); singl.: singletons; π : mean differences between all pairs of haplotypes in the sample; N_h : number of haplotypes.

	Gene fragment name	L	All sites			Non-synonymous sites			Silent sites			N_h
			SNPs (singl.)	Indels	π	SNPs (singl.)	Indels	π	SNPs (singl.)	Indels	π	
Positional candidates	PIIN-T	757	8(0)	0	2.7	1(0)	0	1.1	7(0)	0	3.43	5
	OX-RED-1	587	14(5)	3	4.95	1(0)	0	1.1	13(5)	3	6.93	7
	OX-RED-2	617	12(2)	5	8.01	0(0)	0	0	12(2)	5	8.67	4
	OX-RED-3	470	19(2)	0	12.89	0(0)	0	0	19(2)	0	16.31	9
	TR-INH2	520	10(0)	0	5.65	5(0)	0	4.52	5(0)	0	9.13	9
Expressional candidates	HYP-PROT3	546	3(0)	0	0.51	1(0)	0	0.26	2(0)	0	0.98	3
	PatRelProt	807	26(1)	1	12.16	0(0)	0	0	26(1)	1	22.61	9

Using previous data on *Populus* spp., candidate genes were chosen either for their vicinity to resistance genes and QTLs (8 positional candidates) or on the basis of expression studies (15 expressionnal candidates). Fragments of these genes were amplified from 21 French *P. nigra* individuals and amplicons were directly sequenced. We detected an average of 1 SNP every 78 bp and 1 indel every 713 pb.

The level of polymorphism and diversity for some gene fragments is shown in table 1. The ratio of non synonymous/synonymous polymorphic sites is low. However, this ratio varied between gene fragments suggesting that these genes were not submitted to the same levels of selective constraints.

Rust resistance variability

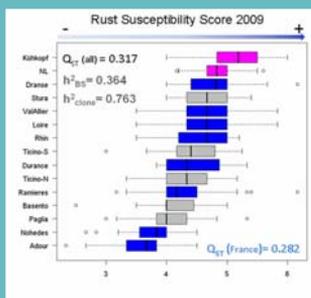


Figure 3. variability for field rust susceptibility within and across metapopulations (clonal means). Susceptibility was scored in September on a 1 to 8 ranking scale on the most infected leaf in a 6-complete-block design.

The estimated broad-sense heritabilities (h^2_{ES}) for field and laboratory resistance descriptors were indicative of reliable phenotyping.

Significant differences between metapopulations were found for field resistance with a possible contrast between Northern and Southern populations. Adour and Nohède metapopulation ranking may be indicative of a peculiar source of rust resistance in the French Pyrenees.

This geographical structure was consistent with the one observed in laboratory, although the latter was significant for uredinia size only.

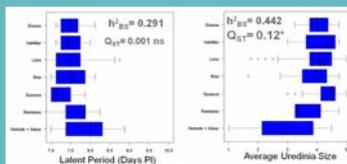


Figure 4. variability for latent period (days after inoculation) and uredinia size (scored on a 1-5 ranking scale) in laboratory excised-leaf-dise bioassay with rust strain 98AG9 (clonal means). Analysis was restricted to a subset of clones from the 6 French metapopulations.

Conclusions and prospects

These preliminary results revealed significant variability at both phenotypic and molecular levels. They also provide guidelines to implement association studies in *P. nigra* for rust resistance. First, the intra-population phenotypic variability is sufficient to perform the association analysis at this level. In this perspective, the size of extreme metapopulations such as Adour should be increased. Second, low numbers of non-synonymous polymorphic sites in the small set of studied genes suggest to extend the SNP discovery to a larger number of genes.

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