BSA-Seq : An efficient tool to characterize loci involved in the Poplar leaf rust resistance.

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INTRODUCTION

The efficiency of Bulk Segregant Analysis (BSA) has clearly been demonstrated to detect genomic regions and genes involved in diverse traits**.** It allows for large experiments reducing the cost and time and preserving the power of full individual's population analysis. These past few years the combination of BSA and Next Generation Sequence (NGS) data (BSA-Seq) gave a new accuracy and depth to the discovery on many traits of interest, mainly on crop and model species (1).

In our study, we applied BSA-Seq to narrow down *Populus* genomic regions involved in the resistance to *Melampsora larici populina* (*Mlp*) leaf rust. First, as a proof of concept, we focused on R_{us}, a major gene previously fine-mapped on Chromosome 19 and controlling the uridenia size during the rust-Poplar interaction (2,3). Then we applied the strategy to detect other regions of interest .

MATERIAL AND METHODS

>MATERIAL

- **Phenotyping** for traits associated with the resistance to *Mlp* leaf rust of parents and **1415 progenies** from an interspecific cross :

***Populus deltoides* clone 73 028-62 (*Pd*) x *Populus trichocarpa* clone 101-74 (*Pt*)**

- Independently DNA extractions with Qiagen Kit **and genotyping** (2,3).

>METHOD – BULK CONSTITUTION

The selection of 62 progenies, based on genotyping of markers physically linked to R_{us} and the phenotypic information, was realized as described in **Figure 1**. Then, the corresponding DNA were pooled equimolarly into 4 bulks.

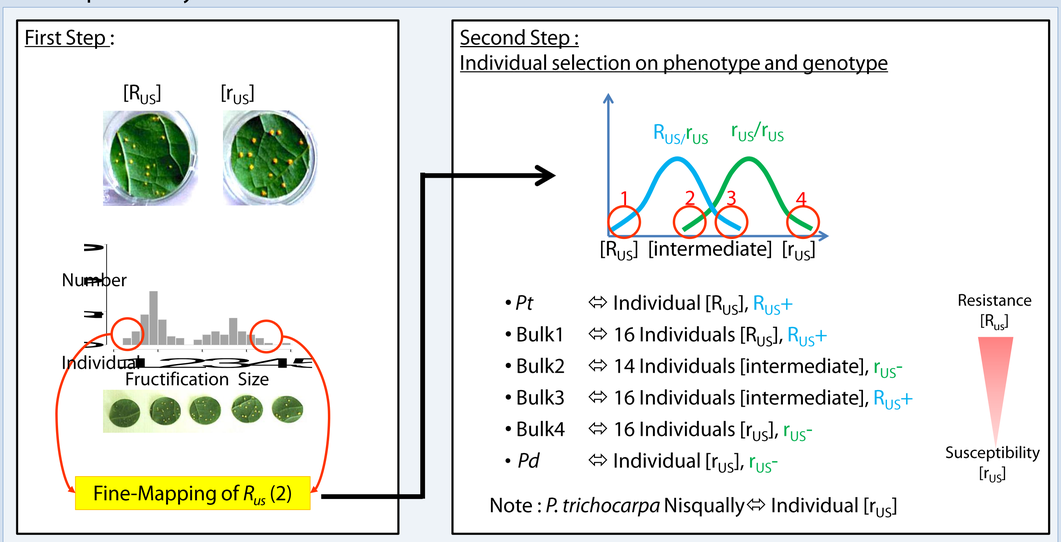

Figure 1. Population phenotyping and genotyping to select progenies for extreme and intermediate Bulks.

>METHOD – BULK COMPARISON

The bulk comparison was done independently for each marker. Three types were observed :

(i) The **RUS** markers are expected to be polymorphic between the 2 parents and to cosegregate with the *P. trichocarpa* [R_{US}] allele and R_{US}+ phenotype. More precisely, its *P. trichocarpa* allele must be present in bulks 1 and 3 and absent in bulks 2 and 4 and *P. deltoides*.

(ii) The **OTH** markers (for « other ») segregate in conformity with the resistance leaf rust and not as **RUS** markers.

(iii) The **NS** markers have no specific allele or do not segregate with the resistance phenotype.

>METHOD – ANALYSIS WORKFLOW (4)

Illumina DNA TrueSeqA kit was used to provide the NGS data. Sequences were trimmed with Trimmomatic/0.32 (5) and mapped with BWA/0.7.12 (6,7) either on the reference *P. trichocarpa* Nisqually v3.0 softmasked (8) or on *P. deltoides* WV94_445 v2.0 softmasked. The variants were obtained simultaneously for the 2 parents and 4 bulks with Freebayes/0.9.21 (9) and filtered with home made Perl scripts on quality and adequation of allelic frequencies with the Mendell's rules. Bulks genotypes comparisons followed to characterize the type of each marker. One region is considered as a **RUS** or **OTH** one if respectivly, almost 2 consecutive variants are **RUS** or **OTH** type. Automatic design of PCR primers in the **RUS** and **OTH** regions was performed with Primer3 (10).

CONCLUSION & PERSPECTIVES

BSA-seq method allows identification of *P. trichocarpa* and *P. deltoides* specific variants for complex traits in a diploid and heterozygous context even if the mapping reference does not carry the searched region of interest. BLAST results showed that genomic regions obtained in the scaffolds can be related to chromosome interest regions and must be studied.

Next steps are first to proceed with the PCR experiments with the new markers on the parents and progenies to enrich the R_{us} fine-map and characterize the other regions in segregation with the resistance to leaf rust. Second to use these markers to describe the diversity of *P. deltoides* and *P. trichocarpa* populations under the QTL and/or genomic regions. Finally we have to study the impact of the diversity on the genes functionality.

Moreover this pipeline, usable on any heterozygous species, releases to the scientific community a high-confidence set of variant positions based on the conformity of the allele frequencies within the bulks.

RESULTS

>INTERVALS NUMBER AND SIZES

References	*P. trichocarpa* Nisqually v3.0 softmasked		*P. deltoides* WV94_445 v2.0 softmasked	
Number of Intervals>50pb (Size) [**>2kb** (Size)]	92 (670kb)	[**12** (143kb)]	43 (188kb)	[**11** (180kb)]
OTH	24 (52kb)	[**2** (47kb)]	14 (22kb)	[**2** (20kb)]
RUS	68 (618kb)	[**10** (96kb)]	29 (166kb)	[**9** (160kb)]
Number of RUS primer pairs	60	[33]	-	[37]
Number of OTH primer pairs	-	[6]	-	[11]

Table 1. Number and size of intervals obtained on each reference.

>FOCUS ON THE RUS REGION

Legend

P. trichocarpa Nisqually v3.0 softmasked
- Chromosome 19 or scaffolds
- Intervals > 50pb

BLAST (11) results
- 1 partial new marker : one primer and partial amplicon
- 1, 2 or 3 new marker(s) : 2 primers and amplicon

P. trichocarpa 101-74 BACs related to :
- R_{us} allele — r_{us} allele

Anchored or **NON anchored** by new markers on one scaffold and Chromosome 19

Previous genetic and physical markers linked to R_{us} (2).

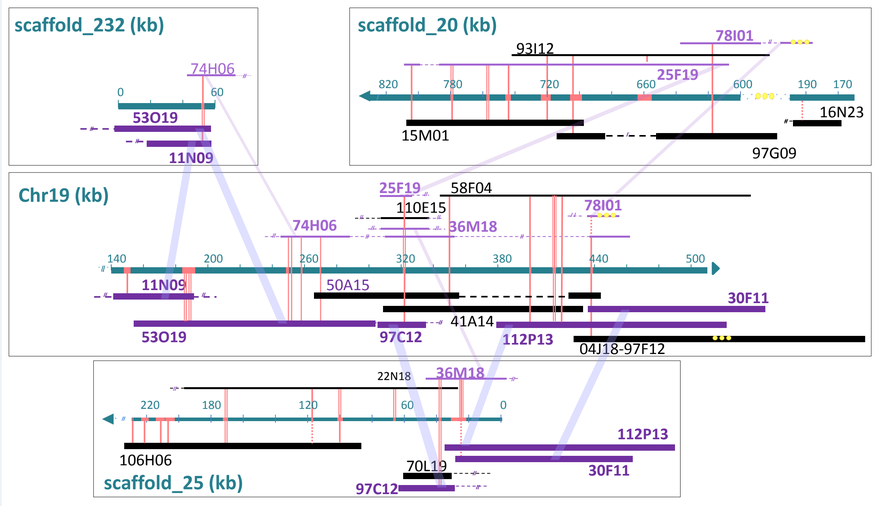

Figure 2. Description of the R_{us} gene environment.

>LOCATION OF THE INTERVALS

Legend

Chromosomes et scaffolds :
P. deltoides WV94-45
P. trichocarpa Nisqually

Variants Number / 100kb :
Heatmap NS Variants
Heatmap and curve of RUS Variants
Heatmap and curve of OTH Variants

QTL :
S Uridinia Number de sores ; **T** Uridinia Size; **L** Latence

Intervals Types (>2kb) :
RUS ; OTH

Primers BLAST results :
Species — Pdelto Ptricho
Links between position
Unique initial Position
Repeated primers

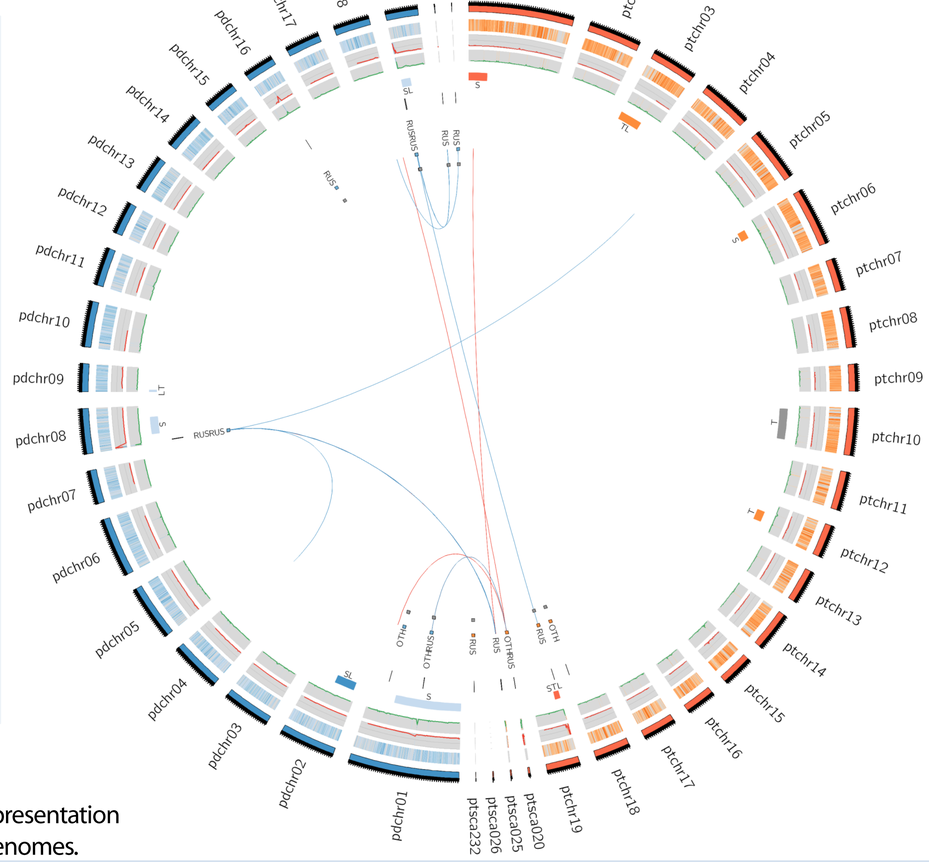

Figure 4. Location of the intervals and representation of the BLAST links between the reference genomes.

>INTERVAL GENES CONTENT

After extraction from Phytozome website (12), of the gene annotations of the *P. trichocarpa* Nisqually genomic region larger than 2kb, a first and rapid automatic term GO enrichment was realized on the agriGO website. (13, **Figure 3** and **Table 2**)

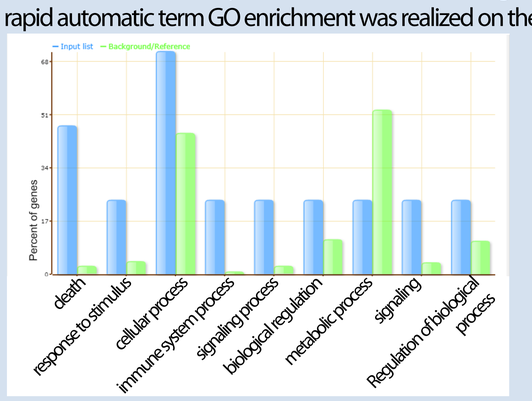

Figure 3. Description of GO annotation.

GO term	Ontology	Description	Number in input list	Number in BG/Ref	p-value	FDR
GO:0043531	F	ADP binding	10	554	3,60E-11	2,30E-09
GO:0006915	P	apoptosis	10	565	4,40E-11	1,20E-09
GO:0012501	P	programmed cell death	10	565	4,40E-11	1,20E-09
GO:0016265	P	death	10	592	6,80E-11	1,20E-09
GO:0008219	P	cell death	10	592	6,80E-11	1,20E-09
GO:0045087	P	innate immune response	5	195	1,20E-06	1,10E-05
GO:0002376	P	immune system process	5	195	1,20E-06	1,10E-05
GO:0006955	P	immune response	5	195	1,20E-06	1,10E-05
GO:0006952	P	defense response	5	251	3,90E-06	3,30E-05
GO:0005524	F	ATP binding	13	3601	4,10E-06	6,30E-05
GO:0032559	F	adenyl ribonucleotide binding	13	3601	4,10E-06	6,30E-05
GO:0004888	F	transmembrane receptor activity	5	263	4,80E-06	6,30E-05
GO:0004872	F	receptor activity	5	265	5,00E-06	6,30E-05

Table 2. Description of most signifiant GO terms.

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