Development of resources for comparative physical mapping between *Muscadinia rotundifolia* and *Vitis vinifera*

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A cost-effective and environment friendly alternative to the use of chemicals is the use of varieties resistant to pathogens. However, for *Vitis vinifera* L. (2n=38), the cultivated grapevine, the resistance needs to be introduced from other Vitaceae through breeding programs ensuring wine quality. Among them, *Muscadinia rotundifolia* (2n=40) is closely related to the *Vitis* genus and is a source of efficient resistance to several pathogens used as a genitor in breeding programs at INRA. However, despite its importance for grapevine breeding, our knowledge about genetics/genomics of *M. rotundifolia* is very limited. Comparative mapping in both species would speed up the identification and isolation the different resistance genes from *M. rotundifolia* and a better understanding of the mechanisms associated to the introgression of genome segments from *M. rotundifolia* in *V. vinifera*. For this purpose, two resources are under development in *M. rotundifolia* cv Regale: a genetic map in a full sib progeny and a Bacterial Artificial Chromosome (BAC) library for physical mapping.

Material and methods

A BAC library of *Muscadinia rotundifolia* cv Regale was constructed according to a protocol modified from Adam-Blondon *et al.* (2005) using *Hind*III and *Bam*HI digested nuclear DNA. The average size of insert was estimated for each sub-library, according to Adam-Blondon *et al.* (2005). The BAC-end sequences (BES) were obtained as described in Lamoureux *et al.* (2006). The percentage of inserts corresponding to chloroplastic DNA was estimated through *in silico* analysis of the BES: when the two BES of a clone were aligned on the grapevine chloroplast sequence, the insert was counted as derived from chloroplastic DNA. The parameters for the alignment of the BES on the *Vitis vinifera* genome were the following: the two BES from a single clone had to show a unique match of 500bp length minimum to the reference genome sequence, the two matches have to be on the same chromosome and their distance is above 20kb or below 150kb.

Results

Four sub-libraries were obtained, 3 using the *Hind*III digested DNA and 1 using the *Bam*HI digested DNA and stored in one hundred and twelve 384 plates. The BAC library thus consists of 54,174 clones. The characteristics of each sub-library are given in table 1. The average size of inserts was rather low compared to previous libraries (Adam-Blondon *et al.*, 2005): 59 to 82kb. The percentage of empty clones was quite high (6% to 14% depending of the sub-library) whereas the chloroplastic contamination was comparable to the one observed for other grapevine libraries by Adam-Blondon et al 2005. Taking into account all these parameters, this BAC library may represent 7X the *M. rotundifolia* genome, giving a 91.49% probability of identifying a clone corresponding to any *Muscadinia rotundifolia* DNA sequence.

Table 1: Characteristics of the *Muscadinia rotundifolia* cv Regale. The number of clones does not take into account the empty clones.

Library (CNS name)	Enzyme	Average size of the inserts	Empty clones (%)	Chloro-plastic clones (%)	Clone number
AEMOAAA	*Hind* III	75 kb	6.27	3.1	15774
AEMOAAB	*Hind* III	82 kb	6.81	2.4	10368
AEMOAAC	*Bam*HI	59 kb	13.97	2.4	13440
AEMOAAD	*Hind* III	73 kb	9	2.4	14592

BAC library**.** A total of 86,810 BES were obtained and aligned on the *V. vinifera* reference genome sequence as a starting point for physical comparative mapping (Figure 1). Thirteen thousand and thirty-two BES of the 86810 BES have showed a unique match to the reference genome sequence and the two BES from a clone have been on the same chromosome.

The ongoing work is now focusing on two regions, one on chromosome 12 and one on chromosome 18 where QTL for resistance to powdery or downy mildew have been detected and containing clusters of NBS-LRR (Moroldo *et al.*, 2008). *Run1*, a single dominant gene present in *M. rotundifolia*, has been introgressed into *V. vinifera* and genetic and physical mapping allowed to construct a BAC contig (made from an introgressed individual) between the SSR markers VMC4f3.1 and VMC8g9 on chromosome 12 (Barker *et al.*, 2005; Figure 2). This contig of BACs still contains a gap and correspond to a region with a cluster of NBS-LRR encoding genes (Donald *et al.*, 2002, Barker *et al.*, 2005 and Dry *et al.*, 2010). The SSR markers VMC4F3-1, VMC8G9 and UDV-058 could be aligned on the *V. vinifera* genome sequence which was not the case for any of the BAC end sequence-derived markers developed by Barker et al (2005) CB46.49, CB13.14 and 49MRP1.P2. This shows that, as expected, the microsynteny is not very good in regions containing clusters of NBS-LRR.

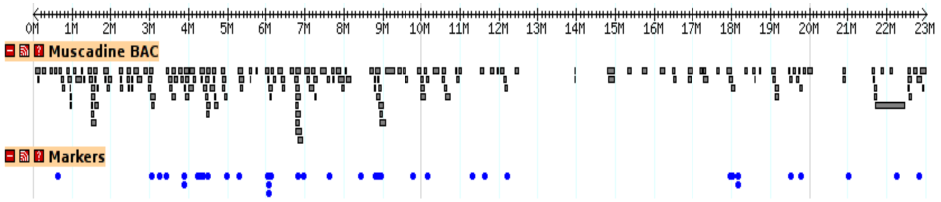

Figure 1. Snapshot of a view on the genome browser showing the alignment of the *M. rotundifolia* BACs on chromosome 1 of the grapevine reference genome.

In parallel, a *M. rotundifolia* genetic map is under development, using SSR markers already mapped in *V. vinifera* (Doligez *et al.* 2006; Cipriani *et al.* in prep). A total of 351 SSR markers have been tested for amplification on Regale and 12 S1 individuals, 144 yielding successful amplification and heterozygosity. The markers are currently scored on 175 individuals of a Regale S1 population.

Perspectives

A complete contig of BAC from the *M. Rotundifolia* library will be constructed by PCR screening of the library with markers developed by Barler *et al.* (2005) and new markers developed from BES. The BAC will be sequenced. The same approach will be carried out on chromosome 18 along a QTL of resistance to downy mildew (Bellin *et al.*, 2009).

Finally, the alignment of the *M. rotundifolia* genetic map to the grapevine reference genome will enlight the macrosynteny between the two genomes.

Literature cited

Adam-Blondon A-F, Bernole A, Faes G, Lamoureux D, Pateyron S, Grando MS, Caboche M, Velasco R, Chalhoub B. 2005. Construction and characterization of BAC libraries from major grapevine cultivars. *Theor. Appl. Genet.* 110:1363-1371.

Barker CL, Donald T, Pauquet J, Ratnaparkhe A, Bouquet A, Adam-Blondon A-F, Thomas MR, Dry I. 2005. Genetic and physical mapping of the grapevine powdery mildew resistance gene, *Run1*, using a bacterial artificial chromosome library. *Theor. Appl. Genet.* 111:370-377.

Bellin D, Peressotti E, Merdinoglu D, Wiedemann-Merdinoglu S, Adam-Blondon AF, Cipriani G, Morgante M, Testolin R, Di Gaspero G. 2009. Resistance to *Plasmopara viticola* in grapevine 'Bianca' is controlled by a major dominant gene causing localised necrosis at the infection site. *Theor. Appl. Genet.* 120:163-176.

Doligez A, Adam-Blondon AF, Cipriani G, Di Gaspero G, Laucou V, Merdinoglu D, Meredith C, Riaz S, Roux C, This P. 2006. An integrated SSR map of grapevine based on five mapping populations. *Theor. Appl. Genet.* 113:369-382.

Donald T, Pellerone F, Adam-Blondon A-F, Bouquet A, Thomas M, Dry I. 2002. Identification of Resistance Gene Analogs linked to a powdery mildew resistance locus in grapevine, *Theor. Appl. Genet.* 104:610-618.

Dry IB, Feechan A, Anderson C, Jermakow AM, Bouquet A, Adam-Blondon A-F, Thomas MR. 2010. Molecular Strategies to enhance resistance of grapevine to powdery mildew. *Australian Journal of Grape and Wine Research* 16:94-105.

Jaillon O, Aury JM, Noel B, Policriti A, *et al.* 2007. The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Nature*, 449:463-468.

Lamoureux D, Bernole A, Le Clainche I, Tual, S, Thareau V, Paillard S, Legeai F, Dossat C, Wincker P, Oswald M, Merdinoglu D, Vignault C, Delrot S, Caboche M, Chalhoub B, Adam-Blondon AF. 2006. Anchoring a large set of markers onto a BAC library for the development of a draft physical map of the grapevine genome. *Theor. Appl. Genet.* 113:344-356.

Moroldo M, Paillard S, Marconi R, Legeai F, Canaguier A, Cruaud C, De Berardinis V, Guichard C, Brunaud V, Le Clainche I, Scalabrin S, Testolin R, Di Gaspero G, Morgante M, Adam-Blondon AF. 2008. A physical map of the heterozygous grapevine 'Cabernet Sauvignon' allows mapping candidate genes for disease resistance. *BMC Plant Biol.* 8:66.

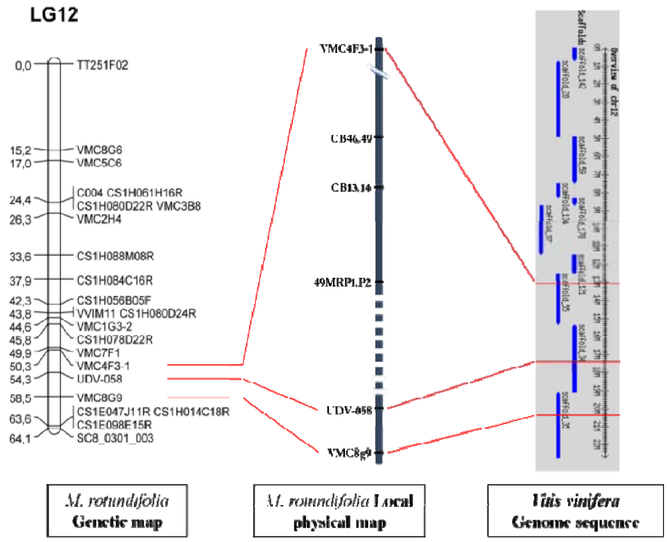

Figure 2. Alignment of the *V. vinifera* reference genetic map (Cipriani et al in prep), the physical map of an introgressed individual carrying the Run1 gene (Barker et al 2005) and the *V. vinifera* reference genome sequence.