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Efficiency of Single Nucleotide Polymorphisms to improve a genetic map of complex pedigree grapevines

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Summary

A set of 47 SNP (single nucleotide polymorphisms) markers (Cabezas *et al*. 2011) was tested for their usefulness to improve a genetic map from the cross of GF.GA-52-42 x 'Solaris' previously established with SSR markers (Schwander *et al.* 2012). 55.3 % of the SNPs showed informative segregation and 26 SNP markers were localized on 16 of the 19 linkage groups of grapevine. Five chromosome regions with large gaps of recombining SSR markers could be equipped by positioning a SNP marker there. One SNP marker, VV10992, was found linked to the major resistance locus *Rpv*10 and should be applicable for marker-assisted selection.

K e y w o r d s : genetic mapping, SNP markers.

Introduction

Grapevine breeding for improved resistance and quality traits recently shifted from traditional empirical methods to marker-assisted analysis of introgression of genetically defined loci. This progress has been achieved by development of genetic maps in segregating populations followed by QTL analysis of simultaneously segregating phenotypic traits to identify trait-linked markers. The approach has proven to be robust and reliable, if the phenotypic data are environmentally stable and reproducibly scored. In the last years genetic mapping relied mostly on linkage/recombination analysis of length polymorphic dinucleotide repeats. These are amplified by flanking primer pairs anchored in neighboring unique DNA sequences through PCR (polymerase chain reaction). Such "microsatellite" or "SSR- (simple sequence repeat)" markers yield co-dominant information and -depending on the parental genotypes- up to four alleles with different lengths may segregate after a controlled cross. Publication of the *Vitis* reference genome sequence of 'Pinot noir' inbred line PN40024 (Jaillon *et al.* 2007) and its further improvement as publicly available (http://www.genoscope.cns.fr) paved the way to develop additional SSR markers targeted to particular genomic regions of interest. However recent progress in grapevine genomics motivates to test the new type of SNP (single nucleotide polymorphisms) markers for their usefulness to improve the saturation of genetic maps and the precision of QTL mapping studies.

Genome-sequencing of a heterozygous `Pinot noir´ clone (Velasco *et al.* 2007), additional *Vitis vinifera* cultivars and accessions of *Vitis sp*. with "next generation sequencing" techniques revealed a high level of sequence diversity (Myles *et al.* 2010), especially a huge number of single nucleotide polymorphisms (SNPs). Such SNPs are bi-allelic variants of two alternative nucleotides at a specific genomic position, scored as co-dominant markers. They can be analyzed with various adapted techniques including local mini-sequencing or allele-specific PCR. They exhibit reduced genetic information (maximally two alleles) as compared to SSRs (maximally four alleles) but this fact is outweighed by their high frequency of occurrence. Transferability of SNP markers between *Vitis vinifera* cultivars and *Vitis sp.* hybrids or wild accessions, however, remains a matter of debate. Transferability is crucial for genetic analyses after crosses and for the characterization of germplasm material. Addressing this issue, contradicting results have been obtained so far. The transferability of SNPs identified in the heterozygous 'Pinot noir' clone to non-*vinifera Vitis* accessions was observed to be only 2.3 % (Vezulli *et al.* 2008). In contrast, SNPs identified by comparison of 10 *V. vinifera* cultivars, 6 wild *Vitis* accessions and the homozygous model genome sequence of PN40024 were shared by 24.3 % between *V. vinifera* and wild *Vitis* species (Myles *et al.* 2010). To get a clearer picture about the usefulness of SNP markers for genetic mapping and QTL analyses, we decided to check a set of SNP markers for their efficiency of integration into an existing genetic map based on SSR markers. The SNPs tested here had been pre-selected for genotyping success in *V. vinifera* cultivar identification trials as described in Cabezas *et al.* (2011).

Material and Methods

The test population was a progeny from the cross of GF.GA-52-42 ('Bacchus' x 'Villard blanc') x 'Solaris' 'Merzling' x 'Geisenheim 6493') as analyzed with SSR markers before (Schwander *et al.* 2012). 134 randomly selected individuals and both parental types were used for SNP genotyping applying 47 markers of the set published (Cabezas *et al.* 2011). A control reaction for a chloroplast SNP (Hunt *et al.* 2010) was included to validate the analytical system.

PCR assays for SNP detection were designed based on up to 250 bp of flanking upstream and downstream ge-

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nomic DNA sequence as deduced from the 12x PN40024 sequence (http://www.genoscope.cns.fr) in service (FluiDigm SNP Type assays). Primer pairs were developed to amplify the target region first using 10 ng of genomic DNA in a 48 primer pair multiplex to harmonize DNA quality and quantity. In a second step, a FAM- resp. HEX fluorescently labeled forward primer carrying the discriminative nucleotide at its 3´-end was applied together with an unlabeled reverse primer to resolve the genotype. Analysis was performed on re-usable 48.48 Dynamic Array™ IFC Genotyping chips processed with FluiDigm SNPtype™ reagents in a BioMark™ nano fluidics system according to the specifications of the supplier. Each run of 48 samples contained one or two non-template controls. Results were read as ratios of fluorescence intensities and converted into genotypes homozygous of either alternative nucleotide or heterozygous with both alternative nucleotides present.

The SNP detection system was tested by checking the results for a chloroplast SNP marker. Both parents and all the progeny analyzed carry a T chlorotype at SNP marker cp4527 (Hunt *et al.* 2010) that was clearly scored as "homozygous" marker throughout all runs, validating the technology.

The genotypic data were coded according to the requirements of the JoinMap4.1® mapping program (Van Ooijen 2006) and processed for genetic mapping by regression analysis employing the Kosambi function for determination of genetic distances, together with SSR marker data described in Schwander *et al.* 2012. The QTL analysis was performed using Map QTL6® (Van Ooijen 2009).

Results and Discussion

From the 47 SNP markers tested, 26 (55.3 %) showed informative segregation. Eight markers (17 %) were double heterozygous (coded hk x hk) and segregated co-dominantly from both parental types. Eleven markers (23.4 %) segregated from the heterozygous maternal (code lm x ll) and seven markers (14.9 %) from the heterozygous paternal (coded nn x np) genotype (Table). Sixteen markers were found homozygous with both parents carrying the same (14 cases, type aa x aa) or both alternative (2 cases, type aa x bb) alleles, yielding no segregation. Two markers completely failed in amplification and the remaining three were difficult to score and excluded from genetic mapping. The resulting genetic map is shown in the Figure. The 26 SNP markers mapped to 16 of the 19 linkage groups in

Table

Characteristics of SNP markers mapped. SNP designation, linkage group, genetic position, maternal and paternal genotype, segregation type according to JoinMap4.1® codes and physical position in the 12x reference genome sequence of PN40024 are indicated

#	SNP	linkage group	genetic position (cM)	genotype Gf.Ga-52-42	genotype `Solaris´	segregation type	PN12x physical position*
1	SNP1453_40e	01	0	AG	AG	<hkxhk>	729514
2	SNP269_308e	01	13.9	AA	AG	<nnxnp>	5948674
3	SNP829_281	02	0	AG	AA	<lmxll>	415342
4	SNP613_315	03	0	CT	TT	<lmxll>	1348328
5	VV10113e	05	45	AG	AG	<hkxhk>	6744629
6	SNP1471_179	05	46.5	CT	CT	<hkxhk>	5773320
7	SNP1027_69	05	65.4	CT	CT	<hkxhk>	1785979
8	SNP945_88	06	0	AA	AG	<nnxnp>	327200
9	SNP873_244	06	9.2	TT	CT	<nnxnp>	4258638
10	SNP1015_67	07	32.7	AG	GG	<lmxll>	8839239
11	SNP593_149e	08	12.6	CT	TT	<lmxll>	3320936
12	SNP1323_155e	08	33.4	CC	AC	<nnxnp>	13401437
13	VV10992	09	47.4	AT	AA	<lmxll>	3123999
14	SNP447_244	10	31.4	TT	CT	<nnxnp>	5489212
15	SNP197_82e	11	0.5	AC	AA	<lmxll>	311765
16	SNP1119_176	12	12.3	AC	AA	<lmxll>	22228357
17	SNP1215_138e	12	58.2	CT	CT	<hkxhk>	739916
18	SNP1035_226	14	2.1	CT	CT	<hkxhk>	29590769
19	SNP1411_565e	14	19.8	TT	AT	<nnxnp>	23135445
20	SNP1349_174	16	13.4	AA	AG	<nnxnp>	21202286
21	SNP1079_58	16	40.1	AG	GG	<lmxll>	13454358
22	SNP879_308	17	0	AG	AG	<hkxhk>	12206201
23	VV9920	18	49.8	AG	GG	<lmxll>	11138668
24	SNP1003_336	18a	5.3	AC	AC	<hkxhk>	3829207
25	SNP819_210e	19	29.1	AT	TT	<lmxll>	7217380
26	SNP1127_70	19	42.8	GT	GG	<lmxll>	17751334

* Genomic positions are according to Cabezas *et al.,* 2011.

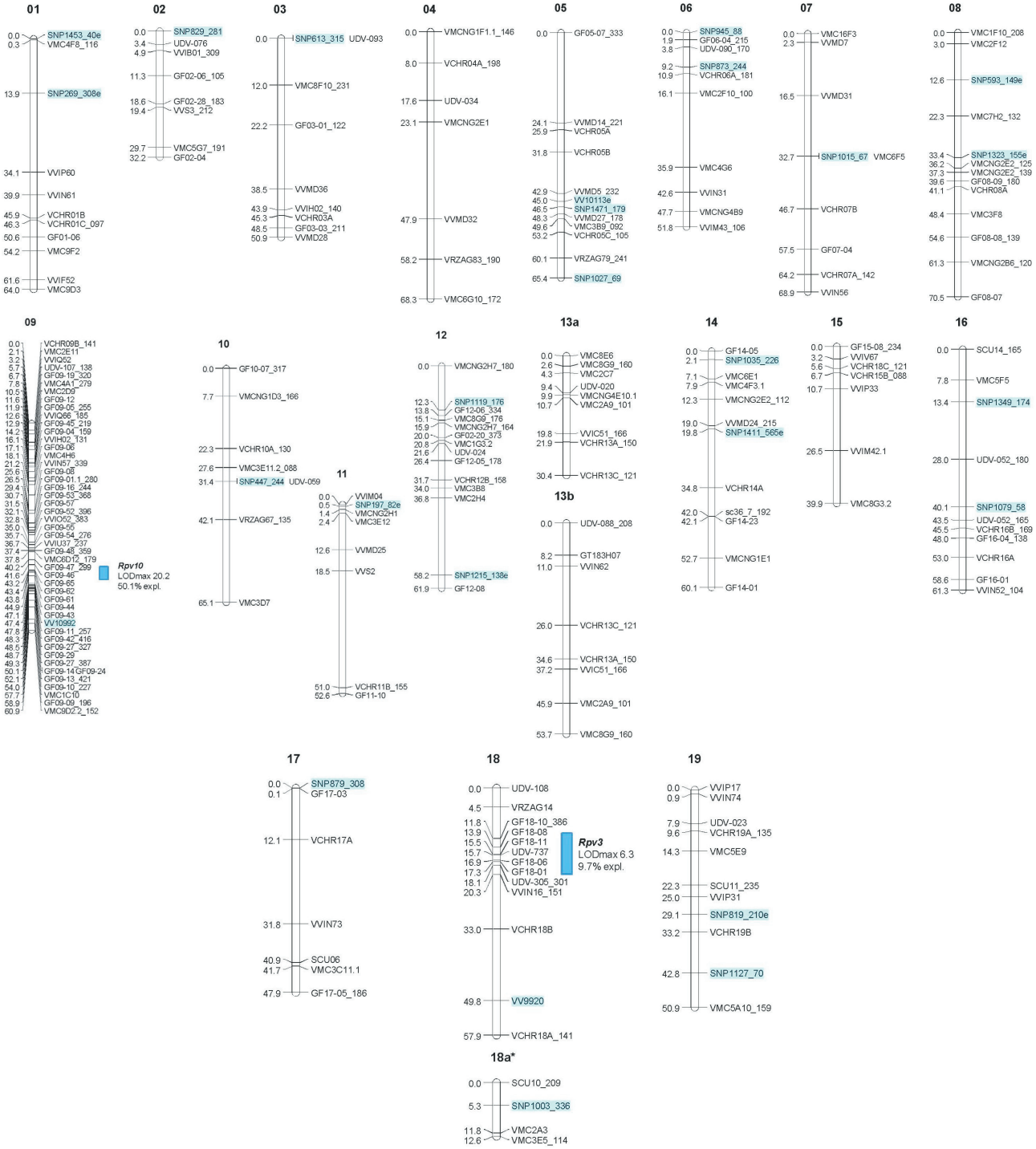

Figure: Consensus map from the progeny of 'GF.GA-52-42' x 'Solaris' as extended by the integration of SNP markers (indicated by grey shading). *Group 13 was split into two alternative maps (a and b) due to insufficient linkage phase determination. In LG18 a satellite 18a was mapped due to limited linkage between the markers.

Vitis sp. and are embedded in the previous map based on 212 SSR markers with a particularly high density (46 SSR markers) on LG9 to saturate the *Rpv10* region containing a newly identified major QTL for *Plasmopara viticola* resistance from Asian *Vitis* wild species origin in more detail (Schwander *et al.* 2012).

Three SNP markers co-segregated with SSR markers. On linkage group 3, SNP613_315 maps to the same position as UDV-093. Comparing the physical positions of both markers based on the reference genome (1348328 for SNP613_315 and 1335060 for UDV-093) reveals a distance of 13 kb that remained genetically unresolved in this experimental population of 134 individuals. Similarly, on linkage group 10, SNP 447_244 (position 5489212) exhibits no distance to UDV-059 (position 5457979), both separated physically according to the PN40024 genomic sequence by 31 kb. On linkage group 7, SNP 1015_67 co-segregated with VMC6F5. The latter is described to be localized on linkage group 17, however the forward and reverse primer sequences amplifying locus VMC6F5 show better homology to an unassigned scaffold (GenBank Loci NW 003724210 and FN595521.1 of PN40024 scaffold

189) with better (smaller) E-values than to linkage group 17 (E = $2e^{-04}$ for the forward primer sequence and $6e^{-04}$ for the reverse primer sequence on scaffold 189 as compared to E = 0.038 for both forward and reverse primer sequences on chromosome 17). This indicates that scaffold 189 is part of chromosome 7. VMC6F5 had already been mapped to linkage group 7 in the previous study based solely on SSR markers (Schwander *et al.* 2012) using a higher number of 265 individuals from the same population.

The genetic map was improved by the integration of SNP markers. Several large gaps present in the SSR-marker based map on chromosomes 1, 8, 16, 18 and 19 could be equipped with a SNP marker (Figure). QTL analysis for *P. viticola* resistance was thus repeated as described previously (Schwander *et al.* 2012) to check newly marker tagged chromosome regions that had not been covered in previous analysis and hence may have gone undetected to carry additional QTL regions. It confirmed the major QTL on linkage group 9 centered at marker GF09-46 (*Rpv10*) and the minor QTL on linkage group 18 (*Rpv3*) in Kruskal Wallis analysis, interval mapping and MQM mapping. A SNP marker was detected that is linked to the major QTL locus for resistance to *Plasmopara viticola.* SNP VV10992 maps close to *Rpv10*, while SNP VV9920 is in the vicinity of *Rpv3* on linkage group 18, a QTL locus specifying *P. viticola* resistance from American origin (Welter *et al*. 2007). However, the latter is localized in large genetic distance to the *Rpv3* locus and hence will be of restricted usefulness for marker-assisted selection. Quite in contrast, SNP marker VV10992 may be applicable for marker-assisted selection. The *Rpv3* and *Rpv10* loci are indicated with their confidence intervals (LOD_{max} +/- 1 LOD) in the Figure.

In conclusion this study showed the usefulness of SNP markers for genetic mapping and QTL analysis. SNP markers were informative to more than 50 % and helpful to improve the marker coverage of the genetic map. First SNPs in linkage to important traits were identified. This example shows that SNPs will become useful as novel tools for marker-assisted selection in grapevine breeding.

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