

## SSR AND SRAP MARKER BASED LINKAGE MAP OF *VITIS AMURENSIS* RUPR.

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### Abstract

SSR (Simple Sequence Repeat) markers and SRAP (Sequence Related Amplified Polymorphism) markers were used to construct a genetic linkage map of *Vitis amurensis* Rupr. The mapping population consists of 94 individuals derived from the selfing of Beibinghong, a *V. amurensis* cultivar. The map covers a total length of 1123cM on 21 linkage groups with an average distance between adjacent markers of 4.5cM and 10.9 markers per linkage group on average. The order of SSR markers in the map presented in this paper was mainly consistent with the previous published *Vitis* linkage maps except for few inversions. The efficiency of SSRs to anchor homologous linkage groups from different *Vitis* maps and the ability of SRAPs in saturating molecular linkage maps were observed. This map can serve as a fundamental tool for a series of further studies in *V. amurensis*, including resistance trait QTL (quantitative trait locus) detection and MAS (marker assisted selection).

### Introduction

Grape is one of the most important horticultural crops in the world, and it is cultivated in almost every climatic region. However, in many areas, such as Northeast China, the extremely low temperatures create additional challenges of grapevine survival through the long winter period. In cold regions in China, the ever lasting burying the vines under the soil becomes the most important viticultural practice in winter, which costs a great amount of labor force and money, thus limits the development of grape and wine industry.

*V. amurensis* Rupr. is native to Northeast China and parts of Northeast Inner Mongolia. This genetically diverse *V. amurensis* is widely distributed, and it is the only species within *Vitis* found in this native region where the lowest winter temperature can reach -35 to -40°C (Wan *et al.*, 2008). Therefore with an extraordinary cold tolerance, *V. amurensis* becomes the most important resource for grape cold hardiness breeding all over the world (Liu *et al.*, 2004). Grape cold hardiness breeding dates back to the year 1952 in China (Lin, 2007). During this over half a century period, especially after China's reform and opening up since the year 1978, we've made some progress in grape cold hardiness breeding, including the investigation of *V. amurensis* in Northeast China, the selection and breeding of several good *amurensis* cultivars (Song *et al.*, 1999), and the interspecific hybridization between *vinifera* and *amurensis* resulting in some good quality cultivars with cold tolerance (Song *et al.*, 2005; Song *et al.*, 2008; Fan *et al.*, 2009; Wang *et al.*, 2009) and a number of potential materials for further research etc. However, these achievements are not enough and the efforts in this field should not be ended, because most of the existing hardy cultivars could not satisfy the need of production, especially in North and Northeast China. In the future, hardy cultivars with excellent quality but without the need of being berried under the soil over winter in cold areas are to be bred using whatever conventional method or biotechnological method.

SSR is a classic molecular marker technique based on PCR (Polymerase Chain Reaction) technique, which has many advantages, such as abundance, high polymorphism,

co-dominant and primer transferability. SSR marker is widely used in genetic diversity analysis (Hu *et al.*, 2012), genetic map construction (Riaz *et al.*, 2004; Adam-Blondon *et al.*, 2004; Yu *et al.*, 2012), QTL mapping (Kongjaimun *et al.*, 2012) and gene characterization (Rasheed *et al.*, 2012).

SRAP is a PCR-based molecular marker described by Li & Quiros (2001), which aims for the amplification of ORFs (open reading frames). The primers are specially designed according to the genomic characteristic that the exons are rich in GC, while the promoters and introns are rich in AT. Polymorphism is generated because of the length differences of introns, promoters and intervals of different individuals. Having the advantages of simplicity, high polymorphism and primer generality, SRAP has already been used in genetic diversity analysis (Uzun *et al.*, 2011; Yildiz *et al.*, 2011; Guo *et al.*, 2012; Abedian *et al.*, 2012), genetic map construction (Xie *et al.*, 2011; Lu *et al.*, 2012), fungi identification (Ren *et al.*, 2012), QTL detection (Zhang *et al.*, 2011) and gene mapping (Luo *et al.*, 2011).

As a perennial woody crop, grapevine has a series of typical characteristics, such as high heterozygosity, large plant body and long life cycle etc. So compared with annual crops, it's difficult to quickly create an ideal mapping population so as to restrict the genetic map study in the genus *Vitis*. However, after Hemmat *et al.*, (1994) proposed the 'double pseudo-testcross' theory, the genetic map research in fruit trees has been in progress. After Lodhi *et al.*, (1995) reported the first *Vitis* map in 1995, mapping research on *Vitis* had been representing a great development. Genetic maps have been constructed in not only *Vitis vinifera* (Grando *et al.*, 2003), but also in other species, such as *V. rupestris* and *V. arizonica* (Doucleff *et al.*, 2004), *V. riparia* and *V. champinii* (Lowe & Walker, 2006), and *V. amurensis* (Blasi *et al.*, 2011). The co-dominant SSR markers, whose high conservation and transportability within the genus *Vitis* has been proved (Di Gaspero *et al.*, 2000), is used in these maps which allowed a good comparison among them. Therefore, the genetic bases of some non-*vinifera* species could be well studied which may realize the possibility of discovering excellent genes carried by them.

Here we report a genetic linkage map for *V. amurensis* Rupr. based on SSR and SRAP markers, aiming to provide technical support for further studies, such as QTL detection for grape resistance traits and MAS.

### Materials and Methods

The construction of the map was based on the study of an F2 population (named B population) consisting of 94 individuals from Beibinghong selfing (Beibinghong is a hybrid derived from the cross between *V. amurensis* Rupr. and *V. vinifera* L.). The population was cultivated in grape nursery, Shenyang Agricultural University, Shenyang, China. Genomic DNA was extracted from the young leaves by a modified CTAB method described by Hanania *et al.*, (2004). SSR markers were mainly from three large series: VMC (*V. Microsatellite Consortium*, Agrogene SA, Moissy Cramayel, France), VVI (Merdinoglu *et al.*, 2005) and UDV (Di Gaspero *et al.*, 2005). Detailed information can be found at [http://www.ncbi.nlm.nih.gov/mapview/map\\_search.cgi?taxid=2976](http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=2976). A total of 345 primer pairs were tested on Beibinghong, and 174 markers were chosen for map construction, based on their heterozygosity in Beibinghong. PCR amplifications were performed in 10 $\mu$ L reactions consisting of 10ng template DNA, 1.5mM MgCl<sub>2</sub>, 0.5U Taq polymerase, 150 $\mu$ M dNTP, 1  $\times$  Taq polymerase buffer and 5pM of each primer. All reactions were run on BIO-RAD S1000 Thermal Cycler. Annealing temperatures were optimized individually for each marker. All markers were tested on Beibinghong at 8 annealing temperatures ranged from 50 to 63°C programmed by S1000TM Cycler, keeping all other conditions of the amplification protocol constant (4 min at 94°C followed by 25 cycles of 1 min at 94°C, 1 min at optimized annealing temperature, 1 min at 72°C followed by a final step of 7 min at 72°C). Amplification products were separated by 10% native PAGE (polyacrylamide gel electrophoresis) and visualized by silver staining.

SRAP primer information is from Li & Quiros (2001). 6 randomly chosen individuals (including the parent, Beibinghong) were used for testing a total of 200 primer pairs, among which 57 primer pairs amplified stable, clear and polymorphic bands. These 57 primer pairs generated 279 segregating loci, which were used for map construction. PCR amplifications were performed according to the former results published by us (Guo *et al.*, 2011). PCR products were separated by 7% PAGE and visualized by silver staining.

Linkage analysis was performed with JOINMAP 3.0 (Van Ooijen & Voorrips, 2001) under the following parameters: LOD=3.0, maximum recombination rate=0.4. Data were organized according to its data entry notation. SSR data and SRAP data were combined into one single data set for linkage analysis. A linkage map was then drawn with the software Mapchart 2.2.

### Results and Discussion

**SSR markers:** Of the 345 SSR primer pairs tested on Beibinghong, 36 did not show any amplification and 48

showed low quality results and were discarded. The remaining 261 markers amplified 174 heterozygous loci and 87 homozygous loci, which resulted in a heterozygosity percentage of 66.7%. 66.7% is nearly the same heterozygosity percentage with 69% according to Adam-Blondon *et al.*, (2004). The high level of heterozygosity or polymorphism of *Vitis*-derived SSR markers has been repeatedly described in *vinifera* species (Thomas & Scott, 1993; Bowers *et al.*, 1996, 1999a, 1999b; Sefc *et al.*, 1999; Adam-Blondon *et al.*, 2004) as well as in non-*vinifera* species (Blasi *et al.*, 2011) which allowed a good comparison among *Vitis* maps and stressed the efficiency and reliability of SSR marker in grape genetic studies.

**SRAP markers:** The present work allowed us to map 128 SRAP markers on the *V. amurensis* genome (Fig. 1). These SRAP markers were not mapped to any of the previously published *Vitis* maps. It presents an average of 6.1 SRAP markers per linkage group with a maximum of 14 markers (LG4) per group. Compared with co-dominant SSR markers, although dominant markers are less powerful on covering longer genome length (Riaz *et al.*, 2004), the SRAP markers used in this present work did lengthen the map constructed by only SSR markers (data not shown). Some linkage groups of this map, LG3, LG4, LG5 and LG10 are even longer than those of the 2 integrated maps (Doligez *et al.*, 2006; Vezzulli *et al.*, 2008). The SRAP markers on these linkage groups contributed much to their lengths and made the map more saturated, so the SRAP markers used in our work did play a role of compensation to a large extent.

### Map construction

Of the 174 SSR markers and 279 SRAP markers scored on the B population, 100 SSRs and 128 SRAPs, allocated into 21 linkage groups, allowed us to construct a map of a total length of 1123 cM with an average distance of 4.5 cM between markers and 10.9 markers per linkage group on average (Table 1, Fig. 1). Linkage groups were numbered according to the reference map of Riaz *et al.*, (2004) with the modifications of Adam-Blondon *et al.*, (2004). The largest group, LG5, consisted of 9 SSR markers and 10 SRAP markers covering 119cM, while the smallest, LG9, only contained 2 SSR markers covering no distance. 5 gaps were larger than 20cM. The largest gap was on LG4, where the distance between marker m1e8-205 and m20e11-150 was 35cM.

Two linkage groups, LG7 and LG18, were both split into two parts because of weak linkages of markers. Blasi *et al.*, (2011) also encountered the same question, but the new developed SSR markers Chr7V001, Chr7V003 and Chr3V004 permitted their improvement of the construction of LG7. So the new developed SSR markers from the 12 $\times$ grapevine genome sequence (<http://www.genoscope.cns.fr/externe/Genome-Browser/Vitis/>) must be also valuable for us to rejoin the split linkage groups.

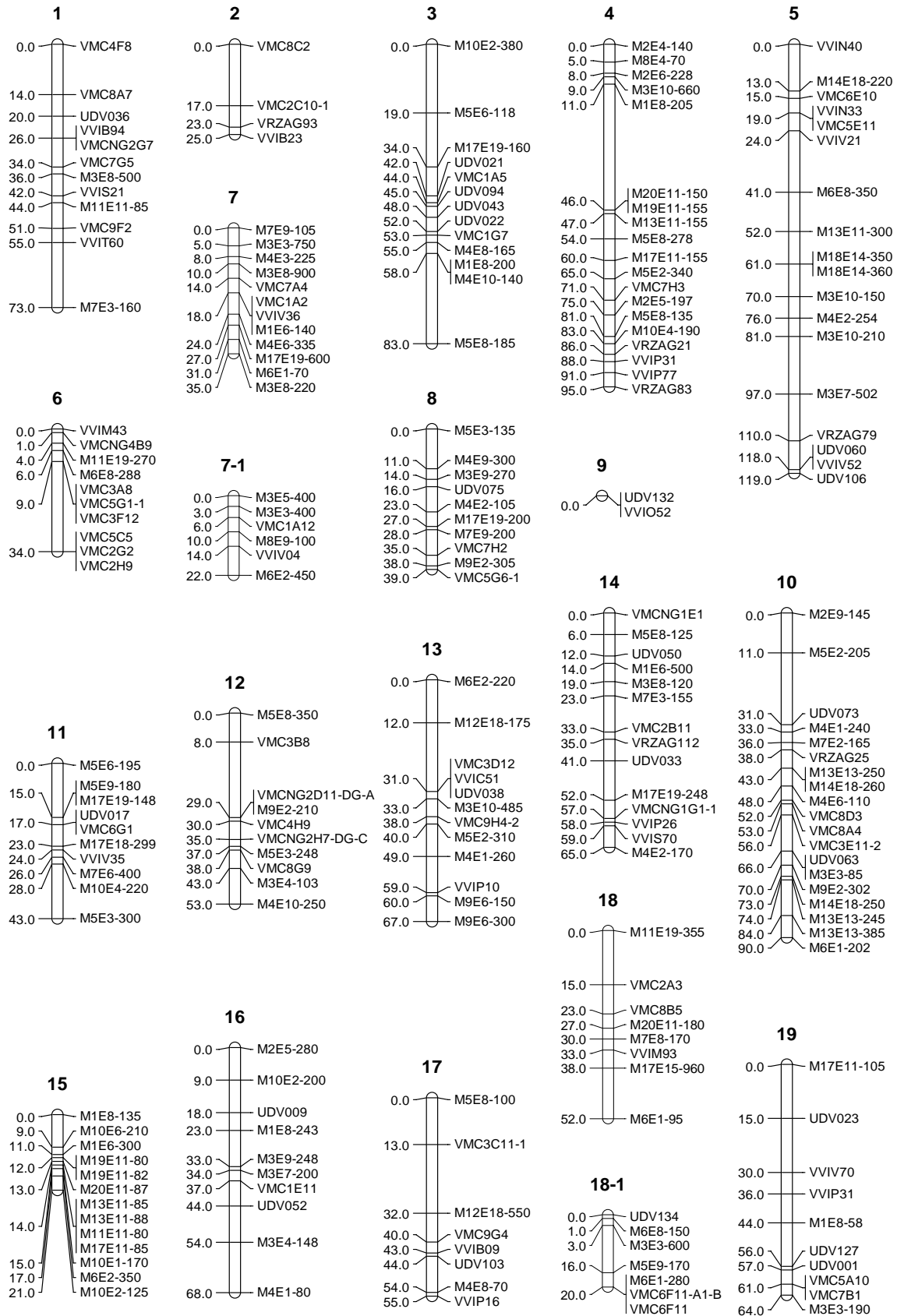


Fig. 1. Linkage map of Beibinghong.

Table 1. Main characteristics of the linkage groups.

Linkage group no.	Number of SSR markers	Number of SRAP markers	Total marker number	Size	Gaps (> 20 cM) number
1	9	3	12	73	0
2	4	0	4	25	0
3	6	7	13	83	1
4	6	14	20	95	1
5	9	10	19	119	0
6	8	2	10	34	1
7	3	9	12	35	0
7-1	2	4	6	22	0
8	3	7	10	39	0
9	2	0	2	0	0
10	6	12	18	90	1
11	3	7	10	43	0
12	5	5	10	53	0
13	5	7	12	67	0
14	8	6	14	65	1
15	0	13	13	21	0
16	3	7	10	68	0
17	5	3	8	55	0
18	3	4	7	20	0
18-1	3	5	8	52	0
19	7	3	10	64	0
Total	100	128	228	1123	5
Average	4.8	6.1	10.9	53.5	

Concerning the order of SSR markers, there existed only small differences between our map and the two reference maps (Riaz *et al.*, 2004 & Adam-Blondon *et al.*, 2004), being the inversions of VVIT60 and VMC9F2 on LG1, UDV106 and VVIT52 on LG5, VMC3E11-2 and VMC8A4 on LG10, VMC8G9 and VMCNGEH7-DG-C on LG12, VVIS70 and VMCNG1G1-1 on LG14, and VVIP31 and VVIV70 on LG19, which might resulted from the small population we used for map construction. In the future, population size is to be enlarged to break the close linkages and to find more segregating loci, especially near the telomeric regions where the combination is easy to be suppressed (Riaz *et al.*, 2004), and more co-dominant SSR markers are to be supplemented to fill in the gaps on the map.

As a whole, the Beibinghong map covers a total length of as long as 1123cM of *V. amurensis* genome and the density of the map is also satisfactory. The map can be used for analyzing QTLs of excellent traits carried by *V. amurensis* in the future.

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