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Grapevine DNA polymorphisms revealed by microsatellite-derived markers from soybean and rice

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Summary

We report detection of DNA polymorphisms in grapevine by the use of microsatellite-flanking primer pairs from soybean and rice. These "cross species" microsatellite-derived markers were checked for their inheritance patterns in controlled grapevine crosses. They produced multiple bands that segregated and can be scored as individual genetic markers of dominant type. Employed in genetic mapping studies they offer advantages such as improved reproducibility in comparison to commonly used multi-locus marker systems like RAPDs and AFLPs.

K e y w o r d s : "Cross-species" markers, simple sequence repeats (SSRs), *Vitis* sp.

Introduction

Grapevine genetics has advanced tremendously through the last decade. Genetic maps based on molecular markers have been established by several research groups. These maps serve to determine genetic regions for traits important to improve this ancient crop through breeding approaches accelerated by marker-assisted selection and investigation of the underlying molecular mechanisms. These maps have been prepared based on various molecular marker types (Doligez *et al.* 2002; Doucleff *et al.* 2004; Fischer *et al.* 2004). Considerable progress concerning marker systems in grapevine has been achieved through the joint efforts of 20 research groups in the international Vitis Microsatellite Consortium (VMC, organized by AGROGENE S.A., Moissy Cremayel, France) that generated a high number of new microsatellite markers. The development of such markers however, requires substantial cloning and sequencing work (Thomas and Scott 1993; Bowers *et al.* 1999) and incurs considerable costs. Some of the techniques employed in genetic mapping work are encumbered by physical and logistical limitations, such as need of a special gel apparatus (AFLPs, Vos *et al.* 1996), handling and disposal of radioactive materials (RFLPs, Shappley 1996), and reproducibility of the method (RAPDs, Büscher *et al.* 1993). Simple Sequence Repeats (SSRs), also known as microsatellites, have proven to be very important sources of length-polymorphic markers and have been used very successfully in plant genetic studies. Genetic maps of various species including grapevine using this marker system have been constructed (Zulini *et al.* 2002; Fischer *et al.* 2004; Adam-Blondon *et al.* 2004; Riaz *et al.* 2004). However, to overcome some of the aforementioned limitations, we decided to investigate, if the advantages of this marker system can be gained by using publicly available microsatellite-flanking primer sequences from across the species. Hence, instead of grapevine microsatellites, markers available from 4 different and taxonomically diverse plant species (*Arabidopsis thaliana*, hard pine, soybean and rice) were investigated for their ability to amplify useful markers in grapevine. To the best of our knowledge, application of such "cross species" microsatellte markers in grapevine genotying has not been reported so far.

Material and Methods

Three pairs of grapevine cultivars and genotypes used as parents of controlled crosses [Regent x Lemberger; Gf.Ga-47-42 (Bacchus x Seyval blanc) x Villard blanc; V3125 (Schiava grossa x Riesling) x Börner] and 5 randomly chosen individuals from their progeny were selected for the present study.

DNA templates were prepared from young leaf tissue of the parental genotypes and five individual progeny plants of each cross (DNeasy Plant Mini Kit, Qiagen, Hilden, Germany). Commercially available primer pairs (Invitrogen MapPairs) flanking microsatellite sequences in *Arabidopsis* (JV57/58, JV 61/62 and JV65/66, Vogel 2002; www.arabidopsis.org), soybean (*Glycine max*, SATT 102, SATT 114, SATT 115, Cregan *et al.* 1999), rice (*Oryza sativa*, RM 112, RM 113, RM 117, Temnykh *et al.* 2000) and hard pine (*Pinus radiata*, NZPR4, NZPR5, Fisher *et al.* 1998 and PR4.6, Smith and Devey 1994) were obtained from Invitrogen (Karlsruhe, Germany) and tested under standard PCR conditions at annealing temperatures specified by the supplier. The generation of amplification products and their size range was first checked by agarose gel electrophoresis. DNA fragments easily detectable by staining with Ethidium bromide in agarose were further analyzed on denaturing 8 % polyacrylamide/urea gels and visualized by silver staining according to the protocol of the Promega Silver Sequencing Kit (Promega, Madison, Wi, USA). Band sizes were determined in comparison to size standard fragments derived from lambda-DNA cut with *Pst*I.

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Results and Discussion

Application of molecular markers based on polymerase chain reaction (PCR) in a range of techniques is currently the method of choice for genetic mapping due to their ease of generation. Microsatellite-derived markers are typically co-dominant, represent a single locus and are considered to be conserved within plant species (Di Gaspero *et al.* 2000; Van de Ven and Nicol 1996). In the present study, three randomly chosen microsatellite-flanking primer pairs each from *Arabidopsis* and hard pine did not produce any amplification product detectable on agarose gels when tested on grapevine genomic DNA. In contrast, three microsatellite-flanking primer pairs from rice and soybean produced informative amplification products of multilocus-type in grapevine. Some of the products were monomorphic, while others were polymorphic and showed segregation depending on the paternal combination in three different grapevine crosses (Table). Although these markers have lost their codominance and single-copy characteristics typical of microsatellites, they were useful for mapping when the multiple bands obtained were scored as individual dominant markers. These could easily be reproduced under stringent PCR conditions (Figure). The molecular nature of the amplificates was not further investigated and it is unknown, if the products correspond to microsatellite-type sequences as in the original rice and soybean genomes and could be conserved between the species. In some cases, a strong but monomorphic product was obtained, that could be a conserved SSR locus. The majority of weaker, but polymorphic bands possibly represent amplification products from priming of polymorphic sequences of grapevine DNA through random or incomplete complementarity. As microsatellitic sequences typically are rather short in length (100-300 bp including the flanking primers), the wide size range of amplification products obtained in this study from 144 bp to 1700 bp (cf. Table) supports this hypothesis. Employed in mapping approaches, these "cross-species" markers offer the advantages of improved reproducibility as compared to commonly used multi-locus marker systems such as AFLPs and RAPDs. They have been tested in a large cross population of roughly 200 individuals and found genetically informative as dominant markers (Agrawal and Zyprian, data not shown). These results confirm the applicability of "cross species" markers in detecting DNA polymorphisms in grapvine.

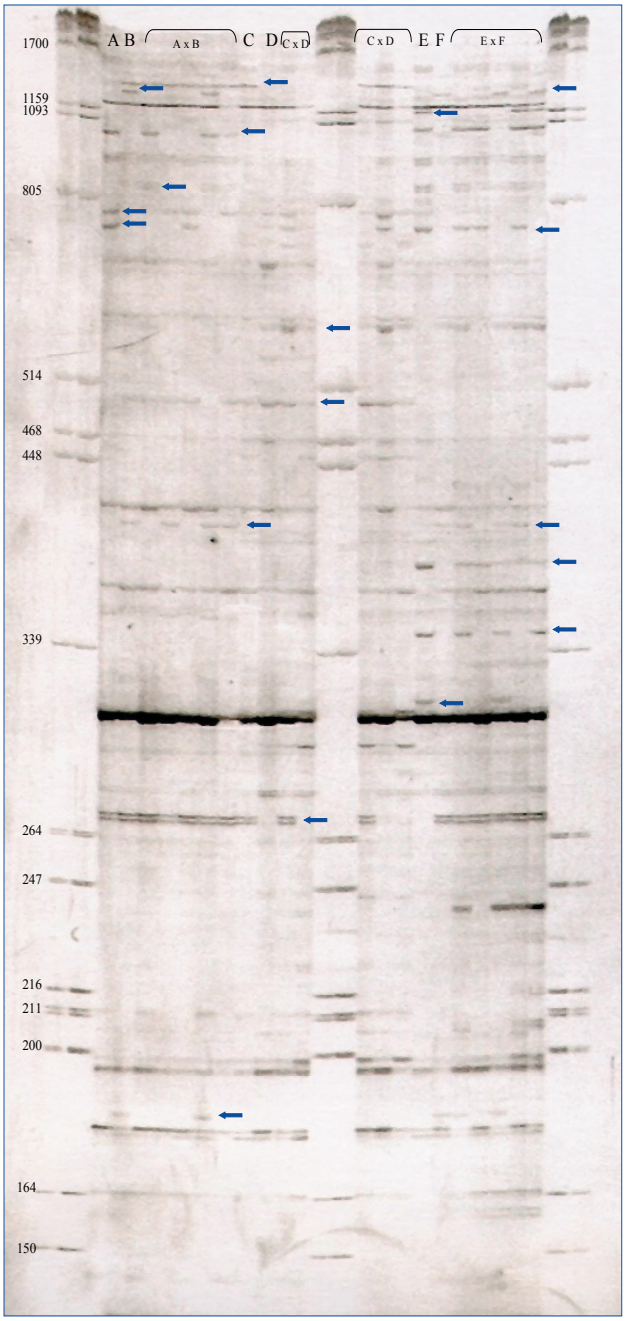

Figure: Segregation of polymorphic amplification products (highlighted by arrows) of primer pair SATT 114 from a soybean microsatellite in the three grapevine crosses Regent x Lemberger, Gf.Ga-47-42 x Villard blanc and V3125 x Börner. A = Regent, B = Lemberger, A x B = marker patterns in 5 progeny plants from Regent x Lemberger; C = Gf.Ga-47-42, D = Villard blanc, C x D = marker profiles in 5 progeny plants from Gf.Ga-47-42 x Villard blanc; E = V3125, D = Börner, E x F = marker profiles in 5 progeny plants form V3125 x Börner. Size standard fragment sizes are indicated on the left-hand side.

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T a b l e

Sizes of polymorphic amplification products and segregation of the "cross-species" markers tested on genomic DNA of parental lines and progeny from grapevine DNA crosses. A = Regent, B = Lemberger, A x B = inheritance patterns in 5 progeny plants from Regent x Lemberger; C = Gf.Ga-47-42, D = Villard blanc, C x D = inheritance patterns in 5 progeny plants from Gf.Ga-47-42 x Villard blanc; E = V3125, D = Börner, E x F = inheritance patterns in 5 progeny plants form V3125 x Börner

Cross species microsatellite marker tested	Amplification products obtained (bp)	A	B	A x B	C	D	C x D	E	F	E x F
Soybean SATT 102	280	-	-	-	-	-	-	x	-	seg
SATT 102	226	-	-	-	x	-	seg	-	-	-
Soybean SATT 114	1330	x	x	monom	x	-	seg	-	-	-
SATT 114	1250	-	x	seg	-	-	-	-	x	seg
SATT 114	1160	-	-	-	-	-	-	x	-	seg
SATT 114	1080	-	-	-	-	-	-	x	-	seg
SATT 114	1020	x	-	seg	-	-	-	-	-	-
SATT 114	850	-	x	seg	-	-	-	x	-	monom
SATT 114	780	x	-	seg	-	x	seg	-	-	-
SATT 114	760	x	-	seg	-	x	seg	x	-	seg
SATT 114	600	-	-	-	-	x	monom	-	x	seg
SATT 114	500	-	x	seg	x	x	monom	-	-	-
SATT 114	410	-	x	seg	-	-	-	-	x	seg
SATT 114	385	-	-	-	-	-	-	x	-	seg
SATT 114	348	-	-	-	-	-	-	x	-	seg
SATT 114	320	-	-	-	-	-	-	x	-	seg
SATT 114	270	x	x	monom	x	-	seg	x	-	monom
SATT 114	184	-	x	seg	-	-	-	-	x	seg
Soybean SATT 115	1700	-	-	-	-	-	-	x	-	seg
SATT 115	442	-	-	-	-	-	-	x	-	seg
SATT 115	418	x	x	seg	-	x	seg	-	x	seg
SATT 115	408	-	-	-	-	-	-	x	-	seg
SATT 115	406	-	-	-	x	-	seg	-	-	-
SATT 115	376	-	-	-	x	-	seg	-	-	-
SATT 115	368	-	x	seg	x	-	seg	-	-	-
SATT 115	342	x	x	seg	-	-	-	-	-	-
SATT 115	316	-	-	-	x	-	seg	-	-	-
SATT 115	144	-	-	-	x	-	seg	-	-	-
Rice RM 112	980	-	-	-	x	-	seg	-	x	seg
RM 112	970	-	-	-	-	-	-	x	-	seg
RM 112	690	-	-	-	-	-	-	x	-	seg
RM 112	544	-	-	-	-	-	-	x	-	seg
RM 112	512	x	-	seg	-	-	-	-	x	seg
RM 112	340	x	-	seg	-	-	-	-	-	-
RM 112	266	-	-	-	-	-	-	x	-	seg
RM 112	176	x	-	seg	-	-	-	-	-	-
Rice RM 113	480	x	-	seg	-	-	monom	-	-	-
RM 113	478	-	x	seg	-	-	-	-	x	monom
RM 113	476	x	-	seg	-	-	monom	-	-	-
RM 113	474	-	x	seg	-	-	-	-	x	monom
Rice RM 117	1060	-	-	-	x	-	seg	x	-	seg
RM 117	450	-	-	-	-	x	seg	x	x	monom
RM 117	264	-	-	-	-	-	-	?	?	seg
RM 117	256	x	-	seg	-	-	-	?	?	seg

bp = base pair; x = amplification product detectable, - = amplification product of that size not detectable; seg = segregating in five progeny individuals; monom = monomorphic in five progeny individuals; ? = amplification questionable in the parental lines

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