Chapter

Reconstruction of Parental SSR Haplotypes from a Single Grape Seed

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Abstract

Microsatellite ('single sequence repeats', SSR) markers were widely used in the last decade for the identification of parents of a given grapevine variety or for pedigree reconstruction as well. By now the pedigree of the majority of the most important varieties is established. At the same time, knowing both of the parents gives information about which one was the mother plant and which one was the pollinator. Analyses of archaeological grapevine seeds can give new opportunities in the research of the evolution of varieties. In most of the angiosperms, the endosperm is triploid with two genome equivalents from the maternal line and one from the paternal line. Our presumption was that this numeral difference in the maternal and paternal alleles causes measureable difference in the amplification of SSR alleles from grapevine seeds. To validate our method, pre-experiments were carried out on 12 'Pinot gris' seeds, which verified our theory.

Keywords: grapevine, single sequence repeats (SSR), double fertilisation

1. Introduction

Microsatellite (SSR) markers were widely used in the last decade for the identification of parents of a given grapevine variety or for pedigree reconstruction as well. By now the pedigree of the majority of the most important varieties is established. For example, large-scale parentage analyses were carried out by Lacombe et al. [1]. At the same time, knowing both of the parents gives information about which one was the mother plant and which one was the pollinator only in special cases, for example, if one of the parents is female flowered. Based on only SSR analyses, no information is available about the time, when the given variety was born.

Analyses of archaeological grapevine seeds can give new opportunities in the research of the evolution of varieties. Over the analyses of seed morphology, the genetic analyses of the seeds can provide interesting data.

Angiosperms have a complex fertilisation process, the so-called double fertilisation (**Figure 1**). It is double, because two female cells, the egg cell and the central cells, are fertilised by two sperm cells forming the embryo and endosperm, respectively. This unique procedure was discovered parallel by Nawaschin [3] and Guignard [4]. Due to the double fertilisation, in such species, where the mass of the endosperm is significantly higher than the mass of the embryo, the haplotype of the

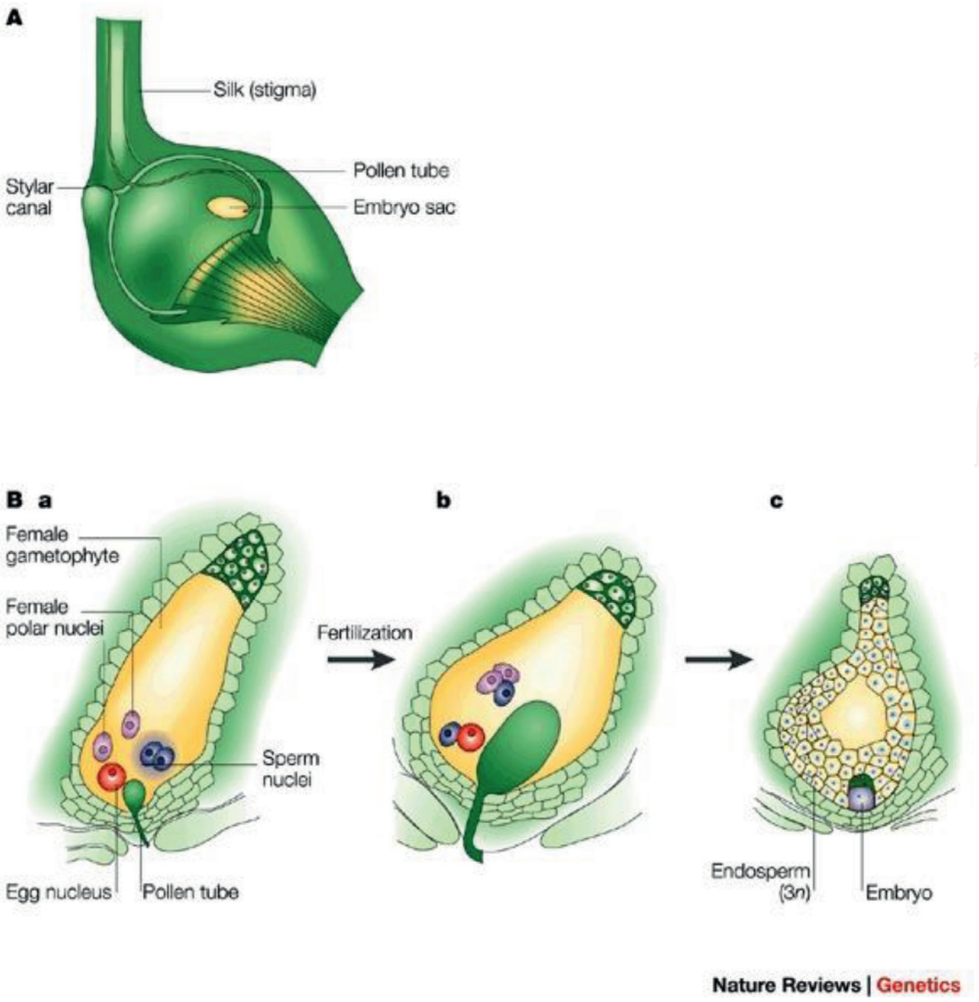

Figure 1.
Process of double fertilisation [2].

parents can be determinable. The main point of the idea is based on the fact that in most of the angiosperms, the endosperm is triploid with two genome equivalents from the maternal line and one from the paternal line [5]. Our presumption was that this numeral difference in the maternal and paternal alleles causes measureable difference in the amplification of SSR alleles.

To validate our method, pre-experiments were carried out on 12 Pinot gris seeds.

2. Materials and methods

Fully matured berries were collected from Pinot gris (clone B. 10) stocks in September 2016. Seeds were removed from the berries, washed in tap water and dried in room temperature. Only fully matured, brown-coloured seeds were used in the analyses. Pinot noir, Pinot gris (clone B. 10.), Chardonnay and Cabernet sauvignon DNA (extracted formerly from leaves) were also used as controls.

DNA was extracted from 12 single seeds. Prior to extraction the seeds were crushed in a mortar to powder; then, this powder was moved to a tube. Qiagen Plant Mini Kits were used for DNA extraction following the instructions of the manufacturer. The amount and quality of DNA were determined spectrophotometrically. The DNA was diluted to a concentration of 10 ng/ml.

The SSR analysis was performed at 19 loci (see **Table 1**). PCR reaction mix was the following: 0.2 mM of each primer, 12.5 ml of Hot Start Master Mix (Quiagen) and 50 ng of template DNA, completed to the total volume of 25 ml with DNA- and

Linkage group[a]	SSR locus	Annealing temp.
1	VMC8A7	64°C
2	VMC7G3	60°C
3	VVMD28	62°C
4	VrZag21	62°C
5	VrZag79	60°C
6	VMC4G6	50°C
7	VVMD7	50°C
8	VMC1F10	57°C
9	VMC1C10	60°C
10	VrZag25	67°C
11	VVS2	60°C
12	VMC2H4	57°C
13	VMC3D12	57°C
14	VMCNG1E1	58°C
15	VMC5G8	58°C
16	VVMD5	53°C
17	Scu06vv	60°C
18	VVIM10	57°C
19	VMC5E9	58°C

[a]*Linkage group numbers according to Adam-Blondon et al. [7].*

Table 1.
List of SSR loci [6].

RNA-free distilled water. The following thermal profile was used: (1) 94°C for 45 min; 35 cycles of: (2) 94°C for 1 min, (3) annealing temperature (see **Table 1**) for 1 min, 73°C for 1 min; and (4) 73°C for 7 min. Each forward primer of the primer pairs was fluorescently labelled with 6FAM on the 5′ end of the DNA chain. Separation of the amplified products was carried out through capillary electrophoresis in a PE-Applied Biosystems 3100 Automated Capillary DNA Sequencer; the molecular sizes of the products were determined using Peak Scanner Software (v. 1.0; Applied Biosystems) [8]. Allele sizes and peak area were also recorded for every single allele. Data were stored in Microsoft Excel [8].

3. Results

DNA extraction and amplification in 18 loci (out of the 19) were successful in all of the 12 seeds. In VVMD5 the amplification was weak, so the results were unevaluated. In all of the remaining 18 loci, the maternal and parental alleles were determined (**Figure 2**); according to our presumption, the allele with the higher area value was supposed to be maternal. Ratio of the quantity of maternal and paternal alleles was computed based on area values. In some loci (where 'Pinot gris' has a homozygote genotype and the majority of the seeds showed also homozygote genotype—VMC4G6 and VMCNG1E1), this ration was excluded from the further analyses.

Based on the remaining 16 loci, the average ratio of maternal and paternal alleles ranged from 1.89 (VMC5E9) to 2.58 (VrZag25), which confirms our presumption.

Maternal and paternal haplotypes were determined by the separation of maternal and paternal alleles based on the method described previously (**Table 2**).

Based on our results, it can be established that most of the seeds are originating from the selfing of 'Pinot gris', but cross-fertilisation is appearing in some cases, such as in the case of seed no. 4, where in six loci the paternal allele is absent from

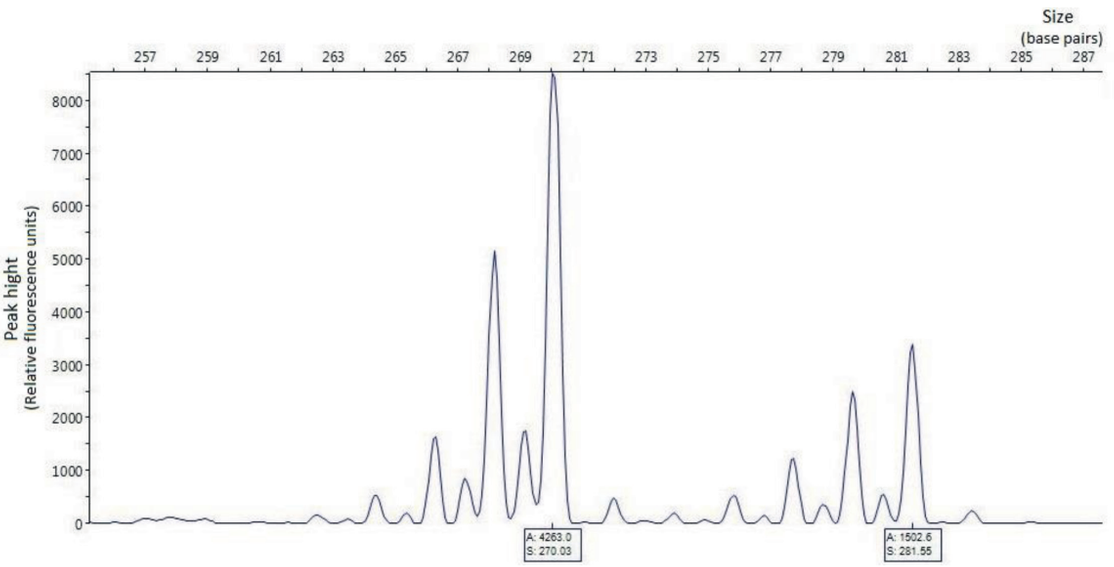

Figure 2.
Example for the determination of maternal and paternal allele in the case of seed no. 11 and VMC4A1 locus: maternal allele, 270; paternal allele, 282 (maternal genotype, 270:278).

Locus	Maternal haplotype	Paternal haplotype	'Pinot gris' genotype
VMC8A7	**158**	**158**	158:158
VVMD28	**236**	**236**	218:236
VrZag79	**242**	**248**	242:248
VVMD7	**240**	*238*	240:244
VMC1C10	**157**	**157**	157:157
VVS2	**150**	**134**	134:150
VMC3D12	**236**	*234*	200:236
VMC5G8	**315**	**311**	311:315
Scu06vv	**164**	**172**	164:172
VMC5E9	**220**	*218*	216:220
VMC7G3	**116**	*114*	116:116
VrZag21	**205**	*195*	199:205
VMC4G6	**122**	**122**	122:122
VMC1F10	**190**	**208**	190:208
VrZag25	**237**	**225**	225:237
VMC2H4	**206**	*204*	206:224
VMCNG1E1	**124**	**124**	124:124
VVIM10	**335**	**335**	335:339

Table 2.
Determination of parental haplotypes in the case of seed no. 4. (Alleles of 'Pinot gris' are red coloured, bold letters; alleles not in 'Pinot gris' genotype are blue coloured, italic letters) [9].

'Pinot gris' (blue coloured, italic letters in **Table 2**). These alleles were inherited from the pollinator, which is surely different from 'Pinot gris'.

4. Discussion

Such amount of DNA can be extracted from a single grape seed, which is suitable for SSR analyses. The amplification of DNA is successful in most of the loci; the PCR reaction optimised for other plant parts can be applied.

Our method is safely applicable for the determination of parental haplotypes. Based on the determined haplotypes, the parental identity could be determined by the use of databases.

The method could be suitable for the analyses of archaeological grapevine seeds, with the following limitations:

The quality and quantity of the extracted DNA could be poor because of the degradation; it depends on the age of the seeds and the environmental effects, e.g. carbonisation processes [10].

Mutations can occur in the SSR loci during the time, which can be manifested in the occurrence of the so-called null alleles. Null alleles result in amplification failures, which can be rid by the use of shortened primers in the PCR reactions ([11, 12]).

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