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Molecular Biology

Plastid DNA Sequence Diversity in a Worldwide Set of Grapevine Cultivars (*Vitis vinifera* L. subsp. *vinifera*)

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ABSTRACT. DNA sequence diversity was investigated at two plastid regions (the *trnH-psbA* intergenic spacer and the *rpl16* intron) in a geographically diverse group of 113 cultivated grape samples. This group included 40 samples from the Republic of Georgia, home to over 500 grape cultivars and the earliest archaeological evidence of grape domestication. The greater Caucasus region in which Georgia lies is widely believed to be the area in which grape domestication began, and the study of genetic diversity in this region is viewed as key to understanding grape domestication in general. Four plastid haplotypes are evident in the 113 samples, and are designated by their character-states at each of the 3 polymorphic positions: (AAA) – 23 samples, (ATT) – 29 samples, (GTA) – 26 samples, and (ATA) – 35 samples. The AAA haplotype was only observed in Georgian samples. The observation that the Georgian cultivars exhibited both unique plastid DNA variation (the AAA haplotype) and all other observed plastid haplotypes is consistent with previous studies that have observed both unique and high levels of genetic variation in wild grape (*V. vinifera* subsp. *sylvestris*) in the greater Caucasus region. *© 2011 Bull. Georg. Natl. Acad. Sci.*

Key words: *Grape DNA, trnH-psbA intergenic spacer, rpl16 intron, PCR amplification, sequencing.*

Plant and animal domestication has been called "… the most important development in the past 13,000 years of human history," [1]. Among domesticated plants, the domestication of grapevine (*Vitis vinifera* L.) for food and drink is an event of tremendous cultural and economic importance. Ensuring the long-term value of cultivated plants like grape, particularly in regards to trait improvements such as disease resistance, requires the retention of genetic variability in the form of seed and rootstock collections [2]. Like many other domesticated plants, cultivated grape (*Vitis vinifera* L. subsp. *vinifera*) has experienced recent large-scale demographic events that affect the level of genetic variability available [reviewed in 3]. Both the Phylloxera and mildew epidemics of the 19th century and the growing dominance of a small number of commercial wine varieties in the 20thcentury are believed to have caused a major reduction in overall genetic variability in cultivated grape [4,5].

Fortunately, recent molecular studies have shown that considerable genetic variation exists within the estimated 6000 grape cultivars both at global [6-8] and local scales [9-20]. These studies also indicated that a portion of the

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genetic variation in cultivated grape is geographically restricted, leading to the suggestion that wild grape (*Vitis vinifera* L. subsp. *sylvestris* (C.C. Gmel.) Hegi) could have been taken into cultivation independently in multiple areas, therefore capturing different subsets of the total variation of the wild ancestor [7].

In this study we examine plastid DNA sequence variation in a geographically diverse group of *V. vinifera* cultivars. To date no study has broadly assessed DNA sequence variation in this way. In addition, this study includes a large number of traditional cultivars from the Republic of Georgia, home to over 500 grape cultivars and the earliest archaeological evidence of grape domestication [21,22]. The greater Caucasus region in which Georgia lies is widely believed to be the area in which grape domestication began [23- 26], and the study of genetic diversity in this region is viewed as key to understanding grape domestication in general [4].

Materials and Methods

Tissues for DNA samples came from three sources. We received 9 DNA solutions and 59 cuttings of grape cultivars from the Institut National de la Recherche Agronomique (INRA) Montpellier, France. Dried leaves of 30 grape cultivars were received from National Clonal Germplasm Repository at the University of California, Davis. Finally, 40 Georgian cultivars were received as cuttings from the Georgian Institute of Horticulture, Vini-

Table 1.

Sample information for the 113 sequenced samples. GIHVO = Georgian Institute of Horticulture, Viniculture, and Oenology; INRA = Institut National de la Recherche Agronomique; UC Davis = National Clonal Germplasm Repository at the University of California, Davis.

Sequence group AAA			Sequence group ATA		
Cultivar	Country of origin	Tissue source	Cultivar	Country of origin	Tissue source
Akhardani	Georgia	GIHVO	Mtsv. Mesk.	Georgia	GIHVO
Aleksandrouli	Georgia	GIHVO	Saper. mesk.	Georgia	GIHVO
Almura shavi	Georgia	GIHVO	Aligote		INRA
Budeshuri tetri	Georgia	GIHVO	Ahmeur bou Ahmeur		INRA
Chinuri	Georgia	GIHVO	Alphonse Lavallee		INRA
Chitiskv. Mesk.	Georgia	GIHVO	Chardonnay		INRA
Chkapa	Georgia	GIHVO	Cinsaut		INRA
Gldanula	Georgia	GIHVO	Clairette		INRA
Gorula	Georgia	GIHVO	Colombard		INRA
Mekrenchkhi	Georgia	GIHVO	Cot		INRA
Meskhuri shavi	Georgia	GIHVO	Fahri		UC Davis
Mtsvane Goruli	Georgia	GIHVO	Ferral Izalva		UC Davis
Ojaleshi	Georgia	GIHVO	Folle blanch.		INRA
Rkatsiteli	Georgia	GIHVO	Gamay		INRA
Sazuravi	Georgia	GIHVO	Gamay de Bouze		INRA
Shaba	Georgia	GIHVO	Gamay de Chaudenay		INRA
Shavi Asuretuli	Georgia	GIHVO	Gamay Freaux		INRA
Shavkapito	Georgia	GIHVO	Gouais blanc		INRA
Sqelkana adr.	Georgia	GIHVO	Kali Sahehi		UC Davis
Tavkveri	Georgia	GIHVO	Kurtelaska		UC Davis
Thethri Gomis	Georgia	GIHVO	Marsonne		INRA
Tkhelkana adr.	Georgia	GIHVO	Mauzac		INRA
Tkvlapha shavi	Georgia	GIHVO	Melon		INRA
			Merlot		INRA
			Monbadon		INRA
			Muscat d'Alexandrie		INRA
			Piquepoul blanc		INRA
			Reine des Vignes		INRA
			Romorantin		INRA
			Roussanne		INRA
			Sahebi		UC Davis
			Sultanine		INRA
			Terret gris		INRA
			Valdiguie		INRA
			Yugosl. 360		UC Davis

Sequence group ATT			Sequence group GTA		
Cultivar	Country of origin	Tissue source	Cultivar	Country of origin	Tissue source
Chitisthvala meskh.	Georgia	GIHVO	Aladasturi	Georgia	GIHVO
Sabatono	Georgia	GIHVO	Chkhaveri	Georgia	GIHVO
Saperavi	Georgia	GIHVO	Kachichi	Georgia	GIHVO
Tsigizi	Georgia	GIHVO	Kamuri shavi	Georgia	GIHVO
Kharisthvala Qartlis	Georgia	GIHVO	Krakhuna	Georgia	GIHVO
Kharistvala meskh.	Georgia	GIHVO	Pirgebuli	Georgia	GIHVO
Aramon		INRA	Shonuri	Georgia	GIHVO
Cabernet franc		INRA	Tsitska	Georgia	GIHVO
Cabernet Sauvign.		INRA	Tsolikauri	Georgia	GIHVO
Carmenere		INRA	Alvarelhao		INRA
Chasselas		INRA	Carignan		INRA
Chenin		INRA	Dattier Beyr.		INRA
Emperor		INRA	Grenache		INRA
Gewürtztraminer		INRA	Himrisnky		UCDavis
Itonychi Mavro		UC Davis	Macabeu		INRA
Muscadelle		INRA	Meunier		INRA
Muscat Hambourg		INRA	Mourvedre		INRA
Muscat petits blanc		INRA	Müeller-Thur.		INRA
Nicolas Horthy #39		UC Davis	Perlette		INRA
Sauvignon		INRA	Pinot noir		INRA
Savagnin blanc		INRA	Pinot gris		INRA
Semillon		INRA	Pinot blanc		INRA
Stambulari		UC Davis	Riesling		INRA
Tallian		UC Davis	Sauvignonasse		INRA
Traminer rot RG		INRA	Servant		INRA
Tannat		INRA	Syrah		INRA
Ugni blanc		INRA			
Veltliner rot		INRA			
Viognier		INRA			

culture, and Oenology, Tbilisi. Variable success with DNA isolation resulted in a final set of 113 samples (62 INRA, 11 UC-Davis, 40 Georgian Institute of Horticulture Viniculture and Oenology) (Table 1). Samples received as cuttings were either grown in water at room temperature until leaves appeared, or the cuttings themselves were homogenized using a pencil sharpener. DNA isolation was carried out according to the CTAB protocol presented in Lodhi et al. [27] or with a Plant Genomic DNA Extraction Miniprep System (Viogene U.S.A., Sunnyvale, CA). When necessary, extracted DNAs were purified with GenElute columns (Sigma-Aldrich, St. Louis, MO).

A preliminary sample set comprising seven Georgian and two French cultivars was investigated by sequencing three non-coding plastid DNA regions (the *trnH-psbA* intergenic spacer, the *rpl*16 intron, and the *accD-psaI* intergenic spacer). The *trnH-psbA* intergenic spacer was amplified with the primers "*trn H*" and "*psb A*" [28]. The *accD-psaI* intergenic spacer was amplified with the primers "ACCD-769F" and "PSAI-75R" [29]. The *rpl*16 intron was amplified with the primers "*rpl*16–5'" (5' TGTTGTTTACGAAATCTGGTTC 3') and "*rpl*16–3'" (5' ATGCTTAGTGTGTGACTGGT 3') (this study).

After evaluating the SNPs present in these three plastid regions for the 9-sample preliminary data set (see results), the entire *trnH-psbA* intergenic spacer and a 367 bp portion of the *rpl*16 intron was sequenced for the remaining 104 samples. The new target *rpl*16 intron region was sequenced with the primers "*rpl*16 internalF" (5' GAATAATACACTGAATCG 3') and "*rpl*16 internalR" (5' ATTGAGTGGGATGGCGGA 3') (this study). PCR conditions included denaturing at 94˚C (1 minute), 30 cycles of 94˚C denaturing (1 minute), 55˚C annealing (1 minute), and 72˚C extension (2 minutes), followed by a final extension step at 72˚C (5 minutes). PCR products were purified with GenElute PCR Clean-Up Kits (Sigma-Aldrich, St. Louis, MO), dye-labeled using a Big Dye Terminator Kit (Applied Biosystems, Foster City, CA) and analyzed on either Applied Biosystems 3100 or 3700 genetic analyzers at either the Biology Department of Washington University (St. Louis, MO) or the Laboratory Services Division of the University of Guelph, (ON, Canada). Sequences were manually aligned in Se-Al [30], and haplotype networks were generated using TCS 1.18 [31].

Results and Discussion

Five polymorphisms (not including polynucleotide length variation) were detected among the nine samples

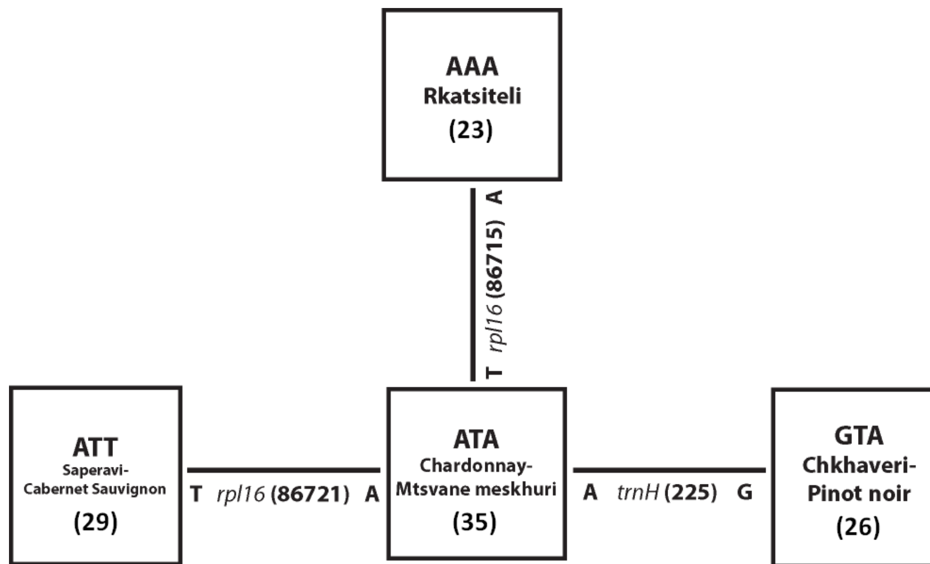

Fig. 1. Haplotype network derived from substitutions observed in the 113-sample *trnH-psbA/rpl*16 dataset. Each haplotype is designated with a three-letter acronym, an informal group name, and the number of samples exhibiting the haplotype. Each branch between haplotypes represents a single mutational step. The substitution type and position in the published *V.vinifera* chloroplast genome [34] are indicated along each branch.

in the preliminary data set, one in the *trnH-psbA* intergenic spacer, and two in both the *accD-psaI* intergenic spacer and *rpl16* intron. Since the substitutions present at both *trnH-psbA* and *accD-psaI* divided the cultivars into the same groups, only *trnH-psbA* and the internal portion of *rpl16* were sequenced for the total 113-sample set. The *trnH-psbA* intergenic spacer was 335 bp in 109 samples. Four samples were 334 bp, owing to either one less nucleotide in a poly-A region (Gouais blanc and Saperavi Meskh) or a poly-T region (Alphons Lavalee and Yugoslavia 360). The targeted portion of the *rpl16* intron was 367 bp in all samples. The haplotype network derived from the 113-sample *trnH-psbA*/ *rpl16* dataset is shown in Fig. 1. Four plastid haplotypes are evident in Fig. 1, and are designated by their character-states at each of the 3 polymorphic positions: (AAA) – 23 samples, (ATT) – 29 samples, (GTA) – 26 samples, and (ATA) – 35 samples (Table 1).

Plastid DNA sequence variation was observed in our geographically diverse set of *V. vinifera* susp. *vinifera* samples, consistent with previous studies of microsatellite and isozyme variation (see introduction). Interestingly, the (AAA) plastid haplotype was found only in the cultivars from the Republic of Georgia. Twenty-three (57.5 %) of the 40 included Georgian cultivars exhibited this haplotype, and in this “Rkatsiteli” group cultivars originated from Eastern Georgia slightly prevails. Contrast this group with the nine cultivars (23%) of the “Chkhaveri-Pinot noir” group (GTA), most of which are cultivated in Western Georgia near the Black Sea coast. Another six of the Georgian cultivars exhibited the “Saperavi-Cabernet Sauvignon” (ATT) haplotype. Among these is the well-known cultivar Saperavi, which is now mainly distribured in Eastern Georgia, but is believed to have originated in south-west Georgia. Only two Georgian cultivars exhibited the “Chardonnay-Mtsvane Meskhuri” group haplotype (ATA), as this group comprises mainly French cultivars.

The observation that the Georgian cultivars exhibited unique plastid DNA variation (AAA haplotype) and all other observed plastid haplotypes is consistent with previous studies that have observed both unique and high levels of genetic variation in wild grape (*V. vinifera* subsp. *sylvestris*) in the greater Caucasus region [7,8,32,33].

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შესწავლილია გეოგრაფიულად დაცილებული 113 კულტივირებული ვაზის ჯიშის დნმ-ის თანმიმდევრობების მრავალფეროვნება ორ პლასტიდურ უბანში (*trnH-psbA* ინტერგენური სპეისერი და *rpl*16 ინტრონი). შესწავლილი ჯგუფი შეიცავდა ვაზის 40 ქართულ ნიმუშს. საქართველო ცნობილია როგორც 500-ზე მეტი ვაზის ჯიშის სამშობლო, აქ აღმოჩენილია ვაზის დომესტიკაციის დამამტკიცებელი უძველესი არქეოლოგიური ნიმუშები. კავკასიის რეგიონი, რომელსაც საქართველო მიეკუთვნება, ითვლება იმ ადგილად, სადაც ვაზის დომესტიკაციის პროცესი დაიწყო, შესაბამისად ამ რეგიონის გენეტიკური მრავალფეროვნების შესწავლა წარმოადგენს ზოგადად ვაზის დომესტიკაციის გარკვევის ერთ-ერთ აუცილებელ გასაღებს. 113 ნიმუშში აღმოჩენილია ოთხი პლასტიდური ჰაპლოტიპი, რომლებიც მონიშნულ იქნა მათი დამახასიათებელი 3 პოლიმორფული უბნის მიხედვით: (AAA) – 23 ნიმუში, (ATT) – 29 ნიმუში, (GTA) – 26 ნიმუში, და (ATA) – 35 ნიმუში. AAA ჰაპლოტიპი აღმოჩენილ იქნა მხოლოდ ქართულ ნიმუშებში. ის ფაქტი, რომ ქართულ ჯიშებში ვლინდება როგორც უნიკალური ჰაპლოტიპი (AAA), ასევე ყველა სხვა დანარჩენი ჰაპლოტიპი, კარგად ეთანადება ადრეულ დასკვნებს იმის თაობაზე, რომ კავკასიის რეგიონისთვის დამახასიათებელია ველური ვაზის (V. *vinifera* subsp. *sylvestris*) უნიკალური და მაღალი დონის გენეტიკური მრავალფეროვნება.

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