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AUTOCHTHONOUS GRAPEVINE BIODIVERSITY OF THE CAUCASUS REGION

Abstract: The Caucasus region, a geographical and historical centre of grape domestication, hosts a largely untapped genetic diversity for cultivated grape and its wild relative *Vitis sylvestris*. Today, new challenges like climate change, diseases, environmental concerns and market demand call for a renewed interest in local genetic resources for selection and breeding while — similarly to other crops, a large share of modern viticulture relies on a small pool of grape varieties.

Several international and national projects have run in the last decades: the first being the "Conservation and use of grapevine genetic resources of the Caucasus and Northern Back Sea Area" (Bioversity International, 2003–2008) and the latest being

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the ongoing "Research project for the study of Georgian grapes and wine culture" (National Wine Agency of Georgia).

A continued effort has allowed researchers to establish a large collections of local genetic resources, characterized for many traits (morphology, phenology, anthocyanins, resistances, wine). The identity of part of these resources have been certified using molecular fingerprinting and made available in the European *Vitis* database. New DNA technologies have also permitted to analyze these resources using genome-wide approaches, allowing the understanding of domestication and gene variant discovery for traits of interest.

This wealth of genetic resources and information attracts the international interest because of its diversity, accessibility and the possibility to carry out phenotypic analysis for complex traits. The Caucasian countries demonstrate the efficiency of simultaneous genotyping and phenotying programs in several experimental collections (with the INRA Vassal Grapevine Resources Center and the University of Milan), providing useful data and training possibilities for researchers and professionals.

Key words: *ampelography, DNA fingerprinting, database, resistance, wild grape*

INTRODUCTION

Geographically the Caucasus is a region located at the border of Europe and Asia situated between the Black Sea and the Caspian Sea and occupied by Russia, Georgia, Azerbaijan and Armenia. The Caucasus mountains trending generally from northwest to southeast and consist of two ranges-the Grater Caucasus in the north and the Lesser Caucasus in the south. The Caucasus region is separated between northern and southern parts — the North Caucasus (Ciscaucasus) and South Caucasus (Transcaucasus), respectively — the north is within the Russian Federation, while in the south is occupied by the independent states of Georgia, Armenia and Azerbaijan [1].

The territory of great ecological importance. It harbors some 6400 species of higher plants, 1600 of which are endemic to the region [2].

The Caucasus is recognized to be one of the oldest region started agricultural activities and domestication of plants and animals. The Caucasus region, a site of grape domestication, hosts a largely untapped genetic diversity for cultivated grape and its wild relative *Vitis sylvestris*. Since the end of the 19th century the researchers V. Hehn [3] and A. de Candolle [4] indicates the South Caucasus as the Center of wine origin and grape domestication, confirmed after by numerous authors during the 20th and 21st centuries [5, 6].

Based on result of investigation wild and cultivated grapevine of the Caucasus N. Vavilov [7], the author of the theory of the centres of origin for cultivated plants, concludes that "All existing data indicates that the Caucasus is the main hearth of origin of wild and cultivated grapevine. Great number of various autochthonous varieties in Azerbaijan, Armenia and Georgia,

which have striking diversity of colour and shape of berries and seeds indicate about concentration the processes of form origin here".

The 21st century demonstrated new evidences for confirmation of preliminarily of wine making activities in the South Caucasus with new finding in Georgia provide the earliest biomolecular archaeological evidence for grape wine and viniculture from the Near East, at ca. 6,000–5,800 BC [8]: The chemical findings are corroborated by climatic and environmental reconstruction, together with archaeobotanical evidence, including grape pollen, starch, and epidermal remains associated with a jar of similar type and date. They are the most numerous pottery type at many sites comprising the so-called "Shulaveri- Shomutepe Culture" of the Neolithic period, which extends into western Azerbaijan and northern Armenia.

Another recent important conformation of wine making activities from the South Caucasus comes from Aremenia: Archaeological excavations in the Areni-1 cave complex in southeastern Armenia revealed installations and artifacts dating to around 4000 cal. BCE that are strongly indicative of wine production [9]. A positive result was observed for two of the samples from the Areni-1 cave complex, adding evidence supporting the hypothesis that wine was produced in the Near Eastern highlands in the Late Chalcolithic Period.

The oldest evidences of wine making and viniculture from the South Caucasus indicate to the process of grape domestication started by these early civilisations, using Euroasian wild grape: *V. vinifera* ssp. *sylvestris* (Gmelin) Hegi, a wild ancestor of the cultivated grapevine *V. vinifera* ssp. *sativa* D. C., is a typical representative of the Caucasus flora. This plant is a lodger in almost all woody regions, in forests on lowlands and rivers' banks up to 1200 m above sea level here. Fr. Kollenati [10] was the first researcher who initiated investigation of wild grape of the South Caucasus following by the numerous authors until novel days [11].

As a result of long time breeding activities wide number of authoctonous varieties were originated in the Caucasus region, part of which have been described in the local and international ampelograptic books like 'Ampelography of the Soviet Union' [12] and others [13, 14]. This genetic resources are the main sources for cultivation within regional countries or initial breeding programs for sucesfull development of new varieties.

Today, new challenges such as climate change, diseases, environmental concerns and market demand call for a renewed interest in local genetic resources for selection and breeding while — similarly to other crops, a large share of modern viticulture relies on a small pool of grape varieties.

The aim of this work is to provide general information about autochthonous grapevine biodiversity of the Caucasus region and demonstrate research activities for their investigation and conservation during last years.

MATERIALS AND METHODS

Several national and international projects have run in the last decades, focused on study and conservation of grapevine genetic resources of the Caucasus region. The first international project (2003–2008) being the "Conservation and use of grapevine genetic resources of the Caucasus and Northern Back Sea Area" leaded by the Bioversity International [15], following by the French ECO-NET (2006–2007) project "Molecular characterization of grapevine genetic resources from the Caucasus", the Eropean GrapeGen06 (2008–2011) project "Conservation, characterization and management of grapevine genetic resources in Europe" [16], the second European GRAPENET project (2010–2014) "East-West Collaboration for Grapevine Diversity Exploration and Mobilization of Adaptive Traits for Breeding" [17]. The activities for description of grape genetic resources from the Caucasus has been supported by the Vitis working network of the "European Cooperative Program for Crop Genetic Resources Network" leaded by the Bioversity International. Among the regional project can be stated the ongoing "Research project for the study of the Georgian grapes and wine culture" leaded by the National Wine Agency of Georgia since 2014 [18].

With the goal of multidisciplinary characterization and evaluation of grapevine germplasm including autochthonous varieties and wild vines from the Caucasus by using modern techniques of ampelography and molecular genetics it was doen the following activities: I) Ampelographic description and compilation of ampelographical cards of the varieties based on the descriptors of OIV and Bioversity International; II) Cytological analyses; III) Ripening profiling of grape; IV) Phenology; V) Chemo-taxonomical characterization of berry skins and anthocyanins and polyphenols profile of varieties on the basis of High Performance Liquid Chromatography (HPLC) and spectrophtometry; VI) DNA fingerprinting to detect genetic variation using 9 to 20 Simple Sequence Repeats (SSR) markers for identification and parentage analysis, and 10.000 SNPs for structure and genetic association analysis.

The local autochthonous varieties available in the collections of regional countries, also in the collections of Italy and France are under investigation.

RESULTS AND DISCUSSION

DIVERSITY OF GRAPE GENE POOLS

Armenia: About 400 indigenous grape varieties are originated in Armenia according to the local references; 70 autochthonous grape varieties are preserved in the collections; 55 varieties in total — as local as well introduced

— are recommended for commercial use, among which 21 are table grape varieties. The country has 17.000 ha of vineyards [19].

Georgia: There are 525 indigenous grape varieties in Georgia and most of those are confirmed by ampelography and DNA markers; 437 autochthonous grape varieties are preserved in the Saguramo collections; 32 autochthonous wine grape cultivars are commercially used in nowadays; more than 60 breeding varieties and clones have been originated in the country, which has 48.000 ha of vineyards [20].

Azerbaijan: The country has about 400 indigenous grape varieties according to local references. Approximately 200 native grape varieties among those are preserved in the collections; 42 table grape and 22 wine cultivars (local and inroduced) are recommended for cultivation; the country has 16.000 ha of vineyards [21].

Russian Federation: There are 200 indigenous grape varieties available in the country; among those 110 local varieties are preserved in the collection, 140 local and introduced cultivars are recommended for usage containing many local breeding varieties and clones; the country has 62.000 ha of vineyards.

Characterization. Description of the grape varieties from the Caucasus initiated since 19th century by the Fr. Kolenati [10], who described autochthonous varieties of Azerbaijan and Georgia and provided information about wild grapevine of the Caucasus. In the second part of the same 19th century Downy and Powdery mildew and Phylloxera infestation rich to the Caucasus region. With idea to preserve local diversity of viticulture and winemaking it was established Phylloxera Committee of the Caucasus in December 1880 by the Ministry of Property of the former Russian Empire, in which the Caucasus countries were included in that time [22]. The Committee had responsibility to describe local viticulture and winemaking traditions including characterization of local varieties: this very rich information about the regions of South and North Caucasus were published in several volumes of the Proceedings of the Caucasus Phylloxera Committee [23]. In the same period was published the one of the first ampelography of local varieties of Western Georgia, published by the Staroselskii in 1893 [24].

The 20th century passed under the leadership of N. Vavilov and the members of his school including collecting and preserving grapevine genetic resources in all over regions of the former Soviet Union, making the network of the available field collections, Research Institutions of Viticulture and Winemaking, and agricultural universities together with their research stations. A. Negrul [12, 25] elaborated his well-known theory about classification of cultivated grapevine varieties. The book of "Ampelography of the Soviet Union"

in 10 volumes printed during 1949–1970 became one of the main publication describing rich genetic richness of autochthonous, local, breeding and introduced varieties. Before and after of this edition different local ampelographies were published based on the study this genetic resources. The local varieties of grapevine were selected for cultivation in the regional countries and were included in breeding programs for obtaining of new varieties [24, 26–30].

The past years of 21st century became very important milestone for study of grapevine genetic resources of the Caucasus countries due to increasing interest of West European researchers to this gene pools. Since 2003 the regional countries are participants of the various international projects listed above in the Materials and Methods together with the leader European institutions and did significant progress for investigation of local varieties of the region.

To reach this milestone it was adapted internationally adopted protocols for ampelographic study based on the descriptors of the OIV list [31], to the BBCH scales for phenology observation [32], classification according to anthocyanins and polyphenols based on the agreed eno-carpological method [33] and application of standard methods for the grapevine phenotypic diversity exploration on phenological traits [34].

Based on the results of investigation it was published several ampelographic books of the grapevine varieties of the Caucasus [13, 14, 35–37]. Various results of these multidisciplinary study have been published in the international journals [18, 33, 34, 38–49].

Resistance. The discovery of resistance genes against powdery mildew in autochthonous *Vitis vinifera* accessions [50] opens up new interests in germplasm screening and utilization. Screening of genetic resources from the Caucasus region initiated within the COST project [17] and wide number of autochthonous varieties were tested. Among those some Georgian [51–53] and Azerbaijan varieties [54] demonstrated positive results. More — the Georgian varieties also show a range of symptoms severity in case of infection of phytoplasma disease like Bois noir [29, 55]. Based in this information Failla et al. [54] indicate grape genetic resources of the Caucasus as a font of resistance to diseases and grape quality.

Wild grapevine. The knowledge of the situation for grape wild relatives (*Vitis* species as well as the subspecies *sylvestris*, the wild compartment of *Vitis vinifera)* is one of the main course for study due to the lack of information on the relative importance of the wild individuals in Europe and was stimulated in the Caucasus countries since 2003 [14]. A 'Protocol for the inventory of *V. vinifera* subsp. s*ylvestris*' was defined and applied for the inventory of *V. sylvestris* wild sites in Europe and the inventory of *V. sylvestris* wild

plants [16]. Some important population characters were taken into account: the location of population by GIS mapping, the number of plants in the population, the sex of these plants, the presence in the proximity of other *Vitis* which could intercross with the wild population, the status of preservation, the risk of loss of the populations and the owner of the land. Various aspects of study of wild grapevine of the region are described in the articles [11, 18, 56–59] and in the "Ampelography of the Caucasus" [14].

DNA study. DNA study of local genetic resource became one of the main technique to investigate local grape gene pool — cultivated and wild — of the Caucasus countries. The identity and genetic characterization of these resources have been certified using molecular fingerprinting and made available in the European *Vitis* database [6, 60–66].

Plastid DNA sequence diversity in wild grapevine samples (*Vitis vinifera* subsp. *sylvestris*) from the Caucasus region was conducted [67].

New DNA technologies have also permitted to analyze these resources using genome-wide approaches, allowing the understanding of domestication and gene variant discovery for traits of interest [61,68].

The main objective for last COST action was to define a core collection which could both represent and preserve the highest genetic diversity with the lowest number of accessions [17]. The prospect to design core collections including east European germplasm is now really accessible — the Core Collection referred to East European germplasm it includes 63 accessions, out of which 45 are from Caucasus — this means that 71.4% of varieties are from the Caucasus. The average expected heterozygosity in the entire sample was 0.87.

Database. The European Vitis database website (www.eu-vitis.de) is open for accumulation of data on varieties with different levels of access. The website database adopted common data standards, which is a prerequisite for searching for data. With respect to passport data, the FAO / IPGRI Multi-crop Passport Descriptor (MCPD) format used by EURISCO has been adopted. Descriptors of the OIV descriptor list for grapevine varieties and species [31] have been used to establish a standardized format. The 9 markers of Genres081 and GrapeGen06 had been recommended as a standard set for grapevine genotyping [16].

The first collaborative project "Conservation and Sustainable Use of Grapevine Genetic Resources in the Caucasus and Northern Black Sea Region" (2003–2007) has started to incorporated information avout regional varieties in it. As a results it turned out that 2,654 accessions from autochthonous cultivars maintained by Armenia, Azerbaijan, Georgia, Moldova, Russian Federation and Ukraine in ten grapevine collections may belong to

1,283 cultivars, but trueness to type assessment by morphology and genetic fingerprinting of collected materials still needed to be done [69].

The last European COST Action FA1003 "East-West collaboration for grapevine diversity exploration and mobilization of adaptive traits for breeding" (2010–2014) a first step in that direction was initiated. The following countries participated: Albania, Armenia, Austria, Azerbaijan, Bulgaria, Croatia, Georgia, Hungary, Latvia, Moldova, Romania, Slovakia, Slovenia and Ukraine. 1098 *Vitis vinifera* accessions and 76 *Vitis sylvestris* individuals were analyzed by nine SSR-markers. Cultivar identity confirmation/rejection was attempted for 306 genotypes by comparison of the generated genetic profiles with international SSR-marker databases and ampelographic studies. The outcome proved unambiguously the necessity of morphologic description and photos (a) for comparison with bibliography, (b) for a clear and explicit definition of the cultivar and (c) the detection of sampling errors and misnomers. According to the Maul et al. [38] from the 1,098 analyzed accessions, 997 turned out to be indigenous to the participating countries. The remaining 101 accessions were Western European cultivars. The 997 fingerprints of indigenous accessions resulted in 658 unique profiles/cultivars. From these 353 (54%) are only maintained in the countries of origin and 300 (46%) unique genotypes exist only once in the Eastern European collections. For these 300 genotypes duplicate preservation needs to be initiated.

The benefits of this database can be address to the follwoing stacekholders like curators of grapevine germplasm repositories, wine growers, breeders, researchers and industry using it as a tool to know the varieties, their ampelographic and agronomic feature, wine characteristics, availability for conservation and crossing purposes.

Germplasm Conservation. A continued effort has allowed researchers to establish a large collections of local genetic resources, characterized for many traits (morphology, phenology, anthocyanins, resistances, wine). A continued effort has allowed researchers to establish a large collections of local genetic resources, characterized for many traits (morphology, phenology, anthocyanins, resistances, wine). For example,

— The project "Conservation and Sustainable Use of Grapevine Genetic Resources in the Caucasus and Northern Black Sea Region" (2003–2007) stimulated establishement of two new collections in Armenia and Georgia and guaranted conservation of varieties in available collections of the project partners;

— The National Amelographic Collection of Russia (NACR), located in Anapa district of Krasnodar region, became one of the largest collection due

to last years activities having 3320 accessions [28]. This result has achieved due to collaborative activities of various domestic and foreign collections.

— Saguramo germplasm collection in Georgia, established in 2008, comprises 420 local varieties and 100 wild grapes, not available elsewhere. Having about 1.000 *Vitis* accessions in total make this center suitable for ampelographic study, breeding activities and promotion of varieties among local viticulturists.

CONCLUSIONS

— Rich grapevine genetic resources of the Caucasus region are an unique germplasm, having diverse ampelographic and genetic background and are linked with the history of domestication.

— Some traits of quality, adaptation and resistance available in it can be suitable for breeding purposes and winemaking for diverse ecological and marketing reality.

— It is a modern platform with conservation, documentation, testing, genotyping, pehenotyping, experimental vinification...

— The value of Caucasus cultivars is confirmed as in their countries of origin as well in other winemaking countries too.

— The germplasms of the Caucasus grapes is documented and conserved in the field collections in the region and in the collections of various countries.

— The research cooperation for studying autochthonous grapes is in progress together with the leader World institutions and new partners are also welcome.

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AUTOHTONI BIODIVERZITET VINOVE LOZE U KAVKASKOM REGIONU

Sažetak

Kavkaski region, mjesto domestikacije vinove loze, posjeduje uglavnom neiskorišćeni genetički diverzitet kultivisane vinove loze i njenog divljeg srodnika *Vitis silvestris*. Danas, novi izazovi, kao što su klimatske promjene, bolesti, zaštita životne sredine i potražnja na tržištu, zahtijevaju ponovno interesovanje za lokalne genetičke resurse za selekciju i gajenje, dok se, slično kao i za druge kulture, veliki dio modernog vinogradarstva oslanja na mali diverzitet sorti vinove loze.

U posljednjih nekoliko decenija realizovano je nekoliko međunarodnih i nacionalnih projekata: prvi je bio „Konzervacija i upotreba genetskih resursa vinove loze Kavkaza i područja Sjevernog Crnog mora" (Bioversity International, 2003–2008), a najnoviji je tekući „Istraživački projekat za proučavanje gruzijskih sorti vinove loze i vinske kulture" (Nacionalna agencija za vino Gruzije).

Neprekidni napori omogućili su istraživačima da uspostave veliku kolekciju lokalnih genetičkih resursa, opisanih za mnoge osobine (morfologija, fenologija, antocijani, otpornost, vino). Identitet dijela ovih resursa je potvrđen molekularnim „otiskom prstiju" i dostupan je u evropskoj Vitis bazi podataka. Nove DNK tehnologije su takođe omogućile analizu ovih resura, koristeći široke pristupe genomskih tehnologija, razumijevanje domestikacije i otkrivanje varijanti gena za osobine od interesa.

Ovo bogatstvo genetskih resursa i informacija privlači međunarodni interes zbog raznolikosti, pristupačnosti i mogućnosti da se izvrši fenotipska analiza za kompleksne osobine. Kavkaske zemlje pokazuju efikasnost istovremenih programa za genotipizaciju i fenotizaciju u nekoliko eksperimentalnih kolekcija (sa INRA Vassal centrom i Univerzitetom u Milanu), pružajući korisne podatke i mogućnosti obuke za istraživače i profesionalce.

Ključne riječi: ampelografija, DNA otisak prsta, baza podataka, rezistentnost, divlja vinova loza