Association Prosperity and Development in Bulgaria
Project:"SOS for endangered traditional vine varieties"
Acronym: "VineSOS"
Project No. 1829

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Challenges and Opportunities in the Greek-Bulgarian Cross-Border Region Related to Local Biodiversity and Vine Varieties

The „SOS for endangered traditional vine varieties" deals mainly with common challenges and problems in the Greek - Bulgarian Cross-border Region (Fig. 1) related to local biodiversity and vine varieties, especially those in the sites of "Natura 2000". Leading beneficiary is the Executive Agency for Vine and Wine. Its partners are: Association "Prosperity and Development in Bulgaria", Business & Exhibition Researches and Development Institute (IEE)), Greece, and the International Hellenic University, Thessaloniki.

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Fig.1 – Cross-border region Greece-Bulgaria

Within the frames of the project "SOS for endangered traditional vine varieties", various activities were implemented, and the effect of conventional, organic and biodynamic systems for the production of traditional vine varieties was evaluated. Experimental fields were created in the area of the Greek-Bulgarian cross-border region, which showed a different approach to determining the most appropriate system of agriculture at both national and local level (Fig. 2).

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Fig.2 – Experimental fields

A unique DNA study of local vine varieties from Bulgaria and Greece was performed. The study achieved its goal of assessing the genetic diversity of eight Greek and nine Bulgarian native vine varieties using seven microsatellite markers.

In Bulgaria, viticulture and wine production date back to ancient Thrace and today commercial varieties consist of old local varieties, widespread European varieties and locally selected varieties.

This activity is necessary as Greece and Bulgaria are neighboring countries and therefore an extensive exchange of germplasm of V. vinifera for breeding has taken place. This has led to the establishment of Greek and Bulgarian local varieties with similar morphological characteristics, called "common".

SSR or STR markers, also known as microsatellites, have been widely used since the early 1990s (Thomas and Scott 1993; Thomas et al., 1994). Nowadays, they are considered one of the best methods for determining the identity of a variety. Due to their high polymorphism,

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microsatellite markers significantly improve the stability of DNA profiling in the analysis of parental origin (BOWERS et al., 1996; BOWERS et al., 1999; VOUILLAMOZ et al., 2003; LACOMBE et al., 2007; VOUILLAMOZ et al., 2007; LAUCOU et al., 2008; ŠTAJNER et al., 2015; BIAGINI et al., 2016) and in the molecular characteristics of grape varieties (LEFORT and Roubelakis-Angelakis 2002; Martin et al., 2003; THIS et al., 2004; VOUILLAMOZ et al., 2006; ŠTAJNER et al., 2008; LAIADI et al., 2009; POBLETE et al., 2011; Arap et al., 2012; DE LORENZIS et al., 2013; ŽULI MIHALJEVIC et al., 2013; Merkouropoulos et al., 2015; Salimov et al., 2015; Popescu et al., 2017; Dong et al., 2018; JIMÉNEZ-CANTIZANO et al., 2018; Popescu and Crespan 2018; MAHMOOD et al., 2019; TAHERI and DARZI RAMANDI 2020).

A total of 384 samples of young leaves were collected from both countries. Genomic DNA was extracted from 20-30 mg of leaf tissue using the NucleoSpin Plant II DNA kit (Macherey-Nagel, Germany) and according to the manufacturer's protocol.

A multiplex PCR response method was applied that amplified seven microsatellite loci simultaneously. Selected microsatellites are recommended by various studies in the scientific literature and have proven to be very successful in deciphering the genetic modification and differentiation of vine varieties. All multiplex PCRs were performed in a 10 ul volume containing 5.5 ul of a 1X KAPA2G fast multiplex PCR kit (KAPABIOSYSTEMS, USA), 3.5 ul of a mixture of primers (0.25 uM for each primer) and 1 ul (~20 ng) from the DNA of the template. The multiplex amplification cycling conditions consist of an initial denaturation step at 95°C for 3 minutes, followed by 30 cycles of 15 s at 95°C, 30 s at 56°C and 30 s at 72° C, with a final extension at 72 ° C for 15 minutes. Fluorescently labeled PCR products were separated on an ABI 3500 genetic analyzer (Applied Biosystems, USA). Alleles were sized and samples genotyped using STRand 2.4.59 software (Tunen and Hughes 2001).

There are two methods of analysis used for the study, and both ampelographic and molecular genetics methods are used to confirm or rule out any presumed synonymy between the varieties coming from these two countries in the cross-border area .

A total of 17 local varieties were studied (9 Bulgarian and 8 Greek). The results of the molecular genetic analysis (i.e. DNA analysis) showed that three (3) species do not make a genetic difference between the two districts. Consequently, out of a total of 17 studied varieties, only 14 are unique. The other 3 varieties appear to have a common genetic origin.

The study showed that most of the Greek and Bulgarian local varieties come from different gene pools, with the exception of three varieties. These three varieties are: the Bulgarian variety Dimyat, which looks genetically similar to the Greek variety Zoumiatiko. In addition, the varieties Pamid (B) or Pamidi (G) and Mavrud (B) or Mavroudi (G) share common names between the two countries and it has been genetically confirmed that they are in fact largely the same varieties.

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Six species [2 Bulgarian (Rubin and Tamyanka) and 4 Greek (Sefka, Limnio, Karnachalas, Bogialamas)] are genetically isolated more than all the others. This seems to be a good starting point for further study of the detailed genetic profile of these varieties, as well as the argonomic advantages and disadvantages of these varieties, but it is more important to further study the advantages of the organoleptic and qualitative characteristics of the wine, produced from these varieties.

It is possible that these varieties offer something different both in the agricultural sector in terms of sustainable genotypes and agronomic practices and in terms of the quality and organoleptic profile of the wine sector. Furthermore, the identification of a very close genetic similarity of three species [Dimyat (B) with Zoumiatiko (G), Pamid (B) with Pamidi (G) and Mavrud (B) with Mavroudi (G)] can be used to resolve arguments when used in wines and their labeling. There may be an exchange of existing empirical knowledge in the agricultural practices and production of wines of these varieties and their inclusion with other varieties in the wine, but also in the process of conservation of these varieties.

A participant in the project has already expressed interest in the wine sector regarding the genetic characteristics of a Bulgarian variety and its genetic comparison with a French variety.

The studied varieties need to be examined more intensively ampelographically, as this process takes more time and moreover at least some of these varieties need to be studied in more detail both at population level (genetic differentiation) with the help of more powerful molecular tools for genetics (i.e. thousands of SNP DNA markers from next generation sequencing methods, etc.) and at a quantitative genetic level (genotype-trait association) to identify unique production and / or qualitative characteristics of these varieties.

In addition, the use of the molecular genetics tools used in this project should be further explored for their use in traceability cases (eg. ability to distinguish single varietal wines from the varieties examined in this project, etc.). Such research can be of great help for the development of PDO and PGI products in both countries.

An experiment is currently underway in Greece to examine whether wines of one variety from three local Greek varieties can be genetically identified and distinguished from each other, and whether these single-variety wines can be uniquely identified as different from commercial wine.

The Greek and Bulgarian authorities can develop with high precision germplasm retention programs for V. Vinifera in relation to the varieties studied and also cooperate in the policies for common varieties between the countries.

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The results of the project can be applied in the short term for the development of conservation programs for breeding and / or germplasm of these varieties and to support the characterization of products with PDO and PGI.

In the long run, with a little more investment in research, any unique production and / or quality characteristics of these varieties could be identified and lead to both increased production, improved quality, reduced agronomic costs and why not produce high quality value added products.

Biodiversity in the cross-border area of Greece and Bulgaria could cooperate through mutual efforts and actions for the benefit of administrations and businesses. Neighboring countries can develop common biodiversity policies and share conservation efforts and actions, and why not promote biodiversity in some areas. An example of this is the design of propagation and / or germplasm programs. From a business point of view, the genetic characteristics of these local varieties can help both to address labeling arguments and to develop the added value of PDO and PGI wine products. In addition, the exchange of agronomic empirical practices would benefit farmers on both sides of the border.

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