PRESENCE OF VIRUSES IN THE POPULATION OF GRAPEVINE CULTIVAR "PROKUPAC" (*Vitis vinifera L.*) IN RASINA DISTRICT, SERBIA

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Abstract

Grapevine cultivar "Prokupac" is officially recognized as Serbian autochthonous variety, It has a long history, tracing to the Middle Ages. This cultivar is used to be the most widespread variety in this part of Balkans and best-rated domestic Serbian wine at international markets. Survey of virus infection incidence in grapevine nurseries, was conducted at 14 location of Rasina district (Republic of Serbia), during 2018. The survey was conducted by Agricultural Service Krusevac, as authorized institution with a professional capacity included into a national phytosanitary system, for implementation of the system of control of prevention of fruit tree and grapevines pathogens (including export and import). It also controls the presence of pathogens in main nurseries. Total number of 17 samples was tested on presence of four viruses using ELISA: *Grapevine fanleaf virus* (GFLV), *Grapevine leafroll-associated viruses 1*, *2* and *3* (GLRaV-1, GLRaV-2, GLRaV-3). The presence of GLRaV-1 was confirmed in one tested sample, and the presence of GLRaV-1, GLRaV-2, GLRaV-3 were also confirmed in one tested sample. Conducted investigation indicates deteriorated viral sanitary status of cultivar "Prokupac" and necessity for intensifying the clonal and sanitary selection program.

Key words: *Virus diseases, Prokupac, Autochthonous grapevine cultivar, ELISA*

Introduction

"Prokupac" is an old autochthonous grapevine (*Vitis vinifera L.*) variety originating in Serbia that belongs to *Convar pontica, Convarietas balcanica* (Bešlić et all., 2012). According to the literary data, besides Prokupac and other autochthonous varieties Smederevka, Plovdina and Začinak have been cultivated in this area since Roman Empire (3rd century B.C.) (Jiriček, 1923). In the past it had much greater significance than it does today. It is grown in Serbia, Macedonia and Bulgaria. The great value of this variety is reflected in the fact that it produces a wine of pink or red color. Grapes are also used for blending, but also for the production of lozovača (rakia made from grapes) and vinjak (a brand of brandy). The trend in wine production is changing and is looking for authentic and specific wines of a certain area, where autochthonous varieties play an indispensable role. In the last few years, work on the prevention of genetic resources and clonal selection of this variety has started, with the aim of its preserving and restoring cultivation on farms. A major problem in its spread is the lack of quality planting material, where by viruses play a significant role since they have a very detrimental effect on the growth and development of the vine (Walter and Martelli, 1998), and, among other things, to a considerable extent may affect the reduction of sugar and increase in acids in freshly crushed must (wine juice) (Besse et al., 2009; Borgo and Angelini, 2002), the reduction of photosynthesis (Bertamini et al., 2004) and, in general, the reduction of grape yields, delay in vegetation and ripening, affect the chemical composition and aromatic profile of must, reduced reception of the rootstock and graft stem and poorer rhizogenesis. Their harmfulness is further enhanced by the fact that as obligate biotrophic

parasites, their development and reproduction requires host living cells, and thus cannot be eliminated by conventional chemical methods (Juretic, 2002).
The aim of this study was to determine the health status of the Prokupac variety in terms of infection with economically the most harmful and the most widespread grapevine viruses by using the ELISA method.

Material and Methods

The research included 14 localities in the Rasina District in Serbia. When testing samples were selecting, those with good viticultural characteristics and satisfactory exuberance were selected which did not show any visible signs of viral infections (leaf curl and deformities, purple color on leaf face, etc.). Samples were collected during the 2018 vegetation resting phase. Sampling was carried out on the presence of the following viruses: Grapevine fanleaf virus / GFLV and Grapevine leafroll virus / GLRaV-1,2,3, in native plantations for the production of the stems of vines. For native plantations of the grapevine for the production of the stems in the gantry system, a visual inspection of all the grapevines in the nursery was carried out in accordance with the Rule Book and mandatory sampling of 10% of the native grapevines from which 1% of the collective samples are formed. A group of 10 adjacent vines or from adjacent rows is selected from which every second (to be marked) is sampled and a group pattern is formed. The specimens contain five central parts of the stems 10-15 cm long. The specimens were packed in plastic bags, labeled and refrigerated at 4 ° C until testing and with the official order of the *competent service – Agricultural Service Kruševac* forwarded to an authorized first-stage diagnostic laboratory (Agriculturaal Service Smederevo), according to the procedure described in the Rule Book on crop health inspection and planting material production facilities (Dulić-Marković, 2008).
Commercial ELISA kits were used in their analysis, and all steps of the analyzes were performed in accordance with the test manufacturer's recommendations. Each sample was tested by ELISA for the presence of four viruses:
- Grapevine fanleaf virus, GFLV,
- Grapevine leafroll-associated virus 1, GLRaV-1,
- Grapevine leafroll-associated virus GLRaV-2,
- Grapevine leafroll-associated virus 3, GLRaV-3.

The following antibody double antibody sandwich ELISA, DAS-ELISA for GFLV, GLRaV-1, GLRaV-2, GLRaV-3 was used for virus presence testing. Monoclonal antibodies were used in GLRaV-2, while polyclonal antibodies and serums were used for other viruses.

Results and Discussion

The conducted analyzes determined a low level of infection of the variety "Prokupac" in the area of Rasina District, Republic of Serbia. Of the 14 samples tested, the presence of GLRaV-1 was detected in one sample, while the presence of GLRaV-2, GLRaV-3 and GFLV was not present in the samples tested. It should also be pointed out that monoclonal antibodies were used in the detection of GLRaV-2, so the possibility of this virus is slightly higher than determined by the conducted research. The main vectors of both GLRaV-1 G and LRaV-3 are in the production of vineyards except humans also the use of infected planting material and different varieties of scale insects. Then the possibility that the plantation can be infected by viruses after vines planting cannot be excluded. From mixed infections, the presence of the combination of GLRaV-1 + GLRaV-2 + GLRaV-3 virus in one tested sample was determined.
The determined health status of native grapevines of the autochthonous variety "Prokupac" is similar to the results of the conducted research on other autochthonous varieties in the Rasina District. According to the research results (Miletaković and Jovanović, 2018) related to the

presence of GFLV, GLRaV-1, T GLRaV-2 and GLRaV-3 on a total of 17 samples in the native vineyard of the autochthonous variety "Smederevka", using the ELISA test, the presence of GLRaV-3 was determined in four tested samples (23.53%), while other viruses did not exist in the tested samples or mixed infections. The occurrence of GLRaV-3 was found in the mixed infection in this Study, so it can be concluded that the infection with this virus of the "Prokupac" variety is much smaller than the infection of the "Smederevka" variety. In both Studies, a large percentage of plants was free from all four studied viruses.

Conclusion

The results of this, as well as similar research, indicate the relatively good health of the native vines of our autochthonous varieties grown in nurseries in the Rasina District. In order to eliminate the presence of viruses in native plants of autochthonous vine varieties, a number of measures should be applied which individually cannot provide major effects. In the future, many varieties, including Prokupac, in order to produce certified planting material, it would be necessary to carry out the thermotherapy procedures and meristem culture to produce virus-free plants. Cured plants could show better production characteristics and thus become more interesting to both nurseries and grape and wine producers. All the above mentioned point to the need to intensify the program of clonal and phytosanitary selection for the purpose of production of certified planting material with the aim of preserving and revitalizing this very valuable perspective and autochthonous variety.

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