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CLONE SELECTON OF AUTOCHTONES AND INTRODUCED VARIETIES IN THE OLD GRAPEVINE PLANTED AREAS OF SOUTH EASTERN AND EASTERN SERBIA AND PRELIMINARY CHECK OF THEIR HEALTH STATUS

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Sivčev B., Z.Rankovic-Vasić, and D. Radovanović (2011): *Clone selection of autochtones and introduced varieties in the old grapevine planted areas of Eastern and South Eastern Serbia and preliminary check of their health status.*- Genetika, Vol. 43, No. 3, 465-475.

Clone and sanitary selection of the grapevine has a fundamental importance in improving the quality and the quantity of the grape production in Serbia. In order to preserve the varieties of the old vineyards, the clone and sanitary selection has begun in 2006 in the South Eastern Serbia vineyard areas, 1048 grapevine plants have been examined in three distant vineyards and 60 grapevine plants have been separated that deserved attention based on their production characteristics. The selected plants have been tested serologically, with the ELISA method, to the presence of 4 grapevine viruses: Grapevine leaf roll-associated virus 1, Grapevine leaf roll-associated virus 2 and Grapevine leaf roll-associated virus 3 (GLRaV-1, GLRaV-2 and GLRaV-3), and grapevine fun leaf virus- GFLV. The infection level of the selected plants was between 10.5% (vineyard III) and 22.2% (vineyard II). We eliminated the infected plants among the selected ones and analyzed only the healthy ones in the 2008. Various potential

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variety clones have been selected for Prokupac, Pamid, Dimyat, Sauvignon blanc, Rosaki, Chasselas, Semillon, Detier de Bayreuth and Riesling. In 2008 we have repeated the same procedure we did in 2006 but in a different region – the Eastern Serbia area on the autochthonous variety of Muscat des roses noir on 400 grapevine plants 40 potential clones have been selected.

The goal of this paper was check out the health status to preserve the autochthonous and introduced varieties of the eastern and south eastern region and to renew the vineyards it's grown in. It was necessary to go on following the selected candidate – clones for other viruses based on EPPO PM 4/1-26 certification scheme in order to identify the virus-free clones to multiply, conserve and maintain in the collection growing areas.

Key words: autochthonous and introduced varieties, clone, sanitary selection

INTRODUCTION

Grapevines are among the most important crops in Serbia, both from the economic and social point of view. The quality of the grape depends on several influences and between the most important production factors is grapevine planting material, the way in which grapevine is multiplied and the varieties of geographical and climatic environmental under which they are grown (MARTELLI, 1999). Serbia is faced with rapid erosion of the autochthonous grapevine germplasm due to the introduction of the famous European cultivars. Unfortunately, clone selection of these cultivars has never been done and there is no planting material of adequate quality standards (RAKONJAC *et al*, 2009).

Technical literature describes a great number of diseases caused by viruses, either individually or in mixed infections with a greater number of viruses. Certain diseases do significant economic damage to viticulture and may be a limiting factor of the successful and economically justified grape production (LEE & MARTIN, 2009). Grapevine propagates vegetative and bears the risk of infection from two sources: rootstock and scion, or both, with the same or different virus/viruses. The infected planting material in the plantings is one of the most dangerous and most important ways of spreading viruses to new destinations, often very distant (PAUNOVIĆ, 2007). Individual plants – vines in a vineyard are exposed to the possibility of infection for many years. Vectors-virus transmitters or pollen (for those transmitted in this way) are potential sources of infection for several viruses, acting together and have increased harmful effects. Furthermore, the existing state of mixed infections in a vineyard increased the risk of creating new strains of viruses through natural recombination of parts of their genomes. The greatest number of viruses – over 40 of them – was detected on grapevine, as individual type of plant. Some of the discovered viruses are significant pathogens on other important cultures such as cucumber, tobacco, potato, artichoke or clover, whereas they rarely occur in grapevine or the damage they do are so small that they can be disregarded. This is indeed the highest number of pathogens encountered in any single wood species. Other viruses, especially members of the *Nepovirus, Closterovirus and Vitivirus*

genera, are variable pathogens and the agents of diseases that have an undoubted negative impact on the quality and quantity of the yield (WALTER & MARTELLI, 1996).

The most important viruses on grapevine cause degeneration and decay of vines (*Grapevine fan leaf virus-GFLV*) and European NEPO viruses (isometric viruses transmitted by nematodes) or American NEPO viruses; the viruses causing the rolling of leaves, among them economically most important ones: viruses associated with grapevine leaf roll 1, 2 and 3 (*Grapevine leaf roll associated viruses -1, -2, -3 –GLRaV-1, GLRaV-2, GLRaV-3*) (KOMAR *et all*., 2008). Viruses from the complex of grapevine stem furrowing: grapevine stem pitting virus (*Grapevine rupestris stem pitting associated virus-GRSPaV*), virus associated with the stem furrowing of root-stock Kober 5 BB (*Grapevine virus A- GVA*), grapevine stem pitting virus (*Grapevine virus B- GVB*), *Grapevine virus C* and *Grapevine virus D*, vitiviruses from the said complex, with still insufficiently known role, as well as grapevine leaf spots virus (*Grapevine flack virus-GFkV*) responsible for the same disease (MARTELLI & WALTER, 1998). Grapevine spots virus is latently present in the grapevine originating from Europe and most American root-stocks, whereas in *Vitis rupestris* L. it causes clear symptoms, so this plant is used as an indicator plant for its detection.

Reliable disease diagnosis, combined with swift and early detection of virus is a crucial step in the development and implementation of successful measures of virus control and the control of diseases they cause, which is defined by the knowledge of epidemiology of detected virus. Each new clone candidate-new potential clone must be registered, i.e. there must be a guarantee by the breeders for maintaining phytosanitary health, vitality of plant and stability of clone's characteristics. In the course of the registration process, time and money have been invested.

Therefore, the breeders should take the initiative, supported by the values of the clone – variety which will be commercialized in the future, making the invested effort and money worth while. Clone selection is based on genetic variability within varieties as well as the way to make certain profit through commercialized production of certified planting material.

The object of this study was to single out the vines of autochthonous and introduced varieties with good bearing potential from the old production plantings, to test them for the presence of most important viruses aimed at selecting potential clones in the region of Eastern and South-Eastern Serbia

MATERIALS AND METHODS

In South-Eastern Serbia, three distant vineyards (I, II and III), raised in the beginning of the twentieth century, were chosen. Table 1 shows basic data, plantings were inspected in the vérasion phase, the selected vines were market varieties were identified by 21 ampelographic characteristics and recorded and leaf and shoot samples were collected for testing for the presence of virus. Selection of vines is based on quantity and quality characteristics: yield, high variety fecundation –

number of bunch per vine, equability in maturation and without discernible simptom of diseases. The selected vines were tested serologically, using the ELISA method, for the presence of four grapevine viruses *Grapevine leaf roll-associated virus* 1, *Grapevine leaf roll-associated virus* 2 and *Grapevine leaf roll-associated virus* 3 - GLRaV-1, GLRaV-2 and GLRaV-3 and grapevine infectious degeneration virus, i.e. *Grapevine fan leaf virus*-GFLV. Stem extract from annual wooded shoots was used for the testing, since all of the above viruses in this type of samples can be detected during the whole year. The extract was prepared by homogenizing stem with two shoots 2-3 internodes long, from the same vine in the solution 1:20 in the suitable buffer. Suitable specific antiserum and configured produced by the company *Bioreba*, in the recommended solutions, as well as their positive and negative controls, were used for testing.

The tests checked the vines for the presence of 4 viruses in the total of 60 selected vines from the locality vineyard I, vineyard II and vineyard III in 2006. In 2008 the testing was repeated in the same vineyards on healthy vines. The new testing, using the ELISA method, was performed for the presence of 11 viruses: ArMV, GVA, GVB, GFLV, GFkV, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-7, TRSV and ToRSV. In 2008 year we expanded the experiment and tested variety Muscat des roses noir in East Serbia. Vineyard was built in 1980, and 400 of the vines, which are characterized by their high content of sugar in a wider (26-34%) identified the 40, tested the 11 viruses. The tests were performed at the Institute of Fruit Growing in Čačak.

Tab.1 Sum data from very old vineyards in South Eastern Serbia (2006/2008)

Variety	Color	Vigor	Ripening	Number of identification varieties		
				I	II	III
Prikupac	N	High	Late	305	161	194
Pamid	Rs	Moderato	Early-Med	119	18	16
Dimyat	B	High	Late	18	11	5
Sauvagnin blanc	B	High	Medseason	8		
Semillon	B	High	Medseason			3
Riesling	B	Moderato	Late		5	
Detier de Bayreuth	B	High	Very late	9		2
Chasselass	B	Medium	Early	8	5	
Garvanka	N	High	Very late	22	19	11
Rozaki	Rs	High	Very late		9	5

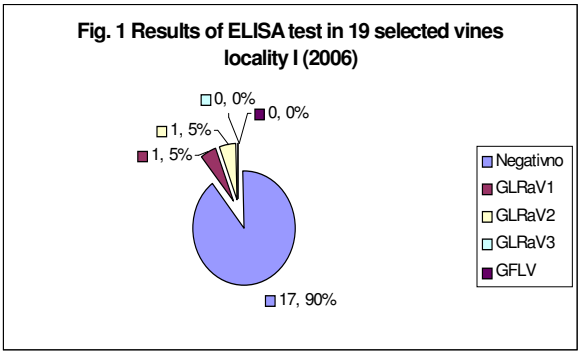
Legend: N-noir, B-blanc, Rs-rose, I. II, III vineyard

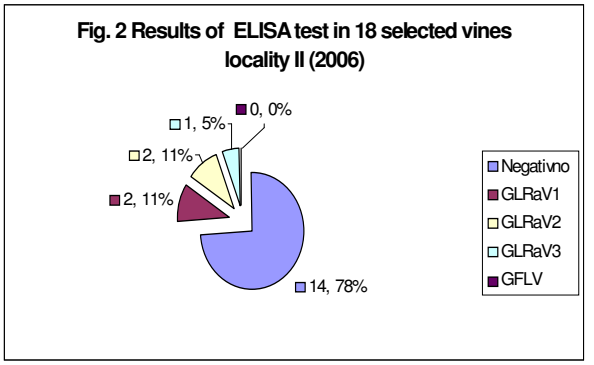
RESULTS AND DISCUSSION

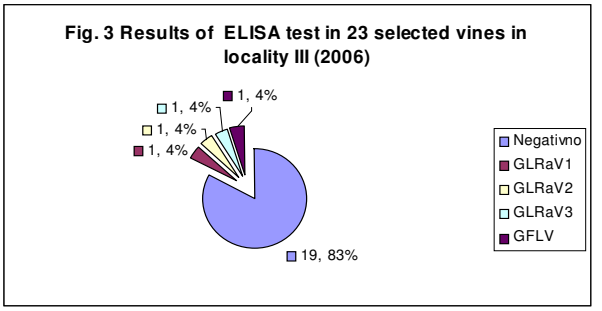
Viral infections may have different effects on the diseased grapevine and they are conditioned by the degree of sensitivity of the grown variety/clone to a certain virus, sensitivity of the root-stock, degree of virus virulence, virus strains present, number of viruses infecting the same host, age of the vine and the moment of infection, as well as the applied agricultural technique in the vineyard. Viruses exhibit greatest damage in the reduced yield and the quality of grapes (CABALLERO *et al.*, 1999; KOVACS *et al*, 2001; WOLPERT & VILAS, 1992).

It is estimated that viruses associated with grapevine leaf roll may cause reduced yield between 20% and 70% with decreased ability to revive the infected plants, as well reduced contents of sugar in the must (DIGIARO et al., 1999). Furthermore, in case of combination of a sensitive variety and root-stock, the infection with viruses from the stem furrowing complex leads to gradual decay and withering of diseased plants, where yields are reduced from 50% (GRSPaV) to 70% (GVB) before total decay.

In 2006, 23 vines were selected in the vineyard on the locality vineyard I, and in 19 of them, or 82.6%, none of the four tested viruses were detected.







No mixed infection with more than one virus was established on the vines. 18 vines were selected in the vineyard on the locality vineyard II, and in 14 selected vines (77.7%) no virus was detected. The presence of mixed infection with two viruses was proven in the sample of the variety Pamid GLRaV-1 and GLRaV-2 (DIGIARO *et al*., 1999). Pathogenic infections, especially viral diseases, cause phenotipe changes within the variety. According to CREDI & BANINI (1997), presence of viruses such as infectious degeneration of grapevine and Leaf roll (GFLV or GLRaV) with varieties Albana and Trebjano romangolo, reduce the yield by 72.9% and 80.4% respectivelly. Due to that fact, it is not about genomic clonial variations but rather the influence of outer conditions. The issue arises when the virus fails to cause visible symptoms, such as yield reduction, but it rather causes considerable changes in phenotypic appearance. Among numerous results during the pre-selective period, there are also «false candidates – potential clones» during the clonal selection. STAROVIĆ *et al*., (2008) discovered the same results, GLRaV is the most widely spread within the GLD (Grapevine Leaf roll Disease) group in Serbia.

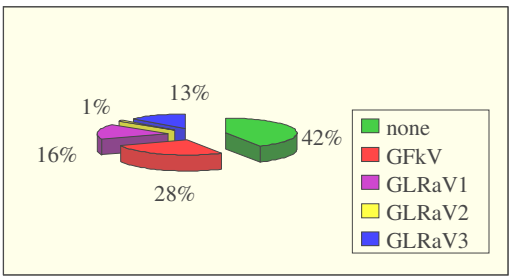


Fig.4 Results of ELISA test on 60 phenotypes in South-Eastern of Serbia (2008)

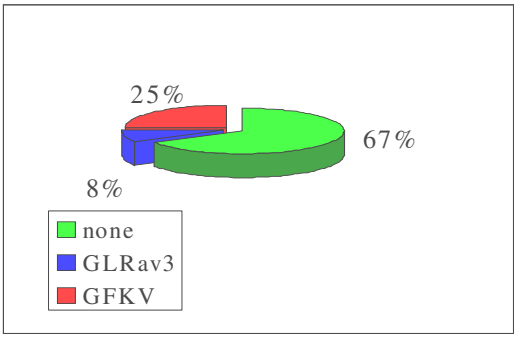


Fig. 5 Results of ELISA test of variety Prokupac in vineyard I (2008)

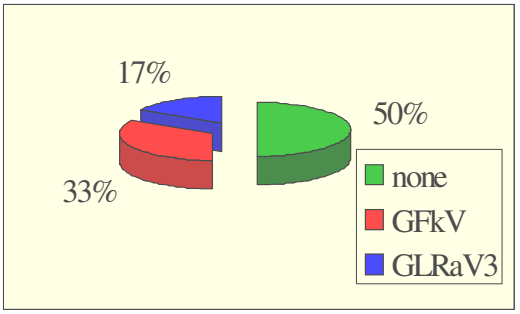


Fig.6 Results of ELISA test of variety Prokupac in vineyard II (2008)

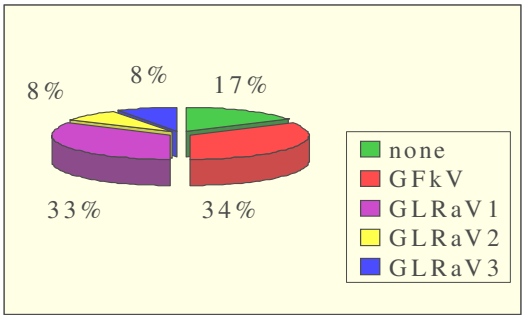


Fig. 7 Results of ELISA test of variety Prokupac in vineyard III (2008)

Two selected vines of the variety Prokupac, characterized by small clusters and relatively large berries, were infected by GLRaV-2 and GLRaV-3. In the locality vineyard III, out of 19 selected vines, viruses were detected only in two vines of the variety Pamid, namely GLRaV-2 and GLRaV-1 in vine, and the remaining 17 vines (89.5%) were free from all 4 tested viruses. This is the oldest planting with the greatest number of varieties, out of 13 in total.

The infection rate was low, but GLRaV-3 has demonstrated to spread rapidly in other wine-growing regions once established, with an infection increase 8-12% per year reported in a Spanish vineyard (CABALEIRO *et al*., 1999, LEE & MARTIN, 2009). Previous studies have reported on the reduced performance like vigor, yield, delayed and uneven ripening, reduced berry pigments, and reduce sugar accumulation on grapevine infection with GLRaVs (CABALERIO *at al.* 1999, GUINDONI *et al.,* 1997, KLIRWER & LIDER, 1976, KOVACS *et al*., 2001, WOLPER & VILAS, 1992).

The total number of samples infected with viruses, regardless of the variety, was 17.4% in vineyard I, 22.2% in vineyard II and 10.5% in vineyard III, in the year 2006.

Only 4 viruses: GFKV, GLRaV-1, GLRaV-2, GLRaV-3 were confirmed present when the ELISA method serological analysis was applied to 11 viruses, and that in 60 separate phenotypes of various autochthonous and introduced varieties, in all three observed localities of the South-Eastern Serbia, in 2008. The most frequent variety of the relevant localities is Prokupac, 27 vines (9 vines in vineyard I, 6 vines in vineyard II and 12 vines in vineyard III). The presence of 2 viruses, GLRaV-3 and GFKV, was confirmed with this variety in vineyards I and III by means of ELISA test. The presence of 4 viruses: GFKV, GLRaV-1, GLRaV-2 and GLRaV-3 were confirmed in vineyard II.

The variety Muscat des roses noir in Eastern Serbia was eaxamined after the experiment had been expanded in the year 2008. Presence of four viruses from the groups GLRaV i GFKV (individual infections) was confirmed within 5 vines, in other words there were 87.5% of healthy plants. There are two varieties Muscat des roses noir, with the feminine-type flower, and the pollinator variety Pinot Noir dominant in the vinyard. The data acquired in the analysis indicate that it is both important and vital to test presence of viruses.

GFLV has no significant influence on the yields of tolerant grapevine varieties, but in sensitive varieties the loss ranges from 50% to 90%. This virus has unfavorable effect on grafting of tissue during the grafting process and on the insufficient development of root during the revival of the cutting.

The control strategies used nowadays are preventive measures which are based on sanitary selection and chemical control of the known vectors (nematodes, mealybugs). Sanitary selection calls for detection techniques the most used being biological indexing and immunochemical techniques such as Elisa. (WALTER, 1998) Clone selection is considered a very important tool for grapevine genetic improvement. The best results are obtained when sanitary and genetic selection are

carried out at the same time in order to propagate only clones naturally free from harmful viruses or made virus-free by artificial techniques (MANNINI, 2000).

CONCLUSION

Clone and sanitary selection of the grapevine has a fundamental importance in improving the quality and the quantity of the grape production in Serbia.

On all three monitored localities in the South-Eastern Serbia, on 60 selected phenotypes of autochthonous and introduced varieties, serological analysis using the ELISA method for testing the presence of 11 viruses, confirmed the presence of only 4 viruses: GFKV, GLRaV-1, GLRaV-2, GLRaV-3. The most frequently found variety on these localities was Prokupac, with the total of 27 vines. ELISA test in this variety determined the presence of 2 viruses GLRaV-3 and GFKV on the localities vineyard III and vineyard I. The presence of 4 viruses was established on the locality vineyard II: GFKV, GLRaV-1, GLRaV-2, GLRaV-3.

Experience in the South Eastern region of Serbia pointed out that the 60 selected vines pose the first year of testing, checked 28 healthy plants that can enter into the procedure for reproduction.

After the two-year investigation, 46.67% healthy vines/potential clones were determined in very old vineyards, containing a large number of various varieties.

In 2008 year expanding experiment in the East Serbia. identified the 40 vines from cv Muscat des roses noir and tested on the 11 viruses confirmed the presence of the 4 virus from the group GLRaV and GFKV (single infection) in 5 of vines, or 87.5% was healthy plants.

Analysis of data obtained once more showed the importance and necessity of testing for the presence of viruses.

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KLONSKA SELEKCIJA AUTOHTONIH I INTRODUKOVANIH SORTI VINOVE LOZE U STARIM ZASADIMA JUGOISTOČNE I ISTOČNE SRBIJE I PRELIMINARNO UTVRĐIVANJE NJIHOVOG ZDRAVSTVENOG STATUSA

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I z v o d

Klonska i sanitarna selekcija vinove loze ima fundamentalni značaj za poboljšanje kvaliteta i kvantiteta proizvodnje grožđa u Srbiji. To se odnosi kako na vodeće tako i manje značajne autohtone i introdukovane sorte. U 2006 godini klonska i sanitarna selekcija u cilju očuvanja sorti iz starih vinograda započeta je u istočnoj Srbiji a u 2008. godini i u jugoistočnoj Srbiji. Pregledano je ukupno 1048 čokota u tri prostorno udaljena vinograda i izdvojeno 60 čokota, koji su po svojim proizvodnim karakteristikama zasluživali pažnju. Selekcionisani čokoti su testirani serološki, ELISA metodom, na prisustvo 4 virusa vinove loze: *Grapevine leafroll-associated virus* 1, *Grapevine leafroll-associated virus* 2 i *Grapevine leafroll-associated virus* 3 - GLRaV-1, GLRaV-2 i GLRaV-3), *Grapevine fanleaf virus*-GFLV. Stepen zaraženosti izabranih čokota virusima kretao se od 10.5% (vinograd III) do 22.2% (vinograd II). Izabrane a zaražene čokote smo eliminisali i u 2008. godini analizirali samo zdrave čokote. Izdvojeno je više potencijalnih klonova sorti Prokupac – 25, Pamid - 11, Dimyat - 2, Sauvagnin blanc - 2, Rosaki - 2, Chasselass, Semillion blanc, Detier de Beyrouth and Riesling po jedan budući klon. U 2008. godini ponovili smo postupak kao u 2006. godini ali u novom lokalitetu – Istočnoj Srbiji na autohtonoj sorti Muscat des roses noir na 400 čokota. Izabrano je 40 potencijalnih klonova koji se odlikuju visokim sadržajem šećera u širi (26-34%).

Cilj ovog rada je bila provera zdravstvenog statusa izabranih klonova autohtonih i inrodukovanih sorti u jugoistočnoj i istočnoj Srbiji. Neophodno je nastaviti praćenje izbaranih kandidata – klonova na druge viruse prema EPPO PM 4/1-26 šemi sertifikovanja, da bi se izdvojili virus-free klonovi za umnožavanje, konzerviranje i održavanje u kolekcionim zasadima.

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