SANITARY STATUS OF SLOVENIAN INDIGENOUS GRAPEVINE CULTIVAR REFOSK

ÉTAT SANITAIRE DE LA VIGNE INDIGÈNE CV. REFOSK EN SLOVÉNIE

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Abstract : Sanitary status of visually selected and nonselected indigenous grapevine cultivar 'Refosk' (*Vitis vinifera* L. cv. 'Refosk') was evaluated. Nine viruses were tested by ELISA: Nepoviruses Grapevine fanleaf virus (GFLV) and Arabis mosaic virus (ArMV), Closteroviruses Grapevine leafroll associated viruses 1 (GLRaV-1), 2 (GLRaV-2), 3 (GLRaV-3), and 6 (GLRaV-6), Grapevine fleck virus (GFkV) and Vitiviruses Grapevine virus A (GVA) and Grapevine virus B (GVB). All viruses except ArMV and GVB were detected. The vines of cv. 'Refosk' passing the visual selection were 50 % free of tested viruses in comparison to only 24 % virus-free vines of non-visually selected material. Visually selected plants revealed the highest incidence of virus GLRaV-1 (37 %) and in addition to that a 15 % incidence of rugose wood (RW) disease symptoms.

Résumé : Une évaluation de l´état sanitaire de la vigne indigène cv. Refosk (Vitis vinifera L. cv. 'Refosk'), selon qu'elle a été sélectionnée visuellement ou non sélectionnée, a été faite. Neuf virus ont été testés par la méthode ELISA: Nepovirus court noué (GFLV) et virus Arabis mosaique (ArMV), Clostérovirus virus de l´enroulement 1 (GLRaV-1), 2 (GLRaV-2), 3 (GLRaV-3) et 6 (GLRaV-6), virus de la marbrure de la vigne (GFkV) et Vitivirus Grapevine A (GVA) et B (GVB). Tous les virus, sauf ArMV et GVB, ont été detectés. 50 % de vins de cv. 'Refosk' qui ont passé la sélection visuelle étaient sans virus contre seulement 24 % de vins sans virus parmi le matériel sélectionné non visuellement. Les vignes sélectionnées visuellement avaient la plus haute incidence de virus GLRaV-1 (37 %) avec en outre des symptômes de la maladie du Bois strié dans 15 % des cas.

Key words : grapevine, *Vitis vinifera* L., indigenous varieties, selection, ELISA, virus disease.

Mots clés : vigne, *Vitis vinifera* L., variété indigène, sélection, ELISA, virus.

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INTRODUCTION

Sanitary selection is an integral part of clonal selection. Selection, the oldest practice for varietal improvement and prevention from virus diseases is still the only efficient way to control the detrimental effect of grapevine viruses. In Slovenia grapevine selection and clone multiplication started after Second World War. The required tests were made to meet the European grapevine certification program (EEC recommendation n°68/193) although we didn't have Slovenian legislation till year 2004 (KOROSEC-KORUZA and KORUZA, 1997; WALTER and Martelli, 1997). The important part of the selection program is also the preservation of the old local indigenous varieties. Since the populations of indigenous varieties are often heavily infected with a number of different viruses (POLJUHA *et al.*, 2004), sanitary selection can exclude them from the commercial propagation. Therefore, they need a special treatment in sanitary selection. Our interest to keep them arises from two main reasons. The old indigenous varieties kept in germplasm collection vineyard are useful for grapevine breeding in future. Several old varieties are highly priced and represent a valuable rarity in the wine market. *Vitis vinifera* cultivar 'Refosk' is the most preferred old red variety of the Karst region in Western Slovenia, and it gives a highly demanded local red wine Teran.

In the present study we show the virus infection status of two populations of cv. 'Refosk': a non-selected group of vines from the old vineyards, and a visually selected group of vines, which represents a potential clone material tested by ELISA for nine grapevine viruses: Nepoviruses Grapevine fanleaf virus (GFLV) and Arabis mosaic virus (ArMV), Closteroviruses Grapevine leafroll associated viruses 1 (GLRaV-1), 2 (GLRaV-2), 3 (GLRaV-3), and 6 (GLRaV-6), Grapevine fleck virus (GFkV) and Vitiviruses Grapevine virus A (GVA) and Grapevine virus B (GVB).

MATERIAL AND METHODS

I - PLANT MATERIAL

In years 1980 - 85 we chose 76 old 'Refosk' vines as mother plants and potential clone material according to their specific, potentially interesting production characteristics and good visual sanitary status. Symptom-less vines have been grafted on standard SO4 rootstock and planted in the collection plot in Komen in Karst region of Western Slovenia. In 1998, with the purpose to preserve the trues to old 'Refosk' type, we further collected 29 old 'Refosk' vines. Most of vines were older than 100 years and they have been preserved in trellis in front of the traditional homes. These vines were not visually inspected for virus infection. 29 non-selecetd old vines and 105 progeny vines from the selection vineyard (randomly selected from each potential elite group) were then tested for the presence of nine grapevine viruses in ELISA procedure: GFLV, ArMV, GVA, GLRaV-1, GLRaV-2, GLRaV-3, GLRaV-6, GFkV and GVB.

II - ELISA TESTING

Viruses GFLV, ArMV, GVA, GLRaV-1, GLRaV-3 GLRaV-6 and GFkV were tested using antisera produced by BIOREBA (Switzerland) in indirect standard DAS ELISA procedure (CLARK and ADAMS, 1977). GVB was tested with antisera produced by AGRITEST (Italy) using DAS-I (double antibody sandwich indirect) ELISA procedure. GLRaV-2 was tested using a serological kit from BIOREBA (Switzerland) in an indirect PTA (direct plate trapping) ELISA procedure. All procedures were performed according to the recommendations of the antisera producers. Reactions were evaluated by measuring absorbance at 405 nm using the MRX Dynex ELISA plate reader. Absorbance was measured several times during the incubation with a substrate. The graphs in figure 1 are presenting an example of the absorbance change over time in ELISA reaction between alkaline phosphatase and substrate para-nitro phenyl phosphate, measured at 405 nm. Same type of graphic analysis of the absorbance change over time was obtained for each microplate using Microsoft Excell program. According to the absorbencies shown in graphs, samples were separated into two groups: group of higher and group of lower absorbance values. Samples in group of higher absorbance were considered virus-positive.

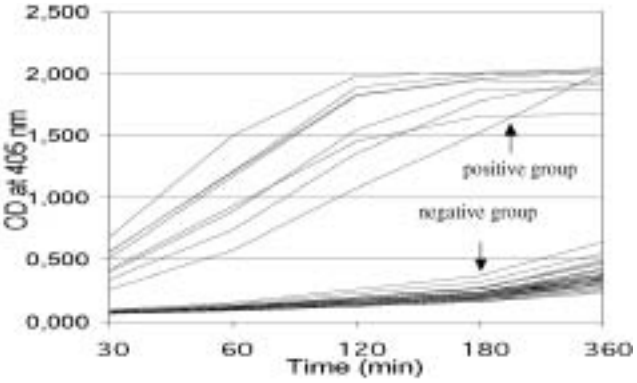

Figure 1 - A graf model used to assess proper distinction between virus positive and virus negative samples, showing an example of the change of absorbance at 405 nm over time during the incubation with the substrate in ELISA reactions.

Modèle graphique utilisé pour noter une propre différence entre les échantillons à virus positif et négatif, montrant un exemple de changement d´absorption à 405 nm au-dessus du temps durant l´incubation avec le substrat dans les réactions Elisa.

III - VISUAL INSPECTION

All of the 1680 vines from selection vineyard were visually inspected for rugose wood symptoms with unknown etiology.

RESULTS AND DISCUSSION

In non-selected vines, ELISA analysis revealed 45 % of the vines with mixed infections (figure 2). 58.6 % of the vines tested GLRaV-1 positive, 41.4 % GFLV positive, 13.8 % GVA positive, 10.3 % GLRaV-6 positive, 6.9 % GFkV positive and 3.4 % positive GLRaV-2 and GLRaV-3 each. Most frequently found mixed infection was with viruses GFLV and GLRaV-1 (24.1 %). Interestingly, the ELISA analysis did not reveal any of the ArMV and GVB virus infection in non-selected material.

The ELISA analysis showed that 50 % vines of cv. 'Refosk', passing standard mass selection, were free of tested viruses in comparison to only 24 % virus free vines of non selected material (figures 2, 3). Present data indicate that the visual selection is effective in eliminating mostly vines with mixed infections of two or more viruses. Visual selection is relatively efficient in eliminating GFLV (41.4 % of positive vines in non-selected group, 4.8 % of positive vines in visually selected group) although the vines of cv. 'Refosk' don't show the most typical symptom of GFLV, called a peculiar leaf malformation. However, in cv. 'Refosk' the shoots are frequently malformed, showing abnormal branching, double nodes or fasciations. The presence of these symptoms during dormant period when scions are collected also contribute to efficiency of selection. On the other hand, the visual selection failed to eliminate the GLRaV-1 infected vines although vines show clear symptoms of infection. The reason could be confusion and wrong interpretation of early foliar reddish discoloration due to virus infection with a variety characteristic of cultivar 'Refosk'.

Among 105 ELISA tested vines, 10.5 % of visually selected vines tested GFkV positive, which was significantly more than in non-selected vines. There could be two main reasons for failing to recognize GFkV in visual selection. The first one is a fact that little is known about the symptoms of GFkV infection, and therefore, there are no detailed descriptions of all possible symptoms. The second reason could be the grafting procedure. According to the graft at random model by Goheen (1989), a 50 % disease incidence in the scion and rootstock sources approaches 100 % infection after the 10th or 12th propagative generation. It seems that the only way for eliminating this virus from cv. 'Refosk' is determination of virus with laboratory tests. The incidence of all other virus is lower among visual selected vines in comparison with non-selected vines. 8.6 % of the vines tested GVA positive and 1.9 % vines tested GLRaV-2. None of them were infected with ArMV, GLRaV-3, GLRaV-6 or GVB (figure 3).

Among all (1680) visually inspected vines 15 % (253) showed rugose wood symptoms on rootstocks or on scion. However, as shown by the ELISA analysis, rugose wood disease could not be associated with the presence of either GVA or GVB viruses. Investigation of rugose wood disease complex in visual selection is more time consuming than investigation of other virus symptoms. For this reason, it is very often left out of the process. Additionally, the visual selection of rugose wood is also questionable because different rootstock could be used for multiplication.

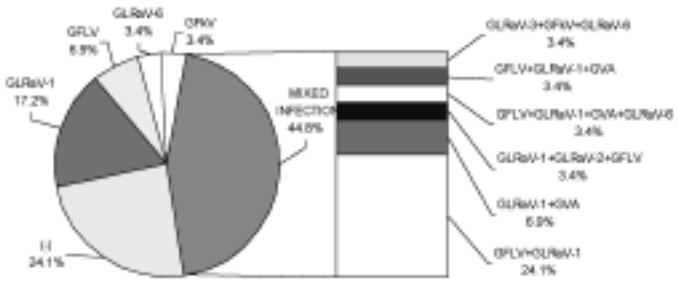

Figure 2 - Incidence of infections by the single virus and mixed infections, as established by ELISA, in old non-selected 'Refosk' vines.

Incidence d´infections avec un seul virus et d´infections mixtes, comme établie par Elisa, sur une vieille vigne de 'Refosk' non-sélectionné.

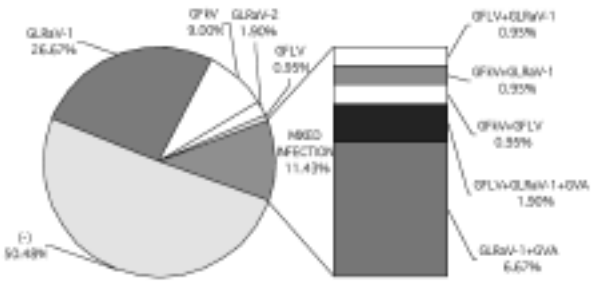

Figure 3 - Incidence of infections by the single virus and mixed infections as established by ELISA, in visually selected 'Refosk' vines.

Incidence d´infections avec un seul virus et d´infections mixtes, comme établie par Elisa, sur une vigne de 'Refosk' sélectionné visuellement.

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CONCLUSIONS

The populations of local varieties that have been propagated for a long time in a small closed circle expressed a high incidence of some viruses and demanded a specific national sanitary control programme.

Sanitary selection is clearly showing a positive effect by improving of improved health conditions and homogenity of the stocks derived from cloning. On the other hand, it could also result in a considerable loss in the biodiversity of extant varieties.

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