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# Influence of viral infection on the quality of grapes at harvest

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## ABSTRACT

**A major concern in the grape breeding programs is represented by the impact of the viral infections in the vineyards performance and the importance of producing virus-free material, measure controlled and regulated also by the governmental institutions. The aim of this study was to identify the presence of the most common and damaging viruses, active in Romanian vineyards, and to evaluate the effect of the viral infection on the selected grapevine plant performance. A total of 21 grapevines belonging to two Romanian cultivars were randomly selected from a plantation located in the Valea Calugareasca viticultural center, in order to be verified for the presence of ArMV, GLRaV-1+3 and GFkV viruses, by using Enzyme-linked immunosorbent assay method. From the total of 21 vines, four vines (FN 3, FN 4, FN 8, FN 14) were identified as having present one virus and five grapevine (FN 1, FN 5, NA 1, NA 3, NA 4) plants showed, in different combination, infection with two viruses. The presence of a single virus respectively, mixed virus combinations, had a visible impact on the results for both types of the analyzed parameters, technological and physicochemical. The current study confirmed the influence of the viral infection presence and highlighted the importance of the virus-free material on the grapevine plant performance.**

**Keywords***:* grapevine, virus, DAS-ELISA, technological parameters, physicochemical parameters

## INTRODUCTION

Viral diseases impact represents a major concern in grapes breeding, presently being a problem widespread throughout all viticultural regions of the world. Several studies reported that simultaneous infection with multiple viruses, significantly increase the symptoms severity respectively can be associated with a reduced cropping capacity and grape juice quality, decrease of the soluble solids, a higher titratable acidity and sometimes can affect the amount of aromatic compounds in muscadine and phenolic substances in red grapes (Gutha *et al*., 2010; Santini *et al*., 2011; Mannini and Digiaro, 2017, Risovannaya *et al.,* 2020). In present, a very high diversity of viral pathogens, with more than 86 viruses from 34 genera, have been identified in grapevine, being also the crop with the largest number of viruses (Martelli, 2014; Fuchs, 2020).

In Romania, for a cultivar/clone multiplication it is necessary to certify that the material is virus-free of the most common and damaging virus infections encountered in this area: Arabis mosaic virus (ArMV), Grapevine fanleaf virus (GFLV), Grapevine leafroll – associated virus 1 (GLRaV-1), Grapevine leafroll – associated virus 3 (GLRaV-3), Grapevine fleck virus (GFkV) Order 1267/2005. The ArMV along with GFLV are two virus species of *Secoviridae*

family, *Nepovirus* genus, which are specifically transmitted from grapevine to grapevine by two distinct ectoparasitic dagger nematodes of the genus *Xiphinema* (Meng *et al*., 2017). The ArMV presence can be expressed by several symptoms like mottling and flecking on the leaves and leaf deformation including enations (bumps), shortened internodes and vine decline (Oliver and Fucks, 2011). The symptoms are similar to those of Grapevine Fanleaf Virus (GFLV) and mixed infections of ArMV and GFLV are common. Infections are often symptomless, and expression varies based on type of rootstock, variety, and environmental conditions. Grapevine leafroll disease (GLD) is the most widespread from the grapevine virus diseases and with major impact economically. The virus species GLRaV-1 and GLRaV-3 are the most prevalent ones and all common cultivars as well as interspecific hybrids and rootstocks are infected, even though not all infected plants develop strong symptoms. As symptoms, yield and quality of the grapevines can be affected with substantial losses. Leafroll symptoms usually include downward rolling of leaves respectively reddening or yellowing of the leaf tissue between the main veins. The main veins may remain green, even in some cultivars the discoloration can affects all the leaf tissue. For the white cultivars, the discoloration may be less obvious (Constable and Rodoni, 2014).
The fleck disease produced by Grapevine fleck virus (GFkV), which belongs to the *Tymoviridae* family of *Maculavirus* genus (Martelli *et al*., 2012), is another of the most important grapevine viral diseases and is expressed by leaves with intense flecking that are wrinkled, twisted, and may curl upward (Hewitt *et al*., 1962, 1972).
The pathogenicity of GFkV it's still unclear but it's direct effect on the viability and productivity of plants lead to deterioration of the main organelles of the cell and, along with other viruses, the chemical composition of berries (Jones *et al.,* 2015; Martelli, 2017; Sabanadzovic *et al*., 2017).
Because direct plant protection measures against viral infections are not available, preventive measures should be applied, these include the use of healthy planting material – virus-free, containment of viral vectors and selection and breeding of resistant or tolerant cultivars (Maliogka *et al.*, 2015). Visual diagnosis of the viral infections is a starting point for establishing further activities and strong identification of pathogenic virus, but methods including biological indicators, as well as ELISA and advanced PCR assays are the most efficient in confirming the virus entity (Zherdev *et al.,* 2018).

## MATERIALS AND METHODS

In this study, 21 red vines (17 grapevines of 'Fetească neagră' and 4 of 'Negru aromat'), were randomly selected to be verified for viral infections presence (single virus and/or multiple-mixed) by using the Enzyme-linked immunosorbent assay (ELISA) method (Clark and Adams, 1977). A number of 3-4 leaves/vine was harvested from the field in June of 2022 and analyzed in the lab in the harvesting day. In order to evaluate if any significant difference can be detected for the virus presence impact, the ELISA results of the infected and healthy grapevines were compared for several technological and physicochemical indicators. The studied material is part of a 34-year-old grapevine plantation located in the Valea Calugareasca viticultural center.
The ELISA method is based on "sandwich" technique, using two specific antibodies (DAS-ELISA = double antibody sandwich ELISA). For the ELISA analyses we used the ArMV Kit Complete 480, the GFLV Kit Complete 480 and the GLRaV-1+3 Complete kit 480. The mixture of the selected antibodies for GLRaV-1+3, allows a broad-spectrum detection of all scientifically recognized and published isolates in a single test (DAS-ELISA).
The reagents were prepared accordingly with the producers' recommendations, certified NUNC MaxiSorp F-96 microtiter plates, 200 µl/well volume were used, and after, the protocol

followed the recommended steps (BIOREBA, Switzerland). The data readings were used to interpret the results by applying the cut-off formula, represented by the three times the mean value of the negative control - NC (all values above this cut-off were regarded as positive):

Cut-off = 3x mean value (NC)

Technological and physicochemical determinations were performed on grapes at harvest, consisting in medium bunch grape (g), weight of 100 grape berries (g), the volume of 100 grape berries (cmc), healthy grape berries/bunch (%), sugar content (g/L), titratable acidity (g/L), pH, total polyphenolic index (IPT), anthocyanin content (mg/L) and total potential anthocyanins (mg/L). The sugar concentration in grape musts was determined by establishing the refractive index at 20°C and the results were expressed as an absolute value and as a percentage by mass of sucrose (OIV-MA-AS2-02 method).

Titratable acidity was determined by titration with 0.1M NaOH, with 1% phenolphthalein and the results were expressed in $g/L^{-1}$ $H_2SO_4$ (OIV-MA-AS313-01 method).

The phenolic potential of grapes at harvest was determined by using the standard ITV (Cayla and Renard, 2008) method based on the following analytical parameters: anthocyanins, total anthocyanin potential and total polyphenol index. An aqueous acid solution was used to extract the phenolic compounds from the grapes. Maceration of the samples was done for one hour at room temperature. Fifteen milliliters of ethanol (95%) and 85 mL of 0.1% HCl were added to the 50 g of grape juice. The samples were shacked for one minute from quarter to quarter. The extracts are filtered through quantitative filter paper in order to clarify the solutions. The dilution of the samples to 1/100 was done in double-distilled water followed by the measurement of the absorbance at 280 nm in a 1 cm quartz vat, against a blank of distilled water and the total polyphenolic index (IPT) was determined as follow:

Total polyphenolic index (IPT) = DO 280 x 100 x [(weight of grape pomace + 100)/ weight of grape pomace].

The samples were diluted 1/20 in 1% hydrochloric acid solution and absorbance was measured at DO 520 nm against a blank of distilled water. The concentration of anthocyanin and the total anthocyanin potential were determined following formulas: Anthocyanins (mg/L) = DO 520 x 22.75 x 20

Total anthocyanin potential (mg/Kg) = anthocyanins (mg/L) x [(weight of grape pomace + 100)/ weight of grape pomace]. The healthy grape berries/bunch ratio was calculated after removing and counting all the healthy and sick berries from each bunch. Data was analyzed by applying the Dunnett test, addressing a special case of multiple comparisons problem — pairwise comparisons of multiple groups with a single control group (Dunnett, 1964). With the principal component plot (biplot) we tried to provide an image to complete the descriptive statistics and to discover underlying patterns. All the statistical analyses were performed with the JMP 16 statistical software.

## RESULTS AND DISCUSSIONS

From the total 21 grapevines analyzed by using DAS-ELISA, four of them were identified as being infected with only one virus (19.1%) and five of the grapevines (23.8%) were detected with simultanous infections, by having different combination of two viruses (Table 1). The positive samples detected after DAS-ELISA, were compared with two specific virus-free controls (one for each cultivar), selected from the analyzed samples, for the technological and physicochemical parameters. The negative controls were selected based on the bunch aspect, respectively the number of healthy berries/grapes. The selected bunch controls had the highest healthy/sick berries ratio from the total samples identified as negative after the ELISA tests.

Table 1. DAS-ELISA results for the analysed material

| Grapevine Code* | Virus | | |
|---|---|---|---|
| | ArMV | GLRaV-1+3 | GFkV |
| FN 1 | - | + | + |
| FN 2 | - | - | - |
| FN 3 | - | - | + |
| FN 4 | + | - | - |
| FN 5 | + | + | - |
| FN 6 | - | - | - |
| FN 7 | - | - | - |
| FN 8 | - | + | - |
| FN 9 | - | - | - |
| FN 10 | - | - | - |
| FN 11 | - | - | - |
| FN 12 | - | - | - |
| FN 13 | - | - | - |
| FN14 | - | + | - |
| FN 15 | - | - | - |
| FN 16 | - | - | - |
| FN 17 | - | - | - |
| NA 1 | - | + | + |
| NA 2 | - | - | - |
| NA 3 | + | + | - |
| NA 4 | + | + | - |

**FN – 'Feteascã neagră'; NA –'Negru aromat'*

The Dunnett test results are presented in Table 2 and Table 3 and was used to compare each number from a group with a single control per cultivar. Table 2 indicate that no pattern in the analyzed material was observed, determined by the presence of a specific virus/virus combination which may affected the quality or quantitative traits of the bunch. From all the infected grapevines, one presented a significative variance ('NA 1') and two of them ('FN 4' and ' NA 3') were distinct significative for only one trait, compared with the virus-free control, rest of the samples being negatively affected at two or three parameters.

Table 2. Technological characteristics of the analyzed material

| Grapevine code & Viral infection (*) | Bunch medium weight (g) | 100 grape berries weight (g) | 100 grape berries volume (cmc) | Healthy grape berries /bunch (%) |
|---|---|---|---|---|
| FN 17 - Control | 124.00 | 121.00 | 107.00 | 92.00 |
| FN 1 [b c] | 119.00** | 97.80** | 90.00 | 79.90** |
| FN 3 [c] | 45.00** | 82.90** | 75.00* | 53.20** |
| FN 4 [a] | 42.60** | 119.80 | 105.00 | 61.00** |
| FN 5 [a b] | 78.50** | 97.30** | 85.00* | 47.50** |
| FN 8 [b] | 86.70** | 114.20* | 100.00 | 85.60* |
| FN 14 [b] | 80.20** | 96.50** | 85.00* | 76.60** |
| NA 2 - Control | 106.00 | 144.89 | 135.00 | 80.00 |
| NA 1 [b c] | 103.40 | 158.50 | 145.00 | 50.70* |
| NA 3 [a b] | 53.30** | 144.50 | 130.00 | 65.20 |
| NA 4 [a b] | 80.30* | 137.60* | 125.00 | 72.40 |

*** distinct significative compared with the control according to Dunnett test; * significative compared with the control according to Dunnett test; (*) [a] ArMV; [b] GLRaV - 1+3; [c] GFkV;*

From Table 3 it can be observed that grape juice quality was affected by the viral infection presence, the most visible impact being noticed for the total polyphenolic index situation in which was observed a decrease of 7-42%, and for the anthocyanin content, respectively for total potential anthocyanins, the values being lower with 32-64% for 'FN' and 24–65% for the 'Na', compared with the control.

Table 3. Physicochemical characteristics of the analyzed material

| Grapevine code & Viral infection | Sugar content (g/l) | Titratable acidity (g/l tartaric acid) | pH | Total polyphenolic index (IPT) | Anthocyanin content (mg/l) | Total potential anthocyanins (mg/l) |
|---|---|---|---|---|---|---|
| FN 17 - Control | 255.67 | 3.30 | 3.65 | 42.00 | 313.95 | 941.85 |
| FN 1 [b c] | 264.00 | 3.68** | 3.55* | 33.00** | 213.85** | 641.55** |
| FN 3 [c] | 221.50** | 3.75** | 3.42** | 27.00** | 159.25** | 477.75** |
| FN 4 [a] | 217.30** | 3.83** | 3.37** | 39.00 | 318.50 | 955.50 |
| FN 5 [a b] | 198.10** | 3.60** | 3.55* | 24.00** | 113.75** | 341.25** |
| FN 8 [b] | 236.40* | 3.83** | 3.51* | 24.00** | 136.50** | 409.50** |
| FN 14 [b] | 191.80** | 4.05 | 3.37** | 27.00** | 172.90** | 518.70** |
| NA 2 - Control | 240.00 | 2.93 | 3.56 | 37.80 | 194.29 | 582.86 |
| NA 1 [b c] | 232.10* | 2.78** | 3.48** | 29.70** | 147.42** | 442.26** |
| NA 3 [a b] | 232.10* | 3.30** | 3.36** | 30.30** | 68.25** | 204.75** |
| NA 4 [a b] | 236.40 | 3.15** | 3.54 | 29.40** | 78.72** | 236.15** |

*** distinct significative compared with the control according to Dunnett test; * significative compared with the control according to Dunnett test;(*) [a] ArMV; [b] GLRaV - 1+3; [c] GFkV;*

From the Figure 1a and Figure 1b, we can observe that the weight and volume of 100 grape berries are positively correlated and the sugar content with the pH values, pairs of vectors being closely localized.

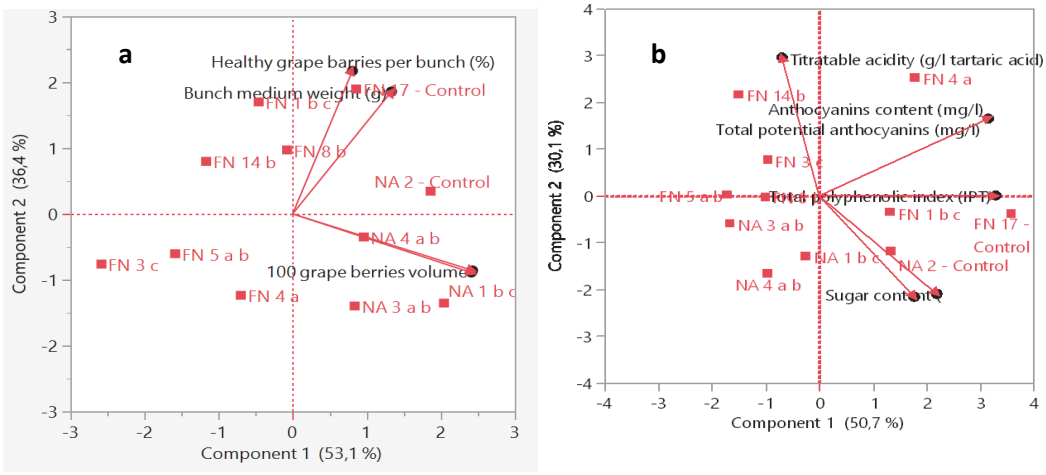


Figure 1. Biplot of principal component scores and loading vectors for the technological determinations (a) and for the physicochemical determinations (b)

The biplots present the observations (as quadrates) formed by the two principal components which expressed more than 89.5% from the variability for the technological characters and 80.8% for the physicochemical ones. The values for both types of observations are distributed at variable distance and only a few of the samples were localized close to the virus-free controls, 'FN1' and for the 'FN' cultivar, respectively 'NA 1' and 'NA 4' for Na cultivars.

## CONCLUSIONS

From the total 21 grapevine analyzed, 42% of them were identified as having one virus (four samples) or combination of two viruses (five samples).

A pattern for a specific virus presence with influence on the technological or physicochemical parameters was not highlighted.

The presence of a single virus respectively, mixed virus combinations, had a visible impact on the results for both types of the analyzed parameters, technological and physicochemical.

The current study confirmed again the influence of the viral infection presence and highlighted the importance of the virus free material on the grapevine performance.

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