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Control of grapevine virus diseases in collections and at the stages of propagation in Ukraine

Nina Muljukina [1], Iryna Kovalova [1], Liudmyla Konup[1], Galyna Lyashenko[1], Nataliia Zelenianska[1], Eustachio Tarasco[2] and Andrii Nenartovych[1]

[1] National Scientific Center "Tairov Institute of Viticulture and Winemaking" NAAS, Odesa, Ukraine
[2] Department of Soil, Plant and Food Sciences, University of Bari "Aldo Moro", Bari, Italy

*Corresponding author: tairmna2005@ukr.net

Abstract. The principles of virological control on different types of grapevine collections and plantations are summarized. Grapevine leafroll virus was most often detected on all studied types of collections and plantations. The lowest risk of virus infection detecting was in the clone repository, the highest in the ampelographic collection, clonal trial plots of the first vegetative progeny and in industrial vineyards where clonal selection is carried out. A trend of influence of temperature and humidity regime indicators on the number of plants with symptoms of grapevine leafroll was revealed. The correlation coefficient R2 between the number of plants with symptoms of grapevine leafroll and indicators of temperature and humidity regime ranges from 0.69 to 0.77.

1. Introduction

Targeted research European grapevine genetic resources began in the late 1970s and is well-established in a methodological sense, including by the International Organization of Vine and Wine (OIV) (resolution "Collections and Conservation of Genetic Resources of Vitis sp" (OIV Resolution No. 2/82). At the same time, it is important to assess the sanitary condition of samples [1], especially in relation to grapevine virus diseases [2, 3].

The study of grapevine virus diseases in Ukraine in previous years allowed us to establish the level of manifestation of visual symptoms and latent infection [4]. This makes their control relevant in breeding and clonal selection, respectively in clone repositories and ampelographic collections, as well as in the process of propagation and obtaining certified grapevine planting material.

The principles of virological control of grapevine in Ukraine are quite well-established in relation to clones of grapevine varieties, as an important component of the grapevine planting material certification system [5]. Currently, in Ukraine, seven viruses are controlled in the system of certified grape planting material producing, among which the first and third serotypes of grape leafroll virus are the most relevant [6]. At the same time, virological control in the process of breeding and in ampelographic collections is not yet systematic.

Grapevine genetic resources of Ukraine, in particular, of the National Scientific Center 'Tairov Research Institute of Viticulture and Winemaking, in the context of European research, represent a certain interest for the general array of genetic resources of the Vitis genus. Over the period of the breeding programs at the Tairov Institute, more than 130 varieties (wine, table grapes and of variousdirections of use have been bred. The ampelographic collection of the institute includes 848 samples of various genetic and geographical origins. Together with the clone repository (109 samples) and a large volume of breeding combinations (52 selections and 2000 seedlings), the ampelographic collection is important for grape breeding not only in Ukraine, but also in wine-growing countries of the world.

The aim of this work was to generalize the principles of virological control in collections and plantations of grapevines for various purposes and to identify the trend of weather and climatic conditions influence on the level of infection by grape leafroll virus.

2. Material and methods

The study was conducted in collections and experimental plots of the NSC "Tairov Research Institute of Viticulture and Winemaking", including the ampelographic collection, clone repository, clonal trial plots, basic mother plantations and industrial vineyards in viticultural regions, where the first stages of clonal selection took place.

To determine the level of infection, visual observation of symptoms, enzyme-linked immunosorbent assay (DAS-ELISA) using diagnostic kits from the company "Agritest" (Italy) and real-time polymerase chain reaction with reverse transcription (RT-PCR-RT) were used. RT-PCR-RT was performed using forward and reverse primers and probes for leafroll viruses 1 and 3. RNA was isolated from leaves or from wooden shoots of grapevine. Reverse transcription was performed at 95 ºC for 10 min. in a thermostat "Dry-block" TDB-120 (Biosan, Latvia). Amplification included the following cycles: at 50 °C for 2 min., 95 °C for 15 min. and 45 cycles of 95 °C for 15 sec. and 57 °C - 1 min., which was performed in a programmable thermal cycler Rotor-Gene 6000 (Corbett Research Pty Ltd., Australia).

The analysis of the temperature and humidity regime in July-August and in August months for the studied years was carried out according to the data of observations at the meteorological station of the National Research Center "Tairov Research Institute of Viticulture and Wine-Making". The following indicators were determined: average daily (Tavg., °C), maximum (Tavg.max °C) and minimum (Tavg.min, °C) temperatures; amount of precipitation (ΣOavg., mm.); number of days with average air temperatures above 25 °C (Tavg.davg.≥25ºC), with maximum temperatures above 35 °C (Tabs.max ≥35ºC) and number of days with relative air humidity below 30% (f≤30%) (Table 2a and b) [7].

3. Results and discussion

3.1. Clone trial plots and clone repository

Visual control of virus diseases symptoms was carried out annually, and latent infection were selectively detected by ELISA and PCR. Most often, at the first stages of clonal selection (P0), leafroll virus serotypes 1 and 3 were detected (Table 1), however, the plants of the clone repository are free from this virus due to sanitary selection. The clone bank is placed in protected soil conditions on a zeolite substrate, which prevents secondary infection due to the activity of insects vectors.

3.2. Ampelographic collection

The ampelographic collection, including registered characteristic collections (characteristics of resistance, quality, seedlessness), is subject to annual visual inspection and once every 5-10 years - to selective laboratory testing for infection by virus diseases. Most often, the analysis revealed the third serotype of leafroll virus (Table 1). The collections are kept in open ground, but the risks of secondary infection are reduced by using a comprehensive system of protection against grape pests (Table 1).

Table 1. Assessment of the virological status of collection plantations and research plots of grapevines

Collection (plantation) type	Visual control of symptoms	Laboratory control (ELISA, PCR)
Clone trial plots	Grapevine leafroll (rarely)	First progeny (P0) – GFLV, GLRaV 1, GLRAV 3, GFKV (rarely)
Clone repository (glasshouses)	Without symptoms	Not identified
Ampelographyc collection	Grapevine leafroll (sometimes occur on red cultivars)	GLRaV 1, GLRaV 3 (rarely), GFkV
Base mother plantations	Grapevine leafroll, grapevine vein mosaic	GLRaV 1, GLRaV 3 (rarely), GFkV
Industrial vineyards	Odessa region – grapevine fanleaf (rarely), grapevine leafroll . Transcarpathia – grapevine fanleaf (rarely), grapevine leafroll symptoms, rugose wood (rarely)	Odessa region – GFLV, GLRaV1, GLRaV2, GLRaV3. Transcarpathia - GFLV, GLRaV1, GLRaV3, grapevine virus A

Note: on all types of plantations, visual virus control was carried out twice a year. Laboratory tests on clones plantations were carried out in accordance with the technological stages of certified grapevine planting material production. On the ampelographic collection, hybrid and selection nurseries, laboratory testing for latent virus infection was carried out selectively or when visual symptoms of viral damage were detected.

3.3. Basic mother plantations

In general, the sanitary condition of basic mother plantations in the wine-growing regions of Ukraine corresponds to the phytosanitary condition of the initial material for propagation, which originally comes from the clone repository.

3.4. Industrial vineyards

In a regional context, industrial plantations in Odessa, Mykolaiv and Kherson regions are characterized by approximately the same complex of viruses and the level of damage (grapevine fanleaf virus - very rarely, more often - grape leafroll virus). Transcarpathia is characterized by several cases of grapevine rugose wood complex and the associated with the disease grapevine virus A infection. Among other viruses, latent damage by the second serotype of leafroll virus [6] was detected in Odessa region. (GLRAV2), which belongs to the Closteroviridae family and is associated with leafroll disease of grapevine (Table 1).

The data we obtained regarding the sanitary condition of grape collections, maintained at the National Scientific

Center "Tairov Research Institute of Viticulture and Winemaking", reflect the features and ways of spreading virus, as well as the level of their control in different types of collections and experimental plots.

It was shown that the degree of grapevine collections damage by viruses depends on both the source of material origin and the severity of the control measures used on it. The lowest risk of virus infection is in the clone repository, the highest is in the ampelographic collection and clonal experimental plots of the first vegetative generation and industrial vineyards.

3.5. Levels of virus infection visual manifestation and weather and climate changes.

Another factor that affects the sanitary condition of grape collections and plantations is weather and climate conditions, as a result of which the degree of visual manifestation of diseases and, under conditions of changing species composition or activity of vectors, the levels of latent infection can change [8].

Changes in weather and climate conditions on the grapevine virus diseases manifestation began to be observed since the 2000s. This was most clearly reflected in the manifestation of grape leafroll virus, GLRaVs.

Grapevine leafroll belongs to diseases, the manifestation of which is significantly influenced by the meteorological conditions of the current year, in particular, hot and dry weather. Detection of leafroll on the Odessa Black variety (first confirmation of the third serotype of GLRaV presence – 1991) in the periods 1987-1991, 2001-2005 and 2010-2015 showed a gradual increase in the number of diseased plants in the vineyards of the state enterprise experimental farm "Tairovskoe" from 1.2 to 4.6% with symptoms of color change – redness between the veins – and curling of the edges of the leaves downwards (Figure 1). In the period from 2010 to 2015 this increase reached 7.2%.

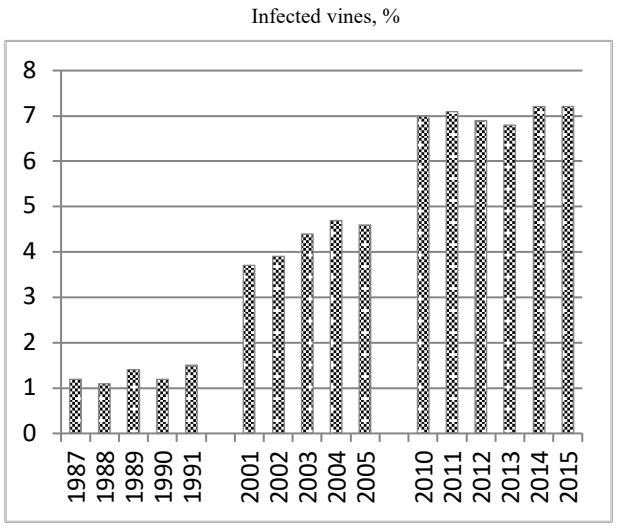

Figure 1. Dynamics of vines with GLRaV 3 symptoms (percentage)

Note: The total number of vines examined is 1000. Enzyme-linked immunosorbent assay (ELISA) confirmation of infection with the GLRaV 3 – 1995, 1998, 2001

As the results of numerous studies show, the manifestation of leafroll is most influenced by heat and humidity regimes during the period of development and manifestation of disease symptoms. The work analyzed many indicators, the most informative of which were the average daily (Tavg., ° C), maximum (Tavg.max ° C) and minimum (Tavg.min, ° C) temperatures; amount of precipitation (ΣOavg., mm.); number of days with average air temperatures above 25 ° C (Tavg.davg.≥25ºC), with maximum temperatures above 35 ° C (Tabs. max ≥35ºC) and number of days with relative air humidity below 30% (f≤30%) ((Table 2a and b).

Table 2. Characteristics of the temperature and humidity regime

a) July - August

Periods / Indicators		1987-1991	2000-2005	2010-2015
Temperature and humidity regimes	Tavg, °C	21,4	22,9	24,4
	Tavg. max, °C	29,8	31,9	34,2
	Tavg. min. , °C	12,7	14,1	15,2
	Precipitations, mm	29	49	22
Days with:	Tavg. dayly≥25°C	17	38	79
	Tabs. max≥35°C	0	2	26
	f≤30%	5	15	27

b) August

Periods / Indicators		1987-1991	2000-2005	2010-2015
Temperature and humidity regimes	Tavg, °C	22,4	23,2	24,5
	Tavg. max, °C	31,0	32,1	33,8
	Tavg. min , °C	13,1	14,7	15,6
	Precipitations мм	79	105	85
Days with:	Tavg. daily≥25°C	54	96	172
	Tabs. max≥35°C	1	9	37
	f≤30%	9	29	36

It was found that in July and August (a) and in August (b) there was an increase in average, maximum and minimum air temperatures and an increase in the number of days with average air temperatures above 25 °C and maximum temperatures above 35 °C from the period 1987-1991 to the period 2010-2015. The greatest amount of precipitation was observed in these months in the period from 2000 to 2005. The variability of the number of days

with relative humidity below 30% repeats the trend of changes in temperature regime indicators in these years, and their maximum number is generally observed in July - August.

4. Conclusions

The degree of virus disease damage to the studied grapevine collections and experimental plots depends on the complex and periodicity of control measures used on them. The lowest risk of detecting virus infection is in the clone repository, the highest is in the ampelographic collection and clonal experimental plots of the first vegetative generation and in industrial vineyards where clonal selection is carried out.

Among harmful viruses, grape leafroll virus was most often detected on all types of collections and plantations, therefore it should be the object of the strictest control given the degree of its distribution, as well as taking into account the high risk of relatively rapid natural transfer by scale insects.

There is a tendency for the influence of temperature and humidity regime indicators on the number of plants with the symptom of grape leafroll. The coefficient of determination R2 between the number of plants with symptoms of grape leafroll and the indicators of temperature and humidity regime varies within 0.69-0.77.

5. References

1. *EPPO Standards. Certification schemes. Pathogen-tested material of grapevine varieties and rootstocks* / EPPO, Paris, France, (2003)
2. P. La Notte, P. Venerito, V. Savino, G. Martelli, *Cost Action FA 1003 materials* (Sophia, 2012)
3. S. Frausin, *Cost Action FA 1003 materials* (Sophia, 2012)
4. G.P. Martelli, J.Plant Pathol., **96** (2014)
5. Ya. Gadzalo, V. Vlasov, N. Muljukina, L. Dzaburiia, M. Tulaeva, V. Chisnikov, I. Kovalova, Bulletin of Agricultural Science, **11** (2014)
6. I. Kovaleva, L. Janse, L. Konup, N. Zelenyanskaya, V. Vlasov, A. Konup, N. Muljukina, N. Kyryk, M. Pikovskyi, Cyt. and Gen., **56,** 6 (2022)
7. G. Lyashenko. *Agroclimatology Workshop:* Textbook, Odesa ODEcU (2014)
8. M. Rienth, R.Walker, S. Castellarin, C. Sweetman, C. Burbidge, C. Bonghi, F.Famiani, Darriet P., Sec. Plant Metabolism and Chemodiversity,**12** (2021)