Bulgarian Journal of Agricultural Science, 29 (No 3) 2023, 452–457

Reaction of newly selected vine forms to gray rot (*Botrytis cinerea* Pers.)

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Abstract

Ivanova, I., Mincheva, R., Dyakova, G. & Ivanova-Kovacheva, G. (2022). Reaction of newly selected vine forms to gray rot (*Botrytis cinerea* Pers.). *Bulg. J. Agric. Sci., 29 (3)*, 452–457

During the period 2014-2018, in Agricultural Academy – Sofia, at the experimental vineyard of the Institute of Agriculture and Seed Science «Obraztsov Chiflik» – Rousse, a study was conducted with breeding wine vine forms to determine their reaction to gray rot, caused by the fungus *Botrytis cinerea* Pers. The following elite wine hybrid forms have been studied: 25/12 (Pamid Ruse 1 x Kaylashki Misket), 5/51 (Naslada x Chardonnay), 5/83 (Naslada x Chardonnay) and 25/54 (Pamid Ruse 1 x Kaylashki Misket). Misket Otonel variety was chosen as control due to the white color and the muscat aroma of the grapes. The phytopathological assessment was performed on the scale of OIV 453, where the resistance was presented in 5 degrees from 1-3-5-7-9, as the lowest degree 1 corresponded to maximum resistance. The following traits were also monitored: grape weight (g), ripening period, sugar content (%), acid content (g/l), grape color. It was found that breeding forms 5/51, 25/12 and 25/54 were resistant to *Botrytis cinerea* Pers., and 5/83 (Naslada x Chardonnay) was medium resistant. The higher resistance, compared to the control, was genetically determined. A strong correlation (-0.844) was found between the yield and the development of the disease.

Keywords: Botrytis cinerea Pers.; gray rot; grapevine; *Vitis* ssp.; sensitivity

Introduction

The grapevine (*Vitis vinifera* L.) is attacked by many phytopathogens, which are the main cause of losses in yield and lowering the quality of grapes (Verhagen et al., 2010; Feechan et al., 2013; Nanni et al., 2013; Agudelo-Romero et al., 2015). Most of the varieties show high susceptibility to main diseases. To solve the problem, varieties have been created in the world and in Bulgaria, mainly via intraspecific and interspecific hybridization, at which a high degree of sustainability has been combined with increased winter hardiness (Valchev et al., 1984; Nikolic et al., 2002; Pernesz, 2003; Zhao et al., 2006; Ivanov & Kostadinova, 2007; Ivanova & Dyakova, 2011). The use of the method of interspecific hybridization in scientific and applied aspects allows new immune and resistant varieties to be created, analogs of *Vitis vinifera* L. (Ivanov, 2009; Ivanov, 2011; Ivanov et al., 2014).

The most important fungal diseases affecting the grapevine are downy mildew (*Plasmopara viticola*), powdery mildew (*Erysiphe necator*) and gray rot (*Botrytis cinerea* Pers.) (Boller & Felix, 2009; Kelloniemi et al., 2015). Gray rot, caused by *Botrytis cinerea* Pers. fungus causes significant losses worldwide and is considered by many authors, to be the most important economic disease in grape production. The susceptibility of the vine to the disease has been the subject of many studies (Jarvis, 1980; Cappellini et al., 1986; Aziz et al., 2004; Fournier et al., 2013; Martinez et al., 2005; Negri et al., 2017; Apolonio-Rodríguez et al., 2017). The fungus develops in a wide temperature range of 10-31°C with an optimum of 25°C. Conidiospores germinate at a relative humidity above 90%, but also infect in the presence of a drop of water. The disease can spread rapidly, causing losses in plantations and after harvest (Oliveira et al., 2009). It appears in cool and wet weather on flowers, flower stalks, formed berries and even shoots, covering them with mold, and then causes their death. Rainfall and lowering of the temperature during the grape harvest, are the reasons for the development of gray rot, but the most characteristic and strong are the damages on the berries during the period of ripening.

The first signs in white varieties are the light brown spots on the skin, which are easily removed and exposes the flesh. There are such spots in red varieties, but they are difficult to notice. The parasite quickly spreads, throughout the whole cluster and spreads to neighboring ones, and at higher humidity the rotten parts are covered with abundant gray mold. According to Kostadinova et al. (2007), the main reason for the harvest damage by gray rot is the excessive rainfall, during the physiological maturity of grapes and note that: "in some years, there may be some relocation of varieties from one group to another".

Some fungal enzymes are likely to be involved in the rapid destruction of fruit integrity (Aguero et al., 2005). In addition to grapes, it can infect other parts, such as leaves and stems (Bezier et al., 2002). Damages from hail, vine moths, oidium, primary infections by other pathogens, mechanical damage, or such caused by insects, frequent rainfall and increase in sugar content, during ripening are favorable conditions for the development of *Botrytis cinerea* Pers. Factors, contributing to the resistance of the disease include morphological and anatomical „barriers". Among the morphological and anatomical features, related to resistance are thickness of the skins of the berries (Sarig et al., 1996), density of the cluster and content of wax (Hill et al., 1981; Marois et al., 1986; Vail & Marois, 1991; Percival et al., 1993; Riberau-Gayon & Lonvaud, 2000; Kostadinova et al., 2007).

Chemicals, such as phytoalexins and pathogenesis-related proteins, are induced in the infectious process (Jeandet & Bessis, 1989; Derckel et al., 1998; Salzman et al., 1998; Renault et al., 2000). According to some authors, gray rot is accompanied with the development of other parasites or saprophytes of the genera *Penicillium* and *Aspergillus* (Hristov, 1976). The infected grapes have an unfavorable composition and the pathogen produces toxic compounds that affect yeast and inhibit the fermentation process of wines (Bocquet et al., 1995; Hong et al., 2011; Hong et al., 2012; Agudelo-Romero et al., 2015).

Material and Methods

Elite wine hybrid forms: 25/12 (Pamid Ruse 1 x Kaylashki Misket), 5/51 (Naslada x Chardonnay), 5/83 (Naslada x Chardonnay) and 25/54 (Pamid Ruse 1 x Kaylashki Misket) were included in the study. The Misket Otonel variety was chosen as control, due to the white color and the muscat aroma of the grapes of the breeding forms.

The main method used to determine the symptoms of the disease was the comparative morphological method at naturally infected plants. Identification of the pathogen was by direct observation of the structures of the pathogen formed on plant tissue, and when cultivated in nutrient media, according combination of morphological and cultural features. The traits with the highest taxonomic value were taken into account: morphology and size of sporulation and morphology of the colonies. Microscopy of temporary preparations was performed. The pathogen was isolated from infected organs, collected from diseased vines in the experimental vineyard. The inoculum for the study was obtained by cultivation of the starting isolates on Chapek nutrient medium at 22°C in dark. Discs with diameters of 5 mm were cut from the periphery of actively growing colonies and placed in the center of petri dishes (d = 90 mm), each containing 25 ml of the respective nutrient medium. The inoculated petri dishes were incubated at 22°C in a thermostat. The shape, profile, periphery and color of the colonies were determined visually with binoculars by microscopy (Figure 1).

During the growing season, attacks of *Botrytis cinerea* on the tested varieties were registered several times. The evaluation of bunches and berries was performed on the scale of OIV 453. Sustainability was presented in 5 levels, as follows: grade 1 – no attacks on bunches; grade 3 – some bunches were weakly attacked, without consequences for the grape harvest; grade 5 – from 20 to 30% of the bunches were attacked with an impact on the yield of grapes; grade 7 – a very large percentage of the bunches were severely damaged or destroyed; grade 9 – all bunches were damaged or destroyed – there was a consequence for the grape harvest. The following traits were also monitored: weight of grapes (g), ripening period, content of sugars (%), content of acids (g/l), color of grapes. The sugar content was determined with a Dujardin saccharometer and the acid content by titration with a decinormal sodium-based solution. The correlation coefficient between the development of the disease and the yield obtained was calculated, according to Mihailova (1982) and was evidence for the relationship between the studied traits.

Results and Discussion

Isolation of the pathogen from diseased plants was performed in the Laboratory of phytopathology of the Institute of Agriculture and Seed Science „Obraztsov Chiflik" – Rousse, Agricultural Academy. Agar nutrient medium Chapek was used as a substrate (Figure 1).

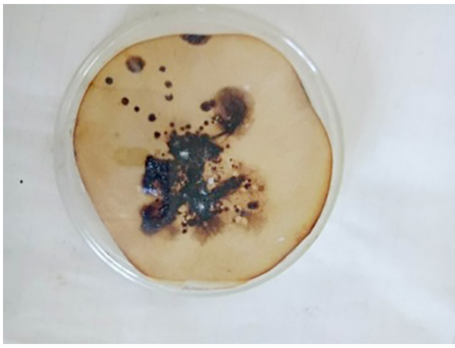

Fig. 1. Isolate with Botrytis cinerea

The development of *B. cinerea* was observed under the conditions of natural infection and depending on the weather conditions. Rainfall and air temperatures during grape ripening had a strong influence on the development of the disease and the susceptibility of the varieties. The presence of excess moisture predisposed to more intensive development of fungal diseases and slowed down the ripening of grapes. Climatic conditions during the observed years were shown in the climatogram in Figure 2.

The period was favorable for the growth and development of the vine and at the same time for the development of the pathogen. The importance of the individual components of the climate consisted in their need for the course of the infectious process and determined the seasonal development of the disease. In 2014, the appearance of the pathogen was observed in the first ten days of August, when the amount of precipitation was 67.3 mm/m^2, at a norm of 67.4 mm/m^2 and a temperature sum of 701.6°C, at a norm for the period 1896-2005 of 697.7°C. In the next year, 2015, favorable conditions for the development of the disease were observed again in August, when the amount of precipitation repeatedly exceeded the norm for the period 49.4 mm/m^2 and was 204.7 mm/m^2, and the temperature sum was close to that of the norm of 715.7°C.

Conditions for the development of the disease in 2016, were observed at the end of July, when the sum of the fallen precipitation was 74.2 mm/m^2, respectively, compared to the norm for the period 80.5 mm/m^2. The temperature was up to 660.7°C, with a norm for a ten-year period of 606.6°C, but it was too early and then almost no complete cluster formation was observed. The greater amount of precipitation in the first half of July, 2017, favored the development of the disease. In the early September, heavy hail damaged 100% the vineyard. As could be seen from the climatic characteristics in 2018, the last year of the study was characterized with average monthly temperatures in the summer months of June and July, in the range of 20-25ºC, and with significant rainfall in the summer months up to 160 mm/m^2, favorable for the development of the pathogen.

From the averaged data for the period of study (2014-2018), it is seen that in three of the breeding forms 25/12, 25/54 and 5/83, the mass of the berries was greater than the reference variety Misket Otonel (1.50 g). The berry of № 5/83 was the heaviest (1.90 g), 0.40 more than Misket Otonel. Along with the development of the disease, differences in technological traits were observed. Regarding the sugar content, most of the analyzed forms 5/51, 25/12, 25/54 had a lower percentage of sugar content in grape juice (from 0.38 to 6 points) than Misket Otonel (23% at maturity). Breeding form 5/83 had the same sugar content as that of Misket Otonel (23%).

The titratable acids in grape juice were 7.51 g/cm^3 in Misket Otonel. The form 5/83 was close to that content. Forms 25/12 and 25/54 were with lower titratable acids, respectively, 6.31 and 6.50 g/cm^3.

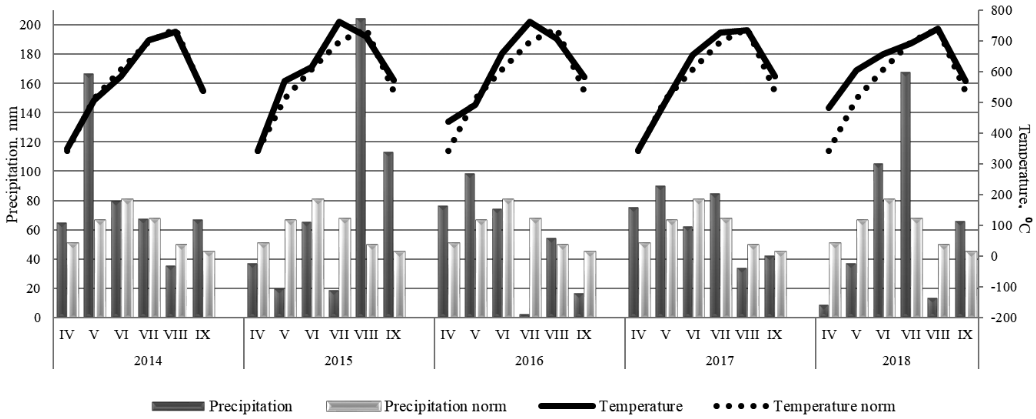

Fig. 2. Climate characterization for 2014–2018

Table 1. Degree of attack by *B. cinerea* for wine-making breeds of hybrid strain, 2014-2018

Variety/hybrid forms	Degree of attack on the scale of OIV 452-453					Average 2014-2018	Degree on the suss. OIV 452/453	Category on sustainability 2014-2018
	2014	2015	2016	2017	2018			
Misket otonel	3	3	3	*	3	3	3	MR Moderately stable
5/51 Naslada /Chardonnay	1	1	0	*	1	1	1	R Resistant
5/83 Naslada / Chardonnay	3	1	1	*	3	3	3	MR Moderately stable
25/12 Pamid Ruse 1/ K. Miskeт	1	1	1	*	1	1	1	R Resistant
25/54 (Pamid Ruse 1/ K. Miskeт)	1	1	1	*	1	1	1	R Resistant

Legend: 1 – resistant, without attack by pathogens; 3 – plants with single necrotic spots on the leaf blade; 5 -limited spots on the leaves, 20 to 30% of the bunches are attacked; 7 – unlimited spots on the leaves, with fruiting bodies of the pathogen and mycelium, a large percentage of the clusters are severely damaged or destroyed. Sensitive, High Sensitive, Resistant, Unstable * – The grapes were destroyed by hail in early September.

Table 1 shows that the sensitivity of the clusters during the years of the experiment was not one and the same. Differences in the stability of breeding forms were due to genetic origin, cluster structure and chemical composition. Examining the results obtained for the chemical composition of grapes, it was found that the more sugars were accumulated, the more susceptible they were to the disease. The study showed that vine forms, that have highly compacted grapes and higher sugar content, were more strongly attacked and reacted as sensitive to gray rot. Their sugar content varieed from 22.67% to 23.82% (Table 2).

The breeding forms with a thicker skin and lower sugar content (5/51 and 25/12) were less attacked. Three forms reacted as resistant, and two as medium sustainable (Table 1). The resistance of grapes to gray rot was directly dependent on the thickness, of the cuticle of the berries.

For the observed period, the highest yield of grapes per plant was obtained in 25/54 breeding form, respectively, 4.900 kg, followed by the forms 25/12, 5/51 and 5/83 with yield per plant, respectively, 4.233 kg, 3.800 kg and 2.763 kg. Misket Otonel variety had the lowest yield during the experimental period – 2.232 kg.

A strong correlation was found between the yield obtained and the development of the disease (-0.744), which proves that the grape yield decrease with the development of the disease.

Table 2. Indicators of wine selection varieties, average for 2014-2018

Indicators	Candidate varieties			5/83 Elite form Naslada/ Chardonnay	Compared variety Misket otonel
	5/51 Naslada/ Chardonnay	25/12 Pamid Ruse1/K. misket	25/54 (Pamid Ruse1/K.misket)		
Grape harvest from the vine, kg	3.800	4.233	4.900	2.763	2.232
Grape: dimensions, cm	17.29/8.73	16.87/10.85	14.18/9.47	12.48/8.45	12.03/7.83
Grape: average mass, g	128	206	176	164	117.4
Grain: dimensions, cm	15.26/14.82	16.15/14.95	14.98/14.98	12.53/11.84	11.33/9.70
Grain: resistance to compression, g	569	755	340	667	560
Grain: average mass, g	1.48	2.85	2.40	1.92	1.50
Grain: tear resistance, g	250	255	280	290	137
Shugar content, %	22.62	21.00	17.00	23.00	23.00
Content of titrable acids, g/l	9.65	6.31	6.50	7.54	7.51
Number of seeds in 100g	190	300	199	180	159
Mass of 100 seeds, g	2.78	3.09	3.53	3.45	2.08

Conclusions

Breeding elite wine forms were characterized with a high degree of resistance to *Botrytis cinerea* and have valuable economic qualities, that can be used to improve the grapevine by creating wine varieties resistant to the disease. The breeding forms 5/51, 25/12 and 25/54 stand out, as promising white elite wine breeding forms. Form 5/83 proved to be moderately resistant.

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Received: **August, 10, 2021**; *Approved*: **December, 10, 2021;** *Published:* **June, 2023**