

THE BEHAVIOUR OF GRAPEVINE UNDER VIRUS INFECTION AND DROUGHT STRESS COMBINATION

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Abstract

Potted Grapevine fleck virus-infected and virus-free grapevine belonging to V. vinifera L., Tămâioasă românească selection, have been subjected to progressive water deficit. At a daily thermal amplitude of 23.7-30.3°C, the soil temperature did not exceed 29.8°C. After 37 days the shoots of the plants were completely dry, the soil moisture reaching 4.8%. The difference in leaf angle values between drought and control plants was higher in infected grapevines as compared to healthy ones, at the beginning of plant wilting. The total water content decreased during the intensification of the wilting degree of the leaves, significant differences comparatively to the control in the virus-free plants being registered. Stomata opening decreased significantly in water stressed virus-infected plants at the onset of wilting, as compared to healthy water stressed plants. The water deficit did not influence significantly the content of assimilating pigments either in healthy or virus-infected plants.

Key words: *Vitis*, GFkV and water stress, leaf angle, stomata, assimilating pigments.

INTRODUCTION

Grapevine (*Vitis vinifera* L.) is an important crop in the temperate climates. Unfortunately, it is considered one of the most affected plant, by a large number of viruses. So far, a list of over 80 distinct viral species has been identified. About half of viruses (31) are associated with the four major disease complexes known as: infectious degeneration (12 Eurasian/European/Mediterranean nepoviruses) and decline (4 American nepoviruses); leafroll (5 viruses), rugose wood (6 viruses), and fleck (4 viruses) (Martelli, 2017). Often, the grapevine plants go through periods of drought with high temperatures, especially because of the current conditions of climate changes. Both drought (one of the major abiotic stress factors) and virus infection (as a biotic stress factor), are limiting factors of the grapes quality and quantity (Malossini et al., 2003). The influence of stress caused by virus infection (Cui et al., 2015) or drought (Zhu, 2002) have been well presented regarding plant growth and development, photosynthetic capacity and grapes production. However, the studies on their combined effects have been limited. Plants grown in the field are

simultaneously exposed to abiotic and biotic stress factors. Abiotic stress has been shown to alter the resistance or tolerance of plants to pathogens. Similarly, biotic stress alters the host resistance tolerance to abiotic stress. Consequently, the plant response to a single stress factor differs from that of several stressors and their simultaneous action can induce complex plant responses (Suzuki et al., 2014). Thus, a better understanding of the effects of combining abiotic with biotic stress has a great importance and would allow the orientation of agricultural management strategies to ensure the durable development of agricultural production.

The study deals with the response of a romanian grapevine variety, Tămâioasă românească selection, under combined stress induced by *Grapevine fleck virus* (GFkV) infection and drought, in the greenhouse conditions.

GFkV has been chosen for investigations having a wide spread in all viticultural regions of the world, with typical symptoms of vein clearing on the sensitive indicator *V. rupestris* St. George and latent infection on *V. vinifera* cultivars, but often identified in mixed

infections with other more dangerous viruses (Buciumeanu et al., 2009; Martelli & Boudon-Padieu, 2006).

MATERIALS AND METHODS

Own-rooted grapevine plants, four years old, belonging to *V. vinifera* L., Tămâioasă românească selection, from two distinct batch (GFkV-infected and GFkV-free) have been subjected to progressive water deficit. Watered plants have been used as control in each batch. The plants were grown in greenhouse, in 10 l pots with a grapevine specific substrate. At the beginning of the experiment, the plants were uniform developed, with canes lengths of 19-22 internodes.

GFkV - infected plants are maintained in the grapevine virus collection belonging to NRDIBH Ștefănești-Argeș. The presence of the virus in naturally infected grapevine have been checked by DAS-ELISA (Clark & Adams, 1977), with commercial reagents (Bioreba, Switzerland). GFkV-free grapevine plants have been obtained by *in vitro* chemotherapy elsewhere (Guță et al., 2014).

Along 6 weeks, at 1 pm every day, the air temperature, the minimum and maximum temperature in the greenhouse, were recorded by Oakton RH/TempLog datalogger, as well as the temperature (with digital thermometer with 15.5 cm probe) and soil moisture (with soil moisture meter TDR 300: rods of 10.5 cm) in the vegetative pots were monitored. The effects of water stress on the grapevine plants were evaluated based on the daily thermal amplitude (Tmax-Tmin) and the values of soil temperature and humidity that were calculated as averages of measurements made in the pots for each variant. To quantify the effects caused by the lack of water, the angle of the leaf, the stomata opening (pore) measurements have been done, total water and chlorophyll pigments were dosed on the leaves at the lower and upper part of the shoots in several phases: at the beginning of wilting, when the shoot was completely wilted and when the plant had half dry shoot.

The angle of the leaf was measured manually with a protractor as the angle formed by the main vein of the leaf and its petiole.

In order to study the stomata apparatus, leaf epidermal imprints were collected (Gokbayrak et al., 2008) from the underside of healthy/GFkV-infected, control/water stress grapevines. Three different regions of a leaf were used (basal, lateral and apical zone of the shoot) and five fields/zone were investigated, using objective of 40x and 10x ocular. The stomata measurements have been made using Quick PHOTO MICRO 2.2 software (OLYMPUS BX 41 microscope equipped with digital camera). The extraction of assimilating pigments from fresh leaf material was realized with 85% acetone (Holm, 1954). The assimilating pigments (chlorophyll *a*, chlorophyll *b* and carotenoids) concentration was expressed in mg/g fresh weight (f.w.).

Statistical significance of differences was analyzed by SPSS 10 for Windows, taking $P < 0.05$ as significant according to one-way ANOVA.

RESULTS AND DISCUSSIONS

The grapevine is a mesophytic plant with high ecological plasticity, which can adapt relatively easily to humidity variations. Grapevine growing on sloping or sandy soils led to the impression that this plant is not moisture demanding. Achieving satisfactory results in a poorer humidity regime is due to the strong root system, which explores a large volume of soil and the high absorption capacity through which it can provide water with the necessary nutrients. What is certain is that the lack of water in the soil decreases the efficiency of photosynthesis and the accumulation of dry matter (Oprea, 2001).

During the study, the minimum temperatures in the greenhouse did not fall below 11°C, most values being in the range of 19-22°C, while the maximum temperatures were in the range 39-52.1°C. It is estimated that, during the vegetation period, the optimal temperature level for grapevine is between 25 and 35°C, the physiological processes taking place with a maximum yield (Oprea, 2001). The studied plants maintained in the greenhouse where the maximum temperatures have exceeded often 40°C were subjected under the influence of both thermal and water stress. Thus, at a daily thermal amplitude of 23.7-30.3°C, the soil

temperature in the vegetation pots in the first 15 cm depth, reached values between 22.6 and 29.8°C. The soil temperature in the pots that did not exceed 30°C being at the level that determines the growth of the roots of grapevine (Țârdea & Dejeu, 1995). This high temperature caused a progressive decrease in soil moisture in the first 10 cm of substrate in vegetation pots subjected to water stress, which led to the dehydration of grapevine plant tissues, expressed by wilting of leaves from the upper to the lower part of the shoot. At a slightly variable level of daily temperature amplitude and soil temperature, the first signs of shoot wilting, visible on the leaves at the upper part, appeared after 10 days, when the average soil moisture dropped from 32.8% at the beginning of the experiment, at 11.3%. After 5 days, when the soil moisture reached an average value of 8.72%, the process of wilting of the leaves covered the entire length of the shoot. The degradations caused by the lack of water continued with the drying of the tendrils and the appearance of the first yellowed leaves, at the base of the plant, when the soil moisture decreased to 6.7%, after another 2 days. The drying of the leaves started from the base of the

plants continued reaching, after another 4 days, the lower half of the shoot (Figure 1).



Figure 1. Wilting and drying symptoms on grapevine (Tămăioasă românească selection), under water deficit

After 37 days from the beginning of the experiment, the grapevines were declared dry. Slight increases in soil moisture in vegetation pots subject to water stress, which deviated from the linear decrease, were determined by variations of air temperature and humidity in the greenhouse, as the studied plant lot was not completely isolated from the control plants, watered periodically as needed (Figure 2).

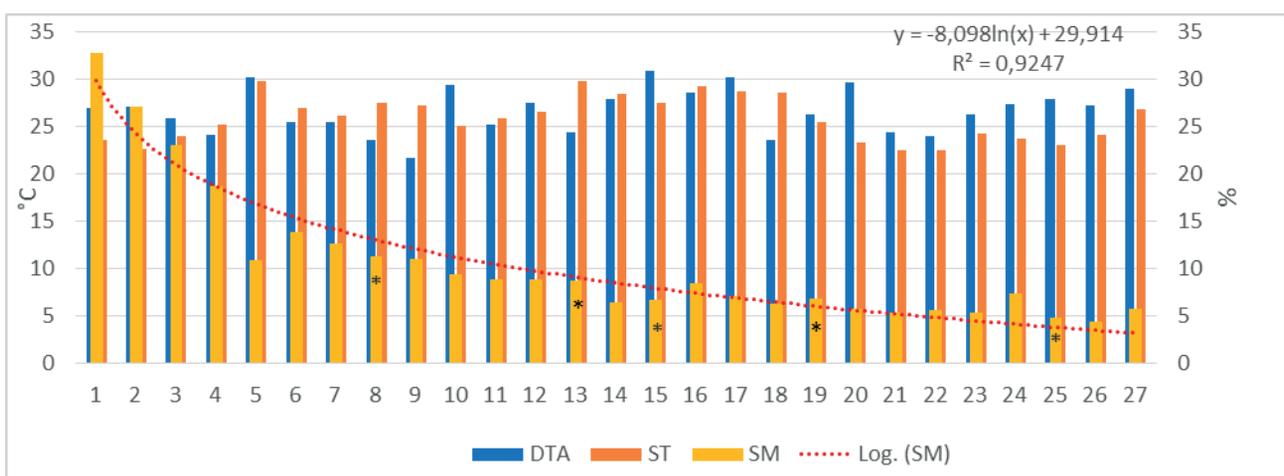


Figure 2. Evolution of soil moisture (SM) depending on the daily thermal amplitude (DTA) and soil temperature in vegetation pots (ST). *Marking the progressive phenomena of grapevine plants degradation subjected to water stress (starting of wilting on the top of the shoot; wilting the entire shoot; drying of the tendrils and the appearance of the first yellow leaves on the base of the shoot; drying of the leaves on the lower half of the shoot; drying of the plant)

When the process of leaves drying reached the lower half of the cane, the others being withered, watering was resumed on some of the plants subjected to water stress. Even the next day, the very young leaves located at the top of the shoots remained withered and those located immediately below began to regain their turgor.

After 15 days from the watering recommencing, the appearance of secondary shoots towards the top of the cane marked the fact that the plants resumed their physiological processes, producing new vegetative organs. The capacity of recovering after water stress

was not influenced by the presence of viral infection.

Surprisingly, *Grapevine leafroll-associated virus serotype 1 and 3* (GLRaV-1, GLRaV-3) have been shown to increase the intrinsic efficiency of water use in the Touriga Nacional grapevine variety under field conditions (Moutinho-Pereira et al., 2012). Also, the interaction between *Grapevine rupestris stem pitting-associated virus* (GRSPaV) and grapevine seems to have improved the plant's tolerance to drought in greenhouse conditions (Pantaleo et al., 2016).

Leaf angle

Studies on the symptoms caused by the water stress in the grapevine have highlighted the decrease in the angle formed by the main vein and the petiole of the leaf (Pool & Lakso, 2000).

The angle of the leaf is an indicator of the plant-water relationship, as it changes depending on the water supply state and tissue turgor (Smart, 1974). Another observation was that changing the angle of the leaves was involved in tolerance to water stress. Thus, variation of leaf angle can reduce the thermo-radiative load on leaves, contributing to water conservation (Gamon & Pearcy, 1989; Palliotti et al., 2008).

Measurements made on the leaves at the base and top of the plants showed a decrease in leaf angle (Figure 3), with increased drought both in healthy and GFkV-infected grapevines at both moments of the study (M1 - leaf wilting at the top of the shoot, M2 - wilting of the leaves along the entire length of the shoot). In the case of healthy grapevines, when the wilting covered the entire plant, the angle of the leaves located at the apical extremity was significantly lower at $P < 0.05$ as compared to the control (Figure 4).



Figure 3. Leaf angle decreasing under the influence of water deficit (Tămâioasă românească, selection)

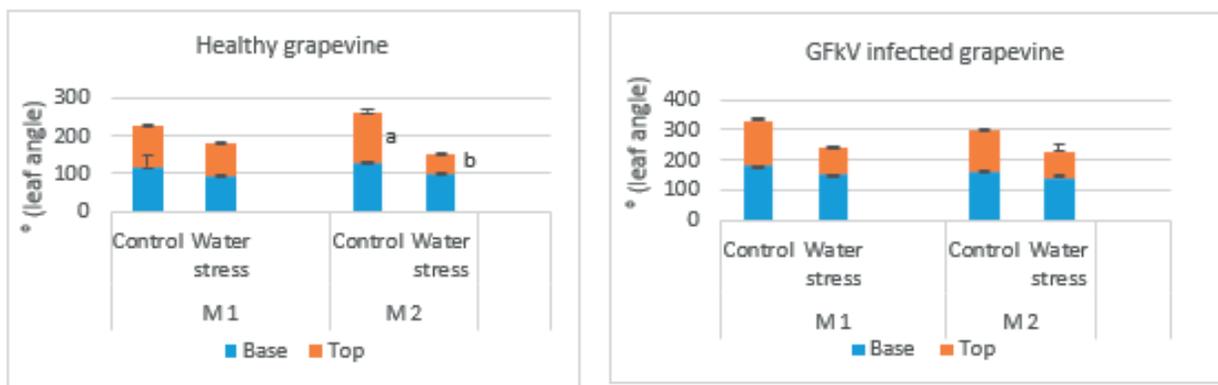


Figure 4. Variations of the leaves angle placed the lower and upper part of the shoots on healthy and GFkV-infected grapevine (Tămâioasă românească selection), subject to progressive water deficit. Bars indicate standard deviations of measurement averages, letters mark the significance of differences at $P < 0.05$ (M1 - leaf wilting at the top of the shoot, M2 - wilting of the leaves along the entire length of the shoot)

Regarding the influence of virus infection on the expression of drought symptoms in grapevines, wilting is more pronounced in infected plants. The difference in leaf angle values between plants subjected to drought and control is higher in GFkV-infected grapevine as

compared to the healthy one, both at the base and at the top of the cane, at the first measurement (M1). When wilting was evident along the entire length of the shoot (M2), this difference could not be maintained (Table 1).

Table 1. The difference between the value of the leaf angle in Tămâioasă românească selection grapevine subject to drought and control (M1 - leaf wilting at the top of the shoot, M2 - wilting of the leaves along the entire length of the shoot)

Leaf position	Grapevine leaf angle (°)			
	Healthy		GFkV - infected	
	M1	M2	M1	M2
Base	24.00	31.20	29.20	16.65
Top	18.35	81.65	64.20	51.80

Other authors have noted in their studies on the effects of drought on plants, the phenomenon of leaf twisting that reduced the area exposed to thermal radiation (Duan et al., 2018). In the studied grapevine genotype, the leaves lost their turgor until they dried, without showing the twisting phenomenon.

Total water

The presence of sufficient water is an essential factor for the survival, growth and development of plants. Depending on the external factors, the water content depends on its absorption and loss by plants.

The existence of a continuous flow of water through the body of the plant is an essential condition of metabolic activity and therefore of plant survival. These processes are modified at the same time by some environmental factors such temperature, humidity and air movements, which strongly influence the degree of hydration of tissues (Boldor et al., 1981).

Despite all the losses, the leaves must maintain a high water content, about 75-85% of their fresh weight (Târdea and Dejeu, 1995).

Evaluation of the total water content in the leaves positioned at the base and upper part of the cane at the time of the first signs of wilting at the end of the plant led to the following observations: water stress does not seem to influence the total water in the leaves at the base or top of healthy plants; in virus-infected plants subjected to water stress, the total water was significantly higher both at the base and at the top of the shoot as compared to the virus-infected control (values are not statistically assured), due to the suction processes intensification as a result of water loss through perspiration (Table 2).

At the time when the grapevine shoots of the plants subjected to water stress showed drying phenomena of the leaves on the lower half, the total water content in the leaves located on the upper half was significantly lower at $P < 0.05$ as compared to the control, to healthy plants. Similarly, the total water content varied in plants infected with GFkV, but the values were not statistically assured (Table 3).

Stomata opening

Losing water in the form of vapor, the grapevine is protected from excessive temperatures, so that the leaves can normally carry out the photosynthesis and other physiological and biochemical processes (Târdea and Dejeu, 1995).

Table 2. Variation of the total water content and stomata opening (SO) to the leaves of plants subjected to drought as compared to the control, depending on the virological status of the plant and the position on the cane, when wilting was visible on the leaves at the top of the cane

Grapevine virological status	Variants	Leaf position on the cane	Total water (%)	SO \pm sd (μ m)
Healthy	Control	Base	74.195 \pm 1.492	5.15 \pm 0.3536
		Tip	76.375 \pm 0.7707	5.35 \pm 0.9192
	Water stress	Base	74.975 \pm 7.77E-02	6.25 \pm 1.0607*
		Tip	76.865 \pm 1.3081	6.10 \pm 1.8385
GFkV- infected	Control	Base	74.440 \pm 0.7071	4.80 \pm 0.000
		Tip	74.750 \pm 1.6405	4.75 \pm 0.212
	Water stress	Base	75.465 \pm 0.5728	4.20 \pm 0.5657*
		Tip	75.780 \pm 0.2546	5.00 \pm 0.4243

*significant difference at $P < 0.05$ between the experimental variants

Table 3. Variation of the total water content, the stomata opening and the content of assimilating pigments, in the leaves of plants subjected to drought as compared to the control, depending on the grapevine virological status and the position on the cane, when the drought induced the dry of the leaves on the lower half

Grapevine virological status	Variants	Total water (%)	SO ± sd (µm)	Chl a (mg/g f.w.)	Chl b (mg/g f.w.)	Carotenoids (mg/g f.w.)	Chl a/Chl b
Healthy	Control	74.6 ± 0.4808*	5.15 ± 0.6364*	1.082 ± 3.111E-02	0.566 ± 1.838E-02	0.820 ± 0.112	1.912 ± 7.071E-02*
	Water stress	69.76 ± 0.3677*	6.75 ± 0.3536*	1.025 ± 5.869E-02	0.601 ± 2.404E-02	0.736 ± 6.858E-02	1.706 ± 2.757E-02
GFkV - infected	Control	73.8 ± 0.4879	5.4 ± 0.1414	1.459 ± 0.20718	0.648 ± 0.1272	0.911 ± 0.128	2.265 ± 0.12445*
	Water stress	72.44 ± 1.6263	6.1 ± 7.071E-02	1.388 ± 0.239	0.680 ± 5.656E-02	0.947 ± 0.115	2.034 ± 0.18314

*significant difference at P < 0.05 between experimental variants at the same indicator

The water elimination takes place mainly through the ostioles of the stomata. Variations in the degree of ostioles opening may be due to the variations in the turgor of the stomatal cells and variations in the turgor of the epidermal cells adjacent to the stomata. The passage of water from the epidermal cells to the stomatal ones determines the increase of the ostioles opening degree. The temperature decisively influences the sweating process in the sense that as the temperature increases, the perspiration intensifies to an optimum, around 30°C. Above the optimum temperature, perspiration decreases sharply to a temperature of 45°C, and the further increase in temperature becomes pathological due to damage of the protoplasm. The cell permeability increases, the stomata increase again the degree of opening, the tissues wither and the plant dies, the water in it continuing to be lost by evaporation. On the other hand, the lack of water in the soil causes the stomata to decrease the degree of opening of the ostiole to limit water loss, increase osmotic pressure, increase respiration, reduce the intensity of photosynthesis (Boldor et al., 1981).

The high temperature in the greenhouse and the progressive water decrease of in the vegetation pots determined an imbalance between the physiological processes in the studied grapevine plants.

When the first obvious signs of wilting of the leaves located at the top of the shoots appear, in healthy plants the water stress seems to cause a higher opening of the stomata along the entire length of the shoot while in virus-infected plants an increase can be observed only in the upper part of the plant (values are not

statistically assured). The degree of stomata opening can be influenced by the presence of virus infection as the average values of ostiole width are higher in healthy plants, even to those under water stress (values are not statistically assured). The average value of stomata opening was significantly lower at P < 0.05 for the leaves at the base of the shoot of virus-infected plants subjected to water stress as compared to the same level of healthy plants without water (Table 2).

When the lack of water in the soil determined the maintenance of the foliar apparatus only in the upper half of the shoots in plants subjected to water stress, there was a higher opening of the stomata both in healthy and virus-infected plants, significant differences as compared to the control at P < 0.05 registering in healthy plants (Table 3).

GRSPaV-infection caused a profound change in the expression of genes involved in hormone metabolism. In addition, a significant overlap of cellular responses was observed between GRSPaV-infection and abiotic stress, such as water deficiency and salinity (Gambino et al., 2012).

Assimilating pigments

Another approach to quantifying the effects caused by the lack of water in the soil on grapevine was the assessment of the photosynthesis process by monitoring the content of chlorophyllous pigments and carotenoids.

Photosynthesis is influenced by the degree of hydration of the assimilating tissues. At water deficits higher than 13%, the process progressively decreases and stops at a water deficit between 40-50%. Research on the influence of the amount of assimilating pigments in

chloroplasts did not lead to a correlation between the amount of pigments and the intensity of photosynthesis. The amount of assimilating pigments becomes a limiting factor only in cases of etiolation of plants caused by lack of light (Boldor et al., 1981).

In terms of eco-physiological performance, the presence of double infection with GLRaV-1 and *Grapevine virus A* (GVA) penalized all the parameters involved, as compared to the healthy plants. The reduction of photosynthetic activity has been evident since the early assessment and has increased during the summer. When only GVA infection was present, the reduction of photosynthesis was delayed when climatic conditions caused water stress. However, the difference between GVA-infected and healthy grapevine in the physiological behaviour of plants was less important as compared to that in which double infection was involved (Couzzo et al., 2018).

In our experiment there were no correlations of the concentration of chlorophyllous pigments and carotenoids in the leaves located in the upper part of the half-dried shoot, with the lack of water in the soil. Viral infection appears to influence the Chl a/Chl b ratio. In GFkV-infected control plants, a significantly higher value was recorded at $P < 0.05$ as compared to healthy control plants. Water stress did not significantly influence this parameter (Table 3). The combination between GLRaV-3 infection and water stress decreased most physiological parameters in vines Banyalbufar and Giro ros Malvasia varieties, but did not increase further the effects on plant development or parameters regarding the gas exchange at the level of leaves, as compared to the individual water stress.

At the metabolic level, responses to combined stress were specific and are not anticipated quantitatively from the sum of responses to each unique stress. Specific adjustment of respiratory metabolism may explain the maintenance of carbon balance in the leaves and the growth of the grapevine under conditions of combined stress (El Aou-ouad, 2017).

Further studies will complete the knowledge regarding the combined drought and virus-infection effects on the quality and quantity of grape production.

CONCLUSIONS

In our study, the degradation processes of the grapevine *V. vinifera* L., Tămâioasă românească selection, subjected to water stress, began with the wilting of the leaves from the upper to the lower part of the cane, followed by the leaves drying that started with the mature ones located at the base of the plant. The lack of water in the soil induced the decrease of the absorption capacity through the roots and the aerial part, continuing to perspire, determined an imbalance expressed by the withering of the leaves, influenced also by the mesophilic structure and the degree of cuticle permeability. The first signs of water stress appeared after 10 days of water stress, the process being reversible if the plant still has enough foliar apparatus to resume physiological processes. The complete drying of the plants was registered after 37 days of water deficit, in a restricted vegetation volume and subjected to maximum daily temperatures of over 40°C.

The soil temperature in the vegetation pots that did not exceed 30°C, being within the range that allows the growth of the grapevine roots.

GFkV infection induced fiziological modification during the progressive water deficit. The wilting process expressed by lowering the leaf angle was more pronounced in virus-infected plants. Also, the loss of turgor of tissues affected the dynamics of the stomata opening, the por being smaller in infected grapevine.

The management of the grapevine crop in the current climate changes involves the interaction between genetic resources and phytosanitary status in order to obtain quantitative and qualitative productions.

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