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Grape variety comparison of different stress tolerance based on the quantitative measurement of carbohydrates

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Summary: The analyses of various host-pathogen relationships have established the response reaction roles of carbohydrates – especially monosaccharides – measurable in the vegetal parts of the host. Published results also provide information concerning the way various pathogens utilize carbohydrates and concerning the carbohydrates pathogens prefer out of the "selection" provided by the host plant. The role of carbohydrates in the response reactions to abiotic stress has been studied on several plant species as well – currently, too, it is an often discussed area of research. The above-mentioned results form the basis of our intention to study the connection between susceptibility to grey mould and the quantity of measurable carbohydrates in the leaves of grape varieties of various stress tolerance levels.

Key words: *Botrytis cinerea,* carbohydrates, chromatographic separation, grape, stress tolerance

Introduction

Abiotic and biotic stress factors cause various changes in the endogenous compounds of plants, for example, quantitative changes in soluble proteins and carbohydrates and quantitative and qualitative changes in certain amino acids and carbohydrates. (*Pius* et al, 1998).

The grape breeding objectives of accentuated importance over the past years have included the production of varieties of higher cold and frost tolerance levels and the production of drought resistant varieties. Due to the numerous factors influencing frost tolerance, it is difficult to evaluate the results of outdoor experiments and to compare the varieties on the basis of these results. Artificial freezing test methods, in consequence of their regulatability and replicability, provide a more reliable possibility for the comparison of the varieties (*Pedryc* et al, 2004). One of the most significant and frequently studied factors is the relationship between carbohydrate transformation, concentration change due to winter or artificial frost and the frost tolerance of the varieties (*Barka & Audran*, 1996; *Korbuly* et al, 2000; *Pedryc* et al, 2006).

The effect of drought on grape plants results in different stress reactions in the different varieties. Lack of water causes the berries to become smaller with thicker skins and, as in the case of other stress effects, endogenous compounds undergo significant quantitative and qualitative changes.

Downy mildew (*Plasmopara viticola*), powdery mildew (*Uncinula necator*), and *grey mould* (*Botrytis cinerea*) are the most widespread grapes diseases of fungal origin, which may cause a significant decrease in the quantity and quality of grape production in susceptible varieties. The extent of damage is primarily determined by the production year and the weather. The most significant factors affecting pathogens are temperature and humidity; infections thrive in warm, humid, rainy weather.

Carbohydrate concentration changes can be related to abiotic and biotic stress effects on the examined plant species. Changes in quantity can be observed during individual plant development and in relation to various environmental influences as well as to resistance mechanisms against various pathogens (e.g.,: *Sárdi* et al, 1996; *Korbuly* et al., 2000; *Pedryc* et al, 2004; 2006).

Results of research reveal how the various pathogens utilize carbohydrates and how they select their preferred carbohydrates from the supply provided by the host plant. *Hevesi* et al (2004) studied how rapidly and which of the examined 49 different carbohydrates could be utilized by Erwinia amylovora. Their results demonstrated that monosaccharides are utilized the most rapidly. *Vercesi* et al (1996) studied the quantitative changes in various carbohydrates and acids in relation to the growth and branching of Botrytis hypha infecting grapes. In the case of higher carbohydrate (glucose, fructose and sucrose) concentrations, the length of growth units of hyphae and their permeation area were significantly enlarged, while high tartaric acid concentrations impeded their growth.

As a result of biochemical and pathological studies of bean plants in different phases of ontogenesis, it has been proved that, in order to correctly interpret the results of experiments concerning host .pathogen relationships, the consideration of the development phase and age of the plant or the examined plant part is of vital importance. These factors influence the manifestation of disease symptoms as well as the response reactions of the plant to stress effects (*Sárdi* et al, 1996). In relation to ontogenesis, the carbohydrate concentration changes in the different developmental phases of the acorn embryos of the pedunculate oak were examined with experiments and carbohydrate fractions of different quantities were identified in the different development phases (*Palada-Nicolau & Hausman*, 2001).

The above published results form the basis of the present experiments addressing the following problems:

- the dependence of carbohydrate concentrations measurable in leaves on the age and development of the studied plant tissues,
- the existence of a connection between the quantity of detectable carbohydrates and the stress tolerance known from production experience of the studied grape varieties,
- the complex purpose of studying the validity of the relationships already proven to exist in regulated circumstances by examining samples from outdoor grape plantations.

Materials and methods

The leaf samples of the varieties susceptible to grey mould (Zwiegelt, Királyleányka, Rizlingszilváni, Chardonnay, Leányka, Mátrai muskotály) and the resistant varieties (Zenit, Zengő, Tramini, Merlot, Cserszegi Fűszeres, Cserszegi Fűszeres) were collected in Nagyréde at the end of August 2007. The leaves were divided into three groups on the basis of their development phases: the youngest leaves of the shoot, young leaves and the lowest leaves (old leaves) of the shoot. The stress tolerance levels of the examined varieties known from production experience are shown in *Table 1*.

Table 1. The stress tolerance levels of the examined varieties

Varieties	Frost tolerance	Drought tolerance	Susceptibility to rot	Other diseases
Zweigelt	mean	mean		
Királyleányka	mean	tolerate	responsive	
Rizlingszilváni	low level	tolerate	responsive	
Chardonnay	fairly above the average	tolerate	responsive	
Leányka	satisfying	tolerate	high responsive	
Mátrai Muskotály	responsive		responsive	responsive to stockdestruction
Zenit	mean or low		slightly responsive	
Zengő	mean		slightly disposed	
Tramini	fairly above the average	tolerate	slightly disposed	
Merlot	frost susceptible	tolerate	response	susceptible to Agrobacterium
Cserszegi Füszeres	tolerance is significant	drought susceptible	relativly well response	slightly resistant to the diseases of fungal
Kékfrankos	fairly above the average	mean	don't decay	

Overpressured Layer Chromatographic separations of carbohydrates. The fresh leaves were frozen with liquid nitrogen, powdered and extracted with methanol (300 mg plant powder/ 800 µl of methanol:H_2O, 80:20, V/V). This suspension was centrifuged at 1500 *g* for 10 minutes at 4 °C. The clear supernatants were used for overpressured layer chromatographic separations (OPLC chromatograph developed by OPLC-NIT Co., Ltd.,Budapest, Hungary). OPLC separations were carried out on TLC and HPTLC silica gel 60 F_{254} (Merck Co.) precoated chromatoplates using acetonitrile:H_2O (85:15, V/V). Staining was performed by aniline – diphenyl amine – phosphoric acid reagent. For densitometric determination a Shimadzu CS-930 TLC/HPTLC scanner (Shimadzu Co., Kyoto, Japan), =540 nm was used.

Results

Under the given measurement conditions, glucose, fructose and sucrose were detectable in the grape leaves. There were significant differences measured in correlation with the ages of leaves and with susceptibility to grey mould. The figures show the order of varieties with the first 6 places occupied by the varieties susceptible to grey mould, while the samples from 7 to 12 represent not susceptible or moderately susceptible varieties. This ordering applies to all of the figures. The data concerning glucose quantities measurable in the leaves of the various varieties are indicated by *Figures 1 and 2*.

A smaller amount of glucose was measured in the young leaves (*Figure 3*) of the varieties not susceptible or moderately susceptible to grey mould than in those of the other group. There is an exception to this observation: in the non-susceptible group, Merlot displays the highest glucose content, in other words it has proved to be "less resistant" in comparison with the other varieties. Considering sensitivity to other stress effects (*Table 1*), it is easy to see that this variety is frost sensitive and it has average drought tolerance, while the other varieties in the group are not frost sensitive and several of them can tolerate drought – in other words the lower level of stress resistance in Merlot in comparison with other varieties of low glucose content, non-susceptible to grey mould is evidenced by production experience as well.

Glucose concentrations measurable in the youngest leaves and the older leaves show no connection with the tolerance level of the examined varieties.

The quantity proportions of fructose among the varieties are similar to those of glucose. Considering the ages of leaves, the tendency is the same in the case of fructose levels as in the case of glucose. The glucose and fructose contents of the youngest leaves

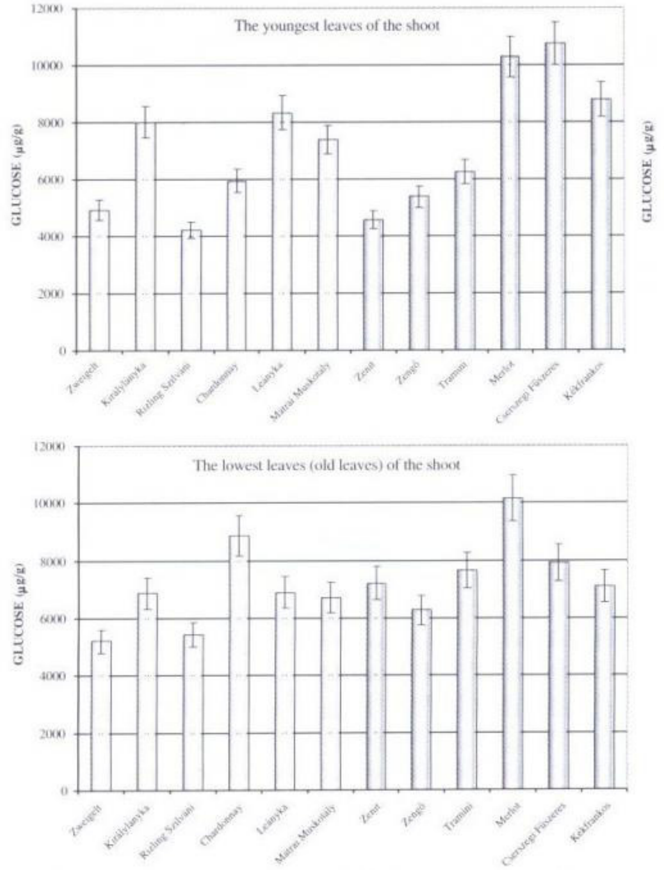

Figure 1 The glucose content measurable in the youngest and oldest leaves of the given shoot

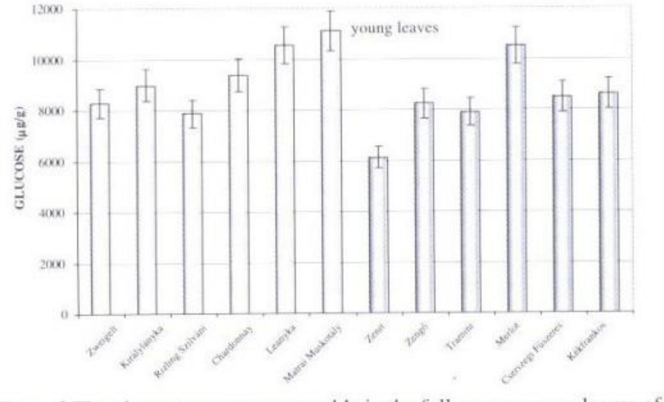

Figure 2 The glucose content measurable in the full-grown young leaves of the given shoot

are lower than in young leaves; and the glucose and fructose quantities measured in the old leaves are higher than those measured in the youngest examined leaves. The amount of sucrose could only be evaluated in the young and old leaves, while its amount in the youngest leaves is not detectable under the given measurement conditions.

Discussion

It has been demonstrated that comparing vegetal parts of identical ages and development phases has vital importance regarding the interpretability of results (*Sárdi and Stefanovits-Bányai,* 2006). In the works cited, individual plant development was studied by the examination of various vegetal parts of young plants grown in plant growing chambers, in regulated circumstances. In these cases, the different development paces of the varieties were not manifested due to the early development phase. In the present research – comparing grape leaves from outdoor plantations – it was difficult to ensure the above conditions, i.e., the identical ages of shoots of the examined varieties and the identical development phases of leaves of different varieties. The samples originated from a plantation that had been producing grapes for several years while sampling was carried out at the same time, which cannot have resulted in leaves of completely identical development phases. Due to the different development paces of varieties, it was not even possible to collect the youngest leaves in completely identical development phases. These circumstances must by all means be taken into consideration when the results are interpreted.

Published results (e.g. *Sárdi* et al, 1996, 1999; *Villanueva* et al, 2004.) confirm that the concentrations of various carbohydrates change during ontogenesis and significantly depend on the development phase of the examined plant. The results of the present research confirmed the same by the examination of grape leaves. It has been established by the results of the present research that the glucose concentration measured in grape leaves of various ages is the highest in the young leaves. Young leaves have higher concenrations of fructose and sucrose as well than the older leaves. In conclusion, the results based on outdoor sampling have successfully demonstrated correlations identical with those resulting from former studies of other plant species grown in regulated circumstances, with optimal conditions of sampling ensured.

The measurement data also demonstrate a correlation between the carbohydrates measurable in grape leaves and the grey mould sensitivity of the varieties or rather their stress tolerance related to production experience. The results indicate that, of the leaves of various ages, the young leaves are the most suitable leaves for the purpose of comparison and the glucose quantities measurable in these leaves correlate with the stress tolerance of the varieties. The young leaves of the varieties susceptible to grey mould are characterised by higher glucose concentration than those of the resistant varieties. The measurement data relating to 9 of the examined 12 varieties completely correspond to the above statement while in the case of 2 varieties the differences can be explained by considering the tolerance levels of the varieties regarding other stress effects. It is important to note that the above correlations resulted from the examination of samples collected form a production site.

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