# STIMULATION OF STILBENE POLYPHENOL BIOSYNTHESIS, UNDER *IN VIVO* CONTROLLED CONDITIONS, IN SOME GRAPE VARIETIES FOR RED AND WHITE QUALITY WINE

## STIMULAREA BIOSINTEZEI POLIFENOLILOR STILBENICI ÎN CONDIȚII CONTROLATE *IN VIVO* LA UNELE SOIURI DE STRUGURI PENTRU VINURI ROŞII ŞI ALBE DE CALITATE

***BEJAN Carmen*[1], *VIŞOIU Emilia*[1]**
e-mail: cabej2003@yahoo.fr

***Abstract.*** *It is known that, in response to stress (physical or chemical elicitors, a parasitic infection), the vine can synthesize natural molecules, generally called phytoalexins, enabling it to adapt to this stress. Stilbene polyphenols are the major constituents of these molecules, with the resveratrol (trans-3, 5, 4-trihydroxystilbene), as the major one. Among the chemical agents able to induce the synthesis of resveratrol in the grape-vine plants they come in contact with, the aluminium chloride is the most effective. The undertaken study aimed to determine the active usable doses of $AlCl_3$, able to stimulate the biosynthesis of polyphenols and implicitly of resveratrol in the vine plants, under in vivo controlled conditions. Several Vitis vinifera L. genotypes for obtaining red and white quality wines were selected for this purpose. Four different experiments, with different doses of $AlCl_3$ aqueous solution, were initiated in order to determine the optimal concentration of aluminium chloride solution, which would lead to the accumulation of large amounts of resveratrol in the vine plants.*

**Key words:** resveratrol, elicitors, (grape-) vine

***Rezumat.*** *Se cunoaşte faptul că, viţa de vie poate sintetiza, ca răspuns la un stres (agenţi elicitori fizici sau chimici, o infecţie parazitară) molecule naturale, denumite generic fitoalexine, care îi permit să se adapteze acestui stres. Polifenolii stilbenici reprezintă constituenţi importanţi ai acestor molecule, resveratrolul (trans-3,5,4-trihidroxistilben), fiind reprezentantul principal. Printre agenţii chimici capabili să inducă sinteza resveratrolului în plantele de viţă-de-vie cu care vin în contact, clorura de aluminiu este cea mai eficientă. Studiul efectuat a avut scopul de a determina dozele active utilizabile de $AlCl_3$, capabile să stimuleze biosinteza polifenolilor şi, implicit a resveratrolului, în plantele de viţă-de-vie, în condiţii controlate in vivo. În acest scop au fost selectate câteva genotipuri Vitis vinifera L. destinate obţinerii de vinuri roşii şi albe de calitate. În vederea stabilirii concentraţiei optime de soluţie de clorură de aluminiu, care să conducă la acumularea unor cantităţi importante de resveratrol în plantele de viţă de vie, s-au iniţiat patru variante experimentale, utilizându-se doze diferite de soluţie apoasă de $AlCl_3$.*

**Cuvinte cheie:** resveratrol, agenţi elicitori, viţă-de-vie.

[1] National Institute of Research and Development for Biotechnology in Horticulture Stefanesti - Arges

## INTRODUCTION

Like the other plants, in response to a biotic or an abiotic stress, the vines can synthesize natural molecules, generally called phytoalexins, enabling them to adapt to that stress. The phytoalexins are antibiotics formed in the vine tissues, as a result of the interaction of the two metabolic systems. The initiation signal for the phytoalexins synthesis is given by the "elicitors". They can be biotic (organic molecules present in parasites, peptides, glycoproteins) or abiotic (some metals, detergents, UV radiation, heat, cold). Stilbene analytical interest was primarily due to their role as natural fungicides. Recent studies show that the trans-resveratrol in physiological concentrations is an effective fungus toxic against *Botritys cinerea* (Adrian M. et al., 1997, Jeandet P. et al., 1995). Moreover, they show that stilbene polyphenols such as resveratrol pterostilbene and ε-viniferine are involved in the vine resistance mechanism against other pathogens too, such as *Plasmopara viticola* (Dai et al., 1995) and *Phomopsis viticola* (Hoos and Blaich, 1990). In this context, it is of interest to stimulate the vine defence mechanisms against the phytopathogenic agents like fungus (*Plasmopara viticola* and *Oidium necator*), by inducing the stilbene polyphenol synthesis of in the grapevine leaves.

In addition, it is also currently highlighted the role of viticultural phytoalexins in the human metabolism (Hain et al., 1993, Jeandet et al., 2002) with stress on the importance of phytoalexins in the phenolic compounds group, whose major representative in the human health is the resveratrol.

The objective of this study is to stimulate the stilbene polyphenols biosynthesis (resveratrol, in particular) in the leaf by elicitors ($AlCl_3$), when the vine is cultivated under *in vivo* controlled conditions, without letting the aluminium salt have a harmful effect on the vine plants.

## MATERIAL AND METHOD

The experiment was conducted in controlled growing conditions (greenhouse), taking into account the fact that this model behaves as the vine plant in the vineyard. Varieties of vines, intended for the red and white wines, were tested during this study, to quantify the amounts of total polyphenols and resveratrol produced by the plant after treatments with different concentrations of aluminium chloride. Schematically, the experimental factors were:

*1. The concentration of $AlCl_3$ solution with the graduations:*

$V_1$ – control plant; $V_2$ - 1% $AlCl_3$; $V_3$ - 2% $AlCl_3$; $V_4$ - 4% $AlCl_3$

*2. The variety:*

Varieties for red wines: $B_1$ - Burgundy, $B_2$ – Busuioaca de Bohotin, $B_3$ - Cabernet Sauvignon, $B_4$ - Cabernet Franc, $B_5$ - Feteasca neagra, $B_6$ - Pinot noir, $B_7$ - Merlot, $B_8$ – Negru aromat;

Varieties for white wines: $S_1$ - Neuburger, $S_2$ - Tamaioasa romaneasca, $S_3$ – Chardonnay.

Plants propagated through one stem cell cuttings were used. The cuttings were invigorated in pots with an improved nutrient substrate (fertilized soil -30%, -30% forest land, 30% peat soil, sand or perlite 10%), according to the vine propagation technology. When plants developed (10-12 leaves), treatments with different concentrations of $AlCl_3$ aqueous solution, were applied by fine spraying. Three

treatments were performed at an interval of one week; the samples, consisting of leaves collected from the same level of the shoots, were taken after the last treatment with $AlCl_3$. They were rinsed with demineralised water and dried in an oven (90ºC) for the biochemical analysis. The biosynthesis of the phenolic compounds from the plant material in the intermediate metabolism was quantified by determining the content of the total polyphenols and resveratrol in the experimental variants.

The working protocol on the quantity analysis of the total polyphenols and resveratrol produced in the leaves involved several steps: extraction of polyphenols from the plant material and purification of the obtained extract and the TPP analysis by UV/Visible spectrophotometer. The resveratrol HPLC dosage was performed with a Merck-Lachrom UV detector-chromatograph for liquids under pressure.

## RESULTS AND DISCUSSIONS

The performed analyses aimed to see the amounts of total polyphenols and resveratrol biosynthesised in the plant as a result of the treatment with $AlCl_3$, used as an elicitor. Since the differences compared to the control sample were not significant in the variants $V_2$ (1% AlCl3) and $V_3$ (2% $AlCl_3$), the results in $V_4$ (4% $AlCl_3$) were interpreted in comparison with the untreated control sample plant. We also point out that no anticriptogamic treatments were applied to the vine cuttings, kept under controlled conditions, during the experiment, even if there were favourable conditions for fungal infection. In this way, the tolerance of the experimental varieties was also tested against the phytopathogenic fungi attack of *Plasmopara viticola* and *Uncinula Necator*. It is noted that the grape-vine plants of the tested varieties had no infections throughout the experiment and up to the leaf fall, and the beginning of their rest period, as seen in Figures 1 and 2, made when sampling leaves for the biochemical analysis were taken.

**Fig. 1-2** - Vine cuttings to which the trans-resveratrol biosynthesis was induced by applying different concentrations of $AlCl_3$ aqueous solution treatments

The histogram shown in figure 3 shows the variation of the total polyphenols in the tested group of the red grape varieties. The TPP content is between 2.27 - 10.9% s.u. in the control plants, the varieties of Negru aromat, Feteasca neagra and Merlot standing out by a high content of polyphenols. The foliar treatments to the elicitor ($AlCl_3$ solution in different concentrations) increased the polyphenols content in all the tested varieties, significant increases occurring in the variants $V_3$ (2% $AlCl_3$) and, especially $V_4$ (4% $AlCl_3$) in the varieties of Negru aromat (12.8%), Merlot (10.85%) and Pinot noir (9.96%).

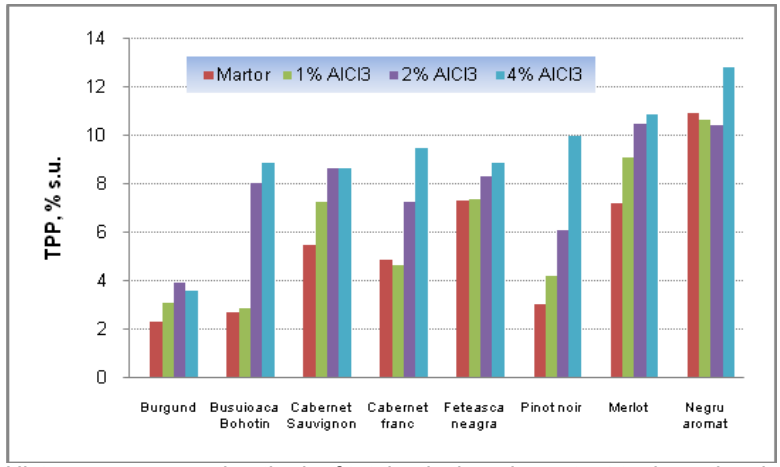


**Fig. 3 –** Histogram representing the leaf total polyphenols content to the red varieties with $AlCl_3$ as elicitor compared with the control plant

Resveratrol (trans-3, 5.4 "-trihidroxistilben) is a low molecular weight compound, a constituent of phytoalexins, belonging to the family of stillbenes polyphenols. As it is known, the stillbenes play an important role in grape-vine protection against fungal infections.

As with polyphenols, the amount of resveratrol produced by the plant as a result of the action of the elicitor, increases according to the vine variety. The histogram in figure 4 shows the trans-resveratrol content of the red varieties with 4% $AlCl_3$ as elicitor, compared with the untreated control plant. The Negru aromat variety has had a nearly four-time increase of resveratrol, and the Pinot noir variety has tripled its content of this compound after the treatment with aluminium chloride. A significant increase, similar to the variation in polyphenols, was also recorded for the Cabernet and Feteasca neagra varieties.

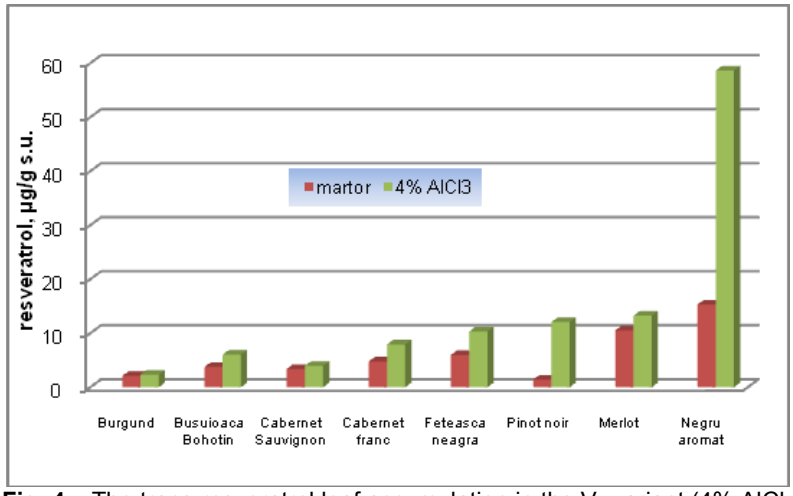


**Fig. 4 –** The trans-resveratrol leaf accumulation in the $V_4$ variant (4% $AlCl_3$) in red grape tested varieties

It may be pointed out that in the group of red grape varieties with elicitors, significant amounts of resveratrol produced in the secondary metabolism under the action of aluminium chloride were found only in five genotypes. They are: Negru aromat, Pinot noir, Feteasca neagra, Cabernet franc and Merlot.

Although the synthesis of resveratrol, as a result of the action of stress has been reported especially for the red grape varieties, the white grape varieties are also capable of producing varying amounts of resveratrol following the use of an elicitor ($AlCl_3$, in this case).

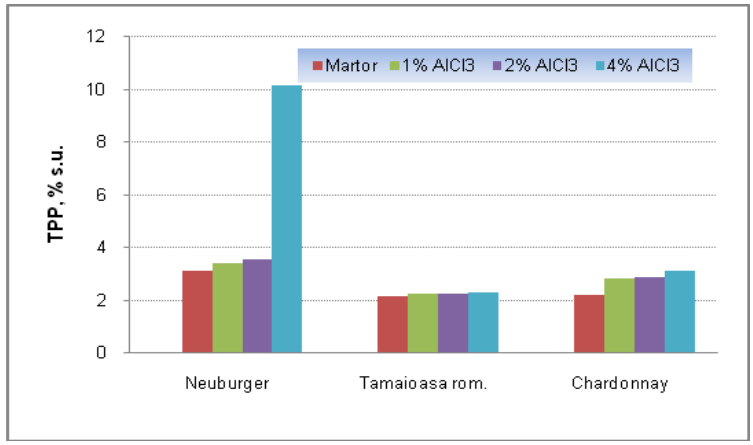


**Fig. 5 –** Histogram representing the total polyphenols leaf content to the white grape varieties with $AlCl_3$ as elicitor compared with the control plant

The histogram in figure 5 shows the variation of total polyphenols in the tested group of the white grape varieties. The TPP content is between 2.14% (Tamaioasa romaneasca variety), and 3.14% (Neuburger variety). The treatments with $AlCl_3$ solution had a noticeable effect on the biosynthesis of polyphenols only for the Neuburger variety ($V_4$ - 10.18% total polyphenols). The accumulations of these compounds to the other white grape varieties were quite low.

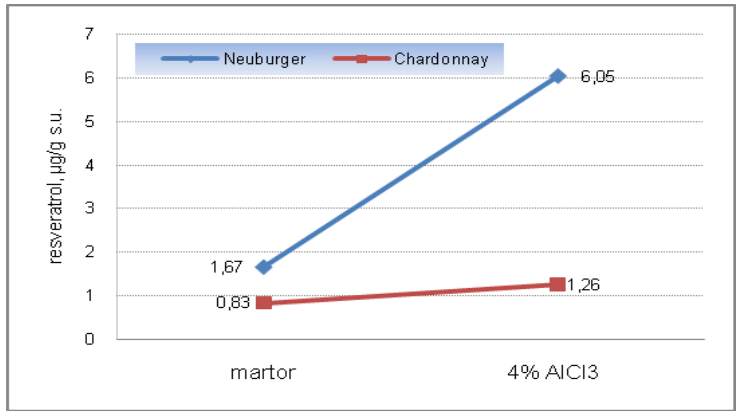


**Fig. 6 –**The trans-resveratrol leaf accumulation in the $V_4$ variant (4% $AlCl_3$) for two white grape varieties

Among the tested control plants, the Chardonnay variety has the lowest content of trans-resveratrol. The control plant for the Neuburger variety had content of 1.67 $\mu g.g^{-1}$ s.u. and after treatments with the $AlCl_3$ solution in concentration of 4%, the resveratrol content tripled, reaching a value of 6.05 $\mu g.g^{-1}$ s.u. Under the same conditions, in the Chardonnay variety (and the other white grape varieties under the experiment) the resveratrol biosynthesis was lower (figure 6).

## CONCLUSIONS

1. The foliar use of 4% solution of $AlCl_3$ to the vine plants intended for the *in vivo* production of the red wines, lead to the generation of large quantities of polyphenols, their levels being higher in the plants with elicitors compared to the control plants;

2. The biosynthesis of resveratrol under the action of an elicitor agent (4% $AlCl_3$) occurred with greater intensity in the Negru aromat variety, the quantity of trans-resveratrol synthesised in the leaves reaching a value of 58.64 $\mu g \bullet g^{-1}$ s.u.;

3. Among the varieties designed to provide high quality red wines, the following varieties have shown a greater capacity to synthesize trans-resveratrol, under the action of 4% $AlCl_3$ solution: Negru aromat, Pinot noir, Feteasca neagra, Cabernet franc and Merlot.

4. The 4% $AlCl_3$ treatments in greenhouse conditions, led to the tripling of the total polyphenols concentration in the leaves of the Neuburger variety;

5. Under *in vivo* culture conditions, the ability of the white grape tested varieties to produce resveratrol is lower compared with the red grape varieties, the Neuburger variety proving to be the most capable in this respect.

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