RESVERATROL BIOSYNTHESIS *IN VITRO* CULTURE CONDITIONS ON GRAPEVINE (FETEASCA NEAGRA AND CABERNET SAUVIGNON) UNDER THE ACTION OF $ALCL_3$ AS ELICITOR AGENT

BIOSINTEZA RESVERATROLULUI ÎN CONDIȚIILE CULTURII *IN VITRO* A VIȚEI DE VIE (FETEASCĂ NEAGRĂ ŞI CABERNET SAUVIGNON) SUB ACȚIUNEA $AlCl_3$ CA AGENT ELICITOR

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Abstract: *Stilbenes are considered the most important phytoalexin group synthesised in grapevine (Vitis vinifera) and they are known to contribute to the protection against various pathogens. The main typical stilbenes of grapevine which show antifungical and pharmaceutical characteristics are resveratrol and his derivates. Recently study prominence also their benefit in human health by their antifungical, anticancerigen, hypolipidemic and antidiabetic properties. Present study was focused on induction of resveratrol biosyntheses in vitro vine culture conditions under effect of $AlCl_3$ as eliciting agent. Specific medium for vine in vitro multiplication (M&S, 1962 + 1 mg/L BAP +0, 5 mg/L AIA) has been supplemented with different doses of 1% $AlCl_3$ (0, 01%; 0, 03%; 0, 05%). Reservation dosage was made by high performance liquid chromatography (HPLC) with pressure liquid chromatography Merck – Lunchrom and UV detector. Using modified mediums resveratrol synthesis has been intensified at Cabernet Sauvignon cv. up to 97, 94 µg/g soluble solids in plant at the 0,05 % $AlCl_3$ concentration.*

Key words: grapevine, elicitor agent, stilbenes, resveratrol

Rezumat: *Stilbenii sunt consideraţi cel mai important grup de fitoalexine sintetizaţi de vița de vie (Vitis vinifera), fiind recunoscută contribuția lor în protecția acesteia împotriva diverşilor agenţi patogeni. Principalii stilbeni caracteristici viței de vie sunt resveratrolul şi derivatii săi, ei atrăgând atenția prin proprietățile lor antifungice şi farmacologice. Studii recente pun în evidență rolul benefic în sănătatea umană prin efectul lor anticancer, hipolipidemic şi antidiabetic, pe lângă însuşirile fungitoxice cunoscute deja. Studiul de față s-a axat pe inducerea biosintezei resveratrolului în condițiile culturii in vitro a vitei de vie, sub acțiunea $AlCl_3$, ca agent elicitor. Mediul de cultură specific multiplicării viței de vie (M&S, 1962 + 1 mg/L BAP+ 0,5 mg/L AIA) a fost suplimentat cu diferite doze de soluție 1% $AlCl_3$ (0,01%; 0,03% şi 0,05%). Dozarea resveratrolului s-a realizat prin cromatografie lichidă de înaltă performanță (HPLC), cu un cromatograf de lichide sub presiune Merck – Lachrom, cu detector UV. Biosinteza resveratrolului a avut loc cu o mai mare intensitate la soiul Cabernet Sauvignon, cantitatea de resveratrol sintetizată în plantă atingând valoarea de 97,94 94µg/g s.u., la o concentrație de 0,05% $AlCl_3$ în mediul de cultură.*

Cuvinte cheie: vița de vie, agent elicitor, stilbeni, resveratrol

INTRODUCTION

It is known that certain plants can synthesize, in response to stress (UV irradiation or a parasitic infection), natural molecules - generically defined as fitoalexines - enabling them to adapt themselves to this stress (Jeandet, 2002). The grapevine produces large quantities of fitoalexines such as polyphenols – the stillbenes being the most important – as a reaction to a physical-chemical stress (UV radiation, ozone) or a biological one (the *Botrytis cinerea, Plasmopara viticola,* etc. fungi attack). The resveratrol, as the main representative of the stillbenes, is synthesized in the leaves or the skin of the grape seeds, becoming a mediator of the plant defence stimulation, thus contributing to the development of a systemic defence of the grapevine (Iriti, 2004). By analogy, the resveratrol has antifungal properties, resisting against the development of microbial infection caused by *Botrytis cinerea*, but also by other pathogens, like *Phomopsis viticola*, *Rhizopus stolonifer* and *Plasmopara viticola*. If all the grapevine varieties of *Vitis vinifera* are capable of producing resveratrol in different quantities, some of them distinguish themselves in its biosynthesis.

Among the chemical agents able to induce the synthesis of resveratrol in the plants that are brought into contact with, the aluminium chloride is the most effective (Adrian, 1996, Adrian, 2000).

Our studies meant to determine the active $AlCl_3$ doses used for the grapevine multiplication in the *in vitro* conditions. The objective of this experiment is to identify the varieties designed to provide high quality red wines able to synthesize remarkable amounts of resveratrol, the compound involved in the defence mechanism of the grapevine against the phytopathogenic agents (*Botrytis cinerea, Plasmopara viticola, Phomopsis viticola).*

MATERIAL AND METHOD

For testing the resveratrol biosynthesis in the *in vitro* culture, under the action of aluminium chloride, two grapevine clones were selected, designed to provide high quality red wines, namely: Feteasca neagra clone 6 St. and Cabernet Sauvignon clone4 Is. To this end, cultures with additional elicitor agent ($AlCl_3$) in their medium were initiated.

Schematically, the experimental factors are:

A. The $AlCl_3$ doses:

M – Control dose (Romanian Control Variant)

A_1 - 0.01% $AlCl_3$

A_2 - 0.03% $AlCl_3$

A_3 - 0.05% $AlCl_3$

B. The grapevine variety:

B_1 - Feteasca neagra clone 6 St.

B_2 - Cabernet Sauvignon clone 4 Is.

The biological material (apexes with intense regeneration of 0,3 to 0,5 cm) used for the multiplication through *in vitro* culture, was taken from plants growing in pots under controlled vegetation conditions. The disinfection of the explants was carried out with calcium hypochlorite ($CaCl_2O_2$ 6%), under sterile

conditions in laminar flow hood for 5 minutes.

The assessment of the regeneration and multiplication processes was carried on the culture medium used for the initiation and multiplication stages and which had in its composition the Murashige-Skoog medium (M&S,1962), supplemented with 1mg/l N 6-benzilaminopurine (BA) and 0,5 mg/l β-indole acetic acid (IAA). During multiplication, the citokinine/auxine ratio changed to 1:1 (0.5 mg/l BA and 0.5 mg/l IAA), in order to balance the multiplication and elongation processes. Sucrose (20 g/l) was used as a carbon source and agar-agar in the amount of 6.2 g/l, was used for the solidification of the culture media.

According to the general recommendations for the *in vitro* grapevine multiplication, the media pH was adjusted before autoclaving to a value in the range 5.7 to 5.8. The inoculation and transfer operations on fresh media were carried out in sterile areas, in laminar flow hoods.

After stabilizing the cultures meaning three subcultures (60-65 days), the explants formed adventitious buds which developed into young shoots groups of about 1.5 - 2 cm, under the influence of hormone components in the culture medium. The young shoots selected in these groups were seized in fragments of 0,5-1cm, and were the biological material used in the culture media, supplemented with aluminium chloride.

In the experimental variants, the aluminium chloride, in 1% aqueous solution, was added in the culture medium in various doses, the final concentration of the medium in Al^{3+} being of 20 ppm (A_1), 60 ppm (A_2) and 100 ppm (A_3). After neutralization with 10% KOH, the culture medium was sterilized at a temperature of 120^oC in an autoclave for 20 minutes (pressure of 1 at).

The regeneration and multiplication processes of the biological material were conducted under controlled conditions, in growth rooms with possibility of adjusting to climate factors (temperature of 25 ± 1^0C; photoperiod and light within 16 hours of light and 3000-3500 lx).

The biosynthesis of the phenolic compounds in the plant material during intermediary metabolism was quantified by determination of the total polyphenols and resveratrol contents in the experimental variants. The presence of the resveratrol was quantitatively shown by liquid chromatography and UV detection at 306 nm.

RESULTS AND DISCUSSIONS

The observations on the vegetative formations regenerated in the explants inoculated in the *in vitro* culture, from both grapevine clones, showed that there is uniformity in obtaining the multiplication indices (adventitious buds, number of primary shoots and shoots) by using the M& S medium as the basic one, improved with 0.5-1mg/l BA and 0.5 mg/l IAA, compared with the medium variants supplemented with aluminium chloride.

The process of the young shoots proliferation was followed periodically. A strong multiplication was remarked in both clones studied (groups of 6-8 primary shoots/young shoots fragment) and a clear elongation in the A_1 variant, with the lowest aluminium salt content (0.01%). The multiplication was lower for the other two experimental variants, but with increased ascension of the young shoots.

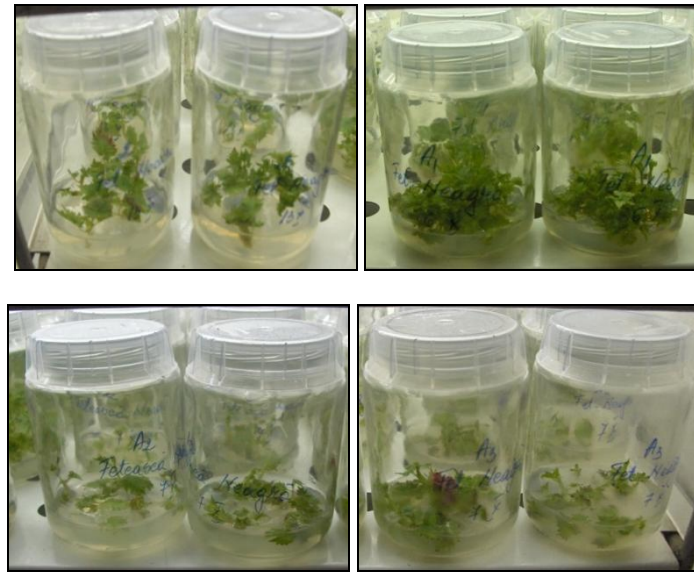

Fig. 1÷4. Evolution of the explants in the four experimental variants - Feteasca neagra clone 6 St. (*detailed*)

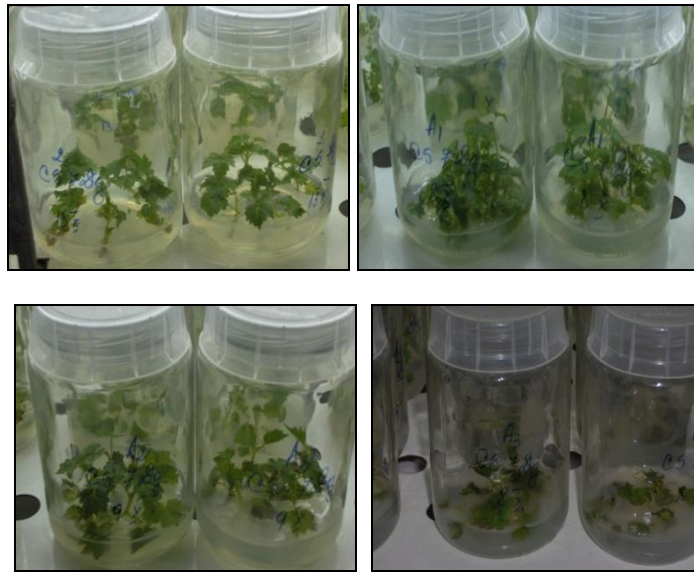

Fig. 5÷8. Evolution of the explants in the four experimental variants - Cabernet Sauvignon clone 4 Is. (*detailed*)

Figures 1÷ 4 and 5÷ 8 shows the detailed results after 20 days the use of the elicitor agent ($AlCl_3$) in the culture medium. The variety of Feteasca neagra clone 6 St. has shown a better multiplication rate in variant A_1 (0.01% $AlCl_3$) compared

with the same variant of Cabernet Sauvignon clone 4 Is.

At higher concentrations of aluminium (60 ppm and 100 ppm Al^{3+}) in the culture medium the rate of multiplication decreased, the elongation being favoured for the young shoots. For the A_2 but especially A_3 variants, a slight reddening of the leaves was also observed, due to the anthocyans biosynthesis.

The chemical analyses conducted on the collected material from the experimental variants showed an increased content of total polyphenols and resveratrol in the plants grown on the medium containing the elicitor agent.

Increasing concentration of $AlCl_3$ in the culture medium for the Feteasca neagra variety led to a significant increase in total polyphenols content in the grapevine plants (figure 9).

Similarly, a content of 0.05% of the elicitor agent in the culture medium almost doubled the content of resveratrol in A_3 variant, compared with the control variant (Mt.) - figure10.

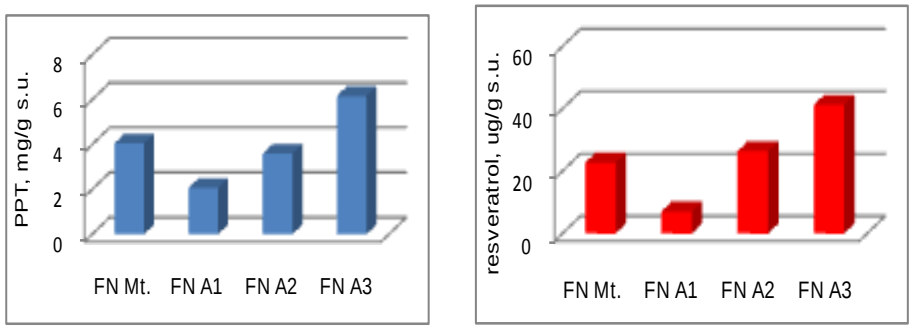

Fig. 9-10. Total polyphenols and resveratrol content in grapevine plants multiplied *in vitro* (Feteasca neagra clone 6 St.)

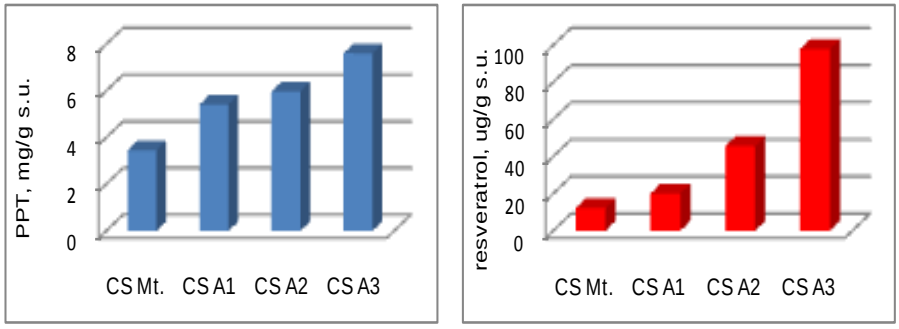

Fig. 11-12. Total polyphenols and resveratrol content in grapevine plants multiplied *in vitro* (Cabernet Sauvignon clone 4 Is.)

The variety of Cabernet Sauvignon had a better response to the treatment with $AlCl_3$, meaning that both total polyphenols and resveratrol contents increased with increasing the dose of elicitor added in the culture medium (figures

11 and 12). Furthermore, the resveratrol biosynthesis was increased in this variety, compared with the variety of Feteasca neagra. Thus, the resveratrol content reached up to 97.94 μg/g d.m. in the A_3 variant compared to 41.3 μg/g d.m. recorded in the similar variant of the variety Feteasca neagra. Is remarkable that, in the plants belonging to the control variant, the resveratrol content was significantly higher in the Feteasca neagra variety compared to the Cabernet Sauvignon one.

CONCLUSIONS

1. To highlight the influence of aluminium chloride in the biosynthesis of resveratrol *in vitro* culture conditions of the grapevine, a laboratory procedure was initiated and the $AlCl_3$ 1% solution was added in the culture medium in various doses;

2. The tested varieties responded to the addition of the elicitor agent in the culture medium by showing an increase of the total polyphenols content, but especially of the resveratrol one according to the used dose;

3. The resveratrol biosynthesis occurred with greater intensity in Cabernet Sauvignon clone 4 Is. compared with Feteasca neagra clone 6 St., the amount of resveratrol synthesized in the plants reaching the value of 97.94 μg/g d.m., at a concentration of 0.05% $AlCl_3$ (100 ppm Al^{3+}).

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