# ANTITRANSPIRANTS FOR A SUCCESSFUL ADAPTATION OF GRAPE MICROCLONES

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Results of research on application of the antitranspirant group preparations intended to adapt grape microclones to in vivo conditions are presented. It is established that 0.3% and 0.4% concentrations of Vapor Gard and 0.4% concentration of EPAA preparations effectively reduce transpiration intensity, increase a number of viable plants and improve their biometric growth indices.

***Key words**: in vitro, antitranspirants, grape microclones, EPAA, Vapor Gard.*

Nowadays the methods of tissue and organs culture in vitro, in particular the method of clonal micro-propagation [1,2,3] are widely applied in the biotechnological science and agriculture. This method has a number of advantages over conventional methods as it ensures high propagation coefficient, production of healthy seeds, propagation of the varieties that are poorly multiplied by customary ways, excludes cross-infection of plants, removes a probability of import and extension of quarantine objects when introducing plants and allows of preserving gene bank for a long period.

The general scheme of the clonal micro-propagation includes the following stages: selection and sterilization of primary explants; introduction of explants in vitro culture; proliferation of buds and induction of shoot development; rooting and propagation in growing media; adaptation of plants to in vivo conditions.

Adaptation of micro-clonal plants to non-sterile conditions is the most important and final stage of this technology. It is on this stage that a high percentage

of plants that either perish or are damaged have been recorded – according to some estimations only 25% of the regenerated microclones take roots in non-sterile conditions [4].

Research results indicate that the stress suffered by plants at replanting from in vitro to in vivo conditions is due to a long chain of anatomic and physiological features of micro-clonal plants: underdeveloped wax cuticles of leaves, inactive respiratory system, poor photosynthetic activity, vitrifaction and deficient vascular link between the root and the shoot, underdeveloped or absent root fibrillae that complicates uptake and transport of water and nutrients [5,6,7]. Such peculiarities of the microclone structure and the conditions of their cultivation in vitro lead to a high level of the plant leaf transpiration and, consequently, their dehydration and decline [4].

Numerous research works have also been devoted to adaptation of grapes microclones. T.M. Cherevata proposes a method of combining the stages of micro-stalking, growing and adaptation. It is necessary to cultivate plants on a compound of Bioni mix and zeolite in the ratio 3:1 [8]. L.V. Ivanova-Khanina recommends, at the stage when grape shoots adapt to in vivo conditions, to use a mix of turf, sand and soil in the ratio 1:1:1 and with the addition of TERAWET hydroabsorbent in the lower substrate layer [9]. A.M. Rebrov asserts that use of the plant growth regulators facilitates better adaptation of the revitalized grape shoots to the environment. He considers that the preparations of potassium lingo-humate, extrasol, emistim, zircon are the most effective preparations in that sense as well as the use of the natural mineral green earth as a substrate [10]. However it should be noted that the practical application of these techniques facilitates adaptation of the root system and, only partially, the vegetative mass.

Thus, considering all the above it is possible to conclude that the general bulk of research studies is devoted to the problem of plant establishment in vitro, including grapes. Still, practical results prove that the perish ratio of grape shoots grown in tubes (depending on the variety) remains rather high at the stage when the shoots adapt to non-sterile conditions and varies from 30% to 70%.

Considering this fact, the objective of this work was to improve a method to adapt the grape microclones to in vivo conditions by using Vapor Gard and EPAA antitranspirants.

**The material and method of research.** The research was conducted on the microclones of the Chardonnay technical grade in the department of nursery garden and propagation of grapes of the National Scientific Centre «Institute of Viticulture and Wine-Making named after V.Ye. Tairov» during 2006–2010.

Stimulation of the auxiliary buds and apical meristems proliferation processes at the first stage of cultivation was achieved by using modified media Murasige and Skuga with addition of cytokinin 6 – benzylaminopurine (BAP).

At the next stage explants were re-planted in culture containers of greater volume filled with fresh nutritive media with addition of auxin phytohormones. The first and next feedings of plants were made after they have reached 8-10 cm. Even-aged micro-stalks were planted in hormoneless nutritive media or in the media containing minimum auxins. The nutrition stage of grapes microclones was combined with their growing and adaptation on Bion ion-exchange substrate.

The physical parameters inside the cultural box were as follows: temperature 25 - 27$^{o}$C, lighting 800-1,000 lux (during the first week), afterwards: 2,000-5,000 lux and 16 hours photoperiod.

The first stage of adaptation was conducted in a cultural box during 7-10 days. Selected for adaptation were those grape microclones that were 6-8 cm high, had 5-8 leaves and well-developed root system without callus formations. Adaptation of plants to low air humidity was accomplished by opening container lids during 3-7 days and gradually increasing the exposure. Before opening the lids for the first time the plants were sprayed with antitranspirants – Vapor Gard and EPAA – of various concentrations, and the nutritive medium surface was poured in with a thin layer of distilled autoclaved water.

At the next adaptation stage the grape microclones were transferred to the adaptation room where they stayed for 5-7 days more. Then they were re-planted in 150 ml vegetation containers filled with a mix of coconut substrate, vermiculite

and agricultural perlite. After re-planting the grape microclones were sprayed with antitranspirant solution anew.

The research scheme concerning application of Vapor Gard and EPAA preparations at the stage while grape microclones were adapting to non-sterile conditions included such variants: 1 – spraying of microclones with 0.3% Vapor Gard solution, 2 – spraying of microclones with 0.5% Vapor Gard solution, 3 – spraying of microclones with 1.0% Vapor Gard solution, 4 – spraying of microclones with 1.5% Vapor Gard solution, 5 – spraying of microclones with 0.2% EPAA solution, 6 – spraying of microclones with 0.3% EPAA solution, 7 – spraying of microclones with 0.4% EPAA solution and 8 – spraying of microclones with distilled water (control group).

Vapor Gard preparation is referred to the group of antitranspirants. The active agents are: resin spirit and emulsifier. Upon application on the plant, the preparation, in response to light, forms a semi-permeable transparent film facilitating transpiration, contributing to the establishment of plants, reducing the impact of abiotic factors and stress after bedding the plants.

Biological gel EPAA is made on the basis of microbe polysaccharides [11]. The preparation is highly soluble in water, gas high adhesive ability, forms strong films on plants and fixes microbial flora which is useful for plants, assists the plants to withstand draughts and other stresses and stimulates growth of plants. This preparation was produced by the Institute of Microbiology and Virology of the Academy of Sciences of Ukraine.

To establish efficiency of antitranspirants in the course of the study, intensity of transpiration of microclones with the natural leaf area and calculated using 10 $cm^2$ [12], establishment of microclones, their height, number, leaf area and foliage of a plant were determined.

**Research results and their discussion.** It is known that the physiological process of evaporating water by a plant – transpiration – is accomplished from a surface of the main transpiration organs – leaves and stoma. As a result, water potential in the leaf cells is reducing (i.e., the suction force) which leads to a

stronger absorption of water from vein xylems by leaf cells and the movement of water along the xylem, from roots to leaves. Consequently, a high suction force of the leave parenchyma cells creates and supports the performance of the «upper end engine» and the movement of water up the plant. The more intensive transpiration occurs, the greater the «upper end engine» force is. Taking into account that the anatomical structure of the grape microclones is imperfect which takes place due to in vitro conditions, it can be stated that the root system exerts a low suction force and, consequently, an insufficient quantity of water that should move up the plant. Moreover, an unlimited quantity of water is evaporated via the leaf apparatus. Therefore, a lack of coordination between the water absorption and evaporation processes in plants under in vitro conditions is accompanied by their quick decline, particularly when in vivo conditions are substituted for in vitro conditions.

In accordance with the literature references spending of water by leaves of the plants cultivated in vitro can be reduced by applying film-forming preparations [4]. As the obtained results indicate, the grape microclones treated by Vapor Gard and EPAA preparations show a reduced intensity of microclones transpiration in all investigated variants as compared to the control group (Fig. 1).

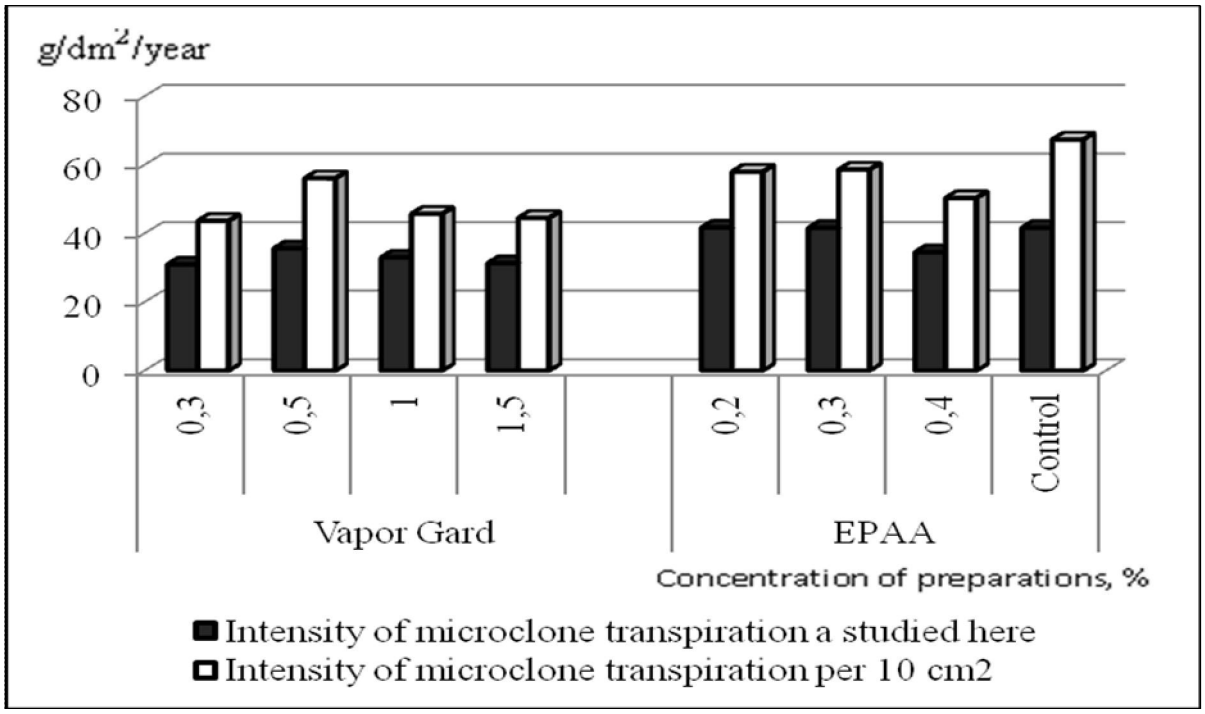


**Fig. 1. Impact of Vapor Gard and EPAA preparations on intensity of grape microclone transpiration**

So, after treating grape microclones with 0.3, 1.0 and 1.5% Vapor Gard solutions the transpiration intensities were at the level of 30.88, 32.93 and 31.27 g/dm$^2$/year. With the control group microclones this index was 1.3 times greater and equalled 41.61 g/dm$^2$/year. After this preparation was applied to the microclone leaf apparatus in a solution of 0.5% concentration, the transpiration intensity was higher, as compared with the first (0.3%), third (1.0%) and fourth (1.5%) variants, by 7.5 – 13.3 %, however it still remained less than the control group index by 14.3%. Following the treatment of grape microclones with 0.2% and 0.3% EPAA solutions, this index was at the control group level. The transpiration intensity was reducing to 34.50 g/dm$^2$/year and equalled the second variant level if the plants were sprayed with 0.4% solution of this preparation.

As the natural leaf area of microclones differed, it can be assumed that the transpiration intensity index depended not only on the preparation or its concentration used but also on the total leaf area of one plant. That is why we calculated this index reducing it to the same leaf area of 10 cm$^2$ (Fig. 1). The obtained results have confirmed the regularity revealed in the study: the least transpiration intensity occurred in the first, third, fourth and seventh variants and amounted to 43.60, 45.69, 44.48 and 50.28 g/dm$^2$/year while in the second, fifth and sixth variants this index was greater by 12.26 – 14.09 g/dm$^2$/year, as compared with the first, third, fourth and seventh variants, and by 8.80 – 11.3 g/dm$^2$/year as compared with the control group.

Effectiveness of any technique is to be appraised by the product output and product quality. In terms of our work we mean establishment of the grape microclones in non-sterile conditions, and their quality (development of a vegetative mass). To this end, in 30 days after re-planting the plants on substrates in cultural containers, we recorded their establishment and obtained the following (Fig. 2).

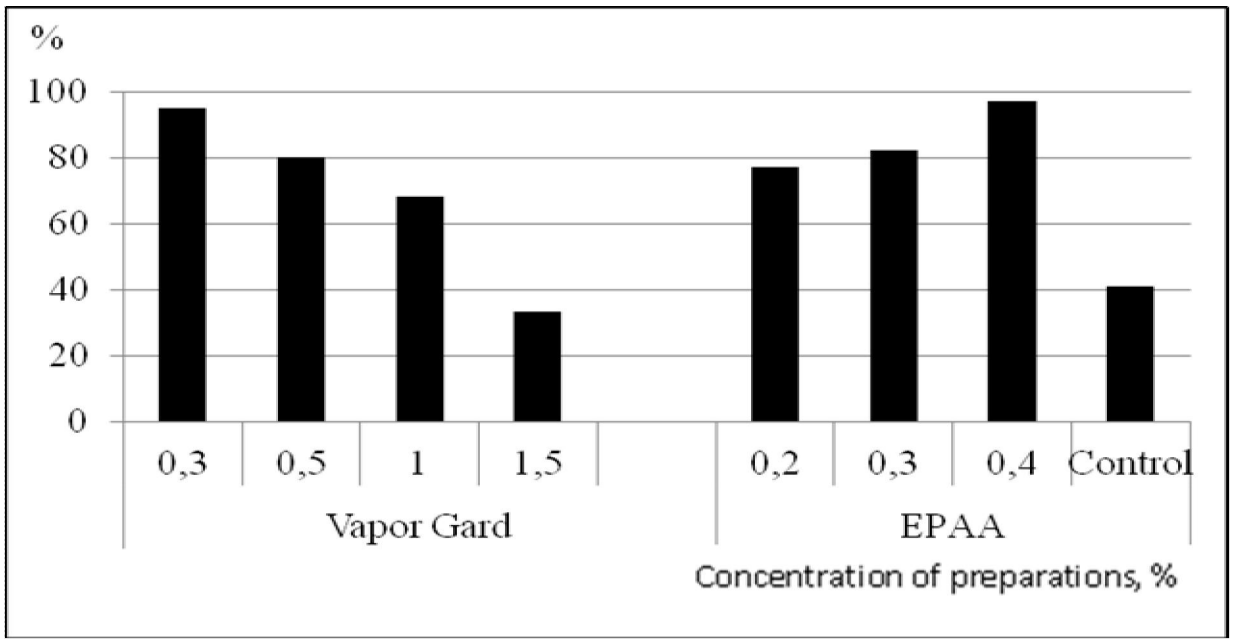


**Fig. 2. Impact of Vapor Gard and EPAA preparations on establishment of grape microclones, (%)**

After treating grape microclones with Vapor Gard solutions of various concentrations use rates the establishment of microclones depended on the concentration of the applied solution. The best establishment was observed with the plants according to variant one, after being treated with 0.3% solution. In variants two and three these indices equalled 80.0 and 68.0%, respectively, which is by 39.1% and 27.1% greater as compared with the control group. Within the control group the grape microclones establishment was the least and occurred at the level of 41%. The least was the index of establishment of the cultural plants, after being treated with Vapor Gard preparation in the greatest use rate of 1.5%, which reached 33.3% - that is by 7.6% less than with the control group. Thus, as the Vapor Gard solution concentration was increasing, the microclones establishment index was reducing. When the plants were treated with EPAA solution, the inverse regularity was noted: the higher concentration of the spray material led to a greater number of viable plants. The best variant according to the grape microclones establishment was observed with the seventh variant (0.4%) where this index equalled 97.0%.

It is known that a nature of growth and a development stage of the assimilation apparatus can influence the basic life-sustaining processes of grapes: photosynthesis, transpiration, breathing, etc. Hence, in the course of cultivation it is required to create the conditions capable of providing active growth and high productivity of leaf apparatus. The conducted research prove that the investigated preparations have essentially impacted these indices with the microclonal grape plants (see Table). The plant height measurements made in 35 days after planting indicated that the highest grape microclones were formed in the control group (their height was 6.7 cm) and in the variants where they were treated with EPAA of all use rates. So, with the fifth variant the plant height was 8.5 cm, with the sixth variant it reached 10.0 cm and with the seventh variant it was 8.5 cm. After being sprayed with Vapor Gard in 0.3-1.0% concentration, the grape microclones height was at the control group level, while with the fourth variant (following the treatment with the greatest use rate solution) the height of the plants was less than with the control group almost twice. Similar regularity of a change in the plant height according to various variants was observed even upon expiration of 90 days after their cultivation.

**Impact of antitranspirants on biometric indices characterizing development of grape microclones**

| Variant | Plant height, cm | Number of leaves, pcs. | Leaf area, $cm^2$ | Microclone leaf surface, $cm^2$ | Microclone leafage, $cm^2/m$ |
|---|---|---|---|---|---|
| 35 days after planting | | | | | |
| 1 | 6.5 | 2.7 | 7.41 | 22. 52 | 346.46 |
| 2 | 6.0 | 2.5 | 7.44 | 18.52 | 308.66 |
| 3 | 6.3 | 2.6 | 6.84 | 20.32 | 322.53 |
| 4 | 3.2 | 2.3 | 4.97 | 11.50 | 359.37 |
| 5 | 8.5 | 3.0 | 10.09 | 33.29 | 391.64 |
| 6 | 10.0 | 3.2 | 10.58 | 37.03 | 370.30 |
| 7 | 8.5 | 3.0 | 10.15 | 30.45 | 358.23 |
| 8 | 6.7 | 3.3 | 8.45 | 27.88 | 416.11 |
| 90 days after planting | | | | | |
| 1 | 13.0 | 4.0 | 14.88 | 69.24 | 532.61 |
| 2 | 12.5 | 3.6 | 14.83 | 63.41 | 507.28 |
| 3 | 12.7 | 4.6 | 13.69 | 60.65 | 447.55 |

| | | | | | |
|---|---|---|---|---|---|
| 4 | 6.8 | 4.3 | 9.94 | 42.66 | 62.450 |
| 5 | 17.0 | 5.0 | 20.18 | 107.03 | 629.58 |
| 6 | 20.1 | 5.6 | 21.17 | 114.43 | 569.30 |
| 7 | 17.1 | 5.1 | 20.30 | 109.67 | 641.34 |
| 8 | 13.7 | 4.8 | 16.91 | 86.22 | 629.34 |

In 90 days after planting microclonal plants were characterized by the maximum size of a leaf and the total leafage area in those variants where EPAA preparation was used. The area of a leaf varied within 20.18-21.17 $cm^2$ and the leafage area from 107.03 to 114.43 $cm^2$, respectively. With the control group these indices comprised 16.91 and 86.22 $cm^2$, respectively. In the variants where Vapor Gard was applied (particularly the fourth variant) the above indices were smaller both when compared with the control group and with the variants where EPAA was applied. It should be mentioned that in the fourth variant a leaf area was 1.7 times less and the total leafage area – 2.0 times less as compared with the control group.

## Conclusions

1. Vapor Gard and EPAA preparations should be applied for foliar treatment of the vegetative mass of grape microclones at the initial stage of microclone adaptation to in vivo conditions. It is advisable to treat the plants twice: the first time when the microclones are in cultural containers prior to the beginning of adaptation, and the second time – after the plants have been planted on nutritious substrates or under cover.
2. The optimum concentrations Vapor Gard are 0.3% and 0.5%, and for EPAA – 0.4%. These concentrations facilitate reduction of transpiration intensity at the beginning of the adaptation period which was characterized by a greater number of viable plants with well-developed vegetative mass.

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## АНТИТРАНСПІРАНТИ ДЛЯ УСПІШНОЇ АДАПТАЦІЇ МІКРОКЛОНІВ ВИНОГРАДУ

*Н.М. ЗЕЛЕНЯНСЬКА*

Наведено результати наукових досліджень із застосування препаратів групи антитранспірантів для адаптації мікроклонів винограду до умов in vivo. Встановлено, що 0,3 і 0,5% концентрації препарату Vapor Gard та 0,4% концентрація препарату ЕПАА сприяють зниженню інтенсивності транспірації мікроклонів винограду, збільшенню кількості життєздатних рослин та покращенню їх біометричних показників росту.

***Ключові слова**: in vitro, антитранспіранти, мікроклони винограду, ЕПАА, Vapor Gard.*

# АНТИТРАНСПИРАНТЫ ДЛЯ УСПЕШНОЙ АДАПТАЦИИ МИКРОКЛОНОВ ВИНОГРАДА

*Н.Н. ЗЕЛЕНЯНСКАЯ*

Приведены результаты научных исследований по применению препаратов группы антитранспирантов для адаптации микроклонов винограда к условиям in vivo. Установлено, что 0,3 и 0,5% концентрации препарата Vapor Gard и 0,4% концентрация препарата ЭПАА способствуют снижению интенсивности транспирации микроклонов винограда, увеличению количества жизнеспособных растений и улучшению их биометрических показателей роста.

***Ключевые слова:*** *in vitro, антитранспиранты, микроклоны винограда, ЭПАА, Vapor Gard*