THE INFLUENCE OF TRACE ELEMENTS AND PGPR ON GROWTH AND PHOTOSYNTHETIC ACTIVITY OF GRAPE SEEDLINGS

INFLUENŢA MICROELEMENTELOR ŞI PGPR ASUPRA CREŞTERII ŞI ACTIVITĂŢII FOTOSINTETICE A BUTAŞILOR VIŢEI-DE-VIE

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Abstract. *The possibility of joint application of a suspension or metabolites of plant growth promoting rhizobacteria (PGPR) and a complex of trace elements Microcom-VA for improving the growth and development of grape seedlings was established. Analysis of the experimental data obtained in controlled and field conditions showed that foliar fertilization of plants by a half dose of Microcom-VA together with bacterial products (suspensions of two- three strains of PGPR) significantly improves the growth of shoots and roots of grape seedlings, content of photosynthetic pigments in leaves, intensity of photosynthesis. This is a consequence of improving the conditions of plant nutrition. The application of PGPR makes possible to improve the quantity and quality of planting material and to reduce the amount of fertilizers (half of recommended dose of trace elements complex) and chemical pressure on the environment.*
Key words: plant growth promoting rhizobacteria, grape seedlings, pigments, intensity of photosynthesis, Microcom-VA

Rezumat. *S-a stabilit posibilitatea aplicării în comun a unei suspensii sau metaboliți ai rizobacteriilor ce promovează creşterea plantelor (PGPR) şi a unui complex de microelemente Microcom-VA pentru îmbunătățirea creşterii şi dezvoltării butaşilor viței-de-vie. Analiza datelor experimentale obținute în condiții controlate şi de câmp a arătat că fertilizarea foliară a plantelor cu o jumătate de doză de Microcom-VA împreună cu produse bacteriene (suspensii de două sau trei tulpini de PGPR) îmbunătăţeşte în mod semnificativ creşterea lăstarilor şi rădăcinilor butaşilor viței-de-vie, conținutul de pigmenți fotosintetici în frunze, intensitatea fotosintezei. Aceasta este o consecință a îmbunătățirii condițiilor de nutriție a plantelor. Aplicarea PGPR contribuie la creşterea cantității şi calității materialului săditor, reducerea cantității de îngrăşăminte (jumătate din doza recomandată de complex de microelemente) şi scăderea presingului chimic asupra mediului ambiant.*
Cuvinte cheie: rizobacterii ce promovează creşterea plantelor, butaşi de viță-de-vie, pigmenți, intensitatea fotosintezei, Microcom-VA

INTRODUCTION

When cultivating perennial crops (vineyards, orchards), intensive removal of nutritive elements from the soil and contamination of soil with heavy metals due to

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multiple treatments of plants against diseases and pests occurs. In agrocenosis are formed the microorganism complexes, poor in species diversity and less resistant to unfavorable environmental factors (Меренюк, 2009). Modern technologies of planting material production must be supplemented by new links and new processes, which will significantly improve the quality and quantity of seedlings.

Studies conducted in recent years show that new biotechnologies, in particular the use of growth-promoting rizobacteria (PGPR), allow a new look at the possibilities of regulating plant nutrition in ontogenesis and plant resistance to unfavourable growing conditions (Bhardwaj *et al*., 2014; Rojas-Tapias *et al*.; Veliksar S. *et al*., 2014; 2015). This is especially important for growing a healthy planting material for the laying of new perennial vineyard plantations.

It is known that the quality of seedlings is largely dependent on the mineral status of the soil and plants. The use of microorganisms can significantly improve plant nutrition, root formation and, accordingly, the quality of seedlings. The mechanisms of the action of PGPB on plants have not yet been fully explored, but their main effect is related to the production of biologically active substances and effects on plant growth (Ahemad and Kibret, 2014).

This paper presents the results of studying the effect of suspensions and metabolites of three strains of saprophytic bacteria on the content of chlorophyll in the leaves of grapes seedlings, photosynthetic activity, growth of shoots and roots, which ultimately determines the quantity and quality of the planting materials obtained in the nursery for the new vineyards.

MATERIAL AND METHOD

The cuttings of 2 grape cultivars (Codrinskii and Presentable) were rooted in distilled water in darkened vessels. After rooting they were placed in 11 L plastic pots filled with soil and grown on the growing platform (vegetation complex) before the autumn. Soil – chernozem carbonate loamy. Another experiment was carried out in the production conditions - in a vine nursery on the same type of soil.

Two-day suspensions of strains of *Azotobacter chroococcum, Bacillus subtilis* and *Pseudomonas fluorescens*, with a titre of 107 cfu / mL were applied to the soil during the cuttings plantation. For foliar fertilization of plants in the process of vegetation the products of the metabolism of bacteria, obtained by centrifugation of concentrated suspensions, were used. The plants were sprayed three times during vegetation period (interval – 15-17 days) by metabolites of microorganisms separately and together with a half of the recommended dose of specially created for grape complex of trace elements Microcom-VA. Microcom-V contains 6 most needed for grape plants trace elements in optimal ratio, and is recommended for foliar fertilization of grape at the critical phases of plants development. Intensity of photosynthesis was determined using a portable LCI device, the content of photosynthetic pigments - in acetone extract.

RESULTS AND DISCUSSION

The content of photosynthetic pigments in leaves is one of the important indications of the plants status during the growing season. Foliar fertilization of plants by Microcom-V increased the amount of chlorophyll (a + b) in the leaves by 119.6 %

compared to the control (tab. 1). The introduction of a suspension of bacteria *Azotobacter chroococcum* and *Pseudomonas fluorescens* (1:1) into the soil together with 0,5 dose of the trace element complex Microcom-V was more effective, sum of the photosynthetic pigments composed 1.34 mg /g f.w. (120.8% compared to the control). The amount of chlorophyll increased mainly due to chlorophyll b. The ratio of the forms *a* and *b* varied according to the variants of the experiments in the range from 3.5 (control) to 2.64 – 2.88 (variants with foliar treatment). The quantity of carotenoides, as a rule, was at the level of the control variant or below it.

Table. 1

Influence of microelements, suspension and metabolites of rhizobacteria on the content of photosynthetic pigments in leaves of grape seedlings, cv. Codrinschii, growing platform , mg /g f.w.

Foliar fertilization	clor. *a*	clor. *b*	*a+b*	carotinoides
Control (H_2O)	0.86 ± 0.01	0.25 ± 0.01	1.11 ± 0.02	0.36± 0.01
Foliar fertilization by Microcom-V 0.5	0.98 ±0.01	0.34 ± 0.02	1.32 ± 0.05	0.35± 0.02
Suspension of *Ag. chroococcum*+ *Ps. fluorescens* (into the soil)	0.92 ±0.03	0.34 ± 0.03	1.27 ± 0.01	0.34± 0.01
Suspension of *Ag. chroococcum*+ *Ps. fluorescens* (into the soil) + Microcom-V 0.5 (foliar)	0.97 ±0.01	0.37 ± 0.01	1.34 ± 0.02	0.35± 0.02
Metabolites of *Ag. chroococcum*+ *Ps. fluorescens* (foliar) + Microcom-V 0.5 (foliar)	0.94 ±0.02	0.37 ± 0.01	1.31 ± 0.05	0.34± 0.04

An increase in the intensity of plants transpiration on the growing platform was noted, especially when the suspension of PGPR was introduced into the soil, followed by foliar fertilization of plants by Microcom-V 0.5. In the same variants a higher stomatal conductance was noted, the lowest - in the control variant.

The intensity of transpiration and the stomatal conductance of plants are closely related to the intensity of photosynthesis and the productivity of plants. The intensity of the photosynthesis of leaves of seedlings in control variant was 6.89 ± 2.31. It was evident higher after the fertilization and varied within 7.71 ± 0.52 and 9.29 ± 2.57 Mmol / M^2 / sec in dependence of applied substances.

In the table 2 the results of the determination of photosynthetic pigments content in leaves of seedlings in a grape nursery (experience in field conditions) are presented, where a consortium of three strains of rizobacteria and a half dose of trace elements complex was applied foliar three times during vegetation. The metabolites of microorganisms *Azotobacter chroococcum* + *Pseudomonas fluorescens* + *Bacillus subtilis* (1: 1: 1) together with Microcom-V in a half dose contributed to an increase in the total amount of chlorophyll (a + b) in leaves by 118.6% compared to control. The ratio of the forms *a* and *b* was in control plants 2.68, and 2.11- in treated plants. The sum of carotenoides was lower than in the control variant.

Table 2

Influence of metabolites of rhizobacteria and trace elements on the content of photosynthetic pigments in the leaves of grape seedlings in the nursery, cv. Codrinskii, mg / g fresh weight

Variants	**clor. *a***	**clor. *b***	***a+b***	**carotinoi-des**
Control	1.02±0.01	0.38±0.01	1.40±0.02	0.20±0.001
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + *B.subtilis* (1:1:1) + Microcom-V, 0.5 (foliar)	1.12±0.002	0.53±0.002	1.66±0.01	0.15±0.002

Improvement of photosynthetic activity of plants is closely connected with the best growth and maturation of shoots of seedlings. Analysis of the main indicators of seedlings quality during their digging (September-October) shows that in both our experiments in all variants with application of bacteria and trace elements the weight and total length of the roots were higher in comparison with control plants. As a rule in the greatest extent the growth and development of the root system was stimulated after fertilization. In the experiment on the growing platform reduced dose of the complex Microcom-V and metabolites of bacteria increased the average length of roots by 186.3 % compared to control (tab. 3). It is also important that these seedlings develop more intensively small roots (of 3 and 4 order), contributing to better nutrition of plants. More evident increase of shoots length was noted after the foliar fertilization of plants by suspension and metabolites of microorganisms together with trace elements (respectively 154,5 and 131.7% to the control). This effect is due to the fact that the main mechanism of action of PGPR on plants is the production of phytohormones, which play the role of chemical messengers and act as regulators of plant growth and development (Martínez-Viveros *et al*, 2010; Rojas-Tapias *et al*, 2012).

Table 3

Influence of trace elements, suspension and metabolites of rhizobacteria on growth and development of grape seedlings, variety Presentable, vegetative complex (average of 20 plants)

Variants	Average length of roots/1 plant, cm	Average length of shoots/1 plant, cm	% to control	
			roots	shoots
Control	246.8±53.87	26.6±2.98	100	100
Foliar fertilization by Microcom-V 0,5	448.5±59.43	33.0±4.23	181.9	123.9
Suspension of *Ag. chroococcum*+ *Ps. fluorescens-(into the soi)l*	358.1±30.26	34.1±2.67	145.1	128.2
Suspension of *Ag. chroococcum*+ *Ps. fluorescens (into the soli)* + Microcom-V 0.5 (foliar)	387.5±47.88	41.1±7.39	157.1	154.5
Metabolites of *Ag. chroococcum*+ *Ps. fluorescens* (foliar) + Microcom-V, 0.5 (foliar)	459.6±24.22	35.1±4.17	186.3	131.7

In the experiment conducted in the grape nursery, the difference in the development of the aboveground part of the seedlings and the root system between the variants (control and three-time fertilization with a complex of trace elements and metabolites of three strains of bacteria) was much more pronounced (tab. 4).

Table 4

Effect of trace elements and PGPR on plant growth in field conditions, cv. Codrinschii, vine nursery

Variants	shoots		roots	
	M±m, cm	% to control	M±m, cm	% to control
Control	26.3	100	232.7	100
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + *B.subtilis* (1:1:1) + Microcom-V, 0,5 (foliar)	54.7	207.9	517.3	222.4

Thus, fertilization of grape seedlings with a small amount of trace elements together with the suspension or metabolites of growth-stimulating bacteria improves their photosynthetic activity and plant growth, which contributes to the improvement of the quality of planting material of grapes. In many cases, application of a suspension of bacteria is more effective and a more technological way to improve the quality of the planting material.

CONCLUSIONS

1. The possibility of joint application of a suspension or metabolites of plant growth promoting bacteria and a complex of trace elements Microcom-V for improving the growth and development of roots and shoots of grape seedlings was established, which is very important for increasing the quantity and quality of planting material.

2. The application of metabolites of rizobacteria makes possible to decrease the total amount of trace elements in the Microcom-V complex (halve of the recommended dose) when growing seedlings, which reduces the possible pollution of the environment by reducing the amount of chemical fertilizers used for plant nutrition.

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