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EFFECT OF JOINT APPLICATION OF TRACE ELEMENTS AND PLANT GROWTH PROMOTING BACTERIA IN THE CULTIVATION OF GRAPE SEEDLINGS

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ABSTRACT

Experiments were carried out on the growing platform and in the production conditions to investigate the effectiveness of plant growth promoting bacteria (PGPB) Azotobacter chroococcum+Pseudomonas fluorescens, Bacillus subtilis and complex of trace elements Microcom-V, specially developed earlier for the grape, in the cultivation of grape seedlings. The schemes of the experiments included treatments with the introduction of bacterial suspensions into the soil when planting grafted or non-grafted cuttings, and foliar fertilization of plants with bacterial suspension and a Microcom-V at the 50 % of the recommended dose. It was established that the joint application of the bacterial products (suspension and metabolites) together with trace elements increase the availability of nutrients for plants, enhance the development of small feeding roots, the photosynthetic activity of leaves, the growth and development of seedlings, and improves the quality of planting material. The use of PGPB in plant nutrition makes it possible to halve the total amount of trace elements in the Microcom-V complex, which reduces the risk of possible environmental contamination when using chemical fertilizers, and the cost of seedlings production.

Keywords: plant growth promoting rizobacteria, complex of trace elements Microcom-V, bacterial suspension, bacterial metabolites, photosynthetic activity, growth and development of grape seedlings.

1. INTRODUCTION

The quality of planting material grown in a fruit nursery depends on the conditions of mineral nutrition in both nursery and plantations, where the cuttings of the graft and rootstock are harvested. It should be also considered that during multiannual cultivating of perennial crops there is an intensive removal of nutrients from the soil, contamination of the soil with heavy metals due to multiple plant treatments with fungicides, formation in the agro enosis of microorganism complexes which are poor in species diversity and less resistant to unfavorable factors [3, 21, 25]. A significant improvement in the quality and quantity of planting material can be facilitated by new technologies that allow providing to the seedlings the optimal regime of nutrition without the use of high doses of fertilizers. The most promising direction in the improvement of existing technologies in nursery farming and improving the quality of planting material is, in our opinion, the integrated use of trace elements and biotechnological products.

The role of trace elements in the viticulture and production of grape seedlings is well enough studied. It has been established that both incorporation into the soil and foliar application of micro fertilizers improve the growth and development of grape bushes and seedlings [23, 24, 25]. It contributes to the increase of the quantity of high-quality seedlings from the nursery. Under the foliar fertilization by micro fertilizers together with macro fertilizers (NPK) the quantity of seedlings from the nursery has increased more than twice as compared to the control. Simultaneously, the quality of seedlings has been improved, both the annual increment of shoots and the number of developed roots.

The creation of microbial biotechnologies is one of the main directions of modern agriculture [5-8, 10, 16, 20]. The use of the potential of saprophytic bacteria, capable of mobilizing nutrients from the soil or the atmosphere, is an important achievement of biotechnology and a factor in increasing the productivity of crops. In the last 5-6 years, significant progress has been made in studying of the mechanisms of interaction between plant growth-promoting rhizobacteria (PGPR) and plants. It allows taking a fresh look at the possibilities of regulating plant nutrition in ontogenesis [17-19].

The stimulating effect of PGPR on plants can be mostly explained with the release of metabolites directly stimulating growth. The mechanisms of the influence of PGPR on plant growth have not been fully explored yet, but presumably it consist of the following: the ability to produce plant hormones such as auxins, cytokinins, gibberellins and inhibit the synthesis of ethylene; non-symbiotic fixation of N2; solubilization of inorganic phosphate and mineralization of organic phosphate and / or other nutrients; antagonism regards to phytopathogenic microorganisms due to the synthesis of siderophores, antibiotics, enzymes and / or fungicidal compounds and competition with undesirable microorganisms [6]. On the basis of microbial metabolites, various biological compositions are created [1, 4, 10]. They are increasingly used in crop production.

Very little data is available on the use of bacteria and their derivatives in horticulture [1, 7, 11-15] and there is practically no information on the use of microbial technologies in the preparation of planting material of grapes. The development of scientifically based technology for the application of small doses of fertilizers and derivatives of saprophytic bacteria in nursery farming enables producers to improve the quality and quantity of planting material and to plant healthy perennial plantations of vine.

The main task of the four-year research presented in the article is to reveal the effectiveness of the joint use of the trace elements containing complex Microcom-V and the products of vital activity of two strains of rhizobacteria in order to improve the quality and quantity of the planting material of grapes and to reduce the chemical load on the environment.

2. MATERIALS AND METHODS

2.1. Plant material and experimental conditions.

The experiments were carried out on the growing platform of the Institute of Genetics, Physiology and Plant Protection of the ASM and in production conditions (in a vine nursery). Dormant cuttings of *Vitis vinifera* L. of cv. Presentable and Kodrinsky with 4 buds were selected after the period of dormancy (on the end of February) from the experimental vineyard at the Institute. Cuttings rooted in nutritive solution (Murasige and Scooge) were annually planted in plastic pots containing 11 kg of soil, 2 plants in each pot, 10 pots in each treatment. Soil - chernozem carbonate loamy. Before planting, a background fertilizer (NPK at 0.63 g / kg air-dry soil) had been added to each pot.

Another experiment has been carried out at the vine nursery in randomized block design at 3 replications with triple foliar fertilization.

2.2. Plant treatment.

In the experiment on the growing platform suspension of bacteria *Azotobacter chroococcum* (CNMN-AzB-01) and *Pseudomonas fluorescens* (CNMN-PsB-04) strains was used for plant treatment. In the condition of production (vine nursery) the consortium of three strains - two above mentioned + *Bacillus subtilis* – was used. Two-day suspensions of bacteria with a titer of 108 CFU / ml were introduced into the soil separately and together while the cuttings were planted into the pots in the area of the roots. For foliar fertilizing of plants during the vegetation period, the products of bacteria metabolism– metabolites - were obtained by centrifugation of concentrated suspensions at 8000 RPM for 20 minutes. Foliar treatment of plants was carried out three times with an interval of 12-14 days after the complete development of the aboveground mass, using metabolites of bacteria separately and together with a half dose of the trace element composition Microcom-V, specially designed for the grape. It includes 6 trace elements in doses and ratios, which correspond to the grape requirements during the critical phases of the vegetative period.

Besides the control (treatment by H2O) schemes of experiments included treatments with the introduction of suspensions of each bacteria into the soil, separately and together; foliar fertilization by the metabolites of each bacteria separately and together, as well as spraying with Microcom-B solution at the recommended and half dose - separately and together with all combinations of derivatives of bacteria (suspension and metabolites).

After the first year of experiment, there were selected 5 best treatments, which were selected in the following 3 years. The schemes of the experiments are given in the tables.

2.3. Plant and soil analyses.

The selection and analyses of the soil and plants samples were conducted in 3 repetitions. Chemical analyzes were carried out by conventional methods. In the samples of soil and plant organs, selected in dynamics according to the variants of the experiments, the following indices were determined: NH4, NO3, P total, P2O5, K2O, K total. The content of trace elements Fe, Mn, Zn, Cu was determined on a Perkin Elmer atomic absorption spectrometer after the dry ashing.

The chlorophyll content in the seedlings leaves was determined in acetone extract on a UV-VIS SP 8001 spectrophotometer. The degree of development of the aerial part and the root system of the seedlings and the total accumulation of biomass were determined after the shoots maturation during the digging of the seedlings. Biometrical measurements were conducted for 20 plants in every treatments. Statistical processing of the obtained results was carried out using the methods of Statistics 7.

3. RESULTS AND DISCUSSION.

3.1. Plant growth and development.

The main indicators of the quality of seedlings are: the development of the root system, the growth and maturing of annual shoots. It is known that a significant number of bacterial species, in particular rhizobacteria, have a beneficial impact on plant growth. We investigated the effect of rhizobacteria and the half dose of a complex of microelements on the development of root-bearing grape seedlings under controlled conditions both on the growing platform and in the vine nursery. Analysis of the obtained data shows that under the treatments which include the use of bacteria and trace elements, the growth of shoots and roots of grapes seedlings was more intensive in comparison with control plants (table 1). The growth-stimulating effect of suspension and metabolites of bacteria was more pronounced on the development of the root system. The total length of the roots of one seedling was greatest when the half-dose of a complex of trace elements and metabolites of bacteria was combined (186.26% comparing to the control plants).

The determination of the architectonics of the seedlings root system has showed that when using bacteria derivatives (suspension and metabolites) with trace elements the number and the length of the smallest rootlets with a diameter up to 3 mm (the so-called feeding or rosy roots) noticeably increased. Stimulating the growth of feeding roots is one of the main mechanisms for the action of PGPR and trace elements on the plant nutrient regime.

Table 1. Influence of Microcom-V, suspension and metabolites of PGPB on growth and development of 1 grape seedling (average of 20 plants), on the growing platform, cv. Presentable

Treatments	Average length of roots, cm	Average length of shoots, cm	% to control	
			roots	shoots
Control	246.8±53.87	26,6±2.98	100	100
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil	448.5±59.43	33,0±4.23	181.94	123.96
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil + Microcom-V 0,5, foliar	358.1±30.26	34,1±2.67	145.13	128.17

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Metabolites of *Az. chroococcum* + *Ps. fluorescens* , foliar	387.5±47.88	41,1±7.39	157.04	154.47
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + Microcom-V 0,5, foliar	459.6±24.22	35,1±4.17	186.26	131.70

The above-ground part of the seedlings was developed better under the both root and non-root (foliar) treatments comparing to the control plants. Average length of the shoots was higher under the foliar fertilization of plants by bacteria metabolites separately and together with trace elements - 154.47 and 131.70% respectively, as compared to the control (table 1). This effect is due to the fact that the main mechanism of PGPR's action on plants is production of phytohormones, which play the role of chemical messengers and act as regulators of plant growth and development [6, 16].

Similar results were obtained in our experiments when testing the influence of the same combinations of bacteria on the seedlings of other grapes cultivars – Codrinschy, Bianka, Hibernal. There were mentioned similar noticeable increase in the total biomass of plants, roots length and diameter and degree of shoots maturation. It identifies a higher viability of the obtained planting material.

3.2. Content of photosynthetic pigments in leaves. During the vegetation period, the content of the photosynthetic pigments in the leaves of seedlings has been determined. The introduction into the soil of a bacteria suspension on the growing platform has increased the total content of chlorophyll in the leaves - by 117.77 % comparing to control. Foliar fertilization with bacteria metabolites was more effective when adding to them a half dose of Microcom-V (108.14% according to control (table 2). The content of carotenoids however has decreased.

Table 2. Influence of Microcom-V, suspension and metabolites of PGPB on the content of photosynthetic pigments in leaves of grape seedlings on the growing platform, cv. Presentable, % to control.

Treatments	**Chlorophyll *a***	**Chlorophyll *b***	**Chlorophyll *a+b***	**Carotenoids**
Control	100	100	100	100
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil	121.05	118.91	117.77	107.89
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil + Microcom-V 0,5, foliar	107.14	102.70	106.66	92.10

Metabolites of *Az. chroococcum* + *Ps. fluorescens* , foliar	104.08	97.29	102.96	89.47
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + Microcom-V 0,5, foliar	109.18	105.40	108.14	92.10

As Bashan Y. [3] wrote, the application of PGPB in the field (apart from rhizobia) has yielded satisfactory results in controlled experiments, although results are less promising under agricultural conditions. The results of our experiment, carried out in the production conditions, are presented in table 3. The effect of three strains consortium of bacteria *Azotobacter chroococcum* + *Pseudomonas fluorescens* + *Bacillus subtilis*, applied together with a half dose of Microcom-V, on the content of photosintetic pigments in the leaves of grape seedlings in the nursery has been studied. Foliar fertilization has contributed to an increase in the total amount of chlorophyll (a + b) in the leaves by 118.57 % comparing to the control. The content of chlorophyll has increased mainly due to chlorophyll *b*. The content of carotenoids in leaves of treated plants was lower than in the control plants. Improving the photosynthetic activity of plants contributes to the acceleration of growth and maturation of seedling shoots.

Table 3. Influence of Microcom-V, suspension and metabolites of PGPB on the content of photosynthetic pigments in leaves of grape seedlings in the nursery, v. Kodrinschi, mg/g f.w.

Treatments	**Chlorophyll *a***	**Chlorophyll *b***	**Chlorophyll *a+b***	**Carotenoids**
Control	1,02±0,01	0,38±0,01	1,40±0.02	0,20±0.001
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + *Bacillus subtilis* + Microcom-V, 0,5 dose, foliar	1,12±0,002	0,53±0.002	1,66±0.01	0,15±0.002

3.3. Content of nutritive elements in soil and plants. Analysis of rhizospheric soil after the digging of seedlings at the end of vegetation period showed significant changes in the content of nutrients. There was observed the tendency to increase the content of ammonia nitrogen in the soil under the introduction of bacterial suspension into the soil and plant foliar fertilization by the solution of the metabolites and Microcom-V (table 4). Bacterial suspension, used both separately and with the addition of trace elements, increased the content of mobile phosphorus in the rhizosphereic soil to 9,3 and 9,4 mg / 100 g (respectively by 112.05 and 113.25% comparing to the control). At the same time, the content of nitrate nitrogen and potassium decreases. The increase in P2O5 content in the rhizosphere results from the fact, that the bacterial strains, used in the experiments, are able to convert insoluble phosphates to soluble ones [2, 6, 9]. It is important, since perennial plants often suffer from a lack of these two forms of mineral nutrition - nitrogen and phosphorus, especially on carbonate chernozem. A significant decrease in the content of nitrate nitrogen in the soil is likely due to a more intensive growth of seedlings after root and foliar fertilization by trace elements and derivatives of PGPB and, accordingly, a more intensive use of nitrates.

Table 4. Influence of Microcom-V, suspension and metabolites of PGPB on the content of of N, P, K in the rhizosphere of grape seedlings, cv. Presentable, growing platform,

mg / 100 g of soil.

Treatments	NH_4	NO_3	P_2O_5	K_2O
Control	0,8	44,3	8,3	24,4
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil	0,9	15,4	9,3	22,0
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil + Microcom-V 0,5, foliar	0.9	23,8	9,4	19,2
Metabolites of *Az. chroococcum* + *Ps. fluorescens* , foliar	1,0	27,3	8,8	20,4
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + Microcom-V 0,5, foliar	1,4	15,4	8,8	20,4

The content of nitrogen, phosphorus, potassium and trace elements Fe, Mn, Zn and Cu has been determined in leaves and roots after the digging of seedlings from pots. As when introducing into the soil suspensions of *Azotobacter chroococcum* + *Pseudomonas fluorescens* both without trace elements and with them, so under the foliar fertilization by bacteria metabolites and trace elements, the content of nitrogen and phosphorus decreased in the roots at the end of vegetation, and content of potassium increased in comparison with the control (table 5). There was no significant difference in the content of macro elements in the leaves between the different treatments of the experiment.

The data of various researchers on the effect of rhizobacteria PGPR on the mineral composition of plant organs are ambiguous. It is noted as a slight increase in the concentration of nitrogen and phosphorus and more essential - potassium in the leaves and roots of plants [5, 10-12], so a decrease in the content of these elements [1]. This is due to the unequal efficiency of different

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strains of bacteria, different growing conditions, the characteristics of a particular crop, and different level of nutrient removal from the soil during the growth and development of seedlings. Our calculation of macronutrient removal from the soil by seedlings, taking into account the accumulated biomass (leaves + shoots + roots), has showed that plants treated by the bacteria suspension or metabolites and trace elements, removed much more macronutrients out of the soil than control seedlings.

Table 5. Influence of Microcom-V, suspension and metabolites of PGPB on the content ofN, P, K in roots and leaves of grape seedlings (after the digging), cv. Presentable, growing platform, %.

Treatments	N total	P_2O_5	P total	K_2O	K total
roots					
Control	1,77	0,92	0,40	1,60	1,33
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil	1,43	0,80	0,35	2,40	1,99
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil + Microcom-V 0,5, foliar	1,29	0,78	0,34	2,10	1,74
Metabolites of *Az. chroococcum* + *Ps. fluorescens* , foliar	1,50	0,69	0,30	2,20	1,83
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + Microcom-V 0,5, foliar	1,36	0,80	0,35	2,20	1,83
leaves					
Control	2,48	1,00	0,44	1,80	1,49
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil	2,52	0,80	0,35	1,80	1,49
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil + Microcom-V 0,5, foliar	2,45	0,85	0,37	1,70	1,41
Metabolites of *Az. chroococcum* + *Ps.*	2,41	1,00	0,44	2,00	1,66

fluorescens , foliar					
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + Microcom-V 0,5, foliar	2,35	0,78	0,34	1,90	1,58

The content of the mobile forms of trace elements Zn, Fe and Mn in the rhizospheric soil at the end of vegetation increased under the introduction of the bacterial suspension of *Azotobacter chroococcum* + *Pseudomonas fluorescens* into the soil, when the cuttings were planted in the vessels, by 368.75, 106.2 and 115.63%, respectively to control (table 6). The foliar treatment by the bacteria metabolites and Microcom-V had no significant effect on the content of trace elements in the rhizosphere at the end of vegetation.

Table 6. Influence of Microcom-V, suspension and metabolites of PGPB on the content of trace elements in the rhizosphere of grape seedlings, v. Presentable, growing platform,mg / kg of soil.

Treatments	**Zn**	**Cu**	**Fe**	**Mn**
Control	1,6	4,2	175,7	6,4
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil	5,9	4,4	186,6	7,4
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil + Microcom-V 0,5, foliar	4,0	4,2	175,2	6,9
Metabolites of *Az. chroococcum* + *Ps. fluorescens*, foliar	2,0	5,5	177,7	5,5
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + Microcom-V 0,5, foliar	1,4	4,3	167,8	6,6

Suspension of bacteria *Azotobacter chroococcum* + *Pseudomonas fluoescens* contributed to an increase in the concentration of trace elements in the roots of seedlings, especially Zn and Fe. Thus, the content of Zn in the roots increased to 12.07 at 10.66 mg / kg in the control, Fe - up to 1018.31 at 873.09 mg / kg in the control (table 7). Changes in the concentration of Cu and Mn in the roots are insignificant. Foliar top dressing with bacteria metabolites and a complex of microelements, which exerted a stronger influence on the growth of seedlings (Table 1), led to a decrease in the concentration of Fe and Mn in the leaves. We assume that there has been a so-called dilution effect, which often occurs when growing perennials with a large growth potential.

Table 7. Influence of Microcom-V, suspension and metabolites of PGPB on the content of trace elements in the roots and leaves of grape seedlings, cv. Presentable, growing platform, mg/kg of d.w.

Treatments	**Zn**	**Cu**	**Fe**	**Mn**
roots				
Control	10,66	5,56	873,09	34,69
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil	12,07	6,53	1018,31	36,83
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil + Microcom-V 0,5, foliar	21,34	9,61	962,92	36,37
Metabolites of *Az. chroococcum* + *Ps. fluorescens* , foliar	13,36	6,36	770,04	35,90
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + Microcom-V 0,5, foliar	11,15	6,10	640,41	36,57
leaves				
Control	10,93	7,33	90,48	104,79
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil	12,38	4,25	170,32	93,57
Suspension of bacteria *Az. chroococcum* + *Ps. fluorescens* into the soil + Microcom-V 0,5, foliar	12,70	7,06	71,02	64,93
Metabolites of *Az. chroococcum* + *Ps. fluorescens* , foliar	13,23	4,91	102,41	95,79
Metabolites of *Az. chroococcum* + *Ps. fluorescens* + Microcom-V 0,5, foliar	12,25	9,66	75,13	70,03

Increased availability of soil nutrients to plants (phosphorus, Zn, Fe, Mn), that has been shown in the different experiments [5, 9, 21, 22], can be the result of a more intensive mineralization of the organic complex and the release of organic acids by plants and bacteria in the rhizosphere. The increase in extraction of nutrients from plants after inoculation by bacteria, especially of the genus *Pseudomonas*, noted by many researchers, perhaps is associated with an increase in exudate of phenolic compounds in the roots under the influence of bacterial indolylacetic acid.

4. CONCLUSION

It has been established the possibility of joint application of a suspension or metabolites of saprophytic bacteria *Azotobacter chroococcum, Pseudomonas fluorescens, Bacillus subtilis* and a half dose of trace element complex Microcom-V, specially created for grapes, for the growth and development of seedlings increase and the quality of the grape planting material improvement. The introduction of bacterial suspensions into the soil and foliar fertilizing of seedlings with PGPB metabolites and trace elements has a positive effect on the nutritional status of plants, the growth of roots, especially of small feed roots, accelerates the growth and maturation of shoots.

Common application of PGPB and trace elements makes it possible to halve the total amount of trace elements for plant feetilization, which reduces the risk of possible environmental contamination when using chemical fertilizers, the cost of seedlings production and viability of new grape plantation.

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