Scientific Bulletin. Series F. Biotechnologies, Vol. XXVII, No. 1, 2023
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

# THE INFLUENCE OF GROWTH-STIMULATING RHYSOBACTERIA (PGPB) ON THE PHYSIOLOGICAL PROCESSES AND PRODUCTIVITY POTENTIAL OF GRAPEVINES

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***Abstract***

*Environmental contamination, deteriorating socio-economic relations and increasingly frequent weather anomalies have complicated the phytosanitary condition of the vineyard complex. The development of organic farming and application of bacterial inoculants have attracted increasing attention in recent years. In this article are presented the results of research on the influence of bacterial products of the group PGPB (plant growth promoting bacteria) in the realization of productivity potential and some physiological processes in grapevines. It was demonstrated that the application of suspensions of Pseudomonas aureofaciens and Bacillus thuringiensis var. kurstak bacteria with the addition of $SiO_2$ at different stages of plant development contributed to the increase of photosynthetic pigment content, transpiration, stomatal conductivity, and increased quantity and quality of grapevine harvest.*

***Key words**: plant growth-promoting bacteria, photosynthetic pigments, productivity, transpiration, stomatal conductivity.*

## INTRODUCTION

Nutrient imbalance, drought as well as long-term cultivation of perennial crops are factors that cause intensive soil depletion, leading to considerable losses in plant productivity. Moreover, it should be noted that with the use of pesticides and fungicides against diseases and pests, heavy metals are accumulating in the soil. As a result, the biomass of micro-organisms in soils under vineyards is considerably reduced, which slows down the plant's absorption of available nutrients. Therefore, plants become more vulnerable to pathogens and adverse environmental conditions, and the quality of grapes and wine decreases. One of the main objectives for the development of integrated sustainable agriculture is the development of new biologically efficient technologies.

A new approach to harnessing plant effectiveness is the use of biologically active compounds. The main goal in this direction is to create biopreparations to increase the availability of nutrients to the plant and increase plant resistance to biotic and abiotic stressors. Achieving this goal will increase plant productivity and reduce the chemical load on the ecosystem. Special attention is given to preparations based on one or more bacterial strains, which is conditioned by the positive influence of microorganisms on plants, and to a large extent by their stimulatory effect on metabolic and biochemical processes in plants. But it is also known that the soil under vineyards and orchards is poor in the diversity of microorganism species. The biological preparations used are based on highly productive strains of microorganisms that populate the rhizosphere of healthy plants. These in turn lead to an increase in the quantity of yield as well as an increase in quality. Fertilizations of plants with bacterial preparations has become increasingly widespread in recent years. The mechanisms of action of microorganism strains in the formation and achievement of plant productivity are insufficiently studied (Asada K., 1990; Avis et al., 2008; Marleny Cadena Cepeda, 2006).

A high level of ecological efficacy and harmlessness of biopreparations does not always have a stable effect on the negative influence of climatic factors on the host plant. To mitigate the negative influence of climatic factors on plants, there are a number of preparations - growth regulators, the use of which mobilizes the plant's defense capacity under outdoor conditions.

This group also includes the preparation silicon dioxide ($SiO_2$), the use of which is known in many fields as well as in agriculture with the following effects on plants: increased permeability in plant tissues, high adhesion, activation of photosynthesis, capacity as an antidote in combination with pesticides and herbicides, alleviation of stress following plant replanting, wounding or trauma (Avis et al., 2008).

The influence of oxides and $SiO_2$ in particular on the production of metabolites of non-pathogenic PGPB organisms is known in microbiological practice:

- The introduction of the preparation into the culture medium when growing bacteria of the genus *Pseudomonas* spp increases the amount of phenazine components in bacterial suspensions which influences the antagonistic properties towards pathogens.
- Silicon dioxide falls into the category of potentially promising substances capable of inhibiting insect defense reactions.

## MATERIALS AND METHODS

The experiments were set up on the experimental field of the IGPPP (Institute of Genetics, Physiology and Plant Protection) in three repetitions, plot dislocation - according to the block method.

Sauvignon vine plants, young plantation (5-6 years old) served as the object of study. The plants were foliar treated with bacterial metabolites during the growing season five times with an interval of 12-15 days (5 true leaves, 3-4 days before flowering, after flowering, in the phase of intensive plant development, before ripening of the berries). On the basis of pre-elimination research, the strains of *P. aureofaciens* and *B. thuringiensis* var. *kurstaki* were selected. Water-treated plants were used as controls.

Bacteria were grown in liquid nutrient medium for 24 h at 27˚C at $10^{10}$ CFU/ml and applied as metabolites. To acquire the bacterial metabolites, the concentrated suspensions were centrifuged at 8 thousand rpm for 20 min in order to precipitate the bacterial cells and obtain the metabolic products. Silicon dioxide ($SiO_2$) was also used.

The content of photosynthetic pigments was determined by the acetone extraction method on the UV-Vis spectrophotometer SP-8001. For analysis, the first three developed leaves were taken from the shoot tip (Avis et al., 2008). The net photosynthetic rate, stomatal conductance and transpiration were determined using the LCpro-SD handheld gas analyzer (ADC biotech-scientific Limited, UK). The results obtained were analyzed according to Statistics-7.

## RESULTS AND DISCUSSIONS

Plant productivity is the result of the integration of several processes, such as photosynthesis, transpiration intensity, metabolite transport, with photosynthesis playing the primary role. One of the main indicators of plant condition is the amount of photosynthetic pigments in the leaves. The overall process of photosynthesis is a continuous series of interdependent photochemical and biochemical events considered to occur in a linear sequence. The content of photosynthetic pigments in leaves is one of the important indicators of the state of plants during the growing season, as the ratio of the sum of chlorophylls and carotenoids is an indicator of the "damaged" state of plants. From our data, it is observed that foliar fertilization of fruiting bushes with the recommended dose contributed to an increase of total chlorophyll content in grapevine leaves by 20%. The highest amount of assimilatory pigments is in the experimental variant where *P. aureofaciens* + *B. thuringiensis* var. *kurstaki*, rhizobacteria were used, and constitutes 20.4%, mainly due to the increase in the concentration of chlorophyll ,,b,, which constitutes 37.4% (Table 1) compared to the control variant. The same effect was also observed in the variants where *P. aureofaciens* + *B. thuringiensis* var. *kurstaki* + $SiO_2$ were used. In these variants the effect of increasing the sum of chlorophyll a+b pigments is probably due to the synergistic effect of rhizobacteria as well as the stimulatory effect of $SiO_2$. An optimal level of pigment content can also be seen in the other treated variants, where it is much higher than in the control variant.

The literature data on the effect of treating plants with microbial metabolites on the carotenoid content of plant leaves are quite contradictory (Martínez-Viveros et al., 2010). The results obtained in our experiments show a trend of decreasing carotenoid content in grapevine leaves after each spraying with bacterial products. This is because carotenoids perform a protective function, keeping various organic substances, especially chlorophyll molecules, from light destruction during photooxidation.

Table 1. Determination of the amount of pigments in grapevine leaves, Sauvignon variety field experiment mg/100 g m.p. Field experiment 2020

| **Experimental variant** | **Chlorophyll *a*** | **Chlorophyll *b*** | | **Chlorophyll *a+b*** | | **Carotenoids** | | |
|---|---|---|---|---|---|---|---|---|
| | **M±m** | **Δ, %** | **M±m** | **Δ, %** | **M±m** | **Δ, %** | **M±m** | **Δ, %** |
| **10.06.2020** | | | | | | | | |
| **1.*** | 0.99± 0.0 | | 0.48±0.01 | | 1.47±0.01 | | 0.21±0.00 | |
| **2.** | 1.33±0.0 | 13.4 | 0.99±0.02 | 20.6 | 2.31±0.02 | 15.7 | 0.18±0.02 | -14.3 |
| **3.** | 1.19±0.01 | 12.0 | 1.31±0.07 | 27.2 | 2.50±0.08 | 17.0 | 0.15±0.01 | -28.5 |
| **4** | 1.34±0.01 | 13.5 | 1.67±0.14 | 34.7 | 3.01±0.14 | 20.4 | 0.13±0.05 | -39.0 |
| **5.** | 1.30±0.01 | 13.1 | 1.02±0.04 | 21.2 | 2.31±0.04 | 15.7 | 0.12±0.02 | -42.1 |
| **6.** | 1.35±0.00 | 13.6 | 1.11±0.00 | 23.1 | 2.45±0.00 | 16.6 | 0.15±0.05 | -0.28 |
| | | | **26.06.2020** | | | | | |
| **1.** | 0.99±0.00 | | 0.49±0.01 | | 1.48±0.01 | | 0.37±0.01 | |
| **2.** | 1.01±0.00 | 10.2 | 0.47±0.01 | 0.22 | 1.48±0.01 | 10.0 | 0.33±0.01 | -10.8 |
| **3.** | 1.13±0.02 | 11.4 | 0.92±0.06 | 18.7 | 2.05±0.06 | 13.8 | 0.24±0.02 | -35.1 |
| **4.** | 1.14±0.02 | 11.5 | 1.00±0.06 | 20.4 | 2.14±0.09 | 14.4 | 0.21±0.03 | -43.5 |
| **5.** | 1.05±0.01 | 10.6 | 0.70±0.02 | 14.2 | 1.74±0.02 | 11.7 | 0.30±0.01 | -18.9 |
| **6.** | 1.13±001 | 11.4 | 0.79±0.04 | 16.1 | 1.93±0.05 | 13.0 | 0.23±0.01 | -37.8 |
| | | | **29.07.2020** | | | | | |
| **1.** | 1.17±0.00 | | 0.45±0,00 | | 1.62±0,00 | | 0.54±0.01 | |
| **2.** | 1.25±0,00 | 10.6 | 0.55±0.01 | 12.2 | 1.80 ±0.01 | 11.1 | 0.53±0.02 | -1.8 |
| **3.** | 1.25±0,00 | 10.6 | 0.56±0.00 | 12.3 | 1.82±0.00 | 11.3 | 0.51±0.03 | -5.5 |
| **4.** | 1.28±0.00 | 10.9 | 0.59±0.00 | 12.7 | 1.87±0.00 | 11.5 | 0.55±0.00 | 10.1 |
| **5.** | 1.31±0.00 | 11.1 | 0.70±0.00 | 15.5 | 2.01±0.00 | 12.4 | 0.52±0.00 | -3.7 |
| **6.** | 1.13±0.16 | 9.6 | 0.74±0.07 | 16.4 | 1.87±0.09 | 11.5 | 0.50±0.04 | -7.4 |

**1.** - Control; 2. - *P. aureofaciens*; **3.** - *P. aureofaciens*+ $SiO_2$; **4. -** *P. aureofaciens*+*B. thuringiensis* var.*kurstaki*; **5. -** *B. thuringiensis* var. *kurstaki* + $SiO_2$; **6. -** *P. aureofaciens*+*B. thuringiensis* var. *kurstaki* + $SiO_2$

Photosynthesis is an important biochemical process in plants, but also one of the most vulnerable to external environmental conditions. In fertilized plants the photosynthesis process takes place with increased intensity (Table 2). The process of carbon (IV) oxide assimilation under suitable conditions is intensified in plants treated with *B. thuringiensis* var. *kurstaki* + $SiO_2$ significantly, and constitutes - 15.7% compared to the control variant, compared to the intensity of the process in the control grapevine plants.

Table 2. Determination of the quantityof pigments in grapevine leaves, Sauvignon variety, field experiment mg/100 g m

| Experimental variant | Chlorophyll *a* | | Chlorophyll *b* | | Chlorophyll *a+b* | | Carotinoids | |
|---|---|---|---|---|---|---|---|---|
| | M±m | Δ, % | M±m | Δ, % | M±m | Δ, % | M±m | Δ, % |
| 15.06. 2021 | | | | | | | | |
| 1.* | 1.14±0.01 | | 0.67±0.02 | | 1.81±0.02 | | 0.28±0.04 | |
| 2. | 1.22±0.01 | 10,7 | 0.73±0.02 | 20.6 | 1.95±0.03 | 10.7 | 0.33±0.01 | 11.7 |
| 3. | 1.08±0.00 | 9.47 | 0.52±0.00 | 27.2 | 1.61±0.00 | 8.8 | 0.33±0.03 | 11.7 |
| 4 | 1.12±0.02 | 9.82 | 0.68±0.01 | 34.7 | 1.80±0.03 | 10.0 | 0.29±0.04 | 10.2 |
| 5. | 1.16±0.01 | 10.1 | 0.76±0.02 | 21.2 | 1.91±0.02 | 10.5 | 0.33±0.01 | 11.7 |
| 6. | 1.14±0.01 | 10.0 | 0.68±0.02 | 23.1 | 1.82±0.02 | 10.0 | 0.31±0.04 | 10.6 |
| 7. | 1.26±0.05 | 11.0 | 0.79±0.02 | 22.3 | 2.09±0.10 | 11.5 | 0.22±0.10 | 7.8 |
| 17.07.2021 | | | | | | | | |
| 1. | 0.81±0.03 | | 0.69±0.06 | | 1.50±0.08 | | 0.21±0.01 | |
| 2. | 0.98±0.00 | 12.2 | 0.66±0.01 | 10.22 | 1.63±0.01 | 10.8 | 0.23±0.00 | 10.6 |
| 3. | 0.91±0.03 | 11.2 | 0.63±0.06 | 18.7 | 1.54±0.08 | 10.2 | 0.24±0.02 | 11.4 |
| 4. | 0.81±0.00 | 10.0 | 0.56±0.01 | 11.4 | 1.38±0.01 | 9.2 | 0.23±0.00 | 10.6 |
| 5. | 1.03±0.01 | 12.6 | 0.85±0.02 | 14.2 | 1.87±0.02 | 12.4 | 0.16±0.01 | 7.6 |
| 6. | 0.80±0.03 | 10.4 | 0.72±0.06 | 16.1 | 1.51±0.09 | 10.0 | 0.22±0.02 | 10.4 |
| 7. | 1.02±0.05 | 12.3 | 0.87±0.02 | 15.6 | 1.89±0.10 | 12.6 | 0.24±0.10 | 11.4 |
| 06.08. 2021 | | | | | | | | |
| 1. | 1.17±0.02 | | 0.45±0.00 | | 1.62±0.00 | | 0.54±0.01 | |
| 2. | 1.25±0.05 | 10.6 | 0.55±0.01 | 12.2 | 1.80±0.01 | 11.1 | 0.53±0.02 | 9.8 |
| 3. | 1.13±0.16 | 9.6 | 0.56±0.00 | 12.3 | 1.82±0.00 | 11.3 | 0.51±0.03 | 9.4 |
| 4. | 1.28±0.03 | 10.9 | 0.59±0.00 | 12.7 | 1.87±0.00 | 11.5 | 0.55±0.00 | 10.2 |
| 5. | 1.31±0.01 | 11.1 | 0.70±0.00 | 15.5 | 2.01±0.00 | 12.4 | 0.52±0.00 | 9.5 |
| **6.** | 1.25±0.01 | 10.6 | 0.74±0.07 | 16.4 | 1.87±0.09 | 11.5 | 0.50±0.04 | 9.2 |

1. - Control (plants treated with distilled $H_2O$);2. - Chemical standard; 3. - $SiO_2$;4. - *P. aureofaciens.*+ $SiO_2$; 5. - *P. aureofaciens* +*B. thuringiensis* var.*kurstaki;* 6. - *B. thuringiensis* var. *kurstaki* + $SiO_2$; 7. - *P.aureofaciens*+*B. thuringiensis var. kurstaki* + $SiO_2$

Closing the stomata reduces water loss through transpiration, but inevitably also leads to decreased photosynthesis due to reduced access of carbon dioxide to chloroplasts *(*Avis et al., 2008*)*. Analysis of the results obtained using the LCpro-SD portable gas analyzer in the experiments shows insignificant differences in plant response to treatment in the other variants.

Table 3. Influence of rhizobacteria on photosynthesis intensity, transpiration and stomatal conductance in grapevine, Sauvignon variety, Field experiment 2020

| Experimental variant | Intensity of photosynthesis, Mm/m$^2$/sec | | Transpiration intensity, Mm/m$^2$/sec | | Stomatal conductance, Mm/m$^2$/sec | |
|---|---|---|---|---|---|---|
| | M±m | Δ, % | M±m | Δ, % | M±m | Δ, % |
| **1.*** | 4.25 ±0.24 | | 1.86 ±0.34 | | 0.16 ±0.04 | |
| **2.** | 5.87 ±0.14 | 13.8 | 2.15 ±0.31 | 11.5 | 0.17 ±0.07 | 10.6 |
| **3.** | 5.86 ±0.25 | 13.7 | 2.43 ±0.26 | 13.0 | 0.19 ±0.01 | 11.8 |
| **4** | 5.43 ±0.18 | 12.7 | 2.49 ±0.21 | 13.3 | 0.18 ±0.02 | 11.2 |
| **5.** | 6.71 ±0.15 | 15.7 | 3.16 ±0.15 | 16.9 | 0.24 ±0.05 | 15.0 |
| **6.** | 5.58 ±0.34 | 13.1 | 2.45 ±0.17 | 13.1 | 0.16 ±0.04 | 10.0 |

**1**. Control; 2. *P. aureofaciens*; **3**. *P. aureofaciens* + $SiO_2$; **4.** *P. aureofaciens*+*B.* thuringiensis var. *kurstaki*; **5.** *B. thuringiensis* var. *kurstaki* + $SiO_2$; **6.** *P. aureofaciens*+*B. thuringiensis* var. *kurstaki* + $SiO_2$

A rather wide aspect of the response of grapevines to fertilizer application, identified by many researchers, is closely related to productivity (Naoko Ohkama-Ohtsu and Jun

Wasaki., 2010). The quality and quantity of the grape harvest is the defining indicator by which the effectiveness of an agrotechnical method is measured. Harvest records according to the variants of the experiments were carried out using the following indices: average quantity of grapes per plant, average weight of a grape, average yield per plant, quality of grapes.

Analyzing the data obtained (Table 4) it was observed that in the variant where bacteria with *P. aureofaciens+B. thuringiensis* var. *kurstaki* strains were used with the addition of $SiO_2$ the average quantity of grapes per plant is 12.1% higher than in the control variant.

The average weight of a grape was higher in the variant where the bacterial strain *B. thuringiensis* var. *kurstaki* + $SiO_2$ was used. This effect is due to the growth stimulating effect these bacterial strains have on the plants, and on the assimilation of nutrients.

Table 4. Effect of foliar fertilisation on the quantity of Sauvignon vine yield IGPPP experimental lot, Field experiment 2020

| **Experi-mental variant** | **Average quantity of grapes per plant** | | **Average weight of 1 grape, g.** | | **Harvest** | | **% of sugar** | |
|---|---|---|---|---|---|---|---|---|
| | | | | | **Average/Plant, kg.** | | | |
| | **M±m** | **Δ, %** | **M±m** | **Δ, %** | **M** | **Δ, %** | **M±m** | **Δ, %** |
| **1.** | 12.2±4.18 | | 104.05±1.16 | | 1.3 | | 22.4±0.05 | |
| **2.** | 12.8±6.59 | 10.4 | 133.0±1.99 | 12.7 | 1.7 | 13.0 | 24.3±0.04 | 10.8 |
| **3.** | 14.0±8.07 | 11.4 | 146.5±3.66 | 14.0 | 2.0 | 15.3 | 24.0±0.04 | 10.7 |
| **4** | 14.0±5.76 | 11.4 | 122.1±3.03 | 11.6 | 1.7 | 13.0 | 24.7±0.05 | 11.0 |
| **5.** | 13.8±4.89 | 11.3 | 133.7±2.06 | 12.7 | 1.8 | 13.8 | 24.3±0.03 | 10.8 |
| **6.** | 14.8±2.72 | 12.1 | 126.1±3.02 | 12.0 | 1.9 | 14.6 | 24.0±0.05 | 10.7 |

**1**. - Control; 2. - *P. aureofaciens*; **3.** - *P. aureofaciens* + $SiO_2$; **4. -** *P. aureofaciens+B.* thuringiensis var. *kurstaki*; **5. -** *B. thuringiensis* var. *kurstaki*+ $SiO_2$; **6.** - *P.aureofaciens+B. thuringiensis* var. *kurstaki* + $SiO_2$

## CONCLUSIONS

It has been shown that the application of suspensions of bacterial strains *P. aureofaciens* and *B. thuringiensis* var. *kurstaki* with $SiO_2$ addition at critical phases of plant development contributed to increase the photosynthetic pigment content in grapevine leaves, increasing the quality and quantity of the harvest.

The use of suspensions of bacterial strains *P. aureofaciens* and *B. thuringiensis* var. *kurstaki* with $SiO_2$ addition contributed to increase the intensity of photosynthesis, transpiration and stomatal conductance, as well as the weight gain of grapes in grapevine plants.

## ACKNOWLEDGEMENTS

This study was supported by the National Agency for Research and Development of the Republic of Moldova through the project 20.80009.7007.16 "Synergism between natural factors and environmentally benign microbiological means of pest population density regulation for crop protection in conventional and organic agriculture".

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