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Victoria Tsygankova
Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, Institute of Bioorganic, Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, 1, Murmanskaya str., Kyiv, Ukraine

Elena Shysha
Department Genomics and Molecular Biotechnology, Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, 2a, Osypovskogo str., Kyiv, Ukraine.

Anatoly Galkin
Department Genomics and Molecular Biotechnology, Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, 2a, Osypovskogo str., Kyiv, Ukraine.

Lyudmila Biliavska
Department of General and Soil Microbiology, D.K.Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154, Zabolotnogo str., Kyiv, Ukraine.

Galina Iutynska
Department of General and Soil Microbiology, D.K.Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine, 154, Zabolotnogo str., Kyiv, Ukraine.

Alla Yemets
Department Genomics and Molecular Biotechnology, Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, 2a, Osypovskogo str., Kyiv, Ukraine.

Yaroslav Blume
Department Genomics and Molecular Biotechnology, Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, 2a, Osypovskogo str., Kyiv, Ukraine.

Correspondence
Victoria Tsygankova
Department for Chemistry of Bioactive Nitrogen-Containing Heterocyclic Compounds, Institute of Bioorganic, Chemistry and Petrochemistry, National Academy of Sciences of Ukraine, 1, Murmanskaya str., Kyiv, Ukraine

Impact of microbial biostimulants on induction of callusogenesis and organogenesis in the isolated tissue culture of wheat *in vitro*

Victoria Tsygankova, Elena Shysha, Anatoly Galkin, Lyudmila Biliavska, Galina Iutynska, Alla Yemets and Yaroslav Blume

Abstract
Impact of microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit on induction of callus formation and regeneration of plantlets of wheat (*Triticum aestivum* L.) of cultivar Zimoyarka on the MS nutrient media supplemented with these biostimulants was studied *in vitro* conditions. The increase of frequency of formation of callus cells (from 43 up to 74 %) was observed on the apical meristem of 4-5-day-old seedlings of wheat placed on the MS media supplemented with microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 60 µl/l in combination with 1 mg/l auxin 2.4-D (experiment) as compared with lower frequency of formation of callus cells obtained on the MS media supplemented with only auxin 2.4-D used at the concentration 1 mg/l (control). The increase of efficiency of regeneration of wheat shoots (from 35 up to 65 %) was observed on the 3-4-week-old callus tissue grown on the MS media supplemented with microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 20 up to 100 µl/l in combination with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA (experiment) as compared with lower efficiency of regeneration of wheat shoots obtained on the MS media supplemented with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA (control). The rooting of shoots was shown on the MS media supplemented with microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 100 µl/l. The practical application of microbial biostimulants for increase efficiency of callusogenesis and organogenesis in the isolated tissue culture of wheat was proposed.

Keywords: microbial biostimulants, callusogenesis, organogenesis, *Triticum aestivum*

Introduction
Wheat (*Triticum aestivum* L.) is an ancient cereal crop widely cultivated in different countries of the world [1-8]. Wheat is a strategically important crop containing essential nutrients such as proteins, lipids and carbohydrates. This cereal crop provides by 30 % of the food calories consumed by the world's population, which accounts for more than 4.5 billion people [3-4]. However, despite the annual increase in the sown area and the yield of wheat there are significant problems with increasing the productivity of this crop since by 2050 the world population is expected to reach 9.7 billion people [7]. Wheat is also used as a source of bioactive compounds for medical industry such as alkaloids, flavonoids, terpenoids, glycosides, steroids, tannins, saponins, and as a raw material for the production of malt and beer [5, 8].

Unfavorable environmental factors (i.e. global climatic changes, cold, heat, drought, soil salinity and contamination by industrial waste, wide spread of pathogens and pests) negatively affect the growth and productivity of wheat [9-15]. Nowadays the various phytohormones, plant growth regulators, biostimulants, herbicides, mineral and organic fertilizers are used in agricultural practices to improve the growth and development of wheat and increase its adaptive properties to stress-factors of environment [16-24].

The alternative approach is application of phytohormones and plant growth regulators of natural origin in biotechnological practice to obtain *in vitro* of new lines of wheat cells with genetically improved commercially important traits such as accelerated growth and development, increased productivity and product quality, and enhanced resistance to a biotic and biotic stress factors such as drought, salinity, cold, pathogenic and parasitic organisms, and soil pollution by anthropogenic factors.

The numerous works devoted to solution of this problem confirm the perspective of this strategy, nevertheless, the elaboration of new effective techniques for cultivation and regeneration of wheat *in vitro* conditions is a very important task for plant biotechnologists [26-43].

Our earlier studies [44] showed the possibility of application *in vitro* conditions of new microbial biostimulants created on the base of metabolism products of soil streptomycetes in the Zabolotny Institute of Microbiology and Virology, NAS of Ukraine, for obtaining of new lines of wheat (*Triticum aestivum* L.) of two cultivars Yatran 60 and Zimoyarka with increased immune-mediated resistance to cereal cyst nematode *Heterodera avenae*, which belongs to one of the most dangerous pests [45-50].

This work was aimed to study of impact of microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit on increase efficiency of callusogenesis and organogenesis in the isolated tissue cultures of wheat (*Triticum aestivum* L.) of cultivar Zimoyarka *in vitro* conditions.

2. Materials and Methods

2.1 Compositions of microbial biostimulants

In this work we studied effect of new polycomponent microbial biostimulants elaborated in the Zabolotny Institute of Microbiology and Virology, NAS of Ukraine on base of selected strains of soil streptomycetes: *Streptomyces avermitilis* IMV Ac-5015 (Avercom, Avercom nova-2), *Streptomyces netroposis* IMV Ac-5025 (Phytovit) and *Streptomyces violaceus* IMV Ac-5027 (Violar) on increase efficiency of callusogenesis and organogenesis in the isolated tissue cultures of wheat plants. The main components of these biostimulants are metabolism products of soil streptomycetes such as phytohormone (auxin such as indole-3-acetic acid, cytokinins such as isopentenyl adenine, zeatin and zeatin riboside, gibberellins and brassinosteroids), free fatty acids, amino acids, vitamins of the B group, lipids, and antiparasitic antibiotics [44, 51-53].

Avercom contains ethanol extract from 7-days biomass of *S. avermitilis* IMV Ac-5015, which produces of antibiotic avermectin. Avercom nova-2 contains 50 ml of Avercom with antibiotic avermectin in concentration 100 μg/ml and 50 ml of supernatant of cultural liquid of *S. avermitilis* IMV Ac-5015 and 0.01 mM of water-soluble chitosan of "Sigma" Company. Violar contains the supernatant of cultural liquid and biomass ethanol extract (4:1) of S. *violaceus* IMV Ac-5025, which produces of antibiotic of anthracycline nature. Phytovit contains the supernatant of cultural liquid and biomass ethanol extract (4:1) of *S. netroposis* IMV Ac-5027, which produces of antibiotic of polyene nature.

2.2 Plant Material

In our researches we used as a plant material seeds of wheat (*Triticum aestivum* L.) of cultivar Zimoyarka. To obtain wheat explants and further their introducing *in vitro* culture wheat seeds were subjected to surface sterilization with 70% ethanol for 2 min. and with 0.02 % solution of $AgNO_3$ for 10-30 min. After sterilization wheat seeds were washed with sterile water 3 times for 30 sec. and 2 times for 10 min. For further germination seeds were placed at the Petri dishes (18-20 seeds per one Petri dish having d=90 mm), each containing 25 ml nutrient MS (Murashige and Skoog) medium [54] supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 50 μl/l. Then the Petri dishes were placed in the chamber for plant tissue cultivation in which seedlings were grown for 4-5 days at the 16/8 h light/dark conditions, at the temperature 22-24^{0}C, the light intensity 1500-2000 lux and air humidity 60-80 %.

2.3 Culture Media for Callus Formation

As explants for formation of callus cells we used apical meristem of 4-5-day-old wheat seedlings that were separated from seedlings and placed on the MS medium supplemented only with auxin 2.4-D used at the concentration 1 mg/l (control) or on the MS media supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 60 μl/l in combination with 1 mg/l auxin 2.4-D (experiment). Then the Petri dishes were placed in the chamber for plant tissue cultivation for 3-4 weeks at the above mentioned conditions.

2.4 Culture Media for Regeneration of Shoots and their Rooting

With the aim to increase efficiency of regeneration of shoots on the 3-4-week-old callus tissue formed on the isolated apical meristems of 4-5-day-old wheat seedlings we used MS media supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations: 20, 40, 60, 80 or 100 μl/l in combination with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA (experiment). As a control we used MS medium supplemented with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA. To obtain root formation on the shoots we used MS media supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 100 μl/l.

2.5 Statistical Analysis

All the experiments were performed in three replicates. The frequency of callus formation was determined according to the number of explants producing callus tissues (in %) formed on the MS media supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 60 μl/l in combination with 1 mg/l auxin 2.4-D (experiment) or on the MS media supplemented only with auxin 2.4-D used at the concentration 1 mg/l (control) relatively to overall number of explants.

The efficiency of regeneration of shoots was determined according to the number of shoots (in %) formed on the explants on the MS media supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 20 up to 100 μl/l in combination with 1-2 mg/l cytokinin BAP and 0,5-1 mg/l auxin IAA (experiment) or on the MS media supplemented only with 1-2 mg/l cytokinin BAP and 0,5-1 mg/l auxin IAA (control) relatively to overall number of explants. Statistical analysis of the data was performed using dispersive Student's-t test with the level of significance at $p<0.05$, the values are mean ± SD [55].

3. Results

3.1 Effect of microbial biostimulants on formation and growth of callus cells

In our experiments as a plant material we used mature seeds of wheat (*Triticum aestivum* L.) of cultivar Zimoyarka for an introduction to the culture *in vitro* [42]. To obtain wheat seedlings the sterilized seeds were placed for germination on

the MS medium supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 50 µl/l (experiment, Figure 1, A) or without adding biostimulants into MS medium (control). As a primary explants for callusogenesis and further regeneration of shoots we used apical meristem of 4-5 days seedlings [25, 32].

To initiate callus formation we used modified MS media containing each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 50 µl/l in combination with 1 mg/l auxin 2.4-D (experiment, Figure 1, B) or without adding biostimulants into MS medium supplemented with only auxin 2.4-D used at the concentration 1 mg/l (control).

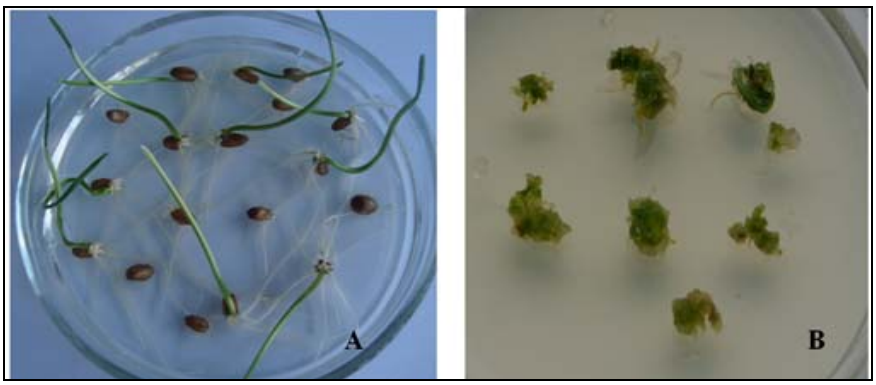

Fig 1: Influence of the biostimulant Avercom on the germination of seeds of wheat of cultivar Zimoyarka and formation of callus cells from apical meristem of 4-5-day-old wheat seedlings. A – 5-day-old wheat seedlings obtained on the MS medium supplemented with 40 µl/l biostimulant Avercom; B – Callus cells obtained on the apical meristems of 4-5-day-old wheat seedlings of cultivar Zimoyarka on the MS medium supplemented with 50 µl/l of biostimulant Avercom in combination with 1 mg/l auxin 2.4-D + 150 mg/l L-asparagine + 10 mg/l $AgNO_3$

It was found that highest frequency of callus formation was observed on the apical meristem of 4-5-day-old wheat seedlings on the MS media supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the most effective concentrations 20 and 30 µl/l in Combination with 1 mg/l auxin 2.4-D (experiment) as compared with lower frequency of callus formation obtained on the MS media supplemented only with auxin 2.4-D used at the concentration 1 mg/l (control) (Figure 2).

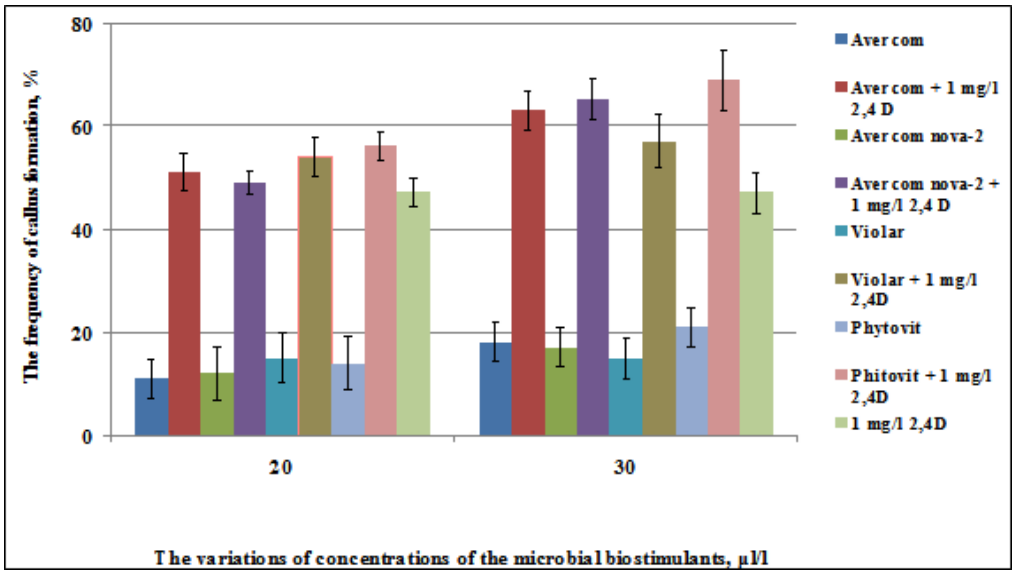

Fig 2: The frequency of callus formation obtained on the MS media supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations 20 and 30 µl/l in combination with 1 mg/l auxin 2.4-D (experiment) and on the MS medium supplemented with 1 mg/l auxin 2.4-D (control)

3.2 Effect of microbial biostimulants on Regeneration of Shoots and their Rooting

To study impact of microbial biostimulants on regeneration of shoots on the 2-3-week-old callus tissue formed on the isolated apical meristems of 4-5-day-old wheat seedlings we used MS media supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the different concentrations: 20, 40, 60, 80 or 100 µl/l in combination with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA (experiment). As a control we used MS medium supplemented only with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA. The more intensive formation of shoots was observed on the 3-week-old callus tissue grown on the MS medium supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentration 60 µl/l in combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA as compared with lower intensity formation of shoots observed on the control MS medium (Figure 3).

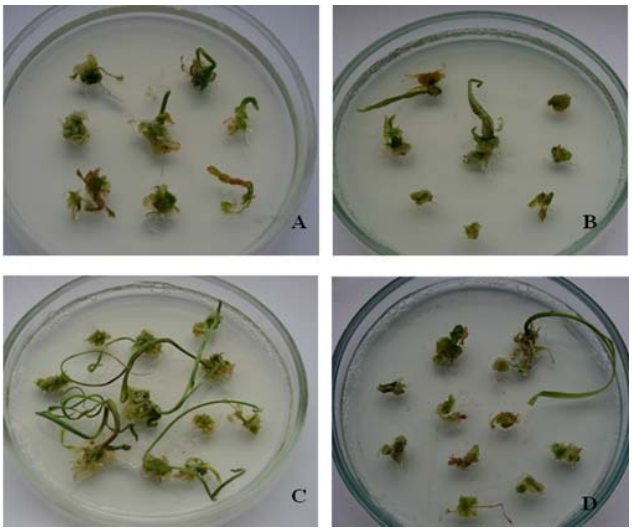

Fig 3: Regeneration of shoots on the 3-week-old callus tissue formed on the isolated apical meristems of 4-5-day-old wheat seedlings on the MS medium supplemented with 1 mg/l BAP + 0.5 mg/l IAA + 150 mg/l L-asparagine + 10 mg/l $AgNO_3$ and each microbial biostimulant used at the concentration 60 µl/l: A - Avercom; B - Avercom nova-2; C – Violar and D - Phytovit

The obtained wheat shoots for their further growth and development were transferred on the nutrient MS media supplemented with each microbial biostimulant: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 100 µl/l in combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA (experiment) (Figure 4).

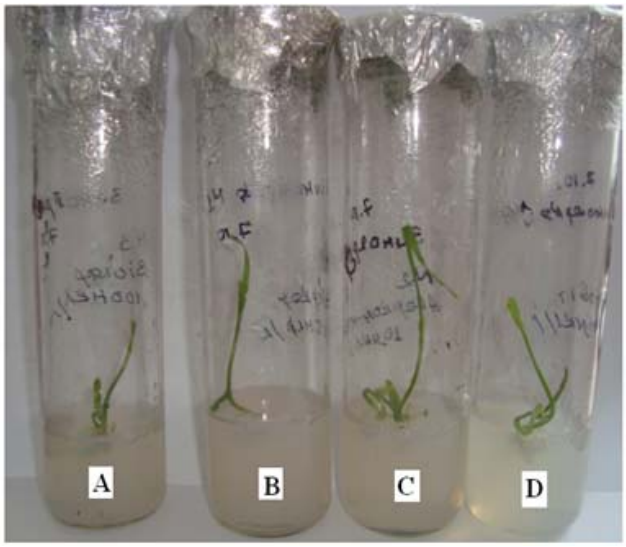

Fig 4: Plantlets of wheat regenerated on the nutrient MS media supplemented with 100 µl/l of each microbial biostimulant: A - Avercom, B - Avercom nova-2, C - Violar or D - Phytovit in combination with 1 mg/l BAP and 0.5 mg/l IAA

It was found that the microbial biostimulants Avercom and Avercom nova-2 supplemented in the MS media at the concentrations 20 and 40 µl/l in combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA showed the highest stimulating effect on the formation of wheat shoots. The lower stimulating effect of microbial biostimulants Avercom and Avercom nova-2 on the formation of wheat shoots was observed on the experimental MS media containing these biostimulants at the concentrations: 60, 80 and 100 µl/l in combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA. At the same time the least formation of wheat shoots was observed on the control MS medium containing only 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA (Figures 5 and 6).

Fig 5: Plantlets of wheat regenerated on the nutrient MS media supplemented with: A - 100 µl/l Avercom + 1 mg/l BAP + 0,5 mg/l IAA + 150 mg/l asparagin + 10 mg/l $AgNO_3$ (experiment), B – 1 mg/l BAP + 0,5 mg/l IAA + 150 mg/l asparagin + 10 mg/l $AgNO_3$(control)

Fig 6: Plantlets of wheat regenerated on the nutrient MS media supplemented with: A - 100 µl/l Avercom nova-2 + 1 mg/l BAP + 0,5 mg/l IAA + 150 mg/l asparagin + 10 mg/l $AgNO_3$ (experiment), B – 1 mg/l BAP + 0,5 mg/l IAA + 150 mg/l asparagin + 10 mg/l $AgNO_3$(control)

At the same time it was found that microbial biostimulants Violar and Phytovit supplemented in the MS media revealed the highest stimulating effect on formation of wheat shoots at their concentrations: 80 and 100 µl/l in combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA as compared with control MS medium supplemented only with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA (Figure 7).

Fig 7: Plantlets of wheat regenerated on the nutrient MS media supplemented with: A - 100 µl/l Violar + 1 mg/l BAP + 0,5 mg/l IAA + 150 mg/l asparagin + 10 mg/l $AgNO_3$ (experiment), B – 100 µl/l Phytovit + 1 mg/l BAP + 0,5 mg/l IAA + 150 mg/l asparagin + 10 mg/l $AgNO_3$ (control)

The obtained data of the statistical analysis of the efficiency of regeneration of wheat shoots (in %) obtained on the experimental MS media supplemented with microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the different concentrations ranging from 20 up to 100 µl/l in combination with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA as well as on the control media supplemented only with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA are shown on the Figures 8-11.

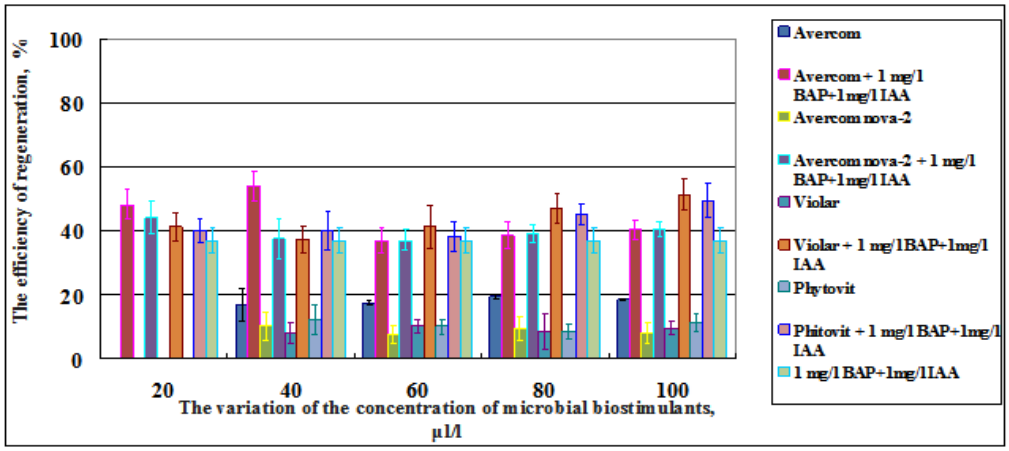

Fig 8: The efficiency of regeneration of wheat shoots obtained on the MS media supplemented with microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 20 up to 100 µl/l in combination with 1 mg/l cytokinin BAP and 1 mg/l auxin IAA (experiment) and MS media supplemented with 1 mg/l cytokinin BAP and 1 mg/l auxin IAA (control)

The obtained data witness that the highest efficiency of regeneration of wheat shoots was found on the experimental MS media supplemented with microbial biostimulants in Combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA as compared with lower efficiency of regeneration of wheat shoots obtained on the control MS media (Fig. 8-11).

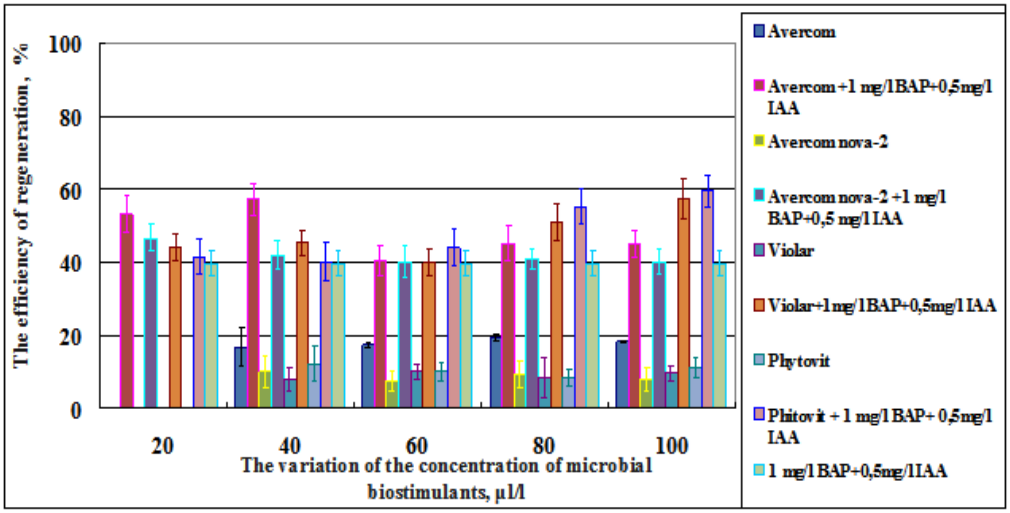

Fig 9: The efficiency of regeneration of wheat shoots obtained on the MS media supplemented with microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 20 up to 100 µl/l in combination with 1 mg/l cytokinin BAP and

0.5 mg/l auxin IAA (experiment) and MS media supplemented with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA (control)

Our researches showed that the microbial biostimulants Avercom and Avercom nova-2 revealed the highest stimulating effect on the regeneration of wheat shoots on MS media at the concentrations: 20 and 40 µl/l. The lower Stimulating effect on the regeneration of wheat shoots was observed on the MS media supplemented with microbial biostimulants Avercom and Avercom nova-2 used at the concentrations: 60, 80 and 100 µl/l (Figures 8-11).

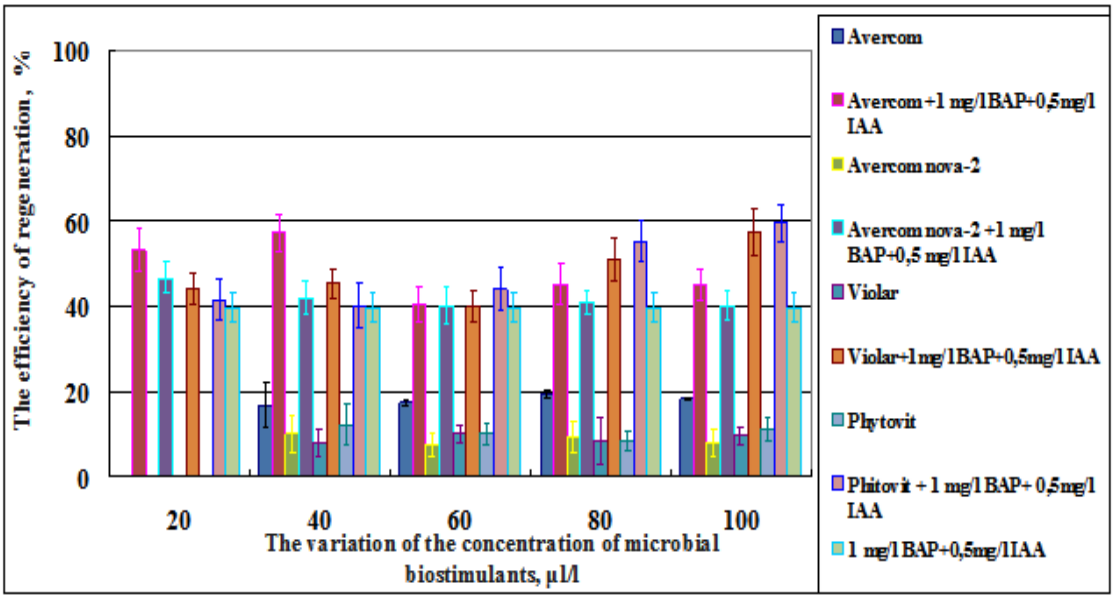

Fig 10: The efficiency of regeneration of wheat shoots obtained on the MS media supplemented with microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 20 up to 100 µl/l in combination with 2 mg/l cytokinin BAP and 1 mg/l auxin IAA (experiment) and MS media supplemented with 2 mg/l cytokinin BAP and 1 mg/l auxin IAA (control)

At the same time the obtained data witness that microbial biostimulants Violar and Phytovit revealed the highest stimulating effect on the regeneration of wheat shoots on MS Media at their optimal concentrations: 80 and 100 µl/l in combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA (Figures 8-11).

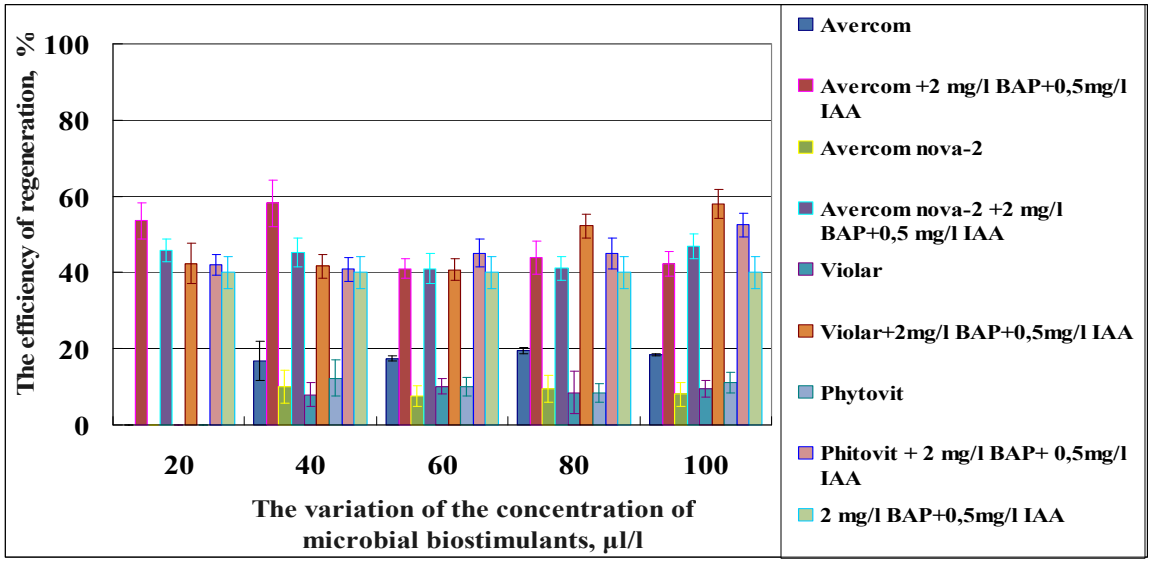

Fig 11: The efficiency of regeneration of wheat shoots obtained on the MS media supplemented with microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 20 up to 100 µl/l in combination with 2 mg/l cytokinin BAP and 0.5 mg/l auxin IAA (experiment) and MS media supplemented with 2 mg/l cytokinin BAP and 0.5 mg/l auxin IAA (control)

The lower stimulating effect on the regeneration of wheat shoots was observed on the MS media supplemented with microbial Violar and Phytovit used at the concentrations: 20, 40 and 60 µl/l (Figures 8-11).

At the further stage the wheat shoots previously regenerated on the MS media supplemented with microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 20 up to 100 µl/l in combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA were rooted on the MS media supplemented with these microbial biostimulants used at the concentrations ranging from 10 up to 100 µl/l in combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA (Figure 12).

Fig 12: The formation of roots on the wheat shoots on the MS medium supplemented with microbial biostimulant Avercom used at the concentration 20 µl/l in combination with 1 mg/l cytokinin BAP and 0.5 mg/l auxin IAA

Taking into account the obtained results it can be concluded that application of new microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 20 up to 100 µl/l in combination with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA on the MS nutrient media sufficiently increases efficiency of callusogenesis and organogenesis in the isolated tissues of wheat (*Triticum aestivum* L.) of cultivar Zimoyarka.

4. Discussion

Today there many studies evidencing that effectiveness of wheat regeneration *in vitro* conditions depends from the morphogenic response of different types of explants (i.e. mature or immature embryo, leaves, petioles, roots), genotypes of wheat, compositions of the phytohormones or synthetic growth regulators and their different combinations in the nutrient media for plant tissue cultivation [25-43].

Among the different cultivars of wheat the cultivar Zimoyarka showed high morphogenic response *in vitro* on the MS media supplemented with synthetic growth regulators of auxin nature: dicamba at different concentrations (0.2, 0.4, 0.6 mg/l), and picloram (0.16, 0.25, 0.5 mg/l) [41]. The obtained data witness that dicamba used at the concentration of 0.2 mg/l was the most effective for production of regenerants. It was also observed that at the concentration of 0.16 mg/l picloram there were the formation of the greatest number of morphogenic zones (60 %) and a significant amount of plant-regenerants [41].

The literature data coincide with the results of our previously published work [44], in which we have elaborated and proposed novel approach in pest management: application of microbial biostimulants created on the base of metabolism products of soil streptomycetes: Avercom, Avercom nova-2, Violar and Phytovit on the nutrient MS media for obtaining *in vitro* conditions of new lines of wheat (*Triticum aestivum* L.) of two cultivars Yatran 60 and Zimoyarka having increased resistance to cereal cyst nematode *Heterodera avenae*, which belongs to one of the most dangerous pests [45-50]. The obtained results confirmed high stimulating effect of microbial biostimulants on induction of both callusogenesis and shoot organogenesis, which were observed on the apical meristem isolated from shoots and roots of 4-5-day-old wheat seedlings placed on the MS media supplemented with microbial biostimulants used at the optimal concentrations ranging from 10 up to 50 µl/l in combination with either 1-2 mg/l auxin 2.4-D or 1-2 mg/l cytokinin BAP [44]. The highest efficiency of shoot regeneration (up to 60 %) was observed on the isolated explants of wheat (*Triticum aestivum* L.) of the genotype Zimoyarka on the MS media supplemented with microbial biostimulants in combination with 1-2 mg/l BAP and 0.5-1 mg/l IAA; the lower efficiency of shoot regeneration (up to 50 %) was observed on the MS media supplemented with microbial biostimulants in combination only with 1-2 mg/l BAP.

The present work was aimed to optimization of compositions of nutrient MS media supplemented with different concentrations of microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit in combination with cytokinin BAP and auxin IAA to increase efficiency of callusogenesis and organogenesis in the isolated tissues of wheat (*Triticum aestivum* L.) of cultivar Zimoyarka.

Our researches showed that addition of microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit in the MS media at the concentrations ranging from 10 up to 60 µl/l in combination with 1 mg/l auxin 2.4-D (experiment) significantly increase of frequency of formation of callus cells (from 43 up to 74 %) on the apical meristem of 4-5-day-old seedlings of wheat as compared with lower frequency of formation of callus cells obtained on the control MS media supplemented with only auxin 2.4-D used at the concentration 1 mg/l.

It was also found that addition of microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit in the MS media at the concentrations ranging from 20 up to 100 µl/l in combination with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA (experiment) significantly increased efficiency of regeneration of wheat shoots (from 35 up to 65%) on the 3-4-week-old callus tissue formed on the apical meristem of 4-5-day-old seedlings of wheat as compared with lower efficiency of regeneration of shoots obtained on the control MS media supplemented only with 1-2 mg/l cytokinin BAP and 0.5-1 mg/l auxin IAA.

The obtained results showed also stimulating effect of microbial biostimulants: Avercom, Avercom nova-2, Violar or Phytovit used at the concentrations ranging from 10 up to 100 µl/l on the formation of roots on the regenerated shoots of wheat.

Thus the data of the present work confirmed possibility of practical application of microbial biostimulants for increase efficiency of callusogenesis and organogenesis on the isolated tissue culture of wheat (*Triticum aestivum* L.) of cultivar Zimoyarka.

5. Conclusion

In vitro conditions impact of microbial biostimulants on initiation of callus formation and induction of shoot organogenesis on the isolated apical meristem of 4-5-day-old seedlings of wheat (*Triticum aestivum* L.) of cultivar Zimoyarka was studied. The obtained results witness that application of new microbial biostimulants: Avercom, Avercom nova-2, Violar and Phytovit used at the concentrations ranging from 10 up to 100 µl/l in combination with 1-2 mg/l cytokinin BAP and 1 mg/l auxin 2.4-D or 0.5-1 mg/l auxin IAA on the MS nutrient media sufficiently increases of efficiency of callusogenesis and organogenesis in the isolated tissues of wheat (*Triticum aestivum* L.) of cultivar Zimoyarka. The new lines of wheat plants obtained *in vitro* conditions on the MS media supplemented with microbial

biostimulants will be further tested on the resistance to parasitic cereal cyst nematode *Heterodera avenae* in the greenhouse conditions.

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