UDC 60:- 634.8: -579.64

N. Tesliuk, N. Tytarenko

IMPROVING OF ADAPTATION OF THE THORNLESS BLACKBERRY MICROCLONES WITH USING OF BACILLUS MEGATERIUM AND ENTEROCOCCUS ITALICUS STRAINS

Odesa I. I. Mechnykov National University, 2, Dvoryanska st., Odesa, 65082, Ukraine, tel.: +38 (0482) 68 79 64, e-mail: alannat@rambler.ru

Summary

The effect of bacterial cultures on the processes of adaptation to the in vivo conditions of the thornless blackberry microclones, which were cultivated in vitro on the Murashige and Skoog medium, was studied. Evaluating the growth and development rates of experimental plants over time, it was possible to establish optimal dilution of pure culture of Bacillus megaterium, which contributed to the increase of survivability of the laboratory plants in the soil and positively influenced on the external characteristics of the adapted blackberry plants.

Key words: *in vitro culture, in vivo adaptation, microclones, Bacillus megaterium, Enterococcus italicus.*

Microorganisms and products of their metabolism find their application in solving of a wide range of different tasks. It is very promising to use microbial preparations as auxiliary agents in plant biotechnology. The convenient and rapid method of microclonal propagation, which allows to get hundreds of genetically homogeneous healthy plants for the short time, is facing with a number of methodological problems today. Different species and plant varieties need correction in the classic propagation technique. The peculiarities arise from the stage of introduction into the culture and cultivation itself [3, 5]. Nevertheless, if these stages are successfully overcome, the last and most difficult period comes for plants then - it is adaptation from in vitro to in vivo conditions.

When growing in a sterile box, plants develop in perfectly comfortable conditions, which can not be achieved in the open field. These are illumination according to the schedule, constant temperature, humidity, nutrients and all necessary compounds from the nutrient medium. Also the intensity of photosynthesis is reduced in plants under such conditions. But, what is the most important, plants are completely deprived of contact with any infections. Thus, the process of adaptation, which means getting used to normal soil conditions with every day changes, is a huge stress for the plants and a possible risk for the researcher to lose a large amount of propagated material [1]. It is expedient in this stage to use bacterial cultures to increase efficiency of adaptation and viability of the planting material. Due to their antagonistic properties to plant pathogens, some microorganisms demonstrate great potential for using them as preparations to improve the resilience of plants on the field. Literature sources [2, 4, 5] indicate a probable positive effect from some strains of the *Bacillus* and *Enterococcus* genus.

The **aim** of our study was to use the strains of *Bacillus megaterium* and *Enterococcus italicus* on thornless blackberry culture *in vitro* to improve the adaptation process to the *in vivo* conditions.

Materials and methods

Our experiment included using of *Bacillus megaterium* ONU500 and *Enterococcus italicus* ONU547 strains that were obtained at the laboratory of the Odessa National University.

Bacillus megaterium are a rod-like gram-positive spore-forming bacteria that are often associated in pairs and chains. Bacteria got their name due to large sizes that far exceed others, especially in comparison with *E. coli*. *B. megaterium* can be found in a large range of environments, but it is traditionally considered to be a soil microorganism. It is also widely used in biotechnology as a producer of recombinant proteins. Some strains have shown themselves to be powerful antagonists to phytopathogens, and some are able to accumulate phosphorus and make it an affordable component of mineral nutrition of plants [2].

For the experiment on improving the efficiency of adaptation, a two-day culture of *B. megaterium* was grown on a liquid nutrient medium LB in a thermostat at a constant temperature of 28°C [4]. Two concentrations of the microbial culture were used. There were 50 percent and 25 percent dilutions with the number of viable cells $4.70*10^7$ in ml and $2.35*10^7$ in ml, respectively. Distilled water was added to the culture just before the experiment to obtain the necessary concentration.

Enterococcus italicus - gram-positive cocci which often can be observed united in pairs and short chains. They are optional anaerobes and typical habitants of intestines of some mammals. These microorganisms can be also isolated from several kinds of cheese - they are involved in maturation processes of this product. *E. italicus* is commensal and does not have a pathogenic effect on humans or animals [3].

In previous studies, the antagonistic activity of the strain *E. italicus* ONU547 against phytopathogens in vitro was detected, but efficiency in application on live plants was not yet verified [4].

A daily culture of *E. italicus*, grown on a liquid MRS medium in a thermostat at a temperature of 36°C, was used for the experiment [3]. Two concentrations of the microbial culture were used as well. There were 50 percent and 25 percent dilutions with the number of cells, on average, $2.94*10^8$ in ml and $1.47*10^8$ in ml, respectively.

The microclones of thornless blackberry *Rubus caesius* (the Thornfree variety) were used as a subject for adaptation. Microclones for the experiment had a height above 3.5-4 cm, 4-6 leaves, and a well-developed root system. They also did not form calluses. The material for adaptation was obtained by cultivating plants in vitro in a nutrient medium of Murashige and Skoog (MS) with addition of 20 g/l of sucrose, 9 g/l of agar, and 1 mg/l of 6-benzylaminopurine (6-BAP).

Adaptation was carried out in two stages. The first one was the habituation of plants to non-sterile conditions. To this end, plants were gradually allowed access to normal air for seven days, removing the covers from sterile containers with microclones [1]. Then the plants were planted into the prepared soil. At this stage, two potentially useful bacterial cultures were tested.

After the first stage of adaptation microclones were divided into three groups. The roots of the first group of plants were kept in a 50 percent solution of bacteria for 30 minutes before planting, the second group was kept in 25 percent solution and the third was a control with sterile distilled water.

After that, the plants were planted in individual containers with prepared soil. Processes of growth and development in experimental plants were observed during 100 days after planting. The experiment was conducted separately for two potentially effective bacterial cultures.

Results

Three-time frequency of the experiments with *E. italicus* did not show a positive effect on the survivability or any other parameters of the researched plants. Both the control and the experimental plants grew almost identically, hence no significant difference was observed. However, considering the positive results of in vitro studies, it can be assumed that *E. italicus* may exhibit its potential at other stages of plant cultivation and adaptation, which requires further research.

The results of experiments with *B. megateruim* revealed significant differences.

On average, 63% of control samples were lost and only 37% survived on the 100th day of the experiment, while viability of experimental plants was at the level

up to 73%. The 25% concentration of *B. megateruim* has shown the best results - when used, the most effective adaptation of plants to the soil was observed (Table 1).

Table 1

Average growth and survivability performance of thornless blackberry microclones during the adaptation process using *B. megaterium* strain ONU 500 (average data according to three replications of the experiment)

Time passed from the planting, days	Culture dilution of *B.megaterium*, %	Average survivability of the microclones, %	Average shoot height, cm	Average number of nodes, pieces	Average number of additional shoots,
14th	50	73,33	3,73	3,76	0
	25	**83,33**	**4,20**	**4,50**	0
	Control (H_2O)	63,33	3,36	3,30	0
30th	50	66,66	4,43	4,90	0,66
	25	**80,00**	**5,56**	**6,33**	0,66
	Control (H_2O)	46,66	3,76	3,63	0,33
100th	50	50,00	7,50	8,30	0,66
	25	**73,33**	**9,73**	**10,36**	1,66
	Control (H_2O)	36,66	6,30	7,20	0,66

Adaptation experiments with *B. megaterium* allowed to establish the following:

- the 25% dilution of the *B. megaterium* culture was the most effective for optimization of the process of adaptation for the studied species.
- the 50% dilution of the *B. megaterium* culture revealed intermediate results between the control and the 25% dilution.

- exposure of roots to the 25% solution of the *B. megaterium* culture before planting allowed to increase the viability of the thornless blackberry plants 2 times compared to the control on 100th day of cultivation.
- experimental plants, which were exposed to the 25% solution, on average, grew 1.3 times more rapidly and formed 1.2 times more nodes than the control did.

Also, the plants, which were exposed to the 25% solution of *B. megaterium*, had the biggest leaves with the most saturated shade of green among all other plants in the experiment (Fig. 1). This fact confirms more intensive photosynthesis in plant tissues and, as a result, the most effective adaptation within our research.

Fig. 1. Appearance of the blackberry plants after 4 weeks of adaptation (on the left - experimental plants, roots of which were kept in the 25% solution of the *B. megaterium* culture, on the right - control).

Thus, the first results of studies on the the thornless blackberry microclones allowed to confirm experimentally that *B. megateruim* strain ONU 500 positively influences on adaptation of laboratory plants to the soil conditions.

Conclusions

In our experiments, a positive effect of the 25% solution of the two-day *B. megaterium* culture on the processes of microclones adaptation of a valuable thornless blackberry variety from in vitro to in vivo conditions was established. The

obtained data testify to the real possibility of using the ONU 500 strain to increase the survivability of the micropropagated plants in the open field conditions and to improve the growth rate and quality of the seedlings.

Literature

1. *Aladin O. M., Akimova S.V., Kovaleva I.S.* Adaptation of raspberries (*Rubus L.*) and lilacs (*Syringa L.*) to non-sterile conditions // Izvestiya TSCA. – 2009. – N. 3. – P. 99–109.

2. *Belyaev A.A., Shpakova T.V., Sternshis M.V.* Influence of strains of bacteria of *Bacillus* genus on adaptation, growth and vegetative reproduction of garden strawberry // Achievements of science and technology of agrarian and industrial complex. – 2012. – N. 3. – P. 16–19.

3. *Gaaloul N., Braiek O. B., Berjeaud J. M., Arthur T., Cavera V. L., Chikindas M. L. Hani K. and Ghrairi T.* Evaluation of antimicrobial activity and safety aspect of *Enterococcus italicus* GGN 10 strain isolated from tunisian bovine raw milk // Journal of Food Safety. – 2014. – P. 1–12.

4. *Merlich A.G., Zhunko I.D., Limanska N.V., Ivanitsya V.O.* Antagonistic activity of products of bacterial metabolism of strains Lactobacillus plantarum and Enterococcus italicus in a concurrent action against phytopathogenic bacteria // Microbiology and Biotechnology. – 2017. – N. 3. – P. 45–54

5. *Murodov S.S., Davarnov K.D.* Complex microbial preparations. Application in Agricultural Practice // Biotechnology ACTA. – 2014 – N. 6. – P. 92-101

6. *Zelenyanskaya N. N., Jaburia L.V., Teslyuk N.I.* Grape breeding technology using tissue culture methods in vitro // VynoGrad. – 2009. – N. 3. – P. 50–53