Osmoprotective properties of sucrose against nodule bacteria inoculants for legumes

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Abstract. In this study, the resistance of soybean nodule bacteria *B. japonicum st.* 634b, *A21*, *H9* to drying on inoculated soybean seeds of the EN Argenta variety was studied and the effectiveness of sucrose as an osmoprotector of rhizobia was determined. To assess the degree of rhizobia resistance to osmotic stress, soybean seeds were treated with an aqueous solution of the corresponding bacterial culture (control variants) or 20% sucrose solution (experimental variants). Further, the treated seeds were periodically washed with sterile water, followed by determining the number of viable cells by sowing a series of 10-fold dilutions on Petri dishes with agarized nutrient medium, followed by counting the characteristic bacterial colonies formed. Washes were performed 1,2,3,4, and 7 days after seed treatment. It was shown that among the studied strains, strain *H9* is the most osmotically stable, and strain 634b is the least. Osmoprotective activity of a 20% sucrose solution was revealed against all three types of rhizobia, which was expressed in a significant slowdown in the rate of reduction of the number of viable cells on inoculated seeds over time.

1 Introduction

In the XXI century, in addition to typical Russian leguminous crops (peas, vetch), there were trends in the growth of acreage due to the cultivation of soybean and chickpea. In recent years, the acreage under leguminous crops has increased from 1 to 1.5-2 million hectares, soybean − from 0.5 to 2 million hectares. [1]. In modern technologies of soybean cultivation, such agricultural practices as pre-sowing treatment of seed material with rhizobial biological preparations are widely used, which ensures the formation of nitrogen-fixing nodules on plant roots [2]. The nitrogen-fixing activity of nodules reduces the dependence of plants on mineral forms of nitrogen, which allows, in some cases, to completely abandon the use of mineral nitrogen fertilizers in the cultivation of legume crops [3]. For the reliable formation of numerous active nodules on the roots of legume crops, it is necessary to ensure the presence of as many viable rhizobia as possible at the time of germination of inoculated seeds. This is achieved by increasing the consumption rates of inoculants, increasing the titer of biological products and increasing the viability of cells on treated seeds [4, 5]. Since a significant increase in the consumption rates of inoculants is not always possible due to a number of economic and technological limitations, to increase the effectiveness of pre-sowing

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inoculation, it remains possible only to increase the titer of preparations and improve the technologies of pre-sowing seed treatment, ensuring greater safety of bacterial cells from the moment they are applied to seeds until it germinates in the soil [6, 7].

At the same time, it has been shown that the viability of nodule bacteria cells on inoculated seeds can be increased by adding some water-soluble polymers [8] or carbohydrates [9].

Sucrose has been shown to have osmoprotective properties for several species of bacteria of the genus *Rhizobium* [10]. Its distinctive feature is that it does not accumulate in cells in the form of cytosolic osmolytes under salt stress [11].

In this regard, the following **purpose of the study** was determined: to determine the osmotic resistance of soybean rhizobia cells *B. japonicum st.* 634b, *A21*, *H9,* applied to soybean seeds of the EN Argent variety in an aqueous solution and in a 20% sucrose solution to study its osmoprotective properties against rhizobia.

2 Materials and Methods

The objects of the study were nodule bacteria of soy *Bradyrhizobium japonicum* strains *634b*, *A21*, and *H9* from the Departmental Collection of Agricultural Microorganisms of the All-Russian Research Institute of Agricultural Microbiology (VNIISHM, St. Petersburg).

Bacterial suspensions were prepared by seeding with a pure culture of flasks with 250 ml of a semi-synthetic nutrient medium based on mannitol, yeast extract, and salts (Table 1), followed by thermostating of the seeded flasks for 7 days at 24°C on a shaking machine at 180 rpm.

Table 1. Composition of the standard nutrient medium (control).

Medium component	Component concentration (g/l)
Mannitol	10
Yeast extract	1
Glycerin	4
K2HPO4	0.5
MgSO4	0.2
NaCl	0.1

The finished bacterial suspensions were stored in a refrigerating chamber at 6°C. To determine the titer of cultures, a series of 10-fold dilutions were made on Petri dishes with agarized nutrient medium (Table 1), followed by their thermostating for 7 days at 30°C. The experiments were carried out in threefold repetition.

To determine the resistance of cells to osmotic stress by assessing the dynamics of their viability, working solutions were prepared on treated seeds with the addition of water and a 4:1 inoculant, which were used to treat soy seeds of EN Argent variety at the rate of 250 µl per 25 g of seeds. After certain time intervals (1,2,3,4,7 days), washes were made from the treated seeds, a series of 10-fold dilutions were prepared and sown on Petri dishes with agarized nutrient medium. The seeded Petri dishes were placed in a thermostat at 30°C, in which the dishes were 6-7 days until the formation of clearly visible white convex mucous colonies characteristic of nodule bacteria.

The experiments were carried out in threefold repetition. The variance analysis of the obtained results was carried out according to the method B.A. Dospekhov [12].

3 Results and Discussion

All three strains studied showed good growth in the mannitol-yeast medium (Fig. 1). In particular, after 7 days after cultivation on rocking flasks, the titer of strain *634b* was 2.56 billion CFU/ml, the titer of strain *A21* was 1.91 billion CFU/ml, and the titer of the *H9* strain was 1.77 billion CFU/ml.

On the basis of the obtained bacterial cultures, their 20% aqueous solutions were prepared, which were used to process soybean seeds of the EN Argent variety at the rate of 250 µl/25 g, i.e. in a ratio of 10 l/t, characteristic of agricultural practice of inoculation of soybean seeds with rhizobial biological preparations. At the same time, for each seed, with a culture titer of $1*10^9$ CFU/ml and an average weight of 1000 seeds in 200 g, there are about 2 million bacteria. But, the determination of the number of viable cells on the treated seeds revealed (Fig. 2) that the seed surface is clearly an unfavorable environment for rhizobia, which is expressed in a noticeable negative dynamic of their number over time. In particular, after 1 day after inoculation, the number of viable cells of strain *634b* per seed was 20 thousand strain *A21* – 270 thousand, strain *H9* – 500 thousand. After 3 days, the number of viable cells of strains *634b* and *A21* on seeds decreased to almost 0, while the number of cells of strain *H9* was about 270 thousand per 1 seed, which allows to characterize this strain as osmotically more stable. At the same time, the complete death of this strain cells on the seeds occurred only 7 days after their inoculation.

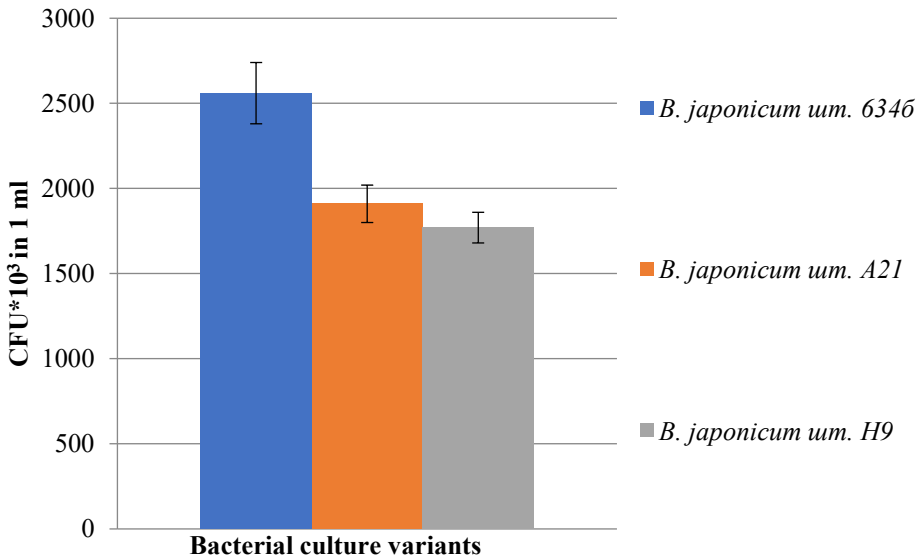

Fig. 1. Titers of bacterial cultures at the time of processing of soybean seeds by them.

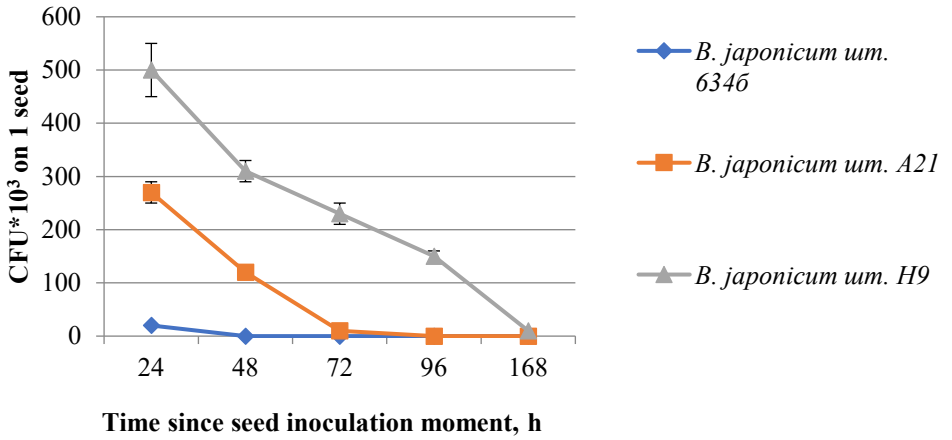

Fig. 2. Dynamics of reduction in the number of viable cells of various strains applied to seeds in an aqueous solution.

Repetition of the experiment with the addition of 20% sucrose to a solution with rhizobia revealed the osmoprotective activity of the latter against nodule bacteria of all three studied strains, which resulted in a significant increase in cell viability on treated seeds. In particular, a day after inoculation, the number of viable cells of strain *634b* per seed was 170 thousand. CFU, strain *A21* – 300 thousand. CFU, strain *H9* – 560 thousand. 7 days after inoculation, 80 thousand seeds were contained on one seed. CFU, strain *634b* - 120 thousand. CFU, strain *A21* - 200 thousand. CFU, strain *H9*.

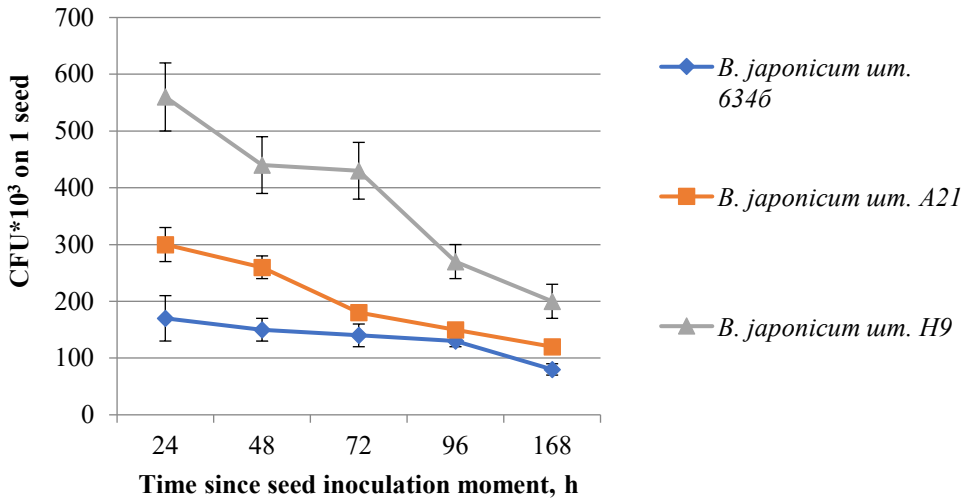

Fig. 3. Dynamics of reduction in the number of viable cells of various strains applied to seeds in a 20% sucrose solution.

4 Conclusions

All three studied strains of soybean nodule bacteria *B. japonicum 634b, A21, H9* differ in their osmotic stability, which is expressed in different dynamics of reduction in the number

of viable cells on inoculated seeds of soybean variety EN Argenta. At the same time, strains *634b* and *A21* can be defined as relatively osmosensitive, and strain *H9* as relatively osmoresistant. It was shown that after 1 day after inoculation, the number of viable cells of strain *634b* per seed was 20 thousand, strain *A21* – 270 thousand, strain *H9* – 500 thousand. After 3 days, the number of viable cells of strains *634b* and *A21* on seeds decreased to almost 0, while the number of cells of strain *H9* was about 270 thousand per 1 seed, which allows to characterize this strain as osmotically more stable. At the same time, the complete death of this strain cells on the seeds occurred only 7 days after their inoculation.

Sucrose has a pronounced osmoprotective activity against nodule bacteria, which is expressed in a significant increase in cell viability on treated seeds. In particular, a day after seed treatment with bacterial cultures in 20% sucrose solution, the number of viable cells of strain *634b* per seed was 170 thousand. CFU, strain *A21* – 300 thousand. CFU, strain *H9* – 560 thousand. 7 days after inoculation, 80 thousand seeds were contained on one seed. CFU, strain *634b* - 120 thousand. CFU, strain *A21* - 200 thousand. CFU, strain *H9*.

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