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GIBBERELLIN ACTIVITY IN THE ISOLATE OF LICORICE *GLYCYRRHIZA GLABRA* L TUBER BACTERIA

Abstract

For the first time, a nodule bacterium was isolated from the root of licorice (*Glycyrrhiza glabra* L) growing in the conditions of Uzbekistan and its effect on increasing the germination of the seed of this plant was revealed. Under salinity conditions, the germination of seeds treated with a solution of bacteria isolated from plant roots was 90%, and in the control - 40%. Using high-performance liquid chromatography, it was found that a bacterium isolated from the root of a licorice plant secretes the phytohormone gibbrelic acid. It was noted that in the future the use of strains of nodule bacteria isolated from licorice root will make it possible to create large plantations of plants in saline conditions.

Key words: symbiosis, bacteria, gibberellin, licorice (*Glycyrrhiza glabra* L), germination, nutrient medium, HPLC, fungi, isolates

АКТИВНОСТЬ ГИББЕРЕЛЛИНА В ИЗОЛЯТЕ БАКТЕРИЙ ИЗ КЛУБНИ КОРНЕЙ РАСТЕНИЯ ЛАКРИЦА *GLYCYRRHIZA GLABRA* L.

Аннатоция

Впервые из корня солодки (*Glycyrrhiza glabra* L) растущей в условиях Узбекистана, выделена клубеньковая бактерия и выявлено ее влияние на повышение всхожести семени, данной растении. В условиях засоленности всхожесть семян, обработанных раствором бактерий выделенного из корня растений составлял 90%, а в контроле - 40%. С помощью высокоэффективной жидкостной хроматографии было установлено, что выделенная из корня растения солодки бактерия выделяет фитогормон гиббрелленовая кислота. Отмечено, что в дальнейшем использование из штаммов клубеньковых бактерий, выделенного из корня солодки, позволит создать большие плантации растений в условиях засоленности.

Ключевые слова: симбиоз, клубеньковые бактерии, гиббереллин, солодка голая (*Glycyrrhiza glabra* L), прорастание, питательная среда, ВЭЖХ, грибы, изоляты.

ШИРИНМИЯ *GLYCYRRHIZA GLABRA* L O’SIMLIGINING TUGANAGIDAN BAKTERIYA IZOLYATIDAGI GIBBERELLEN FAOLLIGI

Annotasiya

Ilk bor O’zbekiston sharoitida o’sadigan (*Glycyrrhiza glabra* L) ildizidan tugunak bakteriyalaridan bakteriya ajratib olingan va uning o’simlik urug’ining unuvchanligiga ta’siri aniqlangan. Mazkur tugunak bakteriyalaridan iborat bo’lgan eritma bilan ishlov berilgan o’simlik urug’ining unuvchanligi 90% teng bo’lib, nazoratda esa 40% ni tashkil etgan.Yuqori samarali suyuqlik xromatografiyasi yordamida *Glycyrrhiza glabra* L.o’simligi ildizidan ajratib olingan tugunak bakteriya o’zidan gibberellin fitogarmonini ajratishi aniqlandi. Kelgusida bakteriyali suyuqlikdan foydalanish asosida shirinmiya urug’ining unuvchanligini oshirish va yirik plantasiyalar tashkil etish imkonini berish qayd etilgan.

Kalit so’zlar: simbioz, tugunak bakteriya, gibberellen,shirinmiya (*Glycyrrhiza glabra* L), unuvchanlik, oziqa muhiti, yuqori samarali suyuqlik xromatografiyasi, zamburug’lar, izolyatlar, eritmalar.

Introduction. In terms of economic importance, legumes are next to cereals. This is explained not only by the nutritional and technical value of the plant, but also by its position in the agrosystem with its property of supplying the form of nitrogen for plants that cannot absorb atmospheric nitrogen. However, the stressful conditions of the external environment: soil salination, high and low temperatures, drought, diseases negatively affect the widespread distribution of legumes in nature.

Salination causes negative affects not only to the elemental composition of the soil, but also to its microflora. In recent years, the improvement of the microflora of saline soils based on legumes has been of interest to many researchers. The formation of the symbiosis of legumes and bacteria, being a chain of morphophysiological processes in both symbionts, largely depends on the success of its initial stages. They are currently not sufficiently studied, especially, in unfavorable conditions.

In this regard, Licorice - *Glycyrrhiza glabra* L one of the promising legume plants. In the territory of Uzbekistan, licorice is considered a typical brush plant and is found mainly in the lower parts of the Syrdarya and Amudarya rivers. As a medicinal plant licorice is known from very ancient times. In Chinese folk medicine, this plant was used in the treatment of various health problems 3000 Years BC. Information is also found in Indian and Tibetan medicine regarding the healing properties of the licorice plant. Abu Ali Ibn Sina, our great-grandfather, a famous scientist and physician, who lived in the Middle Ages, used licorice root parts in his healing practice in a very wide range. In particular, it was used in the treatment of gastrointestinal ulcers, diseases of the lungs and respiratory tract, also as an emictory. Also, in connection with the widespread use in medicine of the physiologically active substances, including glycyrrhizinic acid, contained in the roots of the Licorice, it has been established that a greater accumulation of these physiologically active substances was found in samples growing in saline soil conditions.

Licorice is studied for its resistance to salt by binding to bacteria in the root tubers.

Currently, the intensification of Agricultural production and the creation of microbial biotechnologies that serve to maintain soil fertility are some of the most relevant areas for the development of ecological farming. The intensification of agricultural production and the creation of microbial biotechnologies that serve to maintain soil fertility are one of the most relevant areas for the development of ecological farming. Accordingly, the importance of Licorice in saline soil conditions in view of increasing soil fertility is reflected in the results of a number of studies. The fact that Licorice removes water-soluble salts in addition to its property of improving the reclamation state of saline soils is explained in the results of studies, conducted by scientists [1-2].

The stimulation of microorganisms to plant growth is associated with three main factors: 1) the production of phytogormones that regulate plant growth; 2) the presence of nutrients for plants under the influence of microorganisms (including the presence of water); 3) protection of plants from diseases. These properties can be different or of the same type [3,4]. It is these properties that are characterized by their presence in licorice.

Among bacteria, the ability to synthesize indole-3-acetic acid has been found in many rhizospheric and epiphytic bacteria. Indole 3-acetic acid formation in was identified the following series of soil microorganisms Bacillus, Pseudomonas, Xanthomonas and in yeasts: Saccharomyces, Aureobasidium and in more than 15 categories of fungi, for example, Absidia, Actinomucor Amanita, Aspergillus, Fusarium, Penicillium, Phoma, Phymatotrichum and in other species [5].

The creation of complex-acting microbial preparations, which include compositions consisting of different groups of microorganisms, typical representatives of the soil and rhizospheric microflora, which makes it possible to change approaches in the technology of growing fodder crops is of great importance for Uzbekistan. It is not advisable to use foreign producer microbial preparations to increase soil fertility in Uzbekistan. These preparations are developed on the basis of microorganisms that are not adapted to the soil and climatic conditions of our country. In this regard, it is more effective to use the means developed on the basis of microorganisms extracted from the agrocenoses of Uzbekistan. In studies carried out, it was found that gibberellin is extracted in isolates extractted from the root system of licorice.

Accordingly, this study provides for the study of gibberellin, which is extracted from the isolate extracted from the licorice root tubers and its effect on the germination rate and development of plant seeds.

Material and methods. A well-developed sprout was selected for the extraction of legume bacteria from the licorice rhizome. Pink colour, over 5 mm in size tubers were separated from the plant root system. The tubers were washed several times in sterile water and kept in ethyl alcohol for 2 minutes. Then the tubers were taken in tweezers and passed over a burning gas burner to burn the alcohol out, and the tubers were completely sterilized. The tubers were cracked and mixed in Eppendorf test tubes with 0.5 ml of sterilized water. Then the tuber suspension (bacteroids) was planted in a lawn manner by diluting in a ratio of 1:1000000 to Petri Cups with a nutrient medium of Agar 2% chickpea extract. Tthe tuber suspension planted cups was incubated for 7 days at a temperature of 28o C. From the 2nd day of incubation, on the surface of the medium with agaric feed, the cells of tuber bacterial isolates began to form colonies.

Composition of nutrient medium containing chickpea extract (g/l): 100 g chickpeas extract after boiling for 1 hour contained glucose-4, K2HPO4 – 0,5, KH2PO4 – 0,9, MgSO4 – 0,2, CaCl3 - 0,001 [6,7].

The morphocultural properties of tuber bacterial isolates were studied through the Bergey tester [8].

The plant seeds were treated with diluted concentrations of bacterial isolates. For this, the studied cultures were previously grown in a shaker in a liquid nutrient medium. Microbial isolates were diluted in water; filtered culture liquid was poured into 10 ml cups and 10 plant seeds were placed in each. They were kept in the solution for 4 hours. For the purposes of control, the seeds were kept in water for 4 hours in similar order. The seeds of medicinal plant Licorice - *Glycyrrhiza glabra* L. were used as test seeds. After the seeds were soaked for 4 hours, the seeds were placed in Petri cups with filter paper on moistened cotton wool. All containers were moistened with equal amounts of water. The number of seeds germinated in experimental and control units, the length of the stem and roots were monitored based on appropriate techniques [9,10].

Results obtained and their analysis. To extract the tuber bacterium of the local Licorice variety grown in the Syrdarya oblast, young licorice plants also some pink colour and 2-3 mm diameter root tubers were selected for testing. We extracted the bacterium isolate using the pure strain extraction method, and microscopic study of the extracted isolate showed that the isolate was homogenous isolate and it was composed of tiny rod-like bacteria. It is shown in Figure 1.

Figure 1. The appearance of a bacterium, extracted from licorice *Glycyrrhiza glabra* L root tubers.

A study of the morphology of the tuber bacterium showed that the extracted culture is gram-negative and does not generate spores. One of the main characteristics of tuber bacteria is that they move heavily. When active bacterial cells were seen under the microscope, it was observed that they moved very quickly. The cells of rod-shaped bacteria have rounded two ends and have polar rods. The bacteria are polymorphic, and it has been observed that over time the cell gets a rounded shape. It was noted that optimum growth temperature of bacteria is 28C0and pH is 7.2. Through the morpho-cultural properties of the bacterial isolate, it was found that these bacteria are descendants of the initial tuber bacterium.

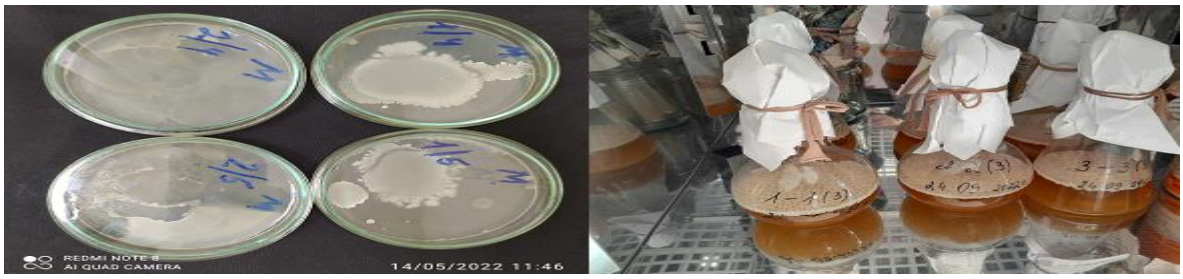

Figure 2. The indicators of the development of bacteria, extracted from licorice root tubers, and grown in am nutrient medium.

In our subsequent work, the effect of the seed germination rate of licorice by growing the extracted tuber bacterium was studied.

The reason of this is that the seed germination rate is considered low due to the fact that licorice is a type of wild plant. In the experiment, the tuber bacterium was planted in a liquid nutrient medium, and after 72 hours of cultivation, the culture liquid was passed through a bacterial filter. It was incubated for 4 hours in the prepared liquid in different proportions (1:1, 1:2 and 1:3). The results obtained are presented in Table 1.

Table 1

The germination indicators of Licorice *Glycyrrhiza glabra* L seeds when processing with bacteria in different proportions

№	Proportions	Number of seeds	Number of germinated seeds	Germination rate %
1	1:1	10	5	50
2	1:2	10	6	60
3	1:3	10	9	90
4	Control	10	4	40

Izoh: Sterile water was used in the control test.

The obtained results show that when processed at a ratio of 1:3, the germination of seeds is 2 times higher than in the control test, and the strength of the germinated sprouts, the acceleration of the physiological processes of seeds incubated with bacteria, also a good effect on the germination of seeds, regardless of their hardness.

Taking into account the fact that the seeds germinate well in the process of bacterial action, we continued our further work on the study of the activity of the gibberellin phytohormone in the bacterial cultural liquid on the high-performance liquid chromatographer (YSSX). The results obtained are shown in Figure 4.

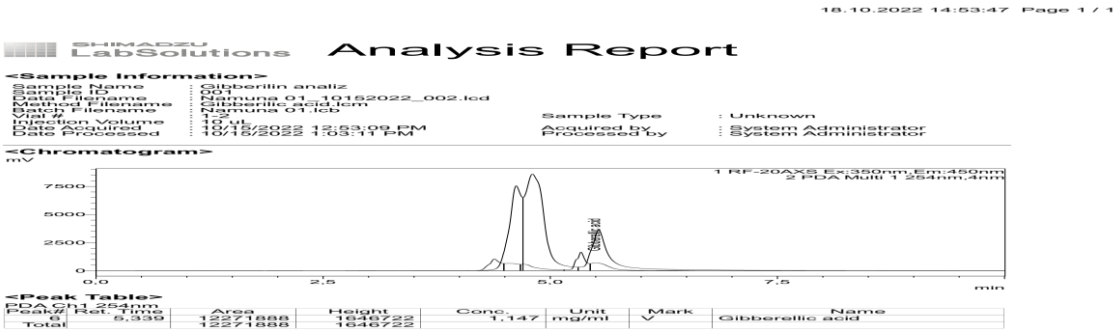

Figure 4. Activity indicator of gibberellin phytohormone in bacterial liquid.

The obtained results show that the data obtained from YSSX is 1.147 mg/mL, and this indicates the presence of 115 mg of gibberellin phytogormone in each liter.

The gibberellen detected on YSSX demonstrated the same activity as the GK-1 Standard. So, it can be concluded that the bacterium, extracted from licorice root tubers accelerates the development of plant seeds along with an increase in the germination rate based on the generration of gibberellen phytogormone from itself.

Conclusion

The tuber bacterium, extracted from licorice rhizome, has the property of generating gibberellin phytogormone from itself. Gibberellin represents the biological significance of the tuber bacterium with the property of accelerating the growth and development of plants. As a result of plant processing using the bacterial cultural liquid in licorice plantations, there will be basis for the plant for developing salt resistance, higher germination rate, body thickness, as well as more accumulation of glycerrizine substance.

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