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# Influence of salinity on growth, development and symbiotic properties of legumes, rhizobia symbiosis of alfalfa in vegetation experiments

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## Abstract
These experiments showed that the salt concentration in 80-100 mm NaCl virtually did not effect on the nodulation of alfalfa plants. Under all conditions, nodulation growth was stimulated in the presence of saline from 80 to 100 mm NaCl. A further increase in the salt concentration inhibits the formation of nodules and color. Nodules have pink color at salination from 80 to 100 mm NaCl, and a white-pink color at salt concentration from 100 to 200 mm NaCl. Critical edge (destructive) concentration of NaCl for the survival of alfalfa plants was 200 mm NaCl, whereas the concentration in the range of 80 to 100 mm NaCl had a negative effect on plant growth and development.
Virulence (formation of nodule and its activity) salt-tolerant rhizobia for alfalfa plant maintains even at high salt concentrations (at 140-160 and even 200 mm NaCl).
In the study of more salt-tolerant alfalfa inoculation strains of nodule bacteria nodulation proceeded more rapidly and effectively than with low salt tolerance strains. If the value of the ratio (fraction) "biomass above-ground parts of plants" / "root biomass" was 2 or more (without the control of salinity), when salinity is reduced to 1 due to the suppression of smaller biomass plant roots. The threshold for the salt tolerance of alfalfa, in which plants lose 50% yield, was 60-80 mm NaCl. Critical (destructive) concentration of NaCl for the survival of alfalfa plants was 160-200 mm NaCl, whereas the concentration in the range of 80 to 100 mm NaCl had already a negative effect on plant growth and development. Conditional threshold salinity for alfalfa depended on the type of salinity - for sulfate, type of salinity was suspended at 30-50 mm. At salinity of 80 mm NaCl ($^{+}$N) - biomass of the aerial part of the plant parts, in both varieties of alfalfa, were similar in their values to the values of the control ($^{-}$N) - biomass (not subjected to salinity) of the aerial part of the plants, in result it can be concluded that the added nitrogen under saline conditions had a positive effect on the growth of alfalfa plants. Nitrogen fixing rhizobium legume symbiosis of alfalfa with using highly salt-tolerant strains of alfalfa nodule bacteria can be used not only for crop rotation in agriculture to irrigated saline soils, but also in the reclamation and restoration of soil fertility, contaminated by industrial pollution (heavy metals, oil products).

**Keywords:** alfalfa, salt tolerance, soil salinity, Rhizobium, Rhizobium-legume, Glycine javanica, nodule bacteria, nodules formation, nitrogen fixation, bacterial isolates.

## Introduction
Biological nitrogen plays an important role in improving soil fertility and a lot of attention on it paid worldwide. Years of experience and manufacturing practices clearly show that all of known nodule bacteria of soil microorganisms that fix nitrogen in symbiosis with leguminous plants play the most important role in enriching the soil with nitrogen and increasing the yield of crops [1-3].
Legumes are one of the most important crops, as they are used as legumes and fodder crops such as pasture component, as well as for the restoration of soil fertility [4-5]. They improve the structure and enhance the fertility of agricultural soils in the crop rotation. These seeds contain about 20-30% fat-based protein, 50% carbohydrates and a small amount (1-2%) of the lipids. Legumes being rich in protein content, also rich in methionine, vitamin B6 (riboflavin), lysine and tryptophan. In Uzbekistan, a prominent place is occupied by crops of alfalfa (Medicago sativa), which in the early 80s was sown in the country on the territory of more than 2 million hectares, and around the world - on the territory of 32 million hectares.
In 2013, the total acreage of agricultural crops in Uzbekistan amounted to more than 3.6 million hectares, of which the area under crops occupied 1.64 million hectares under cotton,

raw - 1.31 million hectares. Harvest crops collected in the country in 2013 increased by 12% compared with 2012 - up to 7.61 million tons, yield of raw cotton remained at the same level as last year - 3.35 million tons [https://regnum.ru/news/economy/1747545.html].

Alfalfa under favorable conditions and optimal growth conditions capable, due to symbiotic nitrogen fixation, to bring up to 200 kg of nitrogen per 1 ha, it has a high forage quality - in 100 kg of green alfalfa biomass contains 21.7 feed units and 4.1 kg of digestible protein (in 100 kg of hay - feed units 45.3 and 10.3 kg of digestible protein). Alfalfa grows in different soil and climatic conditions, which affects its productivity.

Salinization is reducing their bio-productivity, disturbance of soil structure and composition of the soil microflora, suppresses the growth of plants and processes of restoration of fertility and soil structure [6]. Saline soils are characterized with low in organic matter, high alkalinity, and high salinity of the soil solution.

In connection with, it special importance acquire researches, which aimed only at the development of low fertile and saline lands. Soil salinization is one of the most acute problems of agriculture with which to fight for a long time as using traditional methods of soil science and agricultural technology (washing of saline soils, gypsum, organic fertilizers, etc.), and with the help of complex biotechnological methods (creation of transgenic salt tolerant search, use of salt-tolerant crops, etc.).

Physical nitrogen assimilation in legume-rhizobium symbiosis of alfalfa on saline soils may occur in the event, when the root system of alfalfa is infected with active cultures of nodule bacteria that are viable and can survive in saline conditions like soil bacterial population in non-symbiotic position to growing alfalfa crops and have high symbiotic properties in the legume-rhizobium symbiosis with alfalfa (formation of nodule, high nitrogen-fixing activity) under saline conditions.

Therefore, the search and selection of active strains of nodule bacteria with high nitrogen-fixing ability, nodule formation and salt-resistance is of great importance for the further application of the results obtained in nitrogenization of alfalfa.

Nitrogenization (pre-sowing treatment of seeds of leguminous plants with salt-tolerant high-performance strains of nodule bacteria) is one of the areas of the fight against the increase of fertility of saline soils, but the number of works devoted to this area (salt tolerant alfalfa nodule bacteria, its microbiological and symbiotic properties, nitrogen fixation and nodule formation of alfalfa under various types of salinity, etc.) is limited, and research works on rhizobium-legume symbiosis of alfalfa under saline conditions is poorly studied in Uzbekistan.

The purpose and objectives of research. The purpose of this stage of the research was to study the effect of different concentrations of sodium chloride salt on seed germination and development of symbiosis arid and semi-arid varieties of alfalfa in Uzbekistan, inoculated with rhizobia salt-tolerant alfalfa in the absence and in the added nitrogen source.

## Materials and Methods

### The objects of research

The main object of our study were of alfalfa nodule bacteria isolated from the root nodules of alfalfa plants grown in soils with different salinity (Tashkent, Syrdarya, Samarkand regions and Karakalpakstan Republic), and Russian production strain of nodule bacteria SKHM1 [7]. The study used two varieties of alfalfa, "Tashkent-1728" and "Khorezm-2", cultivated in Uzbekistan, the seeds of which were obtained from the collection of Uzbek Research Institute breeding and seed production.

### Micro vegetation experiments

To carry out experiments on selected bacterial isolates of nodule formation in micro vegetation sterile experiments alfalfa seeds were treated for 3-5 minutes with concentrated sulfuric acid H2SO4 and washed repeatedly with sterile distilled water. Washed seeds were placed on filter paper discs in Petri dishes, moistened with sterile distilled water, and germinated in an incubator at 30°C for 1-2 days. After appearance of alfalfa sprouts, every 2 sprouts were transferred into 60 ml agronomic test tubes with 5 g of sterile vermiculite (mica crumb grain size 2-4mm) and 10 ml of the medium-Krasilnikov-Korenyako in the following composition (g/l) [8]: $K_2HPO_4$ -1; $MgSO_4$-1; $Ca_3(PO_4)$ 2-0,2; $FeSO_4$-tracks; solution of trace elements - 1 ml; distilled water - up to 1 liter. The solution of trace elements (g/l): $H_3BO_3$-0,05; $(NH_4)_2MoO_4$-0,05; KCl-0,005; NaBr-0,005; $ZnSO_4 \times 4H_2O$-0,003; $Al_2$ (SO4) 3 × 18 H2O-0,003; MnSO4-0,002; distilled water - 1 liter, pH 7.0 (sterilization at 1 atm, 30 min). Before vermiculite had been thoroughly washed and calcined at 800o C for 2 hours.

For inoculation of seedlings grown in bacterial culture medium with 2% bean broth for 3-4 days at a temperature in the thermostat 28-30oS and in agronomic tubes was added 1 ml of a bacterial suspension grown to a titer of cells 108 cells / ml, with six replication experiments. Symbiotic efficiency was determined by their ability to increase the biomass of plants inoculated as compared to inoculated control plant biomass. The results of the micro vegetation experiments were subjected to statistical analysis using the two vectored variance and correlation analysis, Student's t-criterion. Statistical processing was performed by Microsoft Access program.

## Results

In studying the effect of salinity on seed germination and survival rates at various concentrations of the salt, it was found that increasing salt concentration led to the fact that the growth of seed roots and its length significantly reduced in size - up to 7-10 times at 200 mm NaCl as compared to control options of both sorts of alfalfa Table 1).

The higher concentrations of NaCl have a negative effect on the development and survival of young alfalfa sprouts: approximately 2 times under 200 mm NaCl, and oppress the beginning

("Start"), the effect of salt - "salt tolerance threshold" - is already determined at 60-80 mm NaCl. It should be noted in this connection that the salinity adversely affected in the early stages of development and germination of alfalfa seeds. The salt concentration that is not exceeding 60-80 mm NaCl, able to insignificantly inhibit the vitality of young seedlings of both varieties of alfalfa.

Experiments on germination of seeds under different salinity showed that sort of alfalfa "Khorezm-2", as salt tolerant and arid grade, showed higher rates of germination and survival of seedlings under salt stress compared with the sort of "Tashkent-1728".

Molybdenum by its nature, being a rare earth metal, does not apply to heavy metals, and in contrast to the latter has a

beneficial effect on plants growing in saline conditions. One of the plant growth inhibition reasons under salt stress is the deterioration and violation of their nitrogen nutrition and primary stages of nitrogen assimilation with the participation of molybdenum-dependent enzyme nitrate reductase [9] (in the case of leguminous plants as molybdenum-dependent enzyme biological nitrogen fixation - nitrogenize) due to violations of nitrate and the molecular structure, apparently suppressing nitrogenize activity under the influence of the ionic strength of the salt solution.

The effect, according to the literature, is reversible.

In this view, it was undertaken to study the effect of pretreatment of sodium molybdate (20 mm) on the germination of alfalfa seeds with different concentrations of sodium chloride (table 2). As you can see their data tables, sodium molybdate increased germination and sprouting of alfalfa seeds in the presence of chloride salinity at 9-13%.

Previously, other authors [9] pointed out that the initial stages of ontogeny of legumes to form nodules are very sensitive to salinity, since this period the majority of legumes marked maximum nitrate reductase activity, which with the addition of molybdenum ions restored their activity, and more sensitive to salinity leguminous plant was, the greater was the percentage recovery of nitrate reductase activity.

**Table 1:** Effect of salinity on germination of seeds of local sorts of alfalfa

| Sort of alfalfa | Concentration of NaCl, mm | The incubation time for germination of alfalfa seeds | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 24 h | | 48 h | | 72 h | | 96 h | |
| Khorezm-2 | 0 | 80 | 0.95 | 100 | 2.85 | 100 | 5.0 | 100 | 5.85 |
| | 10 | 70 | 0.95 | 90 | 2.0 | 90 | 3.8 | 90 | 5.4 |
| | 20 | 80 | 0.95 | 85 | 2.25 | 85 | 3.7 | 85 | 5.35 |
| | 30 | 85 | 0.95 | 85 | 2.15 | 85 | 3.7 | 85 | 4.5 |
| | 40 | 85 | 0.8 | 85 | 2.0 | 85 | 3.4 | 85 | 4.5 |
| | 50 | 80 | 0.75 | 80 | 1.7 | 80 | 3.2 | 80 | 4.3 |
| | 60 | 75 | 0.7 | 80 | 1.6 | 80 | 2.7 | 80 | 3.6 |
| | 80 | 80 | 0.65 | 80 | 1.65 | 80 | 2.25 | 80 | 3.6 |
| | 100 | 80 | 0.5 | 80 | 1.0 | 80 | 2.05 | 80 | 2.35 |
| | 120 | 80 | 0.35 | 80 | 0.85 | 80 | 1.75 | 80 | 2.35 |
| | 140 | 80 | 0.3 | 80 | 1.1 | 80 | 1.35 | 80 | 2.25 |
| | 160 | 65 | 0.3 | 75 | 1.1 | 75 | 1.2 | 75 | 2.15 |
| | 200 | 45 | 0.1 | 75 | 0.25 | 75 | 0.5 | 75 | 0.75 |
| Tashkent-1728 | 0 | 100 | 0.9 | 100 | 2.75 | 100 | 4.35 | 100 | 5.1 |
| | 10 | 100 | 0.9 | 100 | 2.0 | 100 | 4.0 | 100 | 4.3 |
| | 20 | 100 | 0.7 | 100 | 2.05 | 100 | 3.8 | 100 | 4.6 |
| | 30 | 100 | 0.7 | 100 | 1.4 | 100 | 3.7 | 100 | 3.45 |
| | 40 | 100 | 0.7 | 100 | 1.55 | 100 | 3.4 | 100 | 3.85 |
| | 50 | 100 | 0.7 | 100 | 1.4 | 100 | 3.6 | 100 | 3.8 |
| | 60 | 100 | 0.65 | 100 | 1.4 | 100 | 2.95 | 100 | 3.95 |
| | 80 | 100 | 0.7 | 100 | 1.2 | 100 | 1.95 | 100 | 3.1 |
| | 100 | 100 | 0.55 | 100 | 1.0 | 100 | 1.7 | 100 | 3.0 |
| | 120 | 100 | 0.4 | 100 | 1.0 | 100 | 1.7 | 100 | 2.25 |
| | 140 | 85 | 0.35 | 100 | 0.7 | 100 | 1.2 | 100 | 1.8 |
| | 160 | 85 | 0.35 | 85 | 0.7 | 85 | 1.15 | 85 | 1.65 |
| | 200 | 60 | 0.1 | 60 | 0.15 | 60 | 0.5 | 60 | 0.7 |

**Table 2:** Effect of pretreatment with molybdenum on seed germination of alfalfa "Khorezm-2" in the presence of different concentrations of salt

| Concentration of salt NaCl, мм | Germination of seeds (96 hours), % | |
|---|---|---|
| | Control (without $Mo^{2+}$) | Processing 20 Мкм $Mo^{2+}$ |
| 0 | 100 | 100 |
| 80 | 80 | 93 |
| 140 | 80 | 89 |
| 160 | 75 | 86 |
| 200 | 73 | 82 |

To study the effect of salinity on growth, development and nodule formation of plants both sorts of alfalfa were used salt-tolerant strain of Sinorhizobium meliloti №10, which can be seen from the results of previous experiments, symbiotic properties of which was excelling the control, the Russian production strain SKHM1.

Research has shown that low (until 30 mm) concentration of NaCl salt significantly affects the biomass growth above ground parts of plants and their roots in comparison with biomasses their control options (without saline) in the absence (- N) and the presence (+ N) of exogenous nitrogen. Further increase of the salt concentration (between 30 and 200 mm NaCl) resulted in a decrease of biomass growth (Table. 3-4, pic. 1-2).

At the same time, the presence of exogenous nitrogen at a salt concentration of 30 mm NaCl resulted in the (+ N) of both cultivars -biomass excelled to corresponding plant biomass 150-200% (- N) - of biomass plants "Tashkent-1728" and "Khorezm-2", respectively. If the value of the ratio (fraction) "above ground biomass of plant parts" / "root biomass" was 2 or more (in the control), when salinity is decreased to 1 because of the lower biomass suppression of plant roots in the concentration range from 60 to 80 mm NaCl. At 80 mm NaCl (+ N) -biomass above ground parts of plants of two sorts of

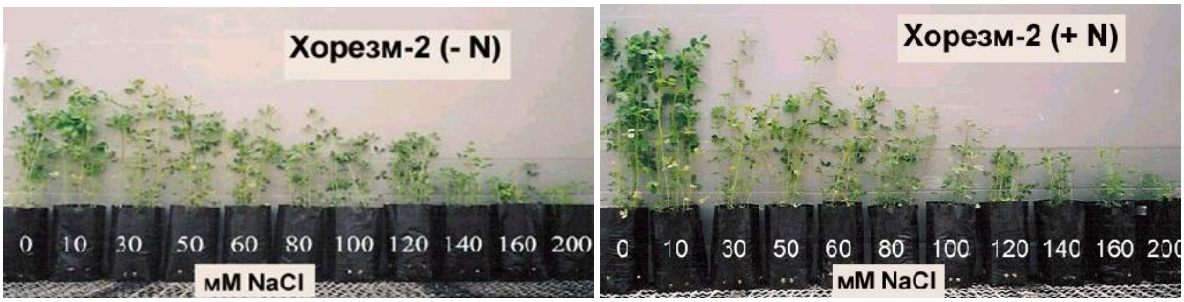
alfalfa were close in their values to the control values (-N) - biomass (not subjected to salinity) above ground parts of plants, from which it can be concluded that the nitrogen is added under saline conditions has a positive effect on the growth of alfalfa plants under salinity.

These experiments showed that the salt concentration 80-100 mm NaCl virtually had no effect on the nodulation of alfalfa plants. Under all conditions of nodulation, growth was stimulated in the presence of saline to 80-100 mm NaCl. A further increase in the salt concentration inhibits the formation of nodules and color.

Pink nodules had salination 80-100 mm NaCl, and a white-pink color at salt concentrations from 100 to 200 mm NaCl. Critical (destructive) concentration of NaCl for the survival of alfalfa plants was 200 mm NaCl, whereas the concentration in the range of 80 to 100 mm NaCl had a negative effect on plant growth and development.

**Table 3:** The effect of salinity on growth, development and nodule formation of alfalfa plant sort "Tashkent-1728", inoculated with a strain of Sinorhizobium meliloti №10 in pot experiments

| Sort of alfalfa | Source of nitrogen | Concentration of NaCl, мМ | Biomass of dry above ground part of the plant, mg | Biomass of dry roots, mg | Height of above ground part, sm | Length of roots, sm | Number of Nodules on the first plant | Biomass of fresh Nodules, on the first plant, mg | Size of nodules, mm | Color of nodules | Symbiosis effect % | Plant surviving, % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Ташкент-1728 | $^{-}$N | 0 | 253 | 120 | 38 | 30 | 13.4 | 29 | 10-70 | Розовый | 100 | 100 |
| | | 10 | 252 | 125 | 35 | 29 | 18.9 | 63 | 1.0-6.0 | | 99.6 | 100 |
| | | 30 | 230 | 106 | 33 | 29 | 17.8 | 61 | 1.0-5.0 | | 90.9 | 100 |
| | | 50 | 210 | 152 | 33 | 25 | 17.3 | 59 | 1.0-6.0 | | 83.0 | 100 |
| | | 60 | 181 | 157 | 28 | 27 | 16.1 | 57 | 1.0-7.0 | | 71.5 | 100 |
| | | 80 | 150 | 130 | 25 | 29 | 15.3 | 52 | 1.0-6.0 | | 59.2 | 100 |
| | | 100 | 140 | 131 | 23 | 30 | 13.5 | 48 | 1.0-6.0 | | 55.3 | 100 |
| | | 120 | 125 | 100 | 23 | 29 | 13.8 | 40 | 1.0-4.0 | White-pink | 49.4 | 100 |
| | | 140 | 100 | 86 | 19 | 25 | 9.5 | 36 | 1.0-7.0 | | 39.5 | 100 |
| | | 160 | 82 | 70 | 15 | 27 | 8.0 | 18 | 1.0-4.0 | | 32.4 | 100 |
| | | 200 | 40 | 43 | 7 | 27 | - | - | - | - | 15.8 | 40 |
| | $^{+}$N | 0 | 387 | 183 | 41 | 24 | 12.9 | 36 | 1.0-4.0 | Pink | 100 | 100 |
| | | 10 | 378 | 175 | 44 | 28 | 16.3 | 60 | 1.0-5.0 | | 97.6 | 100 |
| | | 30 | 320 | 139 | 42 | 27 | 15.7 | 63 | 1.0-5.0 | | 82.6 | 100 |
| | | 50 | 285 | 157 | 39 | 31 | 14.1 | 65 | 1.0-4.0 | | 73.6 | 100 |
| | | 60 | 273 | 148 | 35 | 32 | 12.9 | 48 | 1.0-5.0 | | 70.5 | 100 |
| | | 80 | 280 | 146 | 31 | 32 | 12.7 | 41 | 1.0-5.0 | White-pink | 72.3 | 100 |
| | | 100 | 142 | 129 | 29 | 29 | 12.0 | 35 | 1.0-4.0 | | 36.6 | 100 |
| | | 120 | 133 | 107 | 25 | 30 | 7.9 | 21 | 1.0-4.0 | | 34.3 | 100 |
| | | 140 | 132 | 85 | 23 | 32 | 4.2 | 6.2 | 1.0-1.5 | | 34.1 | 100 |
| | | 160 | 121 | 77 | 23 | 31 | - | - | - | - | 31.2 | 60 |
| | | 200 | 57 | 47 | 13 | 27 | - | - | - | - | 14.7 | 30 |

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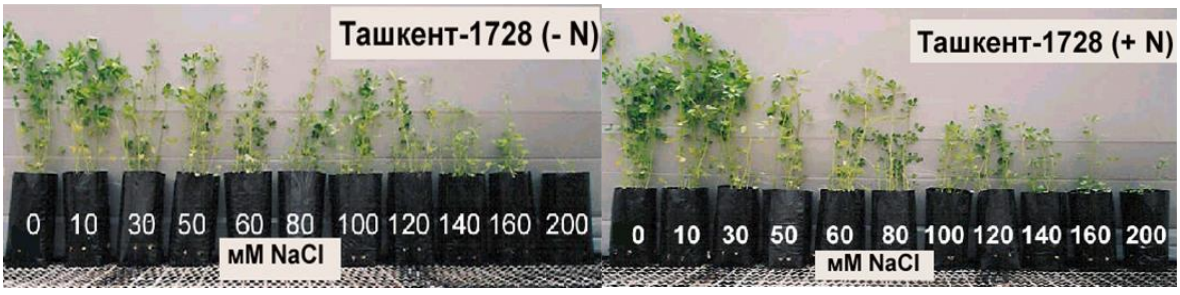
**Pic 1.** Effect of salinity on plants inoculated alfalfa sorts Khorezm-2: A-without added nitrogen-B in the presence of added nitrogen, (nitrogen additive is 1 mm NH4 NO3).

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**Pic 2**. Effect of salinity on plants inoculated alfalfa sorts Tashkent-1728: A-without added nitrogen-B in the presence of added nitrogen, (nitrogen additive is 1 mm NH4 NO3).

**Table 4:** The effect of salinity on growth, development and nodule formation of alfalfa plant sort "Khorezm-2", inoculated with a strain of Sinorhizobium meliloti №10 in pot experiments

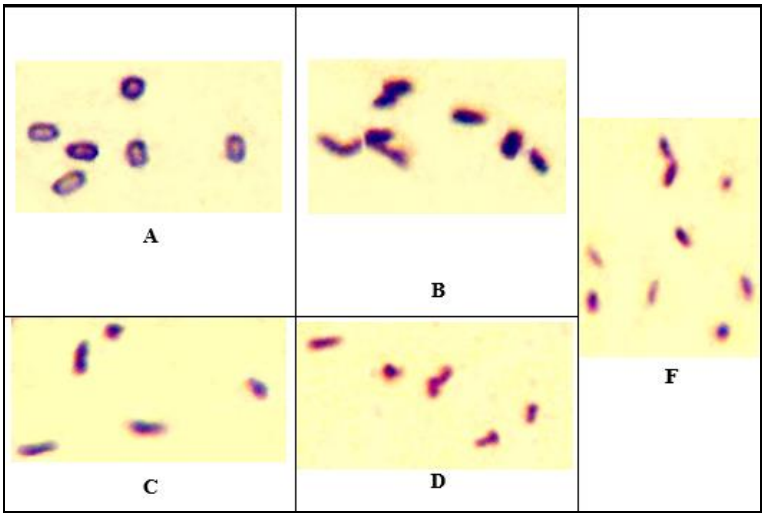
| Sort of alfalfa | Source of nitrogen | Concentration of NaCl, мМ | Biomass of dry above ground part of the plant, mg | Biomass of dry roots, mg | Height of above ground part, sm | Length of roots, sm | Number of Nodules on the first plant | Biomass of fresh Nodules, on the first plant, mg | Size of nodules, mm | Color of nodules | Symbiosis effect % | Plant surviving, % |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Khorezm-2 | -N | 0 | 271 | 128 | 42 | 41 | 16.6 | 44 | 2.0-5.0 | Pink | 100 | 100 |
| | | 10 | 322 | 167 | 52 | 43 | 22.8 | 74 | 1.0-4.5 | Pink | 118.8 | 100 |
| | | 30 | 270 | 152 | 38 | 38 | 17.9 | 83 | 1.0-6.0 | Pink | 99.6 | 100 |
| | | 50 | 261 | 160 | 33 | 37 | 17.6 | 77 | 1.0-5.0 | Pink | 96.3 | 100 |
| | | 60 | 256 | 163 | 33 | 39 | 20.3 | 76 | 1.0-4.0 | Pink | 94.4 | 100 |
| | | 80 | 200 | 174 | 29 | 26 | 23.5 | 70 | 1.0-4.5 | Pink | 73.8 | 100 |
| | | 100 | 200 | 169 | 29 | 30 | 22.8 | 68 | 1.0-5.0 | White-pink | 73.8 | 100 |
| | | 120 | 177 | 125 | 25 | 33 | 18.5 | 70 | 1.0-8.0 | White-pink | 65.3 | 100 |
| | | 140 | 126 | 105 | 22 | 30 | 13.8 | 52 | 1.0-5.0 | White-pink | 46.4 | 100 |
| | | 160 | 107 | 82 | 20 | 26 | 12.3 | 38 | 1.0-5.0 | White-pink | 39.4 | 100 |
| | | 200 | 63 | 62 | 9 | 22 | 6.1 | 12 | 1.0-4.0 | White-pink | 23.2 | 40 |
| | +N | 0 | 530 | 223 | 53 | 35 | 13.4 | 45 | 1.0-5.0 | Dark-pink | 100 | 100 |
| | | 10 | 505 | 217 | 50 | 39 | 15.9 | 53 | 1.0-5.0 | Dark-pink | 95.2 | 100 |
| | | 30 | 336 | 188 | 45 | 38 | 16.2 | 64 | 1.0-5.0 | Pink | 63.3 | 100 |
| | | 50 | 295 | 171 | 45 | 33 | 17.5 | 63 | 1.0-4.5 | Pink | 55.6 | 100 |
| | | 60 | 300 | 216 | 36 | 33 | 17.0 | 57 | 1.0-5.0 | Pink | 56.6 | 100 |
| | | 80 | 293 | 233 | 32 | 31 | 16.5 | 51 | 1.0-7.0 | Pink | 55.2 | 100 |
| | | 100 | 231 | 157 | 27 | 28 | 12.1 | 28 | 1.0-5.0 | White-pink | 43.5 | 100 |
| | | 120 | 186 | 126 | 20 | 27 | 8.9 | 14 | 1.0-4.0 | White-pink | 35.0 | 100 |
| | | 140 | 131 | 101 | 19 | 30 | 5.3 | 8.8 | 1.0-4.0 | White-pink | 24.7 | 100 |
| | | 160 | 122 | 115 | 15 | 29 | 5 | 5.3 | 1.0-3.5 | White-pink | 23.0 | 100 |
| | | 200 | 70 | 63 | 11 | 29 | - | - | - | - | 13.2 | 60 |

"Ability of nodule formation" selected salt-tolerant alfalfa rhizobia for plants maintained even at high salt concentrations: for sort "Khorezm-2", the figure was 200 mm NaCl, while the grade for "Tashkent-1728" - 140 mm NaCl, respectively.

Increasing the NaCl salt concentration in the range from 10 mm NaCl and 100-120 mm NaCl resulted in a significant increase in the number of nodules on the roots of plants without added nitrogen from the source (here the process of nodule formation was more expressive as in the presence of a nitrogen source. Since an increase in salt concentration exceeding 140 mm NaCl in all treatment options was identified suppression of nodule formation process; both varieties (-N) -Option (without nitrogen) at subcritical and critical concentrations of salinity was still possible to detect nodules as opposed to the (+ N) - variants (under nitrogen).

It should be noted also about the color nodules: in the concentration range 10-80 mm NaCl pink nodules were formated, and in added nitrogen source (+ N) -variant grade "Khorezm-2" in the reference embodiment, and at 10 mm NaCl color of nodules were dark pink, with concentrations above 100 mm NaCl nodules color became white and pink.

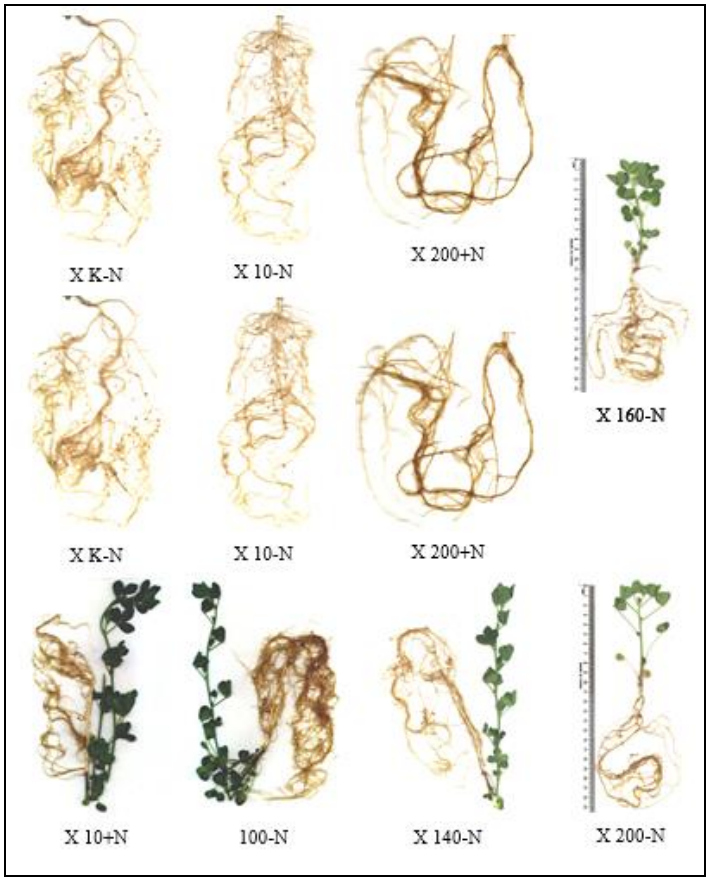
Apparently, leguminous plants, which are exposed to salinity, regardless of the needs for nitrogen, the formation of nodules is adaptive in response to stressful conditions. So-called symbiotic efficiency of inoculated plants of both sorts of alfalfa (change in plant biomass resulting in higher salt concentrations as compared to plants biomass which are not exposed with salinity) decreased from 13 to 39% at 200 mm NaCl (Table 3-4.). At the same time, salinity also influences to morphology of alfalfa root nodule bacteria. Microscopic study of the morphology of alfalfa nodule bacteria cells, in saline conditions, showed that the cells under salinity changed its original form into small rod-shaped cyst (Pic. 3), indicating that there is also impact of adverse conditions of salinity onto nodule bacteria.




**Pic 3:** Morphology of cells of 3 days plants of Sinorhizobium meliloti strains №10 without (A) and with (B-D) of different salinity concentrations: Control A (without saline), B-50 mm NaCl, C-100 mm NaCl, D -150 mm NaCl, F-300 mm NaCl.

Thus, the salinity has a dual effect on the process of nodule formation inoculated of plants of alfalfa:
a) At first, observations showed that stimulation of nodule formation (compared to control) at concentrations ranging from 10 to 120 mm NaCl and enhanced activity of leghemoglobin;
b) The concentration, exceeding 120 mm NaCl, resulted in the suppression of nodulation and leghemoglobin activity.

The presence of the added nitrogen on the one hand supports the growth and development of alfalfa plants and on the other reduces the formation of nodules at subcritical and critical concentrations of salinity. Virulence (nodule formation activity) of salt-tolerant alfalfa plants to rhizobia detected even at high salt concentrations - at concentrations of 140-160 and even 200 mm NaCl (Pic 4.)




**Pic 4:** Effect of sodium chloride salinity on the root system of alfalfa inoculated with rhizobia (sort: "Khorezm-2")

Thus, we can conclude that salinity has a negative impact more on macro symbiont (host plant, alfalfa) on the basis of these studies on salt tolerance of alfalfa nodule bacteria, the effect of salinity on seed germination of alfalfa symbiotic properties of legume-rhizobium symbiosis inoculated plant alfalfa and nodule formation rather than on nodule bacteria,

which are at moderate to high concentrations of salt still retain their virulence (ability of nodule formation).
In this regard, the "fate" of legume-rhizobium symbiosis of alfalfa under saline conditions and prospects for reclamation of saline lands will be determined primarily by the creation of transgenic salt-tolerant sorts of alfalfa plants that are more salt-tolerant and able to maintain an effective nitrogen-fixing symbiosis with more salt-tolerant rhizobia.

**Discussion**

Symbiotic nitrogen fixation in the system "legume - rhizobia" is sensitive to salinity or osmotic stress in contrast to the nitrogen-fixing root nodule bacteria in non-symbiotic free-living state. The concentration of sodium chloride can often reach 300-700 mm NaCl for nodule bacteria of alfalfa or even 850 mm NaCl for rhizobia tropical legume Acacia Nilotic a [10].

There were cases when the inoculation of legumes salt-tolerant strains of rhizobia had a positive impact on the process of nodule formation and symbiotic nitrogen fixation under salt stress. For example, for nitrogen-fixing symbiosis of acacia, it has been shown effective formation of nitrogen-fixing nodules at 80-200 mm NaCl [10].

Drought is one of the adverse factors for nitrogen fixation process. When drought, for example, storage of the tubers of carbohydrate (starch) decreased and hydrolysis of sucrose increased by using a hydrolytic enzyme sucrose synthase and is inhibited nitrogenize activity [11]. Some authors, based on their results, conclude that nitrogen fixation is more sensitive to drought stress than the carbon assimilation [11]. In the study of nitrogen-fixing symbiosis with alfalfa nodule bacteria under saline conditions it was found that, most sorts of nodule formation slowed down for 48-72 hours at salt concentrations greater than 132 mm. In these experiments 29 sorts of alfalfa and 14 bacterial strains were tested, which were tolerant to limiting concentrations of sodium chloride 264 mm NaCl and 528 respectively [12]. At the same time the concentration of sodium chloride, influencing the symbiotic relationship of alfalfa and bacteria were lower than the corresponding concentration of individual alfalfa genotypes, or strains of bacteria affect the growth and survival [12].

Carried out works on obtaining salt-tolerant transgenic alfalfa plants: calluses containing Alfin1-overexpressing gene, encoding putative transcription factor, and associated with resistance of alfalfa to NaCl. Preliminary experiments showed that alfalfa plants were more resistant to inhibition by relatively high concentrations of sodium chloride (171 mm NaCl) and grew well at this concentration [13]. Lucerne is a moderate salt tolerance plant, which reaches an average 8.8 dS / m (1dS/mNaCl-10 mm NaCl, alfalfa at this concentration loses half of its harvest).

Most agricultural land in Uzbekistan affected by salinity (1-2dS/m) and salinization process continues. [13] The literature provides only condition that alfalfa is plant with average salt tolerance with indicating salinity scale at which 50% loss of alfalfa yield.

[14]. Thus, we can conclude that salinity has a negative impact more on macro symbiont (host plant, alfalfa) on the basis of these studies on salt tolerance of alfalfa nodule bacteria, the effect of salinity on seed germination of alfalfa symbiotic properties of legume-rhizobium symbiosis inoculated plant alfalfa and nodule formation rather than on nodule bacteria, which are at moderate to high concentrations of salt still retain their virulence (ability of nodule formation).

In this regard, "fate" of legume-rhizobium symbiosis of alfalfa under saline conditions and prospects for reclamation of saline lands will be determined primarily by the creation of transgenic salt-tolerant sorts of alfalfa plants that are more salt-tolerant and able to maintain an effective nitrogen-fixing symbiosis with more salt-tolerant rhizobia.

**Conclusion**

Study of the effect of salinity on growth, development and nodule formation tested plants sorts of alfalfa inoculated with isolated rhizobia showed that low (30 mm) NaCl salt concentration insignificantly affected the biomass growth of above-ground parts of the plants and their roots in comparison with the corresponding biomasses of control options (no saline) in the absence ($^{-}$N) and presence ($^{+}$N) of an exogenous nitrogen.

Further increase of the salt concentration (between 30 and 200 mm NaCl) resulted in a decrease in biomass growth. At the same time, the presence of exogenous nitrogen at a salt concentration of 30 mm NaCl resulted in that the ($^{+}$N) biomass of both cultivars excelled of corresponding biomass for 150 and 200% ($^{-}$N) -biomass of plants "Tashkent-1728" and "Khorezm-2" respectively.

If the value of the ratio (fraction) "biomass above ground plant parts" / "root biomass" was 2 or more (under control without saline), when salinity is decreased to 1 due to the smaller suppressing plant root biomass at concentrations ranging from 60 to 80 mm NaCl. At 80 mm NaCl ($^{+}$N) -biomass above ground parts of plants of two sorts of alfalfa were close in their values to the control values ($^{-}$N) -biomass (not subjected to salinity) above ground parts of plants, from it can be concluded that nitrogen is added to saline conditions has a positive effect on the growth of alfalfa plants under salinity. The salt concentration at the range 80-100 mm, in fact had no effect on the nodulation of alfalfa plants and even encouraged them in the presence of saline at the range 80-100 mm NaCl. A further increase of salt concentration inhibited the formation of nodules and color.

Virulence (symbiotic nodule formation activity) of salt-tolerant rhizobia for alfalfa plant was maintained even at high salt concentrations - at concentrations of 140-160 and even 200 mm NaCl. Critical (destructive) concentration of NaCl for the survival of alfalfa plants was 200 mm NaCl, whereas the concentration at the range of 80 to 100 mm NaCl had a negative effect on plant growth and development.

Conditional threshold salinity for alfalfa depended on the type of salinity: for sulphate, type of salinity was suspended to 30-50 mm, but for chloride, type was 60-80 mm. Data, obtained on the effect of salinity on the germination and development of alfalfa seeds, as well as on the growth, development and nodule formation of inoculated plant of alfalfa and the impact on them of exogenous nitrogen, are new, which provide specific information of the development of legume-rhizobium symbiosis under saline conditions, indicating the threshold and sub lethal concentrations of sodium chloride and sodium sulphate salinity for alfalfa.

The data on virulence (nodule formation) of nodule bacteria at different concentrations of salinity are brand new, because they indicate that a high salt tolerance of nodule bacteria are most sensitive to salinity link in rhizobium-legume symbiosis is the salt tolerance of alfalfa, but not their nodule bacteria.

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