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Isolation of Salt Tolerant Strains of Rhizobium Trifolii

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Abstract

Strain of Rhizobium trifolii collected from the plant root nodules of Trifolium alexandrinum from different geographical locations were isolated and screened for salt stress tolerance. A total twenty seven strains were checked for different salt tolerance. The results showed that at 3% NaCl salt concentration five strains are resistant and all others were sensitive.

Keywords: Berseem (Trifolium alexandrinum), Natures Farmers, Nitrogen-fixing Bacteria, Rhizobium trifolii, Salt Tolerant.

1. Introduction

Bacteria play an important role in maintaining and increasing soil fertility. The fertility of soil is proportional to its nitrogen content. Nitrogen is an essential ingredient of all living protoplasm. All growing plants therefore require it in their metabolism. Atmosphere is about four-fifths (80%) nitrogen. Green plants generally are unable to use it. They mostly absorb it as nitrates and to some extent as ammonia from the soil. Continuous absorption of these salts results in their exhaustion in the soil. Nearly all fertilizers for soil include a large proportion of such soluble nitrogen compounds to promote plant growth. In nature the presence of a regular supply of these salts is ensured by bacteria of certain types. These bacteria which function as *Natures farmers* belongs to three categories, namely, *ammonifying bacteria, nitrifying bacteria and nitrogen fixing bacteria.* They are the agents of maintaining a continual circulation of nitrogen in nature between the plant world, in soil and the atmosphere. The series of changes through which the nitrogen passes due to the activities of organisms constitute the nitrogen cycle.

Nitrogen-fixing bacteria assimilate atmospheric nitrogen, which green plants cannot utilize, by converting it into organic nitrogen compounds which are subsequently decomposed by the other bacteria and transformed through, a series of

stages into nitrates. The process by which they assimilate in nitrogen is called nitrogen-fixation. Symbiotic nitrogen-fixing bacteria, consisting of species of *Rhizobium*, live symbiotically in small swellings or nodules on the roots of various seed plants chiefly leguminous plants. This group of bacteria tends to increase the amount of nitrogen into the soil. Berseem *(Trifolium alexandrinum)* is a favorite fodder crop in most parts of the country (India) and is grown in large area. *Rhizobium trifolium* bacteria fix nitrogen in symbiotic association with this crop. The present study was undertaking to isolate and screened for salt stress tolerant strain of that bacterial strains.

2. Objectives of the Work

As the soil characteristics and climatic conditions vary from place to place, the yield of berseem crop can be improved by using *Rhizobium trifolii* strains that can tolerate difference stress conditions. The present study was undertaken to obtain such *Rhizobium strains.* The objectives of this work were the following:

Ob.1 To isolate different strains of *Rhizobium trifolii* from different locations.

Ob.2 To select salt tolerant strains of *Rhizobium trifolii.*

Ob.3 To characterize the isolates with respect to colony morphology.

3. Literatures Review

J.B. Boussingault, a French agriculturist in (1884), proposed that legumes can fix nitrogen in soil. What nitrogen source is available to the plant was the title of Hermann Hellreigal's talk just over a century ago (1886) on the 59th session of the meeting of Deutsche Naturforscher and Änzte in Berlin.

Crops grown under saline environment exhibit disturbed metabolism (Ben-Zioni *et al.*, 1967,[1] Ital *et al.* 1968) culminating in stunted growth and poor productivity. Amongest crops, grain legumes are unique because they meet their own nitrogen requirement by fixing atmospheric nitrogen through symbiosis. Their productivity depends on their nitrogen fixation potential in unfavorable environments, but little is known regarding their adaptability and nitrogen fixation potential under saline conditions. Rhizobia are either present in small numbers or are totally absent in saline alkali and acid soils (Genkel and Silin, 1947**[2]**; Mulder *et al.* 1960**[3]**; Burton, 1967**[4]**). Pillai and Sen (1966)**[5]** reported a large area in northern India falls under climatic influences which favor development of salinity in soil. *Trifolium alexandrinum* Justen (Berseem) is a fodder crop in these parts of the country and is grown in large areas on an extensive scale. Holdings and Kings (1963)**[6]** reported a high percentage of in effective strains of *Rhizobium trifolii* in infertile soils. Nitrogen deficiency can be obviated through seed inoculation with an appropriate effective rhizobial strain. The success of the process depends on the survival of the introduced strain (Vincent, 1965)**[7]**.

The presence of high sodium chloride has been reported to reduce the number of rhizobia in legume inoculants (Garg *et al.,* 1984). Filippova (1957)[8] found that some strains of root module bacteria of Lucerne were salt resistant. Some strains of root nodule bacteria of berseem (*Trifolium alexandrinum*) and chinacha *(Sesbania cannabera)* were reported to be salt sensitive (Pillai and Sen 1966[5]; Yadav and Vyas 1971). Both sodium chloride and sodium sulphate at concentration of 0.3N are toxic to *Trifolium alexandrinum*, (Yadav and Vyas, 1971)[9]. Rhizobium is more tolerant towards salt than its host legume and therefore survives in saline (Subha Rao *et al.,* 1972, 1974).

4. Significance of the Study

Salt stress is one of the many environmental constraints which limit the nitrogen fixation. The detrimental effects of salt stress on nodulation, plant growth and nitrogen fixation have been reported for many legumes including alfalfa, berseem, peas, mungbeams etc. Tolerance to salt stress may be an important part of saprophytic competence and competitiveness in Rhizobia. Thus, the exhaustive review of all above aspects shows that despite the efforts made, the Rhizobium legume, interactions process is little understand in terms of the competition ability to stressed conditions and genetic mechanism involved. There is a need to carry out work in this field.

5. Materials and Methods

5.1 Materials

Bacterial Strains: Twenty Seven strains of *Rhizobium trifolli* were isolated from the different localities and maintained on MSY slants.

Medium used for *Rhizobium trifolli*: Complete medium used for growing *Rhizobium trifolli* was Mannitol-Salts-Yeast extract-medium (MSY) (Sikka and Kumar, 1984; Khanuja and Kumar, 1988)

The composition of MSY medium per liter was Mannitol 2.0 g, $K_2HPO_4.3H_2O$ 0.2 g, KH_2PO_4 0.2 g., Yeast extract 1.0g, $MgSO_4.7H_2O$ 0.1 g, $CaCl_2.2H_2O$ 0.05 g, Distilled Water to make 1 liter, Volume, and pH of the medium was adjusted to 7.4. To make solid medium, 1.5% W/V Bactoagar was added per liter. When required, mannitol was used at a final concentration of 10.0 g/L to give yeast extract mannitol agar (YEMA) (Upchurch and Elkan, 1977).

Preparation of Media

Salt Used: MSY medium was used to isolate salt (Sodium Chloride) tolerant strains. The medium was prepared with different concentrations of sodium chloride viz., 0%, 0.25 %, 0.5%, 0.75%, 1%, 2% and 3%.

Methods: The isolation of various strains had been carried out as follows:

Collection of Plants: About three week old Berseem plants (***Trifolium alexandrinum***) were collected from different locations i.e. Roorkee, Haridwar and Muzaffarnagar.

Fig. 1: Map of Area of the Study.

Isolation of Bacteria from Root Nodules of Berseem Plants *(Trifolium alexandrinum)*

To isolate the bacteria following steps were followed:

- Surface sterilization of nodules
- Isolation of Bacteria
- Growth of Bacteria
- Purification of bacterial strains
- Serial dilution and streaking
- Morphological studies of various isolates
- Maintenance of Strains

Test for salt tolerant strains

For this experiment, all the isolated strains were streaked on MSY agar plates containing different concertations of NaCl. Sodium Chloride concentration used were 0%, 0.25%, 0.5%, 0.75%, 1%, 2% and 3%. The pH of medium was adjusted to 7.4 before adding the NaCl. All 27 strains were tested and compared with control. The colonies were observed after 24 to 48 hours of incubation.

6. Results and Discussion

In this study twenty seven strains of *Rhizobium trifolii* HK-1, 2, 3, …….. HK-27 was isolated from different areas. These strains were subjected to various salt stresses and the results obtained have been presented below:

Table 1: Growth pattern of 'Rhizobium Trifolli' strain on MSY medium containing different concentrations of sodium chloride

Strain No.	0%	0.25%	0.5%	0.75%	1%	2%	3%
HK–1	+	+	+	+	+	–	–
HK–2	+	+	–	–	–	–	–
HK–3	+	+	–	–	–	–	–
HK–4	+	+	+	+	+	–	–
HK–5	+	+	+	+	+	+	+
HK–6	+	+	+	+	+	–	–

HK–7	+	+	+	+	+	–	–
HK–8	+	+	+	+	+	–	–
HK–9	+	+	+	+	+	–	–
HK–10	+	+	+	+	+	–	–
HK–11	+	+	+	+	+	+	+
HK–12	+	+	+	+	+	+	+
HK–13	+	+	–	–	–	–	–
HK–14	+	+	+	+	+	–	–
HK–15	+	+	+	+	+	–	–
HK–16	+	+	+	+	+	+	–
HK–17	+	+	+	+	+	+	+
HK–18	+	+	+	+	+	–	–
HK–19	+	+	+	+	–	–	–
HK–20	+	+	–	–	–	–	–
HK–21	+	+	+	+	+	+	–
HK–22	+	+	+	–	–	–	–
HK–23	+	+	+	+	+	–	–
HK–24	+	+	+	+	+	+	+
HK–25	+	+	+	+	+	–	–
HK–26	+	+	–	–	–	–	–
HK–27	+	+	+	+	+	–	–

Note: '+' denotes Tolerant strain and '–' denotes Sensitive strain

Salt Tolerant strains

In order to select the salt tolerant strains all 27 strains of *Rhizobium trifolii* were streaked on the complex medium (MSY) containing different sodium chloride concentration (0%, 0.25%, 0.5%, 0.75%, 1%, 2% and 3%). Single colonies were observed after 24 h to 48 h of incubation at 30°C. It was found that all the strains grew normally on salt concentration of 0% and 0.25% of NaCl (Ref. Table No.1). At 0.5% NaCl concentration most of all the strains were tolerant except only five strains viz., HK-2, HK-3, HK-13, HK-20, and HK-26 were found to be sensitive. At 0.75% NaCl concentration also most of all were tolerant but only six strains viz., HK-2, HK-3, HK-13, HK-20, HK-22, and HK-26 were found to be sensitive. Again at 1% NaCl concentration most of all the strains were tolerant except seven strains viz., HK-2, HK-3, HK-13, HK-19,HK-20, HK-22, and HK-26 were found to be sensitive. But at 2% NaCl concentration only seven strains viz., HK-5, HK-11, HK-12, HK-16, HK-17, HK-21, and HK-24, were found tolerant and rest of all are sensitive. And at 3% NaCl concentration only five strains viz., HK-5, HK-11, HK-12, HK-17, and HK-24 were found tolerant and rest of all were sensitive.

Photo Plates: Selection of Salt Tolerance Strains (at 3% NaCl Solution)

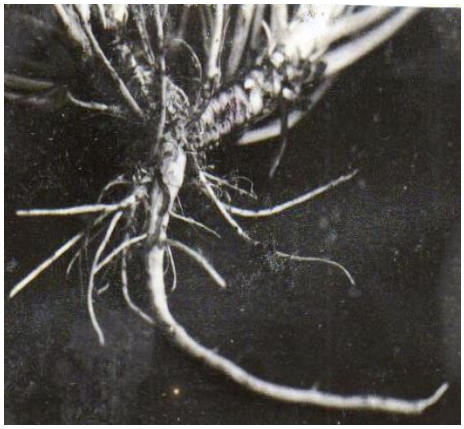

Fig. 2: Three week old Trifolium alexandrinum

Fig. 3: Strain HK-5

Fig. 4: Strain HK-11

Fig. 5: Strain HK-12

Fig. 6: Strain HK-17

Fig. 7: Strain HK-24

7. Discussion

High salt has inhibitory effect on the growth of *Rhizobium* and nodulation of plant but our study indicate that genetic variation is present for tolerance to these stresses. The selection of stress (salt) tolerant strains of *Rhizobium trifolli* can become a good screening criterion for symbiotic nitrogen fixation in unfavorable environment.

8. Conclusion

This study was undertaken with the aim to select salt stress tolerant strains of *Rhizobium trifolli* and these strains can be used to give better results under different stress conditions. If multiplication of these improved strains for biofertilizer production would be done, yield would continue to show the expected enhancement year after year in the waste land and stress condition soil.

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