

# Assessing Tolerance of Rhizobial Lentil Symbiosis Isolates to Salinity and Drought in Dry Land Farming Condition

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## Abstract

Due to nutrient including nitrogen deficiency, vegetable production is restricted in most of semi arid and especially saline lands, and nitrogenous chemical fertilizers in these lands cause the salinity to increase, chemical fertilizer consumption may be economically expensive. So, nitrogen fixation biofertilizer application would be important. In this research 220 rhizobial bacteria were isolated from two lentil dry land farming flat plains of Moghan and Koochin. BTB +YMA culture media with different levels of salinity by NaCl was used in a saline tolerance test of rhizobial lentil symbiont strains. Among 184 rhizobial strains, 101 strains were completely sensitive (EC=10 ds/m) and 10 strains were superior strains which were completely tolerant to salinity. Altogether Koochin flat plain rhizobacteria were more saline tolerant than Moghan flat plain rhizobacteria. Drought tolerance test of rhizobial strains was performed in PEG 6000 +YMA culture media. The drought level tolerance was evaluated based on optical density of bacterial suspension in this test. Rhizobacteria were grouped in 4 levels based on their drought tolerance as completely tolerant, tolerant, sensitive and completely sensitive using their optical density. Among 34 super strains which are completely tolerant, 9 super strains of Moghan flat plain and 25 super strains of Koochin flat plain were selected. At last we hope that the super strains of this research can be used in subsequent studies.

## Key Words

Salinity, drought, NaCl, PEG, *Rhizobium*.

## Introduction

About one-third of the land area of the world comprises arid and semi arid climates and approximately 15% of these land areas are affected by saline. However salinity and drought would suppress rhizobia growth and multiplication but there are some strains which can withstand high levels of drought and salinity and survive. Soil salinity is caused by the accumulation of soluble salts of calcium, magnesium and sodium, mostly as chlorides and sulphates (Singleton *et al.* 1994). About 400-950 million hectare of the world agricultural lands are affected by salinity (Allen *et al.* 1995; Bernstein *et al.* 1983). The whole saline land area of Iran is more than 15 million hectare which comprises 10% of the total area of the country and the salinity is increasing due to the excessive irrigation, saline water utilization and incorrect consumption of chemical fertilizer (Allen *et al.* 1995). Plant production is limited in these arid and semi arid land area because of nitrogen deficit (Worrall and Roughly 1976) and Nitrogen consumption may increase plants tolerance to salinity, however nitrogenous chemical fertilizers consumption would cause more salinity of these land area and it wouldn't be cost effective. So it is important to consider about Biological Nitrogen Fixation and Biological fertilizers utilization especially microbial fertilizers (Cordovilla 1996; Pessaraki and Tucker 2005).

## Material and Methods

### *Sampling and Rhizobial isolates preparation*

To investigate drought and salinity tolerance of rhizobial isolates symbiont of lentil, lentil nodule samples were collected from different agricultural field sites in Moghan and Koochin flat plains in Ardebil and Zanjan provinces, respectively. After washing the root system of the lentil plants, a well formed, healthy pinkish nodule on the tap root of each sample was carefully cut out with a portion of the root attached to the nodule. Then the nodules were transferred to the laboratory for further experiments in small desiccators containing silicagel and were coded individually. The samples related to Moghan flat plain were coded 1-110 and 110-220 for Koochin. To isolate rhizobial strains, the nodules were sown in water and they were surface sterilized for 5 minutes in 0.1% mercuric chloride in water and repeatedly washed with sterile water to get rid of the chemical. The nodules then washed in 70% ethyl alcohol for 3 minutes followed by more washing with sterile water. Then they were crushed with a sterile glass rod in a small aliquot of sterile water. A small aliquot of the suspension was transferred on Congo red incorporated yeast extract mannitol agar medium to obtain sparse and distinct colonies (Subba Rao 1999). At the end of incubation period, macroscopic and

microscopic observations were performed for grown colonies. Gram staining and plant infection test (PIT) were carried out to confirm rhizobia colonies. The purified isolates were transferred to agar slants in 2-5 °C and also preserved in conservation media of YM broth containing glycerol in -80°C (Vincent 1982). 220 rhizobial isolates were totally recovered from the nodules of the collected lentil plant roots.

#### *Evaluation of the isolates tolerance to salinity*

The ability of the rhizobial isolates to withstand salinity was evaluated by their growing at different concentrations of NaCl salt with different electrical conductivities (EC) of 10 to 50 ds/m in bromothymol blue (BTB) incorporated yeast extract mannitol agar medium. Rhizobial strains were inoculated and after incubating, well-grown bacteria which have been changed the colour of medium from blue to yellow were supposed as tolerant isolates.

#### *Evaluation of the isolates tolerance to drought*

The drought tolerance of the isolates was also examined, using Poly Ethylene Glycol 6000 (PEG 6000) in YMB medium with Different concentrations (Burlyn E. Michel & merrill R. Kaufmann, 1973) using the following formula:

$$\text{Water potential (wp)} = -(1018e - 2)c - (1.18e - 4)c^2 + (2.67e - 4)ct + (8.39e - 7)c^2T$$

After inoculating the isolates in sterile condition and incubating on a rotator shaker for 72 hrs in 28°C, their optical density in 570 nm were measured. In fact optical densities were decreased by increasing of PEG amount in the media. The results were analysed in Excel software.

### **Results and discussion**

The number of lentil samples collected from Moghan flat plain in Ardebil province and Koohin flat plain in Zanjan province were equal and total samples number was 220. 214 bacterial strains were isolated on bromothymol blue incorporated yeast mannitol agar media. Isolated colonies of YMA+ Congo red were gram negative, white, translucent, glistening, elevated 3-5mm colonies with entire margins. These colonies changed the colour of media containing BTB to yellow colour. During subculturing, bacterial isolates were purified and refrigerated in 2-5 °C. Plant infection test (PIT) was carried out in tubes with a proper media for lentil legumes in the growth chamber. It was demonstrated that among 214 isolates, 25 isolates were not *rhizobium leguminosarum* bv. *Viciae* (lenti)(*RLV*). The results of salinity tolerance experiment illustrated that among 189 rhizobial strains symbioant with lentil plant (*RLV*), the number of grown strains on different levels of salinity was as followed: 1) EC=10 ds/m, 101 strains(53.44%) which was defined as completely sensitive, 2) EC= 20 ds/m, 93 strains (49.21%), sensitive, 3) EC= 30ds/m, 71 strains (37.57%) defined as partially tolerant, 4) EC = 40ds/m, 35 strains (18.52%) tolerant and 5) EC=50ds/m, 35 strains, completely tolerant (Table 1). Although 25 grown strains in EC=50 ds/m changed the color of their BTB+YMA media from blue to yellow but the colonies didn't grow strongly and typically, while 10 rhizobial strains grew completely were supposed as superior strains. Among 110 isolated Moghan flat plain strains, 46 strains (41.82%) grew on EC= 10 ds/m while the number of grown strains in similar salinity for Koohin flat plain was 55 strains (50%).

It can be resulted that the collected samples of Koohin flat plain had much more salinity tolerant strains than Moghan. However extreme condition suppresses the multiplication and growth of rhizobial strains but there are some strains which can tolerate high amounts of salt and survive (Lakshmi and Subbarao 1984; Vmanchanda and Garg 2008).

Subba rao (1994) had observed a significant difference in the growth of different inoculated rhizobial strains on a salinized YMA medium and reported that rhizobial tolerance to salinity is more than their host plants. Subba rao *et al.* (1999) reported that Rhizobial strains which were able to make an efficient symbiosis in high levels of salinity may not be necessarily originated from saline soils. In return Duca *et al.* reported that the collected isolates from dry and saline soils are more successful to make root nodules in saline conditions. The researches have been proved that some free-living bacteria (Saprophytes) are able to survive in drought or saline stress conditions.

**Table 1. The number of grown rhizobial strains in different levels of salinity.**

Grown rhizobial strains		Different levels of salinity	Tolerance to salinity
Percentage	Number	EC(ds/m)	
53.44	101	10	Completely sensitive
49.21	93	20	sensitive
37.57	71	30	Partially tolerant
18.52	35	40	tolerant
18.52	35	50	Completely tolerant
5.29	10	50	Superior strains

The results from assessing the tolerance of symbioant rhizobacteria showed that among 34 superior tolerant isolates of salinity experiment, 6 strains (17.65%) were completely sensitive to drought stress and 8 strains were sensitive and 15 strains were grouped as drought tolerant and finally 5 strains were defined as completely drought tolerant (Table 2). Drought stress is one of the major environmental factors affecting most crops and decreasing crop yield (Vmanchanda and Garg 2008). The population of soil bacteria decreases along the moisture stress however it would not be lost completely and certain soil bacteria can resist these extreme dry conditions. These microorganisms utilize the water preserved in the micro porous of soil and survive by their minimum metabolic activities.

**Table 2. The tolerance of lentil symbioant rhizobial strains to drought stress.**

Number of strains	Tolerance to drought	Optical density (OD)
6	Completely sensitive	OD<0.3
8	sensitive	OD=0.3-0.4
15	tolerant	OD=0.4-0.5
5	Completely tolerant	OD>0.5

## Conclusion

The results of the experiments demonstrated that we can isolate and purify drought and salinity completely tolerant rhizobial isolates from Iranian dry land farming soils.

Although the strains isolated from Moghan flat plain showed fewer tolerance to salinity and were placed in completely sensitive (46 isolates) and sensitive (42 isolates) groups and they could not tolerate drought condition and the number of all groups from completely sensitive to completely tolerant were less than Koochin flat plain isolates. Hence, it can be concluded that drought tolerance is parallel with salinity tolerance for rhizobia strains. Zahran (1999) also reported that salinity sensitive bacterial strains can not resist against high levels of drought and it is also related to salinity. We hope more supplementary investigations would be conducted to introduce the superior strains to produce industrial and commercial biological fertilizer symbioant lentil.

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