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Research Article

Assessment of Rhizobial and Phosphobacteria biofertilizer to overcome the moisture stress in the Vigna mungo

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Abstract: This study was undertaken to evaluate the property of *Rhizobium* and *Phosphobacterial* biofertilizer in inducing tolerance towards moisture stress. With this objective, the study was conceived by taking up pot culture studies using a mixed culture of biofertilizers (*Rhizobium* of pulses*), phosphobacteria* and silicate solubilizing bacteria. The crop plant taken for this study was Blackgram. The plant Blackgram overcomes the moisture stress by the presence of disaccharide sugar trehalose in the rhizobial cells. The other important factor was the nutrient potassium which is dissolved by silicate solubilizing culture releasing more of potassium in the soil. The increase in potassium content is directly proportional to moisture stress. Both of these enhanced the longevity of plants for few days.

Keywords: Biofertilizer, Blackgram, moisture stress, *Rhizobium*, *Phosphobacteria*, Poatassium, Trehalose

INTRODUCTION

Biofertilizers are widely thought as important owing to their nitrogen fixation or phosphate solubilizing ability. It protects the crop from pathogenic infection by production of Siderophores, thereby it act as a biological control to increase the crop yield and produce growth promoting substances like auxins, Vitamins, Gibberlins etc. The pivotal role played by Biofertilizers is their ability to protect the plants from moisture stress, even though the microbes require water for their multiplication. This study will open a new horizon in the area of biofertilizer application imparting drought tolerance. The presence of trehalose is a non-reducing sugar which accumulates in the bacteroids to overcome moisture stress(6). The second one is nutrient interaction of the plant by uptake of potassium due to silicate solubilizing culture(5).

Materials and Methods

Pot culture study

The pots were filled with soils. Five treatments with five control pots were carried out in different moisture regimes and five seeds were sown on each pot. In each treatment, the pots were filled with *Rhizobium*, Phosphobacterial biofertilizer and silicate solubilizing organisms. The five treatments include soil moisture maintained at Hygroscopic coefficient, Wilting coefficient, Field capacity, Saturation and Super saturation.

Methods for measuring soil moisture

First the different moisture content of soil was determined by the Gravimetric method. A known volume of the moist soil sample was taken and dried in an oven at a temperature of 100-110°C. After drying, the content was weighed again. The moisture lost by heating represent the soil moisture content. It was calculated by

$$\text{Soil moisture \%} = \frac{\text{Loss wt of soil}}{\text{Dry Weight of soil}} \times 100$$

Determination of Potassium

To determine the potassium level in the plant tissue, the fresh tissues from the plant part was collected and decontaminated from dust and another foreign material by washing with detergent solution and deionized water. Then it was subjected to Diacid, Triacid digestion and dryashing. After the preparation of the plant tissue, the potassium level is determined by Flamephotometry.

The level of potassium found in the plant tissue was analyzed by introducing in to the flame photometer in solution form. The atoms get excited by taking energy from flame and the emitted radiation was measured by photocell and photomultiplier tube. The concentration of potassium was measured by comparing the radiation emitted by a known standard with that of the sample.

Estimation of Trehalose

The trehalose sugar present in the rhizobial cells was determined by the following test.

Osazone formation-The sample (rhizobial culture) in the YEMA broth was taken in clean test tube. To this a mixture of phenyl hydrazine hydrochloride and sodium acetate was added and heated in aboiling water bath for 15 minutes.

Hydrolysis-The sample was taken in a test tube and added two drops of 0.1N HCL and iodine solution. The colour change was checked and it was further confirmed by Barfoed's, Fouglars and Fehling's solution.

Result and discussion

In the pot culture study a pulse crop was taken owing to fact that the long tap root system of leguminous crops could help in overcoming moisture stress during the initial period. The five treatments were applied with mixed biofertilizer containing *Rhizobium, Phosphobacteria* and silicate solubilizing bacteria. Then they were evaluated against five similar set of controls without biofertilzer application. The soil moisture percentage and moisture tension was determined by gravimetric method and by using Tensiometer(table-1& table-2).

Table-1 Soil moisture percentage

S.No	Treatment	Moisture level in percentage (%)
1	T1 Hygroscopic coefficient	10.2
2	T2 Wilting percentage	14.4
3	T3 Field capacity	30.2
4	T4 Saturation	52.2
5	T5 Super saturation	86.4

Table-2 Moisture Tension

S.No	Treatment	Moisture tension(bars)
1	Hygroscopic coefficient	33
2	Wilting percentage	16
3	Field capacity	0.4
4	Saturation	0.3
5	Super saturation	0.1

Then the plants were observed for their growth, turbidity and overall performance. The observations made were based on the physical viewing by the crop presentation in both treatments as well as in control. The plants were maintained at optimum moisture level upto 15 days and then the treatment condition was imposed. The duration of the Blackgram crop was 90 days. When results were compared treatment with biofertilizer had prolonged to longevity of Blackgram for a few days (Table-3).

Table-3 Observation on death of plants due to different moisture level

S.No	Parameters	Death of plants(in days)	
		Treatment	Control
1	Hygroscopic coefficient	21	18
2	Wilting percentage	26	24
3	Field capacity	78	75
4	Saturation	79	75
5	Super saturation	65	60

In case of treatment with combined biofertilizers recorded increased potassium status than in control. Hence it is another clear indication that the enhanced potassium content in plant might be one of the reasons for the plant to be more drought tolerant (Table-4).

Table-4 Effect of Nutrient (K) status on Blackgram

S.No	Parameters	Nutrient status in (%)	
		Treatment	Control
1	Hygroscopic coefficient	3.21	2.98
2	Wilting percentage	3.22	3.01
3	Field capacity	3.21	2.98
4	Saturation	3.18	2.95
5	Super saturation	3.15	2.91

Further the effect of trehalose in enhancing drought tolerance is well documented. *Rhizobium* is known for its trehalose production. The utilization of *Rhizobium* in the mixed culture under water stress would release the trehalose from the cell into soil. The presence would have imparted the tolerance for water stress. It was identified by Osazone test here trehalose does not form osazone because it does not contain a functional carbomyl group. There were no crystals formed. It confirms trehalose is one of the nonreducing sugar. On hydrolysis it yields 2 moles of D glucose. It was confirmed by Barfoed's test: The sugar present in the test sample reduced cupric acetate to Cuprous oxide to form reddish brown precipitate.

Conclusion

Biofertilizer application increases the tolerance of Blackgram to water stress compared with control and the plants treated with Rhizobium and Phosphobacterial biofertilizer contain higher level of potassium in their tissues. It might have a role in imparting stress tolerance to water. The next important is the use of Rhizobium, which produces trehalose might be another possible factor to enhance tolerance of the plant to water stress.

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CONFLICTS OF INTEREST

"The authors declare no conflict of interest".