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## Studies on the Physico - Chemical properties of the soil samples collected from different locations of Dharmapuri District

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### Abstract

Soybean (*Glycine max* (L.)) is being a two in one crop supplying about 43.3% protein and 19.5% oil is called "Miracle bean". Legume crops is largely due to their ability to fix atmospheric dinitrogen in a symbiosis with specific bacteria (*Rhizobium* and *Bradyrhizobium*). The *Rhizobium* legume symbiosis is, suggested as an alternative to solving the soil N fertility problem. Soybean performs, nitrogen fixation by establishing a symbiotic relationship with the rhizobia. In this study, the physical chemical properties of soybean soil samples collected from 10 different locations in Dharmapuri district. The soils belonged to textural groups viz., Red loam, Clay loam, Loam and Black soil. Soil organic carbon content ranged from 0.48 to 0.86%; soil pH ranged from 6.55 to 7.75 and EC ranged from 0.10 to 1.45 dsm<sup>-1</sup> Soil available nutrients, nitrogen ranged from 66.00 to 135.60 kg ha<sup>-1</sup> phosphorus ranged from 8.00 to 18.10 kg ha<sup>-1</sup> from potassium ranged from 118.00 to 190.00 kg ha<sup>-1</sup> respectively. The rhizobial population ranged from 0.62 to 7.10 X 10<sup>3</sup> CFU g<sup>-1</sup> soil. The nodulation pattern and rhizobial populations on the rhizosphere soil of soybean plants were studied. Among the soil samples is analysis the pH, EC, Organic carbon and available major nutrients values are estimated.

**Keywords:** Physico-chemical, soybean, Nodulation.

### Introduction

Soybean being a two in one crop supplying about 43.3 percent protein and 19.5 percent oil is called as "Golden bean". Soybean serves as an important fat and protein source for large population residing in Asia and America continents. In, India the area and productivity have been rapidly increasing over the recent years. Besides being a rich source of protein, they maintain soil fertility through biological nitrogen fixation in soil and thus play a vital role in furthering sustainable agriculture. Soybean is indigenous to china and was introduced in India in 1950's (Caldwell, 1973) [5]. Soybean has the ability to reduce atmospheric nitrogen to a biologically usable ammonia, in association with bacteria collectively known as rhizobia (Singleton *et al.*, 1992; Giller, 2001) [15, 7]. Soybean is a promising pulse crop proposed for the alleviation of the acute shortage of protein and oil worldwide (Mahamood *et al.*, 2009). It is basically a pulse crop and gained the importance as an oil seed crop as it contains 20% cholesterol free oil. Nitrogen is a limiting nutrient for growth and yield of soybean, rhizobia have a direct role to play in its supply to the growing plants (Kanimozhi and Panneerselvam, 2010) [9]. Soya protein is the only vegetable source of complete protein, of a quality comparable to meat and eggs, which contains all the essential amino acids required by human and animals. So there is a pressing need to improve the yield of soybean in order to meet the protein malnutrition and the edible oil needs of our country. Nitrogen is one of the major important essential for plant growth. The economic and environment importance of legume crops is largely due to their ability to fix atmospheric dinitrogen in a symbiosis with specific bacteria (*Rhizobium* or *Bradyrhizobium* species). Like most legumes, soybeans performs N<sub>2</sub> fixation by establishing a symbiotic relationship with the rhizobia.

Soybean inoculated with *Bradyrhizobium japonicum* forms highly effective nodules and frequently increased soybean yields, especially in fields where soybeans are cultivated for the first time (Caldwell and Vest, 1970) [4]. The major problem of soybean inoculation is the existing indigenous strains in the field may often suppress the introduced inoculant strains applied to soybeans subsequently. Environmental factors such as temperature, moisture, acidity, salinity and several chemical component of the soil are the limiting factors of the legume-rhizobium symbiosis. Both establishment and activity of the legume-symbiosis are known to sensitive to drought stress (Kirda *et al.*, 1989) [11]. Numerous abiotic and biotic factors are known to influence the competitiveness of specific rhizobial inoculants (Bottomley,

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1992<sup>[3]</sup>; Turco and Sadowsky, 1995)<sup>[17]</sup>.

Semi-Arid Tropical (SAT) regions spread over 11.6 million square kilometers in the developing world and they did not benefit greatly from the green revolution and there is a need for "Grey to Green revolution" in the SAT to feed and provide proper nourishment to the ever increasing population of the developing world (Singh *et al.*, 1999)<sup>[14]</sup>. The main reasons to low productivity of pulses in semiarid tropics of Tamil Nadu, is due to unfavourable weather conditions like erratic and uncertain rainfall, low/high temperature and moisture stress at various crop growth stages. The present study was designed with the following objectives.

Survey on the occurrence of root nodule bacteria, nodulation pattern of soybean grown in semi-arid tropics of Tamil Nadu. Survey of the community population of *Bradyrhizobium japonicum* from each locations in semi- arid tropics of Tamil Nadu. Isolation, identification and characterization of the individual isolates collected from various semi-arid locations of Tamil Nadu.

## Materials and methods

### Soil analysis:

#### Estimation of soil pH

The soil reaction (pH) was determined in Elico model LT-10T pH meter preparing 1:2.5 soil: water suspension and string by means of a glass rod. (Jackson, 1973)<sup>[8]</sup>.

#### Estimation of Electrical conductivity (EC)

The soil suspension was prepared and used to determine the EC using conductivity Bridge and expressed in  $\text{dSm}^{-1}$  (Jackson, 1973)<sup>[8]</sup>.

#### Estimation of organic carbon

The organic carbon content of the soil sample was estimated by Walkely and Black method (1947)<sup>[18]</sup>. One gram of finely ground soil sample was transferred to 500 ml conical flask to which 10 ml 1N  $\text{K}_2\text{Cr}_2\text{O}_7$  solution and 200ml of concentrated  $\text{H}_2\text{SO}_4$  were added and allowed to stand for 30 min. After 30 minutes, 10ml of NaF solution and 2ml of diphenylamine indicator were added. The solution was titrated with the standard  $\text{FeSO}_4$  solution to brilliant green colour from dark blue colour.

#### Estimation of soil available nutrients

Available nitrogen, phosphorus and potassium were estimated by Alkaline permanganate, sodium bicarbonate method vice-versa (Subbiah and Asija, 1956; Watanabe and Olsen, 1965; Toth and Prince, 1949)<sup>[16, 19]</sup>.

## Studies on the occurrence of rhizobia in the soils of Dharmapuri District of Tamilnadu.

### Determination of Rhizobial population

The rhizosphere soil samples from different locations were collected, 10g of the soil samples were serially diluted up to  $10^{-6}$  dilution and 1ml were transferred YEMA petriplates. The plates were incubated at room temperature and the colonies were counted by using colony counter.

### Nodulation pattern of soybean

The nodulation pattern of the soybean is not uniform, so the soybean plants were collected from each location at random without damaging the roots. The average of ten plants were taken to give the nodulation pattern of that place. The total number as well as the number of pink and white nodules were

counted and recorded.

### Isolation of *Bradyrhizobium* from root nodules

Soybean plants aging 40-50 days were removed carefully after watering the surrounding soil and roots were washed with water to remove the soil without disturbing the nodules. Vincent (1970) method was followed for isolation of Rhizobia. Large sized nodules were selected for isolation and were washed to remove surface dirt under water. Washed nodules were stored in silica gel tubes. Then they were rehydrated in sterile water for 1 hour. These were immersed in 95% alcohol for 5 to 10 seconds. Then transferred to a solution of 0.1% mercuric chloride for 5 min. These nodules were then further washed in 10 changes of sterile distilled water. The surface sterilized nodules were then aseptically transferred to test tube containing 1 ml of sterile glass rod to get nodule suspension. A loopful of the nodule suspension was streaked on Yeast Extract Mannitol Agar (YEMA) with congo red plates to obtain isolated colonies. These plates were incubated for 5 to 7 days at  $26^\circ\text{C}$  to  $28^\circ\text{C}$ . The clear or milky white colonies were further purified by repeated streak plate method.

## Results and Discussion

### Physico-chemical properties of soybean soils of semi-arid tropics of Tamil Nadu:

Soybean soil samples were collected from ten different locations of Tamil Nadu and their physico-chemical properties were analysed and the results were presented in Table No.1

Soils of semi-arid tropics of Tamil Nadu belonged to 4 textural types, Red loam, Clay loam, Loam and Black soil. The soil organic carbon contents were low, ranged from 0.48 to 0.86%, soil samples collected from Karimangalam to Dharmapuri District recorded highest pH of 7.75 and EC OF  $1.45 \text{ dSm}^{-1}$ .

Soil available nutrients, Nitrogen ranged from 66.00 to 135.60  $\text{kg ha}^{-1}$ , phosphorus 8.00 to 18.10  $\text{kg ha}^{-1}$  and potassium ranged from 118.00 to 190.00  $\text{kg ha}^{-1}$  respectively.

### Native population of Rhizobium Soybean fields of semi-arid tropics of Tamil Nadu

The different locations were namely Dharmapuri for studying the native rhizobial population of Rhizobium in soybean fields. The results were recorded in table.2

Among the 10 locations, 4 locations were recorded more than  $3 \times 10^3 \text{ CFU g}^{-1}$ , 4 locations were recorded  $1 \times 10^3 \text{ CFU g}^{-1}$  or  $3 \times 10^3 \text{ CFU g}^{-1}$  and 2 locations were recorded less than  $1 \times 10^3 \text{ CFU g}^{-1}$  of rhizobial populations from the rhizosphere of soybean plants.

### Survey on the nodulation pattern of soybean in semi-arid tropics of Tamil Nadu

A survey was taken to study the nodulation pattern of soybean plants grown in district of semi-arid tropics of Tamil Nadu. The total number of nodules as well as number of pinkish white nodules were counted and recorded in Table No. 2. The total number of nodules were ranged from 7.00 to 19.00  $\text{plant}^{-1}$ . 8 locations were 5.00 to 10.00 nodules  $\text{plant}^{-1}$  and pappireddipatti of Dharmapuri District was recorded highest number of nodules of 19.00 nodules  $\text{plant}^{-1}$ . The lowest nodule number was recorded in Karimangalam 5.00  $\text{plant}^{-1}$  of Dharmapuri District. This increase in available N might also be attributed to the greater multiplication of soil microbes

which converts organically bound N to inorganic form as reported by Katkar *et al.*, (2006)<sup>[10]</sup>.

**Table 1:** Physico-chemical properties of the soil samples collected from different locations in Dharmapuri District of Semi-arid tropics of Tamil Nadu

S. No	Locations	Soil type	Soil pH	EC (dsm <sup>-1</sup> )	Organic carbon (%)	Available N (kg ha <sup>-1</sup> )	Available P (kg ha <sup>-1</sup> )	Available K (kg ha <sup>-1</sup> )
1	Mallapuram	Red loam	7.15	0.69	0.48	118.00	12.50	118.00
2	Nallampalli	Red loam	6.55	0.52	0.80	80.20	18.10	118.00
3	Pappiredipatti	Clay loam	6.85	0.35	0.76	126.00	17.50	175.75
4	Adiyamankotai	Clay loam	6.65	0.45	0.86	68.00	18.00	169.85
5	Morappur	Red loam	6.90	0.86	0.68	100.50	11.85	130.10
6	Singarapetai	Clay loam	6.85	0.39	0.62	67.00	17.89	170.25
7	Karimangalam	Red loam	7.85	1.75	0.67	128.00	11.00	98.00
8	Samalpatti	Clay loam	7.80	1.00	0.58	86.00	9.00	98.00
9	Ballurpetai	Red loam	7.10	0.17	0.73	97.35	18.00	190.00
10	Pennagaram	Clay loam	7.70	0.12	0.62	98.65	16.10	158.50

**Table 2:** Survey on the nodulation pattern of soybean grown and native population of Rhizobium in soybean fields of 10 different locations in Dharmapuri district of semi arid tropics Tamil Nadu

S. No	Locations	No. of Green nodules (Plant <sup>-1</sup> )	No. of Pink nodules (Plant <sup>-1</sup> )	Total no. of nodules (Plant <sup>-1</sup> )	Rhizobium Population 1×10 <sup>3</sup> /g <sup>-1</sup> of moisture free soil
1	Mallapuram	2	6	8.00	1.95
2	Nallampalli	3	6	9.00	2.10
3	Pappiredipatti	9	10	19.00	7.10
4	Adiyamankotai	3	6	9.00	2.50
5	Morappur	4	6	10.00	3.12
6	Singarapetai	4	7	11.00	3.50
7	Karimangalam	2	5	7.00	0.70
8	Samalpatti	3	6	9.00	1.75
9	Ballurpetai	2	5	7.00	0.62
10	Pennagaram	3	6	9.00	3.75

The soybean has played a significant contribution to yellow revolution in India and as a food plant it forms an important part of the routine diet of the people of a India. Today, soybean belongs to one of the most important crops in the subcontinent (Appunu *et al.*, 2007)<sup>[2]</sup>. Nitrogen is one of the major important nutrient essential for plant growth. Symbiotic nitrogen fixation resulting from mutual beneficial interaction between soybean and soil nodule bacteria provides a significant boost to N fertilization and additionally, does not cause any hazard to environment. *Bradyrhizobium japonicum* is a slow growing root nodule symbiont, which is widely used as an inoculate in soybean fields through the world (Cardwell and Vest. 1970). Zafar *et al.*, 2012<sup>[20]</sup>. Reported that plant growth promoting rhizobia inoculation positively increased the plant N and P accumulation by improving nitrogen fixation. The highest and lowest value is obtained from SBJ-8 is respectively. The effect of Rhizobium inoculation and nitrogen application on phosphorus accumulation of field pea when compared to uninoculated treatment was non significant. (Anteneh Aragv and Abere Mnalku, 2017)<sup>[11]</sup>.

The beneficial effects of *Rhizobium* and *Bradyrhizobium* in legume in terms of biological N<sub>2</sub> fixation has been a main focus in the recent past (Deshwal *et al.*, 2003)<sup>[6]</sup> as it is an important aspect of sustainable and environmental friendly food production and long term productivity. The common observation that soybean crop nodulates. Poorly in the soils of semi-arid regions of Tamil Nadu. Poor nodulation may be due to various reasons like, unfavourable conditions, Bradyrhizobial population below the threshold level, presence of ineffective native rhizobial population.

Rhizobial population ranged from 0.62×10<sup>3</sup> to 7.10×10<sup>3</sup> CFU g<sup>-1</sup> soil. The number of nodules plant<sup>-1</sup> ranged from 8.00 to 10.00, locations from 19.00 nodules in one location. To improve the nodule status an attempt was made to understand

the limitations and to involve strategy to manage the population of microorganisms. The nutrients status of soil is more important to root nodulation of soybean. Soil available nutrients viz., nitrogen ranged from 66.00 to 135.60 kg ha<sup>-1</sup>, P ranged from 8.00 to 18.10 kg ha<sup>-1</sup> and K ranged from 118.00 to 190.00 kg ha<sup>-1</sup> respectively. The nitrogen content of the semi-arid locations of Tamil Nadu were found to be low. The ability to produce curling of root hair is necessary for a rhizobial strain to be able to cause nodulation (Sahlman and Fahraeus, 1962). The showed on 10 isolates tested, SBJ-8 was found to be the most efficient in increasing the growth, dry weight, nodulation, nitrogen content, Leghaemoglobin content and ARA activity of the nodules of soybean plants.

### Conclusion

Soya protein is the only vegetable source of complete protein, of a quality comparable to meat and eggs, which contains all the essential aminoacids required by human and animals. In this study to evaluate the soybean performs nitrogen relation by symbiotic nitrogen fixation from rhizobia. *Bradyrhizobium japonicum* is the slow grow root nodules symbiont which is used as inoculate in soybean field throughout the world. The strain SBJ-8 was observed recorded highest number of 7.10×10<sup>3</sup> CFU g<sup>-1</sup> soil. The nutrient status of soils is more important of root nodulation of value 19.00 is recorded, NPK content is highest value of 135.60 kg ha<sup>-1</sup>, 18.10 kg ha<sup>-1</sup> 190.00 kg ha<sup>-1</sup> is recorded. Based on result of I will developed the drought tolerant mutant strain for their further study.

### References

1. Anteneh Argav, Abere mnalku. Symbiotic effectiveness of *Rhizobium leguminosarum bv.viciae* isolated from major highland pulses on field pea (*Pisium sativum* L.) in soil with abundant rhizobial population. Annals of

Agarian Science. 2017; 15:410-419.

2. Appunu C, Sen D, Singh MK, Dhar B. Variation in symbiotic performance of *Bradyrhizobium japonicum* strains and soybean cultivars under field conditions. J Centra. Euro. Agri. 2007; 9:185-190
3. Bottomley PJ. Ecology of *Bradyrhizobium* and *Rhizobium*. In: biological nitrogen Eds. Stacey G, Burris RH and Evens, H.J., 1992, 293-348. *New York: Chapman and Hall*, ISBN. 0-41202421.
4. Caldwell BE, Vest G. Effects of *Rhizobium japonicum* strains on soybean yields. Crop science. 1970; 10:19-21.
5. Caldwell D. Soybean (*Glycine max* (L.) Merrill) cultivation as oilseed crop in china. J Agri. Sci. 1973; 28:281-283.
6. Deshwal VK, Duby RC, Maheswari DK. Isolation of plant growth promoting strains of *Bradyrhizobium* species biocontrol potential against *Microphomina phaseoline* charcoal rot of peanut. *Soil Biol. Biochem.* 2003; 47:987-996.
7. Giller KE. Nitrogen Fixation in Tropical Cropping Systems, CABI Publishing, Wallingford, 2001.
8. Jackson ML. Soil chemical analysis. Prentice-Hall of India Pvt. Ltd. New Delhi, India, 1973.
9. Kanimozhi K, Panneerselvam. Studies on isolation and nitrogen fixation ability of *Azospirillum* spp. Isolated from Thanjavur district. *Der Chemica Sinica*, 2010; 1(3):138-145
10. Katkar RN, Turkhede AB, Wankhade ST. Residual effect of organic manures and inorganic fertilizers on soil properties and seed cotton yield. PKV Res. J. 2006; 30:36-41.
11. Kirda C, Danso SKA, Zapata F. Temporal water stress effects on nodulation, nitrogen accumulation and growth of soybean. *Plant Soil.* 1989; 120:49-55.
12. Sadowsky MJ, Rostas K, Sista PR, Bussey H, Verma DPS. Symbiotically defective histidine auxotrophs of *Bradyrhizobium japonicum*. *Arch. Microbiol*, 1986; 144:33-339.
13. Sahlaman K, Fahraeus G. Microscope observation on the effect of Indole 3- acetic acid upon root hairs of *Trifolium repense*. *Kungli Lantbrukshogskolans annaler.* 1962; 28:61-268.
14. Singh SA, Ramakrishnan M, Rao AGA. Optimization of downstream processing parameters for the recovery of pectinase from the fermented broth *Aspergillus carbonarius*. *Process Biochem.* 1999; 35:411-417.
15. Singleton PW, Bohlool BB, Nakao PL. Legume response to rhizobial inoculation in the tropics: myths and realities. In: Lal, R., Sanchez, P.A. (Eds.), *Myths and science of soils of the Tropics. Soil Science Society of America and American Society of Agronomy*, Madison, 1992, 135-155.
16. Subbiah BV, Asija CL. A rapid procedure for estimation of available nitrogen in soil. *Curr. Sci.* 1956; 15:259-268.
17. Turco RF, Sadowsky MJ. The microflora of micromediation, In: *Bioremediation: Science and applications, soil science special publication No.43* Eds. Skipper, H.D. and Turco, R.F. Madison, Wisconsin: Soil Science Society of America. 1995, 87-162.
18. Walkely A, Black IA. Chronic and titration method for determination of soil organic matter. *Soil Sci.* 1947; 63:251.
19. Watanabe I, Olsen SR. Test of an ascorbic acid method

for determining P in H<sub>2</sub>O and NaHCO<sub>3</sub> extracts from the soil. *Soil Sci. Soc. Am. Proc.* 1965; 29:2667-283.

20. Zafar M, Abbasi MA, Khan MA, Khaliq A, Sultan T, Aslam M. Effect of plant growth promoting rhizobacteria on growth, nodulation and nutrient accumulation of lentil under controlled conditions. *Pedosphere* 2012; 22(6):848-859.