STUDIES ON THE NODULATION PATTERN OF BLACK GRAM IN RELATION TO IT'S PHYSICO – CHEMICAL PROPERTIES OF SOIL SAMPLES COLLECTED FROM CUDDALORE AND NAGAI DISTRICT IN TAMIL NADU

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ABSTRACT

Soil deficiencies of essential elements can dramatically decrease legume nodulation, nitrogen fixation, nitrogen accumulation and yield. Eighteen different locations were selected in Cuddalore and Nagai district for the nodulation pattern of Black gram in rainfed areas. The three locations namely Pandaravadai, Vallampadugai and Kokkur supported good nodulation and also supported fairly in high native rhizobial populations of $112x10^6$, $98 x10^6$ and $91x10^6$ cells g^{-1} moisture free soil respectively. The cell count was minimum in the soil sample of Pinnathur ($2 X10^6$ cells g^{-1} moisture free soil). The present study clearly established the existence of a parallelism between the native soil population of rhizobia and natural nodulation. All these eighteen locations viz., Were analysed for the pH, EC nitrogen content, potassium content and phosphorus content. These data were used to correlate the natural population pattern and native rhizobial populations of these locations Pandaravadai having the soil pH - 7.1, EC - 0.60 and NPK 150,30,412.5 kg/ha recorded high nodule number and native rhizobial population the properties of this soil seems to be very congenital for the multiplication's of rhizobia and for the nodulation and nitrogen fixation.

Keywords: *Rhizobium*, Nitrogen, Phosphorus and potassium.

I. INTRODUCTION

All the living organisms on the earth require nitrogen for their life activities but only a Limited number of organisms have the ability to utilize elemental nitrogen. Among them, the *rhizobium* is a symbiotic nitrogen fixer in association with leguminous plants. Soil factors such as salinity temperature, soil moisture are known to influence the multiplication of *rhizobia* in those regions. Soil deficiencies of essential elements can dramatically decrease legume nodulation, nitrogen fixation, nitrogen accumulation and yield. Most of the biological nitrogen fixation is attributed to symbiotic nitrogen fixing system. For the successful exploitation of this system through understanding *legume Rhizobia* symbiosis is highly essential (Madigan et al., 2000). The present trend is aimed to focus on the effective utilization of this phenomenon.

II. MATERIALS AND METHODS

The natural nodulation of Blackgram *Vigna mungo* (Lam. Verde.) varieties and the native rhizobial population of Blackgram *Rhizobium* were studied in different locations of Cuddalore and Nagai districts of Tamil Nadu under rainfed conditions viz., C.Mutlur, Mudasalodai, Thillaividangal, Singarakuppam, Vallampadugai, Pinnathur, Samiyarpettai, Puthuchathiram and AnnamalaiNagar in Cuddalore district, Kuthalam, Tholuthalangudi, Senniyanalore, Thiruvalangadu, Thiruvaduthurai, Pandaravadai, Palayakoodalore, Kokkur and Palaiyur in Nagai district. Five Blackgram plants were collected from each locations at random without damaging the roots. The average number of the nodules of five plants were taken to assess the nodulation of the different locations. The total number of nodules as well as the number of pink and white nodules were counted and recorded separately.

2.1 Occurrence of native rhizobia in different soil samples (MPN) (Thornton, 1983)

Blackgram seeds were surface sterilized with 0.1% mercuric chloride and washed in sterile water for several times. The sterilized seeds were sown on nitrogen free plant nutrient agar plants in test tubes by 4 x 20 cm capacity. On germination, they were inoculated with suspensions (10^{-1} , 10^{-2} ,...10^{-10}) of the soil samples. At regular intervals the moisture contents of the tubes was, checked. The plants were examined and observed for extent of nodulation. The tubes with nodules were recorded as positive tubes and those without nodules as negative tubes - based on this the most probable number (MPN) of Bradyrhizobium was calculated from the tables.

2.2 Determination of soil properties of Blackgram

2.2.1 Soil pH (Jackson, 1956)

Ten gram of soil samples was taken in a beaker, 2.5 ml of water was added and stirred at regular intervals for 20-30 minutes. The pH meter was switched on and allowed for five minutes to warm up. Zero control was adjusted so as to bring the indicator to zero. By using a standard buffer solution the required pH range (0-7 or 7- 14) was made. The temperature dial was adjusted to the temperature of test solution. Control was adjusted to zero. After rinsing and wiping of the electrodes they were dipped into the test soil solution. By adjusting the range switch to pH, the indicator reading was noted and pH value recorded.

2.2.2 Organic matter (Walkey and Black, 1934)

0.5 g of finely ground soil sample was transfered to a 500 ml conical flask. To this 10 ml of the 1N Potassium dichromate prepared by dissolving 49.04 g of pure crystals of Potassium dichromate in one liter of water with the help of pipette. 20ml of concentrated H_2SO_4 was added and the contents of the flask shaken for a minute and set on an asbestos pad for exactly half an hour. At the end of the period, 200 ml of distilled water, 10ml of phosphoric acid, and one ml of the diphenlamine indicator (prepared by dissolving 0.5 g of diphenlamine in a mixture of 100ml of conc. H_2SO_4 and 20 ml of water.

2.2.3 Total nitrogen (Kenny and Bremner, 1960)

It was estimated by converting the combined nitrogen in soil organic matter to ammoniacal form with concentrated sulphuric acid. 100 mg samples were transferred into 50ml pyrex microkjeldhal flask. A quarter teaspoonful of digestion mixture of (10 parts of reagent grade potassium sulphate, 1 part of cupric sulphate and 0.1 part of selenium metal powder) and one ml of salicylic sulphuric acid with a pinch of sodium thiosulphate

were introduced and the contents were slowly heated till frothing ceased and then heated strongly. Completion of digestion was indicated by solution turning bluish green. After cooling about 15 ml of distilled water was added to flask and cooled, the contents were transferred into the distillation unit and 25 ml of the 40 per cent sodium hydroxide added and steam distilled into an excess of 0.1 N sulfuric acid (10ml) Containing 2 drops of methyl red indicator. Distillation was continued for 10 minutes. The contents were back titrated using. 0.1 N potassium hydroxide till the appearance of golden yellow color. Nitrogen in the sample was calculated using the factor 1ml of 0.1 N sulfuric acid = 0.0014g of nitrogen.

2.2.4 Available phosphorus (Olsen *et al*., 1954)

Five gram of soil was taken in a conical flask and one teaspoonful of charcoal powder, 100 ml of sodium bicarbonate solution 0.5 M (prepared by dissolving 42 g of$NaHCO_3$ in 1000 ml distilled water and pH is adjusted to 8.5 with 10.20 per cent NaOH solution) was added to the soil and shaken for half an hour. This was filtered through Whatmann No.40 and 5 ml of filtrate was pipetted out into a 25 ml volumetric flasks and 5 ml of molybdate reagent (15 g of ammonium molybdate in 400 ml of distilled water, filtered and 400 ml of conc. Hcl was added and made upto one litre) Was added, and 1 ml of dilute solution of stannous chloride was added and made upto 25 ml and the phosphorous was determined volumetrically.

2.2.5 Electrical conductivity (Jackson, 1973)

Conductivity cell was immersed into the soil water extract (prepared by adding 20 g of soil to 100ml of distilled water,) the contents shaken, were kept undisturbed and the supernatant liquid filtered through the filter paper. The filtrate kept in a beaker and knob was rotated and dark segment to magic eye was observed till the maximum deflection of the dark segment is magic eye is obtained to get the null point. Dial reading was noted and the range of 'multiply' knob. Electrical conductance was multiplied by cell constant, temperature and correction factor to obtain the specific conductance in dsm^{-1}.

III. RESULT AND DISCUSSION

3.1 Survey on the nodulation pattern of Blackgram in different locations of Cuddalore and Nagai districts

Eighteen different locations were selected in Cuddalore and Nagai districts for the survey of nodulation pattern of Blackgram *Vigna mungo* (Lam verde.) in rainfed areas in such a way that each and every sector of districts is represented in the survey.

The eighteen locations were studied for the nodulation of C.Mutlur, Mudasalodai, Thillai vidangal, Singara kuppam, Vallampadugai, Pinnathur, Samiyarpettai, Puthuchathiram, AnnamalaiNagar, Kuthalam, Tholuthalangudi, Senniyanalore, Thiruvalangadu, Thiruvaduthurai, Pandaravadai, Palayakoodalore, Kokkur, Palaiyur. The observation made on the natural nodulation are given in Table 1.

Table 1: Survey on the nodulation pattern of Black gram in Cuddalore and Nagai district

		No of nodules/plant

S.No	location	No.of Pink nodules	No.of White nodules	Total no.of nodules
1	C. Mutlur	6.4	8.0	14.4
2	Mudasalodai	6.2	6.0	12.2
3	Thillaividangal	6.3	6.9	13.2
4	Singarakuppam	6.5	8.4	14.9
5	Vallampadugai	7.3	11.4	18.7
6	Pinnathur	5.1	4.9	10.0
7	Samiyarpettai	5.2	4.9	10.1
8	Puthuchathiram	6.3	7.6	13.9
9	Annamalainagar	5.8	6.0	11.8
10	Kuthalam	6.9	9.1	16.0
11	Tholuthalangudi	6.7	9.2	15.9
12	Senniyanalore	5.4	4.8	10.2
13	Thiruvalangadu	6.2	6.5	12.7
14	Thiruvaduthurai	6.0	5.9	11.9
15	Pandaravadai	8.4	12.0	20.4
16	Palayakoodalore	6.5	8.9	15.4
17	Kokkur	7.0	9.5	16.5
18	Palaiyur	5.9	5.6	11.5

The total number of nodules was maximum (20.4 nodules/plant) in the locations of Pandaravadai followed by Vallampadugai, Kokkur, Kuthalam, Tholuthalangudi, Palayakoodalore, Singarakuppam, C. Mutlur, Puthuchathiram, Thillaividangal, Thiruvalangadu, Mudasalodai, Thiruvaduthurai, Palaiyur, Annamalainagar, Senniyanalore, Samiyarpettai, Pinnathur. The lowest nodule number was recorded in Pinnathur (10nodules/plant).

3.2 Physico chemical properties of soil samples collected from different locations of Cuddalore and Nagai districts

The physico – chemical properties of soil samples were collected from different locations of Cuddalore and Nagai districts were analysed for pH, EC and NPK. The results were presented in the Table 2. Blackgram soil samples collected from different locations of these two districts viz., Cuddalore and Nagai belonged to 3 textural groups viz., Sandy clay, Sandy loam and Clay loam. Soil organic carbon contents were ranged from 0.20-0.88% , soil pH ranged from 7.10-8.34 and EC ranged from 0.50 to 0.80 dsm^{-1}.

Further soil analysis revealed that the available nitrogen content ranged from 95.17 to 350.00kg/ha and the phosphorous content ranged from 7.80 to 37.5kg/ha, while the potassium content ranged from 116.60 to 425.0 kg/ha. The nitrogen content was maximum (350.00 kg/ ha) in the soils of Palayakoodalore. The lowest nitrogen content was recorded in Pinnathur (95.17 kg/ha) . Phosphorus content was maximum in the soil of Thiruvaduthurai (37.5 kg/ha) potash content was maximum in soil of Kokkur (425.0 kg/ha). The soil Singarakuppam was having the least potassium content (116.60 kg/ha). Soil pH affects all biological, chemical

and physical soil properties (Brade and Weil 2002) and the growth of specific organisms, soil microbial biomass and microbial activity. Organic carbon reaches its optimum value when the soil pH is in neutral (Aciego *et al*., 2008).

Table 2: Physico – Chemical properties of the soil samples collected from different locations of Cuddalore and Nagai district.

S. No	Locations	Soil type	pH	Ec Dsm^{-1}	Organic carbon (%)	N	P	K
						Kg/ha	Kg/ha	Kg/ha
1	C. Mutlur	Sandy loam	7.59	1.38	0.79	110.00	14.89	119.00
2	Mudasalodai	Sandy loam	7.84	1.72	0.57	99.00	9.82	145.10
3	Thillaividangal	Sandy loam	7.74	0.69	0.47	113.85	12.00	128.14
4	Singarakuppam	Sandy loam	7.56	0.66	0.52	115.66	11.59	116.60
5	Vallampadugai	Sandy loam	7.27	0.9	0.88	138.80	9.00	199.00
6	Pinnathur	Clay loam	8.34	0.80	0.59	95.17	18.00	118.00
7	Samiyarpettai	Sandy clay loam	8.23	0.55	0.64	105.00	7.80	175.40
8	Puthuchathiram	Sandy loam	7.72	0.66	0.58	100.50	16.33	152.75
9	Annamalainagar	Clay loam	7.89	0.80	0.49	113.35	17.89	127.00
10	Kuthalam	Sandy loam	7.45	0.70	0.24	162.5	27.5	355.00
11	Tholuthalangudi	Sandy loam	7.50	0.60	0.20	202.5	20.0	325.00
12	Senniyanalore	Sandy loam	8.0	0.70	0.24	187.5	12.5	412.50
13	Thiruvalangadu	Sandy loam	7.75	0.70	0.22	230.0	17.5	350.00
14	Thiruvaduthurai	Sandy loam	7.84	0.70	0.26	125.0	37.5	350.00
15	Pandaravadai	Sandy loam	7.10	0.60	0.88	150.0	30.0	412.50
16	Palayakoodalore	Sandy loam	7.52	0.65	0.23	350.0	27.5	387.50
17	Kokkur	Sandy loam	7.40	0.70	0.50	300.0	17.5	425.0
18	Palaiyur	Sandy	7.85	0.65	0.21	262.5	35.0	362.5

		loam						

3.3 Native Rhizobium population in the Blackgram soils

Native rhizobial population of the different soil samples was estimated by the most propable number (MPN) method the soil collected at Pandaravadai possessed the maximum ($112x10^6$ cell/g) Number of cells followed by the soil sample collected from Vallampadugai, Kokkur, Kuthalam, Tholuthalangudi, Palayakoodalore, Singarakuppam, C. Mutlur, Puthuchathiram, Thillaividangal, Thiruvalangadu, Mudasalodai, Thiruvaduthurai, Palaiyur, Annamalainagar, Senniyanalore, Samiyarpettai, Pinnathur. The native rhizobial population was high, ranging from 70 to 98×10^6 cell/g in the soil collected from Vallampadugai, Kokkur, Kuthalam, Tholuthalangudi. Average ranging from 40-$68x10^6$ cell/g from Palayakoodalore, Singarakuppam, C. Mutlur, Puthuchathiram, Thillaividangal (Table 3). Soil microbial populations and a high microbial diversity commonly detected in the legume rhizosphere (Lupwayi and Kennedy 2007).

Table 3 : Native population of rhizobium in Blackgram fields of twenty four different locations of Cuddalore and Nagai district.

S.No	Location	Population $1x10^6$/g of moisture free soil
1	C. Mutlur	52.00
2	Mudasalodai	35.00
3	Thillaividangal	41.00
4	Singarakuppam	54.00
5	vallampadugai	98.00
6	Pinnathur	2.00
7	Samiyarpettai	3.00
8	Puthuchathiram	46.00
9	Annamalainagar	16.00
10	Kuthalam	87.00
11	Tholuthalangudi	70.00
12	Senniyanalore	7.00
13	Thiruvalangadu	38.00
14	Thiruvaduthurai	28.00
15	Pandaravadai	112.00
16	Palayakoodalore	68.00
17	Kokkur	91.00
18	Palaiyur	26.00

SUMMARY

In the present study the soil collected at Pandaravadai, recorded the maximum nodule number and showed the higher native rhizoidal population of 112 x 10^6 cells/g moisture free soil. The properties of soil samples collected from eighteen locations were analysed. The soil with poor natural nodulation had a high pH, high available nitrogen content, high electrical conductivity and low phosphorus and potassium contents. These factors are considered as main reason for the inadequous population of rhizobia and poor nodulation in these soils. The soil with pH range near phosphorus and potassium content favoured the natural nodulation and also the native rhizobial population. The native Bradyrhizobial population in the soils of eighteen locations varied from $2.0x10^6$ - $112x10^6$cells/g. Strikingly, very low native rhizobial population($2.0x10^6$/g) moisture free soil and natural nodulation (10.00 nodules plant^{-1}) was observed in Pinnathur soil which recorded the pH 8.34 and 0.80 EC.

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