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RESEARCH ARTICLE

NODULATION AND OCCURRENCE OF BLACKGRAM RHIZOBIA IN RELATIUN TO SOIL TYPES AND ITS NUTRIENT STATUS

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ABSTRACT

Soil deficiencies of essential elements can dramatically decrease legume nodulation, nitrogen fixation, nitrogen accumulation and yield. Twenty four different locations were selected in Cuddalore and Nagappattinam district for the nodulation patter of black gram in rainfed areas. The other three location namely C.Mutlur, Pinnathur and Nagapattinam that supported good nodulation and also supported fairly in high native rhizobial populations of 77×10^6 , 85×10^6 , and 60×10^6 , cells g^{-1} of moisture free soil respectively. The cell count was minimum in the soil sample of Thirukkuvalai (10×10^6 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 twenty four locations viz., Pichavaram, Killai, C. Mutlur, Mudasal odai, Thillai vidangal, Singarakuppam, Vallam padugai, Pinathur, Kodyampalayam, Parangipettai, Puthuchathiram, Petiyapattu, Pandaravadi, Thiruvadudurai, Sirkali, Sennialore, Thirukkuvalai, Nagapattinam, Thiruvalangadu, Vedaranyam, Kuthalam, Tharangapadi, Palaiyur, Palayakoodalore. 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 the soil pH 7.52, EC 0.42 and NPK 48.82, 21.20, 210.84 kg/ha^{-1} 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. When the pH, EC and nitrogen content were high and potassium and phosphorus were low, native rhizobial population and natural nodulation were less.

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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. In agriculture soil environment is most important among the many factors which influences the multiplication of *rhizobia* in the spermosphere and rhizosphere regions. Soil factors such as salinity temperature, soil moisture are known to influence the multiplication of *rhizohia* in those regions. Though the legume *rhizobial symbiosis* is highly productive under optimal growing conditions. 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 - Rhizohia* symbiosis is highly essential (Madigan *et al.*, 2000). The present trend is on the effective utilization of this phenomenon.

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 location Cuddalore and Nagapattiam districts of Tamil Nadu. Under rainfed condition. Pichavaram, Killai, C. Mutlur, Mudasal odai, Thillai vidangal, Singarakuppam, Vallam padugai, Pinathur, Kodyampalayam, Parangipettai Puthuchathiram, and Periyapattu in cuddalore district Pandaravadi, Thiruvadudurai, Sirkali, Sennialore, Thirukkuvalai, Nagapattinam, Thiruvalangadu, Vedaranyam, Kuthalam, Tharangapadi, Palaiyur, and Palayakoodalore in Nagapattinam district.

Five blackgram plants were collected from each location 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.

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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.

Determination of soil properties of Blackgram soils Soil pH

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.

Organic matter (Walkey and Black, 1934)

0.5 g of finely ground soil sample was transferred 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 diphenamine indicator (prepared by dissolving 0.5 g of diphenamine in a mixture of 100ml of conc. H_2SO_4 and 20ml of water.

Total nitrogen (Kenny and Bremner, 1962)

It was estimated by converting the combined nitrogen in soil organic matter to ammoniacal form with concentrated sulphuric acid. Hundred mg samples were transferred into 50 ml pyrex microkheldahl 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.

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.

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, 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 $ds\ m^{-1}$.

RESULTS

Survey on the nodulation pattern of black gram in different location of Cuddalore and Nagappattinam districts

1. Twenty four different location were selected in Cuddalore and Nagappattinam 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.
2. The twenty four locations studied for the nodulation were Pichavaram, Killai, C. Mutlur, Mudasal odai, Thillai vidangal, Singarakuppam, Vallam padugai, Pinathur, Kodyampalayam, Parangipettai, Puthuchathiram, Petiyapattu, Pandaravadi, Thiruvadudurai, Sirkali, Senniyalore, Thirukkuvalai, Nagappattinam, Thiruvalangadu, Vedaranyam, Kuthalam, Tharangapadi, Palaiyur, Palayakoodalore. The observation made on the natural nodulation are given in Table 1

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

1. The twenty four soil samples collected from different locations of Cuddalore and Nagappattinam districts were analysed pH, EC and NPK Mudasal odai, Thirukkuvalai and Nagappattinam soils recorded pH of 8.10, 8.60 and 8.3 and EC value of 0.45, 0.89, 0.79 respectively. All other

soils recorded pH values ranging from 7.20, 8.60 most of the soil samples had EC from 0.40-0.89.

Table 1. Survey on the nodulation pattern of blackgram in Cuddalore and Nagapattinam district

S.No	Location	No of Modules plant -I		
		No. of Pink	No. of white	Total No. of modules
1.	Pichavaram	6.9	7.3	14.2
2.	Killai	9.2	12.4	21.6
3.	C. Mutlur	6.0	10.4	16.4
4.	Mudasal odai	5.4	6.8	12.2
5.	Thillai vidangal	5.2	6.4	11.6
6.	Singarakuppam	4.3	6.1	10.4
7.	Vallam padugai	4.0	6.2	10.2
8.	Pinathur	8.3	12.1	20.4
9.	Kodiyampalayam	7.9	6.1	14.0
10.	Parangipettai	4.4	5.8	10.2
11.	Puthuchathiram	4.6	5.3	9.9
12.	Periyapattu	5.2	6.6	11.8
13.	Pandaravadi	5.3	6.9	12.2
14.	Thiruvadudurai	6.9	6.0	12.2
15.	Sirkali	6.2	8.8	15.0
16.	Sennialore	4.1	7.9	12.0
17.	Thirukkuvalai	6.3	6.9	13.2
18.	Nagapattinam	6.3	10.0	16.2
19.	Thiruvalangadu	5.4	6.2	11.6
20.	Vedaranyam	6.4	6.2	12.6
21.	Kuthalam	4.6	5.8	10.4
22.	Tharangapadi	4.0	7.8	11.8
23.	Palaiyur	6.8	7.4	14.2
24.	Palayakoodalore	6.4	5.8	12.2

2. The nitrogen content was maximum ($120.88 \text{ kg/ha}^{-1}$) in the soils of Parangipettai followed by Singarakuppam, Thillai vidangan, Thirukkuvalai, Mudasal odai, Senniyaore, Puthuchathriam, Kodiyampalayam, Vedaranyam, Periyapattu, Pandaravadi, Thiruvaduthurai, Thiruvalangadu, Palaiyur, Pichavaram, Sirkali, Nagapattinam, Tharangampadi, Palaiyur and C.Mutlur lowest nitrogen content was recorded in Killai 48.82 kg/ha^{-1} . Phosphorous content was maximum in the soil of Killai (21.20 kg/ha^{-1}) potash content was maximum in soil of Killai ($210.84 \text{ kg ha}^{-1}$) The soil Thirukkuvalai was having the least potassium content (110.5 kg ha^{-1}) the results are given in Table 2.

Native rhizobium population in the blackgram field soil in the twenty four locations

Native rhizobial population of the different soil samples was estimated by the most propable number (MPN) method the soil collected at killai possessed the maximum ($11 \times 10^6 \text{ cell g}^{-1}$) Number of cells followed by the soil sample collected from Pinnathur, C.Mutlur, Nagapattinam, Sirkali, Pichavaram, Puthuchathiram, Palaiyur, Parangipettai, Thiruvalankadu, Vedaranyam, Kuthalam, Thiruvaduthurai, Mudasalodai, Vallampadugai, Palayam koodalore, Thillaividangan, Tharangampadi, Kodiyampallam, Singarakuppam, Periyapattu, Sennialore and Thirukkuvalai.

The native rhizobial population was high, ranging from 60 to $85 \times 10^6 \text{ cell g}^{-1}$ in the soil collected from Killai, Pinnathur, C.Mutlur, Nagapattinam.

Table 2. Physio-chemical properties of the soil samples collected from different locations of Cuddalore and Nagapattinam district

S.No	Location	PH	Ec dsm^{-1}	N	P	K
1.	Pichavaram	7.68	.59	68.22	18.24	18.39
2.	Killai	7.52	0.42	48.82	21.20	210.84
3.	C. Mutlur	7.40	0.41	58.47	19.33	135.84
4.	Mudasal odai	8.10	0.45	105.61	14.84	145.18
5.	Thillai vidangal	7.74	0.69	113.85	12.00	128.14
6.	Singarakuppam	7.56	0.66	115.60	11.59	116.60
7.	Vallam padugai	7.50	0.58	95.84	14.00	139.25
8.	Pinathur	7.82	0.62	50.56	20.50	202.15
9.	Kodiyampalayam	7.50	0.58	83.36	17.00	179.50
10.	Parangipettai	8.50	0.70	120.88	10.82	112.51
11.	Puthuchathiram	7.72	0.66	100.50	16.33	152.75
12.	Periyapattu	7.60	0.59	86.82	16.44	166.53
13.	Pandaravadi	7.40	0.48	98.64	15.32	178.25
14.	Thiruvadudurai	7.84	0.65	75.40	17.84	188.12
15.	Sirkali	7.53	0.59	65.37	18.39	176.53
16.	Sennialore	7.20	0.55	108.77	14.32	182.42
17.	Thirukkuvalai	8.6	0.89	112.18	10.00	110.11
18.	Nagapattinam	8.3	0.69	62.99	13.72	122.8
19.	Thiruvalangadu	7.30	0.52	75.32	15.54	128.30
20.	Vedaranyam	7.91	0.69	82.14	15.20	119.33
21.	Kuthalam	7.43	0.53	56.18	13.86	150.25
22.	Tharangapadi	7.71	0.62	62.76	12.72	140.39
23.	Palaiyur	7.25	0.50	76.42	16.25	132.42
24.	Palayakoodalore	7.31	0.52	69.39	14.33	129.12

Average ranging from 20 to $58 \times 10^6 \text{ cell g}^{-1}$ in soil collected from Sirkali, Palaiyur, Parangipettai were recorded the results are given Table 3.

Table 3. Native population of rhizobium in blackgram fields of twenty four different locations of Cuddalore and Nagapattinam district

S.No	Location	Population $1 \times 10^6/\text{g}$ of Moisture free soil
1.	Pichavaram	42.00
2.	Killai	114.00
3.	C. Mutlur	77.00
4.	Mudasal odai	7.00
5.	Thillai vidangal	4.00
6.	Singarakuppam	2.00
7.	Vallam padugai	65.00
8.	Pinathur	85.00
9.	Kodiyampalayam	3.00
10.	Parangipettai	20.00
11.	Puthuchathiram	6.00
12.	Periyapattu	2.00
13.	Pandaravadi	8.00
14.	Thiruvadudurai	7.00
15.	Sirkali	58.00
16.	Sennialore	2.00
17.	Thirukkuvalai	1.00
18.	Nagapattinam	60.00
19.	Thiruvalangadu	12.00
20.	Vedaranyam	11.00
21.	Kuthalam	9.00
22.	Tharangapadi	4.00
23.	Palaiyur	38.00
24.	Palayakoodalore	5.00

DISCUSSION AND CONCLUSION

1. Twenty four *Bradishizobial* strains of blackgram (*Vigna mungo* (Lam. verde) were isolated from different locations of Cuddalore and Nagapattinam districts of Tamil Nadu to study the efficiency of *Bradizobium* on blackgram symbiosis. The locations selected were Pichavaram, Killai,

- C. Mutlur, Mudasal odai, Thillai vidangal, Singarakuppam, Vallam padugai, Pinathur, Kodyampalayam, Parangipettai, Puthuchathiram, Periyapattu, Pandaravadi, Thiruvadudurai, Sirkali, Senniyalore, Thirukkuvalai, Nagapattinam, Thiruvalangadu, Vedaranyam, Kuthalam, Tharangapadi, Palaiyur, Palayakoodalore. The extent of natural nodulation and the population of native rhizobia were examined in those twenty four locations.
2. The nodulation pattern in plants are relatively good at four locations namely Killai, C.mutlur, Pinnathur and Nagapattinam. The Average Number of nodules per plant was ranging from 16.2 to 21.6. The nodulation was fairly ranging from 11.6 to 15.0 plant⁻¹ at twenty location namely Pichavaram, Killai, C. Mutlur, Mudasal odai, Thillai vidangal, Singarakuppam, Vallam padugai, Pinathur, Kodyampalayam, Parangipettai, Puthuchathiram, Periyapattu, Pandaravadi, Thiruvadudurai, Sirkali, Senniyalore, Thirukkuvalai, Nagapattinam, Thiruvalangadu, Vedaranyam, Kuthalam, Tharangapadi, Palaiyur, Palayakoodalore. The nodulation was found to be poor recording 9.9 nodules per plant at Puthuchathiram.
 3. Attractively. In the present study the soil collected at killai, recorded the maximum nodule number and showed the higher native rhizoidal population of 114 x 10⁶ cell per g moisture free soil
 4. The properties of soil samples collected from twenty four 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 inadequate 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.

5. The native *Bradyrhizobial* population in the soils of twenty four locations varied from 1x10⁶.
6. Strikingly, very low native rhizobial population (1.0x10⁻⁶/g moisture free soil and natural nodulation (9.9 nodules plant⁻¹) was observed in Puthuchathiram soil which recorded the pH 8.60 and 0.89 EC.

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