Twenty four different locations were selected in Cuddalore and Nagapattinam Districts of Tamilnadu to study natural nodulation of blackgram plants. The nodulation was found to be good (16.2 to 21.6 plant$^{-1}$) in 4 locations, moderate (12.0 to 15.0 plant$^{-1}$) in 19 locations, the lowest nodule number of 9.9 plant$^{-1}$ was recorded in plants collected at Puthuchathiram.

The physico-chemical properties of blackgram soil samples collected from these 24 different locations were studied. Three textural types, namely Sandy clay, Sandy loam and clay loam were recognised. Soil organic carbon content ranged from 0.35 to 0.97 percent. The pH ranged from 7.2 to 8.60 and EC ranged from 0.40 to 0.89 ds m$^{-1}$.

Native rhizobial population of different soil samples estimated by Most Probable Number (MPN) method ranged from 1.00 to 114.00 x 10$^6$ g$^{-1}$.

Twenty four blackgram root nodule isolates obtained from blackgram soils were designated as BGR CN-1 to BGR CN-24 and screened for its efficiency based on IAA production, EPS production and effectiveness of symbiosis with blackgram. Indole acetic acid production in tryptophane supplemented yeast extract mannitol broth ranged from 1.89 to 6.31 µg ml$^{-1}$. The isolate BGR CN-6 produced maximum IAA
of 6.31. EPS production ranged from 16.35 to 361.50 µg ml\(^{-1}\). The isolate BGR CN-6 produced highest EPS of 360.50 µg ml\(^{-1}\).

- The inoculation effect of twenty four rhizobial isolates on the growth, nodulation, nodule ARA, nodule leghaemoglobin, and N content of blackgram plants were recorded on 15, 30, and 45 DAS. BGR CN-6 inoculated Blackgram plant recorded higher values of these parameters at all sampling periods. On 45\(^{th}\) day sampling the percent increase observed was 68.13 for root and shoot length, 68.31 for plant dry weight, 16.0 for nodules, 204.58 n moles C\(_2\)H\(_4\) formed h\(^{-1}\) g\(^{-1}\) nodule, 2.17 for nodule leghaemoglobin, 73.16 for plant nitrogen content in plants inoculated with BGR CN-6. The performance of the isolate BGR CN-10 on blackgram was closely followed.

- The phenolate type of siderophore production by the isolates ranged from 1.82 mg l\(^{-1}\) to 4.84 mg l\(^{-1}\). The isolate BGR CN - 6 produced maximum siderphore of 4.84mg l\(^{-1}\) which was followed by 4.64 mg l\(^{-1}\) with BGR CN – 10.

- The intrinsic antibiotic resistance (IAR) of the isolates was determined against five antibiotics. The isolates BGR CN-6 possessed antibiotic tolerance upto 200ppm for kanomycin and rifampicin, and 300ppm for streptomycin and pencillin. The isolates were highly sensitive to gentamycin at all concentrations tested. The isolates viz., BGR CN-6 and BGR CN-10 were found to grow upto
2.0 percent salt concentration, interestingly the strain BGR CN-6 tolerated up to 2.5 percent salt concentration.

- The two isolates of blackgram rhizobia viz., BGR CN-6, BGR CN-10 were selected for developing salt tolerant mutant using nitrosoguanidine (NTG) as mutagen. The effect of the chemical mutagen NTG on the surviving population and mutation frequency was studied by exposing them to varied concentrations. Mutation frequency of 48% for BGR CN-6 and 42% for BGR CN-10 was obtained at 150ppm. The LD$_{50}$ value was 175ppm for BGR CN-6 and 200ppm for BGR CN-6. The reversion frequency was found to be minimum for cells grown at 150ppm concentration. BGR CN-6M recorded 15.23 percent and BGR CN-10M recorded 11.5 percent.

- The drug resistant character of the halotolerant mutants of BGR CN-6M, BGR-10M and their wild parents BGR CN-6S and BGR CN-10S was studied in comparison with reference strain BGR-AU-1 was against 5 antibiotics.

- The mutant BGR CN-6M and BGR CN-6S possessed same level of antibiotic tolerance i.e., up to 200ppm for kanomycin and rifampicin and 300ppm for streptomycin. Interestingly, the level of tolerance to pencillin was 500ppm for BGR CN-6M and only 300ppm for BGR CN-6S. All the isolates were highly sensitive to gentamycin.

- The effect of different salts namely Na$_2$SO$_4$, NaCl, MgSO$_4$, MgCl$_2$, 

K₂SO₄ KCl and CaCl₂ each at concentrations of 1.0, 2.0 and 3.0 percent on the growth of halotolerant mutant rhizobial strains BGR CN-6M, BGR CN-10M and their wild parents BGR CN-6S and BGR CN-10S along with the reference strain BGR-AU-1 was studied. The halotolerant mutant BGR CN-6M showed growth upto 3.0 percent concentration with the salts tested. The isolates BGR CN-10M, BGR CN-6S BGR CN-10S and BGR-AU-1 showed growth only upto 2.0 percent.

- The efficiency of halotolerant mutants and their wild parents to produce indole acetic acid (IAA) and exopolysaccharides (EPS) in the medium containing NaCl upto 3.0 percent, level was studied. At 3.0 percent no detectable IAA, and EPS produced by the isolates tested except BGR CN-6M (mutant) which recorded 5.00 µg ml⁻¹ of IAA and 292.00 µg ml⁻¹ of EPS.

- The halotolerant mutant BGR CN-6M inoculated blackgram recorded highest number of 21 nodules plant⁻¹, ARA of 195 n moles C₂H₄ formed h⁻¹ g⁻¹ and nodule nitrogen content of 6.00 percent at 3.0 percent salt concentration.

- The number of cells adhered to the blackgram roots proportionately decreased with increase in NaCl concentrations from 0.0 to 3.0 percent. The mutant BGR CN-6M showed appreciable root adherence of 254 x 10⁴ g⁻¹ root dry weight at 3 percent concentration. On the other
hand, the BGR-AU-1 reference strain BGR CN-10M, BGR CN-6S, and BGR CN-10S were less efficient in root adherence.

- The chemotaxis of halotolerant mutant BGR CN-6M, BGR CN-10M, their wild parent BGR CN-6S, BGR CN-10S and the reference strain BGR-AU-1 towards ADT-3 blackgram root exudate was studied. The recombined anionic + cationic + neutral fractions of blackgram root exudate elicited higher chemotactic response towards the rhizobial strain than the crude root exudate as well as individual and recombined fractions of any two of them.

- Blackgram root exudate contained seven amino acids in cationic fractions and the relative occurrence of these amino acids was in the order of glutamic acid (11.00 µg) > alanine (8.40 µg) > aspartic acid (8.20 µg) > glycine (7.40 µg) > serine (5.20 µg) > valine (1.80 µg) > proline (1.40 µg). Five organic acids in the anionic fraction and the relative occurrence of these organic acids was in the order of citric acid (12.10 µg) > oxalic acid (10.40 µg) > malic acid (7.10 µg) > succinic acid (4.40 µg) > gluconic acid (3.40 µg), and five sugars in the neutral fraction and the relative occurrence of sugars was in the order of fructose (9.50 µg) > glucose (9.10 µg) > arabrinose (5.50 µg) > ribose (3.50 µg) > maltose (2.90 µg).

- The performance of halotolerant mutant BGR CN-6M was better than BGR CN-10M and their wild parents and the reference strain, BGR-
AU-1 in saline soil. The plant height of 60.35 cm, plant dry weight of 6.85 g plant\(^{-1}\), 36.34 nodules plant\(^{-1}\) nodule ARA of 252.00 n moles C\(_2\)H\(_4\) \(\mu\)g\(^{-1}\) of nodule nodule leghaemoglobin content of 1.347 mg g\(^{-1}\) and N content of 271.00 mg plant\(^{-1}\) was recorded in saline soil. The values of these parameters observed in the inoculated blackgram plants grown in normal soil although slightly higher it was found to be on par with values obtained in the saline soil.

- The ureide metabolism of BGR CN-6M inoculated blackgram was significantly more pronounced than other strains in saline soil. Allantoin content of 46.00 fig ml\(^{-1}\), xanthine oxidase of 14.50 \(\mu\)M of uric acid formed h\(^{-1}\) g\(^{-1}\) of nodule, Uricase of 100.00 uM of uric acid removed h\(^{-1}\) g\(^{-1}\) of nodule, allatoninase of 36.60 uM glyoxyla formed h\(^{-1}\) g\(^{-1}\) of tissue, allantoicase of 36.50 M glyoxalate removed hg\(^{-1}\) of tissue, urease of 25.00 mg NH\(_3\) g\(^{-1}\) tissue in saline soil was recorded. The values of these parameter observed in the normal soil grown blackgram although slightly by more it was statically to the on par with the values obtained in saline soil.

- Application of pesticides viz., furadan, endosulphan and monocrotophos, fungicides viz., captan, thiram and mancozeb; herbicides viz., alachlor, chloramben and nitrofen decreased the rhizobial population, nodulation and nodule ARA of halotolerant mutants, their wild parents and the reference strain inoculated ADT-3 blackgram plants. The decrease
observed with halotolerant mutant BGR CN-6M inoculated blackgram plants was 5.54 percent of rhizobial population, 5.00 percent of nodulation and 1.12 percent of nodule ARA due to monocrotophos and found to be less significant compared to control, 5.51 percent rhizobial population 6.29 percent in nodulation and 3.65 percent in nodule ARA due to thiram, and 5.37 percent of rhizobial population, 5.71 of percent nodulation, and 2.24 percent nodule ARA due to nitrofen. The pesticide monocrotophos, fungicide thiram and herbicide nitrofen showed more toxic effect than other agro chemicals tested.

- The inoculation of BGR CN-6M to blackgram in saline soil, significantly increased the growth parameters such as plant height and dry matter production and yield parameters such as number of branches per plant, no. of pods per plant number of clusters per plant, test weight and grain yield at all levels of N tested. The inoculation effect was more pronounced at 50% N level than at 75 % and 100% N levels. The values of growth parameters and yield parameters observed with 100% N alone and 50% N + BGR CN-6M mutant inoculation was found to be on par.

- The treatment with 100% N recorded plant height of 64.12 cm plant$^{-1}$, dry matter production of 71.28 g plant$^{-1}$ 4.97 branches plant$^{-1}$, 77.85 pods plant$^{-1}$ 22.96 clusters plant$^{-1}$, and test weight (1000 seed weight)
of 50.11 g and grain yield of about 14.83 q ha\(^{-1}\) and protein percentage of 22.98, however it was found to be on par with the values observed at BGR CN-6M + 50% N, which recorded plant height of 64.82 cm plant\(^{-1}\), dry matter production of 69.01 g plant\(^{-1}\), 3.92 branches plant\(^{-1}\) 60.72 pods plant\(^{-1}\), 21.43 clusters plant\(^{-1}\) and test weight (1000 seed weight) of 49.51 g and grain yield of 13.76 q ha\(^{-1}\) and protein percentage of 24.85. In normal soil, both the halotolerant mutant BGR CN-6M and the reference strain BGR-AU-1 showed equal performance, however in saline soil the performance of BGR CN-6M was better than that of reference strain.

Based on the result of the study it could be concluded that the salt tolerant mutant BGR CN-6M can be used as a potential biofertilizer for blackgram grown in salt affected tracts of Cuddalore and Nagapattinam districts of Tamil Nadu under rainfed condition, with moderate level of nitrogen i.e., 10 kg N ha\(^{-1}\) which recommended dose. The application of biofertilizer helps in reducing the fertilizer N by 50% (10 kg N ha\(^{-1}\)) and the input cost by nearly 20 per cent. Besides, the yield of blackgram significantly improved in saline soil.