ABSTRACT

Chemical fertilizers increase crop production but their overuse has already proven serious challenges to balance and sustain growth. Biological N2 Fixation and PGPR (Plant Growth Promoting Rhizobacteria) are potential alternative for harmful effects of inorganic fertilizers. The capability of rhizobia to fix nitrogen reduces considerably the use of chemical fertilizers in agriculture. Rhizobia are able to multiply in soil quickly surrounded by root portion under the impact of legume plant “rhizosphere” and form specialized structure called nodule inside which it reduces atmospheric nitrogen into its usable form to legume plants throughout fixation of nitrogen. Transposon mutagenesis is an easy and highly effective method for generating bacteria with improved characteristics and gene knockouts. Mung bean (Vigna radiata) is leguminous economically important crop and is the third most pulse crop of India cultivated an over 3.5 million hectare of land in 6 major mung cultivating states of India.

The present study involves a transposon mutagenesis in Rhizobium japonicum forming symbiotic association with mung bean. Suicide plasmid pK03 was introduced transposon Tn3 in Rhizobium japonicum via Escheriachia coli mediated conjugation and successfully generated 800 mutants with frequency of 3.7 x 10^6. These 800 mutants along with rifampicin resistant mutant and wild strain were screened for nodulation. Among these 800 mutants 80 were non nodulating, 508 mutants formed poor nodulation, 112 produce white coloured nodules and 100 mutants formed pink coloured nodules on root system of mung bean plant. The mutants which had more nodule dry and fresh weight, shoot fresh and dry weight were selected and subjected for the determination of nitrogenase activity. Out of 10
mutants 2 were promising with respect to nitrogen fixation. The mutant AVR040 showed nitrogenase activity 12.4µmol/hr/mg fresh weight of nodule which is more than wild 9.61µmol/hr/mg fresh weight of nodule, while AVR063 showed higher nitrogenase activity 19.4µmol/hr/mg fresh weight of nodule than AVR040. These two mutants AVR040 and AVR063 were further studied for Tn3 specific sequence amplification and DNA sequencing of amplified PCR product. BLAST alignment of generated sequences of strains compared with *Bradyrhizobium japonicum* indicates that Tn3 occurred in the genes Aldehyde Oxidase (AO) of mutant strain AVR040 and RND transporter of AVR 063. These two genes have special role in N₂ fixation and nodulation and transposition in these genes enhances N₂ fixation and nodulation of strain AVR 040 and AVR063.