ABSTRACT

The formation of nitrogen-fixing nodules involves complex molecular interactions between rhizobia and legume plants, particularly through the Nod Factor (NF) dependent pathway. Recent studies indicate that *Bradyrhizobium* utilizes a needle-like protein complex known as the Type III Secretion System (T3SS) to regulate nodule formation and host plant specificity. This study investigated the wild-type *Bradyrhizobium elkanii* USDA61, BErhcj T3SS mutant strain, and constructed Tn5 mutants as inoculants for *Lupinus pilosus*. Symbiosis analysis included assessments of nodulation phenotypes, plant biomass, nitrogen-fixing activity, and Tn5 insertion in mutant DNA. Six mutant samples were used in this research, namely Tn5-4, Tn5-5, Tn5-6, Tn5-7, Tn5-8 and Tn5-9. USDA61 served as the negative control, while the BErhcj mutant acted as the positive control. Our results showed that all Tn5 mutant strains exhibited distinct outcomes. Among six mutant samples, significant differences ($p < 0.05$) were found in total plant, nodule, and root fresh weight, with p-values of 0.004**, 0.01*, and 0.00003***, respectively. Stem weight did not show significant differences due to the early developmental stage of the nodules. Notably, the nodulation phenotype positively correlated with nitrogen-fixing activity ($R^2 > 0.5$). Tn5-4, Tn5-6, and Tn5-8 displayed larger, pink nodules and higher nitrogen-fixing activity, in contrast to Tn5-5, Tn5-7, and Tn5-9, which had small, white nodules and very low nitrogen-fixing activity. Furthermore, nitrogen-fixing activity influenced leaf color, indicating chlorophyll content; however, a weak correlation was observed between nitrogen-fixing rate and total plant fresh weight. Mutant strains capable of fixing nitrogen shared similar Tn5 insertion sequence sizes, whereas nodules with negative fixing activity exhibited different sizes.

Keywords: *Bradyrhizobium*, *Lupinus pilosus*, nitrogen-fixing activity, Tn5 insertion, nodulation phenotype