

STRESS TOLERANT RHIZOBIAL STRAINS INHABITING *Clitoria ternatea* L. IN ANURADHAPURA DISTRICT, SRI LANKA

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Biological Nitrogen Fixation (BNF) is a process in which atmospheric nitrogen gas is fixed by microorganisms such as rhizobia that live in nodules of the legume plant roots. *Clitoria ternatea* L. is a perennial herbaceous legume that grows naturally in a wide range of soil and gets successfully nodulated even under harsh climatic and soil conditions. This study was conducted to isolate rhizobia from *C. ternatea* grown in different locations of Anuradhapura district, Sri Lanka and to characterize their stress tolerance to identify highly tolerant strains. The root nodules from *C. ternatea* were collected from seven sites in the Anuradhapura District. Twenty-eight pure rhizobial colonies were isolated in ½ Lupin Agar medium. They were separately grown in ½ Lupin broths and subjected to four physiological conditions of pH (3 – 9), temperature (25 – 45 °C), salinity (NaCl concentrations of 0.1 – 3.0%) and drought [Polyethylene glycol-8000 (PEG) concentrations of 0.1 – 0.4%]. The rhizobial strains which showed high tolerance to extreme physiological conditions were subjected to a combination of physiological stress conditions such as pH (8.0), temperature (36 °C), NaCl (3.0%), and PEG (0.4%) to mimic the prevailing environmental conditions in the Anuradhapura District. DNA was extracted from these stress-tolerant rhizobial strains and subjected to PCR-based ERIC fingerprinting, and the genetic diversity was assessed. Of the 28 pure rhizobial colonies, 12 isolates showed high tolerance to at least two extreme physiological conditions. The optimum pH, salinity and temperature range for the growth for the isolates were found between 5 – 8, 0.1 – 1.0% and 30 – 35 °C, respectively. Almost all the isolates grew well in the 0.2% PEG concentration. AP-2 (Anuradhapura urban site) showed the highest tolerance at pH 3.0 and 9.0 and 3.0% NaCl concentration. The isolates KH-3 (Kahatagasdigiliya site) and MH-3 (Mihintale site) showed the highest survival at 45 °C and 0.4% PEG concentration, respectively. PG-1 (Palugaswewa site) showed the highest tolerance to combined physiological stress. The ERIC profile confirmed the high genetic diversity, and 11 clusters were found at 69.9% of the similarity level. These stress-tolerant strains could be used for further studies on cross inoculation of crop legumes as a solution for the high usage of chemical nitrogen fertilizers.

Keywords: BNF, *Clitoria ternatea*, ERIC fingerprinting