Diversity of Kura clover-nodulating rhizobia from the Lower Caucasus

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Kura clover (*Trifolium ambiguum* M.B.) is a perennial rhizomatous forage legume whose use is currently limited by establishment difficulties in part attributable to nodulation problems. Kura clover has long been reported to have very specific rhizobial requirements. It is not nodulated by rhizobia that nodulate other *Trifolium* species and its rhizobia do not effectively nodulate other *Trifolium* species. Specificity was also reported among various ecotypes and ploidy levels within *T. ambiguum* (i.e., diploid, tetraploid, and hexaploid). These observations are, however, based on a limited number of rhizobia most originating from only two regions in the Caucasus, namely the Krasnodar region in Russia and Northeastern Turkey. Soils outside the natural distribution range of Kura clover (i.e., the Caucasus and Eastern Europe) rarely contain indigenous rhizobia that can effectively nodulate this species. Therefore, when Kura clover is introduced in other regions, rhizobia must also be introduced. Only a limited number of Kura clover-nodulating rhizobial strains are currently available and many have a limited effectiveness.

In this study, 128 rhizobia were isolated from four sites in the center of origin of Kura clover (i.e., two in Azerbaijan, one in Armenia, and one in Northwest Iran) using the three ploidy levels of Kura clover, red clover (*T. pratense* L.), and white clover (*T. repens* L.) plants as trap hosts. Rhizobia were fingerprinted using Rep-PCR (BOXA1R primer) and their genetic diversity was measured using the Shannon-Weaver diversity index. The nodulation specificity and phenotypic diversity of a subset of 13 isolates was determined. Finally, the symbiotic effectiveness of three isolates when used as inoculants with Kura clover and white clover was evaluated in growth chamber. Isolates were compared to highly effective strains used as inoculants for Kura clover (CT1-2) and white clover (UMR6906).

Genetic diversity among the 128 isolates was large and similar for rhizobia grouped according to their geographic origin or original host plant. Isolates did not cluster according to their original host, with significant overlap observed between isolates from different ploidy levels or species. Phenotypic diversity was significant; percentage of similarity among 13 isolates ranging between 38 and 92%. Nodulation specificity of the Kura clover-nodulating rhizobial isolates studied was less complex and not as clearly delineated as previously reported. Some strains originally isolated from Kura clover could effectively nodulate more than one ploidy level of Kura clover and even one or both of two other *Trifolium* species (red clover and white clover). Three isolates (ARH2-1, ART1-2, and IRT10-1) formed effective nodules on both Kura clover and white clover, however none promoted plant growth of both species to levels currently obtained with commercial inoculants when evaluated in growth chamber. Rhizobial isolates that are highly effective with both species remain yet to be identified.