Genetic Diversity and Identification of Common Bean (*Phaseolus vulgaris* L.) Isolated from Soils of Iran

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Abstract:

Nitrogen availability is often a problem for production of common bean, particularly in developing countries where nitrogen fertilizers are either unavailable or unaffordable. Beans can nodulate with various fast-growing *Rhizobium* sp. Despite their unusual ability to enter into intimate association with nitrogen-fixing symbionts, productivity of *Phaseolus vulgaris* is often limited by nitrogen deficiency under agronomic conditions.

To date, five species of *Rhizobium*, *R. leguminosarum* bv. *phaseoli*(Jordan, 1984), *R. tropici* (Martinez-Romero *et al*., 1991), *R. etli* bv. *phaseoli*(Segovia *et al*., 1993), *R. gallicum* and *R. giardinii*(Amarger et al., 1997) have been recognized as microsymbionts of *P. vulgaris*. Characterization and identification of *Rhizobia* nodulating beans is a critical factor influencing inoculation strategies in the field. Limited understanding or incorrect identifying of *Rhizobia* could leading to misrecommendations of inoculants. This could results to poor nodulation of bean plants and lowers seed yield. However, the diversity of rhizobia nodulating common bean in Iran has not been studied yet. Strains collected from different geographical regions may offer the chance to select high effective rhizobia for inoculants.

Material and Methods

Fifty-three isolates of rhizobia were sampled from root nodules of common bean cultivated in 12 different provinces in Iran. The rhizobial isolates were obtaind by routine methods, purified and were maintained on slopes of YMA at 4°C and each isolate was given a Rb prefix and a number for identification. Eight rhizobium type strains were used as references in this study.

Total DNA of isolates was extracted and the concentration of DNA was determined by comparison with known concentrations of λ DNA in 1.5% agarose gel electrophoresis.

The genetic diversity of the isolates was analyzed by PCR-RFLP of the 16S-23S rDNA internally transcribed spacer (ITS) region. Different patterns created after digestion with restriction enzymes HaeII and MspI were analysed by Bionumerics ver. 5.10 and dendrograms showing diversity of the isolates created by this software.

For identification of isolates, 16S rDNA gene of the isolates was amplified by PCR using primers fD1 and rD1. PCR-RFLP was carried out on 16S rDNA products and some representative isolates were sequenced and the sequences were use to blast with known reference rhizobial species.

Isolates recognized as rhizobium were further studied by PCR-RFLP of recA, nodC and nifH genes. Different parrerns were observed following restriction with enzymes and Bionumerics was similarly used for analysis of the results.

Results

After digestion with restriction enzymes, 43 ITS-RFLP patterns were identified among 53 isolates and 8 reference strains. A dendrogram was constructed based on the UPGMA algorithm by analysing the similarity between different RFLP patterns (Fig. 1). Results showed that all isolates could be clustered into 9 groups at a similarity of 50%. There were significant diversity between the isolates regarding their ITS-RFLP analysis.

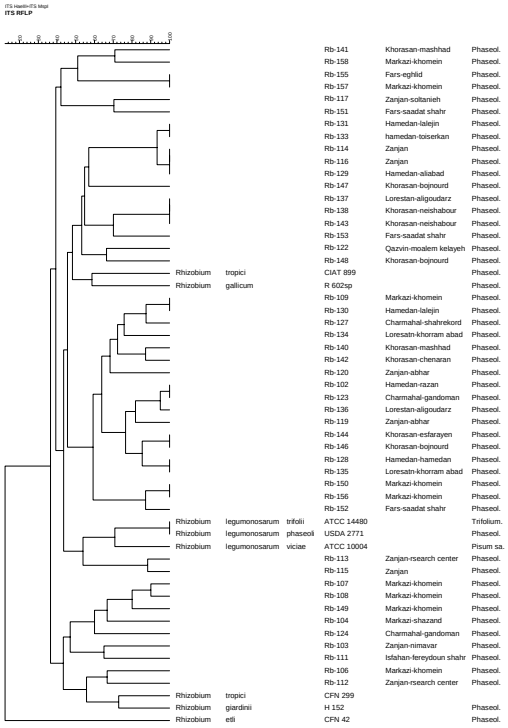

Fig.1 Dendrogram based on the UPGMA cluster analysis of Dice index of normalized RFLP patterns of 16S-23S ITS

Results of 16S rDNA RFLP and sequencing showed that from total of 53 isolates, 6 were *Pseudomonas fluorescens*, 29 were *Agrobacterium tumefaciens* and only 18 isolates were identified as *Rhizobium etli* and *R. leguminosarum* bv. viciae.

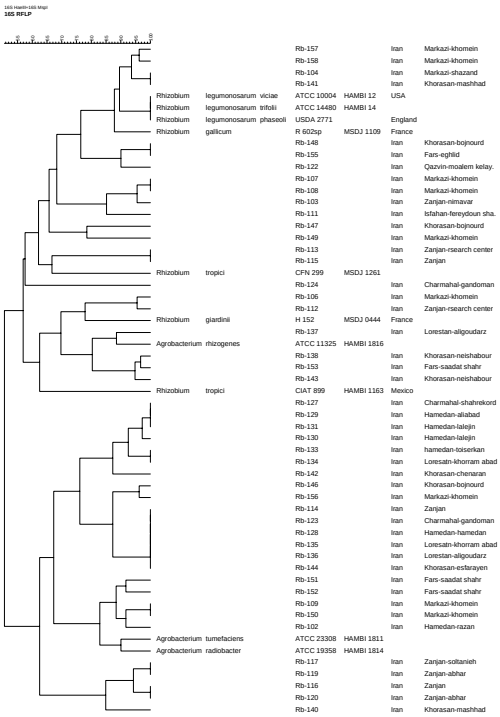

Fig.2 Dendrogram based on the UPGMA cluster analysis of Dice index of normalized RFLP patterns of 16S rDNA

RFLP of the nifH gene showed that all the isolates except Rb-113 and Rb-115 have similar nifH gene. The diversity of the isolates was greater regarding their nodC gene. The results of recA PCR-RFLP was similar to that of 16S rDNA.

This study showed that the rhizobia nodulating common bean in Iran belong to the *R.etli* and *R. leguminosarum* bv. viciae species.

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