Molecular and Isoenzymic Characterization of Indian bean (*Lablab purpureus* L.)

**ABSTRACT**

The present experiment was conducted at Department of Biotechnology, Junagadh Agricultural University, Junagadh with objectives to analyze molecular diversity of 20 Indian bean genotypes using various molecular markers (RAPD, ISSR, and SSR) and to examine the isoenzymes pattern of Indian bean genotypes by polyacrylemide gel electrophoresis.

The genomic DNA was isolated from ten to twelve days old seedlings of 20 Indian bean genotypes and their purity was in the range of 1.7 to 2.08. Thirteen RAPD primers generated total of 78 polymorphic bands/alleles in showing 100% polymorphism. The average band per primer was 6.00. The polymorphic information content (PIC) ranged from 0.58 (OPA-09 and OPA-17) to 0.89 (OPA-20). Similarly RAPD primer index (RPI) ranged from 1.00 to 10.56 with an average of 4.52 per primer. The highest RPI value was obtained by OPB-04 and the lowest was obtained by OPA-09. Jaccard’s coefficient of similarity of 20 Indian bean genotypes ranged from 10.0% (between GP-155 and GP-159) to 62.2% (between GP-157 and GP-161). The phylogenetic tree constructed by UPGMA method generated two main clusters which was again sub-grouped in their respective sub-clusters.

Ten ISSR primers produced 61 polymorphic bands/alleles showing 100% polymorphism with an average of 6.10 bands per primer. The polymorphic information content ranged between 0.49 (ISSR-888) and 0.88 (Oligo-03). Likewise, ISSR primer index (IPI) ranged from 1.96 to 8.8 with an average of 4.54 per primer. The maximum IPI value was obtained by Oligo-03 and the minimum was obtained by UBC-888. Jaccard’s coefficient of similarity between 20 Indian bean genotypes ranged from 3.80% (between GP-158 and GP-165) to 57.8% (between GP-153 and GP-154). The cluster analysis of ISSR revealed two main clusters, which was further divided into various sub-clusters.

Total 20 Indian bean genotypes were subjected to SSR analysis using 10 SSR primers. The polymorphic information content ranged between zero and 0.75. The highest PIC value of 0.75 was noticed in GATS911 AF483842, while lowest PIC value of zero was noticed in BM-142 and PVgaat001 with an average of 0.14 per primer. Likewise, SSR primer index (SPI) ranged from zero to 4.80 with an average of 1.71 per primer. The
maximum SPI value was obtained by AGB-8 and the minimum was obtained by BM-142 and PVgaat001. Jaccard’s coefficient of similarity between 20 Indian bean genotypes ranged from 57.1% (between GP-140 and GJIB-11) to 96.4% (between GJIB-2 and GJIB-11). The cluster analysis of ISSR revealed two main clusters, which was further divided into various sub-clusters.

The pooled study of molecular markers through RAPD, ISSR and SSR was done to confirm the differences and similarity among 20 Indian bean genotypes. Jaccard’s similarity coefficient and UPGMA method showed the highest (66.3%) similarity between GP-157 and GP-161 and the lowest (21.8%) similarity between GP-140 and GJIB-2. The cluster analysis on pooled basis revealed two main clusters, which was further divided into various sub-clusters.

Among the studied techniques, RAPD and ISSR primers gave 100% polymorphism. However, more number of polymorphic fragments, more PIC and higher percentage polymorphism per primer were amplified by RAPD and ISSR as compared to SSR markers. Both RAPD and ISSR markers gave distinct clustering patterns.

Isoenzymes were used for the characterization of 20 Indian bean genotypes. Peroxidase generated two polymorphic bands at 15 DAG with Rm value of 0.841 and 0.856. Four polymorphic bands of esterase isoenzyme were detected at 15 DAG with Rm value of 0.146, 0.205, 0.291, and 0.593. Two polymorphic bands were generated though Polyphenol oxidase isoenzyme at 15 DAG with Rm value of 0.261 and 0.535.

*Key words:* Indian bean, RAPD, ISSR, SSR, Isoenzymes.