CHAPTER - I

INTRODUCTION

Indian bean (*Lablab purpureus* L.; 2n = 2x = 22) is an important herbaceous annual plant and edible food legume in the world representing 50% of grain legumes for direct human consumption (McClean *et al.*, 2004) especially in Latin America and eastern and southern Africa. It is a New World crop with worldwide significance for human nutrition. Indian bean is seed propagated, true diploid (2n = 22) and have a relatively small genome (650 Mb) (Broughton *et al.*, 2003). Indian beans were domesticated in at least two major centers in Mesoamerica and the Andes (Gepts, 1988) and possibly in a third minor centre in the northern Andes (Islam *et al.*, 1997). The crop is consumed principally for its dry (mature) beans, shell beans (seeds at physiological maturity) and green pods. When consumed as seed, beans constitute an important source of dietary protein (22% of seed weight) that complements cereals. The leaf is occasionally used as leafy vegetable and straw is used for fodder as an animal feeds.

Botanically, the Indian bean is classified as a dicotyledonous. Beans are a legume and thus acquires their nitrogen through an association with rhizobia, a species of nitrogen-fixing bacteria. Total 30 million hectares of dry beans and 2.22 million hectares of green beans were produced worldwide during 2016-17, while 3.89 million tonnes of dry beans and 0.66 million tonnes of green beans were produced in India during 2016-17. In India, pulses are grown in about 23.1 million tonnes area with a production of 22.6 million tonnes and productivity of 755 kg/ha. (Anonymous, 2017).

Beans display a wide range of growth habits (Van and Pastor, 1987) from determinate bush types to indeterminate upright or vine bush types, to vigorous climbers. Bush types are the most widely grown, and are a relatively short season crop, maturing in as little as 60 days from seeding in a tropical climate and yielding from 700 to 2000 kg/ha on average. On the other hand, in smallholder agriculture where land is scarce, labour-intensive, high-yielding climbing beans enjoy continuing or even expanding popularity. Climbing beans can mature in 100 to 120 days at mid-elevations, but can delay as long as ten months at higher elevations and can produce the highest yields for the crop up to 5000 kg/ha. These features have significant implications for breeding programmes.

The Indian bean is highly nutritious source of starch, protein and dietary fiber and is an excellent source of iron, potassium, selenium, molybdenum, thiamine, vitamin B₆ and folic
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acid. Dried beans are important sources of protein in vegetarian diets, and in areas where animal protein is scarce or expensive. However, this protein is incomplete (does not contain all essential amino acids), so seeds (which provide the missing amino acids) must also be a significant part of the strictly vegetarian diet or, small amounts of dairy products, meat, poultry, or fish (which contain complete proteins) must be part of the diet. In the areas where Indian beans originated (Central America and Southern Mexico) corn supplied the missing amino acids, and squash was an additional source of vitamins.

*Lablab purpureus* seeds have carminative, aphrodisiac, anthelmintic, antispasmodic, astringent, febrifuge and stomachic properties. Flowers considered as semenagogue antivinous, alexiteric. n-Hexaneand choloform extracts Dolichos lablab (*Lablab purpureus*) exhibited significant antimicrobial and antifungal activity against *B. subtilis*, *S. aureus*, *P. aeruginosa*, *E. coli* and *C. albicans*. As natural coagulants (Bioremediation), *Moringa oleifera*, *Cicer arietinum*, and *Dolichos lablab* significantly improved the removal of turbidity and total coliforms from synthetic raw water. The natural coagulants reduced 89-96% of total coliforms (Sonal *et al.*, 2012).

Hyacinth bean (*Lablab Purpureus*) is also grown as forage for livestock and as ornamental plant. In addition, it is cited both as a medicinal plant and a poisonous plant. The fruit and beans are edible if boiled at 110°C. Otherwise, they are toxic due to presence of cyanogenic glycosides, glycosides that are converted into hydrogen cyanidewhen it consumed. Seed contain flavonoids kievitone and genistein, which play an important role in prevention and treatment of cancer. It has been shown that there is a wide range of cyanogenic potential among the varieties (Guretzki *et al.*, 2014).

The knowledge of genetic diversity in a crop species is fundamental to its improvement. However, morphological traits have many limitations, including low polymorphism, low heritability, late expression, and may be controlled by epistatic and pleiotropic gene effects (Cramer and Havey, 1999).

Molecular marker techniques are used to detect genetic diversity at the molecular level. Molecular data can be gathered via molecular techniques and are more abundant compared to morphological and biological data. Even morphologically diverse species can be compared because of genotypic data showed higher conservation as compared to morphological data which is not only related with genotypes but also affected by environments (Li, 1997 and Raven *et al.*, 1999).

Molecular investigations are essential for collection, conservation and its utilization in future breeding programmes. The knowledge of genetic diversity in a crop species is
fundamental to its improvement. The use of various biochemical methods like isoenzymatic pattern, molecular marker methods which are independent of environmental conditions such as RAPD, ISSR and SSR offer significant advantages for species identification in that they are rapid, relatively cheap and eliminate the need to grow plants up to maturity. DNA markers are mainly classified into three classes based on their detection: 1) Hybridization based DNA markers 2) PCR based DNA markers. 3) DNA sequence based DNA markers (Singh, 2003).

Molecular techniques have altered the way plant breeding is being done. Molecular markers have great potential to help breeders to develop new improved varieties since they may be used to estimate the genetic diversity and level of heterozygosity among plants and animals (Dani et al., 2008; Kumar et al., 2008). Molecular markers have been used for genetic mapping (Grisi et al., 2007) marker-assisted selection (Ender et al., 2008), and to measure spatial and temporal gene flow within and among populations (Papa and Gepts, 2003). Now a days very powerful PCR-based techniques have also emerged which are very fast, reliable and require minimal amount of tissue for investigation. The use of these techniques for genetic diversity estimation is well documented in many crop plants.

The use of molecular markers for the evaluation of genetic diversity is receiving much attention than morphological characterization. The various marker tools are Randomly Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), Inter Simple Sequence Repeats (ISSR) and Sequence Related Amplified Polymorphism (SRAP). They have large number of applications like characterization of gene pool, DNA fingerprinting, phylogenetic analysis, molecular dissection of complex traits, and characterization of genome organization.

Random amplified polymorphic DNA (RAPD) is PCR based which was developed by (Williams et al., 1990). In the RAPD technique, DNA polymorphisms are produced using a single arbitrary primer that binds to the opposite strands of the genomic DNA template (Tingey et al., 1992). Compared to RFLPs, RAPDs are faster and easier to generate because of the fewer numbers of steps involved namely, extraction, amplification, and separation. The major advantages of RAPDs are the utility of universal primers (Williams et al., 1990) and DNA sequence information or radioactive chemicals are not required (Ragot and Hoisington, 1993). Random amplified polymorphic DNA has been used in the genetic identification of Indian bean cultivars in the recognition of phylogenetic relationships among Phaseolus species (Wilkie et al., 1993).

Inter-Simple Sequence Repeat (ISSR) involves amplification of DNA segment present at an amplifiable distance in between two identical microsatellite repeat regions oriented in
opposite direction. Inter-Simple Sequence Repeat usually 16-25 bp long as primers in a single primer PCR reaction targeting multiple genomic loci to amplify different sizes of inter-SSR sequences. The microsatellite repeats used as primer can be either di-nucleotides or tri-nucleotides. ISSR markers are highly polymorphic ISSR segregate mostly as dominant markers following simple Mendelian inheritance. However, they have also been shown to segregate as codominant markers in some cases, thus enabling distinction between homozygote and heterozygote (Reddy et al., 2002).

Micro-satellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability, they are, therefore, favored in population studies (Smith and Devey, 1994) and for the identification of closely related cultivars (Vosman et al., 1992) The ISSRs are DNA fragments of about 100-3000 bp located between adjacent, oppositely oriented microsatellite regions. The ISSRs are amplified by PCR using microsatellite core sequences as primers with a few selective nucleotides as anchors into the non-repeat adjacent regions (15-18 bp). These DNA markers offer several advantages over traditional phenotypic markers, as they provide data that can be analyzed objectively. The knowledge acquired through this investigation may play a pivotal role in the application of molecular markers in common bean improvement programmes.

Molecular markers have extensively been used for studying genetic diversity and genetic relationship in different types of beans, especially PCR-based markers, such as random amplified polymorphic DNA (RAPD) (Horejsi and Staub 1999), amplification fragment length polymorphism (AFLP) (Li et al., 2004), inter-simple sequence repeat (ISSR)(Wang et al., 2007) and simple sequence repeat (SSR) (Danin-Poleg et al., 2001). The polymorphism derived from EST-SSR is associated with the coding regions of the genome and reflects the genetic diversity available inside or adjacent to the genes (Varshney et al., 2005), which can provide more useful information on cross breeding. To date, EST-SSR markers have been developed for a wide range of plant species and used for genetic studies with multiple purposes.

Keeping in view the above, the present investigation was planned to study the genetic diversity through molecular markers of Indian bean (Lablab purpureus L.) genotypes
Objectives of research work

1. To analyze molecular diversity of different Indian bean genotypes using various molecular markers viz., Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR) and Simple Sequence Repeats (SSR).
2. To examine the isoenzymes pattern of Indian bean genotypes by polyacrylamide gel electrophoresis.
3. To find out the phylogenetic relationship among different Indian bean genotypes.