SUMMARY
**VI SUMMARY**

*Lablab purpureus* L. (Sweet) is an important pulse crop in India. Though it is being one of the ancient crops with rich genetic diversity and its ready adaptability to varied types of edaphic factors, it has not attained the level of agricultural significance as expected. One of the major reason is severe competition by other crops with better economics and other reason is limited number of varieties and production technologies. It is a classical case of limited research; hence efforts are required to promote this crop in India. Therefore to facilitate the breeding activities the present study was conducted in relation to marker and population development with the aim of providing tools for the study of Indian *Lablab* genetic and phenotypic variability.

In the present investigation, the field experiments pertaining to the morphological characterization of 224 germplasm accessions, development of F$_2$ mapping populations and their morphological characterization were conducted at the Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bengaluru, Karnataka, India. The studies on Molecular diversity of selected *Lablab* using COS legume markers and molecular characterization of 224 germplasm accessions were carried out in the Kirkhouse Trust funded Laboratory, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, Bengaluru, Karnataka, India. Further, the screening of selected *Lablab* accessions to identify polymorphic markers and their validation in F$_2$ mapping populations were carried out in the Laboratory of Professor T.H. Noel Ellis, Associate Head, Department of Crop Genetics, John Innes Centre, Norwich Research Park, Colney Lane, Colney, Norwich, United Kingdom.

The objectives of the study was phenotypic and molecular characterization of germplasm, selection of distant genotypes for wide crosses based on molecular and phenotypic data, development of F$_2$ populations for construction of genetic map and molecular and phenotypic characterization of F$_2$ populations for best markers.
The salient features of the experiments are summarised below:

- The diversity analysis showed that *Lablab* accession of Indian origin (Genotypes) grouped into one single cluster while genotypes from (African origin) grouped into second cluster. The clustering of 224 germplasms into 7 clusters revealed diverse relationship between the genotypes. Germplasm from the different eco-geographic location clustered into same group.

- Principal component analysis for 71 traits in 224 germplasms revealed the 81% of variation in the population by first four principal components. The clusters formed based on molecular data were not in agreement with the clusters formed based on morphological data, indicating the presence of discrepancy between these two methods.

- Frequency distribution for different traits (Fig.3) revealed normal distribution for all the traits studied except pod width (in cross HA4 × CPI31113), Number of seeds per pod (in cross HA4 × CPI60216) and total seed yield per plant (in both the crosses), which are skewed towards Parent2. It revealed the suitability of F2 as mapping population to growth, yield and associated traits.

- Mean values differed for all traits among the F2 in the crosses HA4 × CPI31113 and HA4 × CPI60216. The difference was mainly due to contrast genotypic background of parents used in hybridization. High phenotypic and genotypic coefficient of variability (PCV and GCV) was observed for first flowering initiation and total seed weight per plant in both the crosses. Pod length, pod width, number of seeds per pod and test weight showed moderate GCV and PCV in both the crosses. The differences between PCV and GCV values were narrow for all the traits studied, which indicates the low level of environmental influence in the expression of these traits. High heritability was noticed for all the traits studied in both the crosses. Genetic advance as per cent mean was high for all the traits studied in both the crosses. High genetic advance as per cent mean for pods per plant, pod yield per plant and number of productive pods per plant indicate the influence of additive gene effects in expression of these characters.
In the cross HA4 × CPI31113, the test weight exhibited significant positive correlation with total seed yield per plant and pod width. But negative correlation with number of seeds per pod indicates the share of resources between seeds in pods which reduced the seed size and weight in turn reduced the test weight. Wherein, the cross HA4 × CPI31113 number of seeds per pod exhibited significant positive correlation with total Seed yield per plant and pod width exhibited significant negative correlation, which indicates the increase in the number of seeds per pod increase the total seed yield per plant and increased pod width negatively related to the number of seeds per pod reduce the total yield per plant.

In the cross HA4 × CPI31113, test weight recorded highest positive direct effect towards seed yield followed by seeds per pod and pod length. Significant correlation of test weight with seed yield per plant was due to high direct effect whereas other characters had low indirect effect. Positive correlation of NSP with seed yield was mainly due to positive direct effect and negative indirect effect through test weight. In the cross HA4 × CPI60216 seeds per pod had the highest positive direct effect towards seed yield followed by seed yield per plant and first flower initiation. Positive correlation of seeds per pod and test weight with seed yield was mainly due to its high positive direct effect and other characters had very low indirect effect. Negative association of pod width with seed yield was mainly due to its high negative direct effect and effects of other characters were negligible.

The segregation pattern for quantitative traits in F2 populations of both the crosses HA4 × CPI31113 and HA4 × CPI60216 showed continuous variation. The qualitative traits viz., growth habit, branch orientation, flower bud colour, pod fragrance and stem pigmentation segregated in 3:1, 13:3, 3:1, 3:1 and 9:7 ratios in both the crosses HA4 × CPI31113 and HA4 × CPI60216 respectively. Pod curvature is segregated in the ratios of 13:3 and 3:1 in the cross HA4 × CPI31113 and HA4 × CPI60216, respectively. Pod constriction and seed colour in 13:3 ratio in the cross HA4 × CPI60216 whereas in the cross HA4 × CPI31113 segregated in 15:1 and 3:1 ratios.
High rate of amplification of cowpea genic SSR primers in *Lablab* indicates high marker transferability between these two species, whereas the lack of amplification in some of the genotypes could be due to sequence variation at primer binding regions. Four polymorphic markers (CP2, CP8, CP29, and CP435) were identified in parental genotypes when separated on 5% horizontal polyacrylamide gel while these were monomorphic on 2% agarose gel.

The sequencing 18 genic fragments viz., Met1, Met2, D13557, AF 287258, AF 151961, SSR RNA, CP2, CP5, CP8, CP16, CP32, CP215, CP431, CP171, CP403, CP435, CP181, and Y31, fril, PDLL, CP23, CP15 and CP117 among nine parental genotypes revealed 60 SNPs and 16 InDels from genic fragments. Using SNPs and InDels information of genic fragments viz., Met 2, AF 287258-1 AF285278-2, AF 151961, D 13557, CP 5, CP16 and CP 8 allele specific markers were developed. Three primer and tetra-primer AS-PCR method was used to genotype SNPs and InDels. The alleles were successfully distinguished in tetra primer AS-PCR method unlike three primer AS-PCR method.

Four molecular marker systems viz., sequencing of genic fragments, SNPs, allele specific PCR markers and InDel markers were used successfully to genotype the F2 populations of the crosses HA4 × CPI31113 and HA4 × CPI60216 and they exhibited 1:2:1 ratio, as expected in case of co-dominant markers. It confirms the suitability of mapping population for the construction of genetic map.

There were few F2 individuals those flowered earlier in the cross HA4 × CPI60216 than their parents and those can be used for breeding programme to develop the varieties for early type. F2 populations of both the crosses HA4 X CPI31113 and HA4 × CPI60216 exhibited 50 % and 60% transgressive segregants for total seed yield per plant, respectively. These transgressive segregants can be used for breeding programme to develop the varieties with high yield.