"Improvement of pigeonpea for plant type, pod borer resistance and moisture stress tolerance"

The Indo-Swiss collaboration in Biotechnology (ISCB) initiated in Phase IV a pigeon pea network considering the importance of pulses in the food and nutrition security of India. Pulses are grown on rainfed marginal lands with meagre nutrient and pest managements. Pigeon pea (*Cajanus cajan* L.) is the second largest pulse crop and is an important source of dietary proteins in India. India contributes more than 85 per cent to the global pigeon pea production but yield stagnation for decades at about 700 kg/ha has been the main concern. The major constraints to enhancing pigeon pea productivity are inadequate availability of seeds of improved varieties, and a range of biotic and abiotic stresses. The Gene Bank at NBPGR, New Delhi and ICRISAT, Hyderabad hold over 12,000 pigeon pea accessions, but the potential of these resources has not been adequately exploited in varietal improvement. Apart from breeding for grain yield and early maturity, tolerance to biotic and abiotic stresses are equally important for yield stability. Two pod borers, *Helicoverpa armigera* and *Maruca vitrata* are major pests of pigeon pea causing huge yield losses. Water logging at early growth stage and water deficit at the reproductive stage also result in poor crop establishment and yield losses. Further, the pigeon pea genome sequence and a range of molecular markers are now available but not exploited for pigeon pea improvement. Combining conventional and molecular breeding approaches, as envisaged in the ISCB pigeon pea network, will accelerate the development of much needed modern high-yielding, semi-dwarf, synchronous early maturing pigeon pea varieties. Dwarf varieties will help farmers in easy farm operations including mechanical harvesting while early maturity will make the land available for the second crop. The program also envisages strategies for developing insect resistant pigeon pea.

The main challenges for research and development are to bridge the gap between actual and attainable yield by enhancing farmers’ access to quality inputs, improved technologies and information; and also improve the competitiveness of pulse crops. Hence, a unique socio-economic component was included in the ISCB pigeon pea network to analyse three major aspects of pigeon pea production environment. Firstly, tracing changes in the traditional pigeon pea production areas and reasons therein, identifying emerging production centres and yield gaps at the district level. Second, identification of socio-economic constraints faced by farmers in pigeon pea production and traits preference of the stakeholders. Finally, ex-ante evaluation of improved pigeon pea varieties.

The vision of ISCB pigeon pea network is ‘Improved nutritional security and economic status of stakeholders by achieving self-sufficiency in pigeon pea production in India with reduced environmental footprints’. For this the network has embarked upon a mission-mode project for development and testing of semi-dwarf pigeon pea cultivars with high yield, early maturity, pod borer resistance and moisture stress tolerance, compatible with the cropping system of small and marginal farmers. The ISCB pigeon pea network has five specific objectives or outcomes: (i) to develop high yielding semi-dwarf early synchronous maturity pigeon pea varieties compatible with the stakeholders’ preferences; (ii) to understand the molecular mechanism of resistance to pod borer *Helicoverpa armigera* in the wild relatives of pigeon pea; (iii) to develop host-delivered RNAi in pigeon pea for resistance to legume pod borer, *Maruca vitrata*; (iv) to identify donor lines and markers for productivity and moisture stress tolerance traits in the pigeon pea germplasm; (v) to describe socio-economic conditions stakeholders’ preferences for successful introduction of improved pigeon pea varieties developed through to outcomes I to IV. The progress made in achieving these outcomes during the ISCB phase IV is outlined below:

**Biotechnology component:** Re-sequencing of 48 diverse pigeon pea varieties was carried out and the sequence reads were aligned to the reference genome of pigeon pea variety ‘Asha’. This identified 645,662 single nucleotide polymorphisms (SNPs) in 17,125 selected genes, including single-copy, disease resistance and other agronomically important genes. A high-density 62K SNP genotyping chip was designed and fabricated using Affymetrix GeneTitan platform. The chip was used on 384 pigeon pea genotypes, including a set of 96 diverse cultivars and three different trait-mapping populations. The data so generated have been used for delineating the population structure of pigeon pea cultivars and mapping of fertility restorer gene for the A2 cytoplasmic male sterility (CMS) system, which will be useful in hybrid seed production. A high-density genetic map of more than 2000 marker loci has been completed and trait-mapping studies are in progress. Further, we have selected several new plant type advance generation high-
yielding early-maturing semi-dwarf recombinant inbred and back-cross inbred lines, which will be taken to multi-location yield trials during the next two years, and eventually the best of these will be released for commercial cultivation by the farmers.

Wild relatives of pigeon pea were used for understanding the mechanism of pod borer resistance and identification of candidate genes for resistance to pod borer Helicoverpa armigera. For this four wild pigeon pea species, viz., C. scarabaeoides, C. albicans, C. platycarpus and C. serecius were screened and C. scarabaeoides and C. platycarpus showed higher resistance to pod borer than C. cajan cultivars. Differential gene expression analysis was carried out between C. platycarpus and cultivar TTB7 at different time points after challenge with the insect larvae and herbivory-specific genes with >2-fold differential expressions were identified. Genes with probable role in (i) insect structural destruction; (ii) interference in digestion; (iii) reduction in availability of nutrients; and (iv) insect toxic products were shortlisted. Extensive inputs were provided by the Swiss partners in understanding the molecular mechanism of pod borer resistance, particularly by high throughput proteomics analysis. The flavonoids and the phenylpropanoid biosynthesis pathways are known to generate volatile and non-volatile metabolites that are used in pest management. Integration of this information with the transcriptome data will aid in better understanding of the resistance phenomena existing in the wild relative.

Towards the control of second important pod borer, Maruca vitrata, the extent of genetic diversity in the insect population collected from different agro-climatic zones of India was analysed using gene-based markers viz., translation elongation factor-1, ITS and cytochrome oxidase I. Insect population originating from Warangal district was the most, while Delhi population represented the most prevalent form of M. vitrata and it was further used for transcriptome and proteome analysis with the help of Swiss partners. Growth conditions and artificial diet were standardized for the rearing of insect in laboratory. Stage-specific RNA transcripts were identified and annotated by Gene Ontology and Pathways for Biological Enrichment analysis. Eighteen genes were selected for silencing using host delivered RNAi, gene fragments successfully made and mobilized into Agrobacterium. Primary transformants have been developed by apical meristem-targeted in planta transformation and analysis of transformants is in progress.

After evaluation of over 2000 germplasm accessions, we have identified 25 high yielding, 65 extra early, 350 early maturing, 7 sterility mosaic disease tolerant, 17 moisture deficit stress tolerant and 7 highly drought susceptible accessions. Multi-location evaluation of identified promising lines is in progress. Further, differential gene expression analysis has been carried to identify candidate genes for these traits, and 50 of these lines have been genotyped using 62K SNP chip for trait association studies.

The socio-economic component was aimed at identifying market preferred traits and understanding of seed supply systems along with the use of quality seeds of improved varieties. Baseline focus group discussions and detailed survey of all the stakeholders were conducted in central, southern, north-eastern and north-western plain zones of the country. The analysis clearly revealed concentration of pigeon pea production in the central and southern states of Maharashtra, Karnataka, Madhya Pradesh, Telangana and Gujarat. There has been a shift in the pigeon pea cultivation from the northern plain zones to the southern and central zones of the country, but consumption is spread over the entire country. Intercropping of pigeon pea with soybean, cotton and sorghum and sole cropping of old and improved varieties are the dominant production system in central and southern zones. Farmers were receptive to adoption of improved varieties but the results indicated very low varietal diversity and prevalence of old varieties in all the regions. The majority of the farmers identifying pod borer & pod fly as the major pests, and wilt as the major disease. Drought was identified by almost all the farmers as a major production constraint. Farmers preferred high grain yield with drought tolerance, medium to early maturity, pod borer resistance, tolerance to wilt disease, moderate plant height and ease of threshing without compromising other preferred attributes. The millers preferred uniform size, oval shape, orange coloured seed and most importantly moderately hard seed coat for high dal recovery. A study was also conducted by the Swiss partners to assess consumers’ perception and preferences of pigeon pea varietal traits and consumption pattern in two urban centers, namely Delhi and Hyderabad. These findings underlie tremendous scope for improving pigeon pea productivity by introduction of improved cultivars well suited for intercropping systems, timely availability of quality seeds, improved crop management, and remunerative prices with effective procurement are addressed adequately.

An important component of ISCB phase IV was the training of Indian scientists and students in the area of proteomics and bioinformatics data analysis at ICAR-NRCB, New Delhi and ETH, Zurich, and the support of the participation of two students in the PAG 2017.