**Pigeonpea (Cajanus cajan (L.) Millspaugh)**

- Pigeonpea is diploid (2n = 22) and primarily grown for food (dry seeds or green vegetables).
- Sixth most important food legume crop and good source of protein; enriches soil; provides fodder and fuel wood; and arrests soil erosion.
- Grown in over 82 countries worldwide as a field and/or backyard crop.
- As a regular crop, grown only in 19 countries on 4.6 million hectares producing 3.5 million tons of grain (FAOSTAT, 2005).

**Pigeonpea composite collection**

- Developed considering the phenotypic diversity present in the entire collection held at the ICRISAT genebank (Upadhyaya et al., 2005).
- Collection consists 1000 accessions comprising mini-core (Upadhyaya et al., 2006), comparator mini-core and trait-based accessions along with 62 accessions of seven wild Cajanus species.

**Genetic diversity in composite collection**

- Plant material: DNA extracted from randomly selected 12 plants per accession following a high-throughput procedure and pooled together to capture the within accession variation.

Selection of SSR markers

- Pigeonpea SSR markers being less polymorphic, all the available markers at ICRISAT initially screened on 15 diverse accessions (8 cultivated and 7 wild) that are included in the composite collection.
- Thirty-three SSR markers showed polymorphism between at least two of the tested accessions.
- A series of artificial pools having different proportions of DNA from two genotypes developed and tested with 33 SSR markers for polymorphism.
- The coefficient of correlations analyzed between different proportion of alleles recorded (corresponding to 12 plants per accession) and proportion of genomic DNA used for the corresponding accession.
- Twenty SSR markers (Table 1) with highly significant correlations (r > 0.9) identified.

**Molecular characterization**

- PCR components of 20 SSR markers optimized following Taguchi method (Taguchi, 1986) as described in Cobb and Clarkson (1994).
- Fluorescent-based multiplex genotyping system used to generate five multiplexes of four markers each.
- Capillary electrophoresis with an automated system (ABI 3700) used to separate the amplified PCR products.
- SSR fragment sizes called to two decimal places using the Genotyper v3.7 software.
- Observed allelic data binned into discrete genetic units using the Allelobin program (http://www.irisat.org/gt/biometrics.htm) developed at ICRISAT based on the least squares algorithm of Idury and Cardon (1997).
- Markers produced allele sizes expected, based on the repeat motif of each of the SSR markers.
- Less than 5% missing data (ie, marker x genotype) in the dataset.

**Data analysis**

- Data analysis completed on 12 SSR markers.
- DARWin 5.0 Structure program (Perrier et al., 2003) used to determine the Mean Polymorphic Information Content (PIC) value was 0.345, ranging from 0.09 to 0.44.
- Principal coordinate analysis done considering the biological status of the crop (cultivated and wild).
- Cervus 2.0 software used to determine the allele frequencies and PIC values.

**Results**

- Preliminary analysis detected 144 alleles, ranging from 5 (PGM109) to 24 (CCB8) (CT)16.
- Mean Polymorphic Information Content (PIC) value was 0.345, ranging from 0.09 to 0.53.
- Phylogenetic tree constructed based on biological status revealed that both cultivated and wild accessions formed distinct clusters (Fig. 1).

**Future plan**

- Further analysis of data with remaining eight markers in progress to fully understand the genetic diversity and population structure of the composite collection.
- Results from genotypic data will be used to identify a reference set of 300 diverse accessions for future use.

**References**


