GCP Sub-Programme 1: 2006 Commissioned Research Project #32

Molecular characterization of pigeonpea [*Cajanus cajan* (L.) Millspaugh] composite collection

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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 ha 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 ($r^2 > 0.9$) identified.

Table 1. List of 20 polymorphic SSR markers selected for genotyping the composite collection

CCB1	PGM109	PGM45	CCB10
CCB9	PGM82	PGM102	PGM16
PGM3	PKS21	PKS30	PKS18
PGM101	PGM5	PKS25	CCB7
PGM106	PGM10	PKS26	CCB8

Molecular characterization

- PCR components of all 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.icrisat.org/gt-bt/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 population structure of the composite collection
- Forty-five accessions with high missing values excluded from data analysis
- 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) with a mean of 12.0 alleles per locus (Table 2)
- Mean Polymorphic Information Content (PIC) value was 0.345, ranging from 0.09 to 0.53, (Table 2).
- Phylogenetic tree constructed based on biological status revealed that both cultivated and wild accessions formed distinct clusters (Fig. 1)

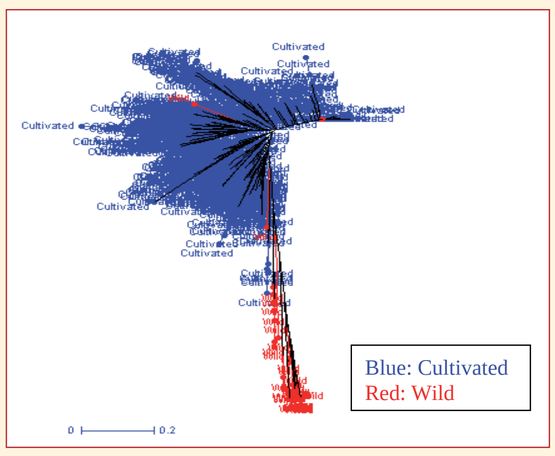

Fig. 1. Tree diagram of 955 accessions with 12 SSR markers.

Table 2. Information on 12 SSR primers

Primer	Repeat Unit	Quality Index	No. of Accessions Genotyped	No. of Alleles	PIC Values
CCB1	(CA)10	0.26	1000	20	0.46
CCB9	(CT)22	0.46	951	18	0.53
PGM3	(GAA)5G (GAA)5	0.42	976	11	0.50
PGM101	(AC)7	0.14	984	8	0.43
CCB7	(CT)16	0.24	1000	17	0.47
PGM106	(AAG)13	0.31	1000	11	0.33
PGM109	(CTT)8	0.18	1000	5	0.44
CCB8	(CT)30	0.27	961	24	0.47
PKS21	(CT)6TT (CT)2	0.23	997	8	0.13
PGM5	(GAA)6	0.23	948	8	0.13
PGM10	(AGA)5	0.22	986	7	0.09
PGM16	(TC)8	0.26	962	7	0.17

- All the accessions belonging to seven wild *Cajanus* species grouped together in a separate cluster except few, which grouped with the cultivated accessions.

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

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