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Diversity and geographical gaps in *Cajanus scarabaeoides* (L.) Thou. germplasm conserved at the ICRISAT genebank

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Abstract

Crop wild relatives are important components of agro-ecosystems as potential gene contributors for crop improvement programmes. *Cajanus scarabaeoides* (L.) Thou., a pigeonpea wild relative is crossable with cultivated pigeonpea and possesses several beneficial traits. Hundred accessions conserved at the ICRISAT genebank were characterized for 13 quantitative and ten qualitative traits to assess the diversity in the collection. Highly significant genotypic variance for leaflet length, days to 5% maturity, seeds per pod, 100-seed weight, seed protein content and trichome density and length was observed. All *C. scarabaeoides* accessions used in the present study are the best sources for extra early (<80 d to 50% flowering) and early maturity (80–100 d to 50% flowering). Eight accessions (ICP 15692, ICP 15696, ICP 15698, ICP 15699, ICP 15712, ICP 15719, ICP 15732 and ICP 15758) and the control ICP 15695 have produced more than 92% healthy pods per plant and higher number of seed per pod (4–6 seeds). Accessions in cluster 2, 3 and 4 with low mean values for days to 50% flowering were found as the best sources for early flowering and maturity. Accessions in cluster 2 and 3 for seeds per pod and cluster 2 for healthy pods per plant were found as promising sources for use in crop improvement. Mean diversity over all clusters was highest ($H = 0.57 \pm 0.01$) for seeds per pod and lowest for days to 50% flowering (0.48 ± 0.02). Significant negative correlation between pods per raceme and healthy pods per plant (-0.213) indicated high pod damage in racemes having more pods. Trichome length had highly significant negative association with healthy pods per plant (-0.293). The probability map generated using FloraMap, a GIS tool, revealed the occurrence of *C. scarabaeoides* quite close to the origin and dispersal of pigeonpea. The probability (>75%) map identified a total of 118 provinces covering 790 districts in Bangladesh, Cambodia, India, Indonesia, Laos, Malaysia, Myanmar, Nepal, Papua New Guinea, Philippines, Thailand and Vietnam as geographical gaps in the collection. Complete passport data including location coordinates should be collected while collecting the germplasm to analyze the spatial aspects of species distribution.

Keywords: characterization; diversity; genetic resources; geographical gap; germplasm; wild relatives

Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh], a major grain legume crop of tropics and subtropics is grown in about 82 countries lying between 30°N and 30°S latitudes. It has wide adaptability to diverse climates

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and is mainly grown for its multiple uses. Crop statistics are reported only for 21 countries and, during 2009, pigeonpea was cultivated in 4.86 mha with an annual production of 4.10 million tons and an average productivity of 844 kg/ha (FAO, 2009). India has the largest area under pigeonpea (3.50 mha), followed by Myanmar (0.57 mha), Kenya (0.19 mha), Malawi (0.12 mha), Uganda (0.09 mha), Tanzania (0.07 mha), Nepal (0.02 mha) and Dominican Republic (0.02 mha) (FAO, 2007).

Crop wild relatives (CWR) are important components of agro-ecosystems as potential gene contributors for crop improvement programmes. When the genetic variability is narrow and levels of resistance to various biotic and abiotic stresses in cultivated germplasm are low, the identification and incorporation of useful genes from wild species becomes key to sustain crop productivity. Wild relatives become more important when they posses traits of agronomic importance in addition to resistance to the biotic and abiotic stresses and can be hybridized easily with cultivated species. Despite their importance as the source for several beneficial genes, CWR have not received due attention. Though considerable progress in pigeonpea improvement has been made, the crop still suffers from several problems such as biotic and abiotic stresses, low pod setting, long duration, etc. Many wild relatives of pigeonpea have evolved to survive drought, floods, extreme heat and cold, and they have become adapted to cope with many natural hazards and often developed resistance to various biotic and abiotic stresses. Some wild species of pigeonpea such as *Cajanus scarabaeoides* (L.) Thou. possess genes for earliness and high pod setting (Upadhyaya, 2006).

Genebank at ICRISAT conserves 13,632 accessions of pigeonpea germplasm from 74 countries, including 555 accessions of wild relatives belonging to 66 species of six genera. The collection of wild relatives includes 102 accessions of *C. scarabaeoides*. *C. scarabaeoides* is crossable with cultivated pigeonpea (Pundir and Singh, 1985) and possess several useful traits such as early flowering (<80 d to 50% flowering) and high pod setting (74%) (Upadhyaya, 2006); tolerance to pod borer (<10% pod damage), pod fly and pod wasp (Sharma, 2006); water logging (Reddy *et al.*, 2000); salinity (Srivastava *et al.*, 2006); resistance to *Fusarium* wilt, sterility mosaic disease (SMD), phytophthora blight and alternaria blight (Reddy *et al.*, 2000) and cytoplasmic male sterility (CMS) (Tikka *et al.*, 1997; Saxena and Kumar, 2003). World genebanks are conserving a limited proportion of wild relatives of total genetic variability and only a small proportion of conserved accessions have been characterized, resulting in limited use of wild species in crop improvement. Because of limitations such as low priority for characterization of wild species germplasm in the past, unavailability of sufficient seeds for characterization, etc., more works were not done using *C. scarabaeoides* germplasm. Increasing threats to natural habitats due to climate change and concomitant natural catastrophes (droughts, floods, fire hazards, etc.), human settlements, overgrazing, irrigation projects, etc. make it imperative to assess the species diversity and identify gaps in the collections before the valuable material is lost for ever (Upadhyaya and Gowda, 2009). Therefore, the present study was aimed at critical assessment of existing *C. scarabaeoides* collection at the ICRISAT genebank for diversity and geographical gaps in the collection for enhanced utilization and possible exploration in future.

Materials and methods

The pigeonpea wild genepool at ICRISAT genebank comprises 555 accessions of 66 species belonging to genus *Rhynchosia* (33 species, 303 accessions), *Cajanus* (19 species, 213 accessions), *Flemingia* (eight species, 18 accessions), *Eriosema* (three species, seven accessions), *Dunbaria* (two species, 12 accessions) and Paracalyx (one species, two accessions). The *C. scarabaeoides* collection comprises 102 accessions from eight countries. Passport and characterization data of 100 accessions in which seed quantity was adequate was considered for the present study. To assess the diversity in the collection, accessions were characterized during 2008 rainy season (June to November) under field conditions in alfisol-Patancheru soil series (Udic Rhodustolf) field at ICRISAT, Patancheru (17.53°N, 78.27°E, 545 m.a.s.l, and 600 km away from the sea), Andhra Pradesh, India. Experiment was laid out in unreplicated augmented design with systematic control after every 20 test accessions. Each plant in all accessions was given support with bamboo pegs to grow as climber to overcome the problem of pod damage due to soil contamination. ICP 15695, an early flowering (52 d) and pod borer [*Helicoverpa armigera* (Hun.)] tolerant (no egg laying, no larvae and <10% pod damage) (Sharma, 2006) accession was used as control. Accessions were grown in a single row of 8-m length with a row-to-row distance of 100 cm and plant-to-plant distance of 50 cm within the row. Crop received a basal dose of Di-ammonium Phosphate (DAP) (150 kg/ha). Life saving irrigation was provided. The crop was protected from weeds, pests and diseases.

Observations were recorded on 13 quantitative traits (leaflet length, leaflet width, days to 50% flowering, days to 5% maturity, pods per raceme, pod length, pod width, seeds per pod, 100-seed weight, seed protein content, trichome density, trichome length and percentage of healthy pods per plant) and ten qualitative traits (growth habit, stem pigmentation, leaflet shape, flowering pattern, flower streak pattern, primary seed colour, seed

coat colour pattern, seed shape, seed strophiole and biomass score). Flower streak pattern was recorded based on the density of streaks on vexillum. Seed coat colour was recorded as primary seed colour. Pattern of seed coat colour was recorded as mottled, plain and plain + mottled. *C. scarabaeoides* serves as forage for goats and other animals. Therefore, accessions were also scored for biomass production on 1–5 scale (1 = poorest and 5 = high biomass producer). Pods without egg layings, larvae, scares and holes on pod wall due to insects and diseases were considered as healthy pods. As per the standard maturity classification developed and being followed for pigeonpea at ICRISAT, accessions that flower in <80 d were considered as extra early and those flower between 80 and 100 d as early maturing (ICRISAT, 1978). Data on trichomes were recorded on 7–10 d old pods. A longitudinal section on middle portion of the pod was made with the help of blade and placed in 45% acetic acid. The vials holding pod sections were placed in oven run at 40°C to soften the tissues. The section was transferred to water in a petri plate. From petri plate, three good sections were picked and placed on diluted glycerol drops on glass slide keeping the trichome side up. The sections were covered with glass cover slip and examined under simple microscope. The trichome density and length could be seen conveniently at 40 × magnification. For measuring density of trichomes, eye piece micrometer was placed in the microscope and the number of trichomes per grid (10 × 10 squares) was counted. For measuring the length of trichome, the number of squares covered by a trichome was counted and converted to microns. Days to 50% flowering, days to 5% maturity, all seed characters and biomass scores were recorded on plot basis and seed protein content was estimated at Crop Quality Unit of ICRISAT (IBPGR and ICRISAT, 1993).

The residual maximum likelihood method (REML; Patterson and Thompson, 1971) was used to analyze the data of 13 quantitative traits. Variance components due to genotype (σ_g^2) and its standard errors (SE) were estimated. Principal component analysis (PCA) of 13 quantitative traits was performed using GENSTAT 13.1. Cluster analysis was performed according to Ward (1963) using scores of first six principal components (PCs). The mean, range and variances were calculated for 13 quantitative characters of each cluster and for the entire collection. The means for different traits were compared using the Newman–Keuls procedure (Newman, 1939; Keuls, 1952). Homogeneity of variances was tested by Levene's test (Levene, 1960). The Shannon–Weaver diversity index (H') (Shannon and Weaver, 1949) was used to measure and compare phenotypic diversity for each trait. The diversity index was estimated for 13 quantitative traits over all the accessions and for each cluster. Phenotypic proportions were estimated for ten qualitative traits (Snedecor and Cochran, 1980).

Gaps in the collection were identified using the GIS tools such as MS Encarta Interactive Atlas, ArcGIS and FloraMap. The basic input in the GIS tools was the geographic coordinates (latitude and longitude) of the sampling site with a unique identifier (accession number). Passport data, particularly the information on precise location of collecting site and corresponding geographic coordinates were updated verifying all related records and collection reports. Using Microsoft Encarta®, an electronic atlas (MS Encarta® Interactive World Atlas, 2000), geographic coordinates were retrieved to fill the gaps for accessions having location information. Finally, the 76 accessions from India (44), Sri Lanka (15), Indonesia (14), Philippines (two) and Myanmar (one) were used to predict the probability of *C. scarabaeoides* occurrence and to identify gaps in the collection. Georeference data were validated by plotting the accessions on world map. Using the FloraMap, a GIS tool developed at Centro Internacional de Agricultura Tropical (Jones and Gladkov, 1999), probability of occurrence of *C. scarabaeoides* was mapped and overlaid the collecting sites of 76 accessions. Multiple accessions having same coordinates were considered as single collection site. Probability more than 75% was considered for interpretation of results and recommendation of gaps for launching collection missions. FloraMap is built on the assumption that climate is a robust indicator of environmental range of wild species. Likely alternative sites for finding a particular species will have climate profiles closely matching to those of the locations where the wild accessions were already collected. FloraMap uses a climate grid (18 × 18 km^2) of monthly climate surfaces to produce probability map showing where else in the world the species might be found. While working on the passport dataset, equal weights were allocated to the climatic variables (monthly rainfall, minimum and maximum temperature and diurnal range in temperature) and an exponential transformation with a power of 0.3 was applied to the monthly rainfall data. More than 95% of total variation was explained by first five PCs. ArcGIS was used to prepare the layout and cartographic outputs. High probability area covering provinces/districts (shaded area in Figs. 2 and 3) in different countries is summarized in Table 5.

Results

Diversity in the collection

Qualitative traits

Growth habit. All accessions of *C. scarabaeoides* have showed creeper–climber growth habit.

Stem pigmentation. Accessions having green stems were found in maximum proportion (60%), followed by dark green (23%) and light green (10%). Remaining 7% accessions had a mixture of green and light green stem plants.

Leaflet shape. *C. scarabaeoides* produced pinnately trifoliate leaves (van der Maesen, 1986). Fifty-two percentage of accessions produced lanceolate leaflets, followed by ovate (25%), broad lanceolate (22%) and linear lanceolate (1%) shaped leaflets.

Flowering pattern. All accessions showed indeterminate flowering habit.

Flower streak pattern. Accessions having few streaks were maximum (32%), followed by dense streaks (22%), plain (12%), mixture of plants with dense streaks and few streaks (20%), plain and few steaks (10%) and plain and dense streaks (4%).

Primary seed colour. Grey colour was predominant (31%), followed by dark grey (26%), grey + grey yellow (23%), dark grey + grey (10%) and dark grey + grey yellow (10%).

Seed coat colour pattern. Most of the accessions produced a mixture of plain + mottled (35%) coat colour pattern, followed by plain (34%) and mottled (31%).

Seed shape. Three clear seed shapes (square = 35%, round = 4% and elongate = 1%) were found in the collection. There were accessions with a mixture of plants producing square and round shaped seeds (58%) and square and elongate (2%) shaped seeds in the collection.

Seed strophiole. This was the important diagnostic trait to identify wilds. Seed strophiole was observed in seeds of all accessions.

Biomass score. Only three accessions (ICP 15685, ICP 14690 and ICP 15706) scored high (5), while nine accessions scored 4, 59 accessions scored 3, 22 accessions scored 2 and 7 accessions scored 1.

Quantitative traits

REML analysis. REML analysis indicated highly significant genotypic variance for leaflet length, days to 5% maturity, seeds per pod, 100-seed weight, seed protein content, trichome density and length indicating the considerable variation in the collection for these traits. Variance for days to 50% flowering was significant at 5% only (Table 1).

Cluster analysis. PCA carried out using standardized data of 13 quantitative traits captured 74% of total variation from first six PCs. The PC1 alone accounted for 19.9% variation, followed by PC2 with 15.6%, PC3 with 11.6%, PC4 with 10.1%, PC5 with 8.6% and PC6 with 8.1%. A hierarchical cluster analysis conducted on the scores of the first six PCs resulted in four clusters (Fig. 1 and Table 2) (Ward, 1963). Clustering of accessions revealed no effect of geographic origin on agronomic performance. Eight accessions from India and one accession from Australia formed the first cluster; 19 accessions from India, 13 from Sri Lanka, four from Indonesia, two from Australia, one from Fiji and one accession of unknown origin formed the second cluster; six accessions from India, three from Indonesia, two from Philippines, one accession each from Australia and Myanmar formed the third cluster and 22 accessions from India, nine from Indonesia, three from Sri Lanka, two from Australia and one accession each from Fiji and United Kingdom formed the fourth cluster (Table 2).

Variances. The homogeneity of variances of the four clusters was tested for all the 13 quantitative traits by Levene's test (Levene, 1960). The variances were heterogeneous for leaf width, pods per raceme and percentage of healthy pods per plant (Table 3).

Range and means. In the entire collection, the important traits such as days to 50% flowering ranged

Table 1. Range, mean, variance and Shannon–Weaver diversity index (H') and variance due to genotypes (σ_g^2) for different characters of *C. scarabaeoides* germplasm assembled and evaluated at ICRISAT genebank, Patancheru, India

Character	Range	Mean	Variance	H'	σ_g^2	SE
Leaflet length (cm)	2.5–3.7	3.1 ± 0.14	0.06	0.615	0.08**	0.022
Leaflet width (cm)	1.6–2.2	1.9 ± 0.11	0.02	0.635	0.03	0.015
Days to 50% flowering	52–100	70.2 ± 6.65	74.12	0.554	118.67*	50.65
Days to 5% maturity	103–167	126.2 ± 2.35	141.73	0.612	156.62**	13.35
Pods per raceme	1.4–2.4	1.8 ± 0.23	0.03	0.599	0.08	0.089
Pod length (cm)	2.1–2.3	2.2 ± 0.08	< 0.01	0.555	0.01	0.01
Pod width (mm)	6.8–7.0	6.9 ± 0.10	< 0.01	0.591	0.01	0.09
Seeds/pod	3.8–5.6	4.9 ± 0.16	0.13	0.551	0.16**	0.03
100-Seed weight (g)	1.5–2.4	1.9 ± 0.12	0.04	0.624	0.05**	0.015
Protein (%)	18.3–23.8	20.3 ± 0.55	1.2	0.597	1.50**	0.35
Trichome density (no./unit area)	28.5–59.2	42.6 ± 3.22	35.64	0.614	46.27**	11.88
Trichome length (μ)	1.9–4.5	3.1 ± 0.17	0.21	0.607	0.24**	0.04
Healthy pods/plant (%)	73–93	86.3 ± 4.52	15.64	0.626	35.98	29.44

* P at 0.05. ** P at 0.01.

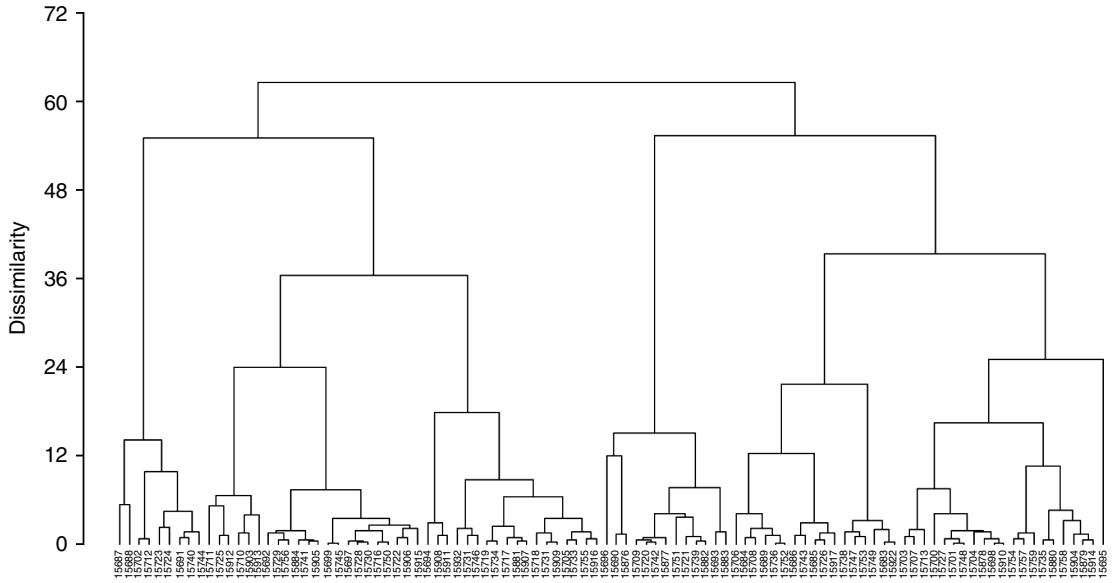

Fig. 1. Dendrogram of 100 accessions of *C. scarabaeoides* conserved at ICRISAT genebank, Patancheru, India, based on scores of first six PCs.

from 52 (ICP 15695) to 100 d (ICP 15723) and days to 5% maturity varied from 103 (ICP 15747) to 167 d (ICP 15723). Seeds per pod varied from 3.8 (ICP 15743) to 5.6 (ICP 15908), seed protein content from 18.3 (ICP 15719) to 23.8% (ICP 15695) and percentage of healthy pods per plant from 73 (ICP 15706) to 93 (ICP 15692) (Table 1). Among the clusters, accessions of cluster 2 varied more for leaf width, days to 50% flowering, days to 5% maturity, 100-seed weight, seed protein content and trichome density. Accessions of cluster 4 varied widely for leaf length, pod length and width, seeds per pod, trichome length and percent healthy pods per plant (Table 4).

Mean overall accessions (70 ± 6.65 d) indicated *C. scarabaeoides* collection as an excellent source for earliness (Table 1). As per the standard maturity

Table 2. Cluster-wise pigeonpea germplasm accessions

Cluster no.	Country	Accession (ICP) no.
1	Australia	15744
	India	15687, 15688, 15691, 15702, 15712, 15723, 15724, 15740
2	Australia	15734, 15745
	Fiji	15737
	India	15692, 15697, 15699, 15705, 15711, 15716, 15717, 15718, 15719, 15722, 15725, 15728, 15729, 15730, 15731, 15732, 15733, 15746, 15884
	Indonesia	15750, 15755, 15756, 15881
	Sri Lanka	15694, 15903, 15905, 15906, 15907, 15908, 15909, 15910, 15911, 15912, 15913, 15915, 15916
	Unknown	15741
3	Australia	15742
	India	15690, 15693, 15709, 15739, 15882, 15883
	Indonesia	15751, 15876, 15877
	Myanmar	15696
	Philippines	15720, 15721
4	Australia	15735, 15743
	Fiji	15736
	India	15683, 15684, 15685, 15686, 15689, 15698, 15700, 15701, 15703, 15704, 15706, 15707, 15708, 15710, 15713, 15726, 15727, 15738, 15747, 15748, 15749, 15922
	Indonesia	15752, 15753, 15754, 15757, 15758, 15759, 15878, 15879, 15880
	Sri Lanka	15695, 15904, 15914
	United Kingdom	15917

Table 3. Variances and Shannon–Weaver diversity index (H') for different characters of *C. scarabaeoides* germplasm clusters, evaluated at ICRISAT genebank, Patancheru, India

Character	Variance[a]						Diversity index (H')					
	Cl1[b]	Cl2	Cl3	Cl4	*F* value	*P*	Cl1	Cl2	Cl3	Cl4	Mean	SE
Leaflet length (cm)	0.09	0.09	0.04	0.10	0.69	0.559	0.43	0.60	0.47	0.59	0.52	0.02
Leaflet width (cm)	0.07	0.04	0.03	0.02	2.71	0.049	0.43	0.61	0.53	0.58	0.54	0.02
Days to 50% flowering	250.00	129.70	39.90	113.30	2.12	0.103	0.53	0.53	0.36	0.50	0.48	0.02
Days to 5% maturity	79.19	127.70	20.69	102.40	1.41	0.246	0.41	0.50	0.43	0.58	0.48	0.02
Pods per raceme	0.08	0.18	0.67	0.19	7.16	<0.0001	0.46	0.58	0.52	0.57	0.53	0.01
Pod length (cm)	0.03	0.02	0.01	0.05	2.52	0.062	0.55	0.53	0.46	0.62	0.54	0.02
Pod width (mm)	0.27	0.14	0.26	0.31	1.50	0.220	0.55	0.57	0.55	0.58	0.56	0.00
Seeds/pod	0.06	0.14	0.18	0.18	0.71	0.548	0.57	0.60	0.55	0.56	0.57	0.01
100-Seed weight (g)	0.07	0.05	0.03	0.07	0.83	0.483	0.53	0.62	0.47	0.60	0.55	0.02
Protein (%)	1.32	1.52	1.39	2.23	0.51	0.678	0.43	0.55	0.47	0.56	0.50	0.02
Trichome density (no./unit area)	57.81	47.45	44.55	51.88	0.05	0.983	0.43	0.56	0.52	0.56	0.52	0.02
Trichome length (μ)	0.12	0.22	0.34	0.18	0.97	0.408	0.42	0.59	0.43	0.51	0.49	0.02
Healthy pods/plant (%)	112.20	46.43	65.32	111.00	3.25	0.025	0.46	0.60	0.55	0.59	0.55	0.02
Mean							0.48	0.57	0.48	0.57	0.53	
SE							0.00	0.00	0.00	0.00	0.01	

[a] Variance homogeneity tested by Levene's test.
[b] Cl1 = cluster 1, Cl2 = cluster 2, Cl3 = cluster 3 and Cl4 = cluster 4.

classification for pigeonpea followed at ICRISAT, mean for days to 50% flowering suggested that 89 accessions were extra early (<80 d to 50% flowering) and 11 accessions were early (80–100 d to 50% flowering) maturing (ICRISAT, 1978). None of the accessions flowered earlier than the control ICP 15695, a promising accession against pod borer flowers in 52 d. All accessions from Australia, Fiji, Myanmar, Philippines and United Kingdom were found to be extra early. About 87% of accessions from India, Indonesia and Sri Lanka were found as extra early. Overall mean for seeds per pod (4.9 ± 0.16) indicated the relatively higher seeds per pod than majority of cultivated accessions. As many as 31 accessions produced more than five seeds per pod. Eight accessions (ICP 15692, ICP 15696, ICP 15698, ICP 15699, ICP 15712, ICP 15719, ICP 15732 and ICP 15758) produced more than 92% healthy pods per plant, which is comparable to that of pod borer tolerant control ICP 15695 (92.08 ± 4.5).

Newman–Keuls test of significance for mean values indicated significant differences among the clusters for one or more traits under study (Tables 2 and 4) (Newman, 1939; Keuls, 1952). Cluster 1 for six traits (leaflet width, pods per raceme, pod length and width, 100-seed weight, seed protein content), cluster 2 for three traits (pods per raceme, seeds per pod and healthy pod per plant), cluster 3 for seven traits (leaflet length and width, pods per raceme, seeds per pod, seed protein content and trichome density and length) and cluster 4 for four traits (days to 50% flowering, days to 5% maturity, pods per raceme and seed protein content) were found as promising sources. Differences were not significant among the clusters for pods per raceme.

Phenotypic diversity. The H' was calculated over all accessions and for each cluster to compare phenotypic diversity among the clusters for 13 quantitative traits (Tables 1 and 3) (Shannon and Weaver, 1949). A low H' indicates extremely unbalanced frequency classes for an individual trait and lack of genetic diversity in the collection. The diversity index values (H') were variable among traits. In the entire collection, diversity index (H') ranged from 0.551 ± 0.00 for seeds per pod to 0.635 ± 0.03 for leaflet width (Table 1). Mean diversity over all traits was maximum in cluster 2 ($H' = 0.57 \pm 0.00$) and it was lowest in cluster 1 ($H' = 0.48 \pm 0.00$). Mean diversity over all clusters was highest ($H' = 0.57 \pm 0.00$) for seeds per pod and lowest for days to 50% flowering ($H' = 0.48 \pm 0.00$) (Table 3).

Character associations. Phenotypic correlations were estimated among all quantitative characters and their significance was tested (Snedecor and Cochran, 1980). Highly significant positive correlation of leaflet length with leaflet width (0.638), pods per raceme (0.255) and pod width (0.297) was observed. Correlation of leaflet

Table 4. Range and mean values for different characters of *C. scarabaeoides* germplasm clusters evaluated at ICRISAT genebank, Patancheru, India

Character	Range				Mean			
	Cl1	Cl2	Cl3	Cl4	Cl1	Cl2	Cl3	Cl4
Leaflet length (cm)	2.6–3.5	2.3–3.7	3.0–3.8	2.3–3.8	3.2^{b}	$3.12^{c,b}$	3.41^{a}	2.93^{c}
Leaflet width (cm)	1.6–2.4	1.4–2.3	1.7–2.3	1.5–2.2	2.13^{a}	1.89^{b}	2.07^{a}	1.78^{b}
Days to 50% flowering	70–118	53–104	60–84	51–88	95^{a}	69.35^{b}	70.31^{b}	65.63^{b}
Days to 5% maturity	140–168	110–160	118–130	102–138	147.8^{a}	126.3^{b}	125.8^{b}	121.5^{b}
Pods per raceme	1.3–2.1	1–2.5	0.6–3.4	1–3.1	1.71^{a}	1.68^{a}	2.1^{a}	1.85^{a}
Pod length (cm)	2.1–2.6	2–2.6	2–2.34	1.8–2.74	2.4^{a}	2.22^{b}	2.17^{b}	2.22^{b}
Pod width (mm)	6.8–8.4	6–7.6	6–7.6	5.6–8	7.76^{a}	6.86^{b}	6.74^{b}	6.96^{b}
Seeds per pod	4.1–4.9	4.1–5.8	4.2–5.8	3.6–5.5	4.6^{b}	5.12^{a}	4.98^{a}	4.67^{b}
100-Seed weight (g)	1.6–2.4	1.3–2.2	1.5–2	1.3–2.6	2.2^{a}	1.8^{b}	1.77^{b}	1.87^{b}
Protein (%)	18.2–21.6	17.8–24.6	19.1–23.6	18.1–24.4	$20.4^{a,b}$	19.78^{b}	21.1^{a}	$20.43^{a,b}$
Trichome density (no./unit area)	29.8–51.2	24.4–64.0	42.6–63.6	27.4–60.8	42.77^{b}	41.37^{b}	51.33^{a}	40.93^{b}
Trichome length (μ)	2.5–3.5	1.8–3.9	2.9–4.9	2.6–4.7	$30.01^{b,c}$	2.76^{c}	3.69^{a}	3.2^{b}
Healthy pods/plant (%)	71–100	72–100	70–97	58–99	86.21^{b}	92.34^{a}	82.19^{b}	81.16^{b}

Cl1 = cluster 1, Cl2 = cluster 2, Cl3 = cluster 3 and Cl4 = cluster 4.
[a,b,c] Means were tested by Newman–Keul test and means followed by different letters are significantly different at $P = 0.05$.

length with pod length (0.230) was significant at 5% level only. Leaflet width had highly significant positive correlation with pod length (0.293) and width (0.319). As expected, days to 50% flowering had highly significant positive association with days to 5% maturity (0.869). Pods per raceme had significant negative correlation with healthy pods per plant (−0.213), indicating the high pod damage in racemes having more pods. Pod length had highly significant positive association with pod width (0.618) and 100-seed weight (0.430) and significant positive correlation with seeds per pod (0.231). Pod width had highly significant positive association with 100-seed weight (0.314). Trichome density had highly significant positive association with trichome length (0.294), indicating pods having more trichomes will produce long trichomes. Trichome length had highly significant negative association with healthy pods per plant (−0.293), suggesting that the selection for trichome length and density may not result in selection for pod borer resistance.

Gaps in the collection

The *C. scarabaeoides* collection at ICRISAT genebank comprises 102 accessions from India (57), Indonesia (16), Sri Lanka (16), Australia (6), Fiji (2), Philippines (2), Myanmar (1), United Kingdom (1) and one accession of unknown origin. The results indicated Asia as the major source with 90 accessions. Eight accessions are from Oceania and one accession from Europe. The collection under study included 72 accessions collected in 26 germplasm collection missions launched by ICRISAT and its partners in five countries for ICRISAT mandate crops germplasm during 1975–93. Maximum collections are from India (37), followed by Indonesia (16), Sri Lanka (16), Philippines (2) and Myanmar (1). A total of 30 accessions were introduced from four countries. All accessions from Australia (6), Fiji (2) and United Kingdom (1) are the introductions in the collection. Overlaying of collection sites (76) on predicted probability map revealed that most of the existing collections are from the primary centre of diversity for pigeonpea (Fig. 2). It also revealed high probability (>75%) areas in Asia and Oceania where no collections were made in the past (Figs. 2 and 3). The high probability areas comprise of 118 provinces covering 790 districts in ten countries, which include 25 provinces in Indonesia, 19 provinces in Cambodia, 18 provinces in Vietnam, 15 provinces in India, 12 provinces each in Laos and Philippines, seven provinces in Myanmar, four provinces each in Malaysia and Thailand and two provinces in Nepal. In Bangladesh, seven districts (district is the sub-nation unit in Bangladesh) were identified as gaps. Papua New Guinea was considered as one unit as there was no information on province and districts (Table 5).

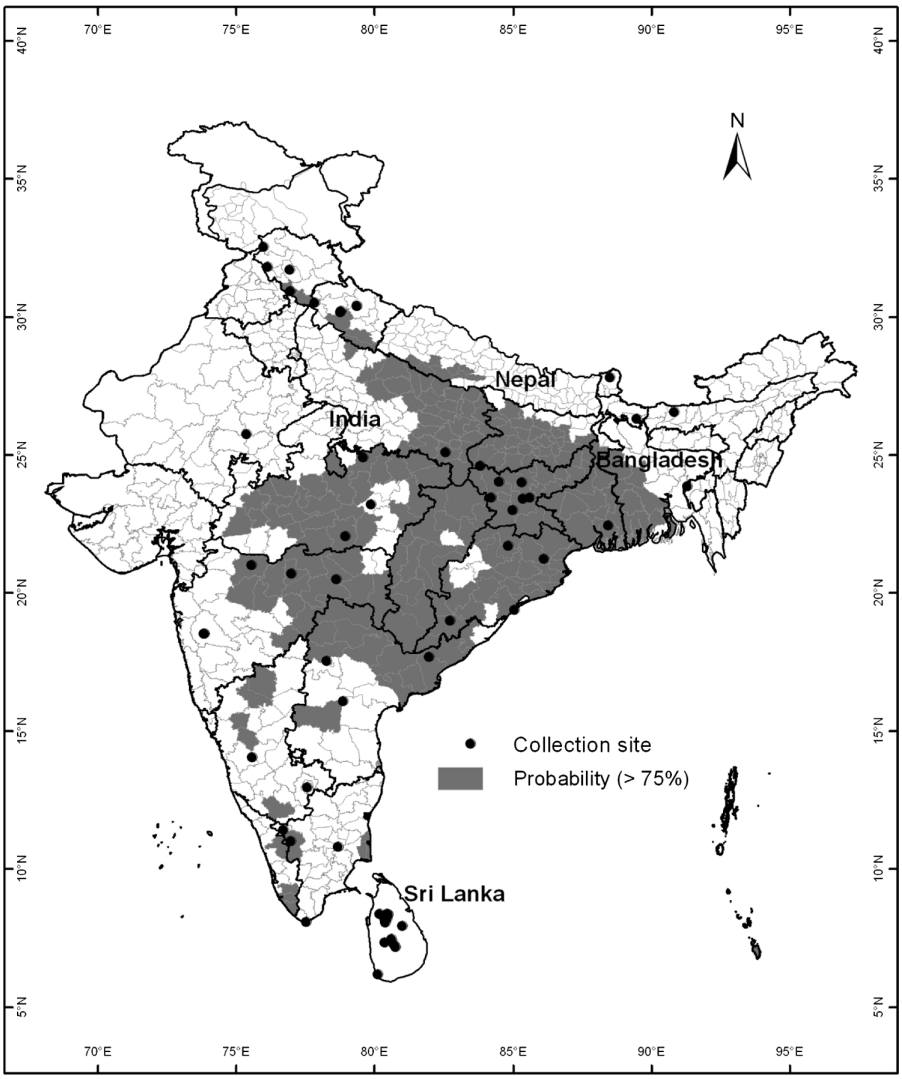

Fig. 2. Geographical gaps (shaded area) identified in Indian subcontinent for *C. scarabaeoides* germplasm assembled at ICRISAT genebank, Patancheru, India.

Discussion

During the year 2009, the average productivity of pigeonpea was 844 kg/ha (FAO, 2009), which is considered as low. Insect pests like pod borer, pod fly, pod wasp, diseases such as wilt, SMD, phytophthora blight, alternaria blight and abiotic stresses such as salinity and water logging conditions are the major causes for low seed yields in pigeonpea. In addition, long duration of crop, low pod setting percent and less number of seeds per pod are the other reasons for low seed yield in pigeonpea. Pod borer alone causes an estimated yield loss of US$1000 million per year globally (Sharma, 2006). Farmers rely upon synthetic insecticides and fungicides to manage the pests and diseases, creating lot of environmental pollution and causing health hazards.

At ICRISAT, efforts of cultivated germplasm screening for resistance sources resulted in identification of 27 accessions resistant to pod borer, 21 accessions resistant to pod fly, 87 accessions resistant to wilt, 362 accessions resistant to SMD, 148 accessions resistant to phytophthora blight, 19 accessions resistant to alternaria blight and 29 accessions tolerant to salinity. *C. scarabaeoides* was found to have higher levels of resistance to pod borer, pod fly, pod wasp, wilt, SMD, phytophthora blight and root-knot nematode, in addition to having high seed protein content (28%) (Kameswara Rao *et al.*, 2003). Upadhyaya (2006) reported early maturity (34 d), high pod setting (74%) and more seeds per pod (4–6 seeds) in *C. scarabaeoides*. Saxena *et al.* (1990) reported near immunity of *C. scarabaeoides* accessions to pod fly. Sharma *et al.* (2001) reported higher levels of resistance to pod

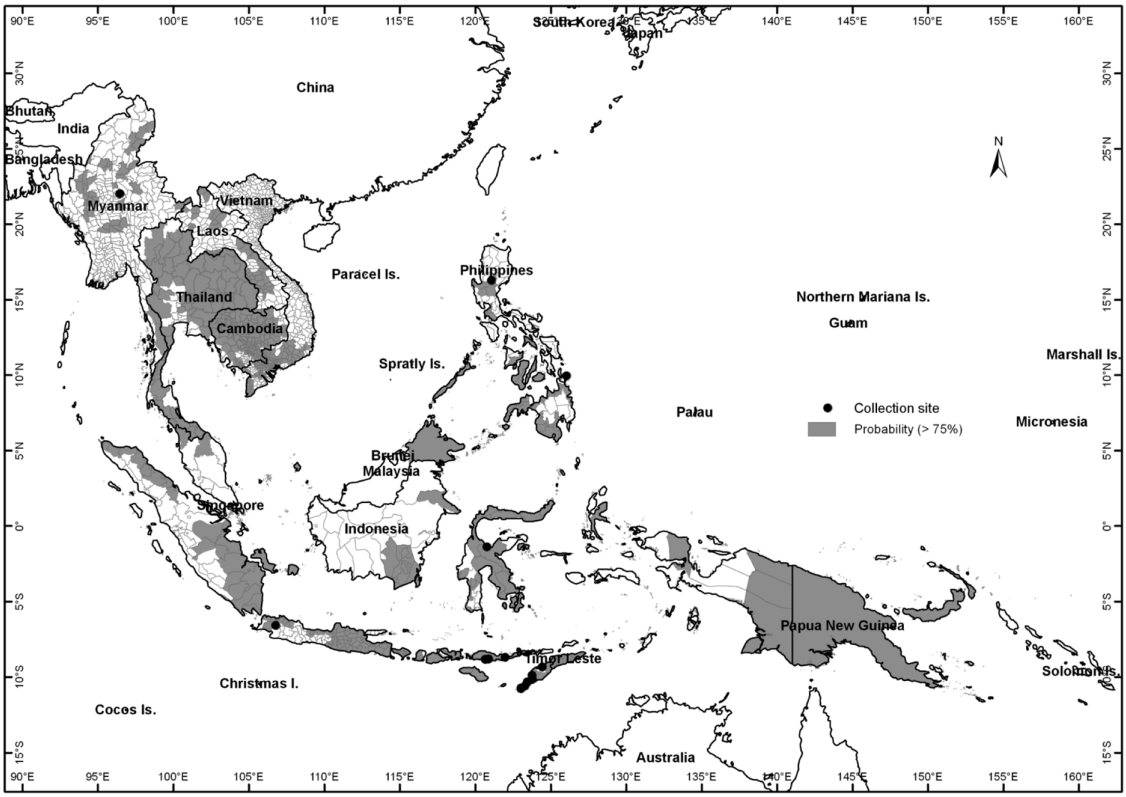

Fig. 3. Geographical gaps (shaded area) identified in East Asian countries for *C. scarabaeoides* germplasm assembled at ICRISAT genebank, Patancheru, India. Is, island.

borer in *C. scarabaeoides*, *Cajanus sericeus* (Benth Ex Bak.) van der Maesen, *Cajanus acutifolius* (F. von Muell.) van der Maesen and *Cajanus Albicans* (W & A) van der Maesen. Sharma *et al.* (1993) reported *C. scarabaeoides* accession ICPW 92 as highly resistant to *Melaidogyne javanica* and ICPW 38 and ICPW 92 as resistant to *Rotylenchulus reniformis* nematode. *C. scarabaeoides* crosses easily with cultivated pigeonpea and many interspecific crosses involving *C. scarabaeoides* and *C. sericeus* have resulted in the development of stable CMS lines (Pundir and Singh, 1985; Tikka *et al.*, 1997). Saxena and Kumar (2003) developed CMS system in pigeonpea using ICPW 89, a germplasm accession of *C. scarabaeoides*. Development of *C. scarabaeoides* based CMS system has shown promise in breaking the yield barriers in pigeonpea. However, issues related to fertility restoration needs to be resolved for making *C. scarabaeoides* based hybrids acceptable to the farmers.

All *C. scarabaeoides* accessions used in the present study are the best sources for extra early (<80 d to 50% flowering) and early maturity (80–100 d to 50% flowering). Eight accessions (ICP 15692, ICP 15696, ICP 15698, ICP 15699, ICP 15712, ICP 15719, ICP 15732 and ICP 15758) and the control, ICP 15695, which produced more than 92% healthy pods per plant, are also found as the good sources for higher number of seeds per pod (4–6 seeds). Accessions in cluster 2, 3 and 4 with low mean values for days to 50% flowering were found as the best sources for early flowering and maturity (Table 2). Accessions in cluster 2 and 3 for seeds per pod and cluster 2 for healthy pods per plant were found as promising sources for use in crop improvement. The genetic potential of CWR in crop improvement is now well demonstrated and is likely to contain the genetic diversity necessary to combat the climate change because of the diversity of habitats in which they grow and wide range of conditions they are adapted to (FAO, 2008). Wide latitudes of collecting sites for accessions under study, varying from 10°75′S in Indonesia to 32°53′N in India, suggested that the *C. scarabaeoides* accessions are from diverse climate and may adapt well to varying climate. For enhanced utilization of *C. scarabaeoides* germplasm in pigeonpea improvement, assembled germplasm needs systematic characterization for morpho-agronomic traits and evaluation for biotic and abiotic stress resistance.

The collection strategy for wild relatives involves difficulties in identifying the geographic distribution of species, precise location, time of maturity, etc. In addition, the wild relatives are rather uncommon in natural vegetation and the available location data are

Table 5. Country-wise provinces showing high probability (>75%) for occurrence of *C. scarabaeoides*

Country	Province	No. of districts
Bangladesh	N.A.	7
Indonesia	Aceh, Bali, D.I Yogyakarta, Dki Jakarta, Irian Jaya, Jambi, Jawa Barat, Jawa Tengah, Jawa Timur, Kalimantan Barat, Kalimantan Selatan, Kalimantan Tengah, Kalimantan Timur, Lampung, Maluku, Nusa Tenggara Barat, Nusa Tenggara Timur, Riau, Sulawesi Selatan, Sulawesi Tengah, Sulawesi Tenggara, Sulawesi Utara, Sumatera Selatan, Sumatera Utara, Timor Timur	184
India	Andaman and Nicobar, Andhra Pradesh, Bihar, Chattisgarh, Himachal Pradesh, Jharkhand, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu, Uttar Pradesh, Uttaranchal, West Bengal	209
Cambodia	Banteay Meanchey, Battambang, Kampong Cham, Kampong Chhnang, Kampong Speu, Kampong Thom, Kampot, Kandal, Koh Kong, Kratie, Mondul Kiri, Phnom Penh, Preah Vihear, Prey Veng, Pursat, Ratana Kiri, Siem Reap, Stung Treng, Svay Rieng	130
Laos	Bolikhamsai, Champassack, Khammouane, Luang Prabang, Namtha, Oudomxay, Phongsaly, Saravane, Savannakhet, Sayabouri, Sekong, Vientiane (Munic.)	44
Myanmar	Chin, Kachin, Magwe, Mandalay, Sagaing, Shan, Tenasserim	35
Malaysia	Kedah, Kelantan, Perlis, Sabah	4
Nepal	Midwest, West	2
Philippines	NCR, Region 1, Region 2, Region 3, Region 4, Region 5, Region 6, Region 7, Region 9, Region 10, Region 11, Region 12	26
Papau New Guinea	N.A.	N.A
Thailand	Central, Northeastern, Northern, Southern	53
Vietnam	An Giang, Ben Tre, Binh Thuan, Can Tho, Dac Lac, Dong Nai, Dong Thap, Ho Chi Minh City, Kien Giang, Lam Dong, Long An, Minh Hai, Ninh Thuan, Son La, Song Be, Tien Giang, Tra Vinh, Vinh Long	96
Total		790

often very old and may not be precise. Physical survey for *C. scarabaeoides* occurrence will be more laborious and require lot of resources. Hence, the use of GIS tools to analyze passport data and corresponding climate data to map the potential distribution of species is a powerful method to assist germplasm collectors and genebank managers. As revealed in the present study, GIS tools have earlier been found to provide more precise cartographic representation of the eco-geographic regions and the germplasm collecting sites (Burle *et al*., 2003). The number of collection sites (76) included in the present study is quite considerable to predict the high probability of *C. scarabaeoides* occurrence. Hernandez *et al*. (2006) reported a 30% prediction success using Bioclim, a GIS tool, with ten samples and this increased to over 80% when 75 samples are used. India is the primary centre of origin for pigeonpea and the probability image generated in the present study for occurrence of *C. scarabaeoides* matched quite close to the origin and dispersal of pigeonpea (Figs. 2 and 3) (van der Maesen, 1990). van der Maesen *et al*. (1984) reported the occurrence of *C. scarabaeoides* in South and Southeast Asia, parts of Oceania, coastal Africa, Madagascar and Jamaica from 0 to 1000 m.a.s.l. It is the most widely spread species of genus *Cajanus* all over India. *C. scarabaeoides* is mostly confined to areas close to the shores of oceans and rivers (van der Maesen, 1980a). New countries identified as potential for *C. scarabaeoides* occurrence include Bangladesh, Cambodia, Laos, Malaysia, Nepal, Papua New Guinea, Thailand and Vietnam. Singh *et al*. (2005) reported that Uttar Pradesh and Uttarakhand (districts of Mirzapur, Bundelkhand region and Tarai), Madhya Pradesh (Rewa, Sidhi, Gwalior, Morena and Bhind region), Maharashtra (Nasik and Dhule) and adjoining North Karnataka, southeastern districts of Bihar, parts of Orissa, Rajasthan, Gujarat, Nilgiri and Ragan hills and the northeast region of India are yet to be surveyed for pigeonpea and its wild relatives. In Uganda, emphasis should be in potential areas such as eastern and northern parts of the country (Singh *et al*., 2005).

There may be some low probability (<75%) areas for the occurrence of *C. scarabaeoides* in countries such as Australia and Fiji. Though Australia was considered as one of the potential countries for *C. scarabaeoides*, it is not adequately represented in the global collection at ICRISAT genbank. Hence, attempts to collect and assemble Australian pigeonpea wild relatives, including *C. scarabaeoides*, should receive high priority before they are lost irretrievably (Singh *et al*., 2005). As per the dispersal of pigeonpea, southeast Africa and Central

American countries, particularly the Caribbean region, are also expected to have pigeonpea wild relatives and countries of these regions are not represented in the world collection (van der Maesen, 1980b; van der Maesen, 1986). Hence, there is a clear need for further collection of *C. scarabaeoides* to cover the full range of geographic diversity.

Many CWR, including *C. scarabaeoides*, are currently threatened with loss of diversity and/or extinction (Stolton *et al*., 2006). Major threats to the CWR include deforestation, logging, plantation agriculture and forestry, industrialization, dryland destruction and desertification, fire hazards, urbanization, civil unrest, mining and quarrying, invasive species and climate change. Therefore, there is an urgent need to assemble as much diversity as possible in *C. scarabaeoides* through introduction of already collected germplasm if any, from different organizations and by launching germplasm collection missions in the potential areas (provinces) identified in the present study as well as in other studies (Table 5). Irrespective of probability of occurrence, collecting for resistant sources should concentrate on areas most favourable for pests and diseases. Disease evaluation data for previously collected *C. scarabaeoides* germplasm should be used to target collecting sites for disease resistance sources (Lenne and David, 1991). Although the information available on *C. scarabaeoides* occurrence through GIS tools is precise, it is important to go through different flora and fauna, catalogues and literature for the distribution of species before embarking on actual germplasm collection. Generally, different species mature at different times. Therefore, the overall knowledge of wild relatives of pigeonpea is essential to collect different species in one collection mission and make the collection mission a success. It is suggested to collect the complete passport data including georeference information while collecting the germplasm. Availability of georeference data is crucial in predicting the probability of occurrence of a particular species in nature, its diversity distribution and the areas of adaptation and identifying geographical gaps in the collection.

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