8 Pigeonpea

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8.1 Introduction

Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is a short-lived perennial shrub that is traditionally cultivated as an annual grain legume crop in tropical and subtropical regions of the world. It is known by various names, such as red gram and congo bean (English), tur and arhar (Hindi), guand (Portuguese), gandul (Spanish), poid d'Angole and poid de Congo (French) and ervilba de Congo in Angola, and is grown primarily as a food crop. Dry whole seed and dehulled and split seed (dhal) are used for cooking various dishes. Besides its use as a food crop, there are also forage, fodder, fuel and medicine uses. The crushed dry seeds are fed to animals, while the green leaves form a quality fodder. In rural areas, dry stems of pigeonpea are used for fuel, thatching, basket-making, etc. The plants are also used to culture lac insects. Pigeonpea has a deep root system which helps it to withstand drought, and is grown on mountain slopes to bind the soil and reduce soil erosion. Due to its deep root system, pigeonpea offers little competition to associated crops and is therefore extensively used in intercropping systems with cereals, such as millets, sorghum and maize; it also provides a good means to improve fertility in fallows. In a cropping season, the plants fix about 40 kg/ha atmospheric nitrogen and add valuable organic matter to the soil through fallen leaves (up to 3.1 t/ha of leaf dry matter) (Rupela, Gowda, Wani, & Ranga Rao, 2004). Its roots help in releasing soil-bound phosphorus to make it available for plant growth. Pigeonpea seed protein content (on average approximately 21%) compares well with that of other important grain legumes. Owing to several unique characteristics and benefits, pigeonpea has become an ideal crop for sustainable agricultural systems in rainfed areas. Because of the large temporal variation (90–300 days) for maturity, four major durations for pigeonpea varieties exist: extra short (mature in <100 days), short (100–120 days), medium (140–180 days) and long duration (>200 days). Each group is suited to a particular agro-ecosystem, which is defined by altitude, temperatures, latitude and day length. Invariably, the traditional pigeonpea cultivars and landraces are long duration types and grown as intercrops with other more early maturing cereals and legumes. Extra short and short varieties have the potential for inclusion as sole crop into rotation as an alternative to rice within the rice–wheat systems of the Indo-Gangetic Plain in

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Asia, especially during periods of water shortage, price incentives and problems of soil fertility. Further, pigeonpea production is affected by several biotic and abiotic stresses. Among biotic factors, important diseases such as sterility mosaic, *Fusarium* wilt (FW), *Phytophthora* blight, root rot, stem canker and *Alternaria* blight in the Indian subcontinent; wilt and *Cercospora* leaf spot in eastern Africa and witches' broom in the Caribbean and Central America cause considerable yield losses. The distribution of these diseases is geographically restricted. For example, sterility mosaic disease (SMD), the most important disease of Indian subcontinent, is not found in eastern Africa. Similarly witches' broom is absent from the two major pigeonpea-growing regions, the Indian subcontinent and eastern Africa. Besides diseases, the seeds and other parts of the plant are fed upon by many insects, with over 200 species having been recorded in India alone. Some of these insects cause sufficient crop losses to be regarded as major pests, but the majority are seldom abundant enough to cause much damage, or are of sporadic or localized importance, and regarded as minor pests. The pod-damaging insects (pod borers and pod fly) cause significant yield losses in pigeonpea and therefore are the most important pests of this crop.

8.2 Origin, Distribution, Diversity and Taxonomy

The name pigeonpea was first reported from Barbados, where the seeds were used to feed pigeons (Plukenet, 1692). There are several theories about the true origin of pigeonpea (reviewed in Saxena, Kumar, Reddy, & Arora, 2003). However, based on the range of genetic diversity of the crop in India, Vavilov (1951) concluded that pigeonpea originated in India. Several authors considered eastern Africa to be the centre of origin of pigeonpea, as it occurs there in wild form. However, based on the large diversity among the crop varieties, the presence of several related wild species, including the progenitor species, linguistic evidence and wide usage in daily cuisine, most of the researchers have agreed on India as the original home of pigeonpea. India is now unequivocally accepted as the primary centre of origin and Africa as the secondary centre of origin of pigeonpea (De, 1974; Royes Vernon, 1976; van der Maesen, 1980). Most probably in the nineteenth century, immigrants from India introduced the crop into East Africa (Hillocks, Minja, Nahdy, & Subrahmanyam, 2000). Thereafter, pigeonpea moved into the Nile valley, then into West Africa and eventually to the Americas (Odeny, 2007). It is now widely grown in the Caribbean region. Further, Reddy (1973) and De (1974) also postulated that the genus *Cajanus* probably originated from an advanced *Atylosia* (now reclassified as *Cajanus*) species through single gene mutation. It is now well known that this advanced species is *C. cajanifolius*, the most probable progenitor of pigeonpea, found only in India. Besides *C. cajanifolius*, 16 species of *Cajanus*, including cultivated species *C. cajan*, occur in India.

At present, pigeonpea is cultivated in the tropical and subtropical areas between 30°N and 30°S latitude on 4.71 million hectares with an annual production of 3.69 million metric tons and productivity of 783 kg/ha (FAOSTAT, 2010). The pigeonpea

Table 8.1 Major Pigeonpea-Growing Countries of the World

Continent	Country	Area (ha)	Productivity (kg/ha)	Production (tonnes)
Asia	Bangladesh	811	951	772
	India	3,530,000	696	2,460,000
	Myanmar	581,200	1246	724,200
	Nepal	21,296	875	18,647
	Pakistan	0		0
	Philippines	684	1244	851
Africa	Burundi	1900	1000	1900
	Comoros	540	592	320
	Democratic Republic of the Congo	10,139	582	5901
	Kenya	158,746	650	103,324
	Malawi	190,437	1013	193,005
	Uganda	98,200	947	93,000
	United Republic of Tanzania	75,000	733	55,000
America	Bahamas	230	565	130
	Dominican Republic	23,461	1068	25,070
	Grenada	640	765	490
	Haiti	7200	333	2400
	Jamaica	723	1036	749
	Panama	4400	447	1969
	Puerto Rico	344	755	260
	Trinidad and Tobago	1300	769	1000
	Venezuela (Bolivarian Republic of)	1900	789	1500
World		4,709,151	783	3,690,488

is widely grown in the Indian subcontinent, which accounts for about 88% of the global pigeonpea production. The major pigeonpea-growing countries in the region are India followed by Myanmar and Nepal. India alone represents about 75% of the area and about 67% of the global pigeonpea production. Africa, including major pigeonpea-growing countries, such as Malawi, Kenya and Uganda, accounts for about 11% of the global production. The Americas and the Caribbean produce about 1% of the total pigeonpea of the world (Table 8.1). Pigeonpea is often cross-pollinated, with an insect-aided natural out-crossing range from 20% to 70% (Saxena, Singh, & Gupta, 1990), with chromosome number $2n=2x=22$ and genome size 1C = 858 Mbp. It belongs to the family Leguminosae, subfamily Papilionoideae, tribe *Phaseoleae* and the subtribe *Cajaninae*. The tribe *Phaseoleae* comprises many edible bean species (*Phaseolus*, *Vigna*, *Cajanus*, *Lablab*, etc.) of which the members of subtribe *Cajaninae* are well distinguished by the presence of vesicular glands on the leaves, calyx and pods. Currently, 11 genera are grouped under the subtribe *Cajaninae*, including *Rhynchosia* Lour., *Eriosema* (DC.), G. Don, *Dunbaria*, W. & A. and *Flemingia* Roxb. ex Aiton, but the cultivated pigeonpea *C. cajan* is the only domesticated species in *Cajaninae*. The word '*Cajanus*' is derived from a Malay word 'katschang' or 'katjang' meaning pod or bean. The members of the earlier

genus *Atylosia* closely resemble the genus *Cajanus* in vegetative and reproductive characters. However, they were relegated to two separate genera mainly on the basis of the presence or absence of seed strophiole. In 1980, van der Maesen revised the taxonomy of both the genera and merged the genus *Atylosia* into *Cajanus* following systematic analysis of morphological, cytological and chemotaxonomical data, which indicated the congenicity of the two genera (van der Maesen, 1980). The revised genus *Cajanus* currently comprises 18 species from Asia, 15 species from Australia and 1 species from West Africa. Of these, 13 are found only in Australia, 8 in the Indian subcontinent, and 1 in West Africa, with the remaining 14 species occurring in more than 1 country. Based on growth habit, leaf shape, hairiness, structure of corolla, pod size and presence of strophiole, van der Maesen (1980) grouped the genus *Cajan* into six sections. The 18 erect species were placed under three sections: seven species in section *Atylosia*, nine species in section *Fruticosa* and two species in section *Cajanu*s, which consists of the cultivated pigeonpea along with its progenitor, *C. cajanifolius*. Eleven climbing and creeping species were arranged in two sections, section *Cantharospermum* (5) and section *Volubilis* (6); the remaining three trailing species were classified under section *Rhynchosoides*. Three *Cajanus* species have been further subdivided into botanical varieties: *C. scarabaeoides* var. *pedunculatus* and var. *scarabaeoides*; *C. reticulatus* var. *grandifolius*, var. *reticulatus*, and var. *maritimus*; and *C. volubilis* var. *burmanicus* and var. *volubilis*.

On the basis of success in hybridization between pigeonpea and its wild relatives, van der Maesen (1990) placed cultigens in the primary gene pool, all 10 cross-compatible species *C. acutifolius*, *C. albicans*, *C. cajanifolius*, *C. lanceolatus*, *C. latisepalus*, *C. lineatus*, *C. reticulatus*, *C. scarabaeoides*, *C. sericeus* and *C. trinervius* in the secondary gene pool, and the cross-incompatible species *C. goensis*, *C. heynei*, *C. kerstingii*, *C. mollis*, *C. platycarpus*, *C. rugosus*, *C. volubilis* and other *Cajaninae* such as *Rhynchosia* Lour., *Dunbaria* W. and A., *Eriosema* (DC.) Reichenb in the tertiary gene pool.

8.3 Erosion of Genetic Diversity from the Traditional Areas

The contribution of landraces as source material for crop improvement has been substantial. In the past, most released pigeonpea varieties have been developed through selection from landraces. To meet the challenges in crop improvement, efforts were made to widen the genetic base by collecting and conserving germplasm across the world before it is lost forever, which led to the assembly of large collections at the national and international gene banks. The gene bank at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), serving as a world repository for genetic resources of its mandate crop including pigeonpea, holds 13,771 accessions from 74 countries. Landraces and wild relatives are the best sources of resistance to the biotic and abiotic stresses and contribute towards food security, poverty alleviation, environmental protection and sustainable development. Plant genetic resources (PGR) are finite and vulnerable to erosion due to the severe threats to world food security of replacement of landraces/traditional cultivars by modern

varieties, natural catastrophes such as droughts, floods, fire hazards, urbanization and industrialization, and habitat loss due to irrigation projects, overgrazing, mining and climate change (Upadhyaya & Gowda, 2009). Therefore, there is an urgent need to assess the existing collection to identify geographical, trait-diversity and taxonomical gaps for planning future collection strategies for pigeonpea.

8.4 Status of Germplasm Resources Conservation

The CGIAR consortium represents the largest concerted effort towards collecting, preserving and utilizing global agricultural resources. CGIAR holds nearly 760,000 samples of the estimated 7.4 million accessions of different crops preserved globally (FAOSTAT, 2010). There are a number of gene banks conserving the pigeonpea germplasm worldwide. ICRISAT has the global responsibility of collecting, conserving and distributing the pigeonpea germplasm comprising of landraces, modern cultivars, genetic stocks, mutants and wild *Cajanus* species. It contains 13,216 accessions of cultivated pigeonpea and 555 accessions of wild species in the genus *Cajanus* from 60 countries. The collection includes 8315 landraces, 4830 breeding materials, 71 improved cultivars and 555 wild accessions. This is the single largest collection of pigeonpea germplasm assembled at any one place in the world. India is the major contributor with 9200 accessions. These accessions came from donations as well as from collecting missions launched in different countries. Other major gene banks holding pigeonpea germplasm are the National Bureau of Plant Genetic Resources (12,900 accessions), New Delhi, India; All India Coordinated Research Project on Pigeonpea (5195 accessions); NBPGR Regional Station Akola (2268 accessions), India; Indian Agricultural Research Institute (IARI; 1500 accessions), New Delhi and the National Gene Bank of Kenya, Crop Plant Genetic Resources Centre (1380 accessions), Muguga, Kenya (Table 8.2).

Table 8.2 Major Gene Banks Holding Pigeonpea Germplasm

Country	Institute	Wild	Cultivated	Total
Australia	Australian Tropical Crops and Forages Genetic Resources Centre	352	406	758
Brazil	Embrapa Recursos Genéticos e Biotecnologia	3	279	282
Colombia	Centro Internacional de Agricultura Tropical	623	135	758
Ethiopia	International Livestock Research Institute	539	143	682
India	All India Coordinated Research Project on Pigeonpea		5195	5195
	Indian Agricultural Research Institute		1500	1500
	ICRISAT	555	13,216	13,771
	National Bureau of Plant Genetic Resources	41	12,859	12,900
	Regional Station Akola, NBPGR		2268	2268

(*Continued*)

Table 8.2 (Continued)

Country	Institute	Wild	Cultivated	Total
Indonesia	National Biological Institute		200	200
Kenya	National Genebank of Kenya, Crop Plant Genetic Resources Centre – Muguga	92	1288	1380
Nepal	Nepal Agricultural Research Council		228	228
Philippines	Institute of Plant Breeding, College of Agriculture, University of the Philippines, Los Baños		629	629
Thailand	Thailand Institute of Scientific and Technological Research		201	201
Uganda	Serere Agriculture and Animal Production Research Institute		200	200

8.5 Germplasm Characterization and Evaluation

Germplasm collection is of little value unless it is characterized, evaluated and documented properly to enhance its utilization in crop improvement. A multidisciplinary approach is followed at ICRISAT gene bank; the data generated in various disciplines are fed to the pigeonpea germplasm characterization database. The characterization was done at the ICRISAT Research Farm in Patancheru on 18 qualitative characters (plant vigor, growth habit, plant pigmentation, stem thickness, flower base colour, streak colour, streak pattern, flowering pattern, pod colour, pod shape, pod hairiness, seed colour pattern, primary seed colour, secondary seed colour, seed eye colour, seed eye colour width, seed shape and seed hilum) and 16 quantitative characters were recorded following the 'Descriptors for Pigeonpea' (IBPGR & ICRISAT, 1993). Observations on all qualitative and six quantitative characters (days to 50% flowering, days to 75% maturity, 100-seed weight, harvest index, shelling percentage and plot seed yield) were recorded on a plot basis. Observations on the remaining 10 quantitative traits (leaf size, plant height, number of primary, secondary and tertiary branches, number of racemes, pod bearing length, pods per plant, pod length, seeds per pod) were recorded on three representative plants from each plot. To realize the true potential of the accessions and to facilitate the selection of genotypes by researchers, sets of selected pigeonpea germplasm, such as core and mini-core collections, were evaluated for important agronomic characters at different locations in India and several other countries in Africa during suitable seasons.

8.5.1 Diversity in the Collection

To study the geographical patterns of diversity in the collection, data of 14 qualitative and 12 quantitative traits of 11,402 accessions from 54 countries were analysed. The accessions were grouped based on geographical proximity and similarity of climate (Reddy, Upadhyaya, Gowda, & Singh, 2005; Upadhyaya, Pundir, Gowda, Reddy,

Table 8.3 Range of Variation for Important Agronomic Traits in the World Collection of Pigeonpea at ICRISAT Gene Bank, Patancheru, India

Character	Mean	Minimum	Maximum
Days to 50% flowering	133.5	52	237
Days to 75% maturity	192.1	100	299
Plant height (cm)	177.9	39	310
Primary branches (no.)	13.5	1	107
Secondary branches (no.)	31.3	0	145.3
Tertiary branches (no.)	8.8	0	218.7
Racemes per plant (no.)	150.3	6	915
Pod length (cm)	5.7	2.5	13.1
Pods per plant (no.)	287.3	9.3	1819.3
Seeds per pod (no.)	3.7	1.6	7.2
100-seed weight (g)	9.3	2.7	25.8
Seed protein (%)	21.3	13	30.8

& Singh, 2005). Large variation was observed in the entire collection for important agronomic traits (Table 8.3). The range of variation for quantitative traits in respect to the different regions was maximum for group AS 4 (south India, Maldives and Sri Lanka) and minimum for germplasm accessions from Europe and Oceania. The region AS 4 encompasses the area of the primary centre of diversity of pigeonpea; therefore, the high variation in the germplasm from that region is not surprising (Upadhyaya et al., 2005). The accessions from Africa were of longer duration, tall and producing large seeds. Accessions from India had medium plant height, high pod number, medium duration and high seed yield. Accessions from Oceania were conspicuous in their short growth duration, short height, few branches, small seeds and low seed yield. Shannon–Weaver diversity index (H') (Shannon & Weaver, 1949) indicates that the accessions from AS 6 (Indonesia, Philippines and Thailand) had the highest pooled H' for qualitative traits (0.349 + 0.059) and accessions from Africa the highest for quantitative traits (0.613 + 0.006) (Upadhyaya et al., 2005). African accessions also had highest pooled H' (0.464 + 0.039) over all the traits. The accessions from Oceania had the lowest pooled H' (0.337 + 0.037). The H' values across the regions were highest for primary seed colour (0.657 + 0.050) followed by flower streak pattern, seed protein content and shelling percentage, whereas it was lowest for flowering pattern (0.087 + 0.026). A hierarchical cluster analysis conducted on the first three PC scores (92.28% variation) resulted in three clusters. Cluster 1 comprised accessions from Oceania (60 accessions), cluster 2 comprised accessions from AS 1–5 containing 9648 accessions and cluster 3 comprised accessions from Africa, America, Caribbean countries, Europe and AS 6 containing 1694 accessions (Figure 8.1) (Upadhyaya et al., 2005). Semi-spreading growth habit, green stem colour, indeterminate (NDT) flowering pattern and yellow flower were predominant among the qualitative traits. Primary seed colour had maximum variability; orange colour

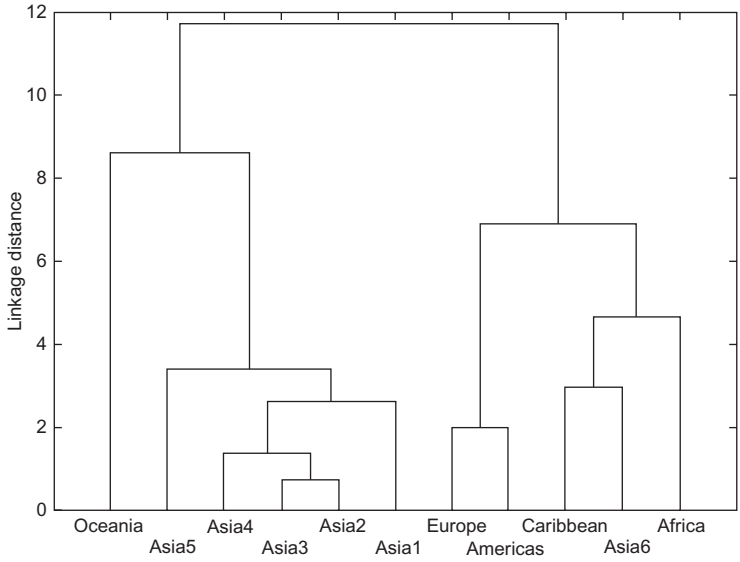

Figure 8.1 Dendrogram of 11 regions in the entire pigeonpea germplasm based on scores of the first three principal components (92.3% variation).

followed by cream were the two most frequent second colours in the collection. At ICRISAT a large number of pigeonpea accessions were tested for biotic and abiotic stresses and promising sources for resistance were identified.

8.6 Germplasm Maintenance

The ICRISAT gene bank ensures maintenance of germplasm at international standards and the continued availability of good-quality seeds of its mandate crops for research and development globally. Maintenance of germplasm includes maintenance of seed viability and seed quantity in the gene bank. Seed viability and quantity of germplasm accessions in medium-term store are monitored at regular intervals. Accessions are regenerated when the seed viability is below 85% and/or seed quantity <100 g in medium-term store. Regeneration is the crucial process in gene bank management. Accessions with poor quality are given top priority. Objectives for regeneration include maximizing seed quality, optimizing seed quantity and maintaining the genetic integrity of accessions. Pigeonpea floral biology favors self-pollination. However, it is considered an often cross-pollinating species without crossing ranging from 20 to 70%, due to visits by bees (Saxena et al., 1990). Therefore, it is essential to preserve the accessions' integrity using effective pollination control methods. Controlling pollination is the most crucial part of the regeneration process. Methods to control pollination include: bagging individual plants, growing accessions in isolation, growing barrier crops, growing under

Figure 8.2 Field view of growing pigeonpea germplasm under insect-proof cages for regeneration.

insect-proof cages, 'polyhouses', etc. But the most common procedure is covering individual plants using muslin cloth bags and growing accessions under insect-proof cages (Figure 8.2). The pollination control method of growing accessions under insect-proof cages was three times cheaper than the traditional method of bagging individual plants. However, the regeneration cost depends largely on method of pollination control, availability and cost of materials in local markets, labour wages, quantity of seed required per accession in one cycle of regeneration, type of material to be regenerated, etc. Due to increased seed yield per plant, we can minimize the regeneration frequency (Reddy, Upadhyaya, Reddy, & Gowda, 2006). Minimizing the regeneration requirement of each accession can reduce maintenance costs of the total collection. Therefore, pigeonpea germplasm accessions are grown under insect-proof cages for regeneration at ICRISAT Research Farm, Patancheru, during the rainy season. In order to minimize the damage to the nylon net used for the cages by reducing the vegetative growth, particularly plant height, accessions are sown later during the crop season, during the first week of August, in Alfisol fields. Remanandan, Sastry, and Mengesha Melak (1988) reported that sowing pigeonpea in Alfisols close to the shortest day of the year results in reduced plant height. Each accession is grown on a single 9-m-long ridge, spaced 75 cm apart. Plant to plant spacing is 25 cm, accommodating about 72 plants in 36 hills. Adequate plant protection measures are taken inside the cage to reduce damage by pests and diseases.

At maturity, individual plants are harvested and an equal quantity of seeds from each plant is bulked to reconstitute the accession.

8.6.1 Regeneration of Wild Pigeonpea Germplasm

Seeds of almost all species require scarification by making a small cut to the seed coat to improve water absorption and germination. Seeds are treated with Thiram or any other appropriate fungicide and initially sown in small cups or pots and transplanted to the field when they have three to four leaves. Climbers, such as *C. albicans*, *C. mollis* and *C. crassus*, are provided support using bamboo sticks or iron poles. At maturity, pods from individual plants are harvested and threshed, and seeds are cleaned. An equal quantity of seed from each plant is bulked to reconstitute an accession (Upadhyaya & Gowda, 2009).

8.6.2 Documentation

All information, such as method of viability test, initial viability, seed quantity, as well as the year of regeneration, pollination control method used, regeneration site, accession, field number, accession verification, number of plants harvested and seed quantity obtained are recorded and documented (Upadhyaya & Gowda, 2009).

8.7 Use of Germplasm in Crop Improvement

The small subsets, such as core and mini-core collections, are now international public goods and used by scientists globally. Many national programmes have shown interest in the mini-core collection and ICRISAT has supplied 19 sets of pigeonpea mini-core to National Agricultural Research Systems (NARS) in India (17), UAE (1) and USA (1). Using the mini-core collection, scientists at ICRISAT and NARS partners have identified several promising sources for agronomic, nutritional, biotic and abiotic traits (Upadhyaya, Dronavalli, Gowda, & Singh, 2012).

8.7.1 Biotic Stresses

8.7.1.1 Resistance to Diseases

Evaluation of a mini-core collection has resulted in the identification of six accessions (ICP 6739, ICP 8860, ICP 11015, ICP 13304, ICP 14638 and ICP 14819) resistant to FW (Sharma et al., 2012) and 24 accessions (ICP 3451, ICP 6739, ICP 6845, ICP 7869, ICP 8152, ICP 8860, ICP 9045, ICP 11015, ICP 11059, ICP 11230 and others) resistant to SMD (Sharma et al., 2012).

8.7.1.2 Resistance to Insects

Evaluation of a mini-core collection has resulted in the identification of 11 accessions (ICP 7, ICP 655, ICP 772, ICP 1071, ICP 3046, ICP 4575, ICP 6128, ICP

8860, ICP 12142, ICP 14471 and ICP 14701) reported moderately resistant to pod borer (damage rating 5.0 as compared to 9.0 in ICPL 87) under unprotected conditions, and also had no wilt incidence as compared to 38.2% wilt in ICP 8266 (ICRISAT Archival Report, 2010).

8.7.2 Abiotic Stresses

8.7.2.1 Waterlogging

Evaluation of a pigeonpea mini-core collection resulted in the identification of 23 accessions (ICP 1279, ICP 4575, ICP 5142, ICP 6370, ICP 6992, ICP 7057 and others) recorded tolerant to waterlogging conditions (Krishnamurthy, Upadhyaya, Saxena, & Vadez, 2011).

8.7.2.2 Salinity

Evaluation of a pigeonpea mini-core collection resulted in the identification of 16 accessions (ICP 2746, ICP 3046, ICP 6815, ICP 7260, ICP 7426, ICP 7803, ICP 8860 and others) selected for tolerance to salinity (Srivastava, Vadez, Upadhyaya, & Saxena, 2006).

8.7.3 Agronomic Traits

Evaluation of a pigeonpea mini-core collection resulted in the identification of eight accessions (ICP 1156, ICP 9336, ICP 14471, ICP 14832, ICP 14900, ICP 14903, ICP 15068 and ICP 16309) for early flowering (<85 days); three accessions (ICP 13139, ICP 13359 and ICP 14976) for large seed size (>15g/100 seed); one accession (ICP 8860) for more primary branches (>29) and three accessions (ICP 4167, ICP 8602 and ICP 11230) for high pod number per plant (>200 pods/plant) (Upadhyaya, Yadav, Dronavalli, Gowda, & Singh, 2010).

8.7.4 Nutritional Traits

Evaluation of a pigeonpea mini-core collection resulted in the identification of six accessions (ICP 4575, ICP 7426, ICP 8266, ICP 11823, ICP 12515 and ICP 12680) for high seed protein (>24%); eight accessions (ICP 4029, ICP 6929, ICP 6992, ICP 7076, ICP 10397, ICP 11690, ICP 12298 and ICP 12515) for high seed iron (>40 ppm) and four accessions (ICP 2698, ICP 11267, ICP 14444 and ICP 14976) for high seed zinc (>40 ppm).

8.8 Limitations in Germplasm Use

Very few germplasm accessions (<1%) have been used by plant breeders in crop improvement programmes (Upadhyaya, 2008). A large gap exists between availability

and actual utilization of the germplasm. This was true both in the international programmes (CGIAR institutes) as well as in the national programmes. Extensive use of fewer and closely related parents in crop improvement could result in vulnerability of cultivars to pests and diseases. The main reason for low use of germplasm in crop improvement programmes is the lack of information on the large number of accessions, particularly for traits of economic importance, which display a great deal of genotype×environment interaction and require multilocation evaluation. To overcome the difficulties with large collections, ICRISAT scientists have developed a 'core collection' consisting of 1290 accessions (about 10% of entire collection), representing the genetic variability of the entire collection (Reddy et al., 2005).

When the entire collection is over 10,000 accessions, developing a core collection will not solve the problem of low use of germplasm, as even the size of the core collection would be unwieldy for meaningful evaluation and convenient exploitation. To overcome this, a seminal two-stage strategy was followed. The first stage involves developing a representative core collection (about 10%) from the entire collection using all the available information on origin, geographical distribution, and characterization and evaluation data of accessions. The second stage involves evaluation of the core collection for various morphological, agronomic and quality traits, and selecting a further subset of about 10% accessions from the core collection. Thus, the mini-core collection contains 10% of the core or approximately 1% of the entire collection and represents the diversity of the entire collection (Upadhyaya & Ortiz, 2001). In pigeonpea, a mini-core collection consisting of 146 accessions was constituted by evaluating a core collection of 1290 accessions for 34 morpho-agronomic traits (Upadhyaya, Reddy, Gowda, Reddy, & Singh, 2006). Due to their greatly reduced size, mini-core collections provide an easy access to the germplasm collections and scientists can evaluate the mini-core collection easily and economically for traits of economic importance.

8.9 Germplasm Enhancement Through Wide Crosses

Narrow genetic diversity in cultivated germplasm has hampered the effective utilization of conventional breeding as well as development and utilization of genomic tools, resulting in pigeonpea being often referred to as an 'orphan crop legume'. A number of wild *Cajanus* species, especially those from the secondary gene pool which are cross-compatible with cultivated pigeonpea, have been used for the genetic improvement of pigeonpea. The most significant achievement is the development of unique cytoplasmic nuclear male sterility systems (CMS). The CMS systems have been developed with cytoplasm derived from cultivated and wild *Cajanus* species. The A_1 cytoplasm is derived from *C. sericeus* (Ariyanayagam, Nageshwara, & Zaveri, 1995). The CMS lines derived from this source are temperature sensitive and the male sterile lines restore fertility under low temperature conditions (Saxena, 2005). The A_2 cytoplasm derived from *C. scarabaeoides* (Saxena & Kumar, 2003; Tikka, Parmar, & Chauhan, 1997) is a stable source of CMS but the fertility restoration (fr) is not consistent across environments, making it unsuitable for hybrid

seed production. A_3 cytoplasm derived from *C. volubilis* (Wanjari, Patil, Manapure, Manjaya, & Manish, 2001) has a poor-quality fr system. The A_4 cytoplasm derived from *C. cajanifolius* (Saxena et al., 2005) is stable across environments with a good fr system and has been used to develop the world's first commercial pigeonpea hybrid, ICPH 2671 (Saxena et al., 2013). The A_5 cytoplasm derived from *C. cajan* (Mallikarjuna & Saxena, 2005) is still under development. The A_6 cytoplasm has been derived from *C. lineatus* and at present this CMS source is in BC_5F_1 generation with a perfect male sterility maintenance system available (Saxena, Sultana et al., 2010). The studies on A_7 CMS system derived from *C. platycarpus* are in progress. Recently, the A_8 CMS system derived from *C. reticulatus* has also been developed, but the detailed studies on this CMS system are in progress at ICRISAT.

Wild *Cajanus* species, especially, *C. scarabaeoides*, *C. acutifolius*, *C. platycarpus*, *C. reticulates*, *C. sericeus* and *C. albicans* have been reported to have resistance to pod borer, *Helicoverpa armigera* (Rao, Reddy, & Bramel, 2003; Sharma, Sujana, & Rao, 2009; Sujana, Sharma, & Rao, 2008). At ICRISAT, utilization of *C. acutifolius* as the pollen parent has resulted in the development of advanced generation population having resistance to pod borer (Mallikarjuna, Sharma, & Upadhyaya, 2007), variation in seed colour and high seed weight. Evaluation of wild *Cajanus* species has identified accessions having resistance to *Alternaria* blight (Sharma, Kannaiyan, & Saxena, 1987), *Phytophthora* blight (Rao et al., 2003), sterility mosaic virus (Kulkarni et al., 2003; Rao et al., 2003), pod fly (Rao et al., 2003; Saxena et al., 1990), pod fly and wasps (Sharma, Pampapathy, & Reddy, 2003), root-knot nematodes (Rao et al., 2003; Sharma, 1995; Sharma, Remanandan, & Jain, 1993; Sharma, Remanandan, & McDonald, 1993), and tolerance to salinity (Rao et al., 2003; Srivastava et al., 2006; Subbarao, 1988; Subbarao, Johansen, Jana, & Rao, 1991), drought (Rao et al., 2003), and photoperiod insensitivity (Rao et al., 2003).

Besides for CMS systems and as resistant/tolerant sources for biotic/abiotic stresses, utilization of wild *Cajanus* species has also contributed significantly towards the improvement of agronomic performance and nutritional quality of cultivated pigeonpea. Some wild *Cajanus* species, namely *C. scarabaeoides*, *C. sericeus*, *C. albicans*, *C. crassus*, *C. platycarpus* and *C. cajanifolius*, have higher seed protein content (average 28.3%) compared to pigeonpea cultivars (24.6%) (Singh & Jambunathan, 1981). A high protein line, ICPL 87162, was developed from the cross *C. cajan*×*C. scarabaeoides* (Reddy et al., 1997). This line contains 30–34% protein content compared to the control cultivar (23% protein). Breeding lines with high protein content have also been developed from *C. sericeus*, *C. albicans* and *C. scarabaeoides*. Utilization of wild *Cajanus* species has resulted in the development of several lines, such as HPL 2, HPL 7, HPL 40 and HPL 51, having high protein and high seed weight (Saxena, Faris, & Kumar, 1987). Recently, scientists at ICRISAT have generated segregants with high seed weight from the crosses between cultivated pigeonpea and *C. acutifolius*. Using wild *Cajanus* species, viable hybrids have been produced between pigeonpea and *C. platycarpus* (Mallikarjuna & Moss, 1995), *C. reticulatus* var. *grandifolius* (Reddy, Kameswara Rao, & Saxena, 2001), *C. acutifolius* (Mallikarjuna & Saxena, 2002) and *C. albicans* (Subbarao, Johansen, Kumar Rao, & Jana, 1990).

8.10 Pigeonpea Genomic Resources

Pigeonpea breeders have developed varieties with several attributes with a major focus on productivity traits and as a result diversity has been lost in the elite gene pool; subsequently yield levels in pigeonpea have been stagnant during the last six decades. In order to meet future challenges and to enhance the yield levels, genomics interventions are required to identify the genes or quantitative trait loci (QTLs) responsible for resistance or tolerance to various economically important traits. A large amount of genomic and genetic resources have been developed by ICRISAT in collaboration with partners and have regularly been used in accelerating the genomics and breeding applications to increase the efficiency of pigeonpea improvement programmes. ICRISAT scientists have developed a number of marker systems and genetic linkage maps and identified marker-trait associations for a few important traits. Recently complete genome sequencing of pigeonpea has been accomplished (Varshney et al., 2012).

8.10.1 Mapping Populations

Genetic diversity among elite pigeonpea cultivars is very low (Saxena, Sultana et al., 2010) and hence selection of crossing parents is the most crucial step. In order to select a diverse set of parents, simple sequence repeats (SSRs) genotyping of elite cultivars was performed and a number of intraspecific biparental mapping populations, segregating for FW, SMD and fr have been developed (Saxena, Prathima et al., 2010; Saxena, Saxena, Kumar, Hoisington, & Varshney, 2010). One interspecific [ICP 28 (*C. cajan*)×ICPW 94 (*C. scarabaeoides*)] mapping population has also been developed (Saxena et al., 2012).

8.10.2 Molecular Markers

Recently several marker systems have been developed and used in pigeonpea (Table 8.4). Prior to PCR technologies, restriction fragment length polymorphisms (RFLPs) (Sivaramakrishnan, Seetha, & Reddy, 2002), protein isoforms and phenotypes were used. However, these markers present challenges for large-scale throughput because they are labour intensive, require large amounts of starting material (genomic DNA or protein) and are less informative as compared to the modern marker systems. The vast majority of markers now used for pigeonpea are PCR based, with the majority being microsatellite markers (SSR) (Bohra et al., 2011; Burns, Edwards, Newbury, Ford-Lloyd, & Baggott, 2001; Odeny et al., 2007; Saxena, Prathima et al., 2010; Saxena, Saxena, Kumar et al., 2010; Saxena, Saxena, & Varshney, 2010). Other potential marker systems, such as random amplified polymorphic DNA (RAPD) markers (Malviya & Yadav, 2010), single strand conformation polymorphisms (SSCPs) (Kudapa et al., 2012), amplified fragment length polymorphisms (AFLPs) (Panguluri, Janaiah, Govil, Kumar, & Sharma, 2006) and DArT (Yang et al., 2006, 2011) are also in use. By using an SSR-enriched library, several genomic DNA libraries enriched for di- and tri-nucleotide repeat motifs

Table 8.4 Available Genomic Resources in Pigeonpea

Resource		References
Simple sequence repeats	29,000	Raju et al. (2010), Saxena, Sultana et al. (2010), Bohra et al. (2011), Dutta et al. (2011) and Varshney et al. (2012)
Single nucleotide polymorphisms (SNPs)	35,000	Saxena et al. (2012) and Varshney et al. (2012)
GoldenGate assays	768 SNPs	Unpublished
KASPar assays	1616 SNPs	Saxena et al. (2012)
Single feature polymorphisms (SFPs)	1131	Saxena et al. (2011)
Diversity arrays technology (DArT) markers	15,360	Yang et al. (2011)
Sanger ESTs	~20,000	Raju et al. (2010) and Dubey et al. (2011)
454/FLX reads	496,705	Dubey et al. (2011)
Tentative unique sequences (TUSs)	21,432	Dubey et al. (2011)
Illumina/454 reads (million reads)	>160	Dubey et al. (2011), Dutta et al. (2011) and Kudapa et al. (2012)

(CT, TG, AG, AAG, TCG, etc.) were also generated (Burns et al., 2001; Odeny et al., 2007; Saxena, Saxena, & Varshney, 2010). This approach involving SSR marker development has provided only 36 SSRs; however, subsequently SSRs were developed from bacterial artificial chromosome (BAC) end sequences (BESs) and found more effective. SSR development from BAC ends avoids the need for prior information about the repeat motifs within a species and offers genome-wide coverage. After examining 87,590 pigeonpea BESs, a total of 18,149 SSRs were identified in 14,001 BESs representing 6590 BAC clones. Excluding the mononucleotide repeats, a total of 3072 primer pairs were synthesized and tested (Bohra et al., 2011). The recent advent of affordable high-throughput technology for single nucleotide polymorphisms (SNPs), together with the reduction in sequencing costs, is resulting in a shift to SNP markers for trait mapping and association studies (Thudi, Li, Jackson, May, & Varshney, 2012). It is expected that within a couple of years the marker-based studies will be dominated by SNP markers. Three approaches were used for the identification of SNPs in pigeonpea. In the first approach, Illumina sequencing was carried out on parental genotypes of mapping populations of pigeonpea. RNA sequencing of 12 pigeonpea genotypes resulted in 128.9 million reads for pigeonpea (Kudapa et al., 2012). Alignment of these short reads onto transcriptome assembly (TA) has provided a large number of SNPs. The second approach, allele-specific sequencing of parental genotypes of the reference mapping population of pigeonpea using conserved orthologous sequence (COS) markers, has provided 768 SNPs for pigeonpea (Table 8.4). As a result, a large number of SNPs has become available for pigeonpea and cost-effective genotyping platforms have been developed.

8.10.3 Genotyping the Germplasm Collection

A composite collection of 1000 accessions was developed and profiled using 20 SSR markers. Analysis of molecular data for 952 accessions detected 197 alleles, of which 115 were rare and 82 common. Gene diversity varied from 0.002 to 0.726. There were 60 group-specific unique alleles in wild types and 64 in cultivated. Among the cultivated accessions, 37 unique alleles were found in NDT types. Geographically, 32 unique alleles were found in Asia 4 (southern Indian provinces, Maldives and Sri Lanka). Only two alleles differentiated Africa from other regions. Wild and cultivated types shared 73 alleles, DT (determinate) and NDT shared 10, DT and wild shared 4, and the NDT and wild shared 20 alleles. Wild types as a group were genetically more diverse than cultivated types. NDT types were more diverse than the other two groups based on flowering pattern (DT and SDT: semi-determinate). Reference sets consisting of the 300 most diverse accessions based on SSR markers, qualitative traits, quantitative traits and their combinations were formed and compared for allelic richness and diversity. A reference set based on SSR data captured 187 (95%) of the 197 alleles of the composite collection. Another reference set based on qualitative traits captured 87% of the alleles of the composite set. This demonstrates that both SSR markers and qualitative traits were equally efficient in capturing the allelic richness and diversity in the reference sets (Upadhyaya et al., 2008).

8.10.4 Linkage Maps and Trait Mapping

The first generation pigeonpea linkage map or reference map was developed using DArT markers for an interspecific mapping population (ICP 28×ICPW 94) of 79 F_2 individuals. The map is available in male and female forms, a total of 121 unique DArT maternal markers were placed on the maternal linkage map and 166 unique DArT paternal markers were placed on the paternal linkage map. The length of these two maps covered 437.3 cM and 648.8 cM, respectively (Yang et al., 2011). Another version of reference linkage map consisted of 239 SSR markers and spans 930.90 cM (Bohra et al., 2011). An interspecific mapping population (ICP 28×ICPW 94) relatively bigger in size (167 F_2s) was used for developing a comprehensive genetic map comprising 875 SNP loci (Saxena et al., 2012). The total length of this map was 967.03 cM with an average marker distance of 1.11 cM. This linkage map was a considerable improvement over the previous pigeonpea genetic linkage maps using SSR and DArT markers.

Construction of genetic maps for intraspecific mapping populations has also been performed and a total of six SSR-based intraspecific genetic maps were developed by using six F_2 mapping populations (Bohra et al., 2012; Gnanesh et al., 2011). Furthermore, all six intraspecific genetic maps were joined together into a single consensus genetic map providing map positions to a total of 339 SSR markers at map coverage of 1059 cM (Bohra et al., 2012). A few trait association efforts have been reported in pigeonpea for SMD and fr by using F_2 mapping populations. For instance, six QTLs explaining phenotypic variations in the range of 8.3–24.72%

(Gnanesh et al., 2011) for SMD and a total of four large effect QTLs explaining up to 24% of phenotypic variations for fr in pigeonpea (Bohra et al., 2012) were identified.

8.10.5 Transcriptomic Resources

To characterize the pigeonpea transcriptome, two NGS technologies, namely 454- and Illumina together with Sanger sequencing technology have been used. By using Sanger sequencing technology on FW and SMD, challenged cDNA libraries for pigeonpea 9888 expressed sequence tags (ESTs) were developed (Raju et al., 2010). To improve these transcriptomic resources further, 454/FLX sequencing was undertaken on normalized and pooled RNA samples collected from >20 tissues, generating 494,353 transcript reads for pigeonpea (Dubey et al., 2011). Cluster analysis of these transcript reads with Sanger ESTs generated at ICRISAT, as well as those available in the public domain, provided the first transcript assembly (TA) of pigeonpea (CcTA v1) with 127,754 transcriptional units (Dubey et al., 2011). 494,353 454/FLX transcript reads generated from Asha genotype and 128.9 million Illumina reads generated from 12 genotypes were analysed together with 18,353 Sanger ESTs and 1.696 million 454/FLX transcript reads (Dutta et al., 2011) with improved algorithms. As a result, an improved TA in pigeonpea referred to as CcTA v2, comprising 21,434 contigs, has been developed (Kudapa et al., 2012) (Table 8.4).

8.10.6 Genome Sequence

NGS (Illumina) was used to generate 237.2 Gbp of sequence that, along with Sanger-based BAC-end sequences and a genetic map, was assembled into scaffolds representing about 73% (605.78 Mb) of the 833 Mbp pigeonpea genome size. Genome analysis has resulted in the identification of 48,680 pigeonpea genes. High levels of synteny were observed between pigeonpea and soybean as well as between pigeonpea and *Medicago truncatula* and *Lotus japonicas*.

The genome sequence was also searched for the presence of tandem repeats and a total of 23,410 SSR primers were designed. Transcript reads from 12 different pigeonpea genotypes were aligned with the genome assembly for the identification of SNPs. As a result 28,104 novel SNPs were identified across 12 genotypes (Varshney et al., 2012). These developed resources will be used for germplasm characterization and to facilitate the identification of the genetic basis of important traits.

8.11 Conclusions

The narrow genetic base of pigeonpea, coupled with its susceptibility to a number of biotic and abiotic stresses, necessitates the use of diverse genetic resources for its improvement. Though a large number of germplasm accessions are conserved in different gene banks globally, only a small fraction (<1%) has been used in crop

improvement programmes. The availability of trait-specific germplasm accessions will provide an opportunity for breeders to use new sources of variations in developing new cultivars with a broad genetic base. The utilization of wild *Cajanus* species has contributed significantly to the genetic enhancement of pigeonpea by providing resistance/tolerance to diseases, insect pests and drought, as well as good agronomic traits. The major contribution of wild relatives includes the development of diverse and unique CMS systems for pigeonpea improvement. The availability of rich genomic resources including genome sequence will further accelerate marker-assisted breeding for pigeonpea improvement.

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