

# **Milestones in Food Legumes Research**

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## Enhancing the Value of Plant Genetic Resources of Chickpea, Pigeonpea and Groundnut

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### INTRODUCTION

Chickpea is the 4<sup>th</sup> and pigeonpea the 7<sup>th</sup> most important grain legume worldwide. Together, they contribute 4.8% to the world grain legume production (10.8 million tonnes from 14 million ha). Both the crops are predominantly grown in Asia, contributing 87% and 92%, respectively, to the world chickpea and pigeonpea production. Share of Africa to the world chickpea and pigeonpea production is 4.5% and 6.9%, respectively. North and Central America contributes about 5% of the world chickpea production (Dwivedi et al. 2005). Worldwide, groundnut is the second most important oilseed crop after soybean. Share of Asia and Africa to the world groundnut production (35.6 million tonnes), is 67% and 25%, respectively while North Central America and South America, respectively, contribute 5.8% and 1.6% (Dwivedi et al. 2007a). Chickpea and pigeonpea are rich source of protein (20-25%) while groundnut has good content of both oil (45%) and protein (20%). Abiotic and biotic constraints to chickpea, pigeonpea, and groundnut production are reviewed elsewhere (Dwivedi et al. 2005; 2007a).

A large collection of chickpea, pigeonpea, and groundnut genetic resources, both cultivated and wild relatives, are conserved in the genebanks globally (Table 1). The RS Paroda genebank in ICRISSAT has the largest collection of chickpea, pigeonpea, and groundnut genetic resources. ICARDA genebank has 12,484 chickpea accessions. Other genebanks holding substantial collection include USDA at Beltsville, Maryland, USA (<http://www.ars-grin.gov/npgs/stats/>).

Table 1. Chickpea, pigeonpea, and groundnut germplasm holdings maintained by CGIAR and national genebanks

Genebank	No. of germplasm accessions		
	Cultivated	Wild	Total
<b>Chickpea</b>			
ICRISAT, India	19,916	224	20,140
ICARDA, Syria	12,216	268	12,484
USDA, USA	6019	190	6209
ATFCC, Australia	7734	226	7960
NBPGR, India	-	-	14,566
VIR, Russia	-	-	2293
<b>Pigeonpea</b>			
ICRISAT, India	13,077	555	13,632
NBPGR, India	9711	122	9833
National Genebank, Kenya	-	-	1232
<b>Groundnut</b>			
ICRISAT, India	14,968	451	15,419
NRCG, India	11,846	-	11,846
USDA, USA	9412	631	10,043
INTA-EEAMANE, Argentina	2042	116	2158
VIR, Russia	1705	-	1705
CENARGEN, Brazil	850	450	1300

ATFCC at Horsham, Victoria, Australia ([http://apps3.fao.org/views/germplasm\\_query.htm?l=EN](http://apps3.fao.org/views/germplasm_query.htm?l=EN)) and NBPGR, New Delhi, India. Of the collections maintained at ICRISAT, 17,124 chickpea, 13,389 pigeonpea, and 14,803 groundnut accessions are available to users under multilateral system following the Standard Material Transfer Agreement (SMTA) as per the International Treaty on Plant Genetic Resources for Food and Agriculture.

The genetic base of legumes including chickpea, pigeonpea, and groundnut is narrow (Kumar et al. 2004; Isleib et al. 2001). In addition to bottleneck associated with the origin of these crops there has been limited use of germplasm in breeding programmes in spite of the large collection maintained in the gene banks and also because of the repeated use of the few germplasm/advanced lines for the development of new cultivars. The chickpea breeding programme at ICRISAT during the period from 1978 to 2004 developed 3548 advanced lines, designated as ICCVs, but only 96 germplasm accessions (including 5 wild *Cicer*) and 490

advanced breeding lines (many containing the genetic base from these 96 germplasm) traced back in the pedigree's of these lines, with L 550 and K 850 the most frequently used germplasm (Upadhyaya et al. 2006e). The pedigree analysis of the 86 chickpea cultivars developed by crossing and selection and released in India traced back to 95 ancestors, with Pb7 the most frequently used in 35 cultivars. In pigeonpea, 57 germplasm lines appeared in the pedigrees of 47 cultivars originating as a result of hybridization, with T 1 and T 190 involved in 16 cultivars (Kumar et al. 2004). The reasons for the underutilization of germplasm include the lack of accurate and precise multi-location evaluation of germplasm, the lack of rational systematic entry points into the vast international collections, and the lack of robust cost-effective tools to facilitate the efficient utilization of exotic germplasm in plant breeding programmes (Dwivedi et al. 2005). Clearly, there is a need to identify germplasm with beneficial traits for the diversification of the genetic base in these crops. This paper aims to document the nature of genetic variability in the pool of germplasm maintained at ICRISAT genebank, germplasm reported with beneficial traits, and discuss new approaches towards enhanced utilization of plant genetic resources in breeding and genomics applications.

## MANAGEMENT AND UTILIZATION OF LEGUME GENETIC RESOURCES

### Assembly and Collection, *Ex-situ* Conservation, and Regeneration

Plant genetic resources are the basic raw materials to meet the current and future needs of crop improvement programmes. The management of genetic resources includes (i) enriching the genetic resources through collections of new germplasm and creation of new genetic variability, (ii) regenerating and conserving genetic resources, and (iii) characterizing, evaluating, and documenting genetic diversity. A large part of chickpea, pigeonpea, and groundnut germplasm maintained in ICRISAT genebank has been assembled through donations from various institutions across the world. ICRISAT scientists also undertook 161 collection missions to 49 countries and collected 10,878 accessions (10,531 cultivated and 347 wild relatives) of chickpea, pigeonpea, and groundnut. ICRISAT genebank maintains over 49,000 accessions of its three mandate legumes from 134 countries.

*Ex situ* seed storage, in the form of storing seeds as active (medium term) and base (long term) collections, is the most convenient and widely used method of

conservation. Active collections are kept in conditions, which ensure that the accession viability remains above 65% for 10-20 years. Different combinations of storage temperature and moisture content can provide this longevity (IPGRI 1996). The active collections of ICRISAT genebank are stored in standard aluminum cans for all crops and in plastic cans for groundnut at 4°C and 30% relative humidity, with 7% to 10% seed moisture. The base collections are maintained at -20°C in vacuum packed standard aluminum foil pouches at seed moisture content 3-7% and with initial seed viability above 85%. The storage conditions maintained for both active and base collections are at par with the preferred standards for international genebanks (FAO/IPGRI 1994).

Seeds lose viability over time and it is necessary to regenerate accessions from time to time, the frequency of regeneration depends on the initial viability, the rate of loss of viability and the regeneration standards (Roberts 1984). Regeneration is costly in terms of resources and time, and it involves the risk to genetic integrity. The methods employed for regeneration considerably vary according to the crop species, and its reproductive system (Breese 1989). Storage conditions determine the extent to which potential longevity can be maximized. Several pre- and post-harvest and seed drying practices influence initial vigour and subsequent longevity of regenerated seed lots (Rao and Sasstry 1998). The Manual of Genebank Operations and Procedures (Rao and Bramel 2000) describes the protocols for different operations as per the Genebank Standards (FAO/IPGRI 1994). Active collection of chickpea and pigeonpea with initial viability >95% is monitored every 10 years while active collection of groundnut with >95% viability is monitored every 8 years; those with 85-95% viability every 5 years, and <85% viability every 3 years. Base collection of chickpea and pigeonpea with >95% viability is monitored every 20 years; between 85% and 95% viability every 15 years, and <85% viability every 10 years. The base collection of groundnut with >95% viability is monitored every 10 years. About 82-86% of chickpea, pigeonpea, and groundnut collections are conserved as *base* collection in ICRISAT genebank. ICRISAT uses Genebank Information Management System (GIMS), a user friendly module designed to integrate various documentation activities that provide information on accessions due for regeneration/viability monitoring at any given point of time.

Pigeonpea is often cross-pollinated (0-40%) and seed increase must preclude cross-pollination. Regeneration is carried out with at least 180 plants in an accession by covering whole plants at the time of flowering with muslin cloth

bags or growing plants in pollination cages. Chickpea and groundnut are self-pollinating species, therefore, no special efforts are made for controlling pollination during regeneration; however, a minimum of 160 plants of groundnut per accession and 80 of chickpea are grown for regeneration.

#### Assessing Diversity using Geographic Information and Morpho-agronomical Descriptors

The use of plant genetic resources to enhance the genetic potential of the crops has been well recognized. However, to achieve that goal, it is necessary that germplasm lines must be characterized for the descriptive traits and also evaluated for agronomic traits including response to biotic and abiotic stresses. At ICRISAT, extensive efforts in the last 30 years have been made towards the characterization, evaluation, and documentation of chickpea, pigeonpea and groundnut genetic resources at Patancheru, India. In this section, we briefly describe the patterns of variation in these crops germplasm as unraveled by using the geographic information and qualitative and quantitative descriptors. A two-stage evaluation procedure was used to characterize the variability in the entire collection. In the first stage, the characterization was based on evaluation of descriptor traits that are diagnostic, generally highly heritable, and usually easily scored in discrete classes; while in the second stage germplasm were evaluated for agronomic and seed quality traits and resistance to biotic and abiotic stresses following standard descriptors for chickpea (IBPGR, ICRISAT, and ICARDA 1993), pigeonpea (IBPGR and ICRISAT 1993), and groundnut (IBPGR and ICRISAT 1992). These descriptors provide an important framework for characterization of genetic resources for various morpho-physiological, reproductive, and biochemical traits.

**Chickpea:** Upadhyaya (2003) assessed 16,820 accessions for phenotypic diversity, using 21 descriptors including reaction to *Fusarium wilt*, from eight geographic regions. The means for different agronomic traits differed significantly between regions and the variances for all the traits among regions were heterogeneous. South Asia contained maximum range variation for all the traits. A variable Shannon-Weaver (*H'*) diversity index was observed in different regions for different traits. Seed colour among morphological traits and days to 50% flowering among agronomic traits showed the highest pooled *H'*. Principal component analysis (PCA), when using the first three PC scores, delineated two regional clusters with collection from Africa, South Asia, and Southeast Asia in the first cluster and the Americas, Europe, West Asia, Mediterranean region, and East Asia in

the second cluster (Fig. 1). Characterizing germplasm from geographic regions also reveals the extent of representation of genetic variability from a given region *vis-à-vis* other regions. For example, the Mediterranean region, which is the primary center of diversity, and Africa, which has the secondary center of diversity in Ethiopia, is poorly represented in ICRISAT chickpea genetic resources collection. ICARDA in Syria has the regional mandate to collect, preserve, characterize, evaluate, and document *Kabuli* chickpea genetic resources.

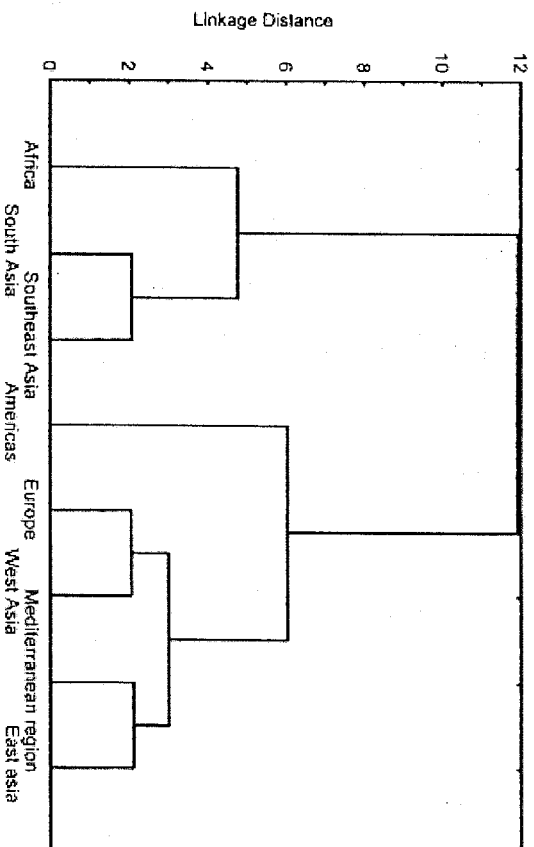


Fig. 1: Dendrogram of eight regions in the entire chickpea germplasm based on first three principal components

**Pigeonpea:** Studying the patterns of phenotypic variability on 11,402 accessions using 26 descriptors, Upadhyaya et al. (2005a) showed that semi-spreading growth habit, green stem colour, indeterminate flowering and yellow flower colour were the primary qualitative traits differentiating the accessions. Primary seed colour had the maximum variability, and the orange and cream were the two most frequent seed colours in the collection. Variances for the traits were heterogeneous among regions. Moreover, the germplasm accessions from Oceania were conspicuous by short growth duration, short height, fewer branches, pods with fewer seeds, smaller seed size and lower seed yields while the accessions from

Africa were of longer duration, taller, with multi-seeded pods and larger seeds.  $H'$  pooled over all traits was highest for Africa ( $0.464 \pm 0.039$ ) and lowest for Oceania ( $0.337 \pm 0.037$ ), thus revealing more diversity in African germplasm. PCA detected three major clusters: cluster 1 includes accessions from Oceania; cluster 2 from India and adjacent countries; and cluster 3 from Indonesia, Thailand, the Philippines, Europe, Africa, America, and the Caribbean countries (Fig. 2). This study further revealed that germplasm from Africa is the best source for growth duration and plant height; germplasm from America for plant height and primary branches; germplasm from Caribbean for growth duration, primary branches, seeds/pod and 100-seed weight; germplasm from AS 4 cluster (Andhra Pradesh, Orissa, Karnataka, Kerala, and Tamil Nadu states of India) for number of primary branches, racemes, higher harvest index, seed yield and seed protein content; and the germplasm from AS 5 cluster (unknown region, India) for increased number of racemes and high seed yield.

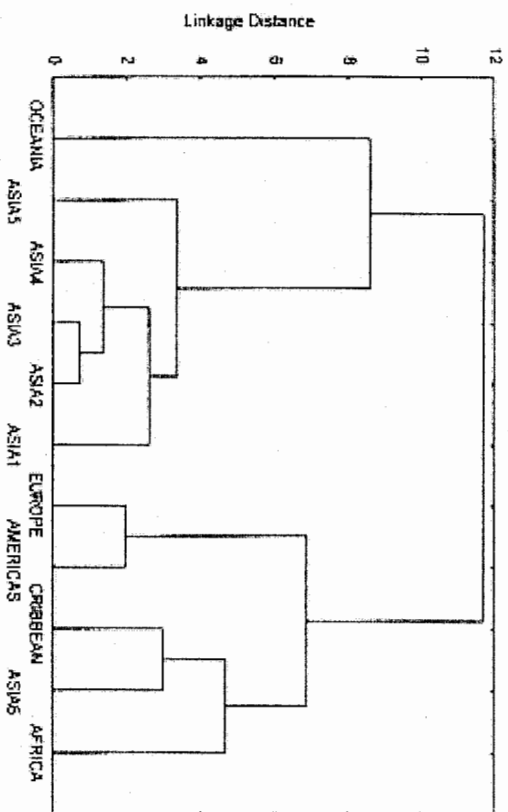


Fig. 2: Dendrogram of 11 regions in the entire pigeonpea germplasm based on first three principal components

**Groundnut:** Upadhyaya et al. (2002a) studied the patterns of phenotypic diversity in the 13,342 accessions using 26 descriptors for two seasons. They detected phenotypic variation for most traits in all the regions, with means significantly

differing for agronomic traits among regions and heterogeneous variances for all the traits among regions,  $H'$  was variable in different regions for different traits. South America among regions, primary seed colour among morphological traits, and leaflet length among agronomic traits showed highest pooled diversity index. Based on botanical types, *aegutatoriana*, *hirsuta*, and *peruviana* were poorly represented in ICRISAT germplasm collection. PCA delineated three regional clusters: North America, Middle East, and East Asia in the first cluster; South America the second cluster; and West Africa, Europe, Central Africa, South Asia, Oceania, Southern Africa, Eastern Africa, Southeast Asia, Central Asia, and Caribbean in the third cluster (Fig. 3).

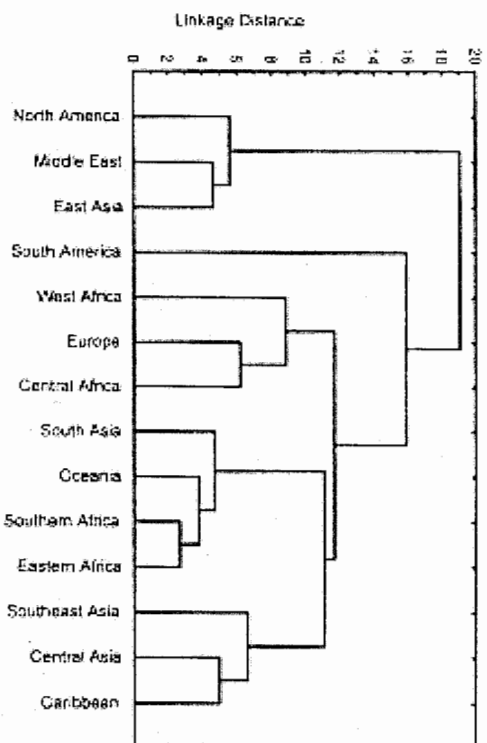


Fig. 3: Dendrogram of 14 regions in the entire groundnut germplasm based on first seven principal components

### IMPACT OF GERMPLASM IN BREEDING PROGRAMMES

The genetic resources of the three legumes in ICRISAT and also for chickpea in ICARDA have been extensively evaluated for agronomic and seed quality traits including resistance to biotic and abiotic stresses. In addition, national programmes in many countries have also been evaluating these germplasm resources for various traits. As a consequence, a large number of accessions, both in cultivated and wild relatives, with resistance/tolerance to biotic and abiotic stresses

(Tables 2 and 4; Dwivedi et al. 2003, 2005, 2007a) and for variation in seed quality traits (Table 3; Dwivedi et al. 2003) have been reported. It is worth noting that many of these resources have contributed resistance to biotic and abiotic stresses

**Table 2. Number of chickpea, pigeonpea, and groundnut germplasm accessions having resistance or tolerance to biotic and abiotic stresses at ICRISAT, Patancheru, India**

Crop	Trait	# accessions screened	# resistant/tolerant accessions identified	
Chickpea	Pod borer	16,346	20	
	Fusarium wilt	15,000	1136	
	Ascochyta blight	3000	192	
	Botrytis grey mold	2400	49	
	Colletotrichum blight	9000	72	
	Pod borer	10,090	27	
	Pod fly	10,090	21	
	Pod borer and pod fly	10,900	6	
	Nematodes	6700	9	
	Fusarium wilt	11,707	107	
	Sterility mosaic	13,201	397	
	Phytophthora blight	7669	152	
	Early leaf spot	10,567	9	
Groundnut	Late leaf spot	9400	76	
	Rust	9400	154	
	Aflatoxin	582	21	
	Rosette	10,590	143	
	Peanut bud necrosis disease	7400	23	
	Thrips	5000	14	
	Jassid	6500	31	
	Termit	520	9	
	Aphid	520	4	
	Leaf miner	930	14	
	Abiotic stresses			
	Chickpea	Drought	1000	11
		Cold	1000	16
Salinity		1000	4	
Pigeonpea	Drought	11,148	7	
	Water logging	11,148	5	
	Salinity	11,148	29	
Groundnut	Drought	742	38	

**Table 3. Genetic variation for 100-seed weight, oil and/or protein contents among cultivated chickpea, pigeonpea, and groundnut germplasm accessions evaluated at ICRISAT, Patancheru, India**

Trait	No. of accessions screened	Range and mean			Top five lines	
		Min	Max	Mean		
Chickpea	100-seed weight (g)	18.791	71.1	17.3	ICG#13778, 13787, 17109, and 17452, 18591	
	Protein (%)	12.973	8.0	29.6	19.5	ICG#1932, 5168, 5912, 8397, and 14315
Pigeonpea	100-seed weight (g)	12.717	2.7	25.8	9.3	CP#12746, 12825, 13144, 13618, and 14163, ICP#2660, 8776, 11488, 11814, and 15135
	Protein (%)	12.105	13.0	30.8	21.2	
Groundnut	100-seed weight (g)	14.380	15.0	140.0	51.5	ICG#8331, 10877, 12064, 12509, and 13264
	Oil (%)	7999	32.0	53.9	21.2	ICG#142, 2378, 2411, 3245, and 7637
Protein (%)	5511	16.0	32.4	25.7	ICG#3509, 7749, 10320, 10600, and 11858	

into improved genetic backgrounds leading to the development and release of cultivars in all the three crops (ICRISAT 2000; Dwivedi et al. 2003, 2007a; Shitraw et al. 2004; Sharma et al. 2007). Additionally, a number of germplasm lines, yielding on par or more than control cultivar, but with beneficial agronomic traits identified: early maturity and/or large seed size in chickpea and groundnut (Upadhyaya et al. 2006a,b; 2007); early maturity and rosette resistance in groundnut (Deom et al. 2006); high yielding large-seeded *kabuli's* (Kumar and Gurha 2005); male sterility sources for hybrid seed production in pigeonpea (Reddy et al. 1995c; Dwivedi et al. 2007b and references therein); pigeonpea accessions with large pod size (Sasstry et al. 2006); better oil quality groundnut (Dwivedi et al. 2003, 2007a); and chickpea and groundnut accessions with drought resistance traits (Kashiwagi et al. 2006; Upadhyaya 2005).

Wild relatives have contributed genes for resistance to diseases and/or adaptation to water limited environment that led to the successful release of few cultivars, for example in groundnut, Coan and NemaTam (resistance to nematode) released in USA and ICGV SM 86715 (resistance to foliar diseases) in Mauritius (Dwivedi et al. 2007a); an interspecific derivative in chickpea, BG-1103 (renamed

**Table 4. Sources of resistance to biotic and abiotic stresses identified in cultivated germplasm of chickpea, pigeonpea and groundnut**

Summary of the germplasm identified with beneficial traits	Reference
<b>Biotic stresses</b>	
<b>Chickpea</b>	
Low to moderate resistance to pod borer in germplasm and advanced lines/cultivars	Sharma et al. 2007
Two large-seeded <i>kabuli</i> types with complete resistance to Fusarium wilt	Gaur et al. 2006
Several sources of resistance to Botrytis grey mold	Pande et al. 2006b
Several sources of resistance to Ascochyta blight both in cultivated and wild <i>Cicer</i> accessions	Pande et al. 2005
10 <i>desi</i> and three <i>kabuli</i> types with multiple resistance to collar rot, dry root rot, and wilt	Gupta and Babbar 2006
Large-seeded <i>kabuli</i> type, ICC 14199, resistant to Fusarium wilt	Kumar and Gurha 2005
Several sources of resistance to Fusarium wilt, dry root rot, Botrytis grey mold, Ascochyta blight, and pod borer	Shiferaw et al. 2004
ILC 10765 and ILC 10766, both resistant to cyst nematode	Malhotra et al. 2002
82 accessions with varying degree of resistance to Ascochyta blight	Iqbal et al. 2002
Five accessions including ICCV2 resistant to Fusarium wilt; with ICCV2 producing twice the grain yield in farmers' field in Sudan	Ali et al. 2002
Barichhola 5, resistant to Fusarium wilt and tolerant to botrytis grey mold	Rahman et al. 1998a
Barichhola 6, resistant to Fusarium wilt	Rahman et al. 1998b
Three <i>kabuli</i> types with multiple resistance to Ascochyta blight, fusarium wilt and cold tolerance	Singh et al. 1997
Six <i>kabuli</i> types resistant to Fusarium wilt races 0 and 1	Singh and Jimenez-Diaz 1996
Three <i>kabuli</i> types resistant to leaf miner	Singh and Weigand 1996
Eight early maturing, large-seeded <i>kabuli</i> types resistant to Ascochyta blight	Singh and Reddy 1994
Two <i>Kabuli</i> and three <i>desi</i> types resistant to Ascochyta blight	Reddy and Singh 1992
ILC 3279 and ILC 482 resistant to Ascochyta blight and cold tolerance	Singh et al. 1992a,b

<b>Pigeonpea</b>	
ICEAP 00068, large white seeds with good grain quality attributes	Gwata et al. 2007
ICP 7035 resistant to sterility mosaic	Rangaswamy et al. 2005
ICEAP 00040 resistant to Fusarium wilt	Silin et al. 2005
Several sources of resistance to fusarium wilt, sterility mosaic, Phytophthora blight, Cercospora leaf spot, powdery mildew, Alternaria blight, pod fly, and pod borer	Shiferaw et al. 2004
ICPM 93003 Fusarium wilt and sterility mosaic resistant genetic male sterile line	Saxena et al. 1998
ICP 9145 resistant to Fusarium wilt	Reddy et al. 1995a
Four pigeonpea germplasm resistant to Fusarium wilt	Reddy et al. 1995b
ICPM#93006, 93007, and 93008 short duration and genetic male sterility	Reddy et al. 1995c
<b>Abiotic stresses</b>	
<b>Chickpea</b>	
Several sources of drought tolerance	Kashiwagi et al. 2005
Several sources of cold and drought tolerance	Shiferaw et al. 2004
Several accessions with freezing and chilling tolerance	Croser et al. 2003
Three cold tolerant <i>kabuli</i> types	Singh and Jimenez-Diaz 1996
FLIP 87-59C tolerant to terminal drought	Singh et al. 1996
ICC 4958, <i>desi</i> type, tolerant to drought	Saxena et al. 1993
ILC#72 and 195, <i>kabuli</i> types, resistant to iron chlorosis and cold tolerance	Singh et al. 1993a,b
ILC 8262, <i>kabuli</i> type, cold tolerant	Singh et al. 1992c
<b>Pigeonpea</b>	
Sixteen pigeonpea mini core accessions tolerant to salinity	Shrivastava et al. 2006
<b>Groundnut</b>	
Several sources of drought tolerance	Upadhyaya 2005
Several sources of cold temperature tolerance	Upadhyaya et al. 2007b
Reddy et al. 1995b Reddy et al. 1995c	

as Pusa 1103) released for commercial cultivation in North India, for its high yield potential, resistance to Fusarium wilt, and tolerance to drought (Abbo et al. 2007).

Some of the germplasm lines outperformed local controls and released as cultivars in several countries: 17 chickpea germplasm in 14 countries (CRISAT 2000; Dwivedi et al. 2005; Siddique and Regan 2005); nine pigeonpea germplasm

in six countries (Dwivedi et al. 2005; Rangaswamy et al. 2005; Gwata et al. 2007), and 11 groundnut germplasm in 15 countries (Dwivedi et al. 2005).

#### NEW APPROACHES TO ENHANCE UTILIZATION OF GENETIC RESOURCES IN CROP BREEDING CORE AND MINI CORE COLLECTIONS

Understanding the range of diversity and the genetic structure of gene-pools is critical for the effective management and use of germplasm resources. Continuous progress in plant breeding depends on the discovery of new sources of genetic variation, accurate identification of lines with beneficial traits, and their judicious use in such a way that a combination of alleles when brought together produces progenies with superior performance. Many of the agronomic traits show considerable genotype by environment interaction, necessitating the need for replicated multi-location evaluation to identify germplasm with beneficial traits for use in crop improvement programmes. However, replicated multi-location evaluation of such a large collection of germplasm for a range of traits is impossible because of the resource limitations and would be of little value due to low precision. A core collection (10% of the entire collection) is a subset of accessions representing at least 70% of the genetic variation in the entire collection of the species (Brown 1989) and can be a gateway for the utilization of diverse germplasm with beneficial traits in crop breeding programmes. However, in crop species with several thousands of germplasm accessions, even a core collection would be unwieldy for evaluation by breeders in the multi-location replicated trials. To overcome this, Upadhyaya and Ortiz (2001) suggested mini-core collection approach which is a core of core (10% of core or 1% of entire collection) representing the species diversity. Mini-core is established after evaluating the core subset for various morphological, agronomic, and seed quality traits, and selecting about 10% accessions from the core subset, using a standard clustering procedure to separate groups of similar accessions.

**Chickpea:** Core and mini core collections have been developed in chickpea, the former consists of 1956 accessions (Upadhyaya et al. 2001b) while the latter 211 accessions (Upadhyaya and Ortiz 2001). Using days to 50% flowering, pods per plant, seed yield and 100-seed weight as a selection criterion, Upadhyaya et al. (2007a) identified 19 *desi*, 15 *kabuli* and five intermediate (pea-shaped) type promising chickpea germplasm lines that originated from 10 countries for use in crop improvement programme. The selected *desi* accessions produced 8.5% more

seed yield and had 32% larger seeds than the control cultivar Annigeri while the selected *kabuli* accessions yielded at par with control L550 but had 84% larger seeds. Extensive and deep root systems have been recognized as one of the most important traits for improving chickpea productivity under progressively receding soil moisture conditions. When chickpea mini core evaluated for the genetic variability for root traits using a cylinder culture system for two seasons at 35 days after sowing, Kashiwagi et al. (2005) detected the largest genetic variation for root length density (RLD) across seasons followed by the ratio of plant dry weight to root length density. They identified nine genotypes including ICC 8261, which had the largest RLD and the deepest root system in comparison to previously identified drought tolerant accession ICC 4958. Moreover, the chickpea landraces from the Mediterranean and the West Asian region showed a significantly larger RLD than those from the South Asian region. In addition, landraces from central Asia (former Soviet Union), characterized by arid agro-climatic conditions, also showed relatively larger RLD. Thus, information on the genetic variability for root traits provides valuable baseline knowledge for further progress on the selection and breeding for drought avoidance root traits in chickpea. Vadez et al. (2007) reported six-fold variation for seed yield under salinity (80 mM NaCl; pot screening), with several accessions yielding 20% more than a previously released saline tolerant cultivar, CSG 8962. The three highly saline tolerant accessions in their study were ICC 1431, ICC 5003, and ICC 15610. Host plant resistance is the major component in the management of fungal diseases in chickpea. When chickpea mini core evaluated for biotic stresses, Pande et al. (2006a) detected high level of resistance to *Fusarium wilt* (FW) in 46 accessions while 3, 55, and 6 accessions, respectively, showed moderate resistance to *Ascochyta blight* (AB), *Botrytis gray mold* (BGM), and dry root rot (DRR). They also identified few accessions with multiple resistances: ICC 11284 to AB and BGM; 4 accessions with resistance to DRR and FW; and 11 with resistance to BGM and FW.

**Pigeonpea:** Reddy et al. (2005) constituted a core collection comprising of 1290 accessions. More recently, a mini core consisting of 146 accessions has been reported (Upadhyaya et al. 2006d). A few accessions with <10% pigeonpea sterility mosaic disease have been identified when pigeonpea mini core subset was evaluated under greenhouse conditions (ICRISAT 2006). Sixteen of the 196 pigeonpea mini core accessions showed tolerance to salinity (Srivastava et al. 2006).

**Groundnut:** A number of core and mini core or even region-specific core collections have been reported in groundnut (Holbrook et al. 1993; Upadhyaya et al. 2002b, 2003; Holbrook and Dong 2005; Dwivedi et al. 2007c). When ICRISAT core collection was evaluated for several morphological and agronomic traits across two seasons at Patancheru, the accessions belonging to subsp. *hypogaea* (var. *hypogaea* and *hirsuta*) and *fastigiata* (var. *fastigiata*, *vulgaris*, *aequatoriana*, and *peruviana*) differed significantly for most of the traits. The *hypogaea* group showed significantly greater mean pod length, pod width, seed length, seed width, yield per plant, and 100-seed weight, whereas the *fastigiata* group had greater plant height, leaflet length, leaflet width and shelling percentage. Similarly when Mallikarjuna Swamy et al. (2003) evaluated Asia region core collection for agronomic traits at four locations in India, they also found that both *fastigiata* and *hypogaea* groups differed significantly for most of the traits. In comparison to chickpea and pigeonpea, the groundnut core or mini core subsets have been extensively evaluated and new sources of variation reported for early maturity (Upadhyaya et al. 2006a,b); high yield, greater shelling percentage and 100-seed weight (Upadhyaya et al. 2005b); drought (Upadhyaya 2005) and low temperature (Upadhyaya et al. 2007b) tolerance; and resistance to root-knot nematode, early leaf spot, pepper spot, tomato spotted wilt virus, and many soil born fungal diseases including pre-harvest aflatoxin contamination (Isleib et al. 1995; Anderson et al. 1996; Holbrook et al. 1998, 2000; Franke et al. 1999; Danicone et al. 2003).

Thus, the new sources of variation identified, from evaluating the chickpea, pigeonpea and groundnut core or mini core subsets, will be of direct relevance to breeders, geneticists, and molecular biologists engaged in improving the genetic potential of these crops and for cloning and elucidating the trait-specific gene(s) function of significant agricultural importance.

#### Assessing Molecular Diversity in Germplasm using DNA Markers

With the availability of core and mini core subsets in chickpea, pigeonpea, and groundnut and the availability of PCR-based markers, it is now possible to dissect the genetic structure of these subsets to identify the genetically diverse germplasm with beneficial traits for diverse uses in genomics and applied breeding of these legumes. Up till now, only few studies dissecting the molecular basis of diversity are reported in these crops.

**Chickpea:** Past studies indicated abundant diversity among wild *Cicer* but limited variation in cultivated chickpea (Kazan and Muehlbauer 1991; Labdi et al. 1996; Trucela et al. 2002; Sudupak et al. 2002; Rajesh et al. 2003; Shan et al. 2005; Rao et al. 2007). More recently, Upadhyaya et al. (2006e) developed a composite collection of 3000 accessions, and profiled these using 50 SSRs. This composite collection showed rich allelic diversity (1741 alleles based on 49 SSR loci data on 2915 accessions) and a number of group-specific unique alleles [*Kabuli's* 114, *desi's* 306, wild *Cicer* 71; Mediterranean 117, West Asia (WA) 120, and South and Southeast Asia (SSEA) 119]. *Kabuli's* as a group were more genetically diverse than other types. SSEA and WA shared 76, Mediterranean and SSEA 33, and Mediterranean and WA regions 39 common alleles. *Desi* and *Kabuli* types shared 450 alleles. A reference set of 300 accessions [211 chickpea mini core (Upadhyaya and Ortiz 2001) and 89 from the remaining 2704 accessions of the chickpea composite collection] has been developed (ICRISAT 2006).

**Pigeonpea:** Of the three ICRISAT mandated legume crops, pigeonpea lagged behind chickpea and groundnut in terms of availability of PCR-based markers and thereupon information on molecular-based genetic diversity. To date, a set of 40 polymorphic SSRs have been reported differentiating cultivated species from wild types, with many also polymorphic among cultivated pigeonpea germplasm (Burns et al. 2001; Odeny et al. 2007). However, less allelic variation was detected within the cultivated species than across the wild species (Odeny et al. 2007), clearly indicating the need to develop more SSRs polymorphic to cultivated species. Using AFLP assay, Panguluri et al. (2006) detected low polymorphism among pigeonpea cultivars and very high polymorphism between cultivated pigeonpea and its wild relatives. More recently, 64 of the 700 DArT markers detected polymorphism in cultivated pigeonpea, whereas many polymorphic among wild relatives or between the wild species and the cultivated pigeonpea. DArT markers revealed genetic relationships among the accessions consistent with the available information and systematic classification (Yang et al. 2006). More recently, a composite collection of pigeonpea (1000 accessions) has been developed that is being genotyped using 20 SSRs and high throughput assay (ICRISAT 2006).

It is hoped that in times to come more numbers of SSRs and DArT markers will be available to pigeonpea research community for dissecting the structure and diversity in pigeonpea germplasm in order to identify genetically diverse and useful germplasm for use in pigeonpea genomics and breeding.

**Groundnut:** Unlike the previous impression of low polymorphism in cultivated groundnut but abundant diversity in wild *Arachis* species, recent work revealed sufficient molecular polymorphism in cultivated groundnut (Dwivedi et al. 2001, 2002, 2003, 2007a; He et al. 2003, 2005; Ferguson et al. 2004a,b; Krishna et al. 2004; Moretzsohn et al. 2004; Mace et al. 2007). A few genetically diverse germplasm resistant to rust, leaf spots, groundnut rosette disease, bacterial wilt, aflatoxin, and for drought tolerance have been reported. A large number of SSR markers are now available in public domain. More recently, 852 accessions of the composite collection (1000 accessions) have been profiled using 21 SSRs that detected 490 alleles, of which 244 were rare and 246 common alleles. Unlike chickpea, wild *Arachis* accessions included in this composite collection detected more number of unique alleles (101) than the cultivated types (subsp. *hypogaea* 11 and subsp. *fastigiata* 50 unique alleles). A reference set of 300 accessions [consisting of 177 groundnut mini core (Upadhyaya et al. 2002b) and 123 from the remaining 675 accessions of the composite collection] has been developed.

#### Expanding Crop Gene Pools using Alien Genetic Resources and Biotechnological Approaches

Wild relatives of the grain legume crops are reported to harbor beneficial alleles and genes for resistance/tolerance to biotic and abiotic stresses, and for increased grain yield and quality. For example, resistance to nematodes in chickpea, pigeonpea and groundnut; to rust, leaf spots and defoliators in groundnut; to Fusarium wilt, leaf miner, pod borer, bruchids, *Ascochyta* blight, Botrytis gray mold, Phytophthora rot in chickpea; to pod borer, pod fly, pod wasp, and sterility mosaic in pigeonpea; to drought and/or salinity tolerance in pigeonpea and chickpea; and to cold tolerance in chickpea. Some of the chickpea and pigeonpea wild relatives are also reported to possess high seed protein content (Dwivedi et al. 2005; Nguyen et al. 2005; Kumar et al. 2005; Sharma et al. 2005, 2007; Abbo et al. 2007). Variable successes have been reported towards transferring resistances to rust, leaf spots and nematodes in groundnut; to cyst nematode and Phytophthora root rot and cold tolerance in chickpea; to pod borer in chickpea and pigeonpea; to cytoplasmic nuclear male sterility, high seed protein content, and cleistogamous flowers in pigeonpea (Dwivedi et al. 2005, 2007b and references therein; ICARISAT 2006). Some of the chickpea interspecific derivatives, involving *C. reticulatum* or *C. echinospermum* in their pedigree, showed high degree of resistance to wilt, foot rot and root rot diseases and produced greater biomass and/or seed yield (Singh et al. 2005a; Dwivedi et al. 2007b and references therein). More recently, using

embryo rescue technique, ICARISAT scientists made breakthrough by crossing *C. bijnorum* with KAK-2 and ICCV 2, and produced viable hybrids for the first time (ICARISAT 2006). *C. bijnorum* belongs to secondary gene pool and has multiple resistances to diseases.

Development of exotic genetic libraries (introgression lines or chromosome segment substitution lines), consisting of marker-defined genomic regions taken from wild species and introgressed onto the background of elite crop lines, is another possibility for the enhanced utilization of wild relatives in crop improvement (Zamir 2001). Once such genetic resources are available they can be evaluated for a range of characteristics to identify alleles associated with beneficial traits that in turn can be introgressed into elite genetic materials. Unfortunately, such introgression lines in chickpea, pigeonpea, and groundnut are not available. However, it is hoped that with the availability of large numbers of PCR-based markers, preferably SSRs and DArT, and associated high throughput assays combined with carefully planned introgression with successive backcrossing to the recurrent parental line it should be possible to develop such useful genetic resources that will be a permanent genetic resource for gene discovery and function and their utilization in crop improvement.

Molecular cloning and subsequent gene transfer through transformation is another approach to interspecific gene transfer in cultigens. However, to date few successful cloning and thereupon transfer exist in cereals and clonal crops (Dwivedi et al. 2007b and references therein). Bhattarai and Fetting (2005) isolated a drought and salinity induced dehydrin gene, *cpdnl*, from *C. pinnatifidum*, which may be introgressed, through genetic transformation, into commercial cultivars to create more stress-resistant chickpeas. Transgenic approach has potential to accelerate introgression of new alleles from wild relatives to crop species compared to conventional breeding, which in many cases slower and less precise due to linkage drag, thus increasing the genetic diversity available for crop improvement.

#### Association Mapping to Identify Genomic Regions with Beneficial Traits

The genomics revolution has provided the scientific community with tremendous opportunities for improving the pace and scale of plant breeding to solve some of the world's agricultural and food security issues. A large collection of germplasm (approx. 0.6 million samples) of the major food crops are housed in genebanks of 11 CGIAR-supported institutions. The Generation Challenge Programme (GCP) aims to utilize molecular tools and comparative biology to

explore and exploit genetic diversity housed in existing germplasm collections, with a focus on improving the drought tolerance in cereals, legumes, and clonal food crops ([www.generationcp.org](http://www.generationcp.org)). A primary goal of the GCP is extensive genomic characterization of global crop-related genetic resources (composite collections); initially using SSR markers to determine population structure and then move onto whole genome scans (including SNP arrays and DART) and functional genomics analysis of subsets of germplasm (reference sets). ICRIASAT established a reference subset of 300 accessions, each in chickpea and groundnut (see section III C) while marker (20 SSRs) profiling of composite collection is in progress to construct a similar reference subset (300 accessions) in pigeonpea. New set of SSRs and orthologous candidate gene markers are being developed. DART marker technology, which is microarray based assay, is actively pursued at ICRIASAT to develop DART markers for chickpea and pigeonpea. It is proposed that these reference sets, which have already undergone initial genome scan, will be saturated with large set of markers to scan for whole genome. The reference subsets will also be evaluated for traits of agricultural importance (drought, salinity, and for extreme variation in temperature) and use the genotyping and phenotyping data, through linkage disequilibrium and association mapping, to mine alleles associated with beneficial traits. It is expected that this exercise will lead to the identification of genetically diverse accessions and alleles associated with beneficial traits for diverse applications in breeding and genomics of these legumes. It is also proposed to develop DNA bank of the chickpea, pigeonpea, and groundnut reference sets, and marker kits that will be freely available to all those interested in using these genetic and genomic resources in improvement of these crops.

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