

## 6. Chickpea germplasm for use in crop improvement: Approaches and way forward

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*Plant genetic resources are the basic raw materials for the genetic gains and an insurance against present and future threats to agricultural production. The ICRISAT genebank at Patancheru, India maintains 20,268 cultivated and wild chickpea accessions. Core (10% of entire collection) or mini core (10% of core or 1% of entire collection), representing diversity of the entire collection of a given species preserved in genebank has been suggested as gateway to enhance use of germplasm in crop breeding. These core and mini core collections consist of 1,956 and 211 accessions, respectively. Further, a global composite collection consisting of 3000 accessions was formed, using data sets and germplasm from ICRISAT and ICARDA genebanks, which was molecularly profiled to form a genotype-based reference set. This reference set consists of 300 genetically diverse accessions, which captured 78% of the 1,683 alleles detected in the global composite collection. Further analysis on this reference set based on 48 and 107 SSRs detected four and nine subgroups. Using mini core collection, a number of trait-specific genetically diverse germplasm with agronomically beneficial traits have been identified for use in chickpea improvement.*

### Introduction

Cereals and legumes together contribute 2616 million tons to global food production (3,742 million tons of cereals, legumes, oilseeds, roots and tubers, and plantains and bananas), of which legumes contribution is only 2.7%

(<http://www.faostat.fao.org>; accessed on April 15, 2014). Globally, chickpea is the 2<sup>nd</sup> most important grain legume (production 11.63 million tons) after bean (production 23.60 million tons) (Fig 1A). South Asia is the leading chickpea producing region, 71.58%, while Africa, Americas, Oceania, and West Asia regions each proportionally contribute 5.47% to 5.99% of the total chickpea production (Fig 1B).

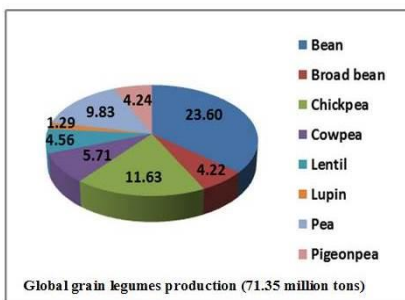


Fig. 1A

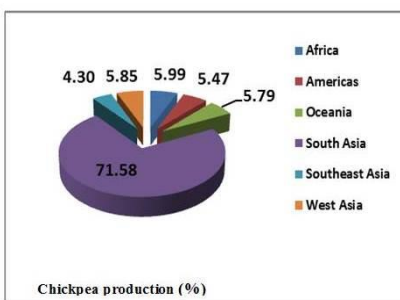


Fig. 1B

Globally chickpea is produced in 55 countries, of which 24 countries (Algeria, Argentina, Australia, Canada, China, Ethiopia, India, Iran, Israel, Italy, Kazakhstan, Malawi, Mexico, Morocco, Myanmar, Pakistan, Russian Federation, Spain, Sudan, Syrian Arab Republic, Tanzania, Turkey, USA and Yemen) in 2012 reported chickpea production of over 10,000 tons. Australia, Canada, Ethiopia, India, Iran, Mexico, Myanmar, Pakistan, Turkey, and USA are the major producers; India being the lead country in chickpea production (Fig 2). Large differences in yield were noted among countries, for example, from 3500 kg ha<sup>-1</sup> in Israel to 250 kg ha<sup>-1</sup> in Kenya (Figure 3).

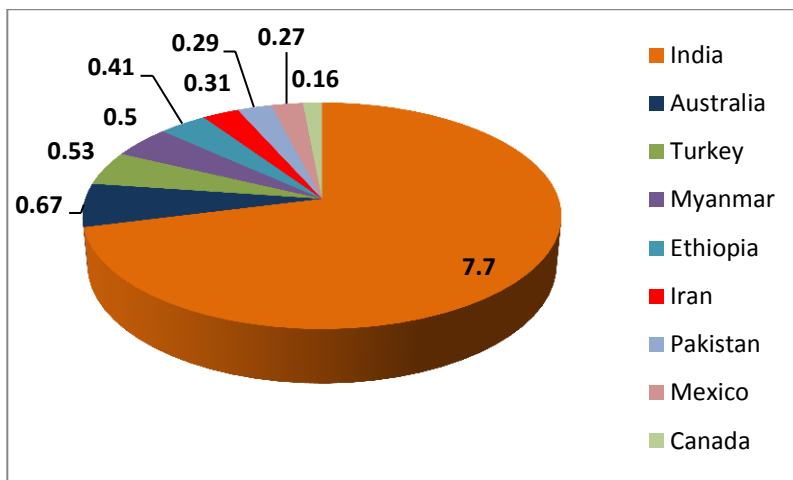


Fig. 2: Contribution (million tonnes) of major producing countries to total global chickpea production ([www.faostat.fao.org/](http://www.faostat.fao.org/); accessed on 15 April 2014)

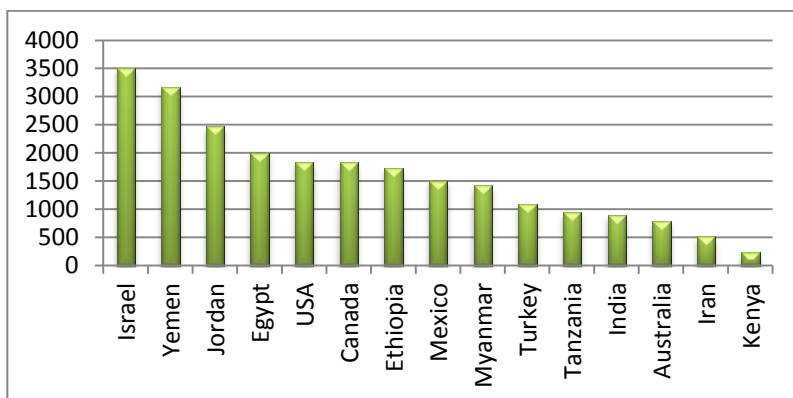


Fig. 3: Variation in chickpea productivity (Kg ha<sup>-1</sup>) in 15 countries ([www.faostat.fao.org/](http://www.faostat.fao.org/); accessed on 15 April 2014)

Several abiotic (drought, heat, salinity, chilling temperature) and biotic (fusarium wilt, dry root rot, ascochyta blight, botrytis gray mold, pod borer and leaf miner) stress adversely impact

chickpea production; together accounting for annual yield loss of US\$ 4.4 billion, of which about one third can be recovered through genetic enhancement of yield potential by augmenting the productivity genes and resistance to biotic and abiotic stress (Ryan 1997).

The two most distinct forms of chickpea are desi (small seeds, angular ram's head shape, and colored seeds with high percentage of fiber) and kabuli (large-seeds, irregular rounded, owl's-head shape, and beige colored seeds with a low percentage of fiber) types. An intermediate pea-shaped type also exists, which is characterized by medium to small and cream colored seeds. Both desi and kabuli are easily hybridized, but there are strong consumer and culinary preferences for the two types (Upadhyaya *et al.* 2002; Upadhyaya *et al.* 2011).

Grain legumes are characterized by low glycaemic index (GI), and food with low GI are generally associated with several long-term health benefits (<http://www.extension.usu.edu>). Diets emphasizing dietary pulses have been reported significantly lowered LDL cholesterol levels compared with control diets (Vanessa *et al.* 2014). Chickpea seeds are rich in protein, starch, fiber, minerals, and vitamins, which make it one of the best nutritionally balanced pulses for human consumption (Jukanti *et al.* 2012).

Cultivated chickpea has a narrow genetic base (Kumar *et al.* 2004; Upadhyaya *et al.* 2011). The breeders are reluctant to use exotic germplasm because of linkage drag and/or loss of coadaptive gene complex, requiring longer cultivars' development time. Thus breeders tend to concentrate on adapted and improved materials avoiding wild relatives, landraces and exotic germplasm available in genebanks (Nass and Paterniani 2000), thereby further narrowing the genetic base as well widening the gap between available genetic resources and their use in breeding programs (Marshall 1989). However, it should be noted that large diversity among chosen parental lines is

essential for the success of any recombinant breeding program, specifically when the traits under improvement are quantitative, highly variable and show high G×E interactions. Identification of trait-specific germplasm and their use in recombinant breeding is, therefore, a critical step to develop crop cultivars that meet adverse effects of climate change and variability on agricultural production.

### **Genebank holding chickpea germplasm globally**

Plant genetic resources (PGR) are the basic raw materials required to power current and future progress in crop improvement programs. The use of PGR in crop improvement is one of the most sustainable ways to conserve valuable genetic resources for the future, and simultaneously to increase agricultural production and food security. To date, 100,852 chickpea germplasm accessions are maintained across genebanks globally, with National Bureau of Plant Genetic Resources (NBPGR), New Delhi, India, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and International Center for Agricultural Research in Dryland Areas (ICARDA) contributing 50.5% of the total chickpea germplasm preserved (Table 1). ICRISAT genebank at Patancheru, India houses 20,268 accessions, including 308 wild relatives from 19 *Cicer* species (Fig 4). Other leading genebanks hosted wild *Cicer* species include ICARDA, Syrian Arab Republic (270 accessions from 11 species), ATFCC, Australia (246 accessions from 18 species) and USDA-ARS, Pullman, USA (205 accessions from 22 species).

Table 1. Number of chickpea germplasm accessions preserved in major genebanks globally (<http://apps3.fao.org/wIEWS/>; accessed on 15 April 2014).

Country	Institution	Wild <i>Cicer</i>		Cultivated (Landrace)	Total	Collection (%)
		# spices	# accession			
Australia	ATFCC	18	246	8,409	8,655	8.6
Ethiopia	IBC	-	-	1,173	1,173	1.2
India	ICRISAT	19	308	19,960	20,268	20.1
	NBPGR	10	69	16,812	16,881	16.7
Iran		-	-	5,700	5,700	5.7
Mexico	IA-Iguala	-	-	1,600	1,600	1.6
Pakistan	PGRP	3	89	2,057	2,146	2.1
Syria	ICARDA	11	270	13,548	13,818	13.7
Turkey	AARI	4	21	2,054	2,075	2.1
USA	USDA	22	205	6,584	6,789	6.7
Total		87	2,136	98,716	100,852	

*ATFCC* (Australian Temperate Field Crops Collection, Horsham Victoria); *IBC* (Institute of Biodiversity Conservation, Addis Ababa, Ethiopia); *ICRISAT* (International Crop Research Institute for the Semi-Arid Tropics Patancheru, India); *NBPGR* (National Bureau of Plant Genetic Resources, New Delhi, India); *NPGBI-SPII* (National Plant Gene Bank of Iran, Seed and Plant Improvement Institute, Karaj, Iran); *IA-Iguala* (Estación de Iguala, Instituto Nacional de Investigaciones Agrícolas, Iguala, Mexico); *PGRP* (Plant Genetic Resources Institute, Islamabad, Pakistan); *ICARDA* (International Centre for Agricultural Research in Dry Areas, Aleppo, Syria); *AARI* (Aegean Agricultural Research Institute, Izmir, Turkey); *USDA-ARS* (Western Regional Plant Introduction Station, Pullman, USA).

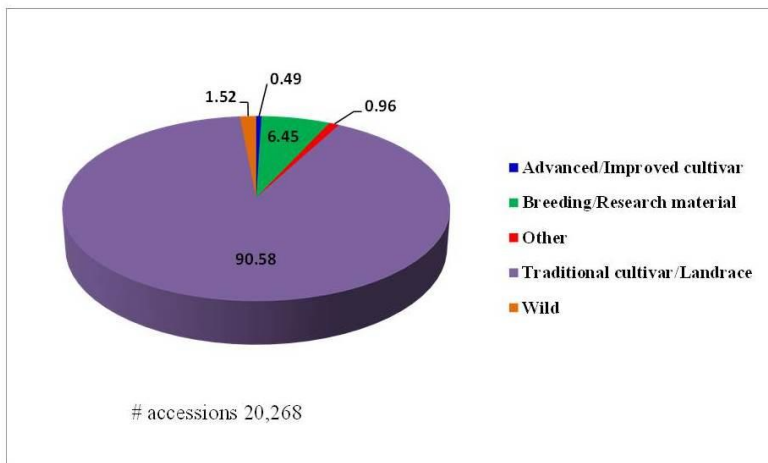


Fig. 4: Biological status (%) of chickpea germplasm conserved at ICRISAT genebank, Patancheru, India. ([www.icrisat.org/what-we-do/crops/chickpea/project1/pfirst.asp](http://www.icrisat.org/what-we-do/crops/chickpea/project1/pfirst.asp))

### **Forming representative subsets to enhance utilization of germplasm in breeding**

To date, only limited number of germplasm have been used in cultivar development in most crops, including chickpea. The reasons for the underutilization of germplasm include non-availability of reliable information on traits of economic importance, linkage load of undesirable genes and assumed risks, restricted access to the germplasm collections and regulations governing international exchange, enhanced role of non-additive genetic variation when diverse germplasm is used by the breeders, and lack of robust, cost-effective tools to facilitate the efficient utilization of exotic germplasm in crop breeding (Dwivedi *et al.* 2009; Upadhyaya *et al.*, 2011). More importantly, breeders are often reluctant to use exotic germplasm, and end up recirculating their own working collection which will lead to narrow genetic base and ceiling on achievable genetic gain, either due to linkage drag or due to loss

of co-adopted gene complex (Ortiz *et al.* 1998) requiring longer generation time in cultivar development.

### ***Core and mini core collections***

Reduced subsets such as corecollection, which represents about 10% of the entire accessions (Frankel 1984) and mini core collection (~1% of the entire accessions or 10% of the accessions of the core collection), representing diversity of the entire collection of a given species preserved in genebank, has been suggested as a gateway to enhance utilization of germplasm in crop improvement programs. Using passport, characterization and evaluation data, the representative subsets have been formed in chickpea. The core collection consists of 1,956 accessions (Upadhyaya *et al.* 2001) and the mini core collection 211 accessions (Upadhyaya and Ortiz 2001). These subsets represent adequate biological (desi, kabuli, and pea-shaped types) and geographical (at regional level and countries within region) diversity.

### ***Reference set***

ICRISAT in collaboration with ICARDA has developed a global composite collection of 3,000 accessions, which included 1,956 accessions of the ICRISAT core collection, 709 ICARDA cultivated genebank accessions, 39 advanced breeding lines and cultivars, 35 accessions with distinct morphological variants, 20 wild *Cicer* species (*C. echinospermum* and *C. reticulatum*) and 241 accessions with unique traits (Upadhyaya *et al.* 2006). Biologically, this composite collection is composed of 80% landraces, 9% advanced breeding lines, 2% cultivars, 1% wild *Cicer* species and 8% accessions of unknown origin. Geographically, 39% of the composite collection originates from South and Southeast Asia, 25% from West Asia and 22% from the Mediterranean region. Africa and the Americas each contributing 5% of the collection. This composite collection was



initially genotyped with 48 polymorphic SSRs to form genotype-based reference set (300 accessions), which captured 78% allelic diversity of the composite collection accessions (1,683 alleles on 2,915 accessions) (Upadhyaya *et al.* 2008). Further analysis with 48 and 107 SSR markers, respectively, detected 4 and 9 distinct subgroups as well variation in allelic richness and diversity (Table 2). Part of this variation could be related to variation in number of SSRs used in the two studies. This reference set, which also included mini core accessions, is thus an ideal germplasm resource for mining allelic variations, association genetics, mapping and cloning of gene(s), and in applied breeding for the development of genetically diverse breeding lines/cultivars with superior yield and enhanced adaptation to diverse environments.

Table 2. Allelic diversity in reference set as detected by 48 and 107 SSR loci in chickpea.

<b>Parameter</b>	<b>48 loci</b>	<b>107 SSR loci</b>
<b>Allelic richness</b>		
Total number of alleles	1212	2254
Polymorphic information content	0.869	0.793
Gene diversity	0.881	812
Heterozygosity (%)	0.009	0.004
Rare alleles	382	666
Common alleles	803	1505
Most frequent alleles	27	83
Unique alleles	476	760
<b>Population structure</b>		
Number of sub-populations	4	9
<b>Biological diversity</b>		
Cultivated species	1160	2147
Desi type	1028	1931
Kabuli type	856	1676
Pea type	339	635
Wild <i>Cicer</i> species	252	499
<b>Regional diversity</b>		
Africa	478	894
Europe	128	230
Mediterranean	857	1589
Russian Federation	218	403
North America	216	403
South America	150	306
South & South East Asia	813	1598
West Asia	914	1695
Unknown	230	427

### ***Discovering new sources of variations using representative subsets***

Chickpea researchers at ICRISAT and elsewhere have extensively evaluated chickpea mini core collection accessions for agronomic traits and resistance to abiotic and biotic stresses and reported a number of new sources with agronomically beneficial traits.

***Variation for early maturity and large seed size:*** early maturity helps chickpea to avoid heat and drought stress and increases its adaptation especially in the sub-tropics. In a previous study, we identified 28 early maturing accessions, representing wide geographical diversity, using core collection approach, which were further evaluated for maturity and agronomic traits in five environments. ICC# 11040, 11180, 12424, 14648, 16641, and 16644 were reported early maturing, similar to or even earlier than controls, and produced on average 22.8% greater seed yield than the mean of four controls (pod yield, 1340 kg ha<sup>-1</sup>), with ICC# 14648, 16641 and 16644 having greater 100-seed weight (Upadhyaya et al. 2007). Kabuli chickpeas' (100-seed weight greater than 40 g) are late maturing types, and to enhance the adaptation of Kabulis' in sub-tropics, it is desirable to identify sources of early maturity in this group. Gowda *et al* (2011) evaluated 65 large-seeded Kabuli's for their agronomic performance and yield stability across 5-7 environments and reported two extra-large-seeded (100-seed weight >50 g) lines originating from Mexico, ICC 17109 and ICC 17452, with high yield potential and moderately stable across environments, which can be used in breeding program to develop large-seeded high yielding Kabuli cultivars or used directly for cultivation after evaluating their performance in large scale trials.

***Variability for crop growth rate and partitioning:*** crop growth rate, crop duration and partitioning coefficient

significantly impact grain yield in crop plants, including chickpea. Krishnamurthy *et al.* (2013b) reported large variability for crop growth rate and partitioning coefficient among 288 chickpea reference set accessions. Accessions with high crop growth rate and partitioning coefficient were ICC# 1392, 4958, 6263, 7441, 8384, 10309, 10399, 13124, 14199, 14669, 15510, 15606, 15618, 15762, 15802, and 16654. Most of these accessions were from mini core collection. The average grain yields among these accessions ranged from 1725 to 2165 kg ha<sup>-1</sup>, averaged 1943 kg ha<sup>-1</sup>; crop growth rate from 2.22 to 2.61 kg ha<sup>-1</sup> d<sup>-1</sup>, averaged 2.41 kg ha<sup>-1</sup> d<sup>-1</sup>; and partitioning coefficient from 0.81 to 1.03, averaged 0.94. In contrast, accessions with lower crop growth rate and partitioning coefficients, grain yield varied from 476 to 1182 kg ha<sup>-1</sup>, averaged 935 kg ha<sup>-1</sup>; crop growth rate from 1.42 to 2.10 kg ha<sup>-1</sup> d<sup>-1</sup>, averaged 1.89 kg ha<sup>-1</sup> d<sup>-1</sup>; and partitioning coefficient from 0.22 to 0.64, averaged 0.48. Accessions with high partitioning coefficient confer greater tolerance to drought and therefore good source of drought tolerance for use in breeding programs.

**Genetic variability for N fixation:** symbiotic nitrogen fixation (SNF) is cost-effective and sustainable strategy for nitrogen supply to agriculture worldwide, and the evidence to date suggests sufficient genetic variability for SNF and its associated component traits in germplasm collections (reviewed in Dwivedi *et al.* 2014). When assessed the SNF potential of 39 genetically diverse chickpea germplasm from USDA global chickpea core collection together with a commercial cultivar UC-5 in a glasshouse experiment, Biabani *et al.* (2011) detected large variation for proportion of plant nitrogen and total nitrogen fixed, which ranged respectively from 47% to 78% and from 0.020 to 0.084 g; an Iraq landrace accession, ILC 235 (PI 254549) being the highest N fixer. More particularly, it fixed, 121% more N than the total N fixed by UC 5 (0.038 g), which suggests that nitrogen fixation in commercial

chickpea cultivar may be improved by introgressing positive alleles from the germplasm. Clearly, there is a need to systematically assay the variation for SNF among the representative subsets (mini core or reference set) reported in chickpea (Upadhyaya and Ortiz 2001; Upadhyaya *et al.* 2008).

**Variability for drought tolerance traits:** terminal drought is the major constraint to chickpea productivity.  $\Delta^{13}\text{C}$  is an important trait conferring drought tolerance, which contribute to grain yield under drought stress conditions. When evaluated 280 chickpea reference set accessions for two seasons under drought stressed conditions, the mini core accessions were reported to have high  $\Delta^{13}\text{C}$  (19.63 - 20.14), with most of these maturing early and producing higher grain yield (1341 – 1992 kg ha<sup>-1</sup>) and harvest index (0.50 – 0.58) (Krishnamurthy *et al.* 2013a). These accessions were previously reported tolerant to drought (Krishnamurthy *et al.* 2010). Furthermore, when used drought response index as a measure of drought tolerance, it showed a positive association with crop growth rate, harvest index and the rate of partitioning but negatively associated with water use efficiency (Krishnamurthy *et al.* 2013b). A chickpea landrace accession from Israel, ICC 7571, was found highly tolerant to drought across seasons (Krishnamurthy *et al.* 2013b).

**Variability for herbicide tolerance:** chickpea is sensitive to many herbicides and therefore use of post-emergence herbicides is limited to manage weeds in chickpeas. Gaur *et al.* (2013) evaluated 278 chickpea reference set accessions for herbicides tolerance and reported large genetic variations among accessions in the mini core collection for tolerance to imazethapyr and metribuzin. In another study, three chickpea mini core collection accessions, ICC# 2242, 2580 and 3325, were reported tolerant to imazethapyr and imazamox herbicides (Taran *et al.* 2010).

**Multiple stress tolerant germplasm meeting breeders need:** Germplasm with multiple resistant traits, both abiotic and biotic stress, offer breeders opportunities to develop breeding and

genetic mapping populations combining multiple resistances into an agronomically improved genetic background. Upadhyaya *et al.* (2013) reported a number of genetically diverse accessions possessing agronomically beneficial traits. For example, a drought and salinity tolerant accession, ICC 3325, possesses resistance to fusarium wilt (FW), legume pod borer (LPB), and herbicide (Odyssey), while ICC 6874, 12155, and 14402, in addition to possessing resistance to drought, heat and salinity, also combine resistance to FW, LPB, or botrytis gray mold (BGM). Likewise, ICC 6279 is resistant to salinity, FW and BGM, while ICC 2580 to drought, salinity and herbicide (Table 3). Further analysis based on 48 SSRs revealed that these accessions were genetically more diverse pairs and agronomically superior than others. Furthermore, they reported a number of genetically diverse germplasm pairs with good agronomic performance and resistance to stress, which may be used in breeding programs to enhance trait values (Table 3).

Table 3. Multiple stress resistance and genetically diverse, agronomically superior chickpea germplasm

<b>Identity</b>	<b>Resistance</b>	<b>Yield (kg/ha)</b>	<b>Diverse pairs</b>	<b>Genetic distance</b>
ICC 2580	Drought, salinity, herbicide	1406	ICC 2580:ICC 3325	0.894
ICC 3325	Drought, salinity, FW, herbicide	1535	ICC3325: ICC6874	0.830
ICC 6279	Salinity, FW, BGM	1351	ICC 6279:ICC 3325	0.894
ICC 6874	Drought, salinity, heat, FW, LPB	1358	ICC 6874:ICC 6279	0.830
ICC 12155	Drought, salinity, heat, FW, BGM	1331	ICC12155:ICC14402	0.851
ICC 14402	Drought, salinity, heat, FW, LPB	1656	ICC14402:ICC3325	0.894

\*based on seven seasons evaluations

### ***Pre-breeding to enhance cultigen's gene pool***

Pre-breeding, the development of semi-finished product, provides a unique opportunity through introgression of desirable gene(s) from exotic germplasm into genetic backgrounds readily used by the breeders with minimum linkage drag (Sharma *et al.* 2013). The genus *Cicer* contains 44 species including 35 perennials and 8 annual wilds, in addition to *C. arietinum*, the cultivated chickpea. Of these, annual wild *Cicer* species hold a great promise for enriching the diversity in cultigen gene pool. For example, resistance to LPB from *C. reticulatum* (Mallikarjuna *et al.* 2007) or BGM from *C. reticulatum* and *C. echinospermum* (Ramgopal *et al.* 2013) have been successfully transferred into cultivated chickpea. Interspecific hybridization has also resulted progenies with improved agronomic performance in chickpea (Upadhyaya 2008). In another study three progenies originating from *C. reticulatum* × *C. arietinum* cross were found resistant to FW and produced 20% higher seed yield than the best-adapted cultivars (Yadav *et al.* 2004). Likewise, a pre-breeding line IPC 71 derived from *C. arietinum* × *C. judaicum* cross showed greater number of primary branches, more pods per plant and green seeds (Chaturvedi and Nadarajan 2010). Researchers at ICRISAT have also extracted several interspecific progenies, originating from cultivated × *C. reticulatum* accession (ICC 17160), which flowered 3-16 days early and yielded similar or greater (3.66 – 3.79 t ha<sup>-1</sup>) than highest yielding control Annigeri (flowered 43 days, seed yield 3.59 t ha<sup>-1</sup>) (ICRISAT, unpublished data).

### ***Impact of germplasm in chickpea breeding at ICRISAT***

With the formation of reduced subsets (mini core collection or genotype-based reference set) and their molecular profiling led to the identification of large number of genetically diverse germplasm accessions with agronomically beneficial traits for

use in chickpea breeding and genomics. For example, germplasm use in chickpea breeding at ICRISAT in comparison to 1993/2002 (177 unique germplasm used to make 1850 crosses) has increased by 18.5% in 2003/2011 (199 unique germplasm used to make 1755 crosses). Further, chickpea breeders at ICRISAT during 1974 to 2008 has so far used 99 unique germplasm or their derivatives (originating through breeding and selection) to develop 3728 advanced breeding lines (designated as ICCVs) with specific attributes, of which 89 have been released as cultivars in 26 countries, while 15 germplasm lines have been directly released as 22 cultivars in 15 countries (Tables 4 &5); all these releases contributing to food, income and nutritional security to millions of farmers engaged in chickpea cultivation in semi-arid tropic regions.

Table 4. List of desi chickpea germplasm released as cultivars.

<b>Country of release</b>	<b>Accession number</b>	<b>Country of origin</b>	<b>Released name</b>
Australia	ICC 14880	India	Hira
Bangladesh	ICC 3274	Iran	Bari Chhola7
	ICC 4998	India	Bina Sola 2
India	ICC 4923	India	Jyothi
Myanmar	ICC 552	India	Yezin 1
	ICC 4944	India	Keyhman
	ICC 4951	India	ICC 4951
Nepal	ICC 6098	India	Radha
Oman	ICC 237	India	ICC 237
USA	ICC 8521	Italy	Aztee



Table 5. List of kabuli chickpea germplasm released as cultivars.

Country of release	Accession number	Country of origin	Released name
Algeria	ICC 11879	Turkey	
	ICC 13816	USSR (former)	Yialousa
Cyprus	ICC 13816	USSR (former)	
Ethiopia	ICC 14808	India	Yelbey
Italy	ICC 13816	USSR (former)	Sultano
Morocco	ICC 11879	Turkey	
	ICC 14911	USSR (former)	
Sudan	ICC 8649	Afghanistan	Shendi
Syria	ICC 11879	Turkey	Ghab 1
	ICC 13816	USSR (former)	Ghab 2
Turkey	ICC 11879	Turkey	
	ICC 14911	USSR (former)	

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