PLANT GENETIC RESOURCES

Development of a Chickpea Core Subset Using Geographic Distribution and Quantitative Traits

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ABSTRACT

Chickpea (*Cicer arietinum* L.) is a major food legume and an important source of protein in many countries in Asia and Africa. Crop productivity continues to be low (0.78 t ha^{-1}). A very small number of the 16 991 accessions in the ICRISAT germplasm collection that contain a high level of genetic variability have been used in the chickpea improvement program. The objective of our research was to develop a core collection of chickpea that will enhance utilization of these resources in improvement programs and simplify their management. Germplasm accessions were stratified by country of origin and the data on 13 quantitative traits were used for clustering by Ward's method. From each cluster, ≈10% of the accessions were randomly selected to constitute a core subset of 1956 accessions. A comparison of mean data using Newman-Keuls test, variance using Levene's test, distribution using the χ^2 test, and Wilcoxon's rank-sum non-parametric test for different traits indicated that the genetic variation available for these traits in the entire collection had been preserved in the core subset. The important phenotypic correlations among different traits, which may be under the control of co-adapted gene complexes were also preserved in the core subset. This core subset will be a point of entry to the proper exploitation of chickpea genetic resources for the improvement of the crop.

Chickpea is a major food legume in many countries including Algeria, Ethiopia, India, Iran, Mexico, Morocco, Myanmar, Pakistan, Spain, Syria, Tanzania, Tunisia, and Turkey. In 1997, it was cultivated on 11.33 million hectares in the world with 8.80 million tonnes produced. Of the world production, 91% is produced in Asia, 3.0% in Africa, 1.0% in Europe, 2.5% in North and Central America (mainly Mexico), and 2.4% in Oceania (mainly Australia). In Asia, India accounts for 70.6% of the area and 74.8% of the production. Other important Asian countries such as Iran, Myanmar, Pakistan, and Turkey account for 26.9% of the area and 22.6% of the production. The productivity in these countries ranges from 0.49 t ha^{-1} in Iran to 0.93 t ha^{-1} in Turkey. The average world productivity of 0.78 t ha^{-1} is rather low. Improving the genetic potential of this crop for yield is the major objective in most improvement programs (Singh, 1987). In addition, resistance to ascochyata blight [caused by *Ascochyta rabei* (Pass.) Labr.], fusarium wilt [caused by *Fusarium oxysporum* Schlechtend.: Fr. f. sp. ciceris (Padwick) Matuo & K. Sato], fusarium root rot [caused by Fusarium solani (Mart.) Sacc. f. sp. Eumartii (C. Carpenter) W.C. Snyder & H.N. Hans.], dry root rot [caused by *Rhizoctonia bataticola* (Taubenhaus) E.J. Butler], and insects such as pod borer (*Helicoverpa armigera* Hubner) and leaf miner (*Liriomyza cicerina* Rondani) are important breeding goals. The importance of increased use of genetic resources to enhance the genetic potential of the crop for yield and in alleviating the biotic and abiotic stresses has been well recognized (Singh, 1987).

Emphasis on the importance of preserving crop germplasm in recent times has resulted in assembling and maintaining very large germplasm collections. The chickpea germplasm collection at the ICRISAT currently contains 16 991 accessions. Like other major crop species the number and size of the chickpea germplasm collection continues to grow. However, even the available diversity has not been adequately evaluated and extensively used in chickpea improvement. The sheer size of the germplasm collection has hindered efforts to enhance the use of this variability because of a lack of proper evaluation. The evaluation of this large germplasm collection is feasible only for the traits which can be scored easily and do not show genotype by environment (G × E) interactions. However, for applied plant breeding research the evaluation often requires replicated field evaluation and the traits of economic importance often display G × E interaction. This implies that the main collection needs to be reduced to a manageable level. Recognizing this, Frankel (1984) proposed that the collection should be pruned to a manageable sample or core collection. The core subset would be designed to minimize repetitiveness within the collection and it should represent the rich genetic diversity of a crop. The core collection could serve as a working collection which could be extensively examined, and the accessions which are not included in the core subset would be designated a reserve collection (Frankel, 1984). The information derived from extensive studies on the core subset could be used to guide more efficient utilization of the much larger reserve collection (Tohme et al., 1995; Brown, 1989b).

Frankel and Brown (1984) and Brown (1989a, 1989b) developed this proposal further and described methods to select a core subset using information on the origin and characteristics of the accessions. In setting the core subset, the first issue was its size. Brown (1989a), using sampling theory of selectively neutral alleles, argued that the entries in a core subset should be ≈10% of the total collection with a ceiling of 3000 per species. This level of sampling is effective in retaining 70% of the

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alleles of the entire collection. The second issue is the degree of the genetic similarity or commonality among accessions and determining groups within the entire collection. The hierarchy of grouping begins with the groupings suggested by taxonomy (species, subspecies, and races) followed by assigning accessions to major geographic groups (country, state), climate, or agroecological regions. The clustering within the broad geographic group could be done to sort accessions into clusters. The number of accessions selected from each cluster will depend on the strategy used.

Since the original proposal of Frankel (1984), core collections have been established for many species including common bean (*Phaseolus vulgaris* L.) (Tohme et al., 1995); barley (*Hordeum vulgare* L.) (Knupffer and van Hintum, 1995); chickpea (Hannan et. al., 1994); annual and perennial medicago species (*Medicago* spp.) (Diwan et al., 1994; Basigalup et al., 1995); perennial glycine (*Glycine* Willd.) (Brown et al., 1987); cassava (*Manihot esculenta* Crantz), (Cordeiro et al., 1995); coffee (*Coffea* spp.) (Dussert et al., 1997); lentil (*Lens culinaris* Medik.) (Erskine and Muehlbauer, 1991); okra [*Abelmoschus esculentus* (L.) Moench] (Mahajan et al., 1996); peanut (*Arachis hypogaea* L.), (Holbrook et al., 1993); quinoa (*Chenopodium quinoa* Willd.), (Ortiz et al., 1998); and sweetpotato [*Ipomoea batatas* (L.) Lam.] (Huaman et al., 1999). Hannan et. al. (1994) selected a core subset of 505 accessions from 3350 chickpea lines maintained at the Western Regional Plant Introduction Station in Pullman, WA. ICRISAT has the world's largest chickpea collection of 16 991 accessions in its genebank. Development of a core subset in the ICRISAT chickpea collection will provide a working collection to chickpea scientists, and this can be extensively evaluated. The information generated will allow the estimation of genetic variation for traits of economic importance. It will further provide an indication of the clusters to be examined in detail for particular traits. The objective of this study was to develop a core subset of the ICRISAT chickpea collection using geographic distribution and quantitative traits.

MATERIALS AND METHODS

We used 16 991 accessions from 44 countries for selecting the core subset for chickpea including 165 accessions where information on country of origin was not available, but data on all 13 quantitative traits was available. Data for all 13 traits were available in 16 264 accessions (Table 1). Data for days to 50% flowering, days to maturity, basal primary branches, apical primary branches, basal secondary branches, apical secondary branches, tertiary branches, and 100-seed weight was available on 16 928 accessions. Data were available in 16 840 accessions for plant height, 16 775 accessions for plant width, 16 879 accessions for number of pods per plant, 16 882 accessions for number of seeds per pod, and 16 356 accessions for seed yield. Sixty-three accessions from 12 countries did not have quantitative trait data available.

The ICRISAT chickpea collection was first stratified by country of origin. The accessions from small and adjacent countries with similar agroclimate were grouped together following Brown (1989a). Therefore, there were 40 sets (including one set with 165 accessions of unknown origin) representing chickpea accessions held at the ICRISAT genebank. The data on 13 traits in each group was standardized using the range of each variable to eliminate scale differences (Milligan and Cooper, 1985). The standardized data was subjected to the hierarchical cluster algorithm of Ward (1963) at an R^2 (squared multiple correlation) value of 0.75, using SAS (SAS Institute, 1989). This method optimizes an objective function because it minimizes the sum of squares within groups and maximizes the sum of squares among groups. The agglomerative procedure starts with n groups (i.e., one observation in one group; maximum among group sum of squares), and proceeds by merging observations in groups so that the between-groups sum of squares decreases and within-groups sum of squares increases. In certain cases the within-groups sum of squares will remain the same, but it will never decrease. From each cluster, ≈10% of the accessions were randomly selected for inclusion into the core subset. At least one accession was included even from those clusters that had less than 10 accessions. From the 63 accessions for which evaluation data was not available, six accessions were selected randomly and included in the core.

Means of the entire collection and core subset were compared using Newman-Keuls procedure (Newman, 1939; Keuls, 1952) for the 13 traits. The homogeneity of variances of the entire collection and core subset was tested with the Levene's test (Levene, 1960). The distribution homogeneity for each of the 13 traits among the entire collection and the core subset was analysed by the χ^2 test. The Wilcoxon (1945) rank-sum non-parametric test was performed with the SAS NPAR1-WAY procedure (SAS, 1989), to determine whether the core subset represents the entire germplasm collection for each of the 13 traits. To know whether these associations, which may

Table 1. Quantitative traits recorded in chickpea accessions held in the ICRISAT genebank.

Trait	Number of accessions†	Description
Days to 50% flowering	16 928	Number of days from planting to the stage when 50% of plants have begun to flower.
Plant height	16 840	Mean canopy height (cm) of five representative plants from soil surface measured at the end of flowering.
Plant width	16 775	Mean canopy spread (cm) of five representative plants at the end of flowering.
Days to maturity	16 928	Number of days from planting to the stage when 90% of pods have matured and turned yellow.
Basal primary branches	16 928	Mean number of basal primary branches on five representative plants.
Apical primary branches	16 928	Mean number of apical primary branches on five representative plants.
Basal secondary branches	16 928	Mean number of basal secondary branches on five representative plants.
Apical secondary branches	16 928	Mean number of apical secondary branches on five representative plants.
Tertiary branches	16 928	Mean number of tertiary branches on five representative plants.
Pods per plant	16 879	Mean number of pods on five representative plants.
Seeds per pod	16 882	Mean number of seeds of 10 pods each from five representative plants at maturity.
Seed yield	16 356	Seed yield at maturity (kg ha^{-1}).
100-seed weight	16 928	Measured on air dried seeds at 10% moisture content.

† Indicates the number of accessions in which the characteristic was recorded.

Table 2. Mean and variance for 13 traits recorded in the entire collection and core subset of chickpea.

Trait	Mean†			Variance†			
	Entire collection	Core subset	Significance	Entire collection	Core subset	F value	*P*
Days to 50% flowering (d)	62.42	62.83	NS‡	140.98	142.13	0.070	0.792
Plant height (cm)	37.52	37.81	NS	81.08	86.94	3.224	0.073
Plant width (cm)	40.47	40.27	NS	125.05	125.25	0.001	0.981
Days to maturity (d)	115.90	116.71	*	181.56	193.01	2.398	0.122
Basal primary branches (No.)	2.73	2.73	NS	0.55	0.58	0.377	0.539
Apical primary branches (No.)	1.38	1.40	NS	1.69	1.84	3.604	0.058
Basal secondary branches (No.)	3.06	3.09	NS	2.60	2.83	3.642	0.056
Apical secondary branches (No.)	4.59	4.52	NS	6.91	6.97	0.024	0.878
Tertiary branches (No.)	0.80	0.86	*	1.92	2.46	12.619	0.000
Pods per plant (No.)	40.46	40.29	NS	566.38	600.30	0.856	0.355
Seeds per pod (No.)	1.23	1.23	NS	0.05	0.06	6.525	0.011
Seed yield (kg ha^{-1})	1216.34	1196.02	NS	378 385.9	393 875.0	0.828	0.363
100-seed weight (g)	16.77	17.15	*	54.00	63.41	7.639	0.006

* Significant at $P = 0.05$.
† NS, nonsignificant at $P = 0.05$.
‡ Differences between mean of entire collection and core subset were tested by Newman-Keuls test and variance homogeneity was tested by Levene's test.

be under genetic control, were conserved in the core subset, the phenotypic correlations among different traits in the entire collection and core subset were estimated independently.

RESULTS AND DISCUSSION

The procedure used to select the core subset for chickpea resulted in the selection of 1956 accessions from the ICRISAT germplasm collection. The composition of the core subset reflected the predominance of germplasm from Asia in the entire collection in the ICRISAT genebank. In the entire collection, 14 393 accessions (84.7%) were from Asia, 1436 (8.5%) from Africa, 619 (3.6%) from America, and 371 (2.2%) from Europe. In the core subset the number of accessions included were 1579 (80.7%) from Asia, 200 (10.2%) from Africa, 87 (4.5%) from America, and 60 (3.1%) from Europe. In Asia, South Asia accounted for 8002 accessions (47.1%) in the entire collection, and 870 accessions (44.5%) in the core subset. Southwest Asia and the Mediterranean, which are the two centers of primary diversity, accounted for 5540 (32.6%) and 402 (2.4%) accessions in the entire collection and for 588 (30.1%) and 53 (2.7%) accessions in the core subset, respectively. Ethiopia, which is the secondary center of diversity for chickpea, accounted for only 928 (5.5%) accessions in the entire collection and 120 (6.1%) accessions in the core subset. Thus, Ethiopia appears underrepresented in the ICRISAT collection. The contribution of India in the entire collection (7174 accessions, 42.2%) and the core subset (734 accessions, 37.5%) reflected the past cooperation of ICRISAT and Indian National Program on the collection and conservation of this crop.

Table 3. Chi-square test and probability for comparison of frequency distribution for 13 traits in core subset with the entire collection of chickpea.

Trait	Number of classes	χ^2	*P*
Days to 50% flowering (d)	8	9.859	0.197
Plant height (cm)	11	11.391	0.328
Plant width (cm)	11	12.614	0.246
Days to maturity (d)	8	12.561	0.835
Basal primary branches (No.)	16	23.862	0.067
Apical primary branches (No.)	10	12.269	0.199
Basal secondary branches (No.)	10	14.588	0.103
Apical secondary branches (No.)	12	9.946	0.535
Tertiary branches (No.)	11	24.901	0.006
Pods per plant (No.)	12	10.287	0.505
Seeds per pod (No.)	12	8.645	0.655
Seed yield (kg ha^{-1})	10	15.488	0.079
100-seed weight (g)	10	25.558	0.002

Differences among means of the entire collection and core subset were found significant only for days to maturity, tertiary branches, and 100-seed weight (Table 2). The variances of the entire collection and core subset were homogeneous for all the traits except tertiary branches ($P = 0.0004$), seeds per pod ($P = 0.011$), and 100-seed weight ($P = 0.006$); (Table 2). Between 92.3 to 100% of the variation range of the entire collection was included in the core subset for plant height, days to maturity, number of pods per plant, number of seeds per pod, and seed yield. In six out of eight remaining traits the variation included ranged from 83.7 to 88.2%. For basal primary branches and apical primary branches the range variation included in the core was 62.3 and 72.5%, respectively. These results indicated that the chosen core subset is representative of the entire collection and that the variation was preserved.

The analysis of frequency distribution, except tertiary branches ($P = 0.006$) and seeds per pod ($P = 0.002$), indicated homogeneity of distribution among the entire and core subset (Table 3). The Wilcoxon rank-sum test also indicated that all the variables except days to maturity ($P = 0.013$) have similar distribution in both the core subset and entire collection. Similarly for the 13 traits, which were not considered for selecting the core subset because of availability of data, the means of 11 traits were not significantly different and the variances of all traits were homogeneous in the core subset and entire collection. The range of the entire collection represented in the core subset was 100% for both fusarium wilt and ascochyata blight resistance, 83.9% for flowering duration, and 79.6% for protein content. For traits like resistance to botrytis gray mold (caused by *Botrytis cinerea* Pers. ex Fr.) and ascochyata blight, the percentage of accessions with a score in the core subset was similar to the entire germplasm. For example, in the entire collection only nine (0.24%) out of the total 3721 accessions evaluated had a rating of three on a 1–to–9

Table 4. Correlation coefficients between 13 phenotypic traits in the entire collection and core subset of chickpea.

	DF†	PH	PW	DM	BP	AP	BS	AS	TB	PN	SN	YLD
PH	0.483 (0.453)‡											
PW	0.132 (0.112)	0.541 (0.518)										
DM	0.665 (0.662)	0.404 (0.392)	−0.023 (−0.035)									
BP	0.098 (0.113)	0.154 (0.129)	0.255 (0.226)	−0.037 (−0.013)								
AP	−0.305 (−0.293)	−0.070 (−0.072)	0.087 (0.073)	−0.207 (−0.213)	0.055 (0.072)							
BS	0.135 (0.169)	0.033 (0.052)	0.034 (0.046)	0.115 (0.158)	0.269 (0.311)	−0.097 (−0.094)						
AS	−0.136 (−0.134)	0.031 (0.033)	0.249 (0.294)	−0.091 (−0.094)	0.246 (0.302)	0.214 (0.216)	0.220 (0.254)					
TB	0.191 (0.202)	0.050 (0.035)	−0.194 (−0.207)	0.276 (0.297)	−0.052 (0.002)	0.057 (0.085)	0.179 (0.223)	−0.040 (−0.043)				
PN	−0.199 (0.169)	−0.048 (−0.068)	0.235 (0.206)	−0.154 (−0.138)	0.279 (0.311)	0.268 (0.261)	0.277 (0.289)	0.554 (0.567)	0.112 (0.148)			
SN	0.047 (0.035)	−0.032 (−0.038)	0.010 (0.001)	0.014 (0.010)	0.024 (−0.053)	−0.067 (−0.023)	0.059 (0.033)	0.014 (0.027)	0.002 (−0.012)	0.002 (−0.003)		
YLD	−0.129 (−0.138)	0.141 (0.121)	0.364 (0.322)	−0.163 (−0.167)	0.263 (0.256)	0.226 (0.230)	0.091 (0.078)	0.384 (0.377)	0.079 (0.075)	0.603 (0.589)	−0.011 (−0.032)	
SW	0.119 (0.081)	0.338 (0.364)	0.075 (0.100)	0.259 (0.256)	−0.041 (−0.045)	−0.068 (−0.093)	−0.043 (−0.034)	−0.077 (−0.099)	−0.036 (−0.035)	−0.282 (−0.291)	−0.323 (−0.309)	−0.133 (−0.124)

† DF, days to 50% flowering; PH, plant height at maturity; PW, plant width at maturity; DM, days to maturity; BP, basal primary branches per plant; AP, apical primary branches per plant; BS, basal secondary branches per plant; AS, apical secondary branches per plant; TB, tertiary branches per plant; PN, pods per plant; SN, seeds per pod; YLD, yield in kg ha^{-1}; SW, weight of 100 seeds.

‡ Figures in brackets are phenotypic correlation coefficients for the core subset.

scale, where 1 = resistant and 9 = very susceptible. Only one of these accessions with a low score was included in the core subset.

A proper and adequate sampling for developing a core collection should consider the conservation of phenotypic associations arising from co-adapted gene complexes (Ortiz et al., 1998). This core collection preserves the phenotypic correlations observed in the entire collection (Table 4). This clearly suggests that the co-adapted gene complexes controlling these associations were properly sampled and that the selection of this core collection was adequate in this regard. The strong correlation among some of the traits like days to 50% flowering, days to maturity (r = 0.665 in entire collection, r = 0.662 in core subset), seed yield, and number of pods per plant (r = 0.603 in entire collection, r = 0.589 in core subset) indicated that future characterization of germplasm may use only days to 50% flowering and seed yield. Both of these traits are less laborious to measure than days to maturity and pods per plant.

This core subset can be used very effectively as a starting point for research projects involving screening of the germplasm collection for sources of desirable traits in chickpea. The information on clusters to which particular accessions with traits of interest belong will assist in looking extensively for more accessions with similar traits. For example, ICC 931 is the only line in the core subset with a score of three on a 1–to–9 (resistant–very susceptible) scale for resistance to ascochyta blight. This accession belongs to cluster 95 from Indian accessions. There are 50 accessions in the cluster containing ICC 931. Theoretically, these accessions may be similar to ICC 931 and also could be valuable sources of resistance. The core subset will also provide an efficient germplasm subset if it is not feasible to screen the entire germplasm collection. For example, in diseases like botrytis gray mold, which is one of most destructive diseases of chickpea, the information on amount of variability present in the germplasm is very limited (only 531 accessions have been screened). Using the currently available screening technique, it will take at least 10 yr to examine the entire germplasm collection. However, the core subset would allow us to determine the amount of genetic variability in the entire germplasm collection and possibly identify new sources of alleles for resistance within ≈1 yr.

The development of the chickpea core subset helps in tackling new constraints that may arise because of new diseases or insect pests. Because the core subset represents the entire germplasm collection and seed of the core accessions are available, resistance sources to the new disease or an insect pest may be identified rapidly. Additional sources of resistance can be found from the reserve collection and examined selectively from the same cluster from which sources in the core subset have been identified.

The resources available for evaluation of germplasm are limited and dwindling steadily. Therefore, extensive evaluations of the entire germplasm collection are not possible. This core subset provides a working collection of chickpea germplasm that can be extensively examined for all economically important traits. The data generated will provide the much needed information on genetic variability in chickpea and possible relationships among traits. This information will assist further in the decision-making process to acquire new variability for a trait showing a very limited variation in the core subset. This chickpea core subset should be revised periodically as additional accessions and information becomes available. The list of chickpea entries included in the core subset with the name of country of origin, ICC number, and the cluster number are available on diskette, free

of charge from the corresponding author. This list is also available on the web site at http://grep.icrisat.cgiar.org/Project1/cpcore_1.html.

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