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Two major genes for seed size in chickpea (*Cicer arietinum* L.)

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Summary

Seed size as determined by seed weight, is an important trait for trade and component of yield and adaptation in chickpea (*Cicer arietinum* L.). Inheritance of seed size in chickpea was studied in a cross between ICC11255, a normal seed size parent (average 120 mg seed^{-1}) and ICC 5002, a small seed size parent (average 50 mg seed^{-1}). Seed weight observations on individual plants of parents, F_1, F_2, and backcross generations, along with reciprocal cross generations revealed that the normal seed size was dominant over small seed size. No maternal effect was detected for seed size. The numbers of individuals with normal, small and medium (average 150 mg seed^{-1}) seed sizes in F_2 population were 1237, 323 and 111 fitting well to the expected ratio of 12:3:1 ($\chi^2 = 0.923$, $P = 0.630$). The segregation data of backcross generations also indicated that seed size in chickpea was controlled by two genes with dominance epistasis. We designate the genotype of ICC 11255 as $Sd_1Sd_1sd_2sd_2$, and ICC 5002 as $sd_1sd_1Sd_2Sd_2$ wherein Sd_1 is epistatic to Sd_2 and sd_2 alleles.

Introduction

Globally, chickpea (*Cicer arietinum* L.) is the third most important food legume crop, which is extensively grown throughout the semi-arid tropics. Presently, it is cultivated in 42 countries spreading over all the continents. The current emphasis on soil heath, environmental quality, and economic consideration has stimulated significant changes in cropping systems throughout the world leading to the expansion of chickpea in countries like Canada and Australia. In 2003, the global production of chickpea was 7.12 million t coming from 10.37 million hectares area with average productivity of 687 kg ha^{-1} (FAO, 2003).

Seed size has always been a trait of consumer preference (Singh, 1987) besides an important component of yield and adaptation (Singh & Paroda, 1986). Screening of more than 16 000 accessions at ICRISAT has revealed a wide range of variation in seed size (40 to 630 mg seed^{-1}) in the world chickpea germplasm collection (Upadhyaya, 2003). More than 60% of the accessions have seed weight of 90–140 mg seed^{-1} and only a few accessions (five in number) with seeds of about 50 mg weight. Variation in seed size has been associated with geographical pattern (Upadhyaya, 2003) and different fitness components of seedling and adult plants (Narayanan et al., 1981; Dahiya et al., 1985). Therefore, seed size is an important breeding objective in chickpea improvement programs. To understand the heritable forces driving phenotypic variation in the seed size, it is important to identify the genes responsible for this variation. Unfortunately, because of its quantitative nature, the genetic analysis of this trait has been restricted mostly to the estimation of heritability.

Earlier studies in chickpea showed low (Sandha & Chandra, 1969) as well as high (Niknejad et al., 1971; Kumar & Singh, 1995) estimates of heritability for seed size. Small seed size was found dominant over large one (Athwal & Sandha, 1967; Smithson et al., 1985; Kumar & Singh 1995; Malhotra et al., 1997).

However, there are reports suggesting reverse to be true as well (Niknejad et al., 1971). Additive gene action has been reported to be predominant for seed size inheritance (Malhotra & Singh, 1989; Singh et al., 1992; Singh et al., 1993; Kumar & Singh, 1995). Seed size inheritance in chickpea was reported to be monogenic (Argikar, 1956), oligogenic (Balasubrahmanyan, 1950; Patil & D'Cruze, 1964), digenic (Ghatge, 1993) and polygenic (Athwal & Sandha, 1967; Niknejad et al., 1971; Kumar & Singh, 1995; Malhotra et al., 1997). Niknejad et al. (1971) reported that one or two genes could explain substantial genetic variation in seed size. Ghatge (1993) identified two additive genes for seed size in chickpea. Recently, a QTL accounting for 52% of the total phenotypic variation for seed size has also been identified (Cho et al., 2002). However, all the above studies have taken normal seed size as small seed parent. Therefore, the present study was taken up to elucidate the genetic control of seed size in chickpea in a cross involving parents with small and normal seed sizes.

Materials and methods

World chickpea germplasm collection at ICRISAT has five accessions with seed weight of about 50 mg seed^{-1} (38–53 mg seed^{-1}). These accessions with small seed size have never been included in genetic studies on seed size in the past. A high proportion of accessions in the germplasm have an average seed weight of about 120–130 mg seed^{-1}. We, therefore, selected an accession ICC 5002 (seed weight of 50 mg seed^{-1}) as small seed parent and ICC 11255 (120 mg seed^{-1}) as normal seed size parent. ICC 5002 is a breeding line from India and ICC 11255 is a landrace cultivar from Pakistan. These two accessions representing limited seed size differences were consciously selected so as to uncover a few major genes responsible for seed size differences among them. The cross, ICC 11255 × ICC 5002 and its reciprocal were attempted in the 1998–1999 postrainy season. The F_1 hybrid and its reciprocal were crossed to both the parents to generate backcross generations (BC_1P_1 and BC_1P_2) and also selfed to produce F_2 populations in the 1999–2000 postrainy season.

Parents, F_1, F_2, and backcross generations of the cross along with its reciprocal generations were evaluated in an unreplicated trial at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, India in the 2000–2001 postrainy season. The plot size varied with the generations. Parents, F_1 and backcross generations were grown in 2-row plots and F_2 generations in as many rows as possible depending on the quantity of seeds available. The rows were 4 m long and 60 cm apart with plants spaced at 20 cm within a row. Seeds were treated with fungicide before planting to protect the crop from soil borne pathogens. Standard cultural practices were followed to raise a successful crop. The experiment was irrigated twice to protect the crop from forced maturity. Weed control was carried out as needed. The experiment was fully protected from insect pests by spraying appropriate chemicals.

Seed weight was chosen as the measure of seed size and observations were recorded on all the plants available, except those on border, in different generations. Mature pods were harvested from each plant separately, and seed size was measured individually in milligrams per seed by dividing the weight of all the seeds of a plant by the number of seeds. Wherever available, a random sample of 100 seeds was taken from each plant to take the seed weight.

The seed size data of different generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2 as well as for their reciprocal generations) were subjected to mean, variance, range and standard error estimation. The means of F_1, F_2, and backcross generations were compared with their reciprocals using '*t*' test to find out the maternal effect. In case of non-significant reciprocal differences, the data were pooled for further analysis. Based on the distribution pattern in F_2 population, the quantitative data of seed size collected from the individual plants were converted into qualitative data using the critical seed sizes of 75 and 140 mg as the break points. The qualitative data were then analyzed using Mendelian ratio. The χ^2 test was performed to determine if the observed ratio for normal, small and medium seed sizes deviate from the expected. The observed ratio was considered to fit the expected ratio if the appropriate χ^2 value had a corresponding probability equal or greater than 0.05.

Results and discussion

Seeds from the parental line ICC 11255 were on average 139% heavier than the seeds of ICC 5002 (Table 1). The average weight of small seeded parent ICC 5002 was 50.8 mg seed^{-1} with a range of 40–54 mg whereas ICC 11255 had an average seed weight of 121.6 mg with a range of 115–127 mg. The F_1 hybrid seeds obtained by using ICC 5002 as female parent were not significantly different in size from the seeds obtained

Table 1. Mean (mg per seed), variance, range and number of plants in different generations of the cross ICC 11255 × ICC 5002 and its reciprocal in chickpea

Generation	Observed mean (mg)	Variance	Range (mg)	Number of plants	Expected mean (mg) in case of 12:3:1
ICC 11255 (P_1)	121.6 ± 0.84	1.76	115–127	25	
ICC 5002 (P_2)	50.8 ± 0.63	9.84	40–54	25	
F_1 (ICC 11255 × ICC 5002)	119.6 ± 1.84	70.80	102–136	21	121.6
F_1 reciprocal (ICC 5002 × ICC 11255)	121.3 ± 1.37	41.11	111–133	22	121.6
F_1 (Pooled)	120.5 ± 1.75	56.30	102–136	43	121.6
F_2 (ICC 11255 × ICC 5002)	105.8 ± 0.87	770.5	30–178	1006	110.1
F_2 reciprocal (ICC 5002 × ICC 11255)	106.4 ± 1.09	787.7	35–198	665	110.1
F_2 (Pooled)	106.0 ± 0.68	771.7	30–198	1671	110.1
BC_1P_1 (ICC 11255)					
F_1 × ICC 11255	127.3 ± 4.10	335.8	90–152	20	121.6
F_1 reciprocal × ICC 11255	127.4 ± 3.86	193.8	108–148	12	121.6
Pooled	127.6 ± 2.95	279.4	90–152	32	121.6
BC_1P_2 (ICC 5002)					
F_1 × ICC 5002	78.6 ± 4.59	441.8	52–131	21	86.2
F_1 reciprocal × ICC 5002	78.7 ± 6.39	735.7	45–121	19	86.2
Pooled	78.6 ± 3.79	561.6	45–131	40	86.2

from the reciprocal cross using ICC 11255 as female parent. Similarly, the reciprocal differences were not observed in further generations (F_2, BC_1P_1 and BC_1P_2) (Table 1). This showed that the maternal genetic factors are not involved in seed size inheritance in chickpea.

The average weight of F_1 seeds was 120.5 mg seed^{-1} with a range of 102–136 mg. The ranges for normal seed size parent, ICC 11255 and F_1 hybrid overlapped. Seeds of both F_1 hybrid and reciprocal were consistently equal in size to the seeds of ICC 11255. This indicated that normal seed size is dominant over the small seed size.

The range and variation in seed size of the segregating populations was much higher than the parents and F_1 generation. The mean seed weight of F_2 generation was 106.0 mg with a range of 30–198 mg. The average seed weights were 127.6 and 78.6 mg in BC_1P_1 (F_1 × ICC 11255) and BC_1P_2 (F_1 × ICC 5002) progenies with respective ranges of 90–152 and 45–131 mg.

Despite the continuous variation in seed size of individual plants in F_2 population, three peaks at 60, 115 and 150 mg, and beginning of two valleys at 75–80 mg and 140–145 mg appeared in the frequency distribution of seed size (Figure 1). Therefore, individual plants with seed size of 30–75 mg, 76–140 mg and 141–200 mg were grouped into three classes of small, normal and medium seed sizes. However, in the backcross generation with ICC 5002, the break point occurred

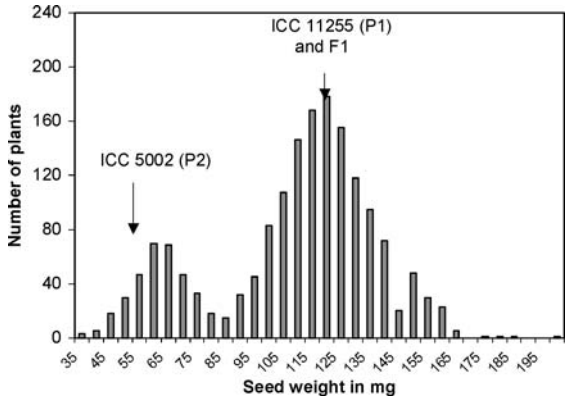

Figure 1. Segregation pattern of seed size in F_2 of a cross ICC 11255 × ICC 5002 of chickpea. The arrows indicate mean values of seed size for parents and F_1.

at 80 mg (Figure 2) and therefore this was considered while classifying the plants having small or normal seed size. The individual plants in the F_2 generation, which were grouped into small seed size class showed average mean of 59 mg, which was not significantly different from the seed weight of small seed sized parent, ICC 5002 (50.8 mg). Similarly, plants that were grouped into normal seed size class had an average seed weight of 115 mg, which was not significantly different from the seed weight of the normal seed sized parent, ICC

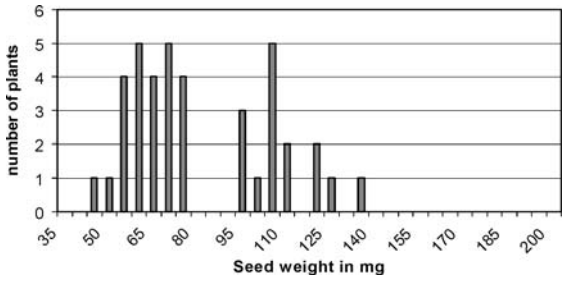

Figure 2. Segregation pattern of seed size in (ICC 11255 × ICC 5002) × ICC 5002 backcross generation.

11255 (121 mg). Another set of individuals having average seed size of 155 mg appeared in the F_2 population as a new combination among the genes responsible for controlling the seed size in both the parents.

Since the segregation relationships within F_1, F_2 and backcross populations were not significantly different in reciprocal crosses, the reciprocal cross data were pooled for further analysis. Observed segregation ratios of large number of progenies for F_1, F_2, BC_1P_1 and BC_1P_2 were tested for goodness of fit to digenic model involving duplicate dominance epistasis (12 normal: 3 small: 1 medium). The numbers of individuals with normal, small and medium seed sizes in F_2 population were 1237, 323 and 111 fitting well to the expected ratio of 12:3:1 ($\chi^2 = 0.923$, $P = 0.630$). This provided an evidence for major qualitative trait loci controlling the seed size in chickpea.

The backcross progeny generated from ICC 5002 parent segregated in 24 normal and 16 small seeds (Table 2). This fitted well with the expected ratio of 1 normal: 1 small ($\chi^2 = 1.600$, $P = 0.206$). The backcross progeny from ICC 11255 parent did not segregate and all plants had normal seeds. These results were consistent with digenic inheritance with duplicate dominant epistasis.

Generation mean analysis of the present data revealed that the dominance and dominance × dominance interaction effects were of opposite signs, indicating classical dominant epistasis with 12:3:1 ratio in the F_2.

Therefore, the segregation data, indicated that seed size in chickpea is controlled by two genes

Table 2. The number of plants in different classes of seed size, chisquare values and probability of goodness of fit for expected ratios in the F_2 and backcross generations in a cross involving ICC 11255 and ICC 5002 chickpeas

	Phenotype in seed size					
Generation	Normal (76–140 mg)	Small (30–75 mg)	Medium (141–200 mg)	Expected ratio	χ^2 value	*P*
Cross ICC 11255 × ICC 5002						
ICC 11255	25	0	0			
ICC 5002	0	25	0			
F_1	21	0	0			
F_2	748	192	66	12:3:1	0.277	0.871
F_1 × ICC 11255	20	0	0			
F_1 × ICC 5002	14	7	0	1:1	2.333	0.127
Reciprocal Cross ICC 5002 × ICC 11255						
ICC 5002	0	25	0			
ICC 11255	25	0	0			
F_1	22	0	0			
F_2	489	131	45	12:3:1	0.795	0.672
F_1 × ICC 5002	10	9	0	1:1	0.053	0.818
F_1 × ICC 11255	12	0	0			
Pooled						
ICC 11255	25	0	0			
ICC 5002	0	25	0			
F_1	43	0	0			
F_2	1237	323	111	12:3:1	0.923	0.630
F_1 × ICC 11255	32	0	0			
F_1 × ICC 5002	24	16	0	1:1	1.600	0.206

with dominance epistasis. We designate the genotypes of ICC 11255 as $Sd_1Sd_1sd_2sd_2$, and ICC 5002 as $sd_1sd_1Sd_2Sd_2$ wherein Sd_1 is epistatic to Sd_2 and sd_2 alleles. Therefore, the individuals that are homozygotes and heterozygotes for the Sd_1 allele will have a normal seed size. Those that are homozygotes or heterozygotes for the Sd_2 allele with recessive homozygote at the first locus will have small seeds, and those homozygotes for both the alleles, sd_1 and sd_2, will have a medium seed size.

This study indicated dominance of normal seed size over small seed size with two major genes while earlier studies indicated partial dominance of normal seed size over large seed size under the control of polygenes with additive effect (Athwal & Sandha, 1967; Smithson et al., 1985; Kumar & Singh, 1995; Malhotra et al., 1997).

The epistatic interaction of the seed size genes to produce medium seed is also of great interest. It shows that a cross between small and normal parents can give rise to a recombinant with better seed size. It would be interesting to involve four remaining small seeded germplasm lines available in the ICRISAT genebank in the genetic studies with ICC 5002 to determine allelic relationship for seed size.

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