RESEARCH NOTE

Major genes with additive effects for seed size in kabuli chickpea (*Cicer arietinum* L.)

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In the present investigation, inheritance of seed size was studied in a cross involving two small-seeded kabuli cultivars, ICCV 2 and L 550. Mean 100-seed weight (100 SW) of parents and that of their F_1 generation were similar. However, transgressive segregants were obtained in F_2 generation in both directions. Considering the segregation pattern, plants in F_2 and backcross generations were divided into three groups, 100 SW less than F_1/parents ($<F_1P_1P_2$), 100 SW similar to F_1/parents ($F_1P_1P_2$), and 100 SW greater than F_1/parents ($>F_1P_1P_2$). The numbers of plants in the three groups in F_2 fitted well to an expected ratio of 5:6:5 ($\chi^2 = 2.15$, $P = 0.34$), and in backcross generations to an expected ratio 1:2:1 ($\chi^2 = 0.94$, $P = 0.33$ in BC_1P_1, and $\chi^2 = 3.89$, $P = 0.14$ in BC_1P_2), which suggested that seed size in the two parents is controlled by two genes exhibiting additive effects with each parent having one pair of alleles with increasing effect at one locus in homozygous form.

Chickpea (*Cicer arietinum* L.) is the third most important food legume crop in the world and is grown over an area of 11.55 million hectare with production of 10.46 million metric tonnes (MT) and productivity of 906 kg ha^{-1} (Food and Agriculture Organization 2009, http://www/FAO.ORG. FAOSTAT database). Of the two types of chickpea, desi (characterized by pink flowers, angular shaped and brown coloured small seeds) and kabuli (white flowers, owl's head shaped, beige coloured large seeds), large-seeded kabuli types are gaining importance, as the market price of kabuli chickpeas is upto twice that of desi chickpea (Upadhyaya *et al.* 2006). Very large (>45 g 100 SW) kabuli chickpeas are being sold at about three times the price of desi chickpea and about two times the price of medium-seeded (~25 g 100 SW) kabuli chickpea in India (Gaur *et al.* 2006). During the last few years, several kabuli cultivars have been developed but majority of these varieties are small-seeded to medium-seeded and do not meet the market preference for large and very large seed types. Hence, there is a need to develop large-seeded kabuli types.

The study of inheritance of seed size (100 SW) is important for adopting appropriate breeding strategies to develop large-seeded kabuli chickpea cultivars. Earlier studies have shown that large-seeded progenies could be obtained from crosses involving parents with small and large seeds (Kumar and Singh 1995; Malhotra *et al.* 1997). The major bottleneck associated with using large-seeded kabuli parents in developing new cultivars is associated with their low yield potential. Hence, in the present investigation, two small-seeded high yielding and well adapted kabuli cultivars, ICCV 2 and L 550 (both having 18–19 g 100 SW) (Kumar *et al.* 1985; Dua *et al.* 2001) were used to test the hypothesis of obtaining progenies having higher 100 SW than the parents. ICCV 2 (ICC 12968) is a breeding line derived through a complex cross, $\{[F_3\ (K - 850 \times Gw - 5/7)\ \times P - 458] \times F_3\ (L - 550 \times Gaumuchil)\ - 2\}$ and L 550 was derived from a cross, Pb 7 × Rabat. The F_1 cross, ICCV 2 × L 550 was performed and the offspring were crossed to both parents to generate backcross generations (BC_1P_1 and BC_1P_2), and also selfed to produce F_2 generation.

Parents, F_1, F_2 and backcross generations of the cross were evaluated in an unreplicated trial at ICRISAT, Patancheru, India, in 2008–09 postrainy season. Parents and F_1 generation were grown in 2-row plots, F_2 in 75 rows, and the backcross generations in 9 rows. The rows were 4-m long, and 60 cm apart with a plant-to-plant spacing of 20 cm.

Data were recorded on five representative plants for parents and on all the available plants, except those on border rows, in the F_1, F_2 and backcross generations. The weight of 100 randomly selected seeds from each plant was recorded.

The mean, variance, range, and standard deviation of different generations (P_1, P_2, F_1, F_2, BC_1P_1 and BC_1P_2) were determined separately. The mean 100 SW of the parents, ICCV 2 (18.6 g) and L 550 (18.3 g) and F_1 (18.7 g) were

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similar (table 1) with overlapping range. The nonsignificant differences for the mean 100 SW between parents and their F_1 showed that 100 SW is controlled by two loci having additive effects that are dispersed in the parents. Thus, each parent carried two alleles with increasing effects at one locus in homozygous form and F_1 had two alleles with increasing effects, one each at both the loci, in heterozygous form. The mean 100 SW of F_2 generation was similar to both parents and F_1 (18.9 g) and had a continuous variation (range 9.8–38.6 g) (table 1; figure 1). However, a proportion of F_2 plants showed 100 SW lesser as well as greater than the either parents or their F_1 generation. This indicated the possibility of presence of alleles causing increase and/or decrease in 100 SW occurring at different loci in the two parents. Therefore, the individual plants in F_2 and backcross generations were divided into three groups: following mean ± 1 standard deviation (s.d.). The plants with 100 SW less than the mean of parents−1s.d. were classified into first group (less than parents/F_1, $<F_1P_1P_2$), those with 100 SW greater than parental mean − 1s.d. and less than mean + 1s.d., into second group (parental/F_1 type, $F_1P_1P_2$), and those with 100 SW greater than parental mean + 1s.d. into the third group (greater than parents/F_1, $>F_1P_1P_2$). Thus, 33% plants in F_2 generation having 100 SW in the range of 17.1–20.3 g were classified into group 2 ($F_1P_1P_2$), 35% plants having 100 SW less than 17.1 g (range 9.8–17.0 g) into group 1 ($<F_1P_1P_2$) and remaining about 33% plants having 100 SW greater than 20.3 g (range 20.4–38.6 g) were classified into group 3 ($>F_1P_1P_2$).

Considering a two loci segregation with additive effects, the expected ratios of the three groups 5:6:5 :: $<F_1P_1P_2$: $F_1P_1P_2$: $>F_1P_1P_2$ in F_2 generation and 1:2:1 in the two backcross generations were tested for goodness of fit using χ^2 test. Following this genetic model (two loci dispersed in parents and having additive effects), the group 2 would have two alleles for increasing 100 SW either at one locus in homozygous form (parental type) or one each at both loci in heterozygous form (F_1 type). Group 1 would have only one allele at either locus or no allele, whereas group 3 will have three (homozygous at one and heterozygous at other locus) or four alleles (homozygous at both loci). The numbers of plants in F_2 generation in the three groups were 321 in $<F_1P_1P_2$, 345 in $F_1P_1P_2$, and 296 in $>F_1P_1P_2$ and fitted well to an expected ratio of 5:6:5 ($\chi^2 = 2.15$, $P = 0.34$) (table 1). In the backcross generation with ICCV 2, BC_1P_1, the population size was small ($n = 9$). Hence, Yate's correction (Yates 1934) was used to account for low frequency of the plants. In BC_1P_1 and BC_1P_2, the frequency of plants in three groups fitted well to an expected ratio of 1:2:1 ($\chi^2 = 0.94$, $P = 0.33$ for BC_1P_1 and $\chi^2 = 3.89$, $P = 0.14$ for BC_1P_2) (table 1). Overall and the χ^2 due to heterogeneity were also nonsignificant ($\chi^2 = 4.69$, $P = 0.10$) confirming the expected segregation of 1:2:1 in the backcross generations (table 1).

Further, the mean 100 SW of the plants in three groups $<F_1P_1P_2$, $F_1P_1P_2$ and $>F_1P_1P_2$ in BC_1P_2 was compared with the same groups in F_2 generation to determine the effect of

Table 1. Mean, variance, range and the number of plants in different groups, chi-square values and probability of goodness of fit for expected ratios in the F_2 and backcross generations in a cross involving ICCV 2 and L 550 kabuli chickpeas.

Generation	Observed mean (g)	Variance	Range	Number of plants in groups			Expected ratio	χ^2	P
				$<F_1P_1P_2$ (less than two alleles with increasing effects)	$F_1P_1P_2$ (two alleles with increasing effects)	$>F_1P_1P_2$ (more than two alleles with increasing effects)			
ICCV 2 (P_1)	18.6 ± 0.43	0.94	17.7–20.2	0	5	0			
L 550 (P_2)	18.3 ± 0.85	0.73	16.9–19.2	0	5	0			
F_1 (ICCV 2 × L 550)	18.7 ± 0.38	2.54	16.7–21.4	0	18	0			
F_2 (ICCV 2 × L 550)	18.9 ± 0.12	13.28	9.8–38.6	321	345	296	5:6:5	2.15	0.34
BC_1P_1 (F_1 × ICCV 2)	20.0 ± 0.86	6.57	16.5–23.6	2	3	4	1:2:1	0.94	0.33
BC_1P_2 (F_1 × L 550)	19.3 ± 0.55	5.54	12.7–25.6	18	51	32	1:2:1	3.89	0.14
χ^2 due to heterogeneity (back cross generations)				20	54	36	1:2:1	4.69	0.10

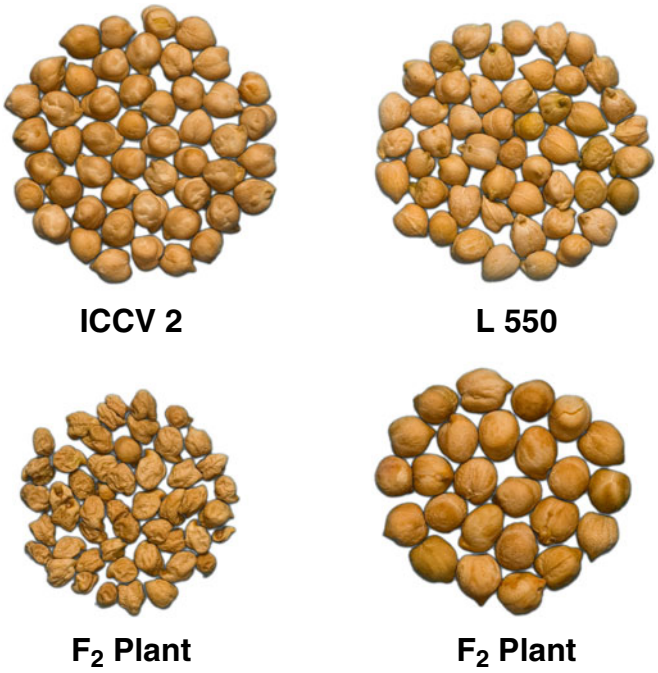

Figure 1. Seed size in parents and F_2 generation (top left are the seeds of parent ICCV 2 (*$Sd_3Sd_3sd_4sd_4$*) and top right L 550 (*$sd_3sd_3Sd_4Sd_4$*). Bottom left are the seeds of F_2 plant having lowest 100 SW (9.8 g; *$sd_3sd_3sd_4sd_4$*) and right the largest 100 SW (38.6 g; *$Sd_3Sd_3Sd_4Sd_4$*).

each locus on mean 100 SW of three groups (table 2). Due to small size of BC_1P_1 generation, the mean 100 SW of this generation was not used for comparison (table 1). Significant differences for 100 SW were observed between BC_1P_2 and F_2 generations for two groups, $<F_1P_1P_2$ and $>F_1P_1P_2$ (table 2). The mean 100 SW of BC_1P_2 was significantly higher for $<F_1P_1P_2$ (16.1 g) and lower for $>F_1P_1P_2$ (22.0 g) than the respective groups in F_2 (15.2 g for $<F_1P_1P_2$ and 23.2 g for $>F_1P_1P_2$) (table 2). The group $<F_1P_1P_2$ had plants having only one allele in BC_1P_2 whereas the same group had plants having no allele (one out of five) and one allele (four out of five) in F_2 generation. In F_2 generation, the plants having no allele in this group resulted in its significantly lower 100 SW. The group $>F_1P_1P_2$ had plants with three alleles in BC_1P_2 generation and plants having three (four out of five) and four alleles (one out of five) in F_2 generation. The plants having four alleles in this group in F_2 generation resulted in its significantly higher 100 SW in comparison to BC_1P_2 generation. The mean 100 SW of the plants in the group $F_1P_1P_2$ was almost similar in the two generations BC_1P_2 (18.8 g), and F_2 (18.5 g), which is due to the same genetic constitution of the plants in this group (plants having two alleles with increasing effects) in both the generations. These results supported the hypothesis of additive effect of each locus in controlling the mean 100 SW in two parents.

As the variation for 100 SW is continuous in F_2 generation, it is difficult to separate the plants having no allele from the plants having one allele with increasing effect in the group $<F_1P_1P_2$ and the plants having three alleles from the plants having four alleles with increasing effects in the group $> F_1P_1P_2$. However, the frequency of the plants classified together in $< F_1P_1P_2$ and $>F_1P_1P_2$ groups would be five each (one genotype with no allele and four genotypes with one allele in $< F_1P_1P_2$; four genotypes with three alleles and one genotype with all four alleles in $>F_1P_1P_2$) out of 16 in the F_2 generation. Out of 962 plants, the group $< F_1P_1P_2$ had 321 plants which fitted well to an expected ratio of 5:11 (321:641) in F_2 generation ($\chi^2 = 2.01$, $P = 0.16$). Similarly, the group $>F_1P_1P_2$ had 296 plants, which also agreed to an expected ratio of 5:11 (296:666) ($\chi^2 = 0.10$, $P = 0.75$) (data not shown). These results confirmed the additive effects of the genes controlling 100 SW in these two parents.

Seed size as determined by seed weight is an important component of trade, yield and adaptation in chickpea. For the development of large-seeded kabuli cultivars, it is important to involve well adapted parental genotypes in the breeding programmes and to investigate the number of genes controlling seed size in such parents to devise appropriate breeding strategies. In the present study, segregation pattern in F_2 and backcross generations indicated that the 100 SW in chickpea is controlled by two genes exhibiting additive effects and each parent carried one pair of alleles with increasing effects at one locus in homozygous form and F_1 plants carried a pair of alleles with increasing effects at different loci in heterozygous form. In a similar study involving ICC 11255 and ICC 5002 having 12 g and 5 g 100 SW, respectively, Upadhyaya *et al.* (2006) obtained about 7% of the F_2 population having 15 g 100 SW and concluded that the seed size in two

Table 2. Mean 100 SW of three groups in F_2 and backcross generations and test of significance.

	F_2		BC_1P_1			BC_1P_2		
Groups	Number of plants	Mean ± SE	Number of plants	Mean ± SE	*t*-test	Number of plants	Mean ± SE	*t*-test
$< F_1P_1P_2$	321	15.2 ± 0.08	2	16.6 ± 0.12	7.04[a]	18	16.1 ± 0.28	2.53[a]
$F_1P_1P_2$	345	18.5 ± 0.05	3	19.1 ± 0.47	1.16	51	18.8 ± 0.12	1.82
$>F_1P_1P_2$	296	23.2 ± 0.15	4	22.3 ± 0.67	1.10	32	22.0 ± 0.27	2.91[a]

[a]Significant at $P \leq 0.05$.

parents was controlled by two genes with dominance epistasis and designated the genotypes of ICC 11255 as $Sd_1Sd_1sd_2sd_2$ and ICC 5002 as $sd_1sd_1Sd_2Sd_2$ where Sd_1 is epistatic to Sd_2 and sd_2 alleles. In the present study, two parents having comparatively higher 100 SW (18.6 g and 18.3 g 100 SW) were involved which resulted into F_1 and about 33% of F_2 plants (group $F_1P_1P_2$) having 100 SW similar to the parents. These findings indicate that the genes controlling the inheritance of 100 SW in these two parents are different from the genes controlling 100 SW in parents, ICC 11255 and ICC 5002 used by Upadhyaya *et al.* (2006). Hence, based on this information, we designate the genotype of ICCV 2 (P_1) as $Sd_3Sd_3sd_4sd_4$ and L 550 (P_2) as $sd_3sd_3Sd_4Sd_4$ and F_1 as $Sd_3sd_3Sd_4sd_4$, in which each Sd_3 and Sd_4 alleles are having increasing additive effect.

In the present study, both the parents involved are well adapted, widely cultivated, having desirable traits such as high yielding and resistance to *Fusarium* wilt, with ICCV 2 having extra-early maturity (85 days). Therefore, this cross would be useful in selecting early maturing progenies having desirable traits. Further, the genetic control of seed size by two genes exhibiting additive effects indicated that the selection for large seed size progenies in early generations of ICCV 2 × L 550 cross would be effective to utilize additive gene effects to manipulate seed size in the progenies of this cross for developing high yielding, large-seeded varieties of kabuli chickpea.

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