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Inheritance of Natural Seed-Coat Cracking in Chickpea

POORAN M GAUR, SAMINENI SRINIVASAN, KAMATAM SURESH, SARIKONDA R DEEPIKA, AND BOYAPATI V RAO

Grain Legumes Research Program, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru, Hyderabad 502 324, Andhra Pradesh, India.

Address correspondence to Pooran M. Gaur at the address above, or e-mail: p.gaur@cgiar.org.

A spontaneous mutant with natural seed-coat cracking, designated "cracked seed-coat mutant (CSM)," was identified in chickpea (*Cicer arietinum* L.) from an F_2 population of a cross ICRISAT chickpea (ICC) 10301 × ICC 12430. The extent of seed-coat cracking (SCC) varied widely from a minute to several wide cracks. Seed coats showed cracks before seeds were fully developed and the plants had reached physiological maturity. However, seed-coat cracks were most visible on dry matured seeds, particularly in desi types. Two loci (*Scc-1* and *Scc-2*) that controlled SCC were identified. F_1 plants from the crosses of CSM with desi genotypes produced seeds with no SCC, whereas F_1 plants from the crosses of CSM with kabuli genotypes produced seeds with SCC. F_2 segregation followed 13:3 and 7:9 ratios for plants without SCC and with SCC in CSM × Desi and CSM × Kabuli crosses, respectively. Three alleles were identified at the first locus (*Scc-1*) from CSM ($Scc\text{-}1^c$), desi ($Scc\text{-}1^d$), and kabuli ($Scc\text{-}1^k$) types, with the dominance relationship being $Scc\text{-}1^d > Scc\text{-}1^c > Scc\text{-}1^k$. At the second locus (*Scc-2*), CSM had the dominant allele (*Scc-2*), whereas both desi and kabuli types had the recessive allele (*scc-2*). The SCC trait showed no linkage with leaf type (pinnate vs. simple) and flower color (pink vs. white) and had no adverse effects on grain yield. The SCC trait may facilitate dehulling and preparation of splits (*dal*), but the cracked seed would be prone to damage by insect pests and unfavorable moisture conditions.

Key words: *Cicer arietinum, inheritance, mutant, seed-coat cracking, seed size*

Chickpea (*Cicer arietinum* L.) is the second most important pulse crop after common bean and contributes to 16% of world's pulse production. During 2010, chickpea was grown on 12.0 million ha across 52 countries (Food and Agricultural Organization [FAO] 2012), spanning the area between latitudes 45°N and 37°S. It is considered to be a hardy crop and is usually cultivated on marginal land with poor soil fertility. There are 2 major types of chickpeas—kabuli and desi. Kabuli chickpeas, also known as garbanzo beans, have larger, cream-colored seeds with thin seed coats and are primarily used in salads and vegetable mixes. On the other hand, desi chickpeas have smaller, darker-colored seeds with thick seed coats and are used whole or split or are milled into flour after dehulling. The seed traits currently considered by the consumers include size, color, shape, and surface.

Rapid cotyledon growth sometimes may not adequately match the expansion of the testa and, as a result, cracks are formed in the seed coat (Agarwal and Menon 1974). Seed-coat cracking (SCC) results from the separation of epidermal (palisade cells) and hypodermal tissues, which exposes the underlying parenchyma tissue (Wolf and Baker 1972). Further, SCC makes the seed more vulnerable to storage problems and field microorganisms, leading to seed rotting or pre- and postemergence damping under high humid conditions. Although the reason for the physical separation of palisade and hypodermal cells is not well known, genetic and environmental factors have been implicated for SCC in other crops such as soybean [*Glycine max* (L.) Merr.] (Stewart and Wentz 1930; Woodworth and Williams 1938; Liu 1949; Schlub and Schmitthenner 1978; Duke et al. 1983, 1986) and watermelon (Hafez et al. 1981). In soybean, apart from genetic control, SCC has been associated with environmental stress factors, such as alternate wetting and drying (Wolf et al. 1981) and occurrence of low temperatures (15 ºC) during the flowering period (Takahashi 1997).

This article describes a natural SCC mutant of chickpea, genetics of SCC, and relationships of SCC with types, seed size, and grain yield of chickpea. To our knowledge, this is the first report on occurrence and inheritance of natural SCC in chickpea.

Materials and Methods

A cracked seed-coat mutant (CSM) was observed in an F_2 population of a cross ICC 10301 × ICC 12430. F_3 seeds harvested from a single F_2 CMS plant were further advanced to develop a pure CSM line. CSM was crossed with noncracked seed types, which included 1 desi (JG 11), 2 kabuli (KAK 2 and ICCV 97311), and 1 intermediate (JGG 1)-type genotypes. Backcrosses with both the parents (BC_1P_1 and BC_1P_2) were also made in crosses of CSM with JG 11 and KAK 2 to further study the inheritance of SCC. Conventional methods for emasculation and pollination were followed to make each cross. F_1 plants were advanced to F_2 by self-pollination in a green house during the rainy season of 2007. Backcrosses were also attempted in the greenhouse in the same season. The segregating generations of crosses (F_2, $BC_1P_1F_1$, and $BC_1P_2F_1$), along with parents and F_1s, were grown under normal field conditions in vertisols during the postrainy season of 2008–2009 at the International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India, for studying inheritance of the SCC trait. Planting was done on 4-m rows, with spacing of 60 cm between the rows and 15 cm between the plants. Standard cultural and plant protection practices were followed to raise a healthy crop. Observations were recorded for each plant on leaf type (normal leaf or simple leaf), flower color (pink or white), grain yield, seed size, and SCC. The data were analyzed using LINKAGE 1 software (Suiter et al. 1983), which tests the goodness-of-fit of the observed segregation with the expected ratios and uses contingency χ^2 test to detect linkages between traits.

Figure 1. **(a)** Cracked seed coat mutant, **(b)** cracked seed coat kabuli segregant, **(c)** normal seed of JG 11 (desi) parent, and **(d)** normal seed of KAK 2 (kabuli) parent.

Results and Discussion

The spontaneous mutant with natural SCC, designated CSM, was isolated from the F_2 of a cross between ICC 10301 and ICC 12430. This mutant bred true in succeeding generations and was developed as a pure line. The seeds of CSM showed SCC in all growing conditions, although the extent of SCC varied widely from a minute or invisible crack to several wide cracks. The seed coat showed cracks before plants had reached physiological maturity and seeds were fully developed. However, seed coat cracks were most visibile on dry seeds, particularly in the desi type (Figure 1a). No difference in the pattern of SCC was observed between desi and kabuli chickpeas. The seed coat cracks in both desi and kabuli types were mostly longitudinal. There were 1–4 cracks per seed, distributed throughout the seed surface. The type of SCC observed in chickpea in this study appears similar to the Type I cracking reported in soybean (Liu 1949). A spontaneous mutation in 1 of the 2 parents or in the F_1 was suspected. However, the possibility for establishment of a rare recombination of 2 interacting recessive genes could not be ruled out.

The inheritance of SCC was studied in the F_2 from crosses of CSM with 1 desi (JG 11), 2 kabuli (KAK 2 and ICCV 97311), and 1 intermediate (JGG 1)-type genotypes (Table 1). SCC segregated differently in crosses of CSM with desi and kabuli types. When CSM was crossed with desi genotype (JG 11), F_1 plants exhibited normal seed coat, indicating the dominance of normal seed coat (NS) over cracked seed coat (CS). The F_2 segregated in a ratio of 13 NS:3 CS, indicating the involvement of 2 genes with dominant suppression epistasis. On the other hand, when CSM was crossed with kabuli genotypes (KAK 2 and ICCV 97311), the F_1 plants expressed CS, indicating the dominance of CS over NS. The F_2 showed a complementary type of gene action (9 CS:7 NS). The inheritance pattern for SCC in cross of CSM with the intermediate-type genotype (JGG 1) was similar to that observed in crosses of CSM with desi types.

Inheritance of SCC was further studied in backcross generations (F_1s of BC_1P_1 and BC_1P_2) of the crosses of CSM with 1 desi (JG 11) and 1 kabuli (KAK 2) genotype. Segregation in backcrosses further confirmed the mode of inheritance observed for the SCC trait in the F_2 of CSM × Desi and CSM × Kabuli crosses. When the CSM × JG 11 F_1 was backcrossed with the desi parent (JG 11), the F_1 produced all NS plants, and in a backcross with CSM, CS and NS segregated in a ratio of 1 CS:1 NS. In a CSM × Kabuli cross, when the F_1 was backcrossed with CSM, all plants produced CS, whereas in backcross with the kabuli parent, CS and NS plants segregated in a 3:1 ratio (Table 1). The chi-square analysis showed that the F_2 and backcross segregations were in agreement with the respective ratios and confirm the digenic mode of inheritance for the CS trait in both desi and kabuli types.

Gene symbols are proposed for the different alleles present at the 2 loci controlling SCC. Three alleles (*Scc-1*^{d} from desi genotypes, *Scc-1*^{c} from CSM, and *Scc-1*^{k} from kabuli genotypes) were identified at the first locus (*Scc-1*) controlling SCC. The allele *Scc-1*^{d} was dominant over *Scc-1*^{c} and *Scc-1*^{k} and the allele *Scc-1*^{c} was dominant over *Scc-1*^{k}, suggesting a dominance relationship of *Scc-1*^{d} > *Scc-1*^{c} > *Scc-1*^{k}. At the second locus (*Scc-2*), the allele was recessive (*scc-2*) in desi

Table 1 Segregation of seed-coat cracking, leaf type, and flower color in different crosses with cracked seed-coat mutant (CSM) of chickpea

		Phenotype class			
Seed-coat cracking	Generation	Noncracked	Cracked	Ratio	χ^2 Probability
CSM × JG 11	F_1	All	—	—	—
	F_2	113	28	13:3	0.736ns
	BC_1P_1	25	34	1:1	0.241ns
	BC_1P_2	100	0	All noncracked	—
JG11 × CSM	F_1	All	—	—	—
	F_2	149	42	13:3	0.250ns
JGG 1 × CSM	F_1	All	—	—	—
	F_2	180	44	13:3	0.732ns
CSM × KAK 2	F_1	—	All	—	—
	F_2	56	84	7:9	0.371ns
	BC_1P_1	0	21	All cracked	—
	BC_1P_2	44	16	3:1	0.766ns
KAK 2 × CSM	F_1	—	All	—	—
	F_2	40	71	7:9	0.102ns
CSM × ICCV 97311	F_1	—	All	—	—
	F_2	46	51	7:9	0.466ns
Leaf type	—	Normal leaf	Simple leaf	—	—
CSM × JG 11	F_1	All	—	—	—
	F_2	115	26	3:1	0.072ns
JG 11 × CSM	F_1	All	—	—	—
	F_2	114	47	3:1	0.900ns
JGG 1 × CSM	F_1	All	—	—	—
	F_2	136	88	9:7	0.864ns
CSM × KAK 2	F_1	All	—	—	—
	F_2	107	35	3:1	0.923ns
KAK 2 × CSM	F_1	All	—	—	—
	F_2	86	25	3:1	0.547ns
Flower color	—	Pink	White	—	—
JGG 1 × CSM	F_1	All	—	—	—
	F_2	169	55	3:1	0.877ns
CSM × KAK 2	F_1	All	—	—	—
	F_2	104	36	3:1	0.845ns
KAK 2 × CSM	F_1	All	—	—	—
	F_2	96	34	3:1	0.761ns

CSM and JG 11 are desi type, KAK 2 and ICCV 97311 are kabuli type, and JGG 1 is intermediate-type chickpea; ns, nonsignificant.

and kabuli types and dominant in CSM (*Scc-2*). The allele from desi type at the first locus (*Scc-1*^{d}) suppressed the activity of the gene at the second locus (*Scc-2*), thus CSM × Desi cross produced F_1s with NS. In CSM × Kabuli crosses, the F_1 produced CS because *Scc-1*^{c} is dominant to *Scc-1*^{k} at the first locus and does not inhibit expression of *Scc-2* allele at the second locus. To the best of our knowledge, this is the first report on the existence and inheritance of SCC trait in chickpea.

Soybean genotypes with natural SCC are common and several reports are available on the inheritance of the SCC trait. SCC in soybean has been classified into 2 types, regardless of environmental conditions. Type I shows irregular SCC (Stewart and Wentz 1930), whereas the Type II shows net-like cracks (Oyoo et al. 2010). Most studies suggested involvement of 2 genes in expression of SCC trait. Stewart and Wentz (1930) reported that Type I SCC is controlled by *T* and *I* genes, and the double-recessive genotype (*i* and *t* or *i-k* and *t*) for these genes produces severely cracked seeds. Nagai (1926) observed 9 cracked and 7 normal seeds and suggested the involvement of 2 complementary genes, in which 2 dominant alleles were responsible for the defective seed coats. However, Liu (1949) found 9 normal and 7 cracked seeds that show the presence of 2 complementary genes, of which 2 dominant alleles were needed to produce normal seed coats.

CSM had simple leaves, whereas all desi and kabuli genotypes used in this study had pinnate leaves (the common leaf type in chickpea). Crosses of CSM (simple leaf) with other chickpea genotypes (pinnate leaf) produced F_1s with normal leaf. The F_2 population segregated in a ratio of 3 (normal):1 (simple) for leaf type (Table 1). Similarly, flower color also segregated in a monogenic ratio of 3 (pink):1 (white) when pink-flowered genotypes were crossed with white-flowered kabuli genotypes (Table 1). These results are in agreement with previous reports on leaf type (Ekbote 1937, 1942; Singh 1962) and flower color segregation (More and D'Cruz 1976; Davis 1991). Joint segregation analysis

Table 2 Yield-contributing characters of parents, F_1, F_2, and backcross populations in direct and reciprocal crosses of cracked seed-coat mutant (CSM) with JG 11 and KAK 2 genotypes

Generation	Genotype/cross	Number of plants	Seed type	Number of seeds ± SE	Seed yield (g per plant)	100-seed weight (g)
Parent	CSM	12	Cracked seed coat	67.7 ± 24.04	18.6 ± 6.51	27.8 ± 2.03
Parent	JG 11	12	Normal seed	125.9 ± 15.58	27.0 ± 3.44	21.3 ± 0.44
Parent	KAK 2	12	Normal seed	83.0 ± 4.33	33.2 ± 1.84	40.0 ± 0.59
F_1	CSM × JG 11	8	Normal seed	91.0 ± 6.57	24.9 ± 1.71	27.4 ± 0.74
F_1	JG 11 × CSM	11	Normal seed	105.4 ± 6.45	29.9 ± 1.69	28.8 ± 1.48
F_1	CSM × KAK 2	11	Cracked seed coat	55.1 ± 6.46	20.8 ± 2.44	38.2 ± 1.26
F_1	KAK 2 × CSM	10	Cracked seed coat	48.0 ± 4.03	17.3 ± 1.32	36.4 ± 0.93
F_2	CSM × JG 11	191	Cracked seed coat	83.4 ± 7.88	27.7 ± 2.49	33.8 ± 0.87
			Normal seed	92.7 ± 5.86	26.9 ± 1.69	30.0 ± 0.82
F_2	JG 11 × CSM	141	Cracked seed coat	109.9 ± 12.92	34.0 ± 3.78	31.3 ± 1.11
			Normal seed	105.5 ± 6.55	28.8 ± 1.83	27.4 ± 0.60
F_2	CSM × KAK 2	142	Cracked seed coat	64.5 ± 3.96	24.6 ± 1.53	38.5 ± 0.51
			Normal seed	61.1 ± 6.32	21.4 ± 1.98	35.5 ± 0.79
F_2	KAK 2 × CSM	111	Cracked seed coat	70.3 ± 4.55	27.2 ± 1.73	39.1 ± 0.46
			Normal seed	55.7 ± 5.72	20.3 ± 2.29	33.4 ± 1.27
BC_1P_1	(CSM × JG 11) × CSM	59	Cracked seed coat	84.9 ± 8.39	27.6 ± 2.28	33.8 ± 0.93
			Normal seed	101.4 ± 14.11	28.1 ± 4.41	26.7 ± 1.41
BC_1P_2	(CSM × JG 11) × JG 11	100	Cracked seed coat	—	—	—
			Normal seed	109.3 ± 6.15	29.4 ± 1.69	27.6 ± 1.01
BC_1P_1	(CSM × KAK 2) × CSM	21	Cracked seed coat	72.5 ± 18.13	26.6 ± 7.03	36.1 ± 1.73
			Normal seed	—	—	—
BC_1P_2	(CSM × KAK 2) × KAK 2	57	Cracked seed coat	82.1 ± 11.83	32.1 ± 4.47	39.1 ± 1.31
			Normal seed	69.9 ± 6.05	26.4 ± 2.44	36.8 ± 0.76

CSM and JG 11 are desi type, KAK 2 is kabuli type, and JGG 1 is intermediate-type chickpea. SE, standard error.

indicated no linkage of CS with leaf type (r = 0.46) and flower color (r = 0.44).

Seed size and plant yield were always higher in CS segregants compared to NS segregants in F_2 (Table 2). In backcross generations, CS segregants also had larger seeds and higher yields than the NS segregants. A positive significant correlation between seed weight and SCC (r = 0.46) has been observed in soybean and it was suggested that genes contributing to seed weight might have minor effects on the intensity of SCC (Nakamura et al. 2003). The yield advantage in CS segregants observed in this study appears to be mainly through increase in seed size by ~11% compared to the size of the normal seed. Further studies are needed to understand the causes of SCC and its association with grain yield in chickpea.

Desi chickpeas are widely used in making splits (*dal*) after dehulling. The SCC trait can facilitate dehulling. The kabuli chickpeas have thinner seed coats than the desi type and therefore removal of seed coat from the kabuli chickpea is difficult than that from the desi type (Ravi and Harte 2009; Wood et al. 2011). This is one of the reasons for not using kabuli chickpea in making splits. The SCC trait can facilitate dehulling and expand the use of kabuli chickpea in making splits and flour. However, SCC makes the seed prone to moisture imbibition and attack by insects and pathogens. Thus, cracked seed coat reflects poor quality of seed and may reduce consumer preference and market value. Further studies are needed for a better understanding of the anatomical and biochemical characteristics of SCC and its possible uses.

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