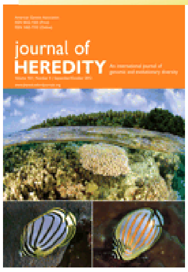

Inheritance of Natural Seed-coat Cracking in Chickpea

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Running title: Natural seed-coat cracking in chickpea

Abstract

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 ICC 10301 × ICC 12430. The extent of seed-coat cracking (SCC) varied widely from a minute to several wide cracks. Seed coat show 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*) were identified to control SCC. F_1s from the crosses of CSM with desi genotypes produced seed with no SCC, while F_1s from the crosses of CSM with kabuli genotypes produced seed 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 of $Scc\text{-}1^d > Scc\text{-}1^c > Scc\text{-}1^k$. At the second locus (*Scc-2*), CSM had the dominant allele (*Scc-2*), while both desi and kabuli types had 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. SCC trait may facilitate de-hulling and making of splits (*dal*) but 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 (FAOSTAT 2012), spanning the area between latitudes 45^0N and 37^0S. It is considered to be a hardy crop and is usually cultivated on marginal land with poor soil fertility. There are two major types of chickpeas - kabuli and desi. Kabuli chickpeas, also known as garbanzo beans, have larger, cream-colored seeds with thin seed coats for primary use in salads and vegetable mixes. On the other hand, desi chickpeas have smaller, darker colored seeds with thick seed coats and are used whole, split, or milled into flour after de-hulling. The seed traits currently considered by the consumers include size, color, shape and surface.

Rapid cotyledon growth sometimes may not adequately match with the expansion of the testa and as a result cracks are formed in the seed coat (Agrawal 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 and field microorganisms leading to seed rotting or pre and post emergence damping off 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 like soybean [*Glycine max* (L.) Merr.] (Duke et al. 1983, 1986; Liu 1949; Schlub and Schmitthenner 1978; Stewart and Wentz 1930; Woodworth and Williams 1938) and watermelon (Hafez et al. 1981). In soybean apart from genetic control, SCC has been associated with environmental stress factors like alternate wetting and drying (Wolf et al. 1981), and occurrence of low temperatures (15^oC) during flowering period (Takahashi 1997).

This paper describes a natural SCC mutant of chickpea; genetics of SCC; and relationships of SCC with types of chickpea, seed size and grain yield. To our knowledge, this is the first report on occurrence and inheritance of natural seed coat cracking 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 seed harvested from a single F_2 CMS plant was further advanced to develop a pure CSM line. CSM was crossed with non-cracked seed types, which included one desi (JG 11), two kabuli (KAK 2 and ICCV 97311) and one 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 inheritance of SCC. Conventional methods for emasculation and pollination were followed to make each cross. F_1 plants were advanced to the F_2 by self-pollination in green house during the rainy-season 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 post-rainy season 2008/09 at International Crops Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India for studying inheritance of 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 on each plant on leaf type (normal leaf or simple leaf), flower color (pink or white), grain yield, seed size and SCC. The data was 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.

Results and Discussion

The spontaneous mutant with natural seed coat cracking, 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 seed coat cracking (SCC) in all growing conditions, although the extent of SCC varied widely from a minute or no visible crack to several wide cracks. The seed coat show cracks before plants had reached physiological maturity and seeds were fully developed. However, seed coat cracks were most visibile on dry seeds, particularly in 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 one to four cracks per seed distributed throughout the seed surface. The kind of SCC observed in chickpea in this study appears similar to Type I cracking reported in soybean (Liu 1949). A spontaneous mutation in one of the two parents or in the F_1 was suspected. However, the possibility for establishment of a rare recombination of two interacting recessive genes could not be ruled out.

The inheritance of SCC was studied in the F_2 from crosses of CSM with one desi (JG 11), two kabuli (KAK 2 and ICCV 97311) and one 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 involvement of two 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 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 one desi (JG 11) and one kabuli (KAK 2) genotype. Segregation in backcrosses further confirmed the mode of inheritance observed for SCC trait in F_2 of CSM × Desi and CSM × kabuli crosses. When the CSM × JG 11 F_1 was backcrossed with desi parent (JG 11), the F_1 produced all NS and in 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; while 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 respective ratios and confirm the digenic mode of inheritance for CS trait in both desi and kabuli types.

Gene symbols are proposed for different alleles present at the two loci controlling seed coat cracking. 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 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 gene at the second locus (*Scc-2*), thus CSM x 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 existence and inheritance of seed coat cracking trait in chickpea.

Soybean genotypes with natural seed coat cracking are common and there are several reports available on inheritance of the SCC trait. SCC in soybean has been classified into two types regardless of environmental conditions. Type I shows irregular seed coat cracking (Stewart and Wentz 1930), while the Type II shows net like cracks (Oyoo et al. 2010). Most studies suggested involvement of two 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 seed. Nagai (1926) observed 9 cracked and 7 normal seeds and suggested the involvement of two complementary genes, in which two dominant alleles were responsible for the defective seed coats. However, Liu (1949) found 9 normal and 7 cracked seeds which show the presence of two complementary genes of which two dominant alleles were needed to produce normal seed coats.

CSM had simple leaves, while 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) ratio 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 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 seed coat cracking (r=0.46) has been observed in soybean and it was suggested that genes contributing seed weight might have minor effects on the intensity of seed coat cracking (Nakamura et al. 2003). The yield advantage in CS segregants observed in this study appears to be mainly through increased seed size by ~11% compared to normal seed. Further studies are needed to understand causes of SCC and its association with grain yield in chickpea.

Desi chickpeas are widely used in making splits (*dal*) after de-hulling. The SCC trait can facilitate de-hulling. The kabuli chickpeas have thinner seed coat 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 the reasons for not using kabuli chickpea in making splits. The SCC trait can facilitate de-hulling and expand use of kabuli chickpea in making splits and flour. However, the seed coat cracking make 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 anatomical and biochemical characteristics of seed coat cracking and its possible uses.

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References

Agarwal K, Menon K. 1974. Lignin content and seed coat thickness in relation to seed coat cracking in soybean. Seed Res. 2:64–66.

Davis TM. 1991. Linkage relationships of genes for leaf morphology, flower color, nd root nodulation in chickpea. Euphytica. 54:117–123.

Duke SH, Kakefuda G, Harvey TM. 1983. Differential leakage of intracellular substances from imbibing seeds. Plant Physiol. 72:919–924.

Duke SH, Kakefuda G, Henson CA, Loeffler NL, van Hulle NM. 1986. Role of the testa epidermis in the leakage of intracellular substances from imbibing soybean seeds and its implications for seedling survival. Physiol Plantarum. 68:625–631.

Ekbote RB. 1937. Mutantions in gram (*Cicer arietinum* L.). Cur Sci. 5:648–649.

Ekbote RB. 1942. Genetics of two mutations in *Cicer*. Indian J Genet. 2:50–65.

FAOSTAT. 2012. http://faostat.fao.org/site (accessed on 04 February 2012).

Hafez AAA, Gaafer AK, Allam AMM. 1981. Inheritance of flesh colour, seed coat cracks and total soluble solids in watermelon and their genetic relations. I. Quantitative traits. Acta Agron Acad Sci. (Hung) 30:82–86.

Liu HL. 1949. Inheritance of defective seed coat in soybeans. J Hered. 40:317–322.

More DC, D'Cruz R. 1976. Genetic studies in Bengal gram (*Cicer arietinum* L.) II. NP-6 x Pusa-83 D.P. J Maharashra Agri Univer. 1:15–17.

Nagai I. 1926. [On the genetics of the soybean.] part 2 (in Japanese). Agri Horti. 1: 107–118.

Nakamura T, Yang D, Kalaiselvi S, Uematsu Y, Takahashi R. 2003. Genetic analysis of net-like cracking in soybean seed coats. Euphytica. 133:179–184.

Oyoo ME, Githiri SM, Benitez ER, Takahashi R. 2010. QTL analysis of net-like cracking in soybean seed coats. Breed Sci. 60:28–33.

Ravi R, Harte JB. 2009. Milling and physiochemical properties of chickpea (*Cicer arietinum* L.) varieties. J Sci Food Agric. 89:258–266.

Schlub RL, Schmitthenner AF. 1978. Effects of soybean seed coat cracks on seed exudation and seedling quality in soil infested with *Pythium ultimum*. Phytopathology 68:1186–1191.

Singh B. 1962. Inheritance studies of new spontaneously originated simple leaf mutant in *Cicer arietinum* L. Sci Cul 28:138–139.

Stewart RT, Wentz JB. 1930. A defective seed-coat character in soybeans. J Am Soc Agron. 22:658–662.

Suiter KA, Wendel JF, Case JS. 1983. LINKAGE 1: a PASCAL computer program for detection and analysis of genetic linkage. J Hered. 74:203–204.

Takahashi R. 1997. Association of soybean genes *I* and *T* with low-temperature induced seed coat deterioration. Crop Sci. 37:1755–1759.

Wolf WJ, Baker FL. 1972. Scanning electron microscopy of soybeans. Cereal Sci Today. 17:125–130.

Wolf WJ, Baker FL, Bernard RL. 1981. Soybean seed-coat structural features: pits, deposits and cracks. Scan Electron Micros. (III):531–544.

Woodworth CM, Williams LF. 1938. Recent studies on the genetics of the soybean. J Am Soc Agron. 30:125–129.

Wood JA, Knights EJ, Choct M. 2011. Morphology of chickpea seeds (*Cicer arietinum* L.): Copmarision of desi and kabuli types. Int J Plant Sci. 172:632–643.

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

	Generation	Phenotype class		Ratio	χ2 Probability
Seed-coat cracking		**Non cracked**	**Cracked**		
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 non-cracked	
JG11 × CSM	F_1	All	-		
	F_2	149	42	13:3	0.250ns
JGG1 × 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
JG11 × CSM	F_1	All	-		
	F_2	114	47	3:1	0.900ns
JGG1 × 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**		
JGG1 × 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

Notes: ns, refer to non-significant; CSM and JG 11 are desi type, KAK 2 and ICCV 97311 are kabuli type and JGG 1 is intermediate type

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 JG11 and KAK 2 genotypes.

Generation	Genotype/cross	Number of plants	Seed type	Number of seeds ±SE	Seed yield (g plant^{-1})	100-seed weight (g)
Parent	CSM	12	Crack seed	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	Crack seed	55.1±6.46	20.8±2.44	38.2±1.26
F_1	KAK 2 × CSM	10	Crack seed	48.0±4.03	17.3±1.32	36.4±0.93
F_2	CSM × JG 11	191	Crack seed	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	Crack seed	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	Crack seed	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	Crack seed	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	Crack seed	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	Crack seed	-	-	-
			Normal seed	109.3±6.15	29.4±1.69	27.6±1.01
BC_1P_1	(CSM × KAK 2) × CSM	21	Crack seed	72.5±18.13	26.6±7.03	36.1±1.73
			Normal seed	-	-	-
BC_1P_2	(CSM × KAK 2) × KAK 2	57	Crack seed	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

Note: CSM and JG 11 are desi type, KAK 2 is kabuli type and JGG 1 is intermediate type

Legend for Figures:

Figure 1. (a) Crack seed mutant, **(b)** crack seed kabuli segregant, **(c)** JG 11 (desi) parent – normal seed, and **(d)** KAK 2 (kabuli) parent – normal seed

Legend for Tables:

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

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 JG11 and KAK 2 genotypes.

Supplementary Table for brief Communication "Inheritance of Natural Seed-coat Cracking in Chickpea" by Gaur et al.

	P1: JG 11 (Desi, normal seed coat)		P2: CSM (Desi, Cracked seed coat)
	Scc-1^{d} *Scc-1*^{d} *scc-2 scc-2*	X	*Scc-1*^{c} *Scc-1*^{c} *Scc-2 Scc-2*
		↓	
F_1		*Scc-1*^{d} *Scc-1*^{c} *Scc-2 scc-2*	Normal seed coat
		↓	
		9 *Scc-1*^{d}*_ Scc-2_*	Normal seed coat
		3 *Scc-1*^{d}*_ scc-2 scc-2*	Normal seed coat
		3 *Scc-1*^{c} *Scc-1*^{c} *Scc-2_*	Crack seed coat
		1 *Scc-1*^{c} *Scc-1*^{c} *scc-2 scc-2*	Normal seed coat
		13 Normal : 3 Crack	
BC_1P_1	*P1 (JG11): Scc-1*^{d} *Scc-1*^{d} *scc-2 scc-2*	X	*F1: Scc-1*^{d} *Scc-1*^{c} *Scc-2 scc-2*
		↓	
		1 *Scc-1*^{d} *Scc-1*^{d} *Scc-2 scc-2*	Normal seed coat
		1 *Scc-1*^{d} *Scc-1*^{d} *scc-2 scc-2*	Normal seed coat
		1 *Scc-1*^{d} *Scc-1*^{c} *Scc-2 scc-2*	Normal seed coat
		1 *Scc-1*^{d} *Scc-1*^{c} *scc-2 scc-2*	Normal seed coat
		All Normal	
BC_1P_2	*P2 (CSM): Scc-1*^{c} *Scc-1*^{c} *Scc-2 Scc-2*	X	*F1: Scc-1*^{d} *Scc-1*^{c} *Scc-2 scc-2*
		↓	
		1 *Scc-1*^{d} *Scc-1*^{c} *Scc-2 Scc-2*	Normal seed coat
		1 *Scc-1*^{d} *Scc-1*^{c} *Scc-2 scc-2*	Normal seed coat
		1 *Scc-1*^{c} *Scc-1*^{c} *Scc-2 Scc-2*	Crack seed coat
		1 *Scc-1*^{c} *Scc-1*^{c} *Scc-2 scc-2*	Crack seed coat
		1 Normal : 1 Crack	

	(Parent 1) CSM (Cracked seed coat)		(Parent 2) KAK 2 (Kabuli, normal seed coat)
	Scc-1^{c} *Scc-1*^{c} *Scc-2 Scc-2*	X	*Scc-1*^{k} *Scc-1*^{k} *scc-2 scc-2*
		↓	
F_1		*Scc-1*^{c} *Scc-1*^{k} *Scc-2 scc-2*	Crack seed coat
		↓	
F_2		9 *Scc-1*^{c}_ *Scc-2*_	Crack seed coat
		3 *Scc-1*^{c}_ *scc-2 scc-2*	Normal seed coat
		3 *Scc-1*^{k} *Scc-1*^{k} *Scc-2*_	Normal seed coat
		1 *Scc-1*^{k} *Scc-1*^{k} *scc-2 scc-2*	Normal seed coat
		9 Crack : 7 Normal	
BC_1P_1	*P1* (CSM): *Scc-1*^{c} *Scc-1*^{c} *Scc-2 Scc-2*	X	*F1: Scc-1*^{c} *Scc-1*^{k} *Scc-2 scc-2*
		↓	
		1 *Scc-1*^{c} *Scc-1*^{k} *Scc-2 Scc-2*	Crack seed coat
		1 *Scc-1*^{c} *Scc-1*^{k} *Scc-2 scc-2*	Crack seed coat
		1 *Scc-1*^{k} *Scc-1*^{K} *Scc-2 Scc-2*	Crack seed coat
		1 *Scc-1*^{k} *Scc-1*^{K} *Scc-2 scc-2*	Crack seed coat
		All with cracked seed coat	
BC_1P_2	P2(KAK 2):*Scc-1*^{k} *Scc-1*^{k} *scc-2 scc-2*	X	*F1: Scc-1*^{c} *Scc-1*^{k} *Scc-2 scc-2*
		↓	
		1 *Scc-1*^{c} *Scc-1*^{k} *Scc-2 scc-2*	Crack
		1 *Scc-1*^{c} *Scc-1*^{k} *scc-2 scc-2*	Normal seed coat
		1 *Scc-1*^{k} *Scc-1*^{k} *Scc-2 scc-2*	Normal seed coat
		1 *Scc-1*^{k} *Scc-1*^{k} *scc-2 scc-2*	Normal seed coat
		3 Normal : 1 crack	