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ORIGINAL PAPER

Genetic analysis and fine mapping of the *Ga1-S* gene region conferring cross-incompatibility in maize

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Abstract Cross-incompatibility genes known as *gametophyte factors* (*ga*) are numerous in maize. Many popcorn strains carry these genes and cannot be fertilized by pollen of dent and flint maize strains although the reciprocal crosses are successful. A Chinese popcorn strain SDGa25 carries the strongest allele of *Ga1* (*Ga1-S*) and the majority of Chinese dent and flint maize germplasm are incompatible with SDGa25. The incompatibility is due to pollen tube growth obstruction 2 h after pollination. The pollen tube is arrested in the silk segment 5.5 cm distal to the pollination area and never reaches the ovule. The *Ga1-S* carried by SDGa25 behaves as a single dominant gene. This gene was mapped between markers SD3 on BAC AC200747 0.827 cM apart on the telomere side and SD12 on BAC AC204382 0.709 cM apart on the centromere side. The genetic region mapped spanning the *Ga1-S* locus was estimated to be 1.5 cM in length and the physical distance is 2,056,343 bp on ctg156 based on the *B73 RefGen_v2 sequence*. *Gametophyte factors* influence gene flow direction and the strongest *Ga1-S* allele is useful for isolating one category of commercial varieties from another. The eight tightly linked markers to *Ga1-S* developed in this study would greatly improve marker-assisted introgression efficiency and the fine mapping would facilitate the isolation of the *Ga1-S*.

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Introduction

Cross-incompatibility factors known as *gametophyte factors* (*ga*) are numerous in maize. When present in pistil, they discriminate against or completely exclude pollen lacking the same allele (Nelson 1994). *Ga*-regulated cross-incompatibility has two features. The first one is that the behaviors of pollen are governed by the genotypes of individual pollen grains rather than the parental sporophytes, hence the locus name, *gametophyte factor*. The second one is that cross-incompatibility is in one direction and selfing is compatible. In general, *Ga* pollen can pollinate *Ga/Ga*, *Ga/ga* and *ga/ga* pistils, *ga* pollen, however, can not pollinate *Ga/Ga* pistil, which can limit gene flow (Kermicle 2006).

The best characterized and probably the most interesting *Ga*s are *Ga1-S* and *Tcb1-S*. *Ga1* was first detected by Correns because of the aberrant F_2 ratios for sugary-starchy. *Ga1-S* is the strongest *Ga1* allele and *ga1* pollen completely fails to function on styles homozygous for *Ga1-S* even in the absence of competing pollen (Schwartz 1950). Crosses of *ga1* pollen on styles heterozygous for *Ga1-S* yield a partial seed set. Many popcorn strains and many Mexican and Central American races carry *Ga1*, while most dent and flint corn lines are *ga1/ga1* genotypes

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(Kermicle 2006). *Tcb1* was identified within teosinte and was named *teosinte crossing barrier-1* (*Tcb1*). *Tcb1-S*, the strongest allele of *Tcb1*, prevents teosinte from being fertilized by maize while promoting its own propagation (Evans and Kermicle 2001; Kermicle and Evans 2010). Homozygous *Ga1-S* is unreceptive both to *Tcb1* and *tcb1* pollens, heterozygous *Ga1-S/ga1* plants are somewhat more receptive to *Tcb1* than to *tcb1*. Reciprocally, homozygous *Tcb1-S* is unreceptive both to *Ga1* and *ga1* pollens. Attenuated *Tcb1* is more compatible with *Ga1-S* than to *ga*1, indicating cross recognition between the two systems of incompatibility (Evans and Kermicle 2001). Another allele of *Ga1*, *Ga1-M*, identified from White Rice popcorn strain 4519, has the male function but lacks the pistil barrier function of *Ga1-S* (Jimenez and Nelson 1965; Ashman 1981). *Ga1-M* pollen can pollinate *Ga1-S* pistil and vice versa, *ga1* pollen also can pollinate *Ga1-M* pistil. Both *Ga1* and *Tcb1* have been mapped on maize chromosome 4 and the two loci are loosely linked with *Tcb1* 6 cM distal to *sugary-1* (Evans and Kermicle 2001).

Since the discovery of *Ga* in the early 1920s, no *Ga* genes have been molecularly isolated. The mechanism of pollen-pistil incompatibility is still unclear. In the case of *Ga1-S*, pollen grains germinate well and pollen tubes can penetrate the stylar tissue when *Ga1-S/Ga1-S* plants are pollinated with *ga1* pollens (Schwartz 1950; Andreas Lausser et al. 2010). Pollen-pistil incompatibility to crossing in maize and teosinte result from incongruity rather than active rejection (Kermicle and Evans 2005). In *Arabidopsis thaliana*, a number of gametophytically important genes have been identified (Howden et al. 1998; Grini et al. 1999; Procissi et al. 2001; Gupta et al. 2002). However, these genes affect the pollen development at early stages, resulting in aberrant pollen grains. They are less likely to be involved in the later stages that have been associated with pollen competition in natural populations. Recently, there has been renewed interest in gametophyte factors in maize due to the utilization of transgenic maize in corn production and the completion of the B73 genome (Schnable et al. 2009). Contamination of conventional corn seed from genetically modified varieties is a constant issue in seed corn production and especially so for organic seed corn. When introduced into maize, unidirectional cross-incompatibilities of *Ga1-S* and *Tcb1-S* are useful for isolating one category of commercial varieties from another. Varieties to be protected might be pure breeding stocks, might possess special quality features or be free of transgenes. A barrier to crossing would suffice for certain purposes even if effective only in one direction. Such is the case whereby the *Ga1-S* system currently is being employed to prevent the pollination of popcorn varieties by dent hybrids. The genetic control of the pollen-pistil interaction is fundamental in the determination of reproductive system of plants. Species diversification and species maintenance are key topics in evolutionary biology. The two barriers are excellent systems for studying signal transduction and pollen–pistil interactions. Mapping and eventual cloning of these barrier genes would provide insight into molecular mechanisms of reproductive isolation. The completion of the B73 genome makes gene fine mapping and cloning relatively feasible.

The present paper reports genetic analysis and fine mapping of a gametophyte factor identified from a Chinese popcorn strain SDGa25. This *Ga* mapped on chromosome 4 turns out to be a strong allele of *Ga1* (*Ga1-S*) and complete cross-incompatible with pollen from the majority of Chinese dent and flint corn germplasm. The tightly linked molecular markers reported here would facilitate marker-assisted *Ga1-S* introgression into dent and flint maize inbreds, and the fine mapping is the prerequisite for map-based cloning of this gametophyte factor.

Materials and methods

Plant materials and mapping populations

Popcorn strain SDGa25 carrying *Ga1-S* was provided by Dr. Baoshen Liu, Shandong Agricultural University. The exact pedigree of this popcorn strain is unknown. For genetic study and fine mapping, four BC_1F_1 populations, (SDGa25/Jing24//Jing24) BC_1F_1, (SDGa25/W22//W22) BC_1F_1, (SDGa25/HN287//HN287) BC_1F_1 and (SDGa25/JKN2000M//JKN2000M) BC_1F_1, were constructed. They were constructed by crossing SDGa25 with Jing24, W22, HN287, and JKN2000F, and their F_1 progenies as male backcrossed to Jing24, W22, HN287, and JKN2000F, respectively. To study the relationship between SDGa25 and the Chinese elite inbred lines, 148 inbred lines were selected and they are listed in Table 1. All materials were planted at the experimental farm of Shandong Agricultural University in 2009 and 2010.

Phenotyping, genotyping and crossability evaluation

We used SDGa25 as tester to phenotype BC_1F_1 segregating population. Pollen from BC_1F_1 individuals were used to pollinate SDGa25. If SDGa25 set seed, the corresponding male then carries the barrier allele; otherwise, the corresponding male does not carry the barrier allele. Leaf tissues of BC_1F_1 individuals were collected for DNA extraction and genotyping. All individual plants were tagged and numbered.

Crossability of the 148 inbred lines with SDGa25 was evaluated by making crosses between them (male) and SDGa25 (female). SDGa25 was planted three times at 7-day intervals to make flowering time meet. The 148

Table 1 Maize inbred collections used for cross-incompatibility study

No.	Material	No.	Material	No.	Material	No.	Material
1	178	38	Fu842	75	Jiu03	112	Shen135
2	374	39	Fu96	76	JKN2000F	113	Shen137
3	444	40	Guan17	77	JKN2000 M	114	Si533
4	48-2	41	H10	78	K10	115	Tai184
5	488	42	H2	79	K12	116	Tangsipingtou
6	785	43	H205	80	K14	117	Tie7922
7	87-20	44	H21	81	K22	118	W22
8	8902	45	H285	82	K36	119	W24
9	A632	46	Han102	83	Liao138	120	Wanxi23
10	B73	47	He344	84	Liao2204	121	Weifeng322
11	B84	48	HN287	85	Liao2345	122	Wu202
12	Chang7-2	49	HR962	86	Liao3053	123	Xing83
13	Chaoxianbai	50	Hua160	87	Liao5110	124	Yan103
14	Cheng18	51	Huang C	88	Liao5114	125	Yan172
15	Chihuang32	52	Huangye4	89	Liao7794	126	Ye478
16	Chuan273	53	Huangyesi3	90	Liaoyu311	127	Ye515
17	Chuan321	54	Huangzaosi	91	Linxi11	128	Ye8112
18	CML292	55	Huobai	92	Lo1067	129	Ying64
19	CML396	56	Huotanghuang17	93	Lo1125	130	Yu12
20	CN165	57	HZ32	94	Longkang1	131	Yuanwu02
21	CN962	58	J001	95	Longkang15	132	Yue20
22	D729	59	J002	96	Lu28	133	Yue267
23	Dan3130	60	Ji412	97	Lu65	134	Yue89E4
24	Dan340	61	Ji419	98	Luyuan92	135	ZaC546
25	Dan598	62	Ji465	99	Luyun133	136	Zao49
26	Dan599	63	Ji495	100	LX9801	137	ZD12F
27	Dan9046	64	Ji81162	101	M3005	138	Zheng22
28	De811	65	Ji818	102	Mo17	139	Zheng58
29	DH212	66	Ji842	103	Moqun17	140	Zhong106
30	DH65232	67	Ji846	104	P138	141	Zhong17
31	Dong237	68	Ji853	105	P28	142	Zhong451
32	Dong46	69	Ji880	106	QB80	143	Zhonghuang204
33	Duohuang29	70	Jing24	107	Qi205	144	Zhonghuang64
34	E28	71	Jingnuo2	108	Qi209	145	Zi330
35	ES40	72	Jinhuang96	109	Qi318	146	Ziyu3
36	Feng273	73	Jinsui54	110	Qi319	147	Zong3
37	FR218	74	Jitian15	111	S7913	148	Zun90110

inbred lines were planted when the second batch of SDGa25 was planted. Three individual crosses were made for each line and seed setting was evaluated at harvest. For those lines that showed cross-compatibility, they were reevaluated in the next season.

Marker analysis and genetic mapping

CTAB was used for genomic DNA extraction from leaf tissues. PCR was performed for 35 cycles of 1 min at 95°C, 30 s at 55°C (for most primers), and 45 s at 72°C, followed by a 5 min extension at 72°C. Annealing temperature of different primers varied based on their T_m value. The 25-μl reaction mix consisted of 5 pmol of each primer, 2.5 mM of $MgCl_2$, 100 μM of each dNTP, and 1 × reaction buffer, 0.5 unit of *Taq* DNA polymerase (Promega), and about 50 ng of template DNA. PCR products were separated on a 12% polyacrylamide gel and visualized with ethidium bromide staining. SSR markers on the short arm of chromosome 4 were initially screened for marker-trait association. The primers were adopted from the MaizeGDB (http://www.maizegdb.org). For further linkage analysis, SSR and STS

markers were developed from the BACs in the target region defined by SSR markers (http://www.maizegdb.org/cgi-bin/locus_lookup_refgenv2.cgi?locus=ga1&id=IBM2). Detailed marker/primer information is listed in Table 2

Pollen tube growth observation

Inbred lines SDGa25 and W22 were used for pollen tube growth observation. We compared pollen tube growth among SDGa25 selfing, SDGa25 pollinated with W22 pollen and W22 pollinated with SDGa25 pollen. For each cross, three individual plants were studied. Fresh pollen was paper-bagged between 9:00 and 11:00a.m. and silks were cut 1 cm above the husks to make silks relatively uniform. Silks were fixed for aniline blue staining at 0.15, 0.5, 1, 2, 5, 10 and 20-h intervals after pollination.

Silks were fixed 48 h in FAA (10:85:5 v/v/v formaldehyde: 95% alcohol: acetic acid) at 4°C. Fixed samples were rehydrated by an ethanol series (75, 50, and 25%) each for 3 min and washed with 0.1 M potassium phosphate buffer at pH 8.0. Subsequently, samples were incubated for 2 h in 8 M NaOH and were washed again with 0.1 M potassium phosphate. The cleared and smoothed silk was then stained for 12 h at 4°C with 0.1% aniline blue solution (water blue, Amresco) prepared with 0.1 M potassium phosphate, and then was put on a slide with a cover slip and observed on a fluorescence microscope (Olympus BX61) with near UV excitation. Because silks are not uniform in length, 10 ~ 15 silks were evaluated for each plant at each time interval and 10 silks that were relatively uniform in length were used for pollen tube growth calculation. 30 silks from three plants at each time interval were averaged.

Table 2 Primer sequences and their BAC positions

Primers	Sequences	BACs
Umc1758	F: CTTCCTCCTCACCTCACCTCCTAT	AC208432
	R: GGTAGCCAATCCTTCCTTCCTATG	
SD1	F: TACCTTTCTGGGAGTTCTT	AC196262
	R: GCCACTGTTTGCTCTTTCG	
SD2	F: CTCTAGTGCATTCAAACACC	AC196057
	R: CCCATTAATGAACGGTGG	
SD3	F: CAGATTCGAAGTACACAGTAGCAGG	AC200747
	R: CCGCAATGATTCTTCGTCATTTGGC	
SD4	F: ACTTTCCATCCAATCCCTC	AC184840
	R: TGTCAGTCTACCCAACCAAT	
SD5	F: CCATCGACTTCATCTTCGGA	AC194254
	R: AGGAGTATATCACAGGCGTG	
SD6	F: GAGAGCTACGCACGACTTAT	AC203042
	R: CTCAACTCTGACCTATGTCG	
SD7	F: GGGAACAAAGGCAACCTTCT	AC190823
	R: GTAGGAGTATATCACAGGCG	
SD8	F: ATATACTCCTACACTAATATAGG	AC205353
	R: TGAGAGCTACACACACGACT	
SD9	F: GAGAGCTACGCACGACTTAT	AC214441
	R: CAAGACTTGCACAATCGAGG	
SD10	F: CATCTCGACCATGATGATGAG	AC214441
	R: TCGGCTTTCGTGTGTGCAAT	
SD11	F: AGAGGACATAGTACATAGATTG	AC184772
	R: CGCGTTGCGATGACACACA	
SD12	F: CATCCTTTTCTCGGCTTGAG	AC204382
	R: ACTACGAGACGGTGTTCAAG	
SD13	F: AACGAACGAGGCCTAAATAGT	AC194666
	R: GATGGGCCACCTCAACGTC	
SD14	F: CAGGAACTAGACTCCTCCG	AC194666
	R: CCTCTTGACGCCGTTAATTC	
Umc1294	F: CCTCTTGACGCCGTTAATTC	AC190633
	R: GCCTCCAGCTCTCTCGTCTCTT	

Results

The majority of Chinese corn germplasm are cross-incompatible with SDGa25

To assess the cross-compatibility of SDGa25 with Chinese corn germplasm, emasculated plants of SDGa25 were hand pollinated with 148 Chinese inbreds representing the major heterotic group in China (Table 1). Three SDGa25 plants for each cross were investigated and the total seed number was counted. Three inbred lines, JKN2000F, ZD12F, and 178 were cross-compatible with SDGa25 producing more than 100 seeds, 129 lines produced 0 seeds, and 15 lines produced 1–5 seeds. JKN2000F, ZD12F, 178 and the 15 lines producing a few seeds were reevaluated the following season. Same results were obtained for JKN2000F, ZD12F, and 178 which full-set seeds were produced. The 15 lines, however, produced no seed, indicating that the first season result was due to pollen contamination and these 15 lines were cross-incompatible with SDGa25.

*ga*1 Pollen tube growth is obstructed in SDGa25 pistil

Cross-incompatibility can be resulted from pollen grain germination failure, pollen tube growth arrest, or fertilization/post-fertilization event failures. To address the above questions of the cross-incompatibility of SDGa25 with incompatible maize pollen, we compared pollen tube growth among three crosses: SDGa25 selfing (compatible), SDGa25 pollinated with W22 pollen (incompatible) and W22 pollinated with SDGa25 pollen (compatible), and the results are shown in Fig. 1. The results indicated that pollen grains germinated well and pollen tubes were able to

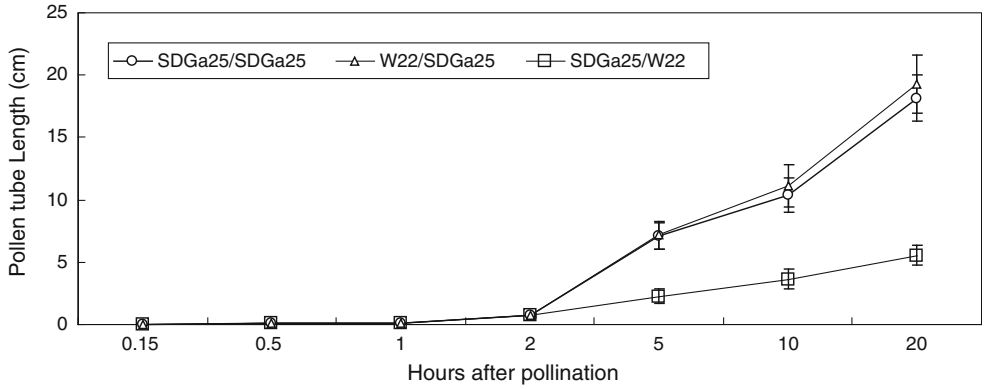

Fig. 1 Pollen tube growth of three different crosses: SDGa25/SDGa25 (compatible, selfing), W22/SDGa25 (compatible, W22 pollinated with SDGa25 pollen), SDGa25/W22 (incompatible, SDGa25 pollinated with W22 pollen)

enter the silk transmitting tract for all three cases. There was no difference of pollen grain germination and pollen tube growth at the 2-h time interval between compatible and incompatible crosses. Significant difference in pollen tube growth occurred 2 h after pollination between the two crosses. Pollen tubes grew much faster in compatible cross than that in incompatible cross. Compatible pollen displayed a linear growth of about 10 mm h^{-1} while incompatible pollen 2.8 mm h^{-1}. At 20-h interval, pollen tubes reached ovule in compatible crosses, while pollen tubes were found in the silk segment 5.5 cm distal to the pollination area in the incompatible cross and no pollen tube ever reached the ovule area. Our results clearly showed that the cross incompatibility of SDGa25 was due to pollen tube growth arrest around 2 h after pollination.

*Ga*1-S behaves as a single dominant gene

Since F_2 population is a distorted segregating population for *Ga1-S*, four BC_1F_1 populations, SDGa25/Jing24//Jing24, SDGa25/W22//W22, SDGa25/HN287//HN287, and SDGa25/JKN2000 M//JKN2000 M, were used for *Ga1-S* study. The four parents used for backcrossing produce 0 seeds when pollinating SDGa25 in the previous crossability studies. To determine the genotype of the BC_1F_1 individuals, each individual was backcrossed to SDGa25. If no seed was set, the individual was lack of the *Ga1-S* allele; if full-set seeds were produced, the individual was then carrying the *Ga1-S* allele. There were plants from Jing24/SDGa25//Jing24 population that set less than five seeds. These individuals were grouped as those lacking the *Ga1-S* allele. Chi-square independence test showed that all four BC_1F_1 populations segregated into 1*Ga1-S*:1*ga1-s* ratio, indicating that *Ga1-S* detected from the respective populations is a single dominant gene (Table 3).

Fine mapping of *Ga1-S*

Since *Ga1-S* has been previously reported on chromosome 4, SSR markers on this chromosome were used to screen the four BC_1F_1 populations. *Ga1-S* was initially detected in all four populations and linked to SSR umc1758 on BAC AC208432 and SSR umc1294 on BAC AC190633. These four populations were then combined as one mapping population for the target gene fine mapping. Consequently, a total of 1,384 individuals were used for the fine mapping. *Ga1-S* was flanked by umc1758 on the telomere side and umc1294 on the centromere side with genetic distances of 9.7 and 4.8 cM, respectively. To fine map the target gene, SSR and STS markers were developed from the BACs in between this region (Table 2). The closest markers we developed was SD3 on BAC AC200747 that was 0.827 cM

Table 3 Chi-square analysis of BC_1F_1 populations for *Ga1-S* and *ga1* segregation ratio

BC_1F_1 Populations	BC_1F_1 individuals			Observed segregating ratio	χ^2
	Number of individuals carrying *Ga1-S*	Number of individuals carrying *ga1*	Number of total individuals		
W22/SDGa25//W22	85	88	173	0.966	0.058
Jing24/SDGa25//Jing24	415	431	846	0.963	0.303
JKN2000 M/SDGa25//JKN2000 M	139	148	287	0.94	0.285
HN287/SDGa25//HN287	35	43	78	0.82	1.07

$\chi^2_{0.05}(1) = 3.84$

to the target gene on the telomere side, and SD12 on BAC AC204382 0.709 cM on the centromere side. There are 16 BACs between AC200747 and AC204382 (AC213790, AC201880, AC184840, AC194254, AC203042, AC191 393, AC184113, AC180823, AC205353, AC214441, AC 196002, AC210943, AC184772, AC201986, AC205010, AC208523). We developed markers from BACs AC18 4840, AC194254, AC203042, AC180823, AC205353, AC214441, and AC184772, and all of these markers were completely co-segregating with the *Ga1-S* locus. The genetic region mapped spanning the *Ga1-S* locus was estimated to be 1.5 cM in length. The above BACs are on ctg156 and the physical distance based on the *B73 RefGen_v2 sequence* is 2,056,343 bp.

The co-segregating markers are efficient tools for introducing *Ga1-S* into *gaga* maize lines

Eight STS markers, SD4, SD5, SD6, SD7, SD8, SD9, SD10, and SD11 were co-segregating with the *Ga1-S* locus in our mapping population and could be efficient markers for introducing *Ga1-S* into *ga1ga1* maize lines. One of our breeding programs was to transfer *Ga1*-S from SDGa25 into our elite waxy corn lines by backcrossing using waxy lines as recurrent parents. To test the usefulness of the above STS markers in marker-assisted selection, two BC_2F_1 populations from our breeding programs were evaluated with the eight STS markers. Fifty individuals from each population were genotyped and also crossed to SDGa25, respectively. The results showed complete association between the eight markers and the *Ga1-S* allele (data not shown). Figure 2 shows the co-dominant nature of marker SD4 that can distinguish *Ga1-S/Ga1-S*, *Ga1-S/ga1-s*, and *ga1-s/ga1-s* genotypes. These tightly linked co-dominant markers prove to be very efficient in our ongoing backcrossing programs.

Discussion

Many popcorn strains carry the strongest dominant gametophytic factor *Ga1-S* and cannot be fertilized by pollen of dent, sweet, and flint strains, although reciprocal crosses are successful (Nelson 1952). No gametophytic factors have been isolated to our knowledge and the exact molecular nature of *Ga1-S* is still unknown. Any event failure from pollen grain germination to double fertilization could lead to cross incompatibility. In the case of SDGa25, *ga1*-pollen grain germinates normally, is able to enter the silk transmitting tract, and shows no difference from *Ga1*-pollen at the very early stage of pollination. However, compatible *Ga1*-pollen tube grows much faster (10 mm h^{-1}) than that of incompatible *ga1*-pollen tube (2.8 mm h^{-1}) 2 h after pollination, and the *ga1*-pollen tube growth is arrested in the silk segment 5.5 cm distal to the pollination area. This is similar to the report that a growth arrest of *ga1*-pollen after 2–3 cm, while compatible pollen displayed a linear growth of about 12 mm h^{-1} (House and Nelson, 1958). Previous studies indicated that maize pollen grains could support around 2 cm of tube growth using exclusively endogenous reserves (Heslop et al. 1984). Pollen tube growth length is critical to overcome crossing barriers between maize and teosinte (Kermicle and Evans 2005; Lausser et al. 2010). All these findings point to the lack of further pollen tube growth by the sporophytic tissues may be the major cause of pollen tube arrest. Pollen tube growth arrest could be caused by pistil active rejection of pollen containing a contrasting allele (incompatibility), or the pistil could require the presence of a matching allele in pollen (congruity). Kermicle and Evans (2005) have shown that pollen-pistil barriers to crossing in maize and teosinte result from incongruity rather than active rejection. Under natural conditions, targeting to the transmitting tract is a prerequisite for pollen tubes to grow through the maize silks completely. Transmitting tract cells attract pollen tubes by secretion of chemotropic signals (Heslop et al. 1984). Pollen tube transmitting tract mistargeting seems not the case for SDGa25 since the majority of *ga1*-pollen can grow ~5 cm in *Ga1-S* silk.

The *Ga1-S* might contribute to reproductive isolation. To do so, it should be widespread in popcorn strains and absent from sympatric dent, sweet and flint maize strains. Many popcorn strains carry *Ga1* (Nelson 1952). Our results suggest that SDGa25 carries the strongest allele of *Ga1* (*Ga1-S*) and the majority of Chinese dent and flint maize germplasm is incompatible with SDGa25. However, three inbred lines, JKN2000F, ZD12F, and 178 were found compatible with SDGa25. 178 was a widely used elite

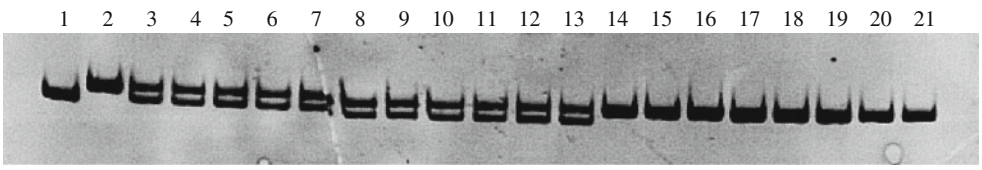

Fig. 2 Banding profie showing polymorphisms identified by SD4 marker among *Ga1-S/Ga1-S*, *Ga1-S/ga1-s*, and *ga1-s/ga1-s* genotypes. *Lane 1* SDGa25 (*Ga1-S/Ga1-S*); *lane 2* recurrent parent (*ga1-s/ga1-s*); lane 3 F_1 (*Ga1-S/ga1-s*); *lanes 4–21* BC_2F_1 segregating individuals, lanes *4–13 Ga1-S/ga1-s* genotypes, *lanes 14–21 ga1-s/ga1-s* genotypes

inbred line in China. Presence of this allele in 178 (*Ga1-M*, Ashman 1981) in maize would neutralize *Ga1-S* in popcorn as a barrier in reproductive isolation.

The key step to map *Ga1-S* is to accurately phenotype BC_1F_1 segregating individuals. To do so, we tagged each BC_1F_1 segregating individual and used SDGa25 as tester to check the presence/absence of the *Ga1-S* allele of the BC_1F_1 segregating individuals. SDGa25 plants were detasseled completely prior to flowering and were then pollinated by tagged BC_1F_1 segregating individuals. If SDGa25 plant set seeds, the corresponding individual then carried the *Ga1-S* allele; otherwise, if SDGa25 plants set no seeds, the corresponding individual then carried the *ga1* allele. 1,384 BC_1F_1 individuals were phenotyped this way and no more recombinants were found within a region of 2,056,343 bp in length. There were individuals from Jing24/SDGa25//Jing24 population that set few seeds. We believe this was due to contamination, either detasseling was not in time or hand washing was not thorough. All these individuals carry the *Ga1-S* allele based on genotyping results by co-segregating STS markers. Recombinant screening from $\sim$15,000 BC_1F_1 segregating individuals is under way.

To our knowledge, tightly linked molecular markers to *Ga1-S* have not been published. The introduction of *Ga1-S* from popcorn to non-crossable maize elite lines has been performed by backcrossing without markers. This is laborious, lengthy and inefficient, especially for *Ga1-S* introduction because of the difficulty of phenotyping of the segregating population. Eight STS markers, SD4, SD5, SD6, SD7, SD8, SD9, SD10, and SD11 co-segregated with *Ga1-S* in our mapping population. Since 50% individuals of the backcrossing population do not contain *Ga1-S* allele, the tightly linked markers can greatly improve the efficiency and reliability of the backcrossing process by eliminating those that do not carry *Ga1-S* allele.

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