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Research Report

Sequence-Characterized Amplified Polymorphism Markers for Selecting Rind Stripe Pattern in Watermelon (*Citrullus lanatus* L.)

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Abstract. The inheritance of foreground stripe pattern in rind of watermelon fruits [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] was evaluated and molecular markers for selecting the Jubilee-type (JT) stripe pattern were developed based on bulked segregant analysis (BSA). Divergence in rind pattern among F_2 progeny derived from crossing Crimson-type (CT) 'Arka Manik' (AM) with JT 'TS34' (TS) indicated that stripe pattern is a quantitative trait controlled by more than one gene. The BSA of F_2 plants (derived from a cross between 'AM' and 'TS') using 60 random amplified polymorphic DNA (RAPD) primers revealed a distinct RAPD band (AT14-900) polymorphic between 'AM' (CT) and 'TS' (JT). The AT14-900 sequence (925 bp) was blasted to the reference watermelon (97103) genome and high sequence similarity (97.8%) was identified on physical location of 26246077 to 26246993 bp on chromosome 6. Two expressed sequence tags (ESTs) designated 'wsbin6-10' and 'wsbin6-11' that were closely linked to AT14-900 on a genetic linkage map (developed using the F_2 population derived from 'AM' x 'TS') were positioned 2,216 kb and 71 kb from AT14-900, respectively on the reference watermelon genome sequence. Marker genotyping of the F_2 population showed that wsbin6-11 was tightly linked to the JT stripe pattern of 'TS' and could be a useful codominant marker for selecting this trait. In a test using 100 breeding lines, 34 of the 36 lines carrying the JT stripe pattern were homozygous for the wsbin6-11 marker (450 bp) derived from 'TS', while other lines (e.g., with no stripe or CT stripe pattern) were homozygous for the wsbin6-11 marker (420 bp) derived 'AM'. These results indicated that wsbin6-11 would be a useful marker in watermelon breeding programs aiming to select for the JT stripe pattern from other various foreground and background rind patterns.

***Additional key words*:** cucurbits, DNA marker, fruit-related trait, marker-assisted breeding, maker-assisted selection

Introduction

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai] belongs to the xerophytic genus *Citrullus* Schrad. Ex Eckl. & Zeyh. (2n = 2x = 22) that exist in Africa and thrives in Old World tropics (Dane and Lang, 2004; Singh, 1990). Watermelon (*C. lanatus* var. *lanatus*) is one of the most popular fruits worldwide, containing water, sugars, flavor, and many nutritional compounds such as lycopene, citrullin, and arginine. Global net production of watermelon is estimated as 102.9 million tons, with Asia as the leading continent (accounting for 83.36% of the production), followed by North America (5.89%), Europe (5.49%), Africa (5.13%), and Oceania (0.14%) (http://www.fao.org).

Watermelon varieties can be differentiated based on fruit shape, fruit size, rind pattern, fruit flesh color, and seed size and seed coat color, amongst other morphological characteristics (Guner and Wehner, 2003; Poole, 1944; Poole and Grimball, 1945). Various watermelon traits are preferred by consumers in different countries and geographical regions; Crimson-type seedless cultivars are popular in the USA and Europe, small sized ice-box-type fruits in Southeast Asia,

Jubilee-type round fruits in China, and Jubilee-type broad elliptic fruits in South Korea (Park and Cho, 2012). In recent years, cultivars with high citrullin, β-carotene, and lycopene content and with tolerance to biotic and abiotic stresses are in increased demand. Also, there is an increased demand for fresh-cut market watermelons with less seeds, diverse flesh colors, and flesh hardness (Park and Cho, 2012). Breeding programs that employ molecular markers could facilitate efficient introgression of these features into elite watermelon cultivars to satisfy seed market demands.

Rind pattern is an important trait in watermelon breeding programs. Rind types are divided by background rind color (light to dark) and foreground stripe pattern (solid to stripe) (Guner and Wehner, 2003). Foreground stripe rind patterns are diverse and well represented in the International Union for the Protection of New Varieties of Plants (UPOV, http://www.upov.int). Rind stripes can differ according to pattern (unicolored, only vein, bicolored, or marbled), width (very narrow to very broad), conspicuousness (inconspicuous to very strong), and margin (diffuse, medium, or sharp). Genetic inheritance has been confirmed for only limited cases of stripe pattern. One example was the study by Weetman (1937) that used genetic populations derived from a cross between 'China 23' (dark green stripe and medium green network) and 'Japan 6' (inconspicuous and panciled stripes on light green background), and determined that the presence of netted rind and dark stripe was controlled by two independent genes. Additional studies are still needed to determine inheritance of the different striped rind characteristics.

The DNA markers tightly linked to rind stripe type could be useful in marker-assisted selection (MAS), intensifying the breeding process by selecting plants carrying targeted rind pattern based on their marker genotype (Collard and Mackill, 2008; Moose and Mumm, 2008). Bulked segregant analysis (BSA) is an efficient approach to screen markers linked to a target trait, especially under circumstances where genetic mapping cannot be easily applied (Kim et al., 2013; Michelmore et al., 1991). To our knowledge, no publicly available molecular markers for MAS of rind stripe pattern in watermelon have been reported.

In this study, we evaluated inheritance of various stripe patterns in a typical Crimson- or Jubilee-type watermelon cultivar. Using BSA procedure (Michelmore et al., 1991), molecular markers for selecting stripe pattern were developed and their chromosomal location was determined using the watermelon genome (Guo et al., 2013) and a genetic linkage map.

Materials and Methods

Plant materials

A Crimson-type (CT) commercialized open pollinated (OP) cultivar 'Arka Manik'(AM) in India (Rai et al., 2008) and a Jubilee-type (JT) inbred line 'TS34'(TS) developed in South Korea were used for the inheritance study and development of DNA markers for foreground rind stripe-type. The stripe patterns of the rinds of 'AM' and 'TS' are shown in Figure 1. F_1 plants were produced by crossing 'AM' as a maternal parent with 'TS' as a pollen donor. The F_1 plants were self-pollinated, and F_2 progenies were produced. All crosses and progeny productions were conducted in the greenhouse at the NH Seed Co. in Ansung, South Korea between 2008 and 2009.

Applicability of the markers for MAS was confirmed using 100 inbred lines provided from two private seed companies, Partner Seed Co. (A) and Nongwoo Bio Inc. (B), in South Korea. Phenotypic information for rind type and leaf samples for DNA extraction of each inbred line was provided by the seed companies.

Phenotypic analysis

The F_2 plants were grown in a breeding field located in Konken, Thailand in 2012, and fruits harvested from 130 F_2 plants were surveyed for rind stripe type. The stripe pattern was classified into nine categories as follows: class 1) stripe pattern of 'AM' (Crimson-type: light green stripe with broad width and diffused margin); class 2) stripe pattern of F_1, (coexpression of the stripe pattern for 'AM' and 'TS'); class 3) stripe pattern of 'TS' (Jubilee-type: dark green with medium width and sharp margin); classes 4 to 9) various stripe patterns that do not belong to 'AM', 'TS' or the F_1 progeny (Fig. 1). Each fruit was scored based on this classification. Fruits were photographed for long-term records and examination. For BSA, only F_2 plants showing the stripe pattern of either 'AM'(1) or 'TS'(3) were considered; fruits showing mixed patterns of other classes were excluded.

Bulked segregant analysis (BSA)

The BSA using random amplified polymorphic DNA (BSA-RAPD) was used to develop a molecular marker that is tightly linked to stripe pattern. Based on the results of progeny test for stripe pattern, DNA samples from six F_2 plants showing the CT light green stripe (class 1) were isolated and mixed together in an equal amount (DNA sample ID: BK1). The same was done for 11 F_2 plants showing a JT dark green stripe (class 3) (DNA sample ID: BK2). Plant genomic DNA extraction was conducted as described previously (Kim et al., 2010).

The RAPDs were evaluated to identify polymorphisms between the bulked DNA samples BK1 and BK2. For the RAPD procedure, 60 10-mer Operon primers that had showed successful PCR amplification for watermelon DNA in a previous study (Kim et al., 2013) were selected and tested following methods described previously (Je et al., 2009).

Fig. 1. Image of stripe pattern as foreground rind type used for the phenotypic classification of 130 F_2 plants derived from 'Arka Manik'(AM) × 'TS34'(TS). Stripe pattern was classified into nine categories as follows: 1, stripe pattern of 'AM' (Crimson-type, light green stripe with broad width and diffused margin); 2, stripe pattern of F_1, (coexpression of the stripe pattern for 'AM' and 'TS'; 3, stripe pattern of 'TS' (Jubilee-type: dark green with medium width and sharp margin); and 4 to 9, various stripe patterns belonging to neither 'AM', 'TS' or F_1.

Marker development

For cloning and sequencing of the PCR amplicons, DNA fragments were eluted from an agarose gel using a GeneAll Gel SV kit (GeneAll, Deajeon, Korea), cloned into pGEM-T Easy Vector (Promega, Madison, WI, USA) and sequenced by the dye-termination method using an ABI3730 capillary DNA sequencer (Applied Biosystems, Foster City, CA, USA). A detailed description of the cloning and sequencing methods can be found in a previous report by Kim et al. (2010). The PCR primer design and sequence alignments were performed using Primer3 (v2.0) and Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) software, respectively. Blastn searches for homologous gene identification were conducted using NCBI BLASTN (version 2.2.29+) software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). A watermelon reference genome sequence (Cucurbit Genome Database, http://www.icugi.org/cgi-bin/ICuGI/genome) (Guo et al., 2013) was used for chromosomal localization of the sequence-characterized amplified polymorphism (SCAR) marker (AT14-900) derived from BSA. An intraspecific genetic linkage map (Park et al., unpublished data) was subsequently used for the identification of the neighboring SCAR markers (wsbin6-10 and wsbin6-11).

Marker evaluation

Genotyping of the SCAR markers (wsbin6-10 and wsbin6-11) was carried out by running PCR amplifications in a total volume of 20 mL containing: 20 ng of genomic DNA, 0.3 mM each of the forward and reverse primer, 1X PCR buffer, 0.2 mM dNTPS, and 0.6 U of *Taq* polymerase (SolGent, Daejeon, Korea). The PCR conditions were as follows: 1 cycle of 5 min at 95°C, 10 cycles of 15 s at 95°C, 30 s at 60°C (in decreasing steps of 0.5°C/cycle for cycles 2 –10), 30 s at 72°C, and 1 min at 72°C, followed by 35 cycles of 15 s at 95°C, 30 s at 55°C, and 1 min at 72°C. Gel electrophoresis was performed using 2.5% agarose gel containing Tris-acetate EDTA (TAE) at 120 V for 2 h and then visualized under ultraviolet light after ethidium bromide staining.

Results and Discussion

Phenotype analysis

Fruits of 'AM' showed green (one colored) stripes with a diffused broad margin, typical to CT watermelons. Whereas the fruits of 'TS' had dark-green stripes (one colored and marbled) with sharp margin and medium width, typical to JT watermelons (detailed information on rind phenotypes of 'AM' and 'TS' is presented in Fig. 1). Fruits of F_1 plants derived from 'AM' × 'TS' showed a hybrid type of CT and JT, demonstrating possible codominance effect of gene loci responsible for stripe pattern (Fig. 1). Stripe pattern was divergent among the 130 F_2 plants tested. Of these, six F_2 plants expressed distinct 'AM'(CT) pattern, 11 F_2 plants expressed 'TS'(JT), 19 F_2 plants expressed F_1 pattern, while all other 94 F_2 plants showed diverse patterns (Fig. 1).

The inheritance of striped and solid green rind has been studied, and a single locus model of three alleles (G, g^s, g) was reported by Weetman (1937) and Poole (1944). In this model, the G allele for dark green rind color is dominant to g for light green rind, while the g^s producing stripes is dominant to g, but recessive to G. However, a hypothesis of two tightly linked loci for controlling dark green rind and striped pattern is also plausible (Weetman, 1937). A dominance of g^s allele conferring the striped pattern was rediscovered by Shimotsuma (1963) against a gray (yellowish-green) background rind. However, allelism and gene interaction for CT and JT stripe has yet to be reported. The divergence among F_2 progeny here suggests that rind stripe pattern is a quantitative trait, controlled by several gene loci.

Bulked segregant analysis (BSA)

The distinct CT and JT stripe pattern in 6 and 11 F_2 plants, respectively, indicates that BSA may still be an advantageous possible approach for the development of markers linked to

these qualities. To identify the genomic region harboring the stripe pattern (CT vs. JT), BSA using RAPD was conducted. For BSA, genomic DNA samples from six F_2 plants with CT fruits were pooled (BK1). The same was done for 11 F_2 plants with JT fruits (BK2). Pooled DNA samples were genotyped using 60 RAPD primers, and a 900 bp band (AT14-900) amplified by RAPD primer AT14 (5'-GTGCCGCCAC-3') was identified to be unambiguously polymorphic between the two pooled DNA samples (Fig. 2). The AT14-900 polymorphic RAPD band was present in bulked DNA samples of CT (BK1), but absent in bulked DNA samples of JT (BK2). The primer AT14 was tested against 130 F_2 plants including each individual plant used in the BSA. AT14-900 was present in 24 of 25 F_2 plants scored as class 1 ('AM') or class 2 (F_1), while it was absent for all 11 F_2 plants scored as class 3 ('TS'). For F_2 plants of classes 4 to 9, 19 and 75 F_2 plants showed the absence or presence of AT14-900, respectively. It was important to note that among 13 F_2 plants scored as class 7 [with a similar stripe pattern to 'TS'(JT) except for a lighter green color], 11 showed an absence of AT14-900, a marker type for 'TS'. Inspection of the rind background color suggests that the light green color stripe characteristic of class 7 arises from a lighter rind background color in those plants, as compared to 'TS'. Thus, assuming that the class 7 fruits also belong to JT accounts for the 71.0% match between marker type and stripe pattern.

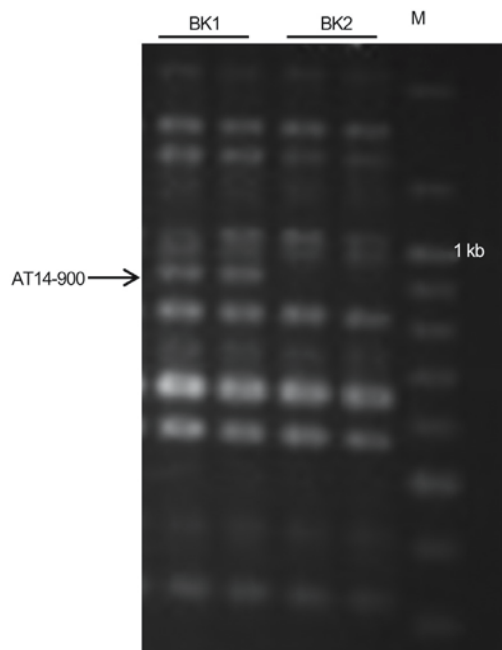

Fig. 2. Agarose gel electrophoresis showing the results of random amplified polymorphic DNA- bulked segregant analysis (RAPD-BSA) for stripe pattern. The PCR band (AT14-900) polymorphic between bulked DNA samples duplicated for Crimson-type (BK1) and Jubilee-type (BK2) stripe pattern is indicated by an arrow. BK1, a pooled DNA sample of six F_2 plants showing Crimson-type stripe pattern (phenotypic class 1); BK2, pooled DNA sample of 10 F_2 plants showing Jubilee-type stripe pattern (phenotypic class 3); and M, 100 bp size marker.

Marker development

The AT14-900 marker was cloned and sequenced, and the 925 bp region showed significant match to mRNA sequences of leucine-rich repeat-containing proteins in *Cucumis melo* (XM008466282.1) and *Cucumis sativus* (XM004170202.1). This sequence was blasted to the reference genome of watermelon (97103) and a genomic region showing 97.8% sequence homology with AT14-900 was identified at the physical location of 26246077 to 26246993 bp on chromosome 6 (Fig. 3 and A1). To develop a codominant SCAR marker, a primer set extended from the AT14 10-mer primer sequence was designed and used for PCR amplification of 'TS' (Table 1). The PCR amplicon was cloned and sequenced, but sequence alignment with AT14-900 revealed insufficient sequence variation for developing a polymorphic SCAR marker.

To further expedite marker development, we used a genetic linkage map derived from an F_2 population of 'AM' × 'TS'. This intraspecific genetic map comprises 15 linkage groups assigned by corresponding chromosome number (Park et al., unpublished data). Most markers in this map are CAPS and SCAR derived from watermelon ESTs annotated for physical locations based on the watermelon reference genome sequence (Guo et al., 2013), and markers closely mapped to the AT14-900 region could be easily identified. The watermelon

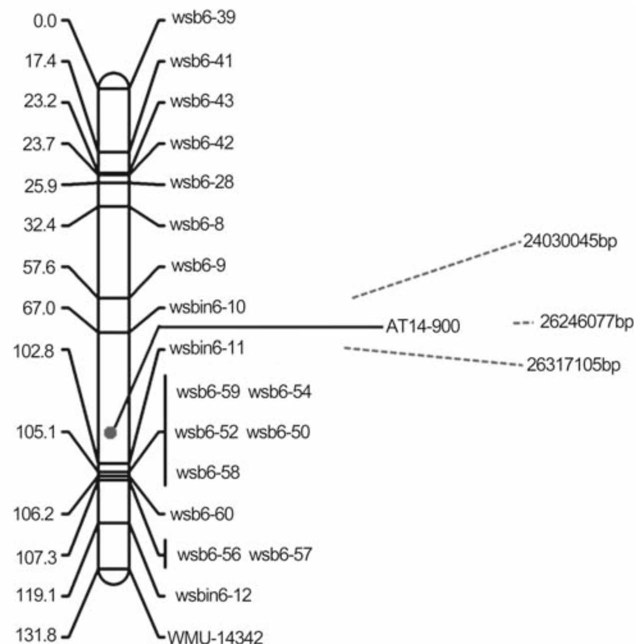

Fig. 3. A genetic linkage map of chromosome 6 composed of 19 molecular markers (Park et al., unpublished data) and approximate chromosomal localization of the DNA sequence of AT14-900 (dot). Physical genomic locations of AT14-900 and the two flanking markers wsbin6-10 and wsbin6-11 are shown in base pair (bp).

Table 1. PCR primers for sequence-characterized amplified region (SCAR) markers used in this study.

Marker		Primer sequence (5'-3')	T_m(°C)	Product size (bp)[z]	
				Arka Manik	TS34
AT14-900	For:	GTGCCGCACTCCTTC	55	925	925
	Rev:	GTGCCGCACTTAGGT			
wsbin6-10	For:	GCTCACCTTCACCTTTCATCA	58	263	257
	Rev:	ATGCCGAGAAGAGACCAAGA			
wsbin6-11	For:	GGTGAAAACTGGGATGGAGA	58	420	450
	Rev:	CATTTTGAGGGTGCATTGTG			

[z]AM, 'Arka Manik'; and TS, 'TS34'.

Table 2. Genotype frequency of the sequence-characterized amplified region (SCAR) markers wsbin6-10 and wsbin6-11 in 130 F_2 plants derived from 'Arka Manik' (AM) × 'TS34' (TS). The shadowed columns in this table indicate F_2 plants showing the Jubilee-type stripe pattern of 'TS34' and linkage to the SCARs.

Marker	Genotype[z]	No. of F_2									
		Phenotypic class[y] (no. of F_2)									Total no. of F_2
		1(6)	**2**(19)	**3**(11)	**4**(9)	**5**(19)	**6**(21)	**7**(13)	**8**(26)	**9**(6)	130
wsbin6-10	A	3	2	0	7	9	3	0	9	2	35
	T	0	3	10	1	1	6	6	3	2	32
	H	3	14	1	1	9	12	7	14	2	63
wsbin6-11	A	2	0	0	8	15	1	0	0	0	26
	T	0	0	11	0	1	0	13	0	0	25
	H	4	19	0	1	3	20	0	26	6	79

[z]A, homozygous for 'AM'; T. homozygous for 'TS'; and H, heterozygous.
[y]1, stripe pattern for 'AM'; 2, stripe pattern for F_1; 3, stripe pattern for 'TS'; and 4~9, stripe patterns that do not belong to the parents and F_1. The image of each phenotypic class is shown in Fig. 1.

reference genome sequence positioned AT14-900 in between wsbin6-10 and wsbin6-11, with a physical distance of 2,216 kb and 71 kb from these two SCARs, respectively. The genetic distance between wsbin6-10 and wasbin6-1 was 35.8 cM (Fig. 3). The codominant SCARs wsbin6-10 and wsbin6-11 (Table 1) were both polymorphic between 'AM' and 'TS' and successfully genotyped for all F_2 plants (Table 3). The frequency of marker genotypes in the F_2 is summarized in Table 2. Notably, for wsbin6-11 all 24 F_2 plants scored as classes 3 and 7 [stripe pattern for 'TS' (JT)] showed the marker genotype T (for homozygosity for the 'TS' allele) and all 19 F_2 plants of class 2 (stripe pattern for F_1) showed the marker genotype H (for heterozygosity) (Table 3). However, no significant agreement between the marker genotype and stripe pattern for 'AM' (CT) was observed. The F_2 population genotyping clearly showed that wsbin6-11 is tightly linked to the Jubilee-type (JT) stripe pattern of 'TS', and could be used as a codominant marker for selecting this trait.

Evaluation of marker usability

Efficacy of wsbin6-11 in MAS of JT stripe pattern was confirmed using a total of 100 inbred lines provided by two private seed companies (A and B). These lines have spherical or oval fruit shape, and various rind patterns, such as non-striped (NS), Crimson-type (CT) or Jubilee-type (JT) stripe pattern, with dark green (DG) or green (G) skin (as shown in Table A1). Breeding lines from company A included 20 lines of JT stripe pattern, 18 of which showed the wsbin6-11 marker genotype (TT) (homozygous for 'TS'). For company B, all 16 lines of JT stripe pattern were genotyped as TT. However, other phenotypes, such as NS and CT could not be discriminated by wsbin6-11, indicating that this marker is useful for selecting JT stripe pattern from other various foreground and background rind types.

In conclusion, the divergence in rind stripe pattern and background color in genetic populations derived from a cross between JT and CT indicates that these qualities might be controlled by several gene loci. The wsbin6-11 SCAR marker that is positioned on chromosome 6 of the watermelon genome and is tightly linked to JT stripe pattern could be used for further identification of gene loci associated with this fruit quality.

Acknowledgements: This work was supported by a grant

Table 3. Evaluation of phenotype for stripe pattern and genotype for RAPD and SCAR markers in an F_2 population derived from 'Arka Manik' (AM) × 'TS34' (TS). F_2 plants showing Jubilee-type stripe pattern and wsbin6-11 marker type of 'TS' were highlighted.

EN	Line	Phenotype class[z]	Marker genotype[y]		
			AT14-900	wsbin6-10	wsbin6-11
1	Arka Manik	1	1	A	A
2	TS34	3	0	T	T
3	F1	2	1	H	H
4	F2-1	5	1	A	A
5	F2-3	6	0	T	H
6	F2-4	4	1	A	A
7	F2-11	4	1	H	A
8	F2-13	4	0	T	A
9	F2-15	6	0	H	H
10	F2-16	5	1	A	T
11	F2-17	3	0	T	T
12	F2-18	8	1	H	H
13	F2-19	5	1	A	A
14	F2-22	8	1	H	H
15	F2-23	8	1	T	H
16	F2-24	6	1	H	H
17	F2-25	9	1	T	H
18	F2-26	5	1	T	A
19	F2-27	6	0	A	H
20	F2-28	7	1	H	T
21	F2-29	9	0	A	H
22	F2-30	7	0	H	T
23	F2-31	2	1	H	H
24	F2-32	2	1	H	H
25	F2-34	2	1	H	H
26	F2-37	8	1	A	H
27	F2-38	6	0	H	H
28	F2-41	8	1	H	H
29	F2-42	6	1	A	A
30	F2-45	5	1	H	H
31	F2-46	6	1	T	H
32	F2-53	5	1	H	A
33	F2-54	8	1	A	H
34	F2-56	1	1	A	H
35	F2-58	2	1	T	H
36	F2-60	8	1	A	H
37	F2-61	2	1	H	H
38	F2-62	1	1	H	A
39	F2-64	7	0	H	T
40	F2-67	2	1	H	H
41	F2-68	7	0	T	T
42	F2-72	3	0	T	T
43	F2-77	2	1	H	H
44	F2-80	6	1	H	H
45	F2-81	4	1	A	H
46	F2-84	7	1	H	H
47	F2-85	8	1	H	H
48	F2-86	4	1	A	A

Table 3. Continued.

EN	Line	Phenotype class[z]	Marker genotype[y]		
			AT14-900	wsbin6-10	wsbin6-11
49	F2-87	8	1	A	H
50	F2-89	9	1	H	H
51	F2-90	5	1	H	A
52	F2-91	9	1	H	H
53	F2-93	3	0	T	T
54	F2-94	8	1	A	H
55	F2-95	4	1	A	A
56	F2-96	7	0	H	T
57	F2-97	6	1	A	H
58	F2-98	9	1	A	H
59	F2-100	1	1	H	H
60	F2-101	6	1	H	H
61	F2-102	4	1	A	A
62	F2-104	5	1	H	A
63	F2-106	5	1	A	A
64	F2-107	5	1	H	A
65	F2-108	5	1	A	A
66	F2-110	8	1	T	H
67	F2-111	8	1	H	H
68	F2-113	8	1	H	H
69	F2-114	5	1	A	A
70	F2-115	5	1	A	A
71	F2-116	2	1	H	H
72	F2-118	7	0	H	T
73	F2-120	2	1	H	H
74	F2-121	6	1	H	H
75	F2-122	3	0	T	T
76	F2-123	1	1	A	A
77	F2-125	7	0	T	T
78	F2-126	8	1	T	H
79	F2-128	8	1	A	H
80	F2-129	5	1	A	A
81	F2-130	6	1	H	H
82	F2-131	2	1	A	H
83	F2-132	6	1	T	H
84	F2-133	6	1	T	H
85	F2-135	9	1	T	H
86	F2-137	8	1	H	H
87	F2-139	7	0	T	T
88	F2-142	5	1	H	H
89	F2-143	8	1	H	H
90	F2-144	6	1	H	H
91	F2-145	5	1	H	A
92	F2-146	8	1	A	H
93	F2-147	8	0	A	H
94	F2-148	6	1	H	H
95	F2-149	3	0	T	T
96	F2-150	3	0	T	T

Table 3. Continued.

EN	Line	Phenotype class[z]	Marker genotype[y]		
			AT14-900	wsbin6-10	wsbin6-11
97	F2-151	2	0	H	H
98	F2-152	6	0	T	H
99	F2-153	3	0	T	T
100	F2-154	7	0	T	T
101	F2-155	6	1	A	H
102	F2-156	7	0	T	T
103	F2-158	3	0	T	T
104	F2-159	7	0	T	T
105	F2-160	7	0	H	T
106	F2-161	2	1	H	H
107	F2-163	6	1	T	H
108	F2-164	2	1	T	H
109	F2-165	2	1	H	H
110	F2-169	8	1	H	H
111	F2-171	2	1	H	H
112	F2-172	8	1	A	H
113	F2-173	2	1	H	H
114	F2-174	1	1	H	H
115	F2-175	2	1	H	H
116	F2-176	4	1	A	A
117	F2-177	6	1	H	H
118	F2-178	2	1	A	H
119	F2-179	4	1	A	A
120	F2-181	6	1	H	H
121	F2-183	8	1	H	H
122	F2-184	8	1	H	H
123	F2-185	2	1	T	H
124	F2-186	1	1	A	H
125	F2-187	8	1	H	H
126	F2-188	5	1	A	A
127	F2-189	5	1	H	H
128	F2-190	3	0	T	T
129	F2-191	8	1	H	H
130	F2-192	8	1	H	H
131	F2-195	3	0	H	T
132	F2-197	5	1	H	A
133	F2-199	3	0	T	T

[z]Stripe pattern was classified into nine classes: 1, stripe pattern of 'AM' (Crimson-type, light green stripe with broad width and diffused margin); 2, stripe pattern of F_1, (coexpression of the stripe pattern for 'AM' and 'TS'; 3, stripe pattern of 'TS' (Jubilee-type: dark green with medium width and sharp margin); and 4 to 9, various stripe patterns belonging to neither 'AM', 'TS' or F_1.
[y]1 or 0, presence or absence of AT14-900 band, respectively; A, homozygous for 'AM'; T. homozygous for 'TS'; and H, heterozygous.

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