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RESEARCH ARTICLE

Analysis of genetic diversity and relationships among waxy maize inbred lines in Korea using SSR markers

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Abstract
Information regarding the genetic diversity and genetic relationships among elite inbred lines is necessary to improve new cultivars in maize breeding programs. In this study, genetic diversity and genetic relationships were investigated among 84 waxy maize inbred lines using 50 SSR markers. A total of 269 alleles were identified at all the loci with an average of 5.38 and a range between 2 and 13 alleles per locus. The gene diversity values varied from 0.383 to 0.923 with an average of 0.641. The cluster tree generated using the described SSR markers recognized two major groups at 32% genetic similarity. Group I included 33 inbred lines while group II included 51 inbred lines. The clustering patterns of most of the waxy maize inbred lines did not clearly agree with their source, pedigree or geographic location. The average GS among all inbred lines was 35.7 ± 10.8. Analysis of waxy maize inbred lines collected from Korea and China at 50 SSR loci revealed higher values of average number of alleles (4.9) and gene diversity (0.638) in Korean inbred lines as compared to Chinese inbred lines (3.5 and 0.563, respectively). The information obtained from the present studies would be very useful for maize breeding programs in Korea.

Keywords Waxy maize; SSR marker; Genetic diversity; Genetic relationship; Inbred lines; Maize breeding program

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Introduction

Information regarding the genetic diversity and the relationship among maize inbred lines has had a significant impact on improvement of new cultivars because it is useful for planning crosses for hybrid and inbred line development, assigning lines to heterotic groups, and protecting the plant variety (Hallauer et al., 1988; Pejic et al., 1998). In conventional breeding, genetic diversity and genetic relationships among maize inbred lines are usually assessed based on the morphological data, the pedigree record of inbred lines and the amount of heterosis expressed by the hybrid. However, these descriptors present several limitations. For example, the morphological characteristics often do not reliably portray the genetic relationships due to environment interactions. Additionally, the pedigree record of inbred lines requires accurate records and testcross designs requiring several testers are extremely expensive and time-consuming. Therefore, to maximize the efficiency of hybrid combinations, the development of new inbred lines and the assignment of inbred lines to heterotic groups, clear assessment of genetic diversity and genetic relationships among breeding materials from different origins is required for maize breeding programs.

Genotyping techniques such as RFLPs, RAPDs, AFLPs and SSRs have allowed genetic discrimination of very closely related inbred lines for purposes of plant variety protection and pedigree validation (Melchinger et al., 1992; Senior et al., 1998; Moeller and Schaal, 1999; Lu and Bernado, 2001; Enoki et al., 2002; Xia et al., 2005; Park et al., 2008; Zhao et al. 2009). Moreover, molecular assessment of the genetic diversity among maize inbred lines has been studied extensively to predict single-cross hybrid performance (Taramino and Tingey, 1996; Smith et al., 1997; Ajmone-Marsan et al., 1998; Pejic et al., 1998; Senior et al., 1998; Reif et al., 2006). Thus, attempts have been made to use molecular markers that directly evaluate genetic differences between maize inbred lines to as-

sess the genetic diversity and genetic relationships among maize inbred lines. Among the various types of markers, microsatellites or SSRs (simple sequence repeats), which are short sequences containing tandemly repeated copies of 1-6 nucleotide fragments (Rafalski et al., 1996), are considered to be one of the most-suitable markers for assessing genetic diversity and genetic relationships among maize inbred lines due to their high level of allelic variation and their potential advantages with respect to reliability, reproducibility and discrimination (Akagi et al., 1997; Smith et al., 1997; Enoki et al., 2002).

Maize is one of the most important crops in the world. In general, maize is divided into two types based on the starch composition of the endosperm in the seed, normal maize (or non-waxy maize) and waxy maize. It is generally considered that the difference between non-waxy maize and waxy maize is the texture or starch content of the grain. For example, the starch produced by non-waxy maize is composed of about 25% amylose and 75% amylopectin, whereas the starch content of waxy maize consists exclusively of amylopectin (Sprague et al., 1943; Nelson and Rines, 1962). In addition, the glutinous phenotype of waxy maize has been shown to occur in response to a reduction in the synthesis of amylose because of mutations or insertions in the Waxy (*Wx*) gene, which encodes a granule-bound starch synthase in maize (Fedoroff et al., 1983; Klösgen et al., 1986). Although normal maize is widely cultivated and used in food and feed worldwide, waxy maize is a special type of cultivated maize that is used in food production in China as well as in Korea. Today, waxy maize is very popular in Korea as shown by the increase in its consumption that is occurring as the population transitions from a traditional diet based on rice to a Western diet based on meat. However, very few systematic studies of the genetic diversity and genetic relationships among Korean waxy maize inbred lines have been conducted to date. In Korea, the Maize Experiment Station operated by the Kangwon Agricultural Research and Extension Services has maintained many waxy maize accessions from different origins and developed a great deal of inbred lines. These waxy maize accessions or inbred lines were collected either from local farmers in various places (including other countries) or were obtained from the Genebank at the RDA (Rural Development Administration) in Korea. Since these accessions and inbred lines have not been or are rarely utilized in breeding programs, genetic characterization is needed to ensure the long-term success of maize breeding programs in Korea.

Therefore, this study was conducted to investigate the genetic diversity and genetic relationships among 84 waxy maize inbred lines (obtained from various parts of Korea and China) with SSR markers, as well as to predict appropriate crossing combinations for the development of new hybrids in Korean maize breeding programs.

Materials and Methods

Plant materials and DNA extraction

Table 1 shows the 84 waxy maize inbred lines and their developed derivations evaluated in this study. With the goal of developing a new waxy maize cultivar and new inbred lines, the following lines were developed: 54 inbred lines developed directly from landraces collected from various areas in Korea and China; 30 inbred lines developed from a cross between either two inbred lines or between a cultivar and an inbred line. These breeding materials were collected from Korea and other countries. To develop an elite inbred line, the inbred lines evaluated here have been cultivated for 6 years (e.g. 97**S6**040), 7 years (e.g. 96**S7**003), 8 years (e.g. 98**S8**004) or 9 years (e.g. 05Y**S9**011) at the Maize Experiment Station, Gangwon Agricultural Research and Extension Service, Hongcheon. The maize genomic DNA was extracted from young leaves with the protocol of Dellaporta et al. (1983), with minor modifications.

SSR analyses

SSR amplifications were performed through PCR in a total reaction volume of 30 μL, which consisted of 20 ng of genomic DNA, 1x PCR buffer, 0.3μM of forward and reverse primers, 0.2 mM dNTPs, and 1 unit of Taq Polymerase (Biotools). The PCR profile consisted of 5-minute initial denaturation period at 94℃ followed by two cycles each consisted of 1-minute denaturation at 94℃, 1-minute annealing at 65℃, and 2-minute extension at 72℃. After the second cycle, the annealing temperature was gradually decreased by 1℃ following every second cycle until a final temperature of 55℃ was reached. The last cycle was then repeated 20 times. Upon completion of the cycles, the extension cycle was extended for 10 minutes at 72℃.

Electrophoresis and fragment detection

Five μl of the final reaction product was mixed with 10 μl of electrophoresis loading buffer (98% formamide, 0.02% BPH, 0.02% Xylene C, and 5 mM of NaOH). After denaturation and immediate cooling, 2 μl of the sample was loaded onto a 6% denaturing (7.5M urea) acrylamide-bisacrylamide gel (19:1) in 1× TBE buffer, and electrophoresed at 1800 volts and 60 watts for 120 min. The separated fragments were then visualized using a silver-staining kit (Promega, USA).

Table 1. Derivation of 84 maize inbred lines used in SSR analysis.

Code No.	Inbred line	Derivation (Pedigree/source)	Country	Code No.	Inbred line	Derivation (Pedigree/source)	Country
1	96S7003	Landrace, Pyeongchang-gun, Gangwon-do	Korea	43	KW7	Landrace, Pyeongchang-gun, Gangwon-do	Korea
2	96S7007	Landrace, Chuncheon-si, Gangwon-do	Korea	44	96S6037	Oh43wx/W9043	America /Korea
3	96S7010	Landrace, Hongcheon-gun, Gangwon-do	Korea	45	98S7007	Chalok 1/W7031	Korea
4	97S6040	Landrace, Pyeongchang-gun, Gangwon-do	Korea	46	98S7017	Chalok 1/KW3	Korea
5	98S7037	Landrace, Gangneung-si, Gangwon-do	Korea	47	98S8006	W8338/A632wx	Korea
6	98S8004	Landrace, Unknown	Korea	48	98S8026	84-8027/A632wx	Korea
7	98S8034	Landrace, Ulleung-gun, Gyongsangbuk-do	Korea	49	98S8044	W84-9067/A632wx	Korea
8	98S8064	Cultivar, Chalok 1	Korea	50	98S8059	Jewon landrace/Boseong landrace	Korea
9	99S8015	Cultivar, Kaset Khao	Thailand	51	98S8007	W9060/A632wx	Korea
10	00S8001	Landrace, Hapcheon-gun, Gyongsangnam-do	Korea	52	99S8003	Chalok 1/W7094	Korea
11	00S8007	IT90185, RDA genebank	Korea	53	01S8025	Mo401wx/KW1	America /Korea
12	01BS8001	Landrace, Unknown	Korea	54	01S8069	Daehakchal/KW14	Korea
13	02S8005	Cultivar, "Daehakchal"	Korea	55	02S8056	Chalok 1/KW7	Korea
14	02S8008	Landrace, Yeongju-si, Gyongsangbuk-do	Korea	56	02S8074	KW2/KW7	Korea
15	02S8014	Landrace, Boeun-gun, Chungcheongbuk-do	Korea	57	03S8001	KW7/KW8	Korea
16	02S8050	Landrace, Unknown	Korea	58	03S8013	KW2/KW7	Korea
17	02S8103	Landrace, Unknown	Korea	59	03S8053	Daehakchal/KW13	Korea
18	02BS8001	Landrace, Muju-gun, Jeollabuk-do	Korea	60	03S8060	KW7/Hoengseong landrace	Korea
19	02BS8005	Landrace, Namwon-si, Jeollabuk-do	Korea	61	03S8064	KW7/Inje landrace	Korea
20	03S8070	Cultivar, "Daehakchal"	Korea	62	03S8102	Daehakchal/KW7	Korea
21	03BS8006	Landrace, Eumseong-gun, Chungcheongbuk-do	Korea	63	03BS8016	Unknown/Yungil landrace	Korea
22	04S8050	Landrace, Samcheok-si, Gangwon-do	Korea	64	04S8008	KW7/Hongcheon landrace	Korea
23	04S8078	Cultivar, "Daehakchal"	Korea	65	05S8004	Daehakchal/Chalok 2	Korea
24	04BS8009	Landrace, Inje-gun, Gangwon-do	Korea	66	05S8036	KW7/98A098	Korea
25	04BS8015	Landrace, Muju-gun, Jeollabuk-do	Korea	67	05BS8005	96A099/96A059	Korea
26	04BS8020	Landrace, Yangyang-gun, Gangwon-do	Korea	68	05BS8010	KW7/98A098	Korea
27	05S8011	Landrace, Danyang-gun, Chungcheongbuk-do	Korea	69	02pum03	Jeongseon landrace/Goseong landrace	Korea
28	02pum08	Landrace, Unknown, Jeollanam-do	Korea	70	02pum04	KW7/Goseong landrace	Korea
29	02pum09	Landrace, Unknown	Korea	71	02pum15	KW7/KW8	Korea
30	02pum47	Landrace, Hoengseong-gun, Gangwon-do	Korea	72	02pum16	Daehakchal/KW7	Korea
31	02pum66	Landrace, Goesan-gun, Chungcheongbuk-do	Korea	73	02pum48	Daehakchal/KW14	Korea
32	02pum95	Landrace, Tongyeong-si, Gyongsangnam-do	Korea	74	04S8038	Landrace, Unknown	China
33	HW1	Landrace, Wonju-si, Gangwon-do	Korea	75	05S8019	Landrace, Unknown	China
34	KL103	Landrace, Gochang-gun, Jeollabuk-do	Korea	76	05YS9011	Landrace, Unknown	China
35	HW3	Landrace, Unknown	Korea	77	05YS9012	Landrace, Unknown	China
36	HW4	Landrace, Anseong-si, Kyunggi-do	Korea	78	05YS9014	Landrace, Changchun	China
37	HW5	Landrace, Unknown	Korea	79	05YS9079	Landrace, Changchun	China
38	HW6	Landrace, Unknown	Korea	80	05YS9098	Landrace, Changbai	China
39	HW7	Landrace, Yanggu-gun, Gangwon-do	Korea	81	05YS9115	Landrace, Yanbian	China
40	HW8	Landrace, Hwacheon-gun, Gangwon-do	Korea	82	05YS9119	Landrace, Longjing	China
41	KW1	Landrace, Gosung-gun, Gangwon-do	Korea	83	05YS9126	Landrace, Longjing	China
42	KW2	Landrace, Hongcheon-gun, Gangwon-do	Korea	84	05YS9129	Landrace, Longjing	China

Data analyses

Fragments amplified using the SSR primers were scored as presence (1) or absence (0) and the gene diversity was calculated according to the following formula described by Nei (1973): gene diversity = $1-\sum P_i^2$, where P_i is the frequency of i^{th} SSR allele present in the examined accessions. Anderson et al. (1993) referred to gene diversity as the polymorphic information content (PIC). The genetic similarities (GS) were calculated for each pair of lines using the Dice similarity index (Dice, 1945). The similarity matrix was then used to construct an UPGMA (un-weighted pair group methods using arithmetic averages algorithm) dendrogram with the help of SAHN-Clustering from NTSYS-pc.V.2.1 (Rohlf, 2000).

Results

Genetic variation and diversity among waxy maize inbred lines

The 50 SSR loci selected in the present study were distributed among all the 10 chromosomes of maize (5 SSRs from each chromosome) and thus ensuring coverage of the entire genome. These loci were used to evaluate the genetic diversity and genetic relationships among 84 waxy maize inbred lines (Table 2). The 50 SSR primer sets detected a total of 269 alleles ranging in size from 75 bp to 265 bp according to their SSR loci in the 84 waxy maize inbred lines. The number of alleles per locus ranged from 2 for umc2056, umc2173 and umc1279 to 13 for umc1012, with an average number of 5.38 alleles per locus (Table 2). The gene diversity values varied from 0.383 for umc1972 to 0.923 for bnlg2228 with an average of 0.641 (Table 2). The loci umc2246, umc1845, umc1012, umc1717, umc2188, umc1680, umc2165, umc1005 and umc1053 showed a comparatively high number of alleles and high values of gene diversity. Conversely, umc1972, umc1454, umc1466, umc2056, umc2173 and umc1279 showed a comparatively low number of alleles and low gene diversity. Overall, a total of 38 SSR loci had more than four allele bands and gene diversity values greater than 0.5. While the remainder of the SSR loci produced only two or three allele bands and had comparatively low gene diversity values.

To clearly understand the genetic variation in waxy maize inbred lines collected from Korea and China, we also analyzed the genetic diversity and allelic numbers in samples collected from group A (43 inbred lines derived from Korean landraces or cultivars) and group B (11 inbred lines derived from Chinese landraces or cultivars) (Table 3). Table 3 shows the gene diversity and allelic numbers at 50 SSR loci of the two groups. The average number of alleles was 4.9 and 3.5 for group A and group B, respectively. Additionally, the average gene diversity values were 0.638 and 0.563 for group A and group B, respectively (Table 3). Of the 50 SSR loci, umc2246, umc1845, umc1027, umc1012, umc1717, umc1550, umc2188, umc1153, umc1006, umc2165, umc1005, umc2366, umc1053 and umc1506 had a higher number of alleles among the two groups, as well as higher gene diversity. Therefore, these SSR loci were highly informative for characterizing and evaluating the genetic diversity of the waxy maize landraces collected from Korea and China.

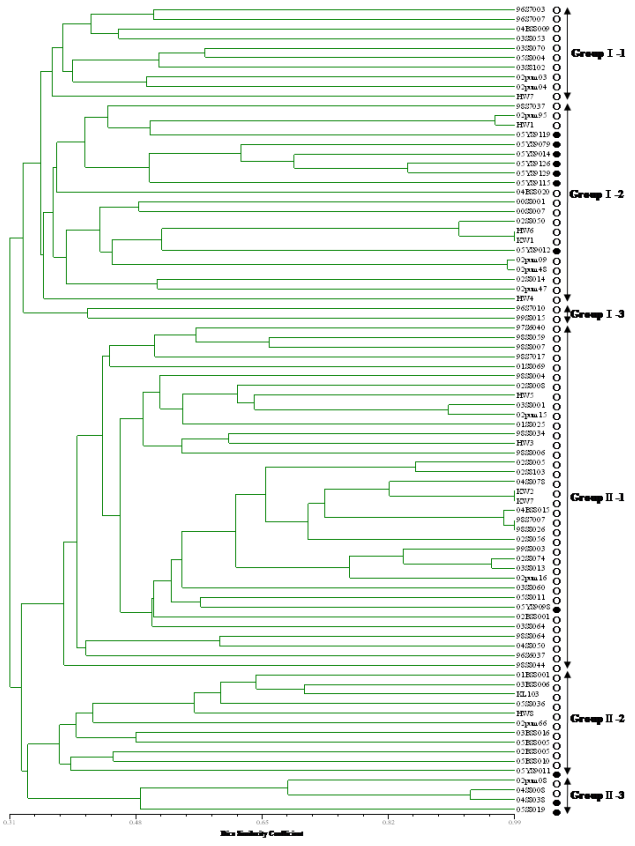

Figure 1. UPGMA dendrogram based on the SSR markers. The waxy maize inbred lines are shown in Table 1. ○: the inbred line was developed directly from Korean landraces, ●: the inbred line was developed from Chinese landraces.

Cluster analysis and genetic similarity (GS) among waxy maize inbred lines

The dendrogram of the 84 waxy maize inbred lines developed by UPGMA analysis is presented in Fig. 1. Cluster analysis resulted in classification of the 84 waxy maize inbred lines into two major groups with a genetic similarity of 0.32. Group I includes 33 inbred lines and group II includes 51 inbred lines. Group I was also clearly subdivided into three sub-

Table 2. Characteristics of the 50 SSR loci including allele size range, allele number and gene diversity in 84 waxy maize inbred lines.

SSR loci	Primer sequence	No. of Chromosome	Allele size range (bp)	No. of alleles	Gene diversity
umc2232	F-CATTCATCCACCATAAATATCCTGC R-CTAGATTGCCTCGGACCTGTAAGA	1	235-260	5	0.679
umc1972	F-ATAGCTCGAGTATTGCGTTGCTCT R-AGTTGTTGGTGATGGTGAAGGTG	1	245-260	3	0.383
umc1701	F-GCGGCAGTACAACACGTAACAATA R-CGCCAATAAACTGGAGGATAAGAA	1	110-120	4	0.666
umc2227	F-ACCTTGAGCGTGGAGTCGGT R-AGCTGAGCCTTCTCTTCTTGGCT	1	270-295	6	0.655
bnlg2228	F-GCAGCAATCGACACGAGATA R-CTTGGATCGCACTCCGTC	1	180-230	5	0.923
umc1024	F-CCTTTTTCGCCTCGCTTTTTAT R-TCGTCGTCTCCAATCATACGTG	2	160-195	6	0.739
umc2246	F-AGGCTCCAGCTCTAGGGGAGT R-GTGAACTGTGTAGCGTGGAGTTGT	2	115-150	9	0.802
umc1756	F-ATCTCAGGTACTCTGCCTACGGG R-AACAGAGGGTAGCTTGTGGCCT	2	155-170	4	0.688
umc1845	F-TGGTTGAACTGTTAAATCTGTCCTGA R-TGGTAACCAGATTCCCACAGATG	2	130-190	10	0.702
umc1454	F-GAGTCTACAATTACCTGGCCGAGA R-ATGTACCCCGCATTTGTGTACCT	2	105-115	3	0.463
umc1600	F-CATATTGATAGGCTAGGCAAATGGC R-CAATACAAGTTTGGTCCCAAATAAGC	3	145-155	4	0.620
umc2266	F-ACGTTGGCCGTTAGTTCTTATCCT R-GGACAGCTTGGCTTCGAGTG	3	130-145	3	0.603
umc1027	F-AACTCTGTCTCCGTCACCGTGT R-GACCTCATCTCGGTGGAAATTG	3	90-105	7	0.607
umc1012	F-TTCTTGCGGACCTCAAACTTGT R-CTCCATCACCACTCAGAATGTCA	3	100-140	13	0.804
umc1717	F-ACGACGAATTCACTAACACAACGA R-TTATCAGAGGAAGGGTTACGTTGG	3	95-120	8	0.749
umc1466	F-CGAATAGTGGTCTCGCGTCTATCT R-GATCCACTAGGGTTTCGGGGT	4	100-110	3	0.490
umc2280	F-TTTTCGTCAACTTGATGTTTATGAGAGT R-AAAAGAAGACGCTTTGTTTGTTGC	4	105-120	3	0.602
umc1550	F-CGGGGTAATTGGGTACATAACCTC R-GTGCCTCCAACGCCTAGTTTTT	4	135-145	7	0.723
umc2188	F-CGCCAACATGATTAACTTGCTATC R-ATTTTCAGTCTGGGTACTTGAGCG	4	150-215	10	0.810
umc1652	F-GAGAGCAGTAGCACTGACCCTTTC R-CACTCGACCTCGATCGGAAC	4	130-150	4	0.494
umc1153	F-CAGCATCTATAGCTTGCTTGCATT R-TGGGTTTTGTTTGTTTGTTTGTTG	5	105-120	7	0.739
umc1355	F-CGATGCTTTTTCTCAATCCGTTAT R-GACTGCTGGGTCTCTCTCTCTTTG	5	115-130	4	0.611
umc1752	F-ATCCTCCTCCATATTCTATCGCGT R-GAAACAGAGCAGGAACCGGAG	5	250-265	4	0.530
umc1680	F-TTAATAAAGGAGAGGGTGGGAACC R-GGGGCTTATATGTCCCTTGAACTC	5	145-190	9	0.848
umc2198	F-CTCTTCACTCGCTTCTCCCAGA R-AGCCCAGAGAAGGGAAGCAG	5	135-170	5	0.596

Table 2. (Continued).

SSR loci	Primer sequence	No. of Chromosome	Allele size range (bp)	No. of alleles	Gene diversity
umc1857	F-TTCCTTGCCAACAAATACAAGGAT R-GTTCATTGCTTCATCTTGGAACCT	6	145-155	6	0.678
umc1006	F-AATCGCTTACTTGTAACCCACTTG R-AGTTTCCGAGCTGCTTTCTCT	6	80-120	7	0.646
umc2165	F-AGAACACCAAATGGTGACGTTATGT R-CTAGCTCGTCTTCCCTGTGGTCT	6	130-165	9	0.646
umc1520	F-AGCAAATATATGAGCAATTAAGAACAGG R-GTGTCGCCACCTATAATTTGATGA	6	75-95	6	0.563
umc2056	F-GCATTAGCAAACAAAGTGGGTTTC R-GGCGAAACTGTGATGAGAAAACAT	6	155-165	2	0.497
umc1904	F-CAGCCACTCGTTTATGGAGGTTTA R-TGTTACTAGTCGATCTGATGCCCA	7	130-150	5	0.562
umc1708	F-GATATGTCGAGCTTCGCTGGAG R-CGCACACTAAAGCATCCTTAACCT	7	75-90	4	0.666
umc1241	F-TGAAGCAAGTCACTGGTAAGAGCA R-TGACACACCCATACTTCCAACAAG	7	140-165	4	0.596
umc1005	F-TTTGATCACAGACTTATCCCTGTT R-CTAATGACGAACCCCTAAAAGGT	7	135-175	8	0.670
umc1159	F-TTCCCATGTTCATTTCAGGTTCTT R-TCATGGGTTTTGAGGCTGTATTTT	7	135-160	5	0.779
umc1457	F-CCCTAGGACACTGGAGGTTACAGA R-GGCTAAGCGTTTTTACAAGTCCAA	8	90-100	3	0.521
umc2366	F-ACATCGATCCAACCGTCATAAATC R-CCTTCTTCCCGTCATTCTTCTTCT	8	200-230	6	0.793
umc2146	F-GTCTCCGTCCACCTCCTGTG R-GTCATGGGAATGTGCTGGATG	8	175-190	5	0.704
umc2173	F-TTCAAAGATCCAGTCAGCAATAAGG R-GAACGTGACGCTCTACATGCTG	8	180-190	2	0.444
umc1268	F-ACGAACAACCTAGCACAGTCCTAAA R-CAAGGCGGTTACCAAGTTTACATC	8	95-100	3	0.530
umc1867	F-TGGTCTTCTTCGCCGCATTAT R-ATAAGCTCGTTGATCTCCTCCTCC	9	105-125	6	0.365
umc1357	F-TAGACATGTTGAAACCAGGACCG R-ACGACGTCAACAACAGCATGA	9	160-180	4	0.564
umc1279	F-GATGAGCTTGACGACGCCTG R-CAATCCAATCCGTTGCAGGTC	9	90-100	2	0.499
umc1271	F-CTCTCCTCGTCCGGTAATTAAGC R-GCTTCTTCTTCTTGCGCTTCTCT	9	150-170	4	0.602
umc1571	F-GCACTTCATAACCTCTCTGCAGGT R-CACCGAGGAGCACGACAGTATTAT	9	95-110	4	0.578
umc1993	F-CTTTTCTGCTACTCCTGCCTGC R-CTAGCTGATGGAGGCTGTAGCG	10	75-100	4	0.683
umc2021	F-AAACTCAAGCTCGGAATGTACTGC R-CGATACTGATCTACTTCACGCTGG	10	120-140	5	0.573
umc1556	F-CCGAACAAAACAAGTAAGCACACA R-GAAGACAGCTAGCCATCATCGG	10	145-165	5	0.720
umc1053	F-CTTGTATCATCAGCTAGGGCATGT R-TCAACTTATGTCAACTGCATGCTT	10	110-150	8	0.783
umc1506	F-AAAAGAAACATGTTCAGTCGAGCG R-ATAAAGGTTGGCAAAACGTAGCCT	10	105-135	6	0.790
Average				5.38	0.641

groups with a similarity of 0.352. The first sub-group (Group I-1) included 10 inbred lines (96S7003, 96S7007, 03S8070, 04BS8009, HW7, 03S8053, 03S8102, 05S8004, 02pum03 and 02pum04) with a similarity coefficient of between 0.366 and 0.574. The second sub-group (Group I-2) contained 21 inbred lines (98S7037, 00S8001, 00S8007, 02S8014, 02S8050, 04BS8020, 02pum09, 02pum47, 02pum95, HW1, HW4, HW6, KW1, 02pum48, 05YS9012, 05YS9014, 05YS9079, 05YS9115, 05YS9119, 05YS9126 and 05YS9129) with a similarity coefficient of between 0.352 and 0.99. The third sub-group (Group I-3) contained only 2 inbred lines (96S7010, 00S8001) with a similarity coefficient of 0.416 (see Fig. 1). Group II was also subdivided into three sub-groups with a genetic similarity of 0.378. The first sub-group (Group II-1) contained 36 inbred lines (97S6040, 98S8004, 98S8034, 98S8064, 02S8005, 02S8008, 02S8103, 02BS8001, 04S8050, 04S8078, 04BS8015, 05S8011, HW3, HW5, KW2, KW7, 96S6037, 98S7007, 98S7017, 98S8006, 98S8026, 98S8044, 98S8059, 98S8007, 99S8003, 01S8025, 01S8069, 02S8056, 02S8074, 03S8001, 03S8013, 03S8060, 03S8064, 02pum15, 02pum16 and 05YS9098) with a similarity coefficient of between 0.382 and 0.99. The second sub-group (Group II-2) contained 11 inbred lines (01BS8001, 02BS8005, 03BS8006, 02pum66, KL103, HW8, 03BS8016, 05S8036, 05BS8005, 05BS8010 and 05YS9011) with a similarity coefficient of between 0.378 and 0.707. The third sub-group (Group II-3) contained 4 inbred lines (02pum08, 04S8008, 04S8038 and 05S8019) with a similarity coefficient of between 0.486 and 0.931 (see Fig. 1).

In this study, we also compared the genetic similarity of the inbred lines of the six groups identified by UPGMA analysis to enable a clear understanding of the genetic diversity within and among the 84 waxy maize inbred lines (Table 4). Table 4 shows the genetic similarities (GS) of a combination of all inbred lines as well as within individual groups. The average GS among all inbred lines was 35.7 ± 10.8. The average GS values for the six groups were 40.1 ± 6.1 for group I-1, 41.1 ± 11.4 for group I-2, 41.6 ± 0.0 for group I-3, 48.2 ± 12.1 for group II-1, 43.1 ± 9.8 for group II-2 and 62.7 ± 16.4 for group II-3, respectively. Therefore, although the average GS values did not differ greatly among the six groups, the inbred lines of group II-3 showed the highest average GS values when the inbred lines of group I-1 showed the lowest average GS values.

Dicussion

Recently, waxy maize has become one of the most important and popular crops in Korea. Therefore, the development of

Table 3. Allele number and gene diversity of 50 SSR loci between two groups of Korean and Chinese waxy maize inbred lines.

Primer	Number of alleles		Gene diversity	
Loci	Korea (n=43)	China (n=11)	Korea (n=43)	China (n=11)
umc2232	5	4	0.662	0.678
umc1972	2	2	0.392	0.124
umc1701	4	4	0.656	0.554
umc2227	6	3	0.655	0.628
bnlg2228	5	3	0.948	0.884
umc1024	6	3	0.735	0.488
umc2246	8	5	0.812	0.620
umc1756	4	3	0.698	0.562
umc1845	8	6	0.748	0.661
umc1454	3	3	0.473	0.430
umc1600	4	3	0.634	0.421
umc2266	3	3	0.586	0.628
umc1027	7	5	0.741	0.760
umc1012	10	7	0.846	0.826
umc1717	8	6	0.783	0.810
umc1466	3	2	0.545	0.298
umc2280	3	3	0.578	0.628
umc1550	7	5	0.741	0.760
umc2188	8	7	0.816	0.785
umc1652	4	2	0.544	0.165
umc1153	6	6	0.723	0.727
umc1355	4	3	0.674	0.628
umc1752	3	3	0.516	0.595
umc1680	9	3	0.840	0.777
umc2198	3	3	0.557	0.562
umc1857	5	2	0.675	0.463
umc1006	9	5	0.637	0.769
umc2165	7	4	0.634	0.446
umc1520	6	2	0.632	0.388
umc2056	2	2	0.482	0.298
umc1904	4	3	0.594	0.405
umc1708	3	4	0.657	0.719
umc1241	4	2	0.526	0.397
umc1005	6	5	0.599	0.818
umc1159	5	3	0.759	0.512
umc1457	3	3	0.452	0.603
umc2366	6	4	0.791	0.702
umc2146	5	2	0.731	0.570
umc2173	2	2	0.394	0.339
umc1268	3	2	0.516	0.463
umc1867	5	4	0.382	0.545
umc1357	4	3	0.562	0.512
umc1279	2	2	0.490	0.496
umc1271	4	3	0.533	0.314
umc1571	4	3	0.573	0.430
umc1993	4	4	0.667	0.694
umc2021	5	4	0.531	0.636
umc1556	4	3	0.670	0.455
umc1053	6	4	0.780	0.719
umc1506	6	4	0.795	0.521
Avg.	4.86	3.5	0.638	0.563

a new waxy maize cultivar is needed to increase the consumption of this crop in Korea. Even though waxy maize inbred lines are not currently used in modern breeding programs in Korea, they are considered to be valuable genetic resources for the development of a new waxy maize cultivar.

SSR markers have many advantages over the other marker systems. Specifically, SSR markers play an important role in establishment of the line identity, protection of the plant variety and the patent protection of genes because they are highly reproducible, polymorphic, generally codominant and abundant in plant genomes (Powell et al., 1996). The results of our study indicated that all SSR loci in our study used were polymorphic and produced a total of 269 alleles, with an average of 5.4 alleles per locus. The majority of the SSR loci differed greatly in the numbers of alleles, ranging from 2 to 13. In this study, the mean number of alleles and the gene diversity per SSR locus (5.4 and 0.641, respectively) detected on the 84 waxy maize inbred lines were similar to those values determined by Senior et al. (1998), Lu and Bernardo (2001), Enoki et al. (2002), Le Clerc et al. (2005) and Xie et al. (2008). A significant correlation between number of alleles and gene diversity was observed at certain loci such as umc2246, umc1845, umc1012, umc1717, umc2188, umc1680 and umc1053 where higher number of alleles were associated with high genetic diversity or at umc2056, umc2173 and umc1279 loci where low number of alleles were associated with low gene diversity. These observations are in agreements with a previous study conducted by Huang et al. (2002). On the other hand, no significant correlation between gene diversity and the number of alleles was observed at some SSR loci such as umc2165 and umc1867, which produced nine and six alleles, respectively but showed low gene diversity (0.646 and 0.365, respectively). Conversely, bnlg2228 showed only five alleles, but had a high gene diversity of 0.923 (Table 2). Similar observations were also reported by Prasad et al. (2000). In our study, we observed an excess of heterozygotes at many SSR loci in maize inbred lines. This result indicated that the flow of genes within and between maize accessions may occur through natural outcrosses, since maize is cross-fertilized species.

Analysis of the waxy maize inbred lines belonging to the two groups collected from Korea and China revealed that higher values of average number of alleles (4.9) and gene diversity (0.638) in Korean inbred lines as compared to Chinese inbred lines (3.5 and 0.563, respectively) (Table 3). Although the number of samples of waxy maize inbred line from China was insufficient for a conclusive analysis, the obtained results implied that the Korean inbred lines produce a greater number of allele bands and gene diversity than Chinese inbred lines. Thus, the results presented here will help expand our understanding of genetic diversity in Korean waxy maize inbred lines. In addition, the SSR loci, which showed high numbers of allelic bands and gene diversity in waxy maize inbred lines from Korea and China may be a useful molecular markers in future genetic studies as well as in the collection and conservation of waxy maize germplasms in maize breeding programs.

Table 4. Genetic similarity [mean, minimum (Min), maximum (Max) and standard deviation (SD) x 100] calculated from SSR data for all lines and for within each group.

Groups	(n)	SSR markers GS x 100			
		Mean	Min	Max	SD
Among All	3486	35.7	11.7	99.0	10.8
Group I-1	45	40.1	29.1	57.4	6.1
Group I-2	210	41.1	26.4	99.0	11.4
Group I-3	1	41.6	41.6	41.6	0
Group II-1	630	48.2	24.0	99.0	12.1
Group II-2	55	43.1	28.8	70.7	9.8
Group II-3	6	62.7	46.0	93.0	16.4

As shown in Fig. 1, the 84 waxy maize inbred lines evaluated in our study were clearly divided into two major groups using the SSR markers. However, the clustering patterns of majority of the waxy maize inbred lines did not clearly agree with their derivations, such as source or pedigree and the geographic locations from which the samples were collected (See Fig. 1 and Table 1). For example, in the case of inbred lines collected from Korea the overall pattern of waxy maize inbred lines did not agree clearly with their collection areas, although a few inbred lines agreed with their collection areas. Specifically, in cluster group I-1, several inbred lines collected from Gangwon-do were found to be associated with their collection area. Furthermore, the waxy maize inbred lines collected from China were mixed with Korean waxy maize inbred lines. These results indicate that the diffusion of waxy maize from China to Korea and within Korea may occur through multiple routes.

Similarly, several inbred lines did not cluster together according to their pedigree data. For example, the inbred lines 98S7007, 98S7017, 98S8064, 99S8003 and 02S8056 were either developed directly from the waxy maize cultivar Chalok 1 or developed from a cross between Chalok 1 and another inbred line during different years. However, these inbred lines were not grouped within the same cluster. Furthermore, some inbred lines such as 01S8069, 02S8005, 03S8053, 03S8070, 03S8102, 04S8078, 05S8004, 02pum16 and 02pum48 were either developed directly from the waxy maize cultivar, Daehakchal, or from a cross between Daehakchal and another inbred line during different years were also not grouped within the same cluster. Similar inconsistencies have also been ob-

served for RFLP, AFLP and SSR markers (Smith et al., 1997; Ajmone-Marsan et al., 1998; Gethi et al., 2002; Xia et al., 2005). In a study conducted to evaluate the SSR variation among maize inbred lines from the US, Gethi et al. (2002) demonstrated that the same inbred lines collected from different institutes showed a certain level of genetic variation among them and suggested that this variation might be generated due to selective pressure occurred during their maintenance for many generation in a particular environment. In addition, for any molecular markers, scoring errors (Messmer et al., 1993), the number and genomic distribution of the loci assayed and the amount of linkage disequilibrium may affect the relationship estimates (Powell et al., 1996).

Clear characterization of the genetic diversity and its relationships among maize inbred lines from different origins will maximize the efficiency of hybrid combinations. Therefore, we also analyzed the genetic diversity among waxy maize inbred lines of the six groups identified during UPGMA analysis to develop a clear understanding of the genetic diversity and its relationships within the 84 waxy maize inbred lines evaluated here (Table 4). Although the average GS values did not differ greatly among the six groups, the inbred lines of group I-1 showed higher genetic variations than the other groups. In addition, the 84 waxy maize inbred lines evaluated in this study may be considered to be useful genetic materials because they showed high genetic variations in a combination of inbred lines as well as within individual groups. Specifically, the inbred lines belonging to groups I and II may be useful in cross combinations with each other and for the planning of crosses because these inbred lines were clearly divided upon cluster analysis and showed higher genetic variation when compared to the other SSR markers evaluated here. In maize breeding programs, this information will be particularly useful for planning crosses for hybrid combinations. In outbreeding crops such as maize, the prediction of hybrid performances and assignment of the inbred lines developed from hybrids to heterotic groups by molecular markers has been extensively studied (Melchinger et al., 1990; Lanza et al., 1997; Ajmone-Marsan et al., 1998; Enoki et al., 2002; Warburton et al., 2002; Xia et al., 2005). In the present study, we used 84 maize inbred lines which have been cultivated for 6 years to 9 years to develop an elite inbred line (See MATERIALS AND METHODS). The results of the present study revealed that the waxy maize inbred lines evaluated here were clearly distinguished into two major groups. Thus, this information will be helpful to maize breeding programs in Korea, particularly in predicting the performance of hybrids and for developing or selecting hybrids from various waxy maize inbred lines.

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References

Akagi H, Yokozaki Y, Inagaki A and Fujimura T (1997) Highly polymorphic microsatellites of rice consist of AT repeats, and a classification of closely related cultivars with these microsatellite loci. Theor. Appl. Genet. 94: 61-67.

Ajmone-Marsan, Castiglioni P, Fusari F, Kuiper M and Motto M (1998) Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor. Appl. Genet. 96: 219-227.

Anderson JA, Churchill GA, Autrique JE, Tanksley SD and Sorrells ME (1993) Optimizing parental selection for genetic linkage maps. Genome 36: 181-186.

Dellaporta SL, Wood J and Hicks JB (1983) A simple and rapid method for plant DNA preparation. Version II. Plant Mol. Biol. Rep. 1: 19-21.

Dice LR (1945) Measures of the amount of ecologic association between species. Ecology 26: 297-302.

Enoki H, Sato H and Koinuma K (2002) SSR analysis of genetic diversity among maize inbred lines adapted to cold regions of Japan. Theor. Appl. Genet. 104: 1270-1277.

Fedoroff N, Wessler S and Shure M (1983) Isolation of the transposable maize controlling elements Ac and Ds. Cell 35: 235-242.

Gethi JG, Labate JA, Lamkey KR, Smith ME and Kresoovich S (2002) SSR variation in important U.S. maize inbred lines. Crop Sci. 42: 951-957.

Hallauer AR, Russell WA and Lamkey KR (1988) Corn breeding. pp 463-564. In G.F. Sprague and JW Dudley (ed). Corn and Corn Improvement. 3rd ed. Agron., Monogr. 18. Madison, WI, USA.

Huang XQ, Börner A, Röder MS and Ganal MW (2002) Assessing genetic diversity of wheat (*Triticum asetivum* L.) germplasm using microsatellite markers. Theor. Appl. Genet. 105: 699-707.

Klösgen RB, Gierl A, Schwarz-Sommer Z, Saedler H (1986) Molecular analysis of the *waxy* locus of *Zea* mays. Mol. Gen. Genet. 203: 237-244.

Lanza LLB, de Souza Jr CL, Ottoboni LMM, Vieira MLC and de Souza AP (1997) Genetic distance of inbred lines and prediction of maize single-cross performance using RAPD markers. Theor. Appl. Genet. 94: 1023-1030.

Le Clerc V, Bazante F, Baril C, Guiard J and Zhang D (2005) Assessing temporal changes in genetic diversity of maize varieties using microsatellite markers. Theor. Appl. Genet. 110: 294-302.

Lu H and Bernardo R (2001) Molecular marker diversity among current and historical maize inbreds. Theor. Appl. Genet. 103: 613-617.

Melchinger AE, Lee M, Lamkey KR, Hallauer AR and Woodman WL (1990) Genetic diversity for restriction fragment length polymorphism and heterosis for two diallel sets of maize inbreds. Theor. Appl. Genet. 80: 488-496.

Melchinger AE, Boppenmmaier J, Dhillon BS, Pollmer WG and Herrmann RG (1992) Genetic diversity for RFLPs in European maize inbreds: II. Relation to performance of hybrids within versus between heterotic groups for forage traits. Theor. Appl. Genet.

84: 672-681.

Messmer MM, Melchinger AE, Herrmann RG and Boppenmaier J (1993) Relationships among early European maize inbreds. II. Comparison of pedigree and RFLP data. Crop Sci. 33: 944-950.

Moeller DA and Schaal BA (1999) Genetic relationships among Native American maize accessions of the great plains assessed by RAPDs. Theor. Appl. Genet. 99: 1061-1067.

Nei M (1973) Analysis of gene diversity in subdivided populations. Proc. Natl. Acad. Sci. USA. 70: 3321-3323.

Nelson OE and Rines HW (1962) The enzymatic deficiency in the *waxy* mutant of maize. Biochem. Biophys. Res. Commun. 9: 297-300.

Park JS, Park JY, Park KJ, Lee JK (2008) Genetic Diversity Among Waxy Corn Accessions in Korea Revealed by Microsatellite Markers. Korean J. Breed. Sci. 40: 250-257.

Pejic I, Ajmone-Marsan P, Morgante M, Kozumplick V, Castiglioni P, Taramino G, Motto M (1998) Comparative analysis of genetic similarity among maize inbred lines detected by RFLPs, RAPDs, SSR, and AFLPs. Theor. Appl. Genet. 97: 1248-1255.

Powell W, Morgante M, Andre C, Hanafey M, Vogel J, Tingey S and Rafalski A (1996) The comparison of RFLP, RAPD, AFLP and SSR (microsatellite) markers for germplasm analysis. Mol. Breed. 2: 225-238.

Prasad M, Varshney RK, Roy JK, Balyan HS and Gupta PK (2000) The use of microsatellites for detecting DNA polymorphism, genotype identification and genetic diversity in wheat. Theor. Appl. Genet. 100: 584-592.

Rafalski JA, Vogel JM, Morgante M, Powell W, Andre C and Tingey SV (1996) Generating and using DNA markers in plants. In: Birren B, Lai E (eds), Non-mammalian genomic analysis. A practical guide. Academic Press, San Diego, pp 75-134.

Reif JC, Warburton ML, Xia XC, Hoisington DA, Crossa J, Taba S, Muminovic J, Bohn M, Frisch M and Melchinger AE (2006) Groping of accessions of Mexican races of maize revisited with SSR markers. Theor. Appl. Genet. 113: 177-185.

Rohlf FJ (2000) NTSYS- pc: Numerical taxonomy and multivariate analysis system. Version: 2.1. Exeter Software, New York.

Senior ML, Murphy JP, Goodman MM and Stuber C (1998) Utility of SSRs for determining genetic similarities and relationships in maize using an agarose gel system. Crop Sci. 38: 1088-1098.

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Mitchell SE, Kresovich S and Ziegler J (1997) An evaluation of the utility of SSR loci as molecular marker in maize (*Zea mays* L.): comparison with RFLPs and pedigree. Theor. Appl. Genet. 95: 163-173.

Sprague GF, Brimhall B and Hixon RM (1943) Some effects of the *waxy* gene in corn on properties of the endosperm starch. J. Am. Soc. Agron. 35: 817-822.

Taramino G and Tingey S (1996) Simple sequence repeats for germplasm analysis and mapping in maize. Genome 39: 277-287.

Warburton ML, Xianchun X, Crossa J, Franco J, Melchinger AE, Frisch M, Bohn M and Hoisington D (2002) Genetic characterization of CIMMYT inbred maize lines and open pollinated populations using large scale fingerprinting methods. Crop Sci. 42: 1832-1840.

Xia XC, Reif JC, Melchinger AE, Frisch M, Hoisington DA, Beck K, Pixley K and Warburton ML (2005) Genetic diversity among CIMMYT maize inbred lines investigated with SSR markers: II. Subtropical, tropical midaltitude, and highland maize inbred lines and their relationships with elite U.S. and European maize. Crop Sci. 45: 2573-2582.

Xie C, Warburton M, Li M, Li X, Xiao M, Hao Z and Zhang S (2008) An analysis of population structure and linkage disequilibrium using multilocus data in 187 maize inbred lines. Mol. Breeding 21: 407-418.

Zhao W, Chung JW, Ma KH, Kim TS, Kim SM, Shin DI, Kim CH, Koo HM, Park YJ (2009) Analysis of Genetic Diversity and Population Structure of Rice Cultivars from Korea, China and Japan using SSR Markers. GENES & GENOMICS 31: 283-292.