Screening of DNA markers suitable for purity test of inbred lines in *Brassica oleracea*

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Summary

Generating inbred lines is the first step in the breeding of F_1 hybrid cultivars in plants. It is generally considered that selfing for more than 5 or 6 generations is necessary for generating genetically identical inbred lines. DNA-marker based purity test of inbred lines is a reliable tool to confirm their genetic homogeneity, but few DNA markers, which are suitable for purity test of inbred lines, are reported in *Brassica oleracea*. In this study, we assessed 175 SSR primer sets to find out the DNA markers, which can detect the polymorphism between parental lines of F_1 hybrid cultivar. The F_2 plants derived from the F_1 hybrid cultivar of cabbage "YR Kinshukyoryoku 152" was used to identify polymorphisms because the F_1 hybrid contains both of the parental genomes. The segregation of the parental genomes in the F_2 population allows detecting the nucleotide sequence differences between the individuals that can be traced back to the parental inbred lines of F_1 hybrid. Twelve primer sets showed polymorphism among F_2 plants, indicating that these DNA markers could be useful for purity test.

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Key words : Purity test, inbred line, simple sequence repeats (SSR) primers, *Brassica oleracea*

The genus Brassica is composed of diploid and allopolyploid species and includes agriculturally important crops and vegetables. *Brassica rapa* (2n=20, AA genome) and *Brassica oleracea* (2n=18, CC genome) are diploid species. *B. oleracea* includes cabbage (var. *capitata*), Chinese kale (var. *alboglabra*), broccoli (var. *italica*) and cauliflower (var. *botrytis*), and *B. rapa* includes Chinese cabbage (var. *pekinensis*), turnip (var. *rapa*), and komatsuna (var. *perviridis*). *Brassica napus* (2n=38) is an allopolyploid species whose genome is composed by AA and CC genome (U 1935) and includes important crops such as oilseed. *B. oleracea* and *B. rapa* are self-incompatible, while *B. napus* is self-compatible because of loss of mutations in genes involved in self-incompatibility (Fujimoto *et al*., 2006; Fujimoto and Nishio 2007; Okamoto *et al*., 2007). Self-incompatibility is the mechanism for preventing self-fertilization and is controlled by the *S* locus (Fujimoto and Nishio 2007). On the *S* locus, there are two key genes, *SRK* (*S* receptor kinase) and *SP11/SCR* (*S*-locus protein 11/*S*-locus cysteine rich protein), which are female and male determinants of self-incompatibility, respectively. These two genes are segregated together with other closely linked genes such as *SLG* (*S*-locus glycoprotein) as a unit, thus this unit is called the *S* haplotype. Many *S* haplotypes have been identified in *B. oleracea* and *B. rapa*, and *S* haplotypes are categorized into two classes, class-I and class-II, by sequence homology (Fujimoto and Nishio 2007). The self-incompatibility is utilized for harvesting the F_1 hybrid seeds in vegetables of *B. oleracea* and *B. rapa*.

In Japan, most vegetables in *B. oleracea* and *B. rapa* are F_1 hybrid cultivars. In the process of breeding for F_1 hybrid cultivars, breeders created the inbred lines with selection for agriculturally important traits. The inbred lines were crossed in various combinations to promote improved growth and yield characteristics such as heterosis/hybrid vigour. In the F_1 hybrid seed production system, contamination of selfed seeds can lead to loss of seed purity and problems such as decrease in uniformity or yield that can devalue the seeds. For this reason, the seed companies evaluate the purity of F_1 hybrid seeds in the field without genetic based confirmation; growth of selfed seeds has lower productivity than F_1 hybrids and lacks commercially important traits. However, this strategy can fail to detect the selfed seeds because environmental factors affect their growth, and moreover, this process is time consuming and laborious. Isozyme analysis has also been used in F_1 hybrid purity test (Arús *et al*., 1985), but this method can be affected by environmental condition and tissue specificity of the isozyme expression. In comparison, DNA-marker-based purity test is advantageous because it can be performed at the seedling stages and this method is not affected by environmental condition. Though

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the genes on the *S* locus (termed *S* locus genes) can be used for purity test of F_1 hybrid seeds as DNA markers (Fujimoto *et al*., 2003; Fujimoto and Nishio 2007), there are few DNA markers suitable for purity test of F_1 hybrid seeds in *B. oleracea* and *B. rapa*.

To evaluate the homozygosity of the inbred lines, DNA marker is also useful as well as purity test of the F_1 hybrid seeds. Higher numbers of DNA markers increase the reliability of determining the homozygosity of inbred lines. There are several types of DNA markers such as restriction fragment length polymorphism (RFLP), simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs) and insertion/deletion polymorphism (InDel) markers. SNPs can easily differentiate individuals among the same species or variety because they are abundant and widely distributed throughout the genome. However, skilful techniques are needed for high-throughput analysis. SSR markers are suitable for high-throughput analysis because it is amplified by PCR from a small amount of DNA. Thus SSR markers have been widely used for detecting genetic diversity and making genetic maps (McCouch *et al*., 2002; Hasan *et al*., 2006). Many SSR markers are available in *B. oleracea* and *B. rapa* (Suwabe *et al*., 2002; Lowe *et al*., 2004; Iniguez-Luy *et al*., 2008; Li *et al*., 2011).

In this study, we screened the SSR markers for the purity test of inbred lines. We used the commercial F_1 hybrid cultivar of cabbage, "YR Kinshukyoryoku 152", and its F_2 plants. Of the 175 SSR primer sets, 12 primer sets reliably detected polymorphisms between parental lines in the F_2 population, indicating that these primer sets can be useful for the purity test of inbred lines or F_1 hybrid seeds.

MATERIALS AND METHODS

Plant materials and DNA extraction

A commercial F_1 hybrid cultivar of cabbage, "YR Kinshukyoryoku 152" (Masuda Seed Co., Ltd) and F_2 plants obtained by buds pollination of this cultivar were used as plant materials. Fresh young leaves harvested from the F_1 and F_2 plants were used for genomic DNA extraction. Total genomic DNA was isolated by the Cetyl-Trimethyl-Ammonium Bromide (CTAB) method (Murray and Thompson 1980).

PCR-RFLP analysis for the identification of *S* haplotypes

DNA fragments were amplified by PCR using class-I and class-II *SLG* specific primer pairs, PS5 (5'-ATGAAAGGGCGTAAGAAAAACCTA-3')+PS15 (5'-CCGTGTTTTATTTTAAGAGAAAGAGCT-3') and PS3 (5'-ATGAAAGGGGTACAGAACAT-3')+PS21 (5'-CTCAAGTCCCACTGCTGCGG-3'), respectively (Nishio *et al*., 1996). The PCR reaction was performed by the following condition: 1 cycle of 94℃ for 3 min, 35 cycles of 94℃ for 30s, 58℃ for 30s, and 72℃ for 1min, and final extension at 72℃ for 3min. The PCR products were electrophoresed on 1.0% agarose gel. DNA fragments digested by *Mbo* I restriction enzyme were electrophoresed on 13% polyacrylamide gel (Kikuchi *et al*., 2003). The gel was stained with a Gelstar solution (0.1 μl/10 ml; Takara Biomedical, Japan).

Detection of DNA polymorphism with SSR markers

Polymorphic detection using the "BoGMS"-SSR marker series was obtained from Li *et al*. (2011). The PCR reaction was performed by the following condition: 1 cycle of 94℃ for 3 min, 40 cycles of 94℃ for 30s, 50℃ for 45s, and 72℃ for 1min, and final extension at 72℃ for 3min. The PCR products were electrophoresed on 2.0% agarose or 10% polyacrylamide gel. The gel was stained with a Gelstar solution (0.1 μl/10 ml). Primer sequences used in this study are shown in Table 1.

RESULTS AND DISCUSSION

We used the RFLP markers of *SLG* to confirm the segregation of parental alleles in the F_2 population derived from commercial F_1 hybrid cultivar "YR Kinshukyoryoku 152". We used class-Ⅰ and class-Ⅱ *SLG* specific primer pairs, PS5+PS15 and PS3+PS21, respectively, and only class-Ⅱ *SLG* amplified in F_1 plants, indicating that F_1 hybrids are heterozygotes of class-Ⅱ *S* haplotypes. *S* haplotypes of six F_2 plants were analysed by PCR-RFLP analysis of class-Ⅱ *SLG*, and identified polymorphisms among them, confirming that *SLG* alleles, *S* haplotypes, have segregated in the F_2 population (Figure 1).

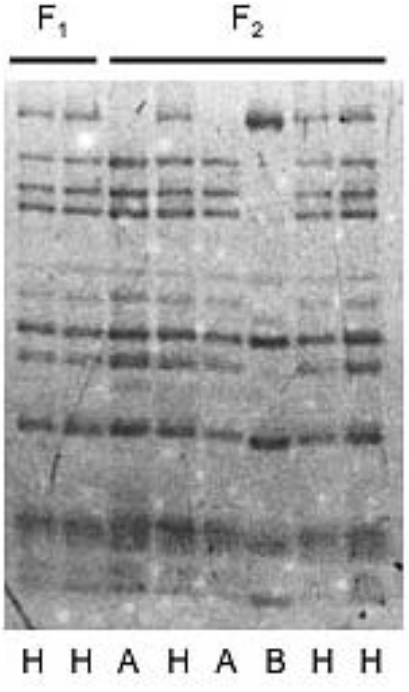

Figure 1. PCR-RFLP analysis of class-II *SLG*. A and B show the parental *S* haplotypes of heterozygous (H) F_1 hybrid.

Table 1 List of SSR markers used in this study

Marker Name	Motif	Left Primer (5' to 3')	Right Primer (5' to 3')	GenBank ID
BoGMS0009	(GA)31(AG)17	AAGACCCGAACCCTAAGTC	TGAACAAATACATAAAGGCAAA	27013782
BoGMS0030	(GA)42	GCTAATCCAGACCCAAACA	AGAAACACCATTCAACTCAAA	23461101
BoGMS0032	(AG)41	GAAGACCCGAACCCTAAGT	GGGCACATCAGAAAGTGA	17643188
BoGMS0037	(GA)40	ACTGCTCTCTCCCTTCATCT	TATCTTCCGCCTCTTCTGT	23453447
BoGMS0038	(GA)40	CCTTTGTTCAGCACCATC	CGTGGATTGAGAAGTAGCAGT	26994130
BoGMS0039	(GA)40	TGGACATTGGACATAAGGTAA	AAGAAGTGTAAATAGGCACAAGA	26745966
BoGMS0088	(AG)33	CAAGAGGGAGAGAGAGAGAGA	GTAGGGAAGTGGAGGCGATAC	23516657
BoGMS0109	(AG)31	ACTGAAGAACAGAACAAACGA	CTCACTAAGACGATACGACAA	17698870
BoGMS0112	(AG)31	ACTGGTCCGAATAGGTGAG	CGATAAATGGTTCAATGTCC	26759227
BoGMS0162	(AG)28	ACTGTTTCTAAGCCATTGTTG	TTCCCTCTCTCAAGGTGTAA	18804642
BoGMS0164	(AG)28	AGAGACACACACACACACACA	AAACATAAACAAACCGAAAGG	23559054
BoGMS0168	(TC)28	GTCTTGTATGAAGCCCAGTAG	AGAGGAAGTGTCGGGAAG	26745947
BoGMS0197	(AT)27	CCCTCTTGATTACAACGAAA	GTATGTTTACCTGGACCTGACT	18717069
BoGMS0208	(CT)24	ACGCTCATCATCCTCTCTC	GGCACCAAATCTAATCTACAA	18704996
BoGMS0254	(TC)21	CTACGGCTTCGTCTCGTC	TACTTCCCAACCATTCTGTAA	17819495
BoGMS0263	(AT)18	GGACACCCTAAACACACATT	TGGATTCTTGCTTCTATGATT	26684898
BoGMS0281	(CT)18	ATCCTTCTGCCTTCTCTG	TGACATCCATCCACACATT	23662902
BoGMS0282	(GA)19	CCCTTGTAGAGAGAGAGAGGA	AAACGAAATAAGATGACGAGA	23441680
BoGMS0299	(GA)16	GCAGGAATCAAACAGAAACT	TCTCTCTCTCTCTCTCTCTCGT	17733174
BoGMS0314	(TGA)12	AGAAAGATGGAGAAGACGAAG	CGAGAAGAAGAGAGGAAAGAA	18751450
BoGMS0327	(CT)15	CCTCTATCTGTGGCTGTGATT	CCTTTCTCTATGAAGTAGGCTG	33775795
BoGMS0342	(AG)15	ATGAATAACCAAGCGACAAG	TAAGCCAGAAGGCACTGTT	17640823
BoGMS0351	(TGAA)7	AGGTGGTGTAGTTGAGGAGAC	TGACAGATGGGATAAGACAAA	23704142
BoGMS0355	(CT)18	GTGAAAGCACGCCAAGAAC	TCTCAACCTAATCAAACACAAA	27040369
BoGMS0364	(AT)16	CCTGTCTTTGCTCATCTATTT	GCGTTAGGTTGTTGTAGGATT	23676894
BoGMS0369	(CT)15	AGGACAGCATCGGTATGA	CTGGATAGTTTCTCTTTCTTGG	33782490
BoGMS0373	(AT)15	CTCTGACGAAGATGGCTG	AAGTCTATGTTTGGATAAGGGA	23537556
BoGMS0394	(GA)67	CCCTTACTTTGTTCAGGTTTC	CATCTCTACCCACCACACA	26731554
BoGMS0405	(TA)33	CATAGATACCACTTCCCTTCA	CACACTCGTCACATAGTCCC	23530083
BoGMS0407	(AAT)21	ATGGTCGCTGCCTTACCC	GCACAATAATACAACTGAAACT	17744468
BoGMS0429	(TA)26	TCATCAAATCTTCTCCTCTTT	TTCATTCTTGTGTCTCTCTTTCT	23399120
BoGMS0456	(AT)25	TAACAAACGGAAAGACGA	GTGAAACCACAGAGGATAGAA	18718698
BoGMS0457	(AACCG)10	TACTTCCTTTCACTTCTGGGT	ATGGACTTTGGTTTGGTTC	18730250
BoGMS0468	(AT)25	TGACAGCAACCAATGATG	CTCTCTGGAACCTTTGAACT	17787617
BoGMS0472	(TA)24	ACATTATCTGTATTTGTGTGAAA	CAAGAAGGAAGAGTGAAGTTAGA	33823812
BoGMS0486	(ATT)16	AAGGAGGAACCAAATGCC	TGATAATGCCACTGATAGGAC	27041016
BoGMS0493	(CT)24	GTTACCTCCGAAATACACCTC	GCCACTTCATCTCTATCACTC	17698340
BoGMS0501	(GA)23	ATGATGAGTTTGCTCGTTAGG	AAATCCTTCCTCCTTTCAC	23441435
BoGMS0505	(CT)23	CTGTGGTGAGTGCTATTGG	TGTTGCTCGTCATTTCTATCT	23642658
BoGMS0507	(TA)23	TACGATTTCTGTTCTATTCTATC	ATCCCTGCGGTTATCAAA	23692968
BoGMS0510	(ATG)15	AAGATGGAGATTGGATGTTG	ATGATTTGTTTATGGTGTGGT	17765236
BoGMS0512	(AG)23	ATTTCGTGAGAAGAGAATGA	CCAACCAATCAAACCCTAA	17634970
BoGMS0514	(GA)23	CTTCTTCCACGCTAAACATC	GGTCTATTCTTTGATTCGGTT	27021177
BoGMS0525	(GAATT)9	AGTCCCATCAAGTCCAAATAC	GTCGTCTTCAGCCATCAG	17072545
BoGMS0545	(AG)22	CCTCTGTTTCTTTGCTCTTTG	GATTCATTGTGTGTGTGATGT	23695120
BoGMS0558	(TA)21	AATGGAGAAAGACGACGAG	ATGAAGACCGAACTCAACAA	23525607
BoGMS0560	(GAA)14	ATGAAGAAGTGTTTGGTGAG	AAGATTGATGGAAGCAAGAA	23652256
BoGMS0574	(AG)21	AACCAACTCTGCTCACGAC	TCTCAGCCTCTCTTTGCC	23582161
BoGMS0582	(TA)21	CCTGAGTCTTGGAGCCTT	TCGTTATTAGATTTGAGTATTTG	26699517
BoGMS0590	(AG)7(GA)14	TGGTTTATCTTCATTCTTTGG	TATTGAGTTGTCGCACTTGA	26766832

Marker Name	Motif	Left Primer (5' to 3')	Right Primer (5' to 3')	GenBank ID
BoGMS0593	(AT)21	TTGTAGCAGCCAAGGTTC	GGAGTATTGAAGCCCAAAG	23433680
BoGMS0594	(TTCGG)8	TTGTTCGGTTTATTTGGTTT	GGTCCATTTACTACTTATTCCT	17699351
BoGMS0596	(TA)5(AT)15	AACGGAAAGTGAAACCTAAGA	GAGCAAGACCATCTCCAAC	18829548
BoGMS0612	(AT)20	TTTGGGCTTATTCTTGTAGG	GAGATAGAGAGAGGGAAGGTG	26668280
BoGMS0616	(TC)20	GGAAGAAACCATACCCTAAATC	TAACAATCCGACTACACAACC	18804018
BoGMS0624	(CT)20	AAGACGAAGTCAAGTCAAGGT	CGTATCATCCAGAGTATCCAG	17067279
BoGMS0627	(AT)20	TGCCACCTTATTTGGAGA	GAGACTACAGGGAGGAAGAAA	23516583
BoGMS0630	(AG)20	TCTCATCTCATACTCTTCCTCA	GAAACCTCTCCAATCTCCTAA	17835139
BoGMS0631	(AG)20	CGTGGAGAAGAAAGTGAGAG	TCAGTATGTGTGTAGGCAAGT	23670388
BoGMS0632	(AG)20	ATCATCGTCCTCTTCTTCTTC	TATCATCCTTATTGGGTCTC	26994558
BoGMS0637	(TCT)13	TGAAAGAAGATGCTGAGACTG	TGGTGGAGAAGATAGTGAATG	23720773
BoGMS0647	(CT)19	TTCTTTCTCACTCGTCTCCTT	GATGTCCCTCTCAATCTCTCT	23627870
BoGMS0660	(TAAA)9	CCTTGTTCTTTGTAGGAAATG	GGTGCTTGTGCTTTGTTT	23541562
BoGMS0661	(GA)19	ATTTGGATAGTGGATGGTG	GAAGACATTAGGGATTGTGAA	18834114
BoGMS0662	(GA)19	CAAAGAAAGTCCAAGGGTT	GATAGAGGATAGGAGCAGGAG	33789522
BoGMS0665	(CT)19	TCAGAGATGAACAAGAAGCAC	GCGAACCTTTCCCAACCT	17749405
BoGMS0674	(AT)19	ATTCTGGTAGTTGATTTGGG	TCTATTCACAGGCTAACGG	23431173
BoGMS0687	(TA)18	GACAACACAACAGACGCA	GCATTTCCCATTACTTCCA	23410752
BoGMS0692	(GA)18	AACTGTGTCTTGGATGTCTTG	AAGGTTAGTCGTGTCGTAAA	18750654
BoGMS0693	(CTT)8(TTC)4	CATCTCCACATCACTACTCCA	ATTACCCAATCCCAACAAA	17855413
BoGMS0702	(AT)18	CGTAATGGTGAAGATACTCGG	TCTAATCAAGAGCGTGTGGT	23638296
BoGMS0705	(AT)18	GTCACCCTATCTCTCTCCATT	TAGCCACCAAGTCCTGTTC	23697345
BoGMS0707	(AG)18	GAGTGTTTATTATCTGGGTCATT	GCATACAGGTTTCATCCC	23417150
BoGMS0717	(GA)13(AG)5	GAGAAACCTATCCTGCTCAC	CTTATCAACGCACAACGAC	23679282
BoGMS0738	(TCT)11	TTGAGGAAGGAACACGAA	GTGGGAGAGTGAGGGTAGTAA	17844169
BoGMS0741	(TC)17	CTCAAACTCCGTCGCTCT	TCCTCCTCACTACTTTCTTCA	17850570
BoGMS0742	(TC)17	TCTCTCTCCTCTTTGCTTTG	GTATCAGACATTATTCACACGA	23427915
BoGMS0756	(GAA)11	AACAAGAAGAAGAAAGGAGGA	GTGGGAGACAGCGAGGAC	23687982
BoGMS0767	(AG)17	AAACAAGTCAGATTCACCAAA	CTCTTCACCACTACCACAGTC	23454260
BoGMS0789	(AT)17	ATGGATAGGTAGGGAGCA	AACCAAAGGAGGAATAGTTAGA	18761397
BoGMS0793	(AT)17	TTCATACACTTGGACTTGCTC	TACACACACACACATACGCAG	27003602
BoGMS0802	(TGA)11	GCCGTTTGTGTTCTGATAAA	ATTCTCCTCGTCCTCGTC	23461611
BoGMS0808	(TC)16	GTCTCCTCCACCATTATCTTT	GACCTCGTGTTTCCTTGA	17830839
BoGMS0811	(TC)16	GCACTGTCAAATCACTCAAA	ACTTCTCCCAATCTCTGTCTC	18748305
BoGMS0812	(TC)16	GCTGGCACATAGTTGTAATG	CTCATCTCCTCTGCTGGA	23430497
BoGMS0819	(TA)16	AGGGAGATGGACACATTTAG	GAGAGAGGGCAAAGAAGATAG	23564897
BoGMS0821	(GA)16	GTAGCCGAAGAAAGCGTC	ATTGACCGAAAGAAAGAAATG	26767912
BoGMS0826	(CT)16	GCAATGATGAAGTTAGGAGAA	AACCAGCGAGAGAGAGGT	18833231
BoGMS0836	(AT)16	CATAAACACACCGAACAAGAC	ACGCAATGACACACATACAC	18718578
BoGMS0845	(AG)16	CCTTTGTCTTCTTCACTCTCC	ACCAGGCTCTTTCTTTCTCT	17837928
BoGMS0847	(AAG)11	CTCATCTTCCCTCTTCCTCT	GGTTCTGCTTGCTTACTTGA	27024525
BoGMS0849	(TTCGG)6	TGTTCGGTTTATTCGGTTT	TACATTTCGGTAGGGCTGT	26736801
BoGMS0868	(CT)11(TC)5	AAATCCCAACGAGATAGGTAG	AGAAAGAAAGGAAGAAAGTGG	23690378
BoGMS0870	(CT)6(TC)10	CCACAGTTCCTAATCTCACAG	GCCTCCTCCACTTTATTTATC	17818539
BoGMS0906	(TA)15	TACCTCTCTGCTTCTCTCTTG	GGTGATTGCCAGTTTCTTT	26768487
BoGMS0927	(AG)15	ACCAGAGAAGGCATACATAGA	CGAAGGAGTTTGTGAGGATAA	23700224
BoGMS0929	(AAG)10	TCAGACCCAAAGCCAGTT	TTGTGGAAGATGAAACCATT	26784913
BoGMS0934	(TTC)10	GGTCAGAGTAAGGGATGGA	TGTAAAGATGTCGGCTAATGT	26706990
BoGMS0941	(TC)15	GTTGAAGAAACTAAGGAGGAAA	GAACGACAGCGAAGAGAG	18810471
BoGMS0949	(TC)15	CTCCTCCTCCTTTCATCTTC	TTCGTCTCCCTTCTCTGTAA	23627860
BoGMS0952	(TCC)10	CAGTGAGTAACATTTGGCTG	CGAGAGAGAAAGTGATGAGAG	26742067

Marker Name	Motif	Left Primer (5' to 3')	Right Primer (5' to 3')	GenBank ID
BoGMS0953	(TCA)10	CCTCGTAAGTAACCGAATCA	AAACAGAAGATGGAGAAGGAG	17668390
BoGMS0961	(TA)9(AT)6	GTTCTCCTTGGTGTTTGTGT	TTCAACCTTCTCCTTCGTC	23442985
BoGMS0965	(GA)15	CACCACAACACCACACAG	GTTCCCTTTACCCTTCCC	17641807
BoGMS0977	(CT)15	TTTGTCTTCCTTCCTCTAAAC	CAGCAATAACCATCTCCTCA	23403284
BoGMS0985	(ATG)10	CAGTTCTCTCATTGGTATGCT	CATCTTGTCATCTGCCTTG	23513850
BoGMS0998	(AT)15	CTCACTTTCTCTGTGGTTGG	GGAACTTTGTGGACTTGGT	17726316
BoGMS1009	(AT)15	CGAAACCAGGATAAGTCA	CAATGCTTCTGTATGCGTC	23675135
BoGMS1017	(AG)15	TTTGTTGTTGTTGTTGTGTTC	TATCTCTCGCTCGTCTCA	17840628
BoGMS1020	(AG)15	CACACCCGCAGTCTCTAC	CCATTCTTCTTCTTCTTCCC	23407424
BoGMS1023	(AGAGA)6	TATCACGACCTAACTTCTCCA	ACCTCTCCAAATCCTTCTTC	23559163
BoGMS1024	(AGAGA)6	ACCTTCTCCACTATGTCACC	ACCTCTCCAAATCCTTCTTC	26699986
BoGMS1025	(AGA)10	TGAAACCCTAATCGTTGACT	ACTACTTGTGAATCGTCGTTG	17612997
BoGMS1028	(AGA)10	ACAAGTTCTCTCTCACCGAA	TTCACAACCCTCTAACATCTC	23616520
BoGMS1031	(TTC)9	CTCGCTCCTTGTCTTCTTC	AAACGATGTCCCATTCTTC	17722449
BoGMS1042	(TC)14	ATAGTGAATAATGGAAGGCTG	GAGAGAGGAGAGAACAGAGGA	17812094
BoGMS1046	(TC)14	CATAACACCATCATCACCATT	ACGAAGAGAAAGAACGAAGAC	23418675
BoGMS1049	(TC)14	CCACGGTCACTTCTCTATTT	CTCTGAACCAACTCCATCTCT	26716641
BoGMS1053	(TA)14	TCGTATTATTCAGTTGCTGTG	AGCGGAAGCGTTAGGAAG	17796025
BoGMS1055	(TA)14	CGGATAGGAGAGGTTTCAG	TGACTTTGCTTCATCTTCCA	18739268
BoGMS1065	(TA)14	GGGTTGATTGGGAAGTGT	CTTAGCACCATTTGTTTGTATT	26764897
BoGMS1066	(TA)14	CTGACAATCCCAGAAATACAC	GGAGAAACAAACATTAGACGA	33816535
BoGMS1071	(GA)14	AGAGACAGATTCGCCATAGA	GTCAACAAGGGAGTGGGT	17689189
BoGMS1076	(GAA)9	CGTTCCTCCTTCTGGTGA	CTCAATCTCAGTCGGTTCTT	17642048
BoGMS1118	(AG)14	AGACATCAAAGGCAAGACAG	TCAACAGAACTACTCTCCTCAA	17632575
BoGMS1131	(TTTG)7	AGAGTTGCTGCTGTGTTTG	ACTTGAGGAGATGGAGATGAG	17678161
BoGMS1145	(TCA)9	CTTCTCTCTTCGCATCATAAC	GTCCCTCTCCTCTCTCTCTCT	27011708
BoGMS1148	(TA)14	TTGTAGAGGTTCCAAAGGG	ATGTTCGTCCGTCCAATC	23522664
BoGMS1162	(GA)14	AAATCTGACAAAGGCGAAA	AGAGAGAAAGGAGAGCCAA	17837739
BoGMS1163	(GA)14	TAAGGGTGAGAAGCAGAGAG	AAGTAAACCGATAGAATACCAGA	23554207
BoGMS1164	(GAA)9	CGATTCAAACTCAAACCAAC	AATAAAGAGACAGGGCGG	23423517
BoGMS1171	(CT)4(TC)10	CAATCCCTAACTCTGTTTCCT	AGCCAATCAATCACACTACAC	17822386
BoGMS1185	(CT)14	ATACAAGATGCGAAGGAGAA	CGGCAAGAGAATAATAGACAG	17607028
BoGMS1186	(CTC)9	GACTGGAACGACAACGACT	GCGGAGGTAGATTAGGGA	17613545
BoGMS1201	(AT)14	GGTAACACCAATCCACTTTCT	CACTTCAAACTTCTCATCTGG	17621049
BoGMS1218	(AG)14	GTAAACCGAAATCAGAATGG	GACGAAGATGGAAGGGTAA	17074958
BoGMS1219	(AG)14	AACACTCACACACACGAAGA	CTCTATCTCCCTTATTACGCAC	17650871
BoGMS1224	(AG)14	TCTGAGCCATTGATTGATTT	CGAGGAAGAGAAGAGAAGAGA	27054984
BoGMS1235	(AGAA)7	ATTCATCATCTCTCTCGGAA	TAAGGCTCATCTCAACTCTCA	18726771
BoGMS1240	(AAGA)7	TCGCTTCTCTCTAATCTTCCT	TCTGGGTCTCGCTTTATCT	23698574
BoGMS1245	(TTTGG)5	TTTGGTTCGGTTCGGTTT	TAAGAAGTAGGTTTAGCCGTG	18721558
BoGMS1258	(TCT)9	TTATTATTCTCCTGCTCCTTG	ACACACAAACGATGCTCAC	17710491
BoGMS1259	(TCT)9	ACAAGGAGAAGGAGAAGACAC	TGAGGAAGATGAAGTTGAAGA	23411110
BoGMS1264	(TC)13	GATTGTGGAGGTGGGTTT	TCAAGAAGAAAGAGAAAGAGAGA	17839945
BoGMS1283	(TCA)9	TTGTCATCATCCTCTTCACTC	TGCTATCCACTCTTCTTCTCA	18836779
BoGMS1287	(TA)13	GTGAAGCAAGGCATAAATAAA	GTAGAAACAGGTCCAACCAA	17705762
BoGMS1305	(GA)13	AATGAACAAAGGTGACGACT	CTGCGATTTCCTCTCTCTC	17716471
BoGMS1307	(GA)13	TGGCGATAAAGAGGAGAAC	CGAAGAGAGAGAAACAAAGAA	23555857
BoGMS1322	(CTTCT)5	CTCTCCAATCCTTCTTCTCAC	CCACCTTCTCCACTAATAACC	18839374
BoGMS1330	(CT)6(TC)7	AGGAGAAGAAGGAAGATACCA	AGAAAGGAAAGAAAGACCAGA	23667519
BoGMS1343	(AT)13	CACCTCTACACGAGATTCATT	TGTTCTTCTTTGTTGCTGG	18725859
BoGMS1360	(AG)13	GAGACCAGAGAAGGAGGAAC	CACTCACTATCACACACACTCA	17736416

Marker Name	Motif	Left Primer (5' to 3')	Right Primer (5' to 3')	GenBank ID
BoGMS1394	(TTC)8	ATTGTGTCAGAGAAGGGTT	GGTGGAAGTAGAGGAAGAAGA	33777311
BoGMS1407	(TC)13	AATCAAGCACACAACACATAA	CGTGAGGAGAGAGAGAGATG	18762190
BoGMS1412	(TC)13	GGTTTAGTGTTGGTTCGGT	GCGAGAGAAAGTTGAGGTT	23461470
BoGMS1413	(TC)13	TGGGCTTCTTCTTACTTACCT	TGCTCTGTCTATTATTCGTTTG	23433060
BoGMS1419	(TC)13	AAAGGTCGTATTTCTCTCTCTC	GATTTGATTCTGGTGTGAAGT	17835351
BoGMS1432	(TA)13	AAGTCGGCACAAGGTGTT	AGGGATTTAGAGTTTCGTGTT	17676301
BoGMS1452	(GA)13	CGGTGGGTGTGTAGTTAGTT	TTCTATCAGTTCCAAGTTCCA	17842832
BoGMS1453	(GA)13	TATCCCAAACCTAAACCCTAA	ATGAAAGCCACTCTCTCTTCT	17671500
BoGMS1464	(GAA)8	CTGATGAACGGAGACACAG	AAGCAAAGCAGAGCATAAAC	23698170
BoGMS1465	(GAA)8	GAGGTGTTGGATACTGTGCT	TGTTGTTGGTGTTAGTGGTG	33802675
BoGMS1467	(CTG)8	ATGGCTTTGTTCTTCTTTCTT	GACTTCAGCACGCCTTTC	27023419
BoGMS1486	(CT)13	AAATGTGTTCTTGGTGATG	AGGAGGGTAAGTTGGTGATT	23537974
BoGMS1493	(CCA)8	CGTAGAGAGTATTGGAAGCA	GTCCTCCTCGTAATGGTGT	23440016
BoGMS1495	(CA)13	TAACACTGAAACACATTGGCT	GTGAGAAAGATGACGAAGATG	17765872
BoGMS1510	(AT)13	GCGAAAGGGTAGAGAAGAGT	TATTTGGTTACAAGTGCGAAG	17633959
BoGMS1530	(AT)13	TTTGGGCAGGTCATAAATAG	GAGATTAGATTCCCTCACC	18827880
BoGMS1539	(AT)13	GTGCTTCACTTGTATGATGACT	ATAATGGGCTGCTCTTCTTC	23449631
BoGMS1561	(AG)13	AGATAACGGATGTGAGGAGA	CTTGAGAAGATTGGCTTGTT	23512899
BoGMS1565	(AG)13	GATGACTTGGCGTTGATT	CTGAGAGAGAAGAAGGAGAAGA	26729121
BoGMS1567	(ACTA)6	TTCAGTAGGGAGGGATAGGT	GGTTATGATGCTCGTCGG	27007423
BoGMS1570	(AATA)6	TCAAGCCAACGCTACTACA	TGATGGGTGAACAACTAAACT	23624681
BoGMS1587	(TTC)8	GCTATCTCGGTCTTGTCTCTC	TGTGTGTTGCTTTAGTGTTTG	17675805
BoGMS1594	(TGA)8	ATCCCACGCTTCTTGACC	CTCCTCACCTTTACTTGCTTT	17804316

Next, we screened the SSR DNA markers suitable for purity test of inbred lines, like DNA markers of the *S* locus genes. We examined the PCR amplification using the reported SSR DNA markers, BoGMS (Li *et al*., 2011) (Table 1). Of the 175 markers, 152 markers (86.9 %) showed clear PCR amplification in agarose gel and 78 markers (44.6 %) showed clearly visible bands in acrylamide gel (Figure 2, Table 2).

Using 78 markers, we examined the polymorphisms between F_1 hybrids and F_2 plants. Twelve DNA markers (6.9 %) (BoGMS0314, 0342, 0394, 0486, 0624, 0693, 0812, 1185, 1235, 1283, 1305, and 1493) showed polymorphism between F_1 hybrids and F_2 plants (Figure 3), and these polymorphisms are due to the difference of the nucleotide sequences between parental lines of F_1 hybrid. These results indicated that 12 markers that we found in this study could be used for the purity test of inbred lines as these DNA markers can detect the polymorphism between parental inbred lines of F_1 hybrid cultivar.

DNA makers are used for cultivar discrimination or genetic diversity in various plants (Barth *et al*., 2002; Deleu *et al*., 2009), thus the 12 DNA markers we found can be applied for these studies in *B. oleracea*. It has already been shown that RFLP markers of the *S* locus genes were useful for detecting polymorphisms between *S* haplotypes and could be amplified in many *S* haplotypes. However, these primer pairs did not amplify the target amplicons in some *S* haplotypes (Fujimoto and Nishio 2007). Another method using *SP11/SCR*, which is highly polymorphic among *S* haplotypes, has been developed (Fujimoto *et al*., 2003; Oikawa *et al*., 2011) using *S* haplotype specific DNA probes or primer sets, but these methods need the sequence information of *SP11/SCR*. Nucleotide sequences of *SP11/SCR* in some *S* haplotypes have not yet been determined. Thus SSR markers that we found in this study could provide another choice for purity test of F_1 hybrid seeds as well as purity test of inbred lines, though further experiments will be required to determine whether these DNA markers allow purity detection in other inbred lines of *B. oleracea*.

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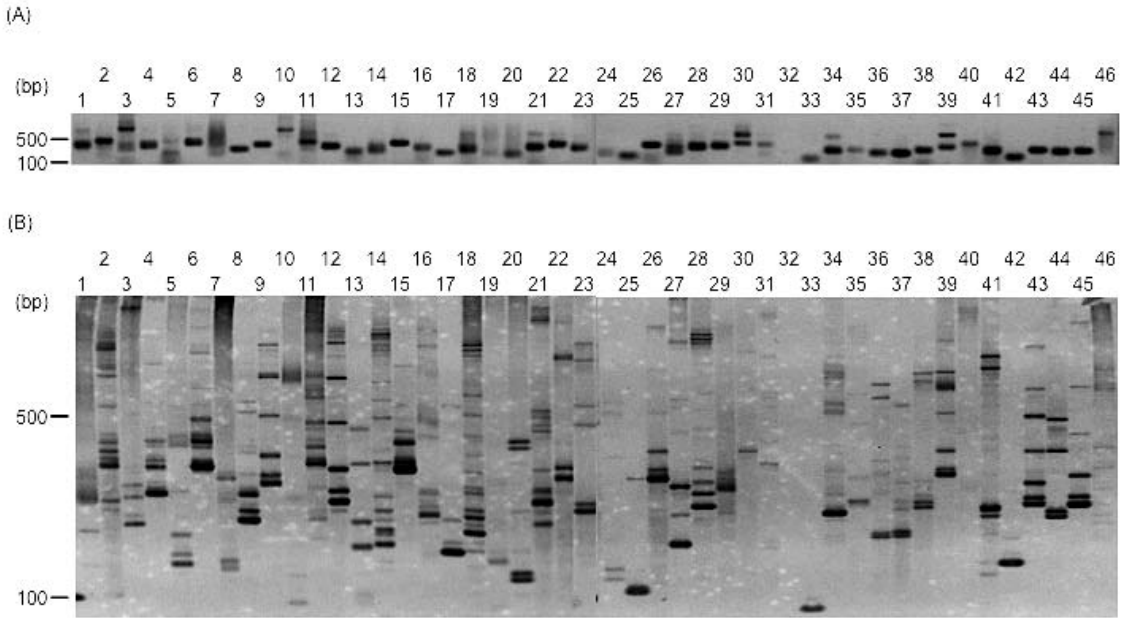

Figure 2. Confirmation of PCR amplification in F_1 plant using SSR markers on agarose (A) or acrylamide (B) gel. Lane numbers are consistent with the numbers shown in Table 2.

Table 2 Summary of the PCR amplification in F_1 plant using SSR markers on agarose or acrylamide gel

	Name	Agarose	Acrylamide		Name	Agarose	Acrylamide
1	BoGMS0009	○	-	89	BoGMS0836	○	○
2	BoGMS0030	○	○	90	BoGMS0845	○	○
3	BoGMS0032	○	○	91	BoGMS0847	○	-
4	BoGMS0037	○	○	92	BoGMS0849	○	-
5	BoGMS0038	-	○	93	BoGMS0868	○	○
6	BoGMS0039	○	-	94	BoGMS0870	○	-
7	BoGMS0088	-	-	95	BoGMS0906	○	○
8	BoGMS0109	○	○	96	BoGMS0927	○	-
9	BoGMS0112	○	○	97	BoGMS0929	○	-
10	BoGMS0162	○	-	98	BoGMS0934	○	-
11	BoGMS0164	○	-	99	BoGMS0941	○	○
12	BoGMS0168	○	○	100	BoGMS0949	○	○
13	BoGMS0197	○	○	101	BoGMS0952	○	○
14	BoGMS0208	○	○	102	BoGMS0953	○	-
15	BoGMS0254	○	-	103	BoGMS0961	○	○
16	BoGMS0263	-	-	104	BoGMS0965	○	○
17	BoGMS0281	○	-	105	BoGMS0977	○	○
18	BoGMS0282	○	○	106	BoGMS0985	○	-
19	BoGMS0299	-	-	107	BoGMS0998	○	-
20	BoGMS0314	○	○*	108	BoGMS1009	○	○
21	BoGMS0327	○	○	109	BoGMS1017	○	○
22	BoGMS0342	○	○*	110	BoGMS1020	○	-
23	BoGMS0351	○	○	111	BoGMS1023	○	-
24	BoGMS0355	-	-	112	BoGMS1024	○	-
25	BoGMS0364	-	-	113	BoGMS1025	○	○
26	BoGMS0369	○	○	114	BoGMS1028	○	○

	Name	Agarose	Acrylamide		Name	Agarose	Acrylamide
27	BoGMS0373	○	○	115	BoGMS1031	○	-
28	BoGMS0394	○	○*	116	BoGMS1042	○	○
29	BoGMS0405	○	-	117	BoGMS1046	○	-
30	BoGMS0407	○	-	118	BoGMS1049	○	-
31	BoGMS0429	-	-	119	BoGMS1053	○	-
32	BoGMS0456	-	-	120	BoGMS1055	○	○
33	BoGMS0457	-	-	121	BoGMS1065	○	○
34	BoGMS0468	○	-	122	BoGMS1066	○	-
35	BoGMS0472	-	-	123	BoGMS1071	○	○
36	BoGMS0486	○	○*	124	BoGMS1076	○	-
37	BoGMS0493	○	○	125	BoGMS1118	○	○
38	BoGMS0501	○	○	126	BoGMS1131	○	○
39	BoGMS0505	○	○	127	BoGMS1145	○	-
40	BoGMS0507	-	-	128	BoGMS1148	-	-
41	BoGMS0510	○	○	129	BoGMS1162	○	○
42	BoGMS0512	○	-	130	BoGMS1163	○	○
43	BoGMS0514	○	○	131	BoGMS1164	○	-
44	BoGMS0525	○	○	132	BoGMS1171	○	○
45	BoGMS0545	○	○	133	BoGMS1185	○	○*
46	BoGMS0558	-	-	134	BoGMS1186	○	○
47	BoGMS0560	○	-	135	BoGMS1201	○	-
48	BoGMS0574	-	-	136	BoGMS1218	○	○
49	BoGMS0582	-	-	137	BoGMS1219	○	-
50	BoGMS0590	○	○	138	BoGMS1224	-	-
51	BoGMS0593	○	○	139	BoGMS1235	○	○*
52	BoGMS0594	○	-	140	BoGMS1240	○	-
53	BoGMS0596	○	○	141	BoGMS1245	-	-
54	BoGMS0612	○	○	142	BoGMS1258	○	○
55	BoGMS0616	○	○	143	BoGMS1259	○	○
56	BoGMS0624	○	○*	144	BoGMS1264	-	-
57	BoGMS0627	○	○	145	BoGMS1283	○	○*
58	BoGMS0630	○	○	146	BoGMS1287	○	○
59	BoGMS0631	○	○	147	BoGMS1305	○	○*
60	BoGMS0632	○	○	148	BoGMS1307	○	○
61	BoGMS0637	○	-	149	BoGMS1322	○	-
62	BoGMS0647	○	○	150	BoGMS1330	○	○
63	BoGMS0660	○	-	151	BoGMS1343	○	-
64	BoGMS0661	-	-	152	BoGMS1360	○	-
65	BoGMS0662	○	○	153	BoGMS1394	○	-
66	BoGMS0665	○	○	154	BoGMS1407	-	-
67	BoGMS0674	○	○	155	BoGMS1412	○	-
68	BoGMS0687	○	○	156	BoGMS1413	○	-
69	BoGMS0692	○	○	157	BoGMS1419	○	○
70	BoGMS0693	○	○*	158	BoGMS1432	○	-
71	BoGMS0702	○	○	159	BoGMS1452	○	-
72	BoGMS0705	-	-	160	BoGMS1453	○	○
73	BoGMS0707	○	○	161	BoGMS1464	○	-
74	BoGMS0717	○	○	162	BoGMS1465	○	-
75	BoGMS0738	○	○	163	BoGMS1467	○	-
76	BoGMS0741	○	○	164	BoGMS1486	○	-
77	BoGMS0742	○	○	165	BoGMS1493	○	○*

	Name	Agarose	Acrylamide		Name	Agarose	Acrylamide
78	BoGMS0756	○	○	166	BoGMS1495	○	-
79	BoGMS0767	○	-	167	BoGMS1510	○	-
80	BoGMS0789	○	-	168	BoGMS1530	-	-
81	BoGMS0793	○	○	169	BoGMS1539	○	-
82	BoGMS0802	○	-	170	BoGMS1561	○	○
83	BoGMS0808	○	○	171	BoGMS1565	○	-
84	BoGMS0811	-	-	172	BoGMS1567	○	-
85	BoGMS0812	○	○ *	173	BoGMS1570	○	-
86	BoGMS0819	○	-	174	BoGMS1587	○	-
87	BoGMS0821	○	○	175	BoGMS1594	○	-
88	BoGMS0826	○	○				

○ shows clear bands or fragments.
- shows no amplification or non-clear bands or fragments.
* shows the primer pair, which can detect the polymorphism between parental inbred lines of F_1 hybrid cultivar.

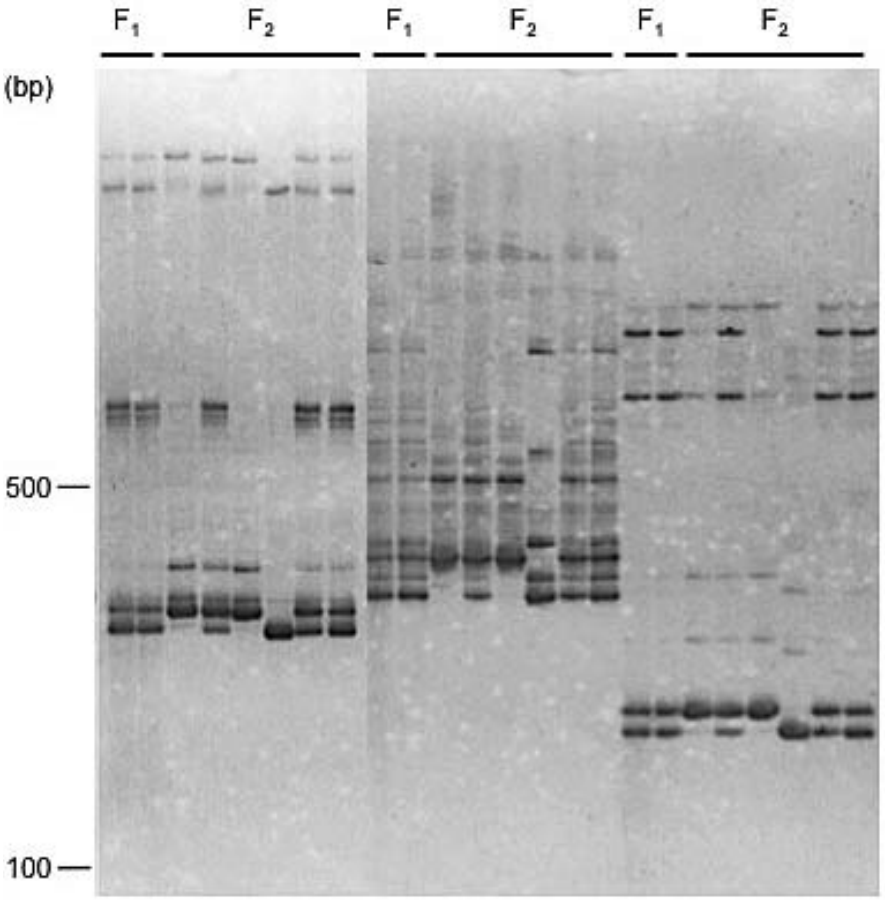

Figure 3. Detection of the polymorphisms between parental alleles of F_1 hybrid cultivar using SSR markers.

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Brassica oleracea における近交系統の純度検定に最適なDNA マーカーのスクリーニング

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（平成25年１月30日受付）

要　約

近交系統の作成は、植物の F_1品種の育成過程の最初の段階である。遺伝的に均一な近交系統の育成には５、６世代以上の自殖が必要であると考えられている。DNA マーカーを利用した近交系の純度検定は信頼性が高いが、*B. oleracea* では、純度検定に有効な DNA マーカーの報告は少ない。本研究では、F_1品種の両親系統間の多型を見分けることができる DNA マーカーを見出すために、175の SSR プライマー対を試した。F_2集団では、両親系統のゲノムが分離することから、両親系統の塩基配列の多型を追跡することが可能となる。そこで、材料にはキャベツの市販 F_1品種である YR 錦秋協力152の F_2個体を用いた。12の DNA マーカーで F_2集団の個体間で多型が見られたことから、これらのマーカーは純度検定に有効的であると考えられた。

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キーワード：純度検査、近交系、SSR プライマー、*Brassica oleracea*

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