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

Kazutaka KAWAMURA[#, 1], Natsumi SAEKI[#, 1], Yusuke EBE[#, 1], Hirofumi ABE[#, 1], Motoki SHIMIZU[1], Kenji OSABE[2], Keiichi OKAZAKI[1, *], Takahiro KAWANABE[1], Ryo FUJIMOTO[3]

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Summary

Developing inbred lines as parental candidates is the first step in breeding F_1 hybrid cultivars of 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 rapa* L.. In this study, we assessed 321 SSR primer sets to identify the DNA markers, which can detect the polymorphisms between the parental lines of F_1 hybrid cultivars. The F_2 plants derived from the F_1 hybrid cultivar of Chinese cabbage "W77" was used to identify the polymorphisms. The F_1 hybrid 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 the F_1 hybrid. Finally 59 primer sets showed polymorphisms among F_2 plants, indicating that these DNA markers could be useful for purity test.

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The genus Brassica is composed of diploid and allopolyploid species and includes important vegetables and oilseed crops. *Brassica rapa* L. (2n=20, AA genome) and *Brassica oleracea* L. (2n=18, CC genome) are diploid species. *B. rapa* vegetables such as Chinese cabbage (var. *pekinensis*), turnip (var. *rapa*), and komatsuna (var. *perviridis*) are widely cultivated in Asia and show morphological diversity. *B. oleracea* includes cabbage (var. *capitata*), Chinese kale (var. *alboglabra*), broccoli (var. *italica*) and cauliflower (var. *botrytis*). *Brassica napus* L. (2n=38) is an allopolyploid species whose genome is composed of the AA and CC genome (U 1935), and includes important crops such as oilseed. *B. rapa* and *B. oleracea* 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).

In Japan, most *B. rapa* vegetables are F_1 hybrid cultivars. In the process of breeding for F_1 hybrid cultivars, breeders selected for inbred lines with agriculturally important traits as candidates of parental lines. 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 can 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 also 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 the environment. The genes on the self-incompatibility locus can be used for purity test of F_1 hybrid seeds as DNA markers (Fujimoto and Nishio 2003; Fujimoto and Nishio 2007). Higher number of DNA markers increases the reliability of determining the homogeneity of inbred lines. However there are limited DNA markers that are available to assess the homogeneity of inbred lines. Simple sequence repeats (SSRs) markers have been used because of high polymorphism, reproducibility, co-dominance inheritance, and genome-wide coverage. In addition, SSR markers are suitable for high-throughput analysis because it can be easily detected 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; Kato *et al*., 2012;

[1] Graduate School of Science and Technology, Niigata University

[2] Plant epigenetics unit, Okinawa Institute of Science and Technology Graduate University

[3] Graduate School of Agricultural Science, Kobe University

[#] These authors equally contributed to this work.

[*] Corresponding author: okazaki@agr.niigata-u.ac.jp

Bagheri *et al*., 2012; Chen *et al*., 2013).

In our previous study, we screened the SSR markers that are suitable for the purity test of inbred lines or F_1 hybrid seeds in *B. oleracea* (Tomita *et al*., 2013). In this study, we screened the SSR markers for the purity test of inbred lines in *B. rapa*. We used the commercial F_1 hybrid cultivar of Chinese cabbage, "W77", and its F_2 plants. Of the 321 SSR primer sets, 59 primer sets reliably detected polymorphisms between the 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 Chinese cabbage, "W77" (Watanabe Seed Co., Ltd) and F_2 plants obtained by buds pollination of this cultivar were used as plant materials. 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).

Determination of S haplotypes

S haplotypes of F_1 and F_2 plants were identified by PCR using two primer pairs, PS5 (5'-ATGAAAGGGCGTAAGAAAAACCTA-3')+PS15 (5'-CCGTGTTTTATTTTAAGAGAAAGAGCT-3') and PS3 (5'-ATGAAAGGGGTACAGAACAT-3')+PS21 (5'-CTCAAGTCCCACTGCTGCGG-3') (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.

Detection of DNA polymorphism with SSR markers

The "BoGMS"-SSR marker series derived from Li *et al*. (2011) was to assess for polymorphisms. 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 "BRAS" (Piquemal *et al*., 2005), "BRMS" (Suwabe *et al*., 2002), "BnGMS" (Cheng *et al*., 2009), "CB" (Radoev *et al*., 2008), "KBr" (developed by NIVTS (NARO Institute of Vegetable and Tea Science)), "Na" (Lowe *et al*., 2004), "Ni" (Lowe *et al*., 2004), and "Ol" (Lowe *et al*., 2004) -SSR marker series were used. The PCR reaction of these marker sets was performed by the following condition: 1 cycle of 94℃ for 3 min, 35 cycles of 94℃ for 30s, 55℃ for 30s, 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 Gelstar solution (0.1μl/10ml; Takara Biomedical, Japan). Primer sequences used in this study are shown in Table 1.

Table 1 Summary of the PCR amplification in F_1 plant using SSR markers on agarose or acrylamide gel

Markers Name	Agarose	Acrylamide	Forward primer	Reverse primer
BRAS019	-	-	CTCAAGACAAACGACCAGTAA	GAGAAGAAATCGCCAAGA
BRAS029	○	-	GTTCAACCTCCCTCGTCTCT	AGGTGCCAACTCATTTCTCAA
BRAS034	○	-	ACGTCGTATTGTCTTTTTATG	GCACTTTTTAGGGTTCTTA
BRAS037	-	-	TCCATTAGCTCTCGTCCTC	TTTTTAGAACAGTGCCTTGAC
BRAS039	-	○*	ACTTACGTGTTCTTGATGGTC	GATGCTCTATGTTTTCTGGT
BRAS044	○	○*	CCCTTCTTTCTTTCCTCACA	TCGCAAAATCTCAACAAAAAC
BRAS050	○	-	CTTTGTGGTGGGTAGTGG	ACTTAGCCTCAATACGGTCTT
BRAS051	-	-	GAATAGCCTCGCAGAAGTAGC	CGACGGCGATAAAACGAA
BRAS061	○	○*	GCAGCCTTCAACTCCCATAGA	TGGGTTCGAGCAGGGTTC
BRAS063	○	-	GACGCTCATTTCACTTC	TCCTAACTAACATCATTTTGC
BRAS065	○	○*	AGCAATCCCGCCTAAATGGTA	GATGCGAGGTCACTGTTGTCC
BRAS069	○	○	CTCGATCTTCCCCTGCTTTC	GTTGAGCCAATCTACGGTTCC
BRAS072	○	○	GCCATCTACACATTTATCCC	CACTAACCTTCTTGCTACCGT
BRAS077	-	-	ACCAGCTCTCTGAGGTTCTTC	GCGAATAAGTGGATGAGGAG
BRAS094	-	-	CACCGGCATCAACAA	CACTTCAAAGGTTCTCAT
BRAS096	-	-	CTGAGAAGGTTTGGGATGCG	CCAGGTTTTCCCAGTCACGAG
BRAS101	○	-	ATTTTCGGACCAACATAGATT	GAAAAAGTAGTCGGTGTGAT
BRAS106	○	-	CTTCTGCCATCTCTTTACTT	ACGACTACAACGATGATGAT
BRAS112	-	-	ATTTCTTAACCGGTGCAG	TGATTTTGATTTACGGTGTT

BRAS114	○	○	TGGGACCTCTATGTCATGCC	CGACGTCGAATTAGCAACC
BRAS115	○	-	TCACCCACAGAACAATCCTC	CAACCAACGGTCACGCCTGC
BRMS005	-	-	ACCTCCTGCAGATTCGTGTC	GCTGACCTTTCTTACCGCTC
BRMS007	○	○*	AAATTGTTTCTCTTCCCCAT	GTGTTAGGGAGCTGGAGAAT
BRMS008	-	-	AGGACACCAGGCACCATATA	CATTGTTGTCTTGGGAGAGC
BRMS014	○	-	CCGTAAGGAATATTGAGGCA	TTCCCAATTCTCAAACGGTA
BRMS017	○	○*	GGAAAGGGAAGCTTCATATC	CTGGAAAGCATACACTTTGG
BRMS026	○	○*	CCTATCCTCGGACTAATCAG	CTTGATGAGTTTCACATTGC
BRMS027	○	○*	GCAGGCGTTGCCTTTATGTA	TCGTTGGTCGGTCACTCCTT
BRMS034	○	-	GATCAAATAACGAACGGAGAGA	GAGCCAAGAAAGGACCTAAGAT
BRMS040	○	-	TCGGATTTGCATGTTCCTGACT	CCGATACACAACCAGCCAACTC
BRMS057	○	○*	CCCGCACCTTCTTCCTTCTCATTTC	TGTCGCCGGAGCTCTCTTTATTGTG
BRMS062	○	-	CTGCTGTGGAGATCGTTCATTGTAT	CCTTTTCTTCTGGGTTTCCTGACTT
BRMS085	○	○*	ACTCCACACTCTCACTTCCTCTATT	TTACGCTTGTTTCTGTTTTTGAATA
BRMS096	○	○*	AGTCGAGATCTCGTTCGTGTCTCCC	TGAAGAAGGATTGAAGCTGTTGTTG
BRMS163	○	○*	CTCTTCCTTCTCGATGTTATTTCAG	CACGTGCCATTCCAATAAGC
BRMS170	○	-	TCACAGCAGCAGCAGCAACAACAAC	CATTTGCCAGGTGGGGCGCAGCTCT
BRMS184	○	○*	AGAACAATCTAACCAAAAAGACTCG	AAACAAAATAGGTCCGAAGAACTTT
BRMS201	○	-	GTAAATAACAGTTCTGCCTCTGCTC	CTGCTGAATTAATTGCTGCTTCT
BRMS226	○	○*	TAAATATGGTGCAAAAGATGACACA	CAACCACTCATCTTGTTCCATTATC
BRMS276	○	○*	GACCGTTTTGCATTTTAAGAGCATT	TCACCACCAGTATCTTCAACAATCA
BRMS315	○	○*	TCTATCTTCCTCAATCACCACCAC	TCCACCCCTAAAACAAATAATACCG
BRMS330	○	○*	TAATTTCCGTTTCGCCTCCACTTAT	TTGCTTTAATCAGGATGCTCTTGTC
BRMS332	○	-	TACAGAAACCAAATACCACCACCAC	TGTGTGCAGAGAACTCTCGGTATCT
BnGMS416	○	-	ACGCGGTATGTAGGAAATAA	GAGACAATGTAGGGAAATGC
BnGMS543	○	○*	TCGAAATCTACGGTTTGACT	ATTATGCTTGTCGTCGACTT
KBrB002E24	○	○*	CAACACTCTCATTCACAGCCAA	TCGATTTGTGCCCACCTCTA
KBrB007I08	○	○*	GAGCTGGCTTAAAGCATGTGCCT	TTACCGGGTCATGTGGTTCGGA
KBrB010O09	○	○	GAGGTTTCCCGGTGACCATG	CAAAAGGTGGTGGTTGGCTT
KBrB010F13	-	-	ACGCACTCATGCAACCAACA	GCTACACGTCAAAAGCTAGCGAAC
KBrB021J09	-	-	TTGACTTCGTTCTTCTGTCACG	TAGCTAACGCAGGACGGAGA
KBrB027J02	○	○	CTCCCAAATGGAAAGAGACGTG	CATATGGCTCCTTTTCCTCAGCT
KBrB052N08	-	-	TAAGGAAGCGCAGAAGCGAT	TGTGGAAGCTTACGGAATCTCGCT
KBrB053E08	○	○	TTAAGCCGGTAAGGCTACTCA	TCCTTCACCAGCAAAAGCTC
KBrB058B22R	○	-	ATTGTCAAGGGAACCGAAACCA	CGTTTGGATTTCACGTCTACAGC
KBrB067H02F	-	-	ACCCATGTAGATTTCCTCTTCCAA	GTTTGAAACGATCTGAGCTTGTTCTGC
KBrB077E04	-	○	ATCGTCAATGATCATCGGTTACCT	GTTTCCAAATAGAAAGCGACCACCACT
KBrB086E02F	○	-	AGTATGGAATTGATCACCATCTTC	GTTTACGGTACCTTCCATACAAACCTGA
KBrB086H24R	○	○	AGTAGACTCCCAGAGGCAAATTCC	GTTTGAAAGCAGAGTTGCGATGACAAGA
KBrB087M18	-	-	AGTTCTAATCGAATCTGGCAACGA	GGAGTCCAAATAAATAAACGGGG
KBrB089H07	○	○	TGCATAACGGTGGTCTTGTG	GTCGAGTCTTCCAGCTAGTGCAA
KBrB091E03	-	-	TAAGGAAGCGCAGAAGCGAT	TAGAAACGCGTGAATCGGTG
KBrH001M22F	-	-	TCACAGCGATGGCTCAGCAAAGCT	GGAATCCCCAGTTTGTCACTCCCA
KBrH005H10R	○	-	ATGCTTCTGAATGCTTTGTGTGTG	CGCTGGTTTCTGGTAAACTCCAT

KBrH011I15	○	○	TCTAGGGTTTTGAAGAGCTGCGT	ACTGACACGTTGTCGGAGTT
KBrH054N12R	○	○*	ACCATGGTAACATCTGGAAGGTGA	GTGCAGGCACTTCTACACCAG
KBrH059N21F	○	○*	ATCGACGCCGTTTATTAGAACTCG	GTTTACGCCACGTCAGCTCACTAACTC
KBrH071B03R	○	○*	AGACCGGCACGTATATTACCTGAA	CATCGAGATCCGAGAAACGAAC
KBrH076K15F	○	-	ATTTTTGGTTTGCCTCAAGTGACC	GTTTCACTGTCATCAATCAGATTCTCCAA
KBrH079A14F	○	-	AGCTTTCCTACCTTTTTCCCCTTC	GTTTGAGTGCGACTTGGAATTTCTCCAT
KBrS001M03	-	-	GGTTTGTGAGCTGGTGAGAGA	GACTTCCCAAGTAAGTCGTCTGA
KBrS011B08	-	-	ATCAGCGTGAATTAGCTCCCCA	CGTATCTGTGATCTGCTCCGT
Na10F08	○	-	AAACTTGCTTTCGAGGATGG	AAACCAGTTGACTCCATCGG
Na12A05	-	-	TGATTGGTGGAAGGTGGAAG	TCATACATCAACCCTCTCTCTCTC
Na12B02	-	-	CGCGTTGTAACTCCAAGACC	TTTTCTAAATCATCCACTGTATCC
Na12B12	-	-	CGTGGACTAACGTTCCCC	CAAGGTTCAGAAGCTGTGGC
Na12C03	-	-	ATCGTTGCCATTAGGAGTGG	ACCAAATTAACCCTCTTTGC
Na12D10	-	-	GCCCTCAAAAAGAGAGTTGC	TTGATGTGGGTGAGGCTAGG
Na12H04	-	-	TTTATCGTCTTTCCCCTCCC	ACAAGGAACTAGAGAGAGAGAG
Na14E02	○	-	ACTGGCTACATGAGTTTCAGTG	GAGGGAAGACAACTGGTCTCA
Ni2B01	○	-	AAGGAGATTGTTTTTGGGGC	AAGACTAATAAACACACGGCG
Ni2E12	-	○	TTATCTGCTTGTCTTGGGGC	AAGGAAATCGTCTCACTTGG
Ni4A10	-	-	CAACGTCTCCCTCACAATCC	TCGCCTCACTCTCAATCTCC
Ni4F09	○	○	CTGTTATGCAAGGTCATCGC	TGTTCCAGGTGAAGAAACCG
Ni4G04	○	○	GAGGCGCGTGGACTAACC	TTACACCCCATCCAAACTCC
Ol10G08	-	○	TGCTTAATTGATTAGGGCAG	TTACCTCATCAGGTGGAGGC
Ol10G09	-	-	TGCTTCCTTTTTCTTCGCTC	GAAGCACGAACGCGAGAG
CB10003	○	-	ACGGTGCCGAATCTCAACG	AAATGGGTCACAGCCGAGAA
CB10004	-	-	AGCCATCTCCTTAAGTTCCT	CAGAGTTACGGCGAGCAG
CB10014	○	○*	TGATACTAAAGGCAAGTCTA	CTGACCATAACCCATCG
CB10015	○	○*	GCTTCTCTCTGTTTTGTTCA	GTAAAAGGTATCCAATTCTA
CB10016	○	-	GGAAGTCTTGTCAATCGC	TGAAGGTCCTGTGATCTATG
CB10018	-	-	GCGCTGACCATGTGTCGTAT	CCGCACCAACGCAGCC
CB10020	-	-	TCTTCTTTCCTCCTGATCTC	TTGATGGTCTCGCTCTCC
CB10023	○	-	TAAGACTTTCCCGCCTAAC	CCGAAGATATAACCGAGAGC
CB10029	○	○	ATTCGGAACCTAAATAGTCAG	GGCGTATAAAACACCTAAAAC
CB10030	-	○*	ATCTCTGTCCTAAATCTCCCA	CTTTATGATGAGTTTCGTCGC
CB10031	-	○	ACAAGTCCTTCACGAGAGCCT	CAAGAGATTGTGTCTGTAGCG
CB10033	○	○*	TCGTCTCAATTCATCATC	GACCATACGTCGTCGTTT
CB10035	○	○	GGCTAGATATTAGACCGTAAG	CACCCTCTTCCTTCATCCTCC
CB10036	○	○	ATTCATCTCCTGCTCGCTTAG	AAACCCAAACCAAAGTAAGAA
CB10037	-	-	ATCCCTGTCCCTTAATAGG	ATCGCCGTTTTCATCGCAC
CB10038	○	○	CGAGGGAATATCTAATTGTAA	GCGACTGTCTTTGTTTTGGTG
CB10041	○	○	GTGGGATTAGGGATTGTGAA	ATAATGCCGTTAGGAAGAAC
CB10042	○	○	GTAATGGGTGTTGCGAC	AGGGAGGGGGTGATGGT
CB10044	○	○	AGAATCACAATCTACACCTTC	GATGTTTCACCACTCCCCAGT
CB10047	○	○	GTAACTGAGCCATCACCTATC	TTTCTTCTTCCACCATTTCTC
CB10051	○	○	CCAAGTGTGCCACCGATG	ACAACACTCCAGGAGCATATC

CB10053	-	-	GACAAGAAAATCTTACTCGAT	ACCAAGTTGGAGGAGAGATAT
CB10055	○	○	GGGAGCATTCCTGTTACTCA	TCAGCCACTGATTGTTCGATT
CB10057	○	○	CTAGGCTAAGGAAGATTGTCA	TAGTTTCTTCCTCCTGCTATC
CB10058	○	○	TTGCATGGCTACCTTAGTTC	GGTGTGGTCGCGCTTTA
CB10059	○	○*	CGCCGTCGTCACCTGAGATG	CGGTGAAGTCGGGATCTTGTC
CB10060	○	○	CTGTACGGCTTCATCTCATAC	AGAATGTTATCTTGCCTTCAC
CB10061	-	-	CTCACGATTAGGGACCG	TCCTTTTCTTTAACAACTCA
CB10062	○	-	GGCTGGTCTTTTCATTATCA	CTCTGTCTTCCTCGTTCATC
CB10069	○	-	GGAAGATAAACCTGACTGGA	ACAAAGCGATGAGTGGGA
CB10071	-	-	CTGAATGGGTTAGCTGAATG	AAACAACCATACACGCACG
CB10074	-	-	TTAACATCTTCTCCACAAAT	AGAAACAAAGAGAGACGAGA
CB10075	○	-	GATTTGGTTTTAGATTTGGA	GAAACAAAAGAGGAACTGC
CB10078	○	-	TGACATCTTGCATACCTTA	CTGCATGTTATCTTTCATAC
CB10079	-	○	TACAGGAAGATTAAATGCC	ACCGTTTGTCAGTGTCATAG
CB10080	○	○	GCCCTCAACCTGTAAAGT	TTGTTGGTGTGTGAATCATA
CB10081	○	○	GGCTTTAGCACTGTGATCCT	TTGGGAGAGAAAACTATACG
CB10083	○	-	GCCTTCGTCATCGGATA	ACAAAGTAAGCCATAACACA
CB10084	○	○	GGGAGAACAAGAGAACCACT	AAACAAACAATGACGACCAA
CB10087	○	○*	TCATGCCGACCGATCAC	TTAAAGAAAATTGGCTACTG
CB10088	-	○	AGGTTAGCGTCCGTCCG	GTGAAAGGGTTGGTTGACAT
CB10089	○	-	AAACATATACACACCCTCAT	CATAACGCTCCTTGGC
CB10092	-	○	TTGATCCGAAATTCTCTGG	AGGCAAGCAATAGATAAAGG
CB10093	○	○*	GACTTGGGAGAGATTAAACA	GGCGATGGTGATTTCCTAGA
CB10095	-	○	CCAAACACCCAACCAACACT	AGGATCTTTGCGTTTTGTAA
CB10096	-	-	CCTATTTTCGCTGACTGTGT	ATGAAGGAGAACCAAACCCA
CB10097	-	-	ACTTCGGTGGTTCTATTTCT	CGACGGTTAATCAAGTTTCT
CB10098	○	-	GCAAAATACTGATCATCCTCG	GCTTCAGTCCAATTTCTCCGTA
CB10099	○	-	CTTCCCCTTTCATCGAACT	TAGAAGCATTTGGAAACGCA
CB10101	○	○*	TGAGGTGTGATAAATGCAGG	CTCTCTCCAACCGCACCGAC
CB10102	-	-	TTTTGTTTGCAGGTAGATGG	ATGAGTCAACGCAATGTGGT
CB10103	○	○	GACGGATGCCTAATAATGAT	TCCTCAAAACTGCCTGTAAG
CB10105	○	○	CGGGTTAACTTGTTACGAG	GGTGTGTGATTTGAGATAGGGT
CB10106	○	○	GTTGGGAGAAGGTTTGGAGT	CTCGGCATTTATGTGTGTTT
CB10107	-	○	TTAATCGGGAATCAAGAAAT	GAGGTATGGGCGGTATGGGA
CB10109	○	○*	GTGTAGCCAGCTTGATCCT	CTTCTTCTGATGCAGCAGTG
CB10111	○	-	CGTCTTCTGGCATTGATCT	CCAGGTCAGCATCATAATTC
CB10112	○	○	ACAGTGACCGTCGTTGATT	CTTCTTCCGTCAAACCAGTA
CB10114	○	○	CCCGTCAACCAAATCACA	CGCAAGGTTGTTATCTAATC
CB10116	○	○	TGCCCTGAACTTAATTGC	CCCAACATCGGAACAAACTG
CB10119	○	-	CAGCCAAGAACTCTCCATAGT	GGAATCAGAGATCCAAGACTC
CB10122	○	○	ACCTGATTTTGAACCGCAGTG	ATTAAGGACTCTGGTGCTAGA
CB10123	-	-	CGAGATTTACTTGCCGATTTG	ATAATCCTGGTAAGTGGTAAC
CB10124	○	○	TATGGGAAGGTTTGTGGTTGC	CACTCCTCGATTACTCTCACT
CB10126	○	-	ATTTCTTCCTCACTTTCAACG	CTCTGTATCATTCCCTTCATC

CB10128	○	-	TCCTGTGCCAAGTTTTACAAG	GGTTACCCTTAGCAAGATATT
CB10132	○	○	CCTGTGGAGACCGTGACTACA	AATTTCGACACAACTGCTTAG
CB10136	○	-	CAGAAGAATCCAGCTTAGTTT	TTGTCTCTTGGGAGGTTACAT
CB10137	○	-	TCCAGCACATTTTCAATCATA	TCTTGGCTTAAAAGGACGGAG
CB10139	-	-	TCTCAAAAGGATATGCGTGAA	CAAAACTCATCAGGGTTGTAG
CB10140	-	○	CTGTTTGCTGCACAAGTGACG	CGCCTCCTCTACCTATACTAT
CB10142	-	○*	ATCTTGGCACTGGTTTGA	TTAACGTGCACACAGCAG
CB10143	○	○	CATGGGAGGCTGTCTAAA	TTGCACCCATACGTTTTC
CB10144	-	-	GCTGCGTGTCTTGGTATC	CAGCTTGTCAGATGCTCC
CB10145	-	-	CTTTGCGACCTTTAACGA	CTGCTCGTTCTCCCTGTA
CB10148	○	○	GCAGTGTCGTTGGATTGT	TCCACGGAAATAGAGCAA
CB10150	-	-	ACGTCAAACTCACATGGC	CCGATGGCATAACCATTA
CB10154	○	○	TCCTTGTGATAGGGTCCA	ATCCCAAGCTGGTCTTTC
CB10155	-	○*	CAGGTATTTCTTGCCCCT	TGCACTCGAGAGGAATGT
CB10158	○	○	TTCGGTTTTGCATTCATT	GGATCCCAAAGGAAGTG
CB10159	-	-	CACAGCTTTACCCAATCG	GATCACCATCTGCACCAT
CB10167	○	○*	TGTGATTTTCTCGTTTTGG	TCATCTTTCACAATTGGCT
CB10170	○	○	GACCCCATGATCCGAATA	AAGACTTGCTTTATTGGAGTT
CB10172	○	-	ATTGGTCTCTTAACCCGC	TTCTCGAATCCCTCGAA
CB10175	-	-	ATCCTCTGCTTCACATGG	TTCCATGACTTCGTCTCC
CB10179	○	-	ACGAAGCAAATAACAAAGA	GAAACCCGAAAGCCTAAG
CB10183	○	-	CGACACCACGAGCTAAAC	AAGCATACATGCCATTCC
CB10184	○	○*	TTTTGTTTTTAGCGCCAC	CAAAGATTCACGGACTGG
CB10186	-	-	CTCCGTGTAAAAGGCTCA	TCATCTTTGCTCCTTCCA
CB10189	○	○	AGAAACATGACACCACCG	GTCCAACTGGTCCTACGTT
CB10192	○	-	GGAGCCACCAATGATAGA	TCGGAGACGATGACATTC
CB10193	○	○*	ATGCCACTTGAACCATTC	CCTGAAGCGCATTTGTTA
CB10195	○	○	CGCTAAGCGTACAAGCAT	GGCTTCTAAACCAGCCTC
CB10196	○	○	TTGTAGGCAATGATGAGGA	GAGAGAAGGGCTCCTTTG
CB10199	-	○	CTCATCATATTCGGCGAC	GCTTGAGTTTCCATGGTG
CB10200	○	-	GGGCACTATGGGAAAATTA	TTGGAAAAATCCGTTCAA
CB10204	○	○	TTATCATATGCTGATCCATT	GACTGTCTAGCTGCTCCAA
CB10205	○	-	CCTCAAAACCTACAAAC	ACACGTTCTTCTCGGTGA
CB10206	○	-	TACAACGCAAACGTTCCT	TTGATGTTCTTGGTGCCT
CB10208	-	-	ACTACTGTTGCGGTTGGA	GGCATTCATTACGTCTGC
CB10209	○	-	CCGCTTAACAACATGACC	TTGCTGTCGTCATTGCTA
CB10212	-	○	TCTTCTCCATCTATCCTTTCA	AGACAGGTTGAATCGCAA
CB10213	○	-	CCTACCTTCCTTTACCAACC	GGTGATGATGATGGGAGA
CB10217	-	-	CCCCATATCATCCCTACC	GCTGAACAACCCACAAAG
CB10218	○	○*	AAATCAAAGGGACAAGGG	CCCCTGATGAACCCTAAC
CB10229	○	-	TTTGGTCTGAATCTGATACT	CCGATTCAACACCTTCAA
CB10232	○	○	TGCAGATTCATAACCAAACT	CAAGCACCATTGAGAAGG
CB10234	○	○	TCTGTTGTTTCTCTCGCC	CTGATGGACTAGGACCCC
CB10235	-	-	GTGGTTACGGTTGTTGGA	CTAACACTTCACACCCGC

CB10236	○	○*	GAGATTTGGTCCAGTCCC	CCAGAGCATGACTTGGAG
CB10242	○	○	TTCCTTTCACGGTTTTCA	TTCCACAGGCACTTTCTC
CB10243	○	-	CGAAGATTTGGAGGGAAT	CAGACATCGTCTCAAGCC
CB10246	○	-	GAAATCAGATGCTCGTCG	CCTATTCCAAGGCGGTAT
CB10248	○	-	TTGGGAAACTTTTGGTGA	TGGTGAGGTCATAAACGG
CB10253	○	-	GGCTTTAAACCGAAAACC	CATCTCTGTTTGGTTGCC
CB10255	○	○	CTGCGCTGCATCTTAGTC	TGAAGAGCAATGCAATCTT
CB10258	-	-	ATGATGCCTAGCATGTCC	AAGCTAAAGCGAAAGAAGC
CB10259	○	○*	CCTTTCGTCCTTATCACC	GCATCAGTCGCCATAACT
CB10262	○	-	AGCAATCAAATCGCAAAG	TCTGAAATCACAAATTCACAA
CB10263	○	-	GCAAGTTGTGAGCTGCTT	CTTGAGAATGGTCAGCCA
CB10265	○	-	CCTGGAGATCGAAGAACA	ACCAAACGCAGATTCCTT
CB10266	○	○*	GTTCTTGACTGCCACCAC	TGTGGTTCACCTGAGCTT
CB10271	○	-	TCCTGACAGGCTGACACT	GGATTCAGAAAGCCAACC
CB10277	○	○*	ACAAATGCTTGAGTGATA	TCTTCGTAAACTTGTTCTTGA
CB10281	○	○	CGAGATGGAGACAGATCG	TAAACTTGACGCAGTCGG
CB10284	○	-	AGCCGTTTAAGACGCATA	GCATTTCCTTAGCTGTGAA
CB10288	-	-	GCAATGCATATCGACCTT	AACCGCGCTATCAAGAAT
CB10290	-	-	CGAGATCCGAGAACAACA	ACCCTAGCCATCAAGACC
CB10291	○	-	TTCAAACACCAATCTGGC	TTCGATGCTGCACCTTAT
CB10297	○	-	CTCATTCCACTGCCAAAC	ACGAGTCACCATGTCAGG
CB10298	○	-	CAACATCCCAGCAGGTAA	CCGTAGCTGTGCTCATTC
CB10300	○	-	CCGCAAATGACAGATAGG	CTCAAATGCGCACCTTAT
CB10302	○	-	CGATACTTGGAGCGTGTC	CTGGTGTCTTAACCACGC
CB10305	○	○	ATAACGCCAAGAACCACC	TGGAATCAAGGTGGAATG
CB10311	○	-	AACGTACCAGAACCGATAGA	AACCCAATTGCATCCTCT
CB10312	○	○*	CCAAATCGAACTGCAAAG	TCTTCCACCGACATGTTT
CB10320	○	-	AGTGCATGATGAAGGCAT	GGGAATCCATGGCTGTA
CB10324	-	-	CTCGAGAGCTACAACCACA	CAACTGATTTTGTTCCCG
CB10326	-	-	CGTGGTAGAGCTGACGAG	ATCTCCACCAGCCTGAAT
CB10329	-	○	AATCATCGAGTGGACGAA	TGAGATACACTCCGGGTC
CB10330	-	○	AGGCGAGTTTACGAGGAT	ACCTGCACCAGTCATTTG
CB10335	-	○	AGACAAGTTGAAGATAGGCTC	GATCGGAGACGGAGAGTT
CB10336	-	-	CAAAACACCCAATTCTCG	GTGGTTGGTTCAGCTTTG
CB10337	-	-	CACCAGCGAATCAACTCT	AGATCCAAGACGAGGAGC
CB10339	-	○	ATCACCTCACCGTCACTG	CCAGGACTACGACGTCAC
CB10347	-	○	ATCTGAACACTTTCGGCA	GGAAGCACCATGTCAGC
CB10348	-	○	CGGAGATTGCTTTTGAAG	ATCATCACCACCAAGTGC
CB10349	-	○	ACCTCCAGATCCAATGCT	TCCTGAGGACAGCGACTA
CB10351	-	○	TCCAGTGAAATGGACTCG	AGCTATCGTCGCTCACAG
CB10352	-	-	CGATGAGTATCGCTTTGC	CCATTGATTCAGGTTTGC
CB10355	-	○	GACGGATTGAGTCGGATA	CCTGCTAGGAAACAGGGT
CB10356	-	○	GCAGATCGGCGACTACTA	GAACCAGAGGAGGCTTGT
CB10357	○	○	CAAAGATTGTGAGCCACC	CCGGATCCCAAATACACT

CB10358	-	○	CGACACAAGAACCGAGTC	CGCATTATCCTCGTCATC
CB10364	-	○*	GAGACGATGCAAAGATCG	TGCAGACACATTCGAACA
CB10367	-	○	GATGCGGCTGATAGCTTA	GCGAGACCGGATAGAGTT
CB10369	○	○	CATTCACAGGACCAGAGC	CAAAGCCAAGACAACCAT
CB10370	-	○	TCCAAGATTGTTGAAAGCA	CCACACGGTGTTCTGTCT
CB10372	-	○	CAGCTTGCTGAACAGCTT	TATGATTGGGATCCGATG
CB10373	○	-	CGGTCAGATTCCAACAGA	GCCATCTCAGAGACGACA
CB10374	-	-	ATGGTGACGAGTTGGATG	CCTCGTCTGGAATCTCCT
CB10377	-	-	CCTGGAAGCTTCTCCTTC	TTTTGGAAGATTCGGACA
CB10378	-	○	GCCAACATGTCACCACTT	ATGATGCTGTGGTGGAAC
CB10379	-	○	TGATTGGTGATGGATGCT	CATTCACTAGACGCCACC
CB10380	○	○	GCTGACTTGCTTCAGCTC	GAAGCACAACTCCAGCAC
CB10382	○	○	TCAACCTCTCACGGGTTA	TGCAATCCTCGAGGTAAA
CB10384	○	-	GTTGACTTGCTTGCCATC	CCTGAAGCTGATTGGTTG
CB10385	○	○	CAGGAGGTTATGCGACAC	TTCAACTCTTCAGGTGCC
CB10386	○	○	ATGTGAGACAACGGATGC	ACTAGCTCTTCATTCACGTTC
CB10388	-	-	GGTGTCGTTCGGACATAA	CTTATCTCTGCTGCTGCG
CB10389	-	○	AGATCCGAATCAAATCCC	ACGAGTTCGAGCATATCG
CB10399	○	○	AGAGTGCATCAGACGTGC	ACCACACTCACATCCTCG
CB10402	-	○	GTCGGTGCTTCGGTTT	TCACATTCTCCACCTTGG
CB10403	○	○	CCACACATGAACCCTGTT	AGGACCAAGATTGGAAGC
CB10413	○	○*	CTTAGCACAACCACTCGG	GGTGATGAAGATGACGATG
CB10415	○	○	GAACTCGTCGCGGTAGTA	TCTCTTTCCTCGCAGATG
CB10416	○	○*	GCTGTTGCTGTAGGTTTGA	GAGCCAGCGTTGATAAGA
CB10418	○	○	TGCGGATTCAATATCACC	CATCCCTGTTGATGGAGA
CB10419	○	-	CTGGAGTCGATGGAACTG	GCCAACATAAGCCAAAGA
CB10420	○	-	CATCAACATCATCAAAACCA	GTTGCTTGTTTCCCCTCT
CB10422	-	-	TCTGATGATTACAGCCAACA	TGTTGACACAGACGTGGA
CB10423	○	-	CAATCATGCATTATGCCC	CAAGGAAAGATGCACCAC
CB10425	○	-	GGTGGCTTGTAGGGACTT	GCTCCCGTAAACTCTTCC
CB10426	○	-	ATTTGGCTAAGGGGAGTG	CCATCCCAGTAAGAGGGT
CB10427	○	-	TCCCAACAAAAGAGTCCA	CAGCGAACCGAGTCTAAA
CB10429	○	○	TGAGGTGATGACGAATCC	TCGGATGACAAAGATGCT
CB10432	○	○*	AGCAACGATTTGCCATAG	AACCAAATCAAAAGTGGGT
CB10433	○	-	CTGTGACTGCATTGCTGA	ACACAATAAAGTGCGGCT
CB10434	○	-	CACGGCATATTAGTTGGG	GAATCGCGGGTTTATCTT
CB10439	○	○	ACCTCGAAGGGTATCTGC	CGTGCAATTTCAACAACA
CB10441	○	-	CTGCGATTGAGGTTTGTC	GACGCAAAACGCACATA
CB10443	○	○*	CAGAAACCATCATAGCCG	TGATTTGGGAGACGAAGA
CB10445	○	○*	CATTGCCTCCATCTTCAG	GGGTCAAGCAGATGAA
CB10448	○	○*	GGGTTTGTCGGAAGAATC	TGTTCGTTCGAAAGGTGT
CB10449	○	-	TTGGATCATTTTGGCATC	TCATCCAGGTAATGTTGTTG
CB10450	○	-	CTTCACCAGCAAAAGCAC	GCTTCGACTCCAAAAACA
CB10454	○	-	CGATTCGAAGGTATCGAG	AGTAATTTCCCAAACCGC

CB10459	○	○*	CCTGCTTTTGCTCTGTTC	GCGATGAAACCAAAGCTA
CB10466	○	○	GAACGGGAGGAGGTTATG	CTTCACCAACACCACCAT
CB10467	○	○*	ATGGATGCAATTTTGGTG	ATTGATCGCCATCTTCAA
CB10471	○	○	CAAGGATCGTCCATGTTC	TCCTTGTAAATGCCCGTA
CB10474	○	-	TCGACAGAGCTAAACCGA	TGTGAAAGATGTGTCGCC
CB10475	○	○	TCAGCCATATAGCTTCGC	CCACTCGCGTCATTTAAG
CB10476	○	-	AGCCAGAATTAAGCAGCA	TGGTAATGGTAGGGATGG
CB10478	○	-	CAATTACAGGAAGAAATGGC	CGTTTCGAACCTTTGTCA
CB10482	-	-	CACGAAAGGTGTATTGGG	ATGCTAACGTGGACAAGC
CB10484	○	○	CGTTACTCGCAAGGAAGA	TTCGAGAGACCGTGAAGA
CB10485	-	-	TGACTGGAGTGGGAACC	ACCAACATATCGACCGTG
CB10487	○	○	ATCCGAGGTTAGGTTTGG	TCCTTGCTCACCCTTGTA
CB10498	○	○	ACTGCGCAGAGACTTGAG	ATCATCATCCCTGGGTTC
CB10501	○	○*	GTACCAGCCGGTTATCAA	CGATGGAGTGGAAGTGAG
CB10502	○	-	TTGAAGAGTGGGGATTCA	GGTGAGCTTCTTCCTTCC
CB10504	○	○*	GGTGTCCCAACTGTTGAA	CATTGGCATAGGAACAGG
CB10506	-	-	GTCCTCTCGTTTCTCTTCG	TCGACTCAATTTCATCCG
CB10508	○	○	GCGGATAAGCCTCTCTTC	ATGGTCTCGGATTGGTCT
CB10509	○	○	TAGTCCCGATCCCTTTTC	TCACTTTGTTGTGGCTGA
CB10511	-	-	AAACCGTCCAAAACTTCC	CCATCGTTCCATCTTCAG
CB10513	○	-	TGTCTTATATGCACAGATCCC	TGCTTGCTCATCAGTGAC
CB10514	○	○	TCCTTGTGATAGGGTCCA	ATCCCAAGCTGGTCTTTC
CB10517	○	-	GGGAATATGTGGTCCTCC	GCATCGAAGCAGTGAGTT
CB10524	○	○	ATGGAAGGCAACGATTCT	TTCTGTGCTAGGTCTGCC
CB10525	○	○	CGGATGAAACCTAAACCC	CTGGGTCCGGTAATTGAT
CB10526	○	○	TTCTTCTTTCCACCACCA	ACTCGGCGGTTAGAGAAT
CB10527	○	○*	AGCGTTTTCCTCCAGACT	CCATGGTTCAAATCCAAA
CB10528	-	-	ATGCTTTCTTTGCACGAG	ACCAGACTGATGGTGTGC
CB10529	○	○	CTTAATCTCCTAGCCGCC	TGTCAGTTGTTTGCACCA
CB10530	○	-	TCCTCGTCCTTTATTCCT	TTTATGGTGATGGGGTGA
CB10533	○	○*	GATGCACGTGTCAATGTG	GGAGGTTGAGGATCTGGT
CB10534	○	-	AGCTGCAACCACAACTCT	GGAGCGCAAGAAAAG
CB10535	○	○	AGAACTCTGGTTTCGCTGT	CTGGACGTTCAGTTCACC
CB10537	-	○*	TTTGACCACTCCATGAGC	AGGCTTCATCAGCAACAG
CB10538	○	○*	TCCGTCTTCAATCTCAGC	TGTTGAAGCTGTGGAGGT
CB10540	○	○*	TCATCAGAATCTTCCACGA	AGCATCACGGGACTTACA
CB10541	○	○	CGTATAGTTTCAGTATTATCA	GGATCACTGGGAGTGTGA
CB10542	○	-	CCTCGCTAGAACCCTTTC	GATGAACCTGACGATCCA

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

RESULTS AND DISCUSSION

Self-incompatibility, the mechanism for preventing self-fertilization, is utilized for harvesting the F_1 hybrid seeds in *B. rapa* vegetables. Self-incompatibility is controlled by the *S* locus (Fujimoto and Nishio 2007) containing *SRK* (*S* receptor kinase)(female determinant), *SP11/SCR* (*S*-locus protein 11/*S*-locus cysteine rich protein) (male determinant), and *SLG* (*S*-locus glycoprotein) as a unit. This unit is called the *S* haplotype, and *S* haplotypes are categorized into two classes, class-I and class-II, by sequence homology (Fujimoto and Nishio 2007). In this study, to identify the *S* haplotype of the commercial F_1 hybrid cultivar "W77", we used class-I and class-II *SLG* specific primer pairs, PS5+PS15 and PS3+PS21, respectively. Both primer pairs amplified in this F_1 hybrid cultivar, indicating that this is heterozygotes of class-I and class-II *S* haplotypes. To confirm the segregation of parental alleles in the F_2 population, *S* haplotypes of six F_2 plants were analysed by class-I and class-II *SLG* specific primer pairs. Among six F_2 plants, five plants were class-I/-II heterozygotes and one plant was class-II homozygote (Figure 1), confirming that *SLG* alleles, *S* haplotypes, have segregated in the F_2 population.

Previously we have screened 12 SSR DNA markers, which show polymorphism between the parental lines of commercial F_1 hybrid cultivar of cabbage (*B. oleracea*), "YR Kinshukyoryoku 152" (Tomita *et al*., 2013), and in this study we assessed these markers on Chinese cabbage (*B. rapa*) cultivar, "W77". Of 12 SSR DNA markers, five markers (41.7%) showed clear PCR amplification in agarose gel, and eight markers (66.7%) showed clearly visible bands in acrylamide gel (Figure 2). Using the eight markers, we examined the polymorphisms between F_1 hybrids and F_2 plants, and two DNA markers (BoGMS0394, BoGMS1185) showed polymorphism between F_1 hybrids and F_2 plants (Figure 3).

Next, we screened the SSR DNA markers, which can detect the polymorphism between parental lines of "W77". We examined the PCR amplification using the reported SSR DNA markers, BRAS, BRMS, KBr, CB, etc. (Table 1). Of the 321 markers, 220 markers (68.5%) showed clear PCR amplification in agarose gel and 167 markers (52.0%) showed clearly visible bands in acrylamide gel (Figure 4, Table 1).

Using 167 markers, we examined the polymorphisms between F_1 hybrids and F_2 plants. Fifty-nine DNA markers (18.4%) showed polymorphism between F_1 hybrids and F_2 plants (Figure 5), and these polymorphisms are due to the difference of the nucleotide sequences between parental lines of F_1 hybrid. These results indicated that 59 markers that we found in this study can be used for the purity test of inbred lines as these DNA markers can detect the polymorphisms between parental inbred lines of the F_1 hybrid cultivar "W77".

Of 59 SSR markers, we predict the positions of 38 SSR markers using Brassica Database (http://brassicadb.org/brad/). Thirty-eight SSR markers were distributed in all chromosomes except for A07 (Table 2), indicating that SSR markers we found covers a wide range of the *B. rapa* genome. Purity test using these SSR marker sets could lead to reliable assessment of genetic homogeneity, as there is little bias of SSR marker positions.

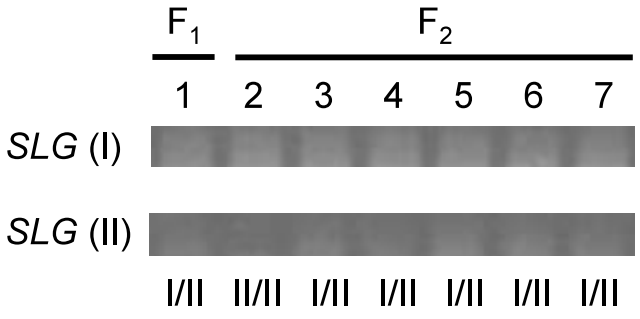

Figure 1. PCR analysis using class-I and class-II specific *SLG*. I and II showed the parental *S* haplotypes in individual F_2 plants derived class-I/-II heterozygous F_1 hybrid.

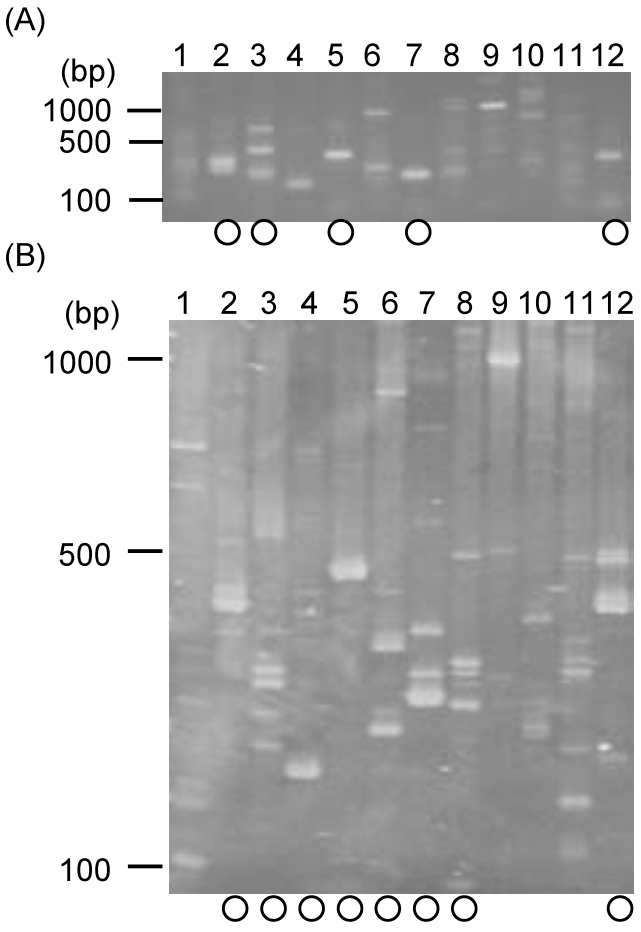

Figure 2. Confirmation of PCR amplification in F_1 plant using previously screened SSR markers (Tomita *et al*., 2013) on agarose (A) or acrylamide (B) gel. ○ shows clear visible bands.

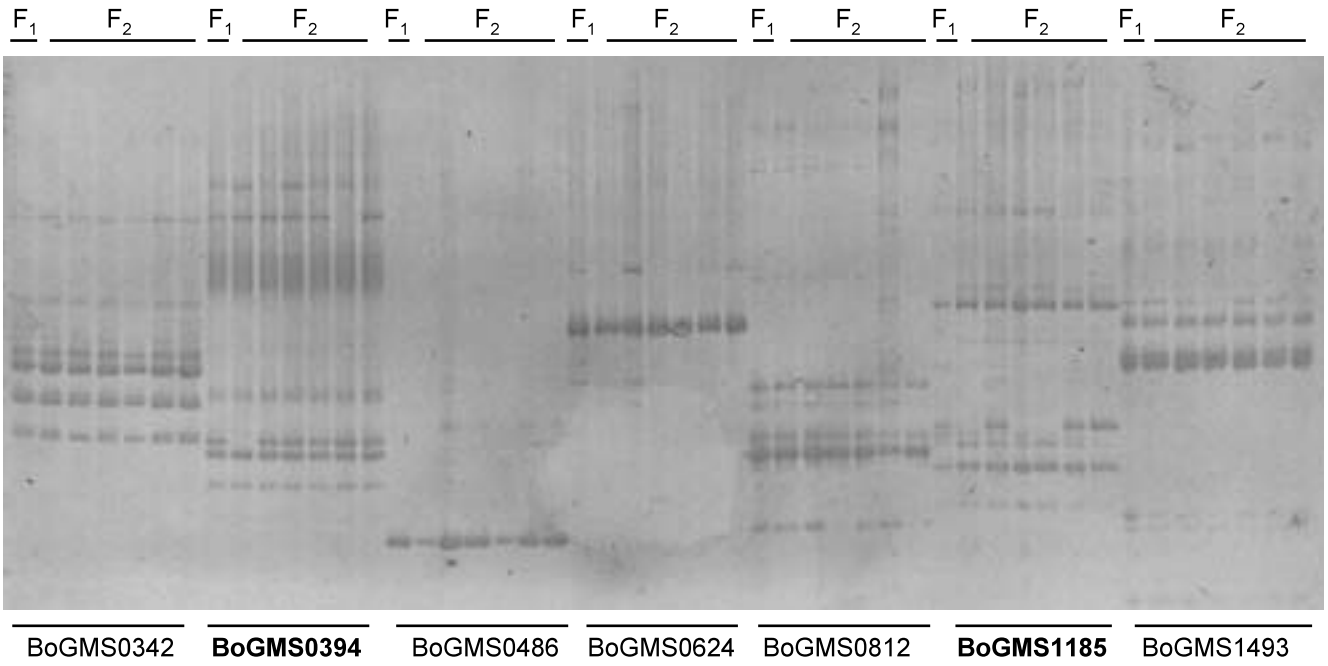

Figure 3. Detection of the polymorphisms between F_1 hybrids and F_2 plants using 7 markers that showed clear visible bands on acrylamide gel shown in Figure 2. Bold letters represent the DNA markers having polymorphism among F_2 plants.

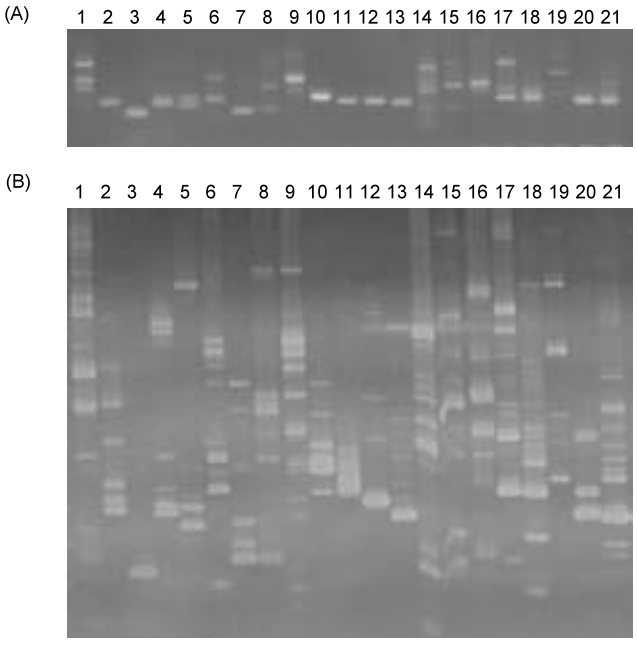

Figure 4. Confirmation of PCR amplification in F_1 plant using SSR markers on agarose (A) or acrylamide (B) gel. Lane numbers are consistent with the numbers shown Table 1.

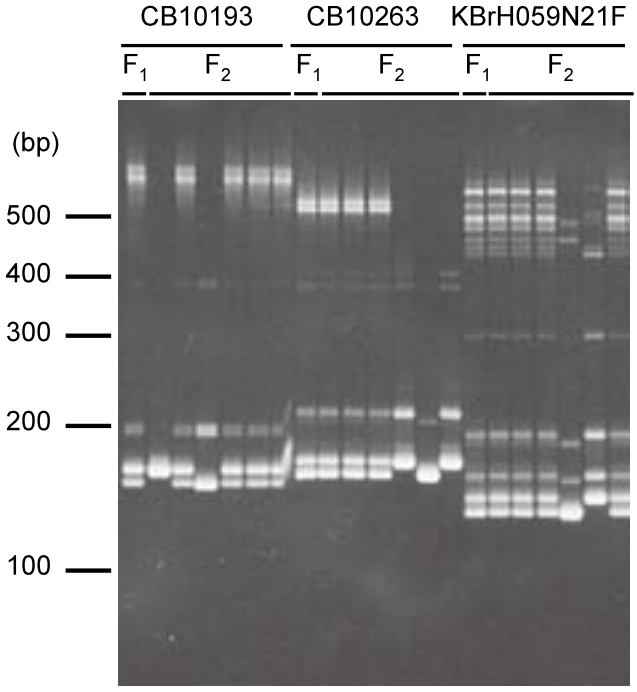

Figure 5. Detection of the polymorphisms between parental alleles of F_1 hybrid cultivar using SSR markers.

Table 2 Putative position of the 39 SSR markers

Marker name	Chromosome	Position (Mbp)	Marker name	Chromosome	Position(Mbp)
BRMS096	A01	5.4	BRMS027	A06	2.0
BRAS039	A01	6.6	BRMS017*	A06	14.2
BRMS184*	A01	21.0	BRMS184*	A06	14.4
CB10093	A02	11.9	CB10432	A06	16.0
BRMS184*	A02	21.3	CB10014	A06	21.0
CB10416	A02	22.8	BRMS226	A06	21.5
CB10540	A02	23.3	CB10101	A06	23.2
BRMS026	A02	24.1	CB10030	A06	23.9
KBrB007I08	A02	-	KBrH071B03Ra	A08	3.5
BRMS315	A02	-	CB10364	A08	6.2
BRMS330	A03	1.7	CB10236	A08	9.3
BRAS065	A03	13.3	CB10445	A08	20.0
KBrB002E24	A03	21.7	CB10459	A09	3.4
KBrH059N21F	A03	24.2	BRMS017*	A09	14.2
CB10413	A03	27.8	CB10501	A09	29.1
BRMS276	A04	6.1	CB10504	A09	34.1
BRMS017*	A05	2.6	CB10533	A09	37.7
CB10193	A05	8.2	CB10109	A10	6.4
BRMS057	A05	15.1	BRMS085	A10	8.3
BRMS163	A05	15.5	CB10167	A10	9.6
BRMS007	A05	24.2	KBrH054N12R	A10	11.3

* shows that these markers are predicted to be mapped to several position.

Two DNA markers (BoGMS0394, BoGMS1185) showed polymorphism between F_1 hybrids and F_2 plants both in *B. oleracea* and *B. rapa*, suggesting some SSR markers are likely conserved in the genus Brassica. In this study we found 59 SSR markers in *B. rapa*, and these may provide preferential choice over other DNA markers when screening for polymorphisms in other Brassica crops.

As SSR makers are co-dominant, multi-allelic, easily detected, hyper-variable, highly reproducible, and abundant in the genome (Morgante and Olivieri 1993), SSR markers are widely used for cultivar identification, genetic diversity, phylogenetic relationship analysis, genetic map construction, and linkage / association mapping of gene / QTL (Quantitative trait locus) (Beckmann and Soller 1990; Gupta and Varshney 2000; Barth *et al*., 2002; Deleu *et al*., 2009). Fifty-nine SSR markers, which can detect the polymorphisms of parental lines of F_1 hybrid, could increase the chance of finding polymorphisms when you need the DNA markers. However, further experiments will be required to determine whether these DNA markers allow purity detection in other inbred lines of *B. rapa*.

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Brassica rapa 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

川村和隆[#,1]・佐伯なつみ[#,1]・江部裕介[#,1]・阿部寛史[#,1]・清水元樹[1]・
長部謙二[2]・岡崎桂一[1,*]・川辺隆大[1]・藤本龍[3]

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要　約

親候補となる近交系統の作成は、植物の一代雑種（F_1）品種の育成過程の最初の段階である。一般的に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、*Brassica rapa* L. 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、321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。材料にはハクサイの市販F_1品種であるW77のF_2個体を用いた。59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DNA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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[1] 新潟大学大学院自然科学研究科

[2] 沖縄科学技術大学院大学植物エピジェネティクスユニット

[3] 神戸大学大学院農学研究科

[*] 代表者 : okazaki@agr.niigata-u.ac.jp