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Introgression of *Sub1* QTL into a rainfed lowland rice variety of Bangladesh using marker-assisted backcross approach

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ABSTRACT

BRRI dhan49 is an early, rainfed lowland and popular rice variety in Bangladesh, but does not possess the flash flooding tolerant QTLSub-1. Molecular markers are tightly linked to submergence tolerant QTL (Sub-1) was utilized in a marker assisted selection program to develop elite breeding lines with broad-spectrum tolerance to flash flood. Sequence tagged site (STS) and simple sequence repeat (SSR) markers were used to detect the submergence tolerant genes. A cross was made between BRRI dhan49 and BRRI dhan52 (a high yielding monsoon rice containing submergence tolerant Sub-1 QTL). High-volume backcrossing techniques were followed to produce large numbers of backcross progeniesfrom the selected plants of different backcross generations. In foreground selection, markers closely linked to Sub-1 QTL were used to select plants possessing the tolerant genes. At BC_4F_1 generation, background selection was done to select plants that have maximum contribution from the recurrent parent genome through polymorphic microsatellite markers between donor and recurrent parents. Three best plants were selected and their recipient genome recovery percentages were 86.84%, 85.13% and 85.0%, respectively. This research work illustrates the successful application of marker-aided breeding to introgress the fla *sh* flood tolerant *QTL into anearly, rainfed lowland, medium fine grain and popular rice variety of Bangladesh.This improved BRRI dhan49-Sub-1 line will be more adaptable under the submergence prone domains of Bangladesh.*

Key words:
Sub1 QTL; Marker-assisted backcross; Rainfed lowland rice

INTRODUCTION

Rice productivity is severely affected by several biotic and abiotic stresses such as salinity, submergence, diseases, insects etc. Flash flood submergence is one of the important hazards to the agriculture of Bangladesh related to climate-change. Submergence stress regularly affects 15 million hectares or more of rainfed lowland rice (RLR) areas in South and Southeast Asia(Neeraja*et al.* 2007). To ensure the rice production by escaping submergence stress in the flash flood affected areas, flash flood tolerant genes or QTLs are necessary to introgress into more number of RLR modern varieties.

BRRI dhan49 is one of the most popular, early RLR variety with high yield potential (5.5 t/ha) possesses one week shorter growth duration compared to another RLR mega variety BR11 and shows lodging tolerance due to shorter plant height (100 cm). This variety is being widely cultivated by the RLR farmers mainly due to its medium fine grain and high value in the market.

Though rice is being cultivated under flooded and irrigated condition, most of the

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ricevarieties are susceptible to flooding if the plants are submerged under water for more than sevendays (Adkins *et al.*1990). A number of submergence tolerant improved breeding lines were developed at IRRI like Swarna*Sub-1* was released in Indonesia and Bangladesh. Among 61 inbred varieties released by Bangladesh Rice Research Institute (BRRI), Bangladesh, BRRI dhan51 and BRRI dhan52 are only two flash flood tolerant those are developed by introgression of *Sub-1* QTL but their growth duration is long. So, the places where more than one time flash flood occurs, the duration of these varieties are so long that flowering becomes affected by cold. That's why BRRI dhan49, an early RLR variety with shorter growth duration (135 days) were selected as a recipient parent for introgression of *Sub-1* QTL through marker-assisted backcrossing (MAB). MAB is used to transfer genes conferring favorable agronomic traits into the genetic background of a recipient parent (Hospital 2005).

This study was dealt with developing BRRI dhan49 into a submergence tolerant variety by incorporating *Sub-1* QTL using marker assisted selection.Under the present investigation, marker assisted foreground selection was conducted for confirmation of *Sub-1* QTL. Recombinant and background selection were used to minimize linkage drag and recover the recurrent parent genome, respectively.

MATERIALS AND METHODS

Plant materials and crossing scheme

BRRI dhan52, the rainfed lowland high yielding rice variety of Bangladesh, carrying the submergence tolerant *Sub-1* QTLserved as the donorparent during the cross . This variety was derived from the cross BR11/IR40931-33-1-3-2 (Iftekharuddaula*et al.* 2011). BRRI dhan49, an early ,popular RLR variety having 135 days growth duration, high yielding with moderately fine type grain was used as a recipient parent in the crossing scheme.BC_4F_1 population developed earlier as a part of varietal development processes of Plant Breeding Division, BRRI, Gazipur, Bangladesh was used for molecular studies. The initial work was started in Monsoon 2009with hybridization between BRRI dhan49 and BRRI dhan52 to introgresssubmergence tolerance*Sub-1* QTL into genetic background of BRRI dhan49.

Molecular marker analyses

DNA was extracted following modified Miniscale method (Zheng *et al* 1995). Miniscale is a standard method for DNA extraction which was followed in most cases. Spectrophotometer method was used and 25 ng/µl concentration of DNA samples was maintained for good amplification. PCR was performed in 10 µl reactions containing 3µl of DNA template, 1 µl 10X TB buffer, 1.35 µl 25 mM $MgCl_2$, 0.2 µl of 10 mMdNTP, 0.5 µl each of 10 µM forward and reverse primers and 0.1 µl of *Taq*DNA polymerase (5 U/µl) using GStorm thermal cycler (Chen *et al.* 1997, Neeraja*et al.* 2007). Ten micro liter of mineral oil was added in each well to prevent evaporation.In all cases template DNA was initially denatured for 4 min at 94°C, each cycle comprises 45 sec denatured at 94°C, 45 sec annealing at 55°C, and 1:30 min extension at 72°C with a final extension for 7 min at 72°C at the end of 35 cycles. The PCR products were mixed with bromophenol blue gel loading dye. The amplified products were analyzed by electrophoresis on 8% polyacrylamide gel using mini vertical gel tank for high throughput manual genotyping (CBS Scientific Co. Inc., CA, USA). The gels were stained in 0.5 mg/ml ethidium bromide and photos were taken under UV.

Genotyping was done using SSR marker RM8300 and gene-based marker Sub1C173 for foreground selection. Recombinant selection was done using four flanking markers SC41, RM23805, RM23901 and RM23915. Background selection was done using 40 SSR polymorphic markers. And finally three plantswere selected on the basis of phenotypic, foreground, recombinant and background selection. BC_5F_1 plants were producedfrom the selected BC_4F_1 plants.

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Details of the used markers are presented in Table1.

Data analyses

The marker data was analyzed using computer software called Graphical Genotyper (GGT 2.0) developed by Dr. Ralph van Berloo, Laboratory of Plant Breeding,Wageningen Agricultural University, Netherlands (Van Berloo 2008). The software provided the high quality graphical illustration of the percentage of recurrent parent chromosomal segments in the selected segregants of the backcross population.

RESULTS AND DISCUSSION

This research work utilized the BC_4F_1 seeds for molecular analyses. An intensive primer survey was carried out prior to MABC scheme for having evenly distributed polymorphic markers.

Foreground Selection

Foreground selection was performed among 62 BC_4F_1 plants in order to detect *Sub-1*QTL. Foreground selection was carried out using a robust tightly linked marker RM8300. The size of the resistant allele of this tightly linked marker, which was obtained from the donor of *Sub-1* QTL viz. BRRI dhan52, was 205 bp and that of susceptible allele obtained from BRRI dhan49 was 195 bp. This marker produced very conspicuous bands and it was possible to identify the genetic constitution of the *Sub-1* locus very easily using polyacrylamide gel electrophoresis. Out of 62 plants, 29 plants were found showing the locus for the tightly linked marker as heterozygous state, 31 plants were found with the locus fixed for recipient allele (susceptible allele) i.e. homozygous state and only 2 plants were found with the locus fixed for donor allele (resistant allele). It was expected that those 29 individuals possessed the submergence resistant allele, so these segregants were selected for recombinant selection and promoted to further selection steps. Fig. 1 shows the gel picture of the foreground selection with the tightly linked marker RM8300 in BC_4F_1 generation.

Recombinant Selection

The target of recombinant selection in BC_4F_1 generation was to obtain single recombinant type segregant at one side of the *Sub-1* QTL. Single recombinant type segregants were those types of segregants which minimized linkage drag at one side of the QTL. In other sense, single recombinant type segregants possessed the recipient allele for both the flanking markers used at any one side of the QTL. These types of segregants were very rare in frequency. Frisch *et al.*(1999) reported that relatively small population sizes could be used for recombinant selection. A total of four flanking markers (SC41, RM23805, RM23901 and RM23915) from the sub-telocentric *Sub-1* region of chromosome 9 were used in BC_4F_1 generation. The description of these flanking markers is presented in Table 2.

SC41 and RM23805 were used as flanking marker for proximal end and RM23901 and RM23915 were used for distal end. The distance between the closest flanking marker and *Sub-1* QTL was 1.8 Mb (2.3 cM) at proximal end and 0.6 Mb (2.4 cM) at distal end (Table 2). The scores for flanking markers viz. RM296, RM8300 & RM23915 were 'B' which indicated that the plants had fixed donor allele at the proximal and distal end of *Sub-1* QTL in BC_4F_1 generation. It was not possible to minimize linkage drag at both sides of the QTL. As the chromosomal background of BRRI dhan52 near *Sub-1* QTL did not affect phenotype of the BRRI dhan52. Hopefully, non-minimization of linkage drag in the genetic background BRRI dhan49-*Sub-1* will not also affect its phenotype. The gel picture of recombinant selection SC41 is shown in Fig. 2.

Background Selection

A total of 40 microsatellite markers were used for background selection over 10 BC_4F_1 plants resulting from foreground, phenotypic and recombinant selection. Maximum number of background markers used was 10 for

chromosome 9 and minimum number of background markers used was 1 for chromosome 3, 4 and 12. Hospital *et al.*(1992) and Visscher (1996) reported that, as a general rule, two to four markers per 100 cM could be efficiently used to accelerate the recovery of the recurrent parent genome in the early generations such as BC_1F_1 or BC_2F_1. The reason for this was that in early generations, few recombinant events occurred so that donor chromosome segments were represented by a few long segments on each chromosome. Neeraja*et al.*(2007) used 56 SSR markers as initial background markers for the development of Swarna-Sub1. However, the average number of background markers over all the 12 chromosomes was 3.5. The percent markers homozygous for the recipient parent ranged from 51.35 to 82.75% in those 10 plants (Table 3).

In BC_4F_1 generation, plant no 42, 47 and 49 were considered as best plants on the basis of phenotype and recipient genome recovery. The percentage of recipient genome recovery in the best plants 42, 47 and 49 were 86.84%, 85.13 and 85.0%, respectively. Inplant number 42 (Table 3& Fig. 3) of BC_4F_1 generation, 28 markers out of 42 markers (73.68%) was like recipient parent type. But if the alleles of the homologous chromosomes were considered, the percentage of recipient alleles in plant number 42 was 86.84%. So, this plant had been considered as the best plant of that population. Fig. 3 shows the graphical genotype of the best plant 1. The red coloured regions on the chromosomes indicated homozygous region for the recipient genome while the yellow coloured regions indicated the heterozygous regions and the gray coloured regions indicated unknown region. The distances were represented in cM based on published map of Temnykh*et al.*(2001).

In BC_5F_1 generation, foreground and background selection will again be applied to identifythe best plant which will be possible to self-pollinate in order to obtain homozygosity in BC_5F_2 generation, winter season (2012-13). In this way, homozygous BRRI dhan49- *Sub-1* line will be produced in Boro 2012-13. Hopefully, newly developed BRRI dhan49-*Sub-1* lines could be useful as a short duration submergence tolerant rice line for the flash flood affected greater Northern region of Bangladesh. The newly developed line will also be suitable in the environment affected by multiple flash floods due to shorter growth duration.

The best plant was identified possessed 86.84% recipient parent genetic background along with target *Sub-1* QTL in the heterozygous state by using foreground, recombinant and background selection through marker assisted backcrossing. The rest of the genome was segregating for the remaining heterozygous background markers. The recovery of recipient parent genome in the best plant of BC_4F_1 generation indicated that one more backcrossing and a final self-pollination in the BC_5F_1 generation would be required in order to develop BRRI dhan49-*Sub-1* homozygous line and it will be completed in BC_5F_2 generation. This research work reflected that the approaches could be routinely used in order to introgress tolerant or resistant genes or QTLs, conferring tolerances or resistances to biotic and abiotic stresses, into the genetic background of high yielding varieties. The newly developed BRRI dhan49-*Sub-1* lines could be useful as short duration submergence tolerant rice line for the flash flood affected area of Bangladesh.

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Table1. Details of polymorphic markers

Sl. No.	Chrom No.	Primers	Position (cM)	Position (Mb)	Band Size	BRRI dhan49	BRRI dhan52	Forward	Reverse	Repeat motif
1	1	RM272	37.3	-	240.00-245.00	B	A	AATTGGTAGAGAGGGGAGAG	ACATGCCATTAGAGTCAGGC	(GA)9
2	1	RM583	58.9	-	193.00-199.00	B	A	AGATCCATCCCTGTGGAGAG	GCGAACTCGCGTTGTAATC	(CTT)20
3	1	RM104	186.6	-	221.44-230.72	A	B	GGAAGAGGAGAGAAAGATGTGTGTCG	TCAACAGACACACCGCCACCGC	(GA)9
4	1	RM414	191.4	-	230.00-235.00	B	A	ATTGCAGTCATGCAGCAGTC	ATATCTCCAATGTGGCAGGG	(ATGC)6
5	2	RM110	6.9	1.32	146.23-165.64	B	A	AAATTCGAAGCCATCCACCAACG	GCCGACGAGGTCGAGTAGAAGG	(GA)15
6	2	RM423	28.7	-	229.27-302.84	B	A	AGCACCCATGCCTTATGTTG	CCTTTTTCAGTAGCCCTCCC	(TTC)9
7	2	RM452	58.4	-	204.86-211.64	B	A	CTGATCGAGAGCGTTAAGGG	GGGATCAAACCACGTTTCTG	(ACG)9
8	2	RM525	143.7	-	108.52-112.33	A	B	GGCCCGTCCAAGAAATATTG	CGGTGAGACAGAATCCTTACG	(AAG)12
9	2	RM341	82.7	-	119.28-152.24	B	A	CAAGAAACCTCAATCCGAGC	CTCCTCCCGATCCCAATC	(CTT)20
10	2	RM208	186.4	35.13	175.07-145.60	A	B	AGTACCACCACCATTCTCTGCAAGC	TCGATTGGCCATGAGTTCTCG	(CT)12
11	3	RM231	15.7	-	179.88-195.20	B	A	CCAGATTATTTCCTGAGGTC	CACTTGCATAGTTCTGCATTG	(CT)16
12	4	RM119	76.1	-	178.00-180.48	A	B	CATCCCCCTGCTGCTGCTGCTG	CGCCGGATGTGTGGGACTAGCG	(GTC)6
13	5	RM153	0.0	0.167	197.77-204.35	A	B	GCCTCGAGCATCATCATCAG	ATCAACCTGCACTTGCCTGG	(GAA)9
14	5	RM574	41.0	3.42	160.17-167.51	A	B	AAACTAGCCACGGTTTGGTAGGG	AGGGTGGCAGGGATGTAATTTCC	(GA)11
15	5	RM598	68.3	-	184.38-203.46	A	B	GAATCGCACACGTGATGAAC	ATGCGACTGATCGGTACTCC	(AGC)9
16	5	RM334	141.8	-	182.28-195.46	A	B	GTTCAGTGTTCAGTGCCACC	GACTTTGATCTTTGGTGGACG	(CTT)20
17	6	RM589	3.2	-	189.54-227.13	A	B	ATCATGGTCGGTGGCTTAAC	CAGGTTCCAACCAGACACTG	(AC)24
18	6	RM136	51.2	-	98.32-104.32	B	A	GAGAGCTCAGCTGCTGCCTCTAGC	GAGGAGCGCCACGGTGTACGCC	(AGG)7
19	6	RM528	121.6		249.78-258.39	A	B	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC	(AGAT)9
20	6	RM340	133.5	-	119.0-157.86	A	B	GGTAAATGGACAATCCTATGGC	GACAAATATAAGGGCAGTGTGC	(CTT)8T3(CTT)14
21	7	RM481	3.2	-	146.00-157.62	A	B	TAGCTAGCCGATTGAATGGC	CTCCACCTCCTATGTTGTTG	(CAA)12
22	7	RM455	65.7	-	130.96-154.67	A	B	AACAACCCACCACCTGTCTC	AGAAGGAAAAGGGCTCGATC	(TTCT)5
23	8	RM256	101.5	24.14	105.20-110.50	A	B	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC	(CT)21
24	8	RM458	121.8	-	175.80-184.51	A	B	GGTGATCTGCATTGTCAACG	TGCAATGGATCTAGCGACTG	(TAG)8
25	9	RM296	0.0	-	122.49-129.60	A	B	CACATGGCACCAACCTCC	GCCAAGTCATTCACTACTCTGG	(GA)10
26	9	RM23778	0.8	3.9	260.70-278.24	B	A	ACACAGCCTAAAGGTGTTCTGAGC	GAGCTTCGGCCCTATAGTCTTCTC	(AAAT)20

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Contd. **Table1.**

Sl. No.	Chrom. No.	Primers	Position (cM)	Position (Mb)	Band Size	BRRI dhan49	BRRI dhan52	Forward	Reverse	Repeat motif
27	9	RM23805	2.1	4.5	164.30-183.02	B	A	GAGACAGATGTGTACGGTTTGGTG	TTGACAAGGGAATTGAAGGAGAAG	(AC)13
28	9	RM23843	2.4-3.2	5.6	298.93-315.61	B	A	TCACAGACATAATTGTTTGGAGAAGG	CCAAAAGCTTTCATCTTTTTGTCC	(AT)40
29	9	Sub1C173	4.4		198.92-213.86	B	A	TGAGCACCATGCAATAACTGTCG	CAGTGTTGGTGTGTGGGATTCG	
30	9	RM8300	6.8-10.0		206.26-217.77	B	A	GCTAGTGCAGGGTTGACACA	CTCTGGCCGTTTCATGGTAT	
31	9	RM23901	6.8-10.0	6.9	252.20-256.78	B	A	CGATTTGTTGTCAGCGTGAATTAG	CAAAAATCATCTATTTTCGCAGGC	(AT)45
32	9	RM23915	10.0	7.2	260.26-273.44	B	A	CACATAGTTTCCATGCTCGTTCAC	GGTAGAATCCATGACCGTCTCATC	(AC)15
33	9	RM219	11.7		210.74-224.41	B	A	CGTCGGATGATGTAAAGCCT	CATATCGGCATTCGCCTG	CT
34	9	RM23958	20.7	7.9	109.40-117.02	B	A	CAACTAGCCCTACCGTGCAT	CGTGTTAAAGCAGCGAAACA	(AG)15
35	9	RM434	57.7	-	195.65-224.75	A	B	GCCTCATCCCTCTAACCCTC	CAAGAAAGATCAGTGCGTGG	(AG)12
36	9	RM245	112.3	-	148.97-154.22	A	B	ATGCCGCCAGTGAATAGC	CTGAGAATCCAATTATCTGGGG	CT
37	10	RM244	15.0	-	159.50-161.75	A	B	CCGACTGTTCGTCCTTATCA	CTGCTCTCGGGTGAACGT	(CT)4(CG)3C(CT)6
38	10	RM216	17.6	-	135.94-149.75	A	B	GCATGGCCGATGGTAAAG	TGTATAAAACCACACGGCCA	(AG)12
39	10	RM536	55.1		242.98-248.44	A	B	CACTCACACGAACGACTGAC	CGCAGGTTCTTGTGAAATGT	(TC)11(CT)5C3(CT)5
40	11	RM229	77.8	18.37	112.0-117.20	A	B	AGCCCCGAATAAATCCACCT	CTGGAGGAGCATTTGGTAGC	(TC)6ATT(CT)11
41	11	RM254	110.0	-	210.36-247.97	A	B			
42	12	RM12	109.1	-	154.48-182.34	B	A	TGCCCTGTTATTTTCTTCTCTC	GGTGATCCTTTCCCATTTCA	AA

A= Indicates bigger allele and B= Indicates smaller allele between two parent

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Table 2. Flanking markers used in BC_4F_1 generation.

Sl. No.	Markers (RM numbers)	Position in Mb	Position in cM
1	SC41	3.3	0.8
2	RM23805	4.5	2.1
3	RM23901	6.9	10
4	RM23915	7.1	10
Position of *Sub-1* QTL		6.3	4.4 – 6.8

Table 3. Results of background selection, BC_4F_1 generation.

Items	Plant`s Number									
	12	15	16	26	31	40	42	47	49	61
Recipient parent (A)	19	22	26	26	27	25	28	26	21	24
Heterozygous (H)	18	16	12	13	10	13	10	11	9	5
Donor parent (B)	0	0	0	0	1	0	0	0	0	1
% A	51.35	57.89	68.42	66.66	72.97	65.78	73.68	70.27	70	82.75
%Recipient allele	77.8	78.94	84.21	83.33	86.48	82.89	86.84	85.13	85.0	91.37
Ranking for best plant selection[1]	8	7	4	5	10	6	1	2	3	9

[1]Lowest values of ranking indicate better than highest value.

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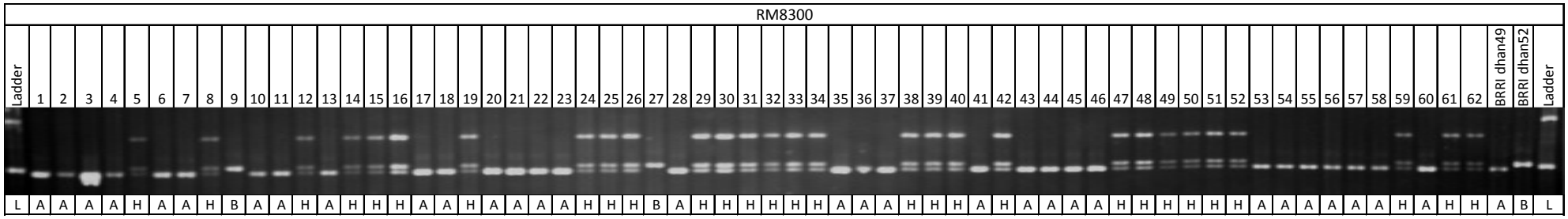

Figure 1. Gel picture of the foreground selection with the tightly linked marker RM8300 in BC_4F_1 generation. (1-62 = BC_4F_1 generations developed between the cross of BRRI dhan49 and BRRI dhan52, L=Ladder marker, A= Recipient parent, B= Donor parent and H= Heterozygous).

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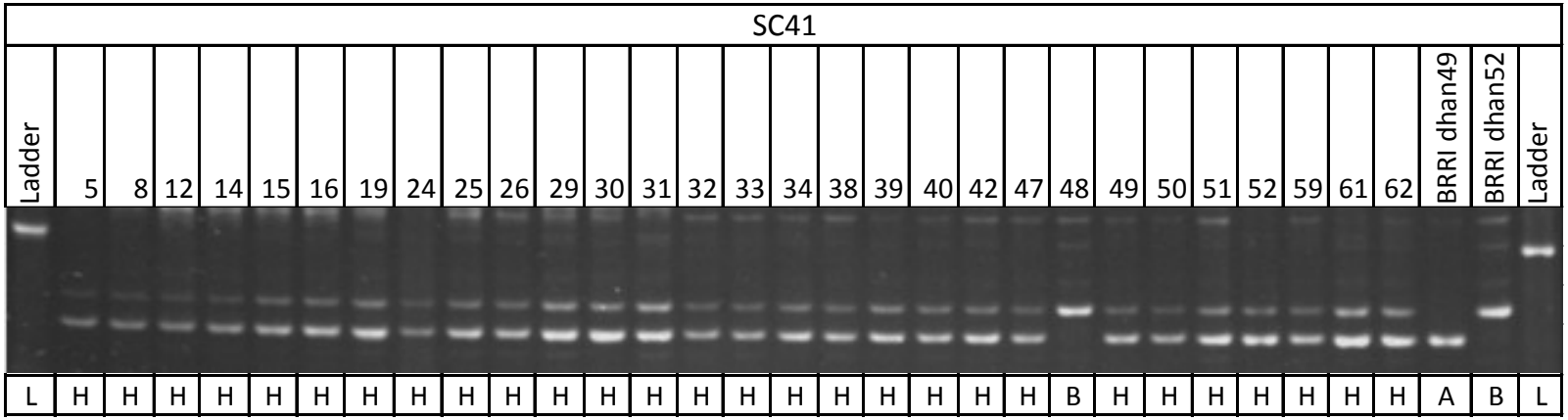

Figure 2. Gel picture shows the partial view of the recombinant selection with the flanking marker SC41. (5, 8, 12, 14, 15, 16, 19, 24, 25, 26, 29, 30, 31, 32, 33, 34, 38, 39, 40, 42, 47, 48, 49, 50, 51, 52, 59, 61 and 62 = recombinants selected by flanking marker, L=Ladder marker, A= Recipient parent, B= Donor parent and H= Heterozygous).

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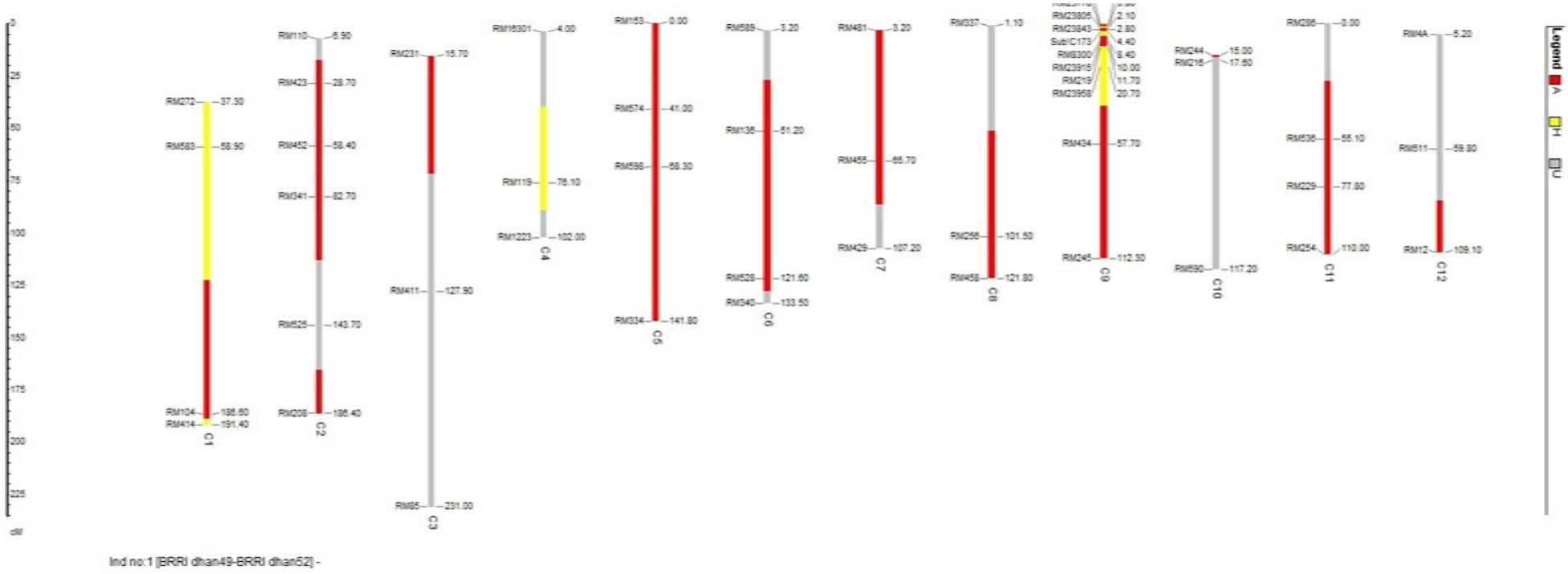

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Figure 3. Graphical genotype of the selected best plant of BC_4F_1 generation. The red colored regions on the chromosomes indicated homozygous region for the recipient genome while the yellow colored regions indicated the heterozygous regions and the gray colored regions indicated unknown region. The distances were represented in cM based on published map of Temnykh*et al.* (2001).