

Original Research Article

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Studies on Marker Assisted Background Screening of Sorghum Downy Mildew Resistant Introgressed Lines (BC₃F₃) in Maize

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ABSTRACT

The present investigation was carried out at Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. The main objective of this research is to retain the recipient parent genome outside the target gene with help of SSR markers. For this purpose, background screening was done in the selected four introgressed lines (BC₃F₃) viz., UMI 79/936-C1-7-7-7-46-2, UMI 79/936-C1-7-7-7-80-17, UMI 79/936-C1-7-7-7-92-1 and UMI 79/936-C1-7-7-7-92-7 with major QTLs for resistance to sorghum downy mildew in order to estimate the recovery of the genome of the recurrent parent. A total of 51 SSR markers, which showed distinct alleles between the parents, were selected for the genome wide background analysis. The graphical genotyping of the BC₃F₃SDMresistant introgressed lines revealed that the complete recovery of UMI 79 genome was present in chromosome 3 in UMI 79/936-C1-7-7-7-46-2 and UMI 79/936-C1-7-7-7-80-17. The complete recovery of UMI 79 genome was present in chromosome 7 in UMI 79/936-C1-7-7-7-92-1 and UMI 79/936-C1-7-7-7-92-7. The genotype UMI 79/936-C1-7-7-7-46-2 has revealed that the 92.45 per cent (maximum recovery) of the genome were derived from the recurrent parent UMI 79, genotype UMI 79/936-C1-7-7-7-80-17 and UMI 79/936-C1-7-7-7-92-7 showed that 89.68 per cent and 80.6 per cent of recovery respectively.

Keywords

Back ground screening, SSR Markers, Sorghum Downy Resistant lines, Maize

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Introduction

Maize (*Zea mays* L.) is an important human food, livestock feed, and an industrial raw material. It is an important cereal in many developed and developing countries of the world. In terms of breeding, it is one of the most studied species and has been used as a model in many situations. It is widely used for animal feed and industrial raw material in the

developed countries where as the developing countries use it in general for feed. In Indian Agriculture, Maize occupies a prominent position and each part of the maize plant is put to one or the other use and nothing goes as waste. In India, about 28 per cent of maize produced is used for food purpose, about 11 per cent as livestock feed, 48 per cent as poultry feed, 12 per cent in wet milling industry and 1 per cent as seed (AICRP on

Maize, 2007). However, demand for maize crop in the developing countries are expected to surpass the demand for both wheat and rice by the year 2020 (Prasanna and Hoisington, 2003).

In India, maize is grown in a wide range of environments, extending from extreme semi-arid to sub-humid and humid regions. The crop is also very popular in the low- and mid-hill areas of the western and northeastern regions. In the tropics, grain is primarily grown for human consumption. The demand for maize as an animal feed will continue to grow faster than the demand for its use as a human food, particularly in Asia, where a doubling of production is expected from the present level of 165 Mt to almost 400 Mt in 2030. (Paliwal *et al.*, 2000). Various biotic and abiotic stresses can constrain maize production with considerable yield loss.

Marker-assisted background selection a term coined by Hospital and Charcosset (1997) was initially proposed by Young and Tanksley (1989). This strategy has been used extensively in commercial maize breeding programmes, particularly for selection of lines carrying transgenes conferring herbicide tolerance or insect resistance (Yu *et al* 1996). Marker assisted background selection is the selection of individuals in advanced generations possessing the maximum genome of the recurrent parent. The main objective is to retain the recipient parent genome outside the target gene and the markers will help to identify the recurrent parent genome constitution of each back cross individual to select the best progeny for further advancement.

Materials and Methods

The experiments were conducted in Centre for Plant Molecular Biology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India during December 2014. Four

BC₃F₃SDM introgressed lines viz., UMI 79/936-C1-7-7-7-46-2, UMI 79/936-C1-7-7-7-80-17, UMI 79/936-C1-7-7-7-92-1 and UMI 79/936-C1-7-7-7-92-7 were used in the present study. It is derived from crossing the inbred UMI 79 which is susceptible for sorghum downy mildew and UMI 936(w) which has resistance for sorghum downy mildew and backcrossing progenies with UMI79. Selfing was done for two generation to get BC₃F₃ Population. Background screening was done in the selected introgressed lines for SDM in order to estimate the recovery of the genome of recurrent parent. For this background survey, the polymorphic SSR marker survey was carried out using 51 polymorphic SSR marker sequences obtained from maizegdb database (www.maizegdb.org). These markers were located among the ten chromosomes of maize genome. The physical position of the polymorphic markers was obtained from the maizegdb database for each chromosome separately. Parental polymorphism for these two parents UMI79 and UMI 936(w) was already done. The markers details are given in Table.1.

Results and Discussion

Marker Assisted Backcrossing (MAB) combines 'Foreground' selection of donor alleles linked to markers and 'Background' selection of recurrent parent alleles in the later generation and become most efficient and feasible breeding approach for introgression of SDM resistant QTLs. Background markers are markers that are unlinked to the target gene/QTL on all other chromosomes, in other words, markers that can be used to select against the donor genome. This is extremely useful because the recurrent parent recovery can be greatly accelerated. With conventional backcrossing, it takes a minimum of six back cross generations to recover the recurrent parent and there may still be several donor chromosome fragments unlinked to the target gene.

Table.1 Skewness and Kurtosis observed in the SDM resistant progenies of BC2F1 generation

Marker	Chrom.	Forward Primer	Reverse primer
bnlg1178	1	ACTACAGTTGAACGCCCTG	GCTCATGTGCAAATGCAAGT
umc2151	1	ATATGTGGTATTTTCTGCAGGCGT	AAAATCCTATACAGAAAACGGGCG
umc2234	1	CAAGATCGTTAGGTTCTAGGCGTC	GACGGACTATAGAGGGGCGATGAG
umc2077	2	AAACTCACTGAACATGATCCTGGC	CTGGTTCGGATGCAAGTAGTCAG
phi127	2	ATATGCATTGCCTGGAACCTGGAAGGA	AATTCAAACACGCCTCCCGAGTGT
umc2214	2	ACCCCTGATTCTCTCTTACGTTT	CTGGATGAGGAGGAAGAATACGAG
umc1552	2	CTCGATAGCTCTGCTGCTTCCTC	CAACACCAGCCCTACCCAGA
umc1256	2	CATCTCGACCTTTGACATTCTCCT	AGAAGACGATGATGATGATGCAGA
bnlg197	3	GCGAGAAGAAAGCGAGCAGA	CGCCAAGAAGAAACACATCACA
umc1158	3	AATGCAACTGCTTCAGCTCCTACT	CGACGAATCGAGAAAAGATATTTGA
umc2101	3	CCCGGCTAGAGCTATAAAGCAAGT	CTAGCTAGTTTGGTGCCTGGTGAT
phi243966	3	CGACCGAAACGAATCAAAA	TACTAGGCTGACACGCACG
phi073	3	GTGCGAGAGGCTTGACCAA	AAGGGTTGAGGGCGAGGAA
umc1594	3	CACTGCAGGCCACACATACATA	GCCAGGGGAGAAATAAAATAAAGC
bnlg1035	3	TGCTTGCACTGTCAGGAATC	CAGCTCTGACACACCACACA
umc2263	3	CGTGCTTATATGGGTTCTTGGGT	GTTTGGTTGCTGCGACCTCTT
umc1608	3	GTGTCGTGTTGGGAGAACATGAG	TAACTACTACACCACTCGCGCAA
umc1231	3	CTGTAGGGCTGAGAAAAGAGAGGG	CGACAACCTTAGGAGAACCATGGAG
umc1136	3	CTCTCGTCTCATCACCTTCCCT	CTGCATACAGACATCCAACCAAAG
umc1030	3	TCCAGAGAATGAGATGACAAGACG	CAGAATAACAGGAGATGAGACGCA

Marker	Chrom	Forward Primer	Reverse primer
umc2104	3	CTGCTGGCAGTGGCAGTATTC	TACTGCTACACCTTTGTCGTCACC
phi053	3	AACCCAACGTACTCCGGCAG	CTGCCTCTCAGATTCAGAGATTGAC
bnlg420	3	CTTGCGCTCTCCTCCCCTT	GGCCAGCTCACTGCTCACT
umc2360	4	TAGCAGCTAGCTTCAGTCACAGGC	CAGATCGGACTACTGGTGGCTAAG
umc1757	4	ATAGGAGGTGAGGTGAGGAGGAAG	TTTTCTGCAGGGATAACATTTGTG
BNLG1601	4	ATCGTGCGCTAGTCCAGAGT	CAGACCAGAGACCATCTGCA
umc2365	4	GAAATCCATTCATTCCTTCGTCC	GTGACCTCTAGCTAGCTGGGCTATT
umc2136	5	CCAGATGCGGAAGTAGACGG	GATTCGGAGGTGATCTGACCTGT
umc1056	5	CGGATCGCTTTTTACCGTCTATAA	AGCAAGAGTAGCGTTCCATTTTCAG
umc2298	5	ATCCACTCCCAAGTCCCAACAC	CTTCTTCCGGTTCTTCTTCAGGC
phi078	6	CAGCACCAGACTACATGACGTGTAA	GGGCCGCGAGTGATGTGAGT
bnlg1702	6	TTATCATCAAATGGAGGACACG	AAAGACACACGCTAATGGGC
umc2165	6	AGAACACCAAATGGTGACGTTATGT	CTAGCTCGTCTTCCCTGTGGTCT
umc1105	6	ATTCCTGCATCATCATCCACTACA	GCCAACTGATCTGCTCTAGCTTC
bnlg1154	6	GGGTGATCACATGGGTTAGG	AAATCAATGCTCCAAATCGC
nc013	6	AATGGTTTTGAGGATGCAGCGTGG	CCCCGTGATTCCCTTCAACTTTC
umc2332	7	GTCGGAGAAGGAGCTACTGAGCTA	CACAGGTACGTCTGGATGCTGT
umc2325	7	CCTAGGAACTCTGATGGCTATGGA	CTACGATATCCACCTCTACCACCG
umc2364	7	AACCTCAAGATCACCAACATCCTC	CACCCTGCTGTCAGATGGATACTT
umc1831	7	TTTCGACTGCTAGTGTACTTGGGG	CTCTACATCTTCAGCGTCTCCACA
bnlg1904	8	AGGAGCATGCACTTGGTTCT	ACTCAACTGATGGCCGATCT

Marker	Chrom	Forward Primer	Reverse primer
phi125	8	ACCGCCGGTGCGAGTTGAAG	CTTGGGATTGCCCTCATCCAC
phi087	8	GAGAGGAGGTGTTGTTTGACACAC	ACAACCGGACAAGTCAGCAGATTG
umc2134	9	TAGTCTAGCGTCGACGAAAAATGC	CAGGCGACGAAGATGAATTGAA
umc1743	9	TGGACTTCGAAAATTCTCTTCAGC	GAGAGGAGGAGCTTCACGAGC
umc2133	9	TTCAGGTGTGCACTGACTCTGACT	ATGCTCAAGCTCAACAGCACTTC
umc2017	9	AGAGGTTACTACGGAGTGTGGCAG	GTCAGGGTACTGCTTCTCGAACTC
umc2021	10	AAACTCAAGCTCGGAATGTACTGC	CGATACTGATCTACTTCACGCTGG
umc2126	10	CAGTTCTGCACTTCTGCTTGCTC	AGGACTGTGAAGAGCGCGAG
bnlg1185	10	CGGTCCAGGCAGGTTAATTA	GACTCGAGGACACCGATTTC
umc2053	10	ATCTCTCCCTCGCTCTCCTTCTC	AGCAGCAGGTTGGTCGAATG

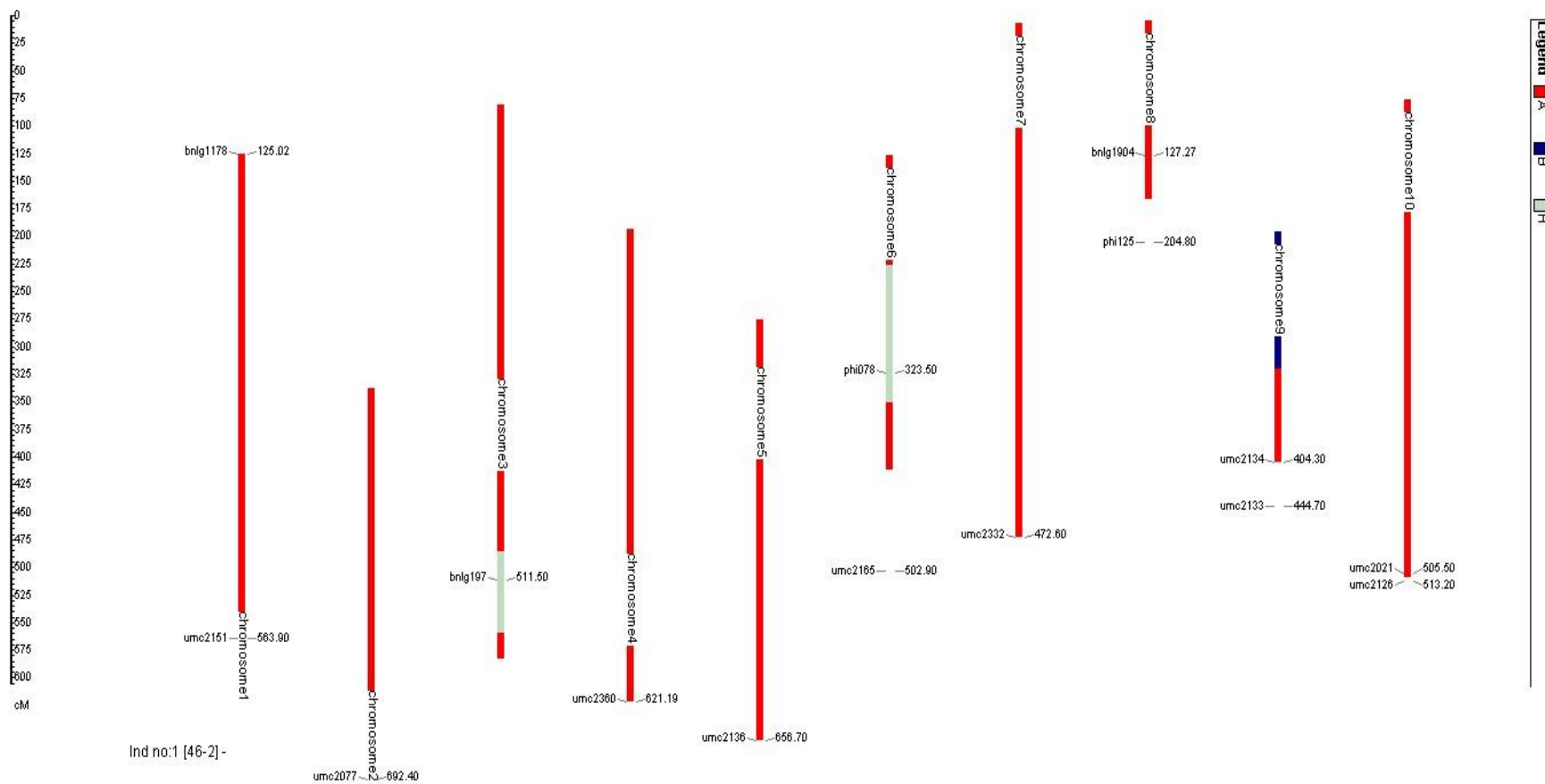


Fig. 1 Graphical representation of background screening in developed SDM introgressed line79/936- C1-7-7-7-46-2

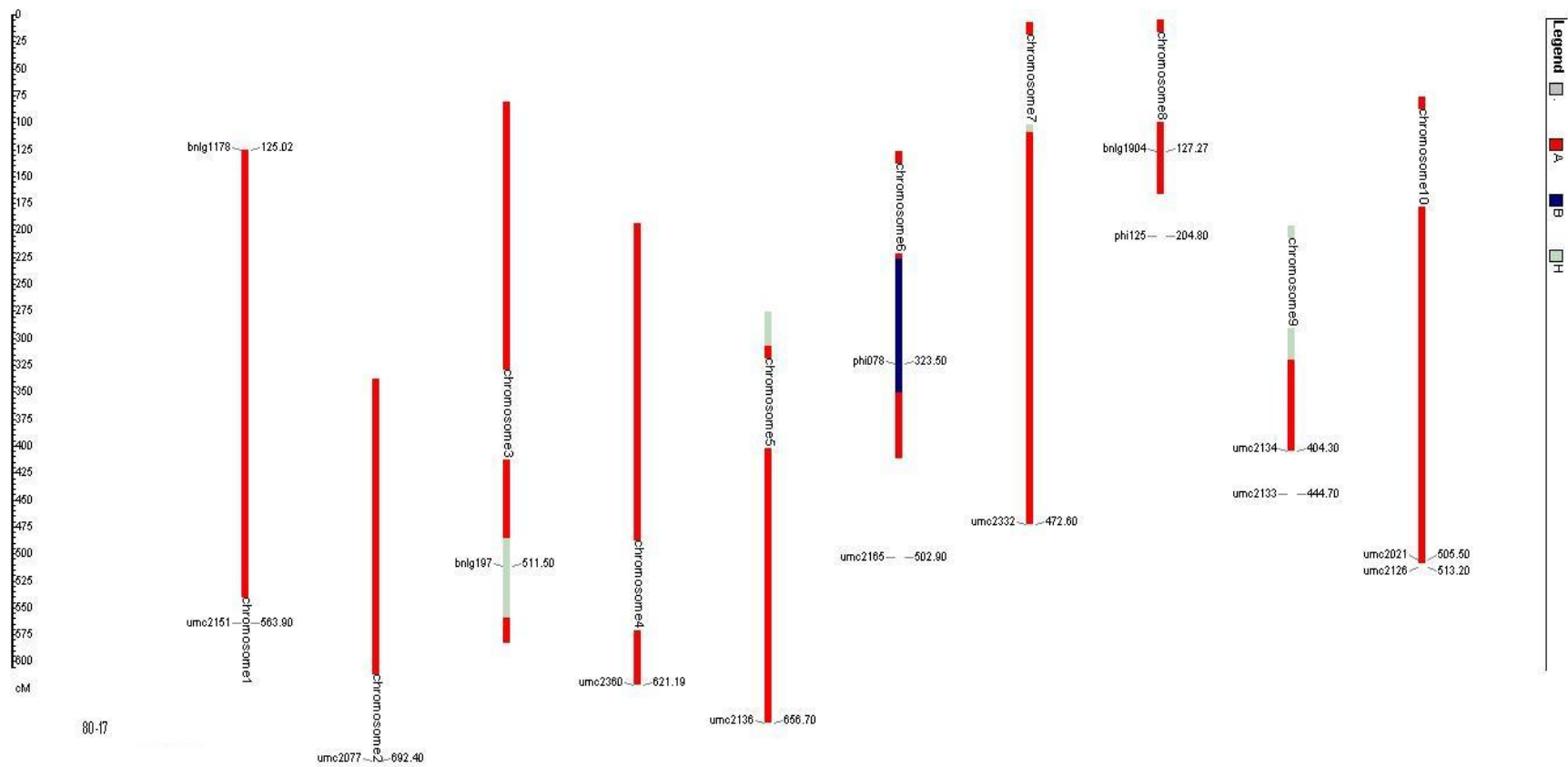


Fig.2 Graphical representation of background screening in developed SDM introgressed line79/936- C1-7-7-7-80-17

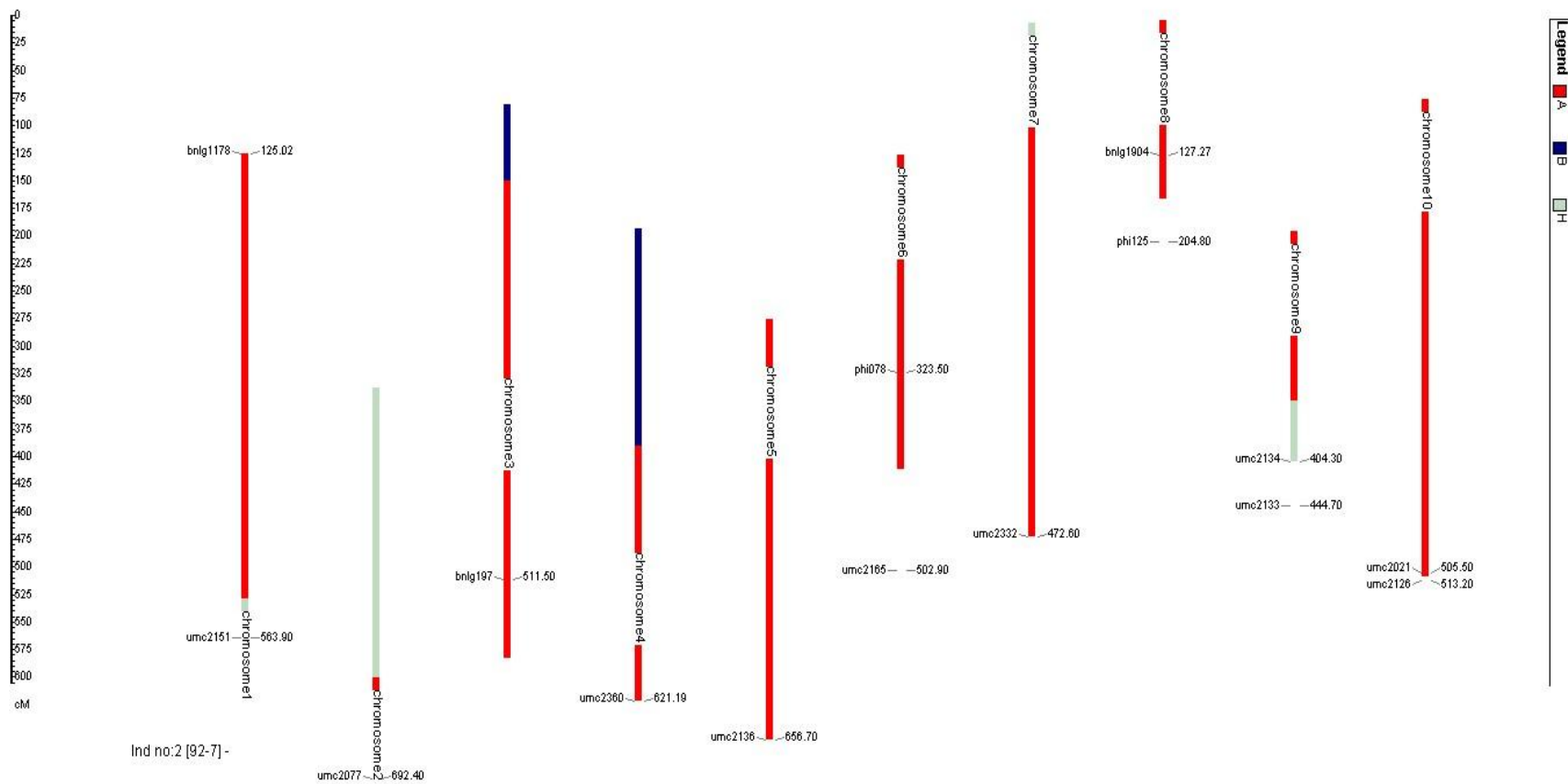


Fig.3 Graphical representation of background screening in developed SDM introgressed line79/936- C1-7-7-7-92-7

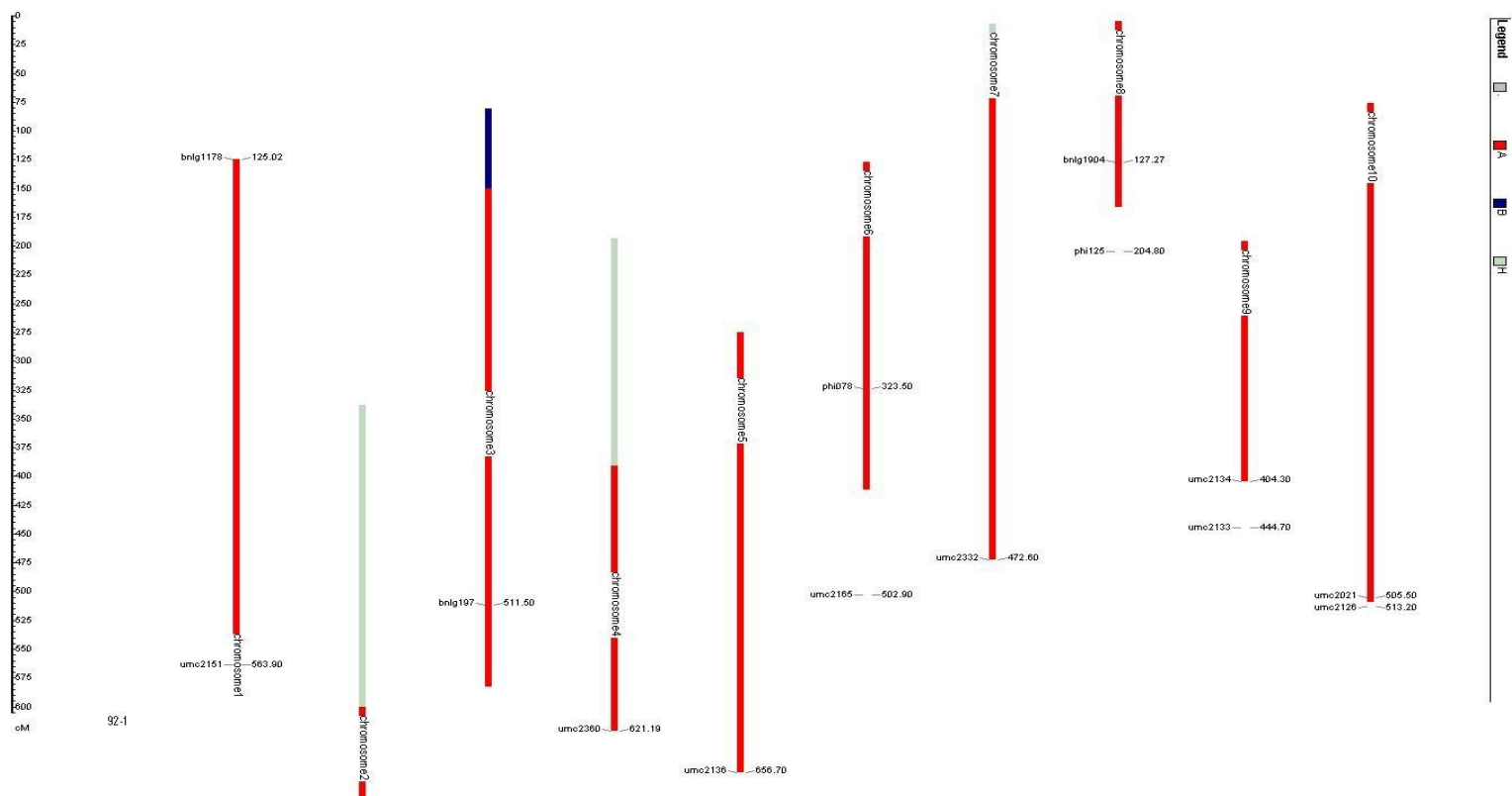
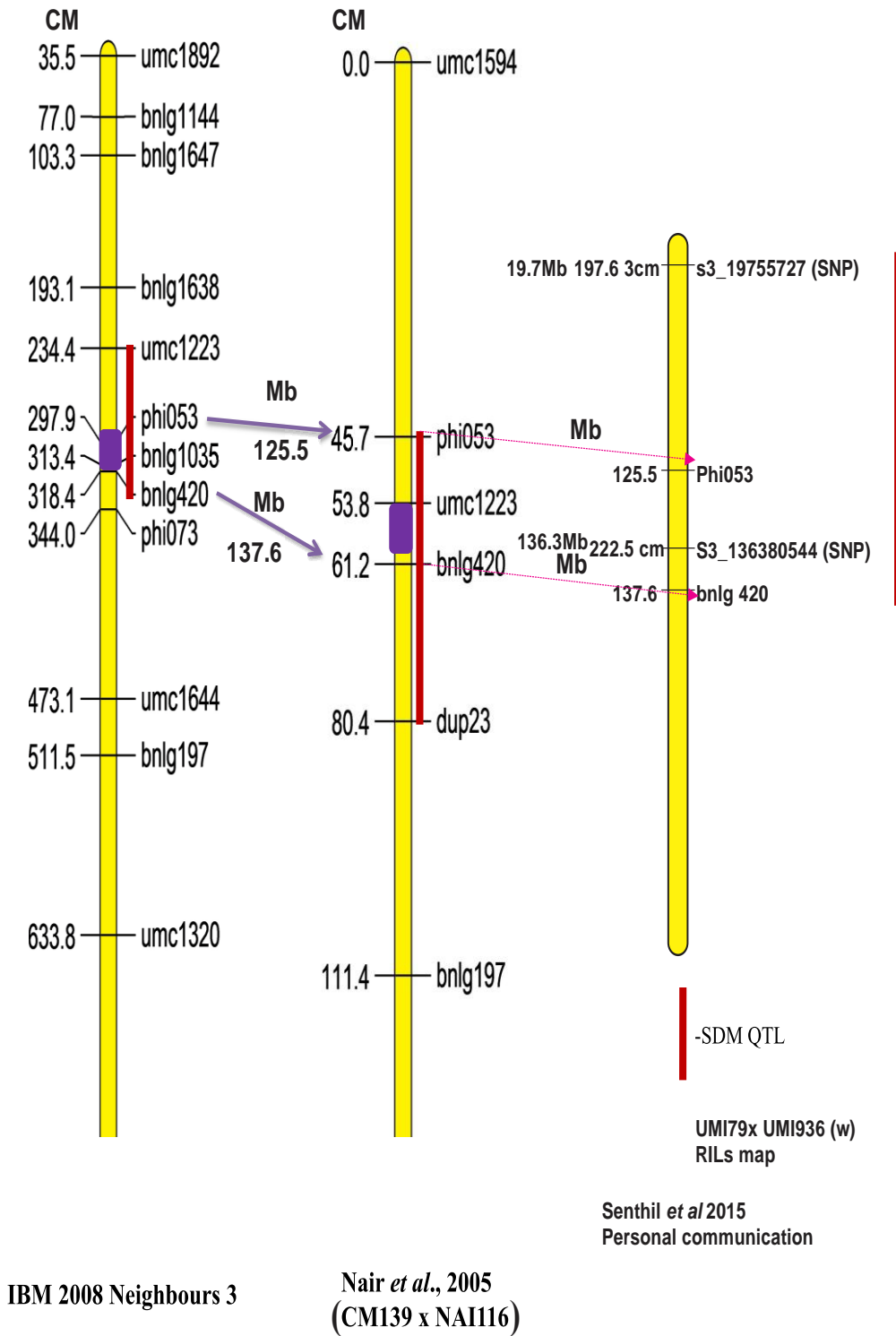
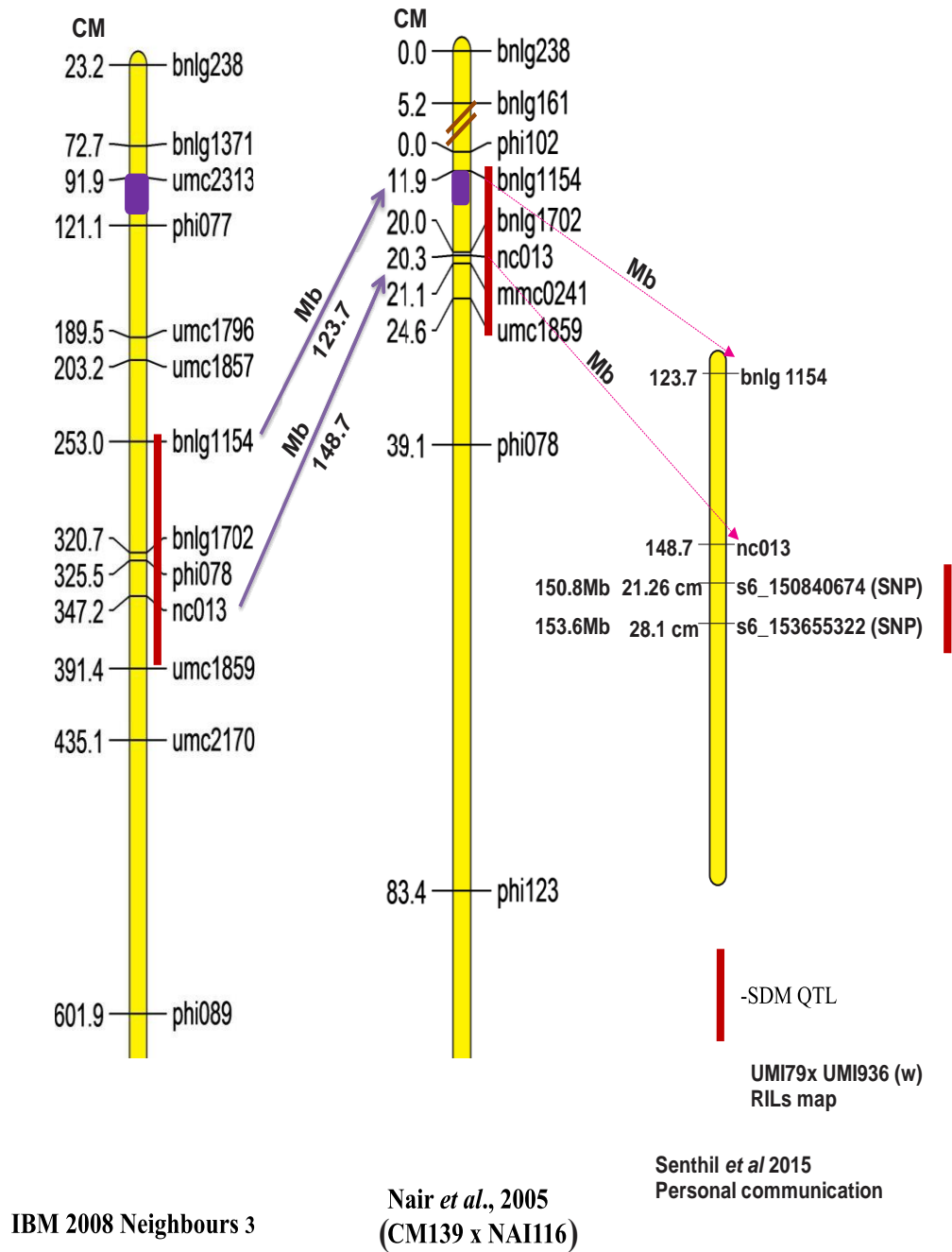


Fig.4 Graphical representation of background screening in developed SDM introgressed line79/936- C1-7-7-7-92-1



Markers phi053 and bnl420 selected for QTLs introgression

Fig.5 Genetic linkage map showing location of SDM QTL on chromosome 3



Markers bnlg1154 and nc013 selected for QTLs introgression

Fig.6 Genetic linkage map showing location ofSDM QTL on chromosome 6

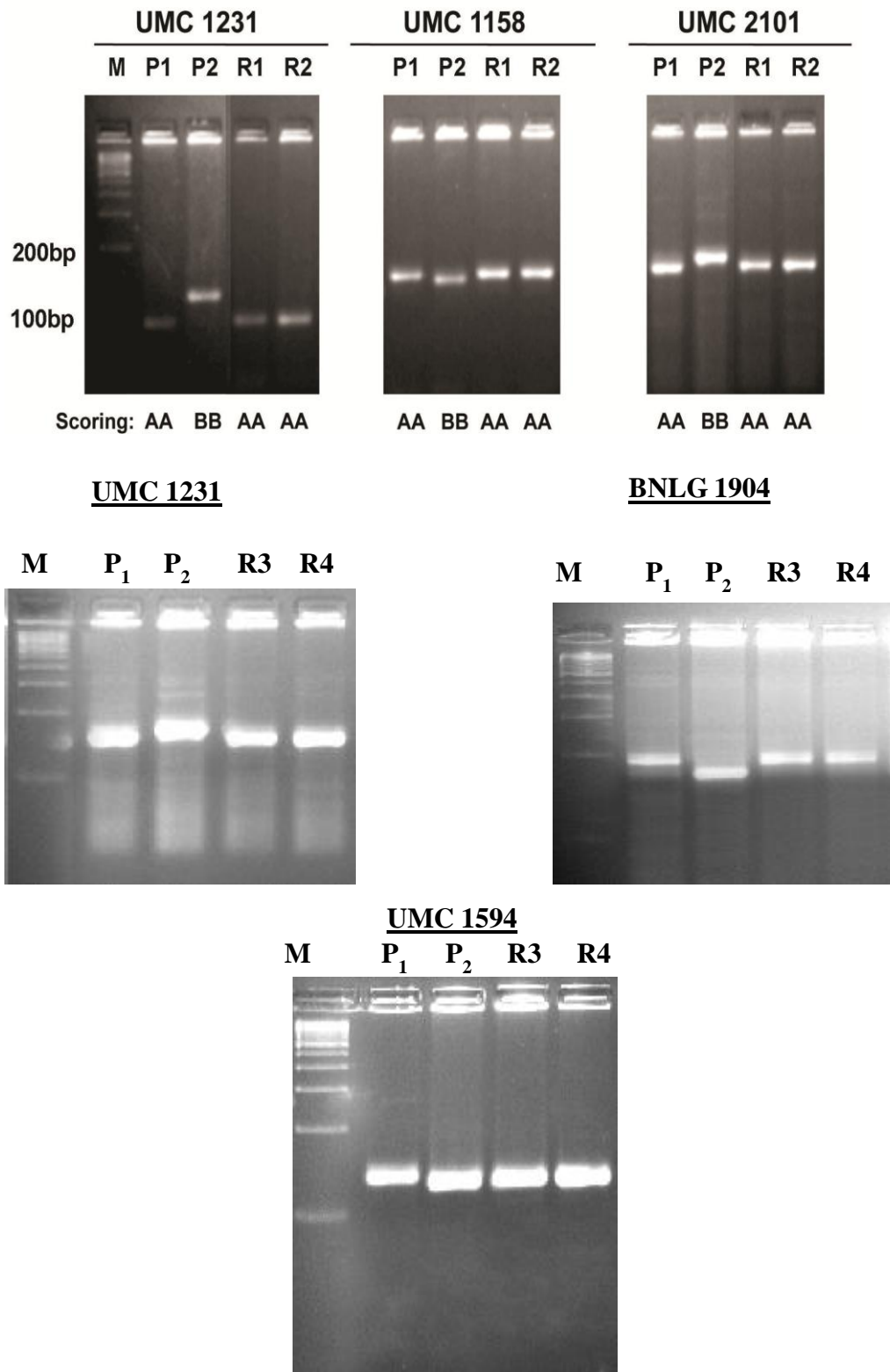


Plate.1 Background screening of SDM introgressed lines (BC₃F₃)

Whereas using markers, it can be achieved by BC₄, BC₃ or even BC₂ (Visscher *et al.* 1996, Hospital & Charcosset 1997), thus saving two to four back cross generations.

Background screenings/survey (i.e., profiling the banding pattern of alleles of background polymorphic codominant SSR markers) were done in the SDM resistant plants UMI 79/936-C1-7-7-S7-46-2, UMI 79/936-C1-7-7-7-80-17, UMI 79/936-C1-7-7-7-92-1 and UMI 79/936-C1-7-7-7-92-7 in order to assess the recovery of the genome of the recurrent parent. This survey was carried out using 51 polymorphic SSR marker sequences (Supplementary Table.1.) obtained from maizegdb database (www.maizegdb.org). These markers were located among the ten chromosomes of the maize genome. The physical position of the polymorphic markers was obtained from the maizegdb database for each chromosome separately. Parental polymorphism for these two parents UMI 79 and UMI 936(w) had already been completed (Plate.19).

Back ground screening of SDM resistant introgressed lines (BC₃F₃ generation)

The 51 SSR markers, which showed distinct alleles between the parents, were selected for the genome wide background analysis. The polymorphic SSR markers were distributed in chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10. These markers were used to survey the four SDM resistant BC₃F₃ progenies (i.e., UMI 79/936-C1-7-7-7-46-2, UMI 79/936-C1-7-7-7-80-17, UMI 79/936-C1-7-7-7-92-1 and UMI 79/936-C1-7-7-7-92-7) for estimating the recovery of the background UMI 79 genotype. The PCR products were screened at 3.5 agarose gel. The plant was scored as AA if it contains UMI 79 allele and scored as BB if it contains UMI 936(w) allele, and scored as H if it contains both UMI 79 and UMI 936(w) alleles. For example, the analysis of

polymorphic markers UMC1231, UMC1158, UMC2101 in parents and two SDM resistant plants are shown in Fig 7.

The graphical genotyping of introgressed lines were performed by using the software GGT 2.0 and the results of graphical genotyping were described. The graphical genotyping of the BC₃F₃ SDM resistant introgressed lines revealed that the complete recovery of UMI 79 genome was present in chromosome 3 in UMI 79/936-C1-7-7-7-46-2 and UMI 79/936-C1-7-7-7-80-17. The complete recovery of UMI 79 genome was present in chromosome 7 in UMI 79/936-C1-7-7-7-92-1 and UMI 79/936-C1-7-7-7-92-7. The genotype UMI 79/936-C1-7-7-7-46-2 has revealed that the 92.45 per cent (maximum recovery) of the genome were derived from the recurrent parent UMI 79, genotype UMI 79/936-C1-7-7-7-80-17 and UMI 79/936-C1-7-7-7-92-7 showed that 89.68 per cent and 80.6 per cent of recovery respectively. A minimum 76.8 per cent of recovery was observed in the genotype UMI 79/936-C1-7-7-7-92-1 (Fig .24 to Fig 27.). Jorboe *et al.* (1994) have used the maize genome as a model for simulation and reported that three backcross generations and 80 markers were needed to recover 99 per cent of the recurrent parent genotype. Naidoo *et al* (2012) recovered of the recurrent parent (92.15 per cent) in the BC₂F₁ generation in marker-assisted selection for low phytic acid (*lpa1-1*) with single nucleotide polymorphism marker and amplified fragment length polymorphisms for background selection in a maize backcross breeding programme.

To conclude that the percentage of the marker alleles in the four SDM resistant BC₃F₃ progenies that shows similarity with that of recurrent parent is used for calculating the recovery of recurrent parent genome. The genome of UMI 79/936-C1-7-7-7-46-2, UMI 79/936-C1-7-7-7-80-17, UMI 79/936-C1-7-7-

7-92-1 and UMI 79/936-C1-7-7-7-92-7 were 92.45 per cent, 89.68 per cent, 76.8 and 80.06 per cent identical to the recurrent parent respectively.

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