Analysis Of Recurrent Parent Genome Recovery In Marker-Assisted Backcross Breeding Program In Watermelon

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ABSTRACT: Marker–assisted backcross (MABC) is a breeding technique used to develop improved varieties by transferring a gene or QTL into the genome background of an elite variety after 2-3 generations. It is an advanced way of overcoming challenges facing conventional backcross methods as it speeds up the recurrent parent genome recovery (RPG). In order to develop a Fusarium wilt resistant watermelon variety, MABC was used to incorporate wilt resistant gene from the resistant inbred line CS-19 into the genome of the high yielding but wilt susceptible inbred line BL-14. There was estimation of RPG recovery in earlier generations with the use of polymorphic simple sequence repeat (SSR) markers. A total of 380 SSR markers were tested to identify polymorphism between the parents and 78 of them were found to be polymorphic. Background analysis revealed 74.7 – 94.4 and 86.6 – 96.8 % recovery in BC1F1 and BC2F1 generations, respectively. In the BC2F2 generation, RPG recovery ranged from 95.1 and 96.9 and the average in the selected lines was 96.14 %. This study led to the selection of plants that are similar to the recurrent parent and it showed the usefulness of MABC for the quick recovery of a parental genome in a backcrossing population.

Index Terms— Fusarium wilt, Marker-assisted backcross, marker-assisted selection, polymorphism, recurrent parent genome recovery, watermelon.

1. INTRODUCATION

Marker-assisted backcrossing (MABC) is a form of marker-assisted selection (MAS) often considered to be the most appropriate and effective means of incorporating desired gene(s) or quantitative trait loci (QTL) into the recurrent plants [1], [2]. MABC consists of three selection levels namely: foreground selection, recombinant selection and background selection [3, 4]. The efficacy of MABC as a strategy in breeding is a function of the ability of foreground markers in the selection of locus of interest, efficient background selection for recovery of recurrent parent genome (RPG) and reduction of linkage drag [5, 6]. Background selection is aimed at ensuring complete recovery of the recurrent parent's genome and to monitor the rate of recovery in each backcross generation. The number of backcrosses needed to have an improved cultivar is a function of the rate at which the genome of the recipient parent is recovered, thus, making recurrent parent genome (RPG) recovery the most important determinant of the success of the application of MABC in breeding [7].

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With efficient MABC, a high percentage of recurrent genome recovery could be attained after 2 or 3 backcross generations [8], [9]. Malaysia is a major grower of watermelon (Citrullus lanatus L.) among the ASEAN countries. In the country, watermelon is regarded as both domestic as well as commercial crop and seeds for its production are imported from Taiwan, China, Korea and Japan. However, the local production of watermelon from the imported seeds is limited by disease outbreak that leads to yield loss [10]. Among these diseases, Fusarium wilt is of high importance. The breeding of disease-free watermelon cultivars have been mainly through the traditional methods and these require more time and high labour as well as chance of transferring other undesirable genes apart from the target gene (gene drag) as its major shortcomings [11], [12]. Thus, there is the use of marker-assisted backcrossing as a viable alternative to the conventional breeding method and its shortcomings. MABC ensures accurate incorporation of the desired gene (s) into recurrent parents, thereby saving time and cost [13]. Unlike in other members of the Cucurbitaceae family, such as cucumber and melon [14], [15] the development of Fusarium wilt disease-resistant varieties of watermelon using SSR markers is still in the juvenile stage. This was due to the lateness in the construction of high-resolution genetic maps for watermelon and lack of much SSR markers linked to Fusarium wilt resistance gene (s) [16]. Early studies on markers linked to FW resistance in watermelon used RAPD and AFLP which are not easy to run, have dominant expression, low reliability and not cost efficient to use [17], [18]. In breeding, background selection aids the recovery of the recipient parent genome outside the target gene and marker-assisted backcross enables the breeder to evaluate the rate of the recurrent parent genome recovery in every backcross generation. This study was therefore conducted to determine the RPG recovery in the progenies at each backcross generation derived by crossing the recurrent parent BL-14 and donor parent CS-19. It is an important step in the breeding for Fusarium resistant cultivar of watermelon through introgression of the Fusarium wilt resistance gene into the genome of the recurrent parent BL-14.

2. MATERIALS AND METHODS

2.1 Planting materials and breeding scheme

Crimson Sweet (CS-19) was used as the donor in this study. It is a resistant inbred line while the recurrent parent; inbred line BL-14 is a susceptible inbred line. B L-14 was reported to be early maturing, medium fruit weight and high total soluble solid contents [19]. Crossing of the parental lines to generate the F1 population was done through hand pollination before 11 am throughout the time of pollination [20]. Two heterozygous F1 plants were backcrossed with the recurrent parent to produce the BC1F1 seeds. Tightly linked marker (BVWS02309) was used in the foreground selection in the BC1F1 generation for the Fusarium wilt-resistant genes. Plants that were confirmed to have the resistant gene and have the highest background recovery and maximum phenotypic similarity to the recurrent parent were backcrossed with BL-14 to generate BC2F1 seeds in each backcross. The crossing scheme used is shown in Figure 1.

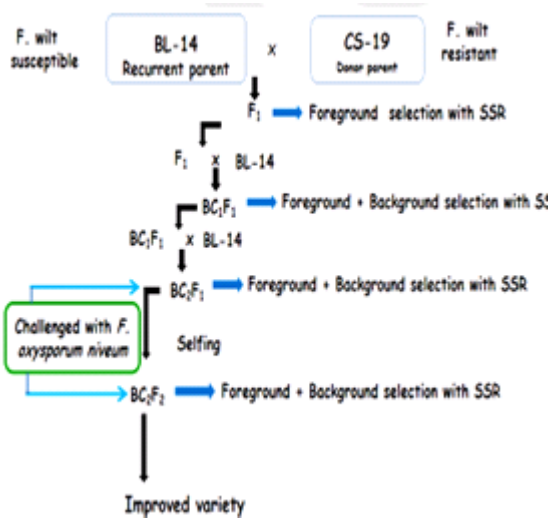

Figure 1: *Crossing scheme for the development of the improved lines*

2.2 DNA extraction, PCR condition and gel electrophoresis

Healthy young leaf samples were collected at the two leaves' stage (about two weeks after planting). Genomic DNA was extracted from between four and six young leaves using the CTAB (hexadecyltrimethylammonium bromide) method of [21]. This was however modified in consonant with that used with [22]. Nano-drop spectrophotometer (ND1000 Spectrophotometer) was used for testing the quality and concentration of the DNA. Polymerase chain reaction (PCR) for the markers was conducted using T100™ Thermal Cycler (Bio-Rad Laboratories, Inc., USA). PCR for all the markers, was performed in a total 15.0 µl consisting of 7.5 µl of DreamTaq Green PCR Master Mix (2×) (Thermo Scientific, USA), 4.5 µl of sterilized water, 1.0 µl of each primer (10 mM) and 1.0 µl of genomic DNA (50 ng/µl). The cycling conditions followed touchdown PCR protocol with the following profile: 94 °C for 3 minutes followed by 10 cycles of 94 °C for 30 seconds, 60 °C for (-1 °C per cycle) for 1 minute, then 72 °C for 1 minute, followed by 30 cycles of 94 °C for 30 seconds, 55 °C for 1 minute, then 72 °C for 5 minutes and, a final extension for 5 minutes at 72 °C followed by rapid cooling to 4 °C prior to analysis both for foreground and background markers. The PCR products were separated by electrophoresis on 2.5% w/v Metaphor agarose gel, stained with Midori green by 1× TBE buffer (0.05M Tris, 0.05M boric acid, 1mM EDTA, pH 8.0). The SSR amplicons were run at 90 V for 1 hour 30 minutes and viewed under the Molecular ImagerR (GelDocTM XR, Bio-Rad). Clear and distinct bands were scored in comparison with a 50-bp DNA Ladder (GeneDireX, USA). The homozygote allele for the resistant parent was represented as A while homozygote allele for the susceptible parent was B; the heterozygous carrying allele from both parents were considered as H.

2.3 Microsatellite marker analysis

Three hundred and eighty microsatellites markers reportedly distributed on watermelon chromosomes [23], [24], [25], [26], [27] were screened to identify the ones that are polymorphic between the inbred lines CS-19 and BL-14. Among these 380, eleven (Table 1) that were reported to be associated with Fusarium wilt resistance in watermelon were selected and used in foreground selection.

2.4. Phenotypic

Plants with Fusarium wilt resistant gene and phenotypically resembled the recipient parent BL-14 were selected during the growing period. Phenotypic selection was carried out over the entire population of BC1F1 and BC2F1. The parameters assessed included days to flowering, days to fruiting, rind thickness, total soluble solid, fruit weight and seed number [28], [29]. In all the generations, plants with the linked gene were selected using this same technique.

2.5 Allele scoring and Analysis of Data

The banding patterns obtained after amplification with the primers were scored in relation to both parents. When data were subjected to foreground selection, the band showing the same level as CS-19 was scored as 'R' which shows the homozygous allele of the resistant parent for a specific microsatellite marker. In similar vein, those showing similar levels with BL-14 were scored as 'S'. Molecular weight of the different alleles was calculated with the Alpha Ease Fc5.0 software. Graphical Genotype (GGT 2.0) software [30] was engaged in determining the recovery of the recurrent parent in the background selection. The analysis of the data that was imported to the GGT-2.0 software program was generated in the Excel file. The results were given as percentages for marker homozygous for recipient parent (%B), the parent donor allele (%A) and heterozygous plant (%H). Goodness of fit test was performed on the BC2F1 population by using Chi-square test calculated using SAS 9.4 software. The level of significance for each chi-square value was ($P \leq 0.05$) if it is greater than 3.84 for single gene model, and for two gene model if it is greater than 7.84 [8].

3. RESULTS

3.1 Markers polymorphism in the parental line

From the 380 SSR markers screened, 78 were found to be polymorphic (Table 1), 250 were monomorphic while 52 did not show any band at all. Thus, the percentage polymorphism among the parents was 20.5%. Figure 2 showed polymorphism with some of the markers. The polymorphic markers were used in background analysis of BC1F1, BC2F1

and BC2F2 populations. The polymorphic markers were used in background analysis of BC1F1, BC2F1 and BC2F2 populations.

Table 1 Details of the polymorphic markers used

Polymorphic microsatellite markers	Chromosome. position
BVWS02309, BVWS00948, BVWS01116, VWS00291, BVWS02003, BVWS00281, BVWS00230, VWS02311, BVWS02290. BVWS02262.	1
BVWS00291,BVWS00314, BVWS00297, VWS01553, BVWS01634, TS82,	2
BVWS01151, BVWS02204, BVWS01685, VWS00485, BVWS00676	3
BVWS00660, MCPI-04, PFW13, BVWS01034, VWS01144, BVWS01427, BVWS00208	4
BVWS00441, BVWS00106, BVWS00658, VWS02445, BVWS02355, BVWS00455	5
BVWS02433, AF074710.1, R7, BVWS02050, VWS02424, BVWS02420, BVWS01944	6
BVWS00433,BVWS00353,BVWS02236, BVWS01915, BVWS01149, BVWS02253	7
BVWS00369, BVWS00826, BVWS01698, VWS00373, BVWS02406, BVWS00193, BVWS02373,	8
BVWS00333, BVWS02389, BVWS01947, VWS02066, BVWS02338, BVWS01133	9
BVWS00236, BVWS00269, BVWS02375, VWS02400, BVWS00079, BVWS02333, BVWS02210, VWS02213, BVWS02396, BVWS02261	10
BVWS00228, EST00675, BVWS02449, BVWS00069, BVWS02310, BVWS01709, BVWS02320, SSRCA4	11

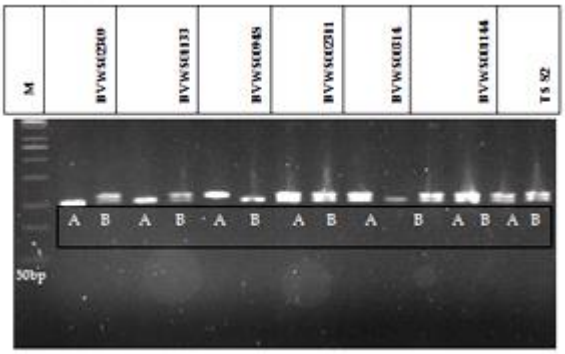

Figure 2: *Screening of parental lines (A: CS-19 and B: BL-14)* for polymorphism using some of the SSR markers. Running on 2.5% metaphor agarose gel stained with Midori green. (M: 50bp Ladder)

3.2. Genotyping F1 generation

The F_1 seeds produced were screened using the 11 tightly linked polymorphic foreground SSR markers (Table 2). Heterozygosity was shown in all the F_1 plants.

Table 2 Linked microsatellite markers screened in this study

S/No	Markers name	Chr	Primer sequence (5'-3')	Product size (bp)	Sources
1	BVWS02309	1	F:AATCTCCACTACAATCCACCAG R:TTCCTCCAAACTCATCATTACC	149	Ren et al., 2012
2	ASSR-23	1	F:TCAAACCGACTGCCATATCA R:AGCTTGTCTTCCTGGCCTTT	160	Singh et al.,2016; 2013
3	SSR 17631	2	F:TTGATTCCAATTTCATTACTTTTCA R:TTCCCTAAGTAGTGACGGATTTTT	204	Zhang et al., 2014
4	TS82	2	F:TCAAGATTGATATTGATTAGATAAAAGC R:CTTTATTTACCACTTGCACAACACTAA	169	Varshney et al., 2014.
5	SSR03084	2	F:GACAAGGGATTCATCCGAGA R:CAGACCCTGAAGCGGATAAA	200	Zhang et al., 2014.
6	SSR06576	2	F:TGATCATGGGAAGAGAGAGACA R:TCAAGAAATGTGATGAATGGAAA	192	Zhang et al., 2014
7	SSR11820	2	F:ACGACGCCGTATTTGCTTAG R:AAGCTCGTTCATTATTACCCAA	244	Zhang et al.,2014
8	SSR06602	2	F:CCCTGCCTTCCTTTCCTATC R:AGAAAGGATCGGATCGAACA	200	Zhang et al., 2014
9	SSR16226	2	F:TTAAAATTCCCAACGGAAACC R:TGATGGGAGAAAGGTAAACAAGA	164	Zhang et al., 2014
10	BVWS01133	9	F:CATCCACCTCAAACTTTAGAAACA R:TTCTATTCCCGTCATTTCATTG	260	Ren et al., 2012
11	BVWS02333	10	F:GGGGGTTTTGGTTTCTTGAT R:ATGATGTCACCATTACGGGG	90.3	Ren et al., 2012

3.3 Genotyping BC1F1 generation

3.3. 1 Foreground selection

Two heterozygous F1 plants were chosen and backcrossed with recurrent parent (BL-14) to raise 90 BC1F1 plants. Of the 90 BC1F1 plants, 50 were heterozygous for the SSR markers BVWS02309 on the major QTL on chromosome 1 (Figure 3) However, all the other 10 foreground markers did not show heterozygous condition and this implies the loss of Fusarium wilt resistance gene in the process of backcrossing to BL-14. Table 3 showed the proportion of the resistant and susceptible plants in BC1F1 generation as well the goodness of fit test to

the expected 1:1 ratio when subjected to Chi2 test (1.11), this confirmed non-significance at a probability level of 0.05.

Figure 3: *Screening of BC1F1 plants using BVWS02309. (A= CS-19, B= BL-14, H- heterozygous and M=50 bp ladder*

Table 3: *Proportion of resistant and susceptible plants in BC1F1 and BC2F1 generation*

Gen.	No. of plants	Observed ratio		Expt. ratio	χ2 value	P value
		resistant	Suscept.			
BC_1F_1	90	50	40	1:1	1.11	0.34
BC_2F_1	150	72	78		0.24	0.69

3.3. 2 Background selection

Total of 78 polymorphic markers were used for the RPG background evaluation of the 50 BC_1F_1 plants. Number of markers that were polymorphic ranged from 5 (on chromosome 3) and 10 (on chromosomes 1 and 10). Others include 6 (chromosomes 2, 5, 7 and 9), 7 (chromosomes 4, 6 and 8) and 8 (Chromosome 11).The extent of recovery of the (RPG) in BC_1F_1 generation plants ranged from 74.7 to 94.4%. Plant P5-5 was the best in BC_1F_1 generation. It has the highest recurrent parent genome recovery (94.4%) and the lowest heterozygous segments together with little or no linkage drag (Table 4). Chromosome-wise RPG recovery of this plant was shown in Figure 5. This finding was similar to that of [8] who reported that RPG recovery for BC_1 generation in rice ranged between 73 and 94%. The recovery in chromosomes 1, 2, 6 and 10 was almost complete in the selected lines (Figure 5).

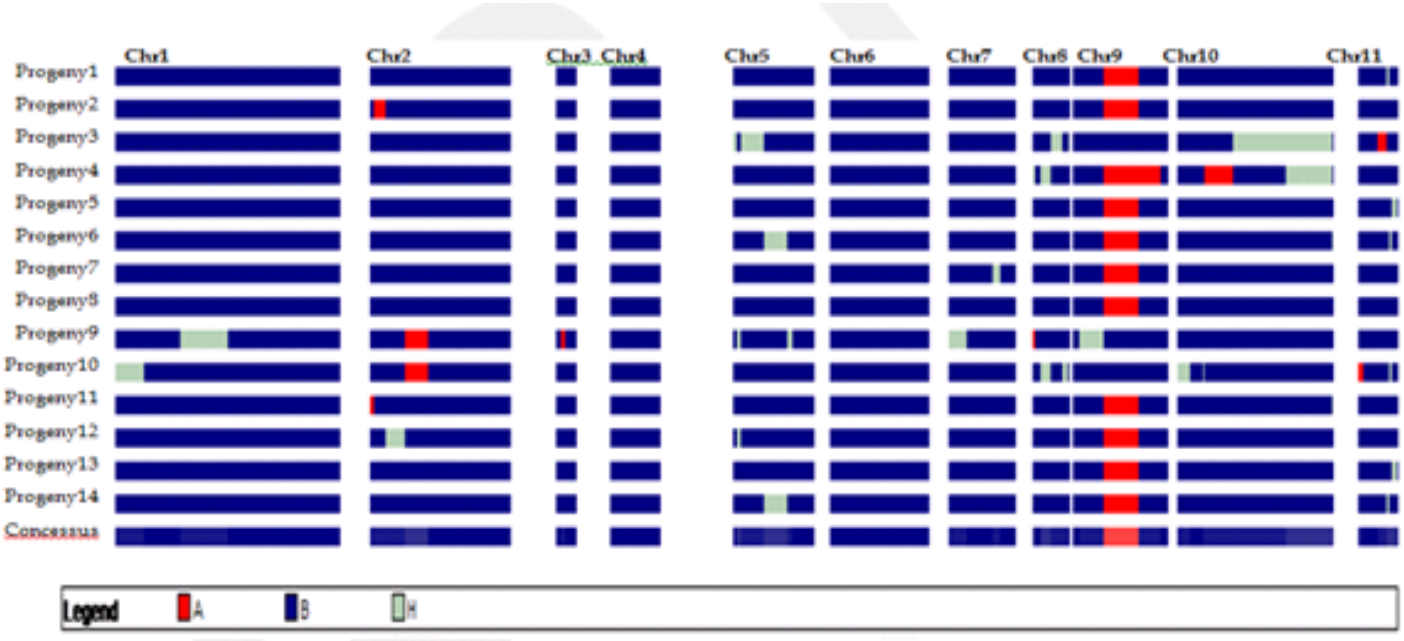

Figure 5: *Chromosome-wise recurrent parent genome recovery of the BC1F1. Red color indicated regions homozygous for CS-19 alleles, blue color indicated regions homozygous for BL -14 alleles and light green color indicated the heterozygous region.*

It showed clearly that most of the residual segment was distributed on chromosome 1 and 10. Being guided by the foreground and background selections, five BC_1F_1 plants whose average recovery was 88.22% were chosen and used to develop BC_2F_1 populations.

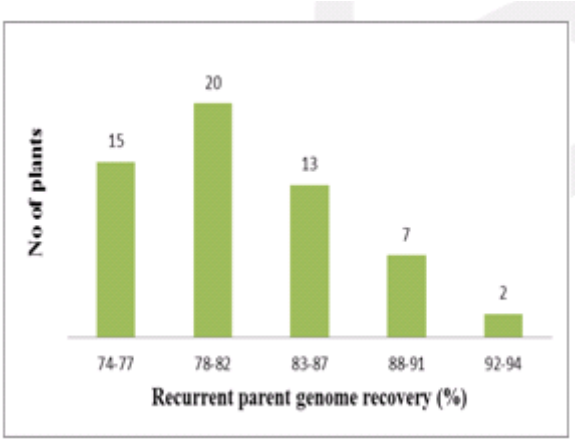

Figure 4: *Frequency distribution of the recurrent parent genome (RPG) recovery in BC_1F_1 generation population derived from cross between BL-14 and CS-19.*

Table 4: *Analysis of background and introgressed segment in selected best lines of BC_1F_1 population*

Selected individuals	B (%)	A (%)	H (%)	Total (cM)	H-segment
BC_1-5-1	87.7	0.1	12.2	600.5	11
BC_1-5-5	94.4	0.0	5.6	600.5	6
BC_1-5-7	90.4	4.1	5.5	600.5	6
BC_1-5-8	85.5	0.0	14.1	600.5	7
BC_1-5-11	83.1	2.5	14.4	600.5	7
Average	88.22	1.34	10.36	600.5	7.4

A= Recurrent, B=Donor, H= Heterozygous, cM= Centimorgan

3.4 Genotyping BC_2F_1 generation

3.4.1. Marker-assisted foreground selection

From the 150 BC_2F_1 plants, the successful introgression of the major QTL into 72 plants was confirmed by the use of linked markers BVWS02309. Figures 6 showed the gel image of 14 of these 72 plants. The numbers of observed resistant and susceptible plants in BC_1F_1 and BC_2F_1 generations are shown in Table 5. This table also confirmed the results of the BC_1F_1 and BC_2F_1 as fitted to the expected 1:1 ratio with chi-square values of 1.11 and 0.25 respectively. Since these values were non-significant at 5% probability level using chi-square test of association, it can be inferred that the markers have an association with Fusarium wilt resistance in watermelon. To develop the BC_2F_2 generation, there was selection and selfing of five BC_2F_1 plants that carried the gene and have the closest resemblance to BL-14.

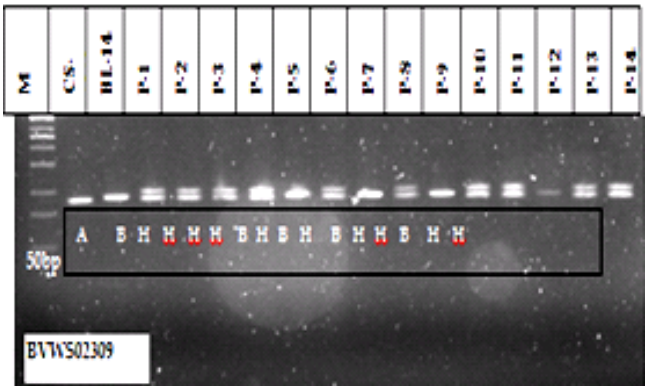

Figure 6 : *Screening of resistant and susceptible plant using BVWS02309 marker in the BC_2F_1 generation (P= Plant no; A= donor parent banding pattern; B= recurrent parent banding pattern; H= heterozygous banding pattern; M= 50bp ladder)*

3.4.2 Background selection for recovery of recurrent parent

The RPG in the BC_2F_1 generation ranged from 86.8 to 96.8% in the (Figure 7). Five best plants (5-5-1, 5-5-5, 5-5-6, 5-5-7, and 5-5-13) with maximum phenotypic resemblance and highest recovery of the RPG were selected. The detail summary of the RPG recovery and heterozygous segment of these best five plants and their average is described in Table 6.

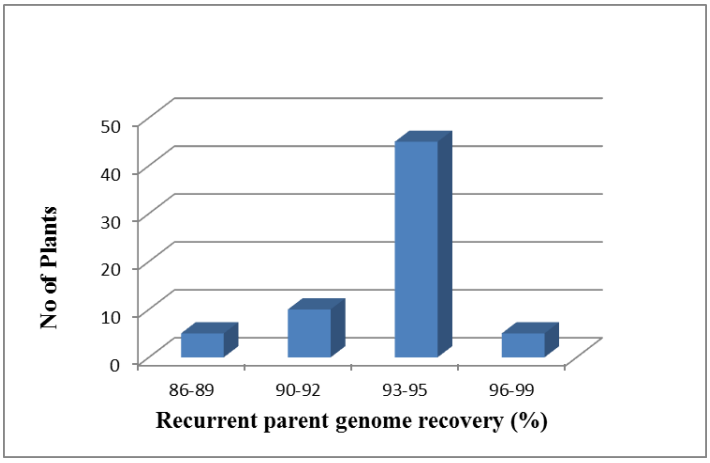

Figure 7*: Frequency distribution of the percentage of the recurrent parent genome in the BC_2F_1 population derived from cross between BL-14 and CS-19.*

Table 6: *Background recovery and introgressed segment analysis in selected best lines of BC_2F_1 population*

Selected best plants	B(%)	A(%)	H(%)	Total (cM)	H-segment
BC_2-5-5-1	93.6	1.8	4.6	600.5	2
BC_2-5-5-5	95.4	0.0	4.6	600.5	4
BC_2-5-5-6	94.7	1.5	3.8	600.5	4
BC_2-5-5-8	96.8	0.0	3.2	600.5	3
BC_2-5-5-13	95.6	0.0	4.4	600.5	5
Average	5.22	0.0	4.78	600.5	3.6

B= Recurrent, A=Donor, H= Heterozygous, cM= Centimorgan

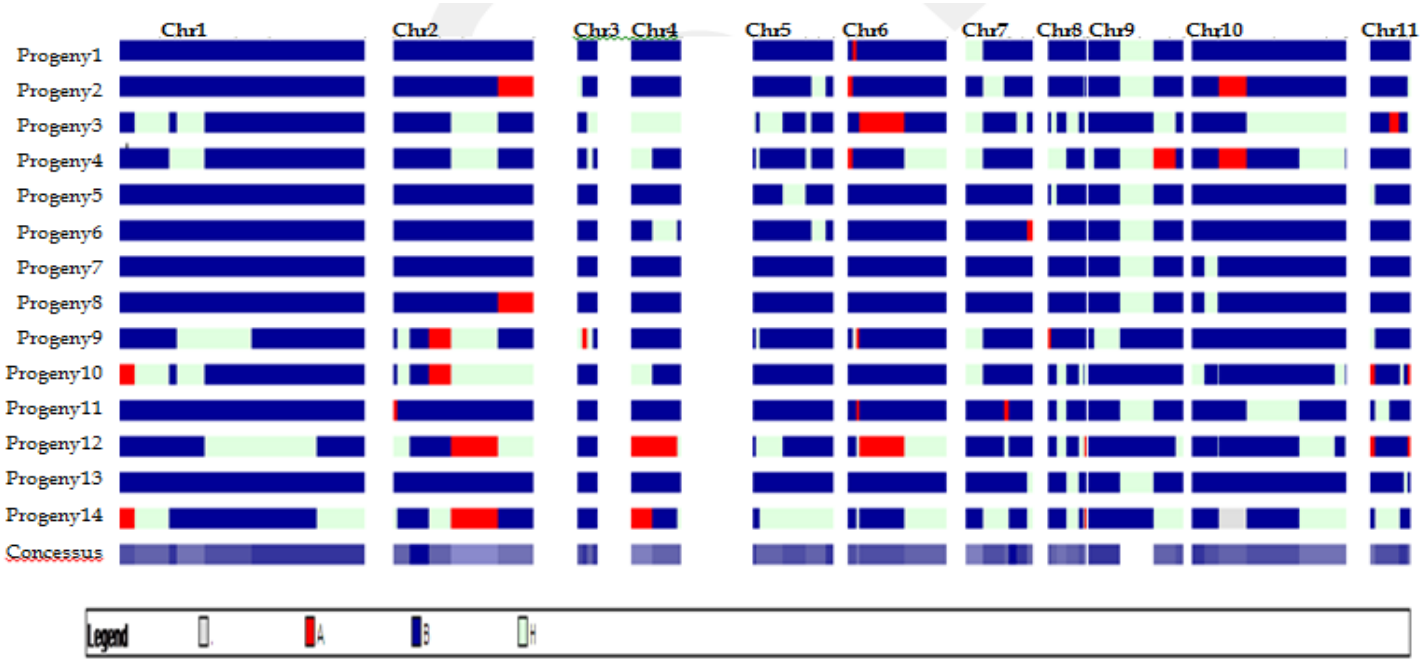

Figure 8: *Chromosme-wise recurrent parent genome recovery of the BC2F1. Red color indicated regions homozygous for CS-19 alleles, blue color indicated regions homozygous for BL -14 alleles and light green color indicated the heterozygous region.*

3.5 Genotyping BC_2F_2 generation

3.5.1 Marker-assisted foreground selection

The BC_2F_2 plants were generated by selfing of the BC_2F_1. Those with maximum phenotypic similarity with BL-14 and possessing target homozygous resistant allele were selected (Figure 9). Table 7 showed the genotypic segregation of the BC_2F_2 population using linked markers BVWS02309. The marker represented a good fit to the expected markers segregation ratio (1:2:1) according to Mendelian expected ratio. From the BC_2F_2 population, 10 lines that possessed the Fusarium wilt resistant allele were selected as the improved lines.

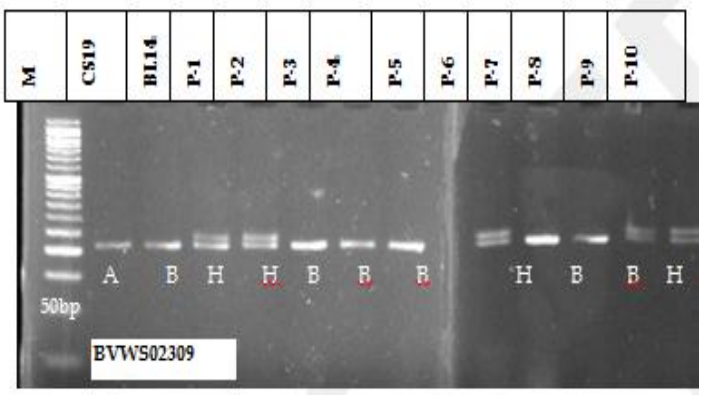

Figure 9: *Fusarium wilt resistant improved homozygous line genotyping using linked marker BVWS02309 (P= Plant no; A= donor parent banding pattern; B= recurrent parent banding pattern; H= heterozygous banding pattern)*

Table 7. *Analysis of markers in BC_2F_2 segregating population*

Marker	Marker segregation analysis			χ2 (1:2:1)	Probability
	AA=R	AB=SG	BB=S		
BVWS02309	54	108	42	2.12	0.347
BVWS01133	44	106	54	1.29	0.525

According to model on single dominant gene, (AA): Resistant; (BB): Susceptible; and (AB): Segregant. df=2; χ2 (0.05, 2) =3.84

3.5.2 Marker-assisted background selection

Seventy- eight microsatellite markers were used for the background selection of improved blast resistant lines; genetic map was constructed covering 600.5 cM with average marker distance of 10.2 cM regions of the genome of Citrullus lanatus. Figure 5.4 showed graphical representation of the chromosome 1 that is bearing the selected improved lines. The use of GGT 2.0 software gave the minimum and maximum recovery of the recurrent parent genome in an improved lined as 95.1% and 96.9 % respectively (Figure 10). It showed clearly that appreciable part of the residual segments of donor genome content were distributed on chromosome 1, 2 and 9 while the remaining 8 chromosomes were almost fully recovered. The percentage of chromosome segment derived from CS-19 in all the 10 improved lines ranged from 2.8 to 4.9% (Table 8). The average proportions of the recurrent parent genome in all 10 improved lines were 96.14%; this indicates the maximum similarity observable between the improved lines and the recurrent parent. Table 9 showed the details of the genome recovery in the selected improved lines.

Table 8: *Background recovery analysis in the selected improved lines*

Improved individuals	A (%)	B (%)	H (%)	Total (cM)	H-segment
5-5-8-21	3.1	96.5	0.3	600.5	1
5-5-8-2	4.9	95.1	0	600.5	1
5-5-8-5	3.1	96.4	0.4	600.5	1
5-5-8-30	3.1	96.5	0.4	600.5	3
5-5-8-7	3.1	95.6	1.2	600.5	1
5-5-8-8	3.1	96.9	0	600.5	0
5-5-8-62	3.8	96.2	0	600.5	1
5-5-8-12	2.8	95.3	1.9	600.5	3
5-5-8-17	3.1	96.4	0.4	600.5	1
5-5-8-53	3.1	96.5	0.3	600.5	2
Average	3.32	96.14	0.48	600.5	1.4

A= Donor, B = Recurrent, H = Heterozygous, cM, Centimorgan

3.6 Comparison of agro-morphological performance of improved lines versus recurrent parent BL-14

The Agro-morphological traits in the selected improved lines were compared with the recurrent parental line BL-14 (Table 9). There was significant variation in the days to flowering between the improved lines and the recurrent parent (BL-14). However, other traits such as days to fruit maturity, fruit weight, rind thickness, total soluble solids (TSS) and number of seeds were not significantly different in BL-14 and the improved line.

Table 9: *Comparison of some agronomic traits in BC2F2 (improved resistant lines) and the recurrent parental line BL-14*

Traits	BC_2F_2	BL-14
Days to flowering (day)	43.3[a]	42.0[b]
Days between pollination and harvesting of the fruit(day)	32.3[a]	32.0[a]
Fruit weight (kg)	2.050[a]	2.031[a]
Total soluble solid (Brix)	10.42[a]	10.26[a]
Rind thickness (cm)	13.15[a]	12.43[a]
Seed number (no)	156.6[a]	161.6[a]
Fruit colour	Deep red	Deep red

Means ±SE followed by same letter in same row are not significantly different (P < 0.05) by t-test.

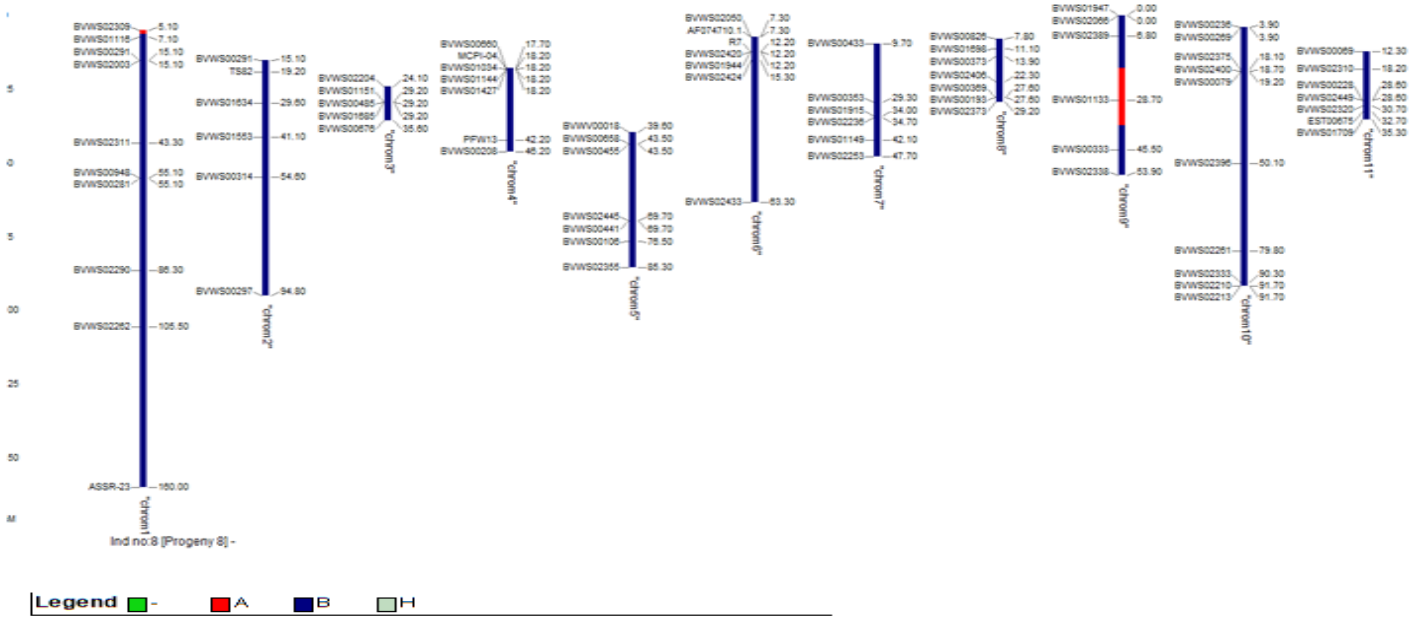

Figure 10: *Graphical genotype of the improved lined with highest recovery among the best 10 improved lines (5-5-8-8). Red color indicates region homozygous for CS-19, blue color indicate region homozygous for BL-14 and light green color indicate heterozygous regions*

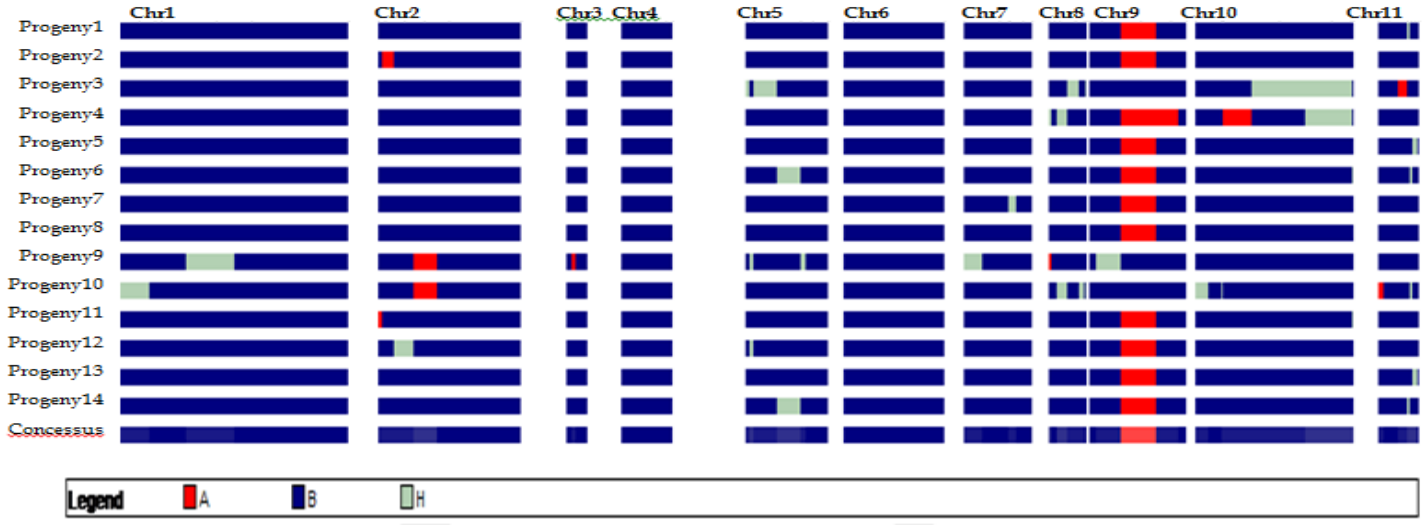

***Figure 11**: Chromosome-wise map of the selected improved lines along with BL-14 background. Red color indicated regions homozygous for CS-19, blue color indicated regions homozygous for BL -14 and light green color indicated the heterozygous region.*

4. Discussion

Marker-assisted backcross breeding has been proved by various researchers to be an effective way of introgressing a gene of interest or QTL to an elite variety [2; 5]. An essential step in marker-assisted breeding is the determination of polymorphism in the markers that are to be used for the parental genotypes. In this study, the percentage polymorphism was found to be 20.5%. Although, the level of polymorphism in the cultivated watermelon was, on an average accepted to be low [23], [31], the result of this study was higher than the five SSR markers found polymorphic from the 107 screened by [18] in a study involving New Hampshire Midget and PI 296341-FR as parents and the F_1 generated from their crossing and far lower than the 86.14% reported by [32]. The differences in percentage polymorphism might be due to differences in the parental populations used in the studies. Molecular markers in breeding work aids selection and speedy recovery of the recurrent parent genome [33]. For instance, [34], using 119 SNP markers recorded over 90% of the recurrent genome recovery after two generations and were able to develop turnip mosaic virus resistant lines of cabbage. This is rarely possible when the selection is based on phenotyping alone. Besides the number of markers used per chromosome, other factors that dictate the selection of non-carrier chromosome for background analysis include saturation of molecular map, technical resource availability and required level of line conversion [35] The use of SSR markers makes foreground selection of the target gene possible and cost effective. Marker-assisted foreground, according to [36] is primarily used to determine the target gene in the progeny (F_1) derived from crossing or selfing of the donor and recipient parent or from the product of backcrossing of the F_1 to the recurrent parent. This will guide the breeder on the progeny that should be kept for subsequent generations or discarded. In this study, SSR markers BVWS02309 showed heterozygous plants for Fusarium wilt resistance gene in the BC_1F_1 generation. The marker BVWS02309 was found by [37] to be closest to the major QTL (Qfon1.1) detected for FON-1 on chromosome 1 through single marker analysis in the 103 RIL developed from the crosses between Chinese elite cultivar 97103 and PI 296341-FR. Although at a much larger interval of 6.1cM, [38] also reported another major QTL for FON-1 on chromosome 1 and gave SNP marker SNP S1_67050 as the closest to this QTL. Based on their findings, by [37] opined that this closely linked marker could be useful in the selection of FON-1 resistant plants. The segregation of the BC_1F_1 generation plants into 1:1 (resistant versus susceptible) with Chi-square value of 1.1 was the same as the one reported by [38]. The result here was also similar to the one by [39] in a backcross involving the progenies derived from the crosses of Mallali; a susceptible cultivar of watermelon and Calhoun gray and “Summit” that were resistant to Fusarium wilt disease. Background selection as an efficient means of speeding up the recovery of recurrent parent genome has been confirmed by [40] and [41]. [42], using computer simulation, further showed that with the use of molecular markers in background selection can speed up the recovery of recurrent genome by 2 or 3 generations. Also, [43] reported shortening of number of backcross up to ten folds through the use of molecular markers in selecting against genetic drag. The estimation of the recurrent parent genome recovery in this study was carried out following the graphical genotypes concept introduced by [44]. The RPG recovery in the BC_1F_1 generation was found to be in the range of 74.4% and 94.4%. This was similar to the one recorded by [34] where 3 of the 75 BC_1F_1 showed over 80% RPG recovery. Similarly, [9] also reported the average RPG recovery of 80.7% in BC_1F_1 while introgressing heat shock protein gene(s) from AVPP0702 (C. annuum L.), into Kulai; a high yielding but heat sensitive chilli variety. The analysis of background recovery indicates that most of the residual segments were distributed over the chromosomes 1, 6 and 10. In the BC_2F_1 generation, the recurrent parent genome recovery was between 86.6% and 96.8%. This result was similar to the RPG recovery of between 89% and 95% mentioned by [45] while using SSR markers to introgress Fusarium wilt resistance locus foc 1 into C 214; an elite cultivar of chickpea. However, [46] recorded 99.1% in the BC_2 population when they used SSR marker Frg13 to introgress Fusarium wilt resistance into elite cabbage line 01-20.It can be deduced that the selection of the best genotype in each

generation is aided by the estimation of the recurrent parent allele in each backcross. It therefore, increases the probability of selecting the best plants carrying target allele and showing highest closeness to the elite recurrent line. The RPG recovery of over 96.8% recorded in this study at the BC_2 generation showed that the analysis of the recurrent genome in each backcross leads to reduction in the likely linkage drag from the donor parent that could have occurred. The use of molecular markers here proved that it can facilitate the quick recovery of RPG with few backcrosses. The speedy recovery of the recurrent genome might be due to the wide spread of between 5 and 10 polymorphic SSR markers spread throughout the whole 11 chromosomes. Even when widely spaced, the location of the markers is equally important [43]. Besides the distribution of the markers on the chromosomes, other factors that determine the success of marker-assisted backcross breeding include; the number of target genes to be transferred, the availability of saturated molecular map, the backcrossing scheme, and selection strategy adopted [6].The genotypic selection, followed by phenotypic selection was proposed by [47]. It was a modification to MAS introduced by [44] and has been confirmed to yield better result in terms of high-RPG recovery. The technique of combining genotypic and phenotypic selections was used in this study. Although, it is generally agreed that the more the number of markers used, the more the expected efficiency of MAS, the 20.5% polymorphic SSR markers used between the parental lines were found adequate for background recovery. This is based on the discovery of [23] and [31] that most watermelon cultivars have low DNA polymorphism. As expected, the combination of background markers with a strong phenotypic selection led to rapid selection of the best plants within the minimum number of backcross generations. It thus gave a reduction in the time required to develop improved lines, saves cost as well as labor.An important condition for the successful implementation of any marker-assisted selection is the strong linkage between the marker and target gene [48]. In this study, the result of the screening for Fusarium wilt resistance, led to the selection of ten BC_2F_2 lines (lines 5-5-8-2, 5-5-8-5, 5-5-8-8, 5-5-8-12, 5-5-8-17, 5-5-8-21, 5-5-8-30, 5-5-8-53, 5-5-8-62 and 5-5-8-7) that showed strong resistance to Fusarium wilt when subjected to artificial inoculation using the virulent Fusarium oxysporun niveum isolate (5p) with accession number:KF493921.1. The disease severity rating of these ten lines was almost equal to that of the donor parent. The success of the introgression was confirmed by the display of phenotypic similarity between improved lines and the recurrent parental line BL-14. Thus, the improved lines had same background as the recurrent parent but with the addition of the major QTL controlling Fusarium wilt disease resistance Seventy-eight SSR markers that showed polymorphism between the parental lines with not less than 5 markers per chromosome were used for genetic background selection. Most of the recurrent parent segments were fully recovered in improved lines with few left not fully recovered. The presence of some heterozygous segments was also found in few improved lines. This was similar to the findings of [49] and [50] who ascribed the presence of some regions not fully recovered in BC_2F_2 generation to the stoppage of the backcross earlier than the BC_3F_2 generations. It can therefore be deduced that the adoption of foreground and background selection for high recovery could lead to saving of cost and time.The comparison of the agro-morphological traits between donor parental inbred line CS-19 and recurrent parent BL-14 showed significant differences in the traits. However, it was noted that even after the introgression of the wilt resistance gene into the recurrent parent, the improved lines still maintained the desired traits found in the recurrent parent. This result showed that the cultivation of the improved Eusarium resistant lines would not have any adverse effect on the yield as well as the quality of the fruits. This assurance is important considering the fact that Fusarium wilt has been a major threat to the production of watermelon worldwide, with Malaysia not an exception. Thus, when a cultivar that is high yielding and wilt resistant is available, it will aid the farmers' income as individuals and safe the resources from millions of Malaysia Ringgit spend annually to import good seeds. The phenotypic-based selection of the agro-morphological traits across the selection stages starting from BC_1F_1 generation, really led to the high recovery of desirable improved plants BC_2F_2 plants of BL-14. The strategies of phenotypic selection along with marker based selection are in agreement with results of [33] and [40] who used the phenotypic based selection for the introgression of Fusarium wilt resistance genes into elite cultivars of melon and cabbage respectively. As shown in Table 9, there was difference in the first day to flower between the improved lines and the recurrent parent. This might be because of the influence of the donor parent that usually takes longer time before the first flowering when compared to the time taken by the recurrent parent (BL-14). For all the other traits measured, there was no significant difference, this was in tandem with what [33] reported about the BC_3 generation of melon plants developed for Fusarium wilt resistance when compared with the recurrent parent.

5. Conclusions

The reliability of SSR markers in marker-assisted selection for the development of Fusarium wilt resistant watermelon cultivar was shown in this study. Marker-assisted backcrossing (MABC) approach using SSR markers combined with phenotypic selection as a potential method of accelerating recipient genome recovery was confirmed. Average recipient allele recovery in the five selected plants from the BC_1F_1 and BC_2F_1 generations were 88.22% and 95.22% respectively. These were higher than the expected values from the conventional approach and thus, showed the possibility of having the total recovery of the recipient genome after three backcrossing. The study led to the development of 10 improved Fusarium wilt resistant lines from a backcross between parental line of BL-14 and CS-19. It is believed that the resistant lines could be utilized as a source of genetic material for Fusarium wilt resistance with high yielding background of BL-14. The introgressed major QTL confers resistance to Fusarium wilt disease in a dominant manner. The study also showed that the introduction of the resistant gene into the developed improved lines have practical breeding value without affecting the yield while conferring resistance to Fusarium wilt disease on the Fusarium oxysporum niveum pathotype in Malaysia. The development of the improved resistant lines will further broaden the genetic resource base of watermelon and eventually assist further research aimed at increasing the local production of watermelon. As a contribution to knowledge, this is first report on successful introgression of Fusarium wilt resistant QTL into elite inbred line BL-14 noted for its high yielding potential in

Malaysia. Author Contributions: All the authors contributed to the paper.

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