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Introgression of Tomato Leaf Curl Virus (ToLCV) Resistant Gene into two Cultivated Tomato (*Solanum lycopersicum* L.) Varieties through Marker Assisted Backcross Breeding

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ABSTRACT: Tomato (*Solanum lycopersicum* L.) is widely grown in the tropical and subtropical regions of the world but its growth and production is limited because of most of the diseases. Among all the diseases ToLCV is a devastating disease worldwide causing complete yield loss in affected area. So far, resistance to begomoviruses in tomato has been achieved using wild species, and at least six resistance genes have been studied. The complex epidemiological factors associated with this disease, such as broad host range, high rates of virus evolution and the migratory behaviour of whiteflies make it difficult to develop effective long-term management strategies. Therefore, breeding resistance to this viral disease in tomato cultivars is an essential element of a sustainable approach in managing the diseases caused by begomoviruses. The present study was undertaken to introgress *Ty-2* conferring resistance to monopartite begomovirus into two cultivated varieties (GPBT-08 and DMT-2) from two donors *i.e.*, CLN2768A and CLN2777H through marker assisted backcross breeding (MABB) by two foreground markers viz., TG0302 and P1-16. Marker-assisted background selection was carried out using 39 polymorphic SSR markers for GPBT-08 and CLN2768A and 35 polymorphic SSR markers for DMT-2 and CLN2777H distributed on 12 chromosomes of tomato genome that helped to reduce non target donor parent genome. In GPBT-08 × CLN2768A maximum RPG recovered is 97.44% in BC_2F_4 and 98.71% in BC_3F_3 and in DMT-2 × CLN2777H maximum RPG recovered is 97.44% in BC_2F_4 and 97.14% in BC_3F_3. The response of these lines for leaf curl resistance was assessed by transplanting artificially inoculated plants to field in a disease hotspot season *i.e.*, summer 2019. The introgressed lines exhibited a high level of resistance to the ToLCV disease tested with minimum percent disease incidence. Stable introgressed tomato lines *i.e.*, NILs of BC_2F_4 and BC_3F_3 generation of both the crosses similar to recurrent parent in fruit morphology and yield potential were developed. The agronomic performance of NILs in field at disease stress condition showed that the yield and yield related traits are well maintained in the plants. The introgressed tomato lines developed in this study could be important genetic resources for sustainable tomato production in areas affected by tomato leaf curl virus disease.

Keywords: tomato, ToLCV, MABB, *Ty-2*, Foreground selection, Background selection.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to the family Solanaceae and it is one of the most important widely cultivated vegetable crop worldwide. Since it has high nutritional value, tomato has become a popular vegetable grown in large scale (Naika *et al*., 2005). Even though it can be grown in wide range of environmental conditions its growth and production is limited by many major and minor disease and pest. Among them leaf curl disease (ToLCV) is one of the most destructive disease caused by whitefly transmitted begomoviruses (Moriones and Navas-Castillo, 2000) which widely affect tomato crop during the summer season in southern parts of India (Saikia and Muniyappa, 1989) and autumn in northern parts of India (Banerjee and kalloo, 1987). In southern India particularly in Karnataka state alone, the incidence of ToLCV in susceptible cultivars caused quality and yield reduction *i.e.*, 90-100% yield reduction (Saikia and Muniyappa, 1989 and Varma and Malathi, 2003).

Over the past 30 years, as a consequence of increasing commercial exchanges and global climate changes, these viruses have emerged as a serious threat for the cultivation of several important crops in different parts of the world, especially in tropical and sub-tropical areas (Hanssen *et al*., 2010). It infect a broad host range *i.e.*, dicotyledonous plants and including many important crops of the families Solanaceae most Severe problem in tomato (Seal *et al.*, 2006). The geographical distribution of tomato-infecting begomoviruses in India indicated that ToLCV isolates from South India constituted a diverse group of monopartite viruses that was distinct from the bipartite tomato begomoviruses of North India (Muniyappa *et al.*, 2000 and Chakraborty *et al.*, 2003).

In many regions of the world, this disease management using several approaches including insecticide applications, physical barriers and cultural practices are

suggested for managing whiteflies. However, these vector management strategies proven to be uneconomical and laborious (Hilje *et al*., 2001 and Palumbo *et al*., 2001). The complex epidemiological factors associated with this disease, such as broad host range, high rates of virus evolution and the migratory behaviour of whiteflies make it difficult to develop effective long-term management strategies. Therefore, breeding resistance to this viral disease in tomato cultivars is an essential element of a sustainable approach in managing the diseases caused by begomoviruses.

Because high levels of resistance to tomato-infecting begomoviruses did not exist in the gene pool of cultivated tomatoes, resistance or tolerance was sought in related wild species. Resistance to tomato-infecting begomoviruses has been successfully introgressed from *Solanum pimpinellifolium, Solanum peruvianum, Solanum chilense and Solanum habrochaites* (Ji *et al.,* 2007b). From these sources, a few resistance genes have been well characterized and mapped on their respective chromosomes i.e., *Ty-1* and *Ty-4* on chromosome 6 (Verlaan *et al.,* 2011), *Ty-2* on chromosome 11 (Yang *et al.,* 2014), *Ty-4* on chromosome 3 (Ji *et al.,* 2009), *ty-5* on chromosome 4 (Hutton *et al.,* 2012 and Levi *et al.,* 2013) and *Ty-6* on chromosome 10 (Hutton and Scott 2013) using molecular markers.

The *Ty-2* gene is derived from *S. habrochaites* provides high levels of resistance to monopartite begomovirus which is highly prevalent in southern India (Chen *et al.,* 2015) which has been successfully used in breeding programs to develop resistant lines or cultivars and its first mapped a dominant resistance gene, *Ty-2*, in *S. habrochaites* derived line H24, to the long arm of chromosome 11 (Hanson *et al.* 2000), recently fine-mapped to a 300 Mb region of chromosome 11 (Yang *et al*., 2011).

Considering above information, the present investigation was conducted to use a gene (*Ty*-2) resistant to ToLCV disease carried by two donors CLN2768A and CLN2777H to introgress into two cultivated varieties GPBT-08 and DMT-2 respectively by Marker assisted backcross breeding approach (MABB) and helped to develop a ToLCV resistant version of GPBT-08 and DMT-2 which could be important genetic resources for sustainable tomato production in areas affected by tomato leaf curl virus disease.

MATERIALS AND METHODS

In the present study two cultivated varieties GPBT-08 and DMT-2 (susceptible to ToLCV) were used as female recipient parent released by University of Agricultural Sciences (UAS), Dharwad for northern Karnataka region, India and two lines CLN2768A and CLN2777H was used as male donor parents carrying *Ty-2* gene resistant to ToLCV disease obtained from AVRDC, Taiwan. Breeding material, F_1 and backcross lines were developed at experimental field, Botany Garden, Department of Genetics and Plant Breeding, UAS, Dharwad, India (Fig. 1). GPBT-08 is crossed with CLN2768A and generated 25 F_1 plants in *Kharif* 2016, finally identified true F_1's were backcrossed to the recurrent parent GPBT-08 to get 34 BC_1F_1 plants in *rabi,* 2016, selected plants which were carrying target gene and similar to the recurrent parent were subsequently backcrossed to get 33 plants in BC_2F_1 and 26 plants in BC_3F_1 generations during summer, 2017 and *Kharif,* 2017 respectively. In BC_2F_1 and BC_3F_1, plants carrying *Ty-2* gene were subjected to background selection and plants with highest recurrent parent genome were selfed to get BC_2F_2 and BC_3F_2 the same procedure was followed to get BC_2F_4 and BC_3F_3 where foreground and background selection was carried out along with phenotypic screening of backcross lines for ToLCV disease during summer, 2019 in field condition and also evaluated for different yield and yield related traits.

DMT-2 is crossed with CLN2777H to get 33 F_1's in *Kharif* 2016. True F_1's were backcrossed to the recurrent parent DMT-2 to get 37 BC_1F_1 plants respectively in *rabi,* 2016, selected plants carrying target gene and similar to the recurrent parent were subsequently backcrossed to get 32 plants in BC_2F_1 and 24 plants in BC_3F_1 generations during summer, 2017 and *Kharif,* 2017 respectively. BC_2F_1 and BC_3F_1 plants carrying *Ty-2* gene were subjected to background selection and the plants with highest recurrent parent genome were selected selfed to get BC_2F_2 and BC_3F_2 population and the same procedure was followed to get BC_2F_4 and BC_3F_3 where foreground and background selection was carried out along with phenotypic screening of backcross lines for ToLCV disease during summer, 2019 in field condition and also evaluated for different yield and yield related traits.

Molecular analysis

For each genotype, DNA was extracted according to Bernatzky and Tanksley 1986 using CTAB (Cetyl Trimethyl Ammonium Bromide) method. Young leaves (about 1-2 g) of each plant were ground to a fine powder using pestle and mortar in the presence of liquid nitrogen and transferred to a sterile polypropylene tube (2 ml) containing 1 ml of preheated (65^{o}C) CTAB extraction buffer (1M Tris-HCL buffer pH 8, 4M NaCl, 0.5 M ethylene diaminetetraacetic acid (EDTA) pH 8, 10 per cent hexadecyltrimethyl ammonium bromide, 1ml mercaptoethanol and 100 mg polyvinylpyrrolidone). The contents were incubated at 65^{o}C for 30-45 min in a water bath with occasional shaking during incubation. The tubes were cooled to room temperature, spinned at 13,000 rpm at 4^{o}C for 8 min in refrigerated centrifuge and the supernatant was transferred to another tube (2 ml). An equal quantity (1 ml) of chloroform: iso-amyl alcohol (24:1) was added to each tube and were firmly capped and shaked vigorously. The tubes were spinned at 13,000 rpm at 4^{o}C for 8 min in a refrigerated centrifuge. The aqueous phase was transferred to another 1.5 ml eppendorf tubes and DNA precipitated using 700 µl pre chilled Iso-propanol alcohol. Tubes were kept at -20^{o}C for overnight. The precipitate was rinsed with 70 per cent ethanol, and re-spun to pelletilize DNA. Supernatant was poured off and allowed to air-dry the pellet. The pellet was then dissoloved in 50 µl of $T_{10}E_1$ (10 mM

Tris pH 8.0 at 25°C: 1 mM EDTA). The quality and concentration of DNA was assessed by using gel electrophoresis (0.8 % agarose) with known concentrations of uncut lambda DNA. Added 1-2 µl of RNase (10 mg/ml) and incubate at 37°C for 40-45 min. Stored at -20°C.

PCR was performed in 20 µl reaction mixture containing 1 µl of template DNA (50 ηg/µl), 2 µl of 10x PCR buffer, 0.8 µl of 1.0 mM dNTPs, 0.8 µl of 5 ρmol forward and reverse primers each, 0.2 µl of 1 U/µl *Taq* polymerase and 13.4 µl of nanopure water each. After initial denaturation for 5 minutes at 95°C, each cycle comprised denaturation at 94°C for 30 seconds, annealing at 58°C for 1 minute, extension at 72°C for 2 minutes and finally final extension at 72°C for 10 minutes at the end of 30 cycles. The PCR products were mixed with bromophenol blue gel loading dye and were analysed by electrophoresis on 3% polyacrylamide gel. The gels were stained with Ethidium bromide (10 µl/ 100 ml of double distilled water) and were documented using BIORAD Gel Doc XR+.

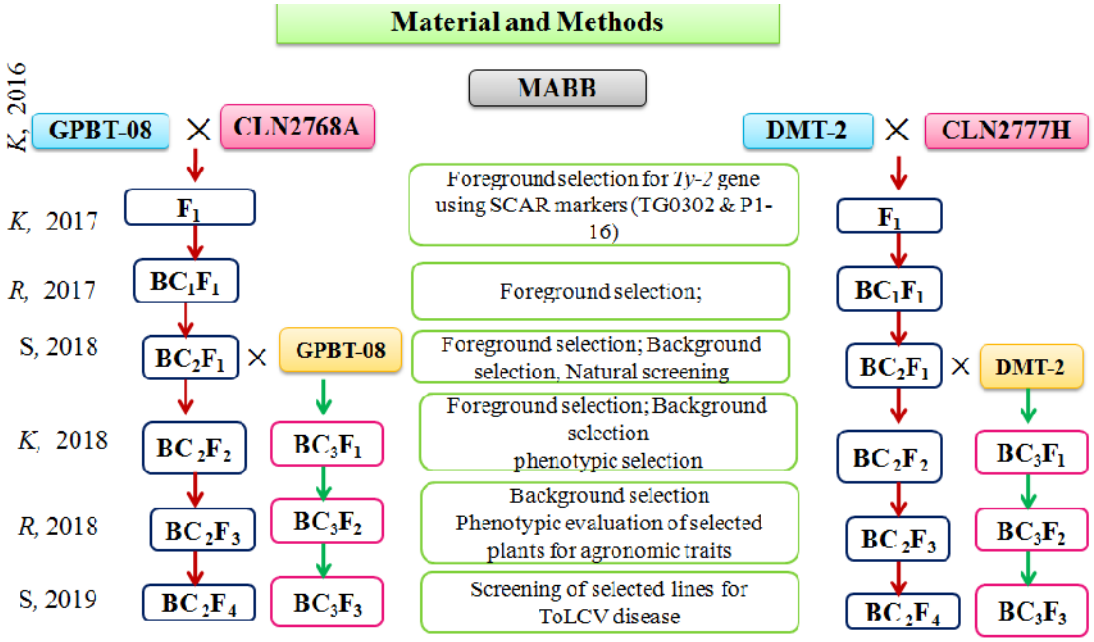

Fig. 1. Flow chart of generation of backcross populations.

Foreground selection

Selection for the specific trait under consideration is known as foreground selection; here for the ToLCV disease resistant gene *Ty-2* from CLN2768A and CLN2777H were selected. This particular gene is located on chromosome 11. For foreground selection, two markers TG0302 (Garcia *et al.*, 2007) and P1-16 (Yang *et al.*, 2014) tightly linked to *Ty-2* gene exhibited parental polymorphism and were subsequently used to generate the genotyping data (Table 1).

Table 1: List of markers used for validation in tomato.

S. No.	Marker name	Type of marker	Forward (F) and Reverse (R)	Reference
1.	TG0302	SCAR	**F:** TGGCTCATCCTGAAGCTGATAGCGC **R:** AGTGTACATCCTTGCCATTGACT	Garcia *et al.* (2007)
2.	P1-16	SCAR	**F:** CACACATATCCTCTATCCTATTAGCTG **R:** CGGAGCTGAATTGTATAAACACG	Yang *et al.* (2014)

Background selection

Background selection for the recovery of the recipient parent is known as background selection. Except target locus, all genomic regions can be selected in background selection using RP marker alleles which are distributed on all chromosomes. This selection is important in order to reduce non target genes (linkage drag) (Fig. 3) from donor parent except target gene. For this purpose, only polymorphic markers between parents which were not linked to the concerned resistant gene *Ty-2* gene and distributed well throughout the genome were used (Somers *et al.*, 2004; Roder *et al.*, 1998; Kadam *et al.*, 2012; Pestsova *et al.*, 2000; Gupta *et al.*, 2002). So, a total of 425 markers for the parents GPBT-08, CLN2768A DMT-2 and CLN2777H were used from the panel of highly polymorphic markers distributed throughout the genome of tomato. Out of these 39 markers for the parents GPBT-08 and CLN2768A and 35 markers for the parents DMT-2 and CLN2777H exhibited polymorphism. Those exhibiting polymorphism were subsequently used for background selection. In each backcross (BC_2F_1 and BC_3F_1) and selfed generations (BC_2F_2, BC_2F_3, BC_2F_4, BC_3F_2 and BC_3F_3), allele replacement rate or recovery of the recurrent parent genome was calculated by using the formula (Neeraja *et al.*, 2007);

$$(RPG\ \%) = \frac{A + 1/2\ H}{N} \times 100$$

A = Number homozygous marker loci for recurrent parent allele

H = Number heterozygous marker loci with alleles of both parents

N = Total number of polymorphic markers used for background screening

For pictorial visualisation of parental segments in BC_2F_4 and BC_3F_3 progenies, resultant genotypic data was subjected to graphical genotypic analysis using GGT v.2.0 software package (Berloo, 2008).

Phenotypic screening

The backcross derived lines i.e., BC_2F_4 and BC_3F_3 generations of both the crosses were phenotypically screened for their reaction to ToLCV disease resistance under field conditions by transplanting artificially inoculated seedlings during summer, 2019. Observation was taken on percent disease incidence (PDI) at 30, 60 and 90 DAT and mean of PDI was calculated.

$$PDI = \frac{\text{Total number of plants infected with ToLCV}}{\text{Total number of plants}} \times 100$$

The disease severity score was based on Saari and Prescott's 0-4 scale for assessing foliar disease and the genotypes were classified on a 5-point scale using the resistance criterion proposed by (Muniyappa *et al*., 1991). Lines with 0 per cent incidence considered as resistant (R), upto 25 per cent incidence considered as moderately resistant (MR), 26-50 per cent incidence considered as tolerant (T), 51-75 per cent incidence considered as susceptible (S) and >75 per cent incidence considered as highly susceptible (HS).

Evaluation of near isogenic lines (NILs):

The developed ToLCV disease resistant NILs i.e., BC_2F_4 and BC_3F_3 of both crosses having maximum recovery of RPG along with phenotypic similarity with the recipient parent were evaluated for yield and yield related traits under disease stress condition along with parents GPBT-08, CLN2768A, DMT-2 and CLN2777H under field condition. Observations were recorded on Plant height (cm), Number of primary branches, Number of secondary branches, Number of clusters per plant, Number of fruits per cluster, Number of fruits per plant, Equatorial length of fruit (cm), Fruit diameter (cm), Fruit shape index, Pericarp thickness of fruit (cm), Number of locules, Total soluble solids (%), Average fruit weight (g) and Yield per plant (Kg). These parameters were recorded from the best selected BLs which are phenotypically resistant to ToLCV disease, along with the parents, GPBT-08, CLN2768A, DMT-2 and CLN2777H.

Statistical analysis:

An analysis for the goodness of fit to the expected ratio of 1:1 was calculated for each BC_3F_1, BC_2F_1 and BC_3F_1 populations using the Chi-square test. All analysis was performed using R studio. The Chi square values were calculated by using the formula;

$$\chi^2 = \frac{\Sigma (o^i - e^i)^2}{e^i}$$

o^i = observed frequency, e^i = expected frequency

The mean difference for the selected best BLs and the recurrent parents GPBT-08 and DMT-2 was analyzed using *t*-test.

RESULTS

A. Foreground and background selection

The inefficiency of selection based on phenotype has been noticed in previous studies emphasizing the limitations associated with traditional backcross breeding for complete recovery of recurrent parent genome (*Yi et al.,* 2009). With the advent of molecular markers gene mapping was achieved, which helped to identify molecular makers linked to gene of interest. Those markers could be used in maker assisted selection. Among different approaches, MABB is one which could be exploited for transfer of specific genomic regions in to recipient parent background with high efficiency (Kumar and Hittalmani, 2000; Jena and Mackill, 2008).

Two SCAR markers TG0302 and P1-16 tightly linked to *Ty-2* gene resistant to ToLCV disease were used in the present study, which clearly differentiated between the donor parents (CLN2768A and CLN2777H) and the two recurrent parents (GPBT-08 and DMT-2). Plate 1 shows an amplified product of marker TG0302 with product size 900bp for donor parents and 800bp for recurrent parents and marker P1-16 with product size 300bp for donor parents and 600bp for recurrent parents.

In BC_1F_1, BC_2F_1 and BC_3F_1 generations, best plants were selected and forwarded to get BC_2F_4 and BC_3F_3. All the backcross lines carrying *Ty-2* gene were selected using foreground markers (Plate 2 and 3). In the cross GPBT-08 × CNL2768A, in BC_1F_1 population out of 620 plants, 303 were heterozygous (H) and 317 were homozygous (B) susceptible similar to GPBT-08 and segregated in the ratio 1:1 with χ^2 value 0.79 (P=0.37). Similarly BC_2F_1 population segregated in the ratio 1:1 (164:149) with χ^2 value 0.72 (P=0.39) and BC_3F_1 population segregated in the ratio 1:1 (106:119) with χ^2 value 0.75 (P=0.38).

In the cross DMT-2 × CNL2777H, in BC_1F_1 population out of 645 plants, 330 were heterozygous (H) and 315 were homozygous (B) susceptible similar to DMT-2 and segregated in the ration 1:1 with χ^2 value 0.72 (P=0.39). Similarly BC_2F_1 population segregated in the ratio 1:1 (172:165) with χ^2 value 0.59 (P=0.44) and BC_3F_1 population segregated in the ratio 1:1 (121:133) with χ^2 value 0.56 (P=0.45).

Background Selection

For background selection, a total of 39 polymorphic SSR markers for the parents GPBT-08 and CLN2768A and 35 polymorphic SSR markers for the parents DMT-2 and CLN2777H distributed over the 12 tomato chromosome were used to screen the backcross population to select the plants carrying maximum recurrent parent genome. The range of recurrent parent genome recovered in both the crosses is shown in the Table 2.

Average recovery of recurrent genome of the cross GPBT-08 × CLN2768A in BC_2F_4 and BC_3F_3 lines is 92.77 % and 93.69 % respectively (Table 3) and for the

cross DMT-2 × CLN2777H in BC_2F_4 and BC_3F_3 lines is 93.82 % and 93.93 % respectively (Table 4). Graphical representation of the RPG recovered for BC_2F_4 and BC_3F_3 lines of both the crosses shown in (Fig. 2, 3, 4 and 5).

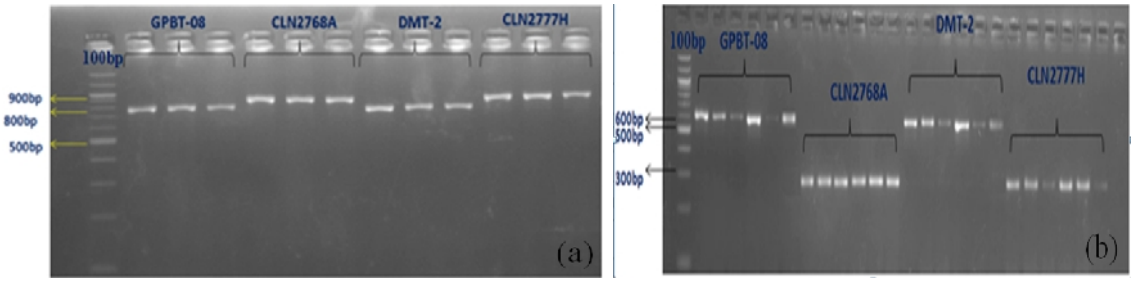

Plate 1. Parental polymorphism between GPBT-08 and CLN2768A and between DMT-2 and CLN2777H using SCAR marker (a) TG0302 (b) P1-16

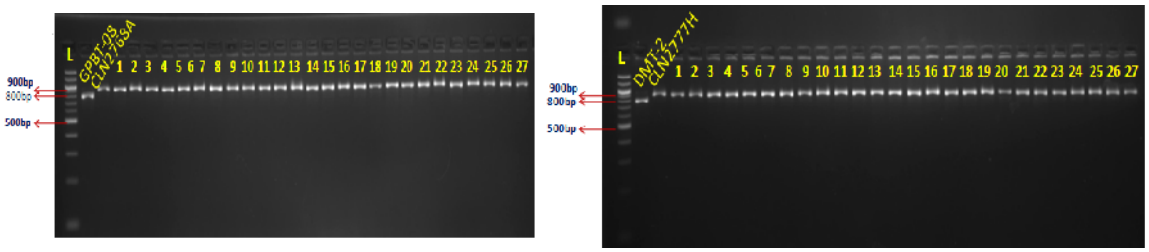

Plate 2. Genotyping of backcross population of the cross GPBT-08 × CLN2768A and DMT-2 × CLN2777H carrying *Ty-2* gene in homozygous condition using SCAR marker TG0302 (L: Ladder; 1 to 27: backcross population).

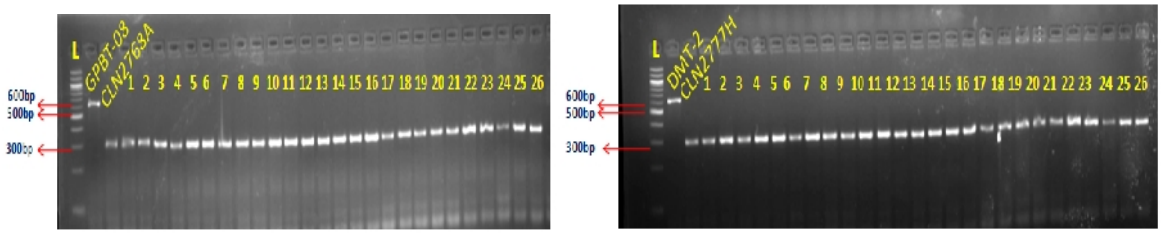

Plate 3. Genotyping of backcross population of the cross GPBT-08 × CLN2768A and DMT-2 × CLN2777H carrying *Ty-2* gene in homozygous condition using SCAR marker P1-16 (L: Ladder; 1 to 27: backcross population).

Table 2: Proportion of recurrent parental genome observed in backcross progenies of two crosses.

Backcross generation	Cross	Number of polymorphic markers	Total number of alleles	Expected recurrent parent genome recovery (%)	Range of Percent recurrent genome recovered
BC_1F_1	GPBT-08 × CLN2768A	39	78	75.00	64.5 – 83.33
	DMT-2 × CLN2777H	35	70	75.00	62.5 – 82.86
BC_2F_1	GPBT-08 × CLN2768A	39	78	87.50	76.5 – 91.03
	DMT-2 × CLN2777H	35	70	87.50	79.5 – 90.43
BC_3F_1	GPBT-08 × CLN2768A	39	78	93.75	84.5-94.87
	DMT-2 × CLN2777H	35	70	93.75	86.5-92.89
BC_2F_2	GPBT-08 × CLN2768A	39	78	87.50	88.57 – 93.59
	DMT-2 × CLN2777H	35	70	87.50	89.33 – 94.44
BC_3F_2	GPBT-08 × CLN2768A	39	78	93.75	87.18-96.15
	DMT-2 × CLN2777H	35	70	93.75	88.57-94.29

Phenotypic screening of parents and NILs

In the present study parents along with selected BC_2F_4 and BC_3F_3 populations of the cross GPBT-08 × CLN2768A and DMT-2 × CLN2777H were subjected to phenotypic screening for ToLCV disease resistance.

In the present study selected BC_2F_4 populations were subjected to phenotypic screening for ToLCV disease resistance during summer, 2019 under natural condition by transplanting artificially inoculated seedlings in the field. Per cent disease incidence of the cross GPBT-08 × CLN2768A of BC_2F_4 lines was ranged from 7.41 to 43.71%, where, 17 lines were moderately resistant and 9 lines were tolerant. Lines GA-7-5-8 (7.41%), GA-20-4-2 (7.41%), GA-20-4-7 (15.83%), GA-3-9-2 (16.20%) and GA-41-7-1 (16.20%) showed minimum PDI (Table 5).

Table 3: Proportion of recurrent parental genome (RPG) observed in NILs (BC_2F_4 and BC_3F_3) progenies of the cross GPBT-08 × CLN2768A using SSR markers in tomato.

Progenies (BC_2F_4)	Total no. of alleles	RP allele	Door parent allele	Percent RPG recovered (1)	Expected RPG recovery (2)	Difference (1-2)	Progenies (BC_3F_3)	Total no. of alleles	RP allele	Door parent allele	Percent RPG recovered (1)	Expected RPG recovery (2)	Difference (1-2)
GA-3-9-2-5	78	72	6	92.31	87.5	4.81	GA-2-4-2	78	66	12	88.46	93.75	1.12
GA-3-9-2-7	78	71	7	91.03	87.5	3.53	GA-2-4-5	78	73	5	93.59	93.75	2.40
GA-5-2-5-3	78	72	6	92.31	87.5	4.81	GA-3-4-6	78	70	8	89.74	93.75	1.12
GA-6-1-4-2	78	75	3	96.15	87.5	8.65	GA-3-5-6	78	72	6	92.31	93.75	-0.16
GA-6-1-4-6	78	75	3	96.15	87.5	8.65	GA-5-6-3	78	70	8	89.74	93.75	-1.44
GA-6-1-7-6	78	71	7	91.03	87.5	3.53	GA-5-6-7	78	76	2	97.44	93.75	2.40
GA-7-5-1-5	78	73	5	93.59	87.5	6.09	GA-6-3-3	78	74	4	94.87	93.75	1.12
GA-7-5-1-3	78	71	7	91.03	87.5	3.53	GA-6-5-6	78	76	2	97.44	93.75	3.69
GA-11-2-4-4	78	71	7	91.03	87.5	3.53	GA-7-3-7	78	71	7	91.03	93.75	-1.44
GA-11-2-4-7	78	72	6	92.31	87.5	4.81	GA-8-5-5	78	74	4	94.87	93.75	2.40
GA-12-6-7-8	78	73	5	93.59	87.5	6.09	GA-9-6-4	78	75	3	96.15	93.75	-0.16
GA-16-9-9-5	78	76	2	97.44	87.5	9.94	GA-11-4-6	78	70	8	89.74	93.75	-0.16
GA-16-9-9-7	78	76	2	97.44	87.5	9.94	GA-12-3-8	78	75	3	96.15	93.75	1.12
GA-23-6-7-3	78	72	6	92.31	87.5	4.81	GA-12-3-9	78	72	2	92.31	93.75	2.40
GA-23-6-7-6	78	71	7	91.03	87.5	3.53	GA-14-6-3	78	75	3	93.59	93.75	3.69
GA-26-2-3-2	78	73	5	93.59	87.5	6.09	GA-14-7-6	78	71	7	91.03	93.75	2.40
GA-26-2-3-9	78	73	5	93.59	87.5	6.09	GA-16-4-4	78	76	2	97.44	93.75	-1.44
GA-27-7-4-5	78	73	5	93.59	87.5	6.09	GA-17-5-6	78	77	1	98.71	93.75	1.12
GA-27-7-4-8	78	75	3	96.15	87.5	8.65	GA-18-8-7	78	69	9	88.46	93.75	2.40
GA-27-7-6-3	78	76	2	97.44	87.5	9.94	GA-20-7-8	78	74	4	94.87	93.75	2.40
GA-27-7-6-5	78	75	3	96.15	87.5	8.65	GA-23-2-3	78	74	4	94.87	93.75	1.12
GA-37-9-5-3	78	73	5	93.59	87.5	6.09	GA-24-5-7	78	73	5	93.59	93.75	1.12
GA-37-9-5-6	78	74	4	94.87	87.5	7.37	GA-26-4-4	78	75	3	96.15	93.75	2.40
GA-37-9-6-4	78	73	5	93.59	87.5	6.09	GA-28-2-3	78	73	5	93.59	93.75	-0.16
GA-40-1-8-3	78	74	4	94.44	87.5	6.94	GA-31-9-8	78	75	3	96.15	93.75	2.40
GA-40-1-8-2	78	74	4	94.44	87.5	6.94				Average	93.69		
GA-40-1-8-6	78	75	3	96.15	87.5	8.65				Minimum	88.46		
GA-41-7-1-5	78	73	5	93.59	87.5	6.09				Maximum	98.71		
GA-41-7-1-9	78	74	4	94.87	87.5	7.37							
GA-41-7-4-3	78	75	3	96.15	87.5	8.65							
GA-41-7-4-7	78	76	2	97.44	87.5	9.94							
			Average	92.77									
			Minimum	86.15									
			Maximum	97.44									

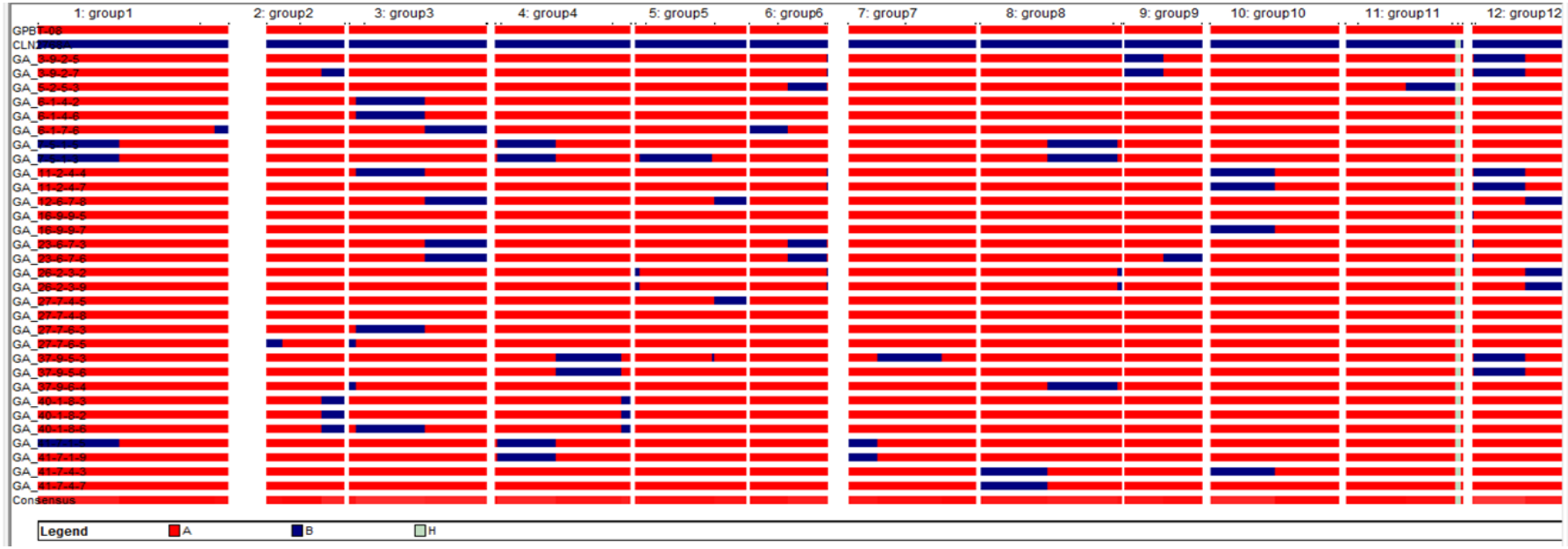

Fig. 2. Genetic constitution of selected BC_2F_4 lines of the cross GPBT-08 × CLN2768A carrying target gene.

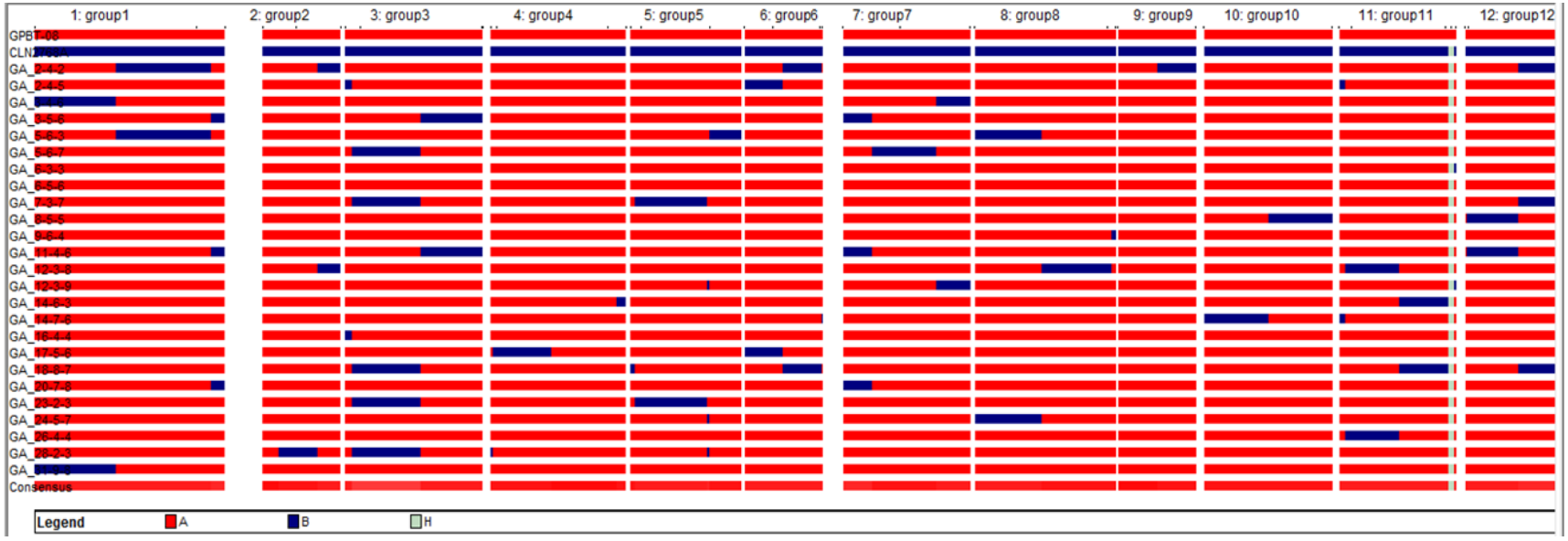

Fig. 3. Genetic constitution of selected BC_3F_3 lines of the cross GPBT-08 × CLN2768A carrying target gene.

Table 4: Proportion of recurrent parental genome observed in NILs (BC_2F_4 and BC_3F_3) of the cross DMT-2 × CLN2777H using tomato SSR markers.

BC_2F_4	Total no. of alleles	Recurrent parent allele	Door parent allele	Percent RPG recovered (1)	Expected RPG recovery (2)	Difference (1-2)	BC_3F_3	Total no. of alleles	Recurrent parent allele	Door parent allele	Percent RPG recovered (1)	Expected RPG recovery (2)	Difference (1-2)
DH_1-4-2-2	70	63	7	90.00	87.5	85.00	DH-2-1-2	70	63	7	90.00	93.75	-3.75
DH_1-4-2-4	70	64	6	91.42	87.5	83.58	DH-3-2-3	70	64	6	91.42	93.75	-2.33
DH_3-5-4-6	70	67	3	95.71	87.5	79.29	DH-4-2-2	70	63	7	90.00	93.75	-3.75
DH_3-5-4-8	70	67	3	95.71	87.5	79.29	DH-4-7-4	70	66	4	94.29	93.75	0.54
DH_4-8-2-3	70	65	5	92.86	87.5	82.14	DH-4-9-6	70	67	3	95.71	93.75	1.96
DH_4-8-2-6	70	65	5	92.86	87.5	82.14	DH-5-6-8	70	68	2	97.14	93.75	3.39
DH_5-1-6-7	70	63	7	90.00	87.5	85.00	DH-7-4-5	70	67	5	95.71	93.75	1.96
DH_9-5-3-5	70	67	3	95.71	87.5	79.29	DH-8-7-6	70	66	4	94.29	93.75	0.54
DH_9-5-3-8	70	68	2	97.14	87.5	77.86	DH-9-5-8	70	64	6	91.42	93.75	-2.33
DH_12-8-6-4	70	68	2	97.14	87.5	77.86	DH-9-9-7	70	63	7	90.00	93.75	-3.75
DH_12-8-6-7	70	68	2	97.14	87.5	77.86	DH-11-4-3	70	67	5	95.71	93.75	1.96
DH_12-8-6-9	70	68	2	97.14	87.5	77.86	DH-12-3-3	70	63	7	90.00	93.75	-3.75
DH_14-5-4-2	70	65	5	92.86	87.5	82.14	DH-12-5-2	70	67	5	95.71	93.75	1.96
DH_14-5-4-6	70	65	5	92.86	87.5	82.14	DH-12-6-3	70	68	2	97.14	93.75	3.39
DH_17-2-7-5	70	66	4	94.29	87.5	80.71	DH-13-2-7	70	66	4	94.29	93.75	0.54
DH_17-2-7-8	70	66	4	94.29	87.5	80.71	DH-13-5-5	70	68	2	97.14	93.75	3.39
DH_18-9-4-5	70	64	6	91.43	87.5	83.57	DH-13-6-3	70	64	6	91.42	93.75	-2.33
DH_18-9-4-9	70	64	6	91.43	87.5	83.57	DH-13-9-5	70	68	2	97.14	93.75	3.39
DH_22-6-2-5	70	65	5	92.43	87.5	82.57	DH-14-3-7	70	67	3	95.71	93.75	1.96
DH_23-9-7-7	70	65	5	92.43	87.5	82.57	DH-15-1-8	70	66	4	94.29	93.75	0.54
DH_23-9-7-9	70	65	5	92.43	87.5	82.57	DH-15-6-9	70	68	2	97.14	93.75	3.39
DH_27-9-1-4	70	67	5	95.71	87.5	79.29	DH-16-2-4	70	64	6	91.42	93.75	-2.33
DH_27-9-1-5	70	67	3	95.71	87.5	79.29	DH-16-6-6	70	63	7	90.00	93.75	-3.75
DH_29-9-3-6	70	63	7	90.00	87.5	85.00	DH-18-4-8	70	68	2	97.14	93.75	3.39
DH_29-9-3-7	70	64	6	91.43	87.5	83.57				**Average**	93.93		
DH_33-5-2-1	70	65	5	92.43	87.5	82.57				**Minimum**	90.00		
DH_33-5-2-7	70	65	5	92.43	87.5	82.57				**Maximum**	97.14		
DH_34-6-1-3	70	65	5	92.43	87.5	82.57							
DH_34-6-1-4	70	66	4	94.29	87.5	80.71							
DH_36-1-8-6	70	63	7	90.00	87.5	85.00							
DH_37-4-4-6	70	64	6	91.43	87.5	83.57							
DH_37-4-4-7	70	64	6	91.43	87.5	83.57							
DH_39-5-6-8	70	62	8	88.57	87.5	86.43							
DH_40-8-3-5	70	68	2	97.14	87.5	77.86							
			Average	93.82									
			Minimum	90.00									
			Maximum	97.44									

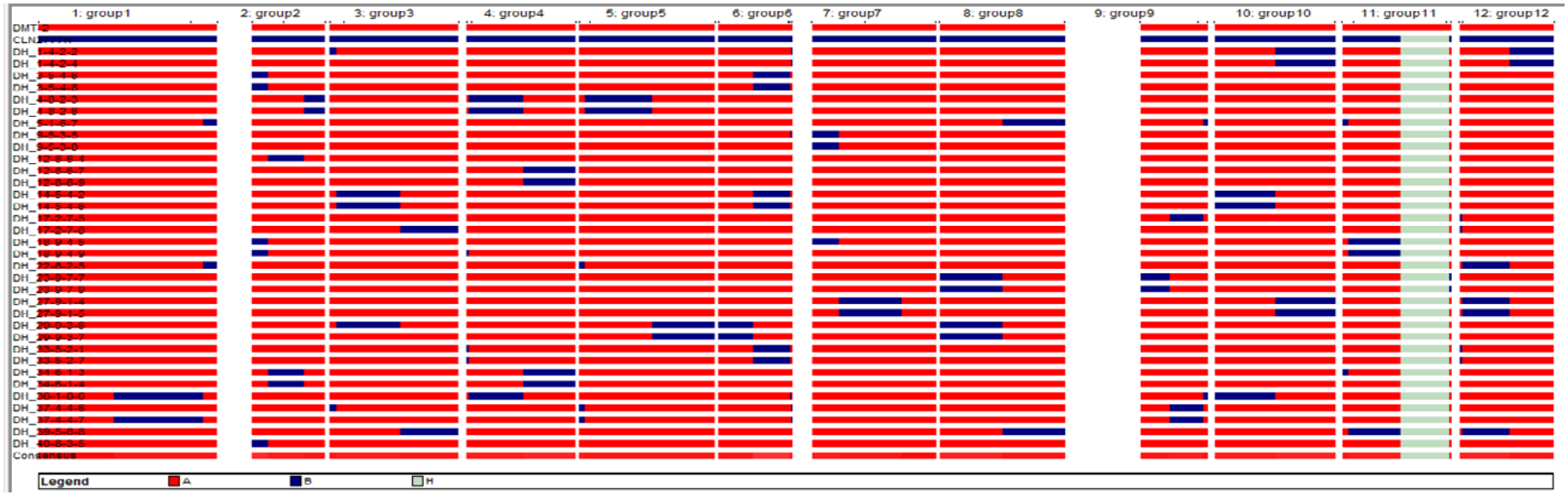

Fig. 4. Genetic constitution of selected BC_2F_4 lines of the cross DMT-2 × CLN2777H carrying target gene.

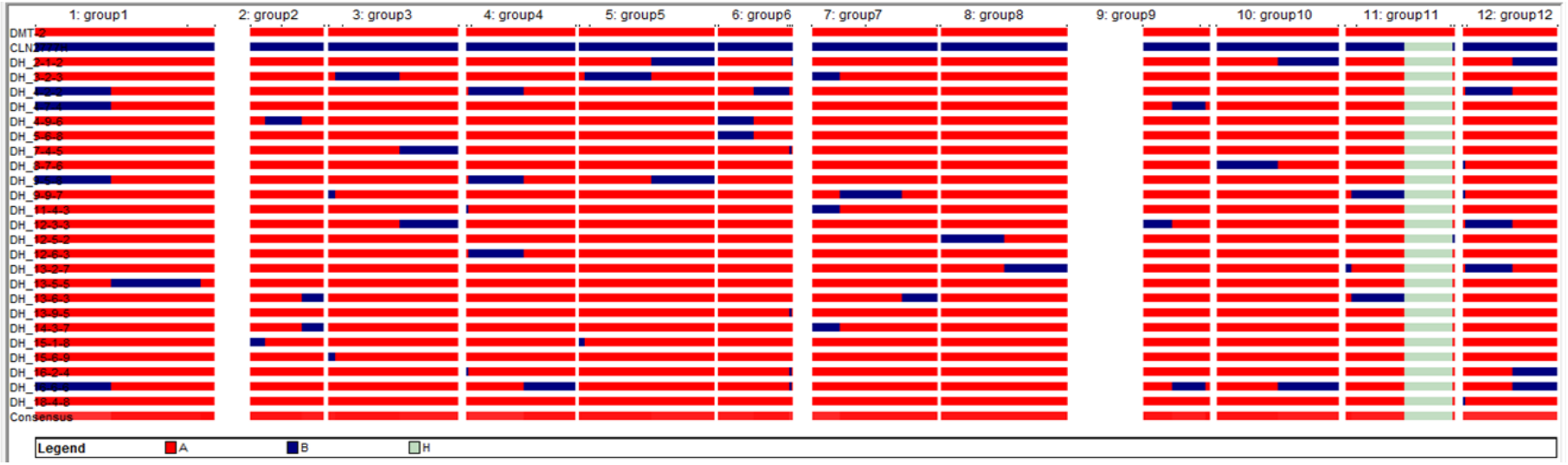

Fig. 5. Genetic constitution of selected BC_3F_3 lines of the cross DMT-2 × CLN2777H carrying target gene.

Table 5: Disease reaction of introgressed lines and parents of the cross GPBT-08 × CLN2768A determined by seedling inoculations in summer 2019.

BC_2F_4	30 DAT	60 DAT	90 DAT	Mean of per cent disease incidence	Disease reaction	BC_3F_3	30 DAT	60 DAT	90 DAT	Mean of per cent disease incidence	Disease reaction
GA-3-9-2	0.00	11.11	37.50	16.20	MR	**GA-2-4**	0.00	11.11	33.33	14.81	MR
GA-5-2-5	0.00	22.22	33.33	18.52	MR	**GA-3-4**	22.22	33.33	42.50	32.68	T
GA-6-1-4	11.11	33.33	77.77	40.74	T	**GA-3-5**	0.00	12.50	28.57	13.69	MR
GA-6-1-7	0.00	14.28	75.00	29.76	T	**GA-5-6**	0.00	37.50	42.85	26.78	MR
GA-7-5-1	0.00	20.00	33.33	17.78	MR	**GA-6-3**	22.22	44.44	62.50	43.05	T
GA-7-5-8	0.00	0.00	22.22	7.41	MR	**GA-6-5**	0.00	33.33	66.66	33.33	T
GA-9-3-7	0.00	22.22	37.50	19.91	MR	**GA-7-3**	0.00	0.00	25.00	8.33	MR
GA-11-2-4	12.50	16.66	37.50	22.22	MR	**GA-8-5**	0.00	11.11	20.00	10.37	MR
GA-12-6-7	0.00	20.00	33.33	17.78	MR	**GA-9-6**	12.50	37.50	42.50	30.83	T
GA-16-9-9	10.00	30.00	77.77	39.26	T	**GA-11-6**	12.50	14.28	28.57	18.45	MR
GA-17-7-3	22.22	37.50	71.42	43.71	T	**GA-12-3**	22.22	33.33	42.85	32.80	T
GA-19-8-5	0.00	25.00	42.85	22.62	MR	**GA-14-6**	0.00	28.57	42.85	23.81	MR
GA-20-4-2	0.00	0.00	22.22	7.41	MR	**GA-14-7**	14.28	28.57	42.85	28.57	T
GA-20-4-7	0.00	22.50	25.00	15.83	MR	**GA-16-4**	0.00	14.28	28.57	14.28	T
GA-21-5-2	22.22	33.33	44.44	33.33	T	**GA-17-5**	22.22	33.33	50.00	35.18	MR
GA-21-5-6	0.00	33.33	42.85	25.39	T	**GA-18-8**	0.00	12.50	28.57	13.69	T
GA-23-6-7	22.22	37.50	42.85	34.19	T	**GA-20-7**	16.66	28.57	42.86	29.36	T
GA-26-2-3	0.00	22.22	33.33	18.52	MR	**GA-23-2**	0.00	11.11	30.00	13.70	MR
GA-27-7-4	0.00	33.33	42.85	25.39	T	**GA-24-5**	0.00	14.28	28.57	14.28	MR
GA-27-7-6	11.11	25.00	42.85	26.32	T	**GA-26-4**	0.00	28.57	42.85	23.81	MR
GA-32-1-7	0.00	22.22	37.50	19.91	MR	**GA-28-2**	11.11	22.22	37.50	23.61	MR
GA-37-9-5	0.00	22.22	33.33	18.52	MR	**GA-31-9**	11.11	22.22	44.44	25.92	T
GA-37-9-6	0.00	22.22	42.85	21.69	MR	**GPBT-08**	66.66	88.88	100.00	85.18	HS
GA-40-1-8	11.11	25.00	37.50	24.54	MR	**CLN2768A**	0.00	0.00	0.00	0.00	R
GA-41-7-1	11.11	12.50	25.00	16.20	MR						
GA-41-7-4	0.00	25.00	37.50	20.83	MR						
GPBT-08	60	100	100	86.66	HS						
CLN2768A	0.00	0.00	0.00	0.00	R						

DAT: days after transplanting; R: resistant; MR: moderately resistant; T: tolerant; HS: highly susceptible

PDI of the cross DMT-2 × CLN2777H ranged from 13.69 to 46.23%, where, 17 lines were moderately resistant and 9 lines were tolerant. Lines DH-14-5-4 (13.69%), DH-23-9-7 (14.28%), DH-5-1-6 (14.81%), DH-4-8-2 (16.67%), DH-9-5-3 (18.52%) and DH-29-9-3 (18.52%) showed minimum PDI (Table 6).

Per cent disease incidence of the cross GPBT-08 × CLN2768A of BC_3F_3 lines was ranged from 8.33 to 43.05%, where, 13 lines were moderately resistant and 9 lines were tolerant. Lines GA-7-3 (8.33%), GA-8-5 (10.37%), GA-3-9 (13.69%), GA-18-8 (13.69%), GA-23-2 (13.70%), GA-16-4 (14.28%) and GA-2-4 (14.81%) showed minimum PDI (Table 5).

Per cent disease incidence of the cross DMT-2 × CLN2777H of BC_3F_3 lines was ranged from 8.33 to 43.05%, where, 13 lines were moderately resistant and 9 lines were tolerant. Lines DH-13-6 (8.33%), DH-16-6 (11.11%), DH-14-3 (12.50%), DH-7-4 (13.69%), DH-5-6 (15.08%), DH-12-3 (15.28%) and DH-2-1 (15.87%) showed minimum PDI (Table 6).

Agronomic performance of the developed ToLCV disease resistant NILs

In order to asses yield performance of stable NILs carrying *Ty-2* gene were tested under disease stress condition along with parents. Collectively, most of the traits showed significant differences ($P < 0.05$) among the populations. In BC_2F_4, GA-26-2, GA-27-6 and GA-40-1 lines of the cross GPBT-08 × CLN2768A (Table 7) and DH-29-9, DH-37-4, DH-4-8 and DH-12-8 lines of the cross DMT-2 × CLN2777H (Table 9) were performed almost similar to the recurrent parents GPBT-08 and DMT-2 respectively for most of the traits.

In BC_3F_3, GA-2-4, GA-8-5 and GA-7-3 lines of the cross GPBT-08 × CLN2768A (Table 8) and DH-16-6, DH-13-6 and DH-2-1 lines of the cross DMT-2 × CLN2777H (Table 10) were performed almost similar to the recurrent parent for most of the traits.

Table 6: Disease reaction of introgressed lines and parents of the cross DMT-02 × CLN2777H determined by seedling inoculations in summer 2019.

BC_2F_4	30 DAT	60 DAT	90 DAT	Mean of PDI	DR	BC_3F_3	30 DAT	60 DAT	90 DAT	Mean of PDI	DR
DH-1-4-2	0.00	33.33	42.50	25.28	MR	**DH-2-1**	0.00	14.28	33.33	15.87	MR
DH-3-5-4	11.11	37.50	33.33	27.31	T	**DH-3-2**	12.50	22.22	44.44	26.39	T
DH-4-8-2	0.00	12.50	37.50	16.67	MR	**DH-4-2**	0.00	28.57	40.00	22.86	MR
DH-5-1-6	0.00	11.11	33.33	14.81	T	**DH-4-7**	0.00	22.22	37.50	19.91	MR
DH-6-3-1	22.22	37.50	42.85	34.19	T	**DH-4-9**	11.11	28.57	42.85	27.51	T
DH-9-5-3	0.00	33.33	42.85	25.39	T	**DH-5-6**	0.00	16.66	28.57	15.08	MR
DH-12-8-6	0.00	22.22	33.33	18.52	MR	**DH-7-4**	0.00	12.50	28.57	13.69	MR
DH-14-5-4	0.00	12.50	28.57	13.69	MR	**DH-8-7**	14.28	44.44	66.66	41.79	T
DH-15-4-3	22.22	37.50	42.85	34.19	T	**DH-9-5**	33.33	37.50	55.55	42.13	T
DH-17-2-7	0.00	37.50	80.00	39.17	MR	**DH-9-9**	0.00	28.57	37.50	22.02	MR
DH-18-9-4	0.00	28.57	42.85	23.81	T	**DH-11-3**	12.50	28.57	44.44	28.50	T
DH-22-6-2	22.22	33.33	37.50	31.02	MR	**DH-12-3**	0.00	12.50	33.33	15.28	MR
DH-23-9-7	0.00	14.28	28.57	14.28	T	**DH-12-5**	22.22	37.50	50.00	36.57	T
DH-24-3-5	22.22	37.50	42.85	34.19	MR	**DH-12-6**	20.00	37.50	55.55	37.68	T
DH-27-9-1	33.33	62.50	62.50	52.78	MR	**DH-13-2**	14.28	28.57	42.85	28.57	T
DH-29-9-3	0.00	22.22	33.33	18.52	MR	**DH-13-5**	33.33	44.44	55.55	44.44	T
DH-33-5-2	0.00	33.33	55.55	29.63	T	**DH-13-6**	0.00	0.00	25.00	8.33	MR
DH-34-6-1	0.00	30.00	40.00	23.33	MR	**DH-13-9**	11.11	22.22	33.33	22.22	MR
DH-36-1-8	22.22	37.50	42.85	34.19	MR	**DH-14-3**	0.00	12.50	25.00	12.50	MR
DH-37-4-4	0.00	28.57	37.50	22.02	MR	**DH-15-1**	0.00	28.50	42.85	23.78	MR
DH-39-5-6	33.33	42.85	62.50	46.23	MR	**DH-15-6**	0.00	33.33	44.44	25.92	T
DH-40-8-3	33.33	44.44	57.14	44.97	T	**DH-16-2**	0.00	33.33	55.55	29.63	T
DMT-2	66.66	100.00	100.00	88.89	HS	**DH-16-6**	0.00	11.11	22.22	11.11	MR
CLN2777H	0.00	0.00	0.00	0.00	R	**DH-18-4**	11.11	22.22	42.50	25.28	T
						DMT-2	66.66	100.00	100.00	88.89	HS
						CLN2777H	0.00	0.00	0.00	0.00	R

DAT: days after transplanting; R: resistant; MR: moderately resistant; T: tolerant; HS highly susceptible

Table 7: Performance of BC_2F_4 lines of the cross GPBT-08 × CLN2768A for yield and its related traits in tomato during summer 2019.

Progenies	Plant height (cm)	No. of branches per plant	No. of clusters	No. of fruits per cluster	Total no. of fruits	Average fruit weight (g)	Polar length of fruit (mm)	Equatorial length of fruit (mm)	Fruit shape index	Pericarp thickness (mm)	No. of locules	TSS (% brix)	Yield per plant (kg)
GA-7-5	42.3*	6.25*	47.55*	2.35	59.02*	34.6	29.15	22.92	1.28	3.61	4.8	4.78	1.81*
GA-9-3	47.75*	5.9*	23.83*	2.7*	47.08*	31.98	38.57	39.92	0.97	4.56	4.91	5.17*	1.47*
GA-20-4	49.85*	5.2*	26.5*	2.33	48.07*	48.37	38.48	30.77	1.25	3.66	5.84*	5.75*	2.27*
GA-26-2	47.05*	7.55*	30.32*	2.6*	64.89*	41.9	36.37	36.02	1.01	3.95	5.3	5.48*	1.5*
GA-27-7	57.13*	6.5*	29.65*	3.1*	54.28*	55.89	42.98	34.92	1.23	5.11	4.59	4.65	2.31*
GA-37-9	46.08*	4.85*	20.01	2.21	45.03*	52.02	39.6	37.07	1.07	4.31	4.76	4.86*	0.87
GA-40-1	44.3*	4.6	24.28*	2.75*	61.09*	56.33	40.96	35.19	1.16	5.14	4.56	4.7	1.21*
GPBT-08	36.09	4.07	17	2.05	22.81	56.84	46.43	38.51	1.21	5.03	4.53	4.35	0.56
CLN2768A	66.87	6.03	21.46	2.26	38.65	52.5	48.8	43.45	1.23	4.07	5.12	4.89	1.05
C.V.	5.14	5.03	5.84	5.12	5.2	6.08	5.11	4.92	7.23	2.39	4.25	4.07	9.62
C.D. at 5%	6.01	0.72	4.13	0.32	6.89	6.83	4.75	4.07	0.2	0.25	0.52	0.5	0.55

*****: Significant at 5% level of probability

Table 8: Performance of BC_3F_3 lines of the cross GPBT-08 × CLN2768A for yield and its related traits in tomato during summer 2019.

Progenies	Plant height (cm)	No. of branches per plant	No. of clusters	No. of fruits per cluster	Total no. of fruits	Average fruit weight (g)	Polar length of fruit (mm)	Equatorial length of fruit (mm)	Fruit shape index	Pericarp thickness (mm)	No. of locules	TSS (% brix)	Yield per plant (kg)
GA-2-4	54.94*	6.16*	30.68*	3.19*	57.26*	55.89	46.51	36.44	1.28	5.8*	4.34	4.38	2.66*
GA-3-5	51.98*	5.71*	22.97*	2.8*	46.99*	31.5	43.39	42.28	1.03	4.78	5.18	5.18*	1.2*
GA-7-3	47.01*	4.59*	27.26	2.42*	44.39*	51.27	38	31.28	1.22	3.87	5.87*	5.8*	2.05*
GA-8-5	38.12	6.87*	31.76*	3.01*	66.01*	38.94	37.93	38.84	0.98	4.19	5.28*	5.48*	2.19*
GA-12-3	48.59*	4.71*	17.13	2.28	46.05*	51.63	35	38.15	0.92	4.34	4.68	4.87	1.87*
GPBT-08	36.09	4.07	17	2.05	22.81	56.84	46.43	38.51	1.21	5.03	4.53	4.35	0.56
CLN2768A	66.87	6.03	21.46	2.26	38.65	52.5	48.8	43.45	1.23	4.07	5.12	4.89	1.05
C.V.	5.4	3.29	7.51	4.51	4.4	5.15	6.1	5.17	7.97	5.11	4.7	4.34	7.98
C.D. at 5%	7.22	0.51	5.41	0.34	6.37	6.56	6.8	5.37	0.24	0.65	0.66	0.62	0.44

*****: Significant at 5% level of probability

Table 9: Performance of BC_2F_4 lines of the cross DMT-2 × CLN2777H for yield and its related traits in tomato during summer 2019 in tomato.

Progenies	Plant height (cm)	No. of branches per plant	No. of clusters	No. of fruits per cluster	Total no. of fruits	Average fruit weight (g)	Polar length of fruit (mm)	Equatorial length of fruit (mm)	Fruit shape index	Pericarp thickness (mm)	No. of locules	TSS (% brix)	Yield per plant (kg)
DH-4-8	46.79	6.65	15.5	2.76	45.37	41.44*	52.02*	0.80	4.39	3.67	4.71	60.52	2.55*
DH-5-1	41.06	6.83	19.21	2.52	41.33	33.8	44.71	0.76	4.55	4.58	5.41	53.11	1.64
DH-12-8	50.56*	5.95	17.88	2.83	49.43*	34.7	41.89	0.83	4.81	5.07*	4.64	50.01	2.21*
DH-14-5	52.91*	8.13*	18.52	2.42	43.6	36.32	46.11	0.79	4.99*	4.21	5.59	61.46*	2.27*
DH-23-9	50.05*	8.92*	21.4	3.23*	56.45*	41.21*	45.99	0.9	5.15*	3.41	5.5	47.54	1.69
DH-29-9	51.61*	8.27*	19.61	2.39	47.78*	31.15	38.21	0.81	4.27	4.76*	4.83	52.55	2.32*
DH-37-4	51.79*	5.55	13.71	3.26*	44.98	36.08	52.49*	0.69	3.56	4.49	4.97	56.97	1.87
DMT-2	42.15	6.75	18.2	2.6	41.2	34.83	43.77	0.8	4.45	4.4	5.58	54	1.67
CLN2777H	48.45	7.76	20.13	2.2	38.76	36.94	42.43	0.75	4.65	2.78	4.86	42.67	1.34
C.V.	5.13	5.03	7.88	5.86	5.34	5.14	4.77	5.9	4.34	5.64	4.87	5.04	9.73
C.D at 5%	6.19	0.88	3.47	0.4	6.14	4.82	5.36	0.11	0.48	0.59	0.61	6.74	0.49

*****: Significant at 5% level of probability

Table 10: Performance of BC_3F_3 lines of the cross DMT-2 × CLN2777H for yield and its related traits in tomato during summer 2019 in tomato.

Progenies	Plant height (cm)	No. of branches per plant	No. of clusters	No. of fruits per cluster	Total no. of fruits	Average fruit weight (g)	Polar length of fruit (mm)	Equatorial length of fruit (mm)	Fruit shape index	Pericarp thickness (mm)	No. of locules	TSS (% brix)	Yield per plant (kg)
DH-2-1	58.14*	5.32	12.22	3.12	44.03	33.05	51.98*	0.64	3.39	4.78	5.39	58.26	2.02*
DH-4-2	42.64	6.54	17.37	2.5	39.44	35	45.66	0.77	4.29	4.4	5.53	52.4	1.87*
DH-5-6	47.65	6.3	17.99	2.7	38.88	33.93	43.43	0.78	4.5	4.45	5.68	53.32	2.05*
DH-12-3	54.5*	8.22*	17.91	2.49	45.63	34.98	47.96	0.73	4.55	3.93	5.82	61.91*	2.3*
DH-13-6	51.42*	9.23*	21.3*	3.48*	58.61*	41.13*	43.38	0.95*	4.88*	3.75	5.07	45.75	2.54*
DH-14-3	49.5*	8.29*	19.67	2.42	45.33	32.41	37.04	0.88	4.05	4.55	4.79	57.17	2.11*
DH-16-6	51.36*	5.57	13.77	3.3*	46.16	34.65	54.09*	0.64	3.4	4.78	5.25	58.37	2.34*
DMT-2	42.15	6.75	18.2	2.6	41.2	34.83	43.77	0.8	4.45	4.4	5.58	54	0.67
CLN2777H	48.45	7.76	20.13	2.2	38.76	36.94	42.43	0.75	4.65	2.78	4.86	42.67	1.34
C.V.	4.88	5.16	6.92	9.6	5.57	4.33	5.82	4.91	5.58	5.38	4.02	5.14	6.22
C.D. at 5%	6.06	0.89	2.91	0.67	6.2	3.71	6.59	0.09	0.57	0.58	0.53	6.96	0.33

*****: Significant at 5% level of probability

DISCUSSION

The effectiveness of different resistance sources in different regions, and consequently against different begomoviruses, has been the main object of several tomato breeding programs. Differential responses of resistance genes to virus isolates suggested the necessity of developing tomato cultivars with resistance to TYLCD isolates from different geographic areas (Pico *et al.*, 1999). Present investigation is to introgress *Ty-2* resistant to ToLCV into two cultivated varieties of tomato *i.e.,* GPBT-08 and DMT-2 by MABB with background selection. Foreground selection was carried out by *Ty-2* linked two SCAR markers TG0302 and P1-16 were successfully used to introgress resistance to monopartite begomovirus (ToLCV) isolate from CLN2768A and CLN2777H resistance sources. Marker assisted foreground selection were in accordance with the investigation of Alam *et al.* (2012), where they used foreground selection for the identification of salt tolerant rice genotypes by marker assisted backcross breeding program. Segregation pattern was observed in BC_1F_1, BC_2F_1 and BC_3F_1 of both the crosses using $\chi 2$ test and results revealed that observed segregation ratio was fit into expected ratio *i.e.,* 1:1 similar results observed by Muthusamy *et al.* (2014). In general, RPG recovery can be accelerated by using markers for background selection (Servin and Hospital 2002). Adaption of background selection allowed to recognize plants which recovered maximum RPG up to 96.88% in BC_2F_4, 96.88% in BC_3F_3 of the cross GPBT-08 × CLN2768A and 96.88% in BC_2F_4, 96.88% in BC_3F_3 of the cross DMT-2 × CLN2777H and itself which was more than expected average recovery. Present results were in agreement with many other MAS studies in rice (Ellur *et al.,* 2016; Rajpurohit *et al.,* 2011). The results obtained by screening the NILs developed by backcrossing revealed that few lines from both the crosses performed similar to the recurrent parent for most of the yield and its related traits and they were shown minimum PDI with high level of resistance without causing yield reduction. Thereby new leaf curl resistant lines could be developed in just 2-3 backcrosses. Similar results were reported by Chen *et al.* (2001); Joseph *et al.* (2004) and Gopalakrishnan *et al.* (2008).

CONCLUSION

The results of the present study, in general, provides evidence of accuracy and reliability for the TG0302 and P1-16 markers to be applied directly to large-scale MABC programmes for the development of high-yielding leaf curl resistant varieties. Within only two backcross generations, at least 90% of the recurrent parents' genomes were recovered, and leaf curl resistant NILs were developed, demonstrating that introgression of *Ty-2* gene with MABC breeding is much faster than that of conventional breeding. For the most part, the developed fragrant NILs showed better yield-related traits than the donor parents CLN2768A and CLN2777H, possessing a similar yield potential with the recurrent parents GPBT-08 and DMT-2. The present study has overall, provided a clear, fast, and yet affordable route to introgressing *Ty-2* gene into tomato lines or varieties, and this would benefit researchers especially those with limited resources.

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REFERENCES

Banerjee, M.K. and Kalloo (1987a). Sources and inheritance of resistance to leaf curl virus in Lycopersicon. *Theoretical and Applied Genetics*, **73**: 707-710.

Berloo, V.R.: GGT2.0: Versatile software for visualization and analysis of genetic data. *Journal of Heridity*, **99**: 232-236 (2008).

Alam, M.S., Salim, M., Moniruzzaman, M., Rashid, J.A. and Islam, M.M. (2012). Marker-assisted Foreground Selection for Identification of Salt Tolerant Rice Genotypes. *The Agriculturists*, **10**(2): 1-8.

Bernatzky, R. and Tanksley, S.D. (1986). Genetics of actin regulated sequences in tomato. *Theoretical and Applied Genetics*, **72**: 314-321.

Brown, J.K., Fauquet, C.M., Briddon, R.W., Zerbini, M., Moriones, E., and Navas-Castillo, J. (2012). Family Geminiviridae. In: Virus Taxonomy: Classification and Nomenclature of Viruses, King, A.M.Q., M.J. Adams, E.B. Carstens and E.J. Lefkowitz (Eds.). Elsevier Academic Press, USA., pp: 351-373.

Brown, J.K., Zerbini, F.M., Castillo, J.N., Moriones, E., Sobrinho, R.R., Silva, J.C.F., Olive, E.F., Briddon, R.W., Zepeda, C.H.N., Idris, A., Malathi, V. G., Martin, D. P., Bustamante, R. R., Ueda, S. and Varsani, A. (2015). Revision of Begomovirus taxonomy based on pairwise sequence comparisons. *Archives of* Virology, **160**: 1593-1619.

Chakraborty, S., Pandey, P.K., Banerjee, M.K., Kalloo, G. and Fauquet, C.M. (2003). Tomato leaf curl Gujarat virus, a new begomovirus species causing a severe leaf curl disease of tomato in Varanasi, India. *Phytopathology*, **93**: 1485-1495.

Chen, J. (2000). Mapping a wild tomato introgression associated with *Tomato yellow leaf curl virus* resistance in a cultivated tomato line. *Journal of the American Society for Horticultural Sciences*, **125**: 15-20.

Chen, H.M., Lin, C.Y., Yoshida, M., Hanson, P. and Schafleitner, R. (2015). Multiplex PCR for Detection of Tomato Yellow Leaf Curl Disease and Root-Knot Nematode Resistance Genes in Tomato (*Solanum lycopersicum* L.). *International Journal of Plant Breeding and Genetics*, **9**(2): 44-56.

Chen, S., Lin, X.H., Xu, C.G. and Zhang , Q. (2001). Improving bacterial blight resistance of '6078', an elite restorer line of hybrid rice, by molecular

marker-aided selection. *Plant Breeding*, **120**: 133-137.

Ellur, R.K., Khanna, A., Yadav, A., Pathania, S., Rajashekara, H., Singh, V.K. (2016). Improvement of Basmati rice varieties for resistance to blast and bacterial blight diseases using marker assisted backcross breeding. *Plant Science*, **242**: 330-341.

Fauquet C.M., Stanley J. (2005). Revising the way we conceive and name viruses below the species level: A review of geminivirus taxonomy calls for new standardized isolate descriptors. *Archives of* Virology, **150**: 2151-2179.

Garcia, B.E., Graham, E., Jensen, K.S., Hanson, P., Mejia, L. and Maxwell, D. P. (2007). Co-dominant SCAR marker for detection of the begomovirus-resistance *Ty-2* locus derived from *Solanum habrochaites* in tomato germplasm. *Report of the Genetics Cooperative*, **57**: 21-24.

Gopalakrishnan, S., Sharma, R.K., Rajkumar, K.A., Joseph, M., Sinah, V.P. and Singh, A.K. (2008). Integrating marker assisted background analysis with foreground selection for identification of superior bacterial blight resistant recombinants in Basmati rice. *Plant Breeding*, **127**: 131-139.

Gupta, P.K., Balyan, H.S., Edwards, K.J., Isaac, P., Korzun, V., Roder, M., Gautier, M.F., Joudrier, P., Schlatter, A.R., Dubcovsky, J., De la Pena, R.C., Khairallah, M., Penner, G., Hayden, M.J., Sharp, P., Keller, B., Wang, R.C.C., Hardouin, J.P., Jack, P. and Leroy, P. (2002). Genetic mapping of 66 new microsatellite (SSR) loci in bread wheat. *Theoretical and Applied Genetics*, **105**: 413-422.

Hanson, P. M., Bernacchi, D., Green, S., Tanksley, S.D., Muniyappa, V., Padmaja, A.S., Chen, H., Kuo, G., Fang, D. and Chen, J. (2000). Mapping a wild tomato introgression associated with tomato yellow leaf curl virus resistance in a cultivated tomato line. *Journal* of the *American Society* for *Horticultural Science*, **125**(1): 15-20.

Hansen, J.W., Mason, S.J., Sun, L. and Tall, A. Review of seasonal climate forecasting for agriculture in sub-Saharan Africa.

Hilje, L., Costa, H. S., and Stansly, P. A. (2001). Cultural practices for managing *Bemisia tabaci* and associated viral diseases. *Crop Protection*, **20**(9): 801-812.

Hutton, S.F. and Scott, J.W. (2013). Fine-mapping and cloning of *Ty*-1 and *Ty*-3; and mapping of a new TYLCV resistance locus, "*Ty*-6", in Tomato Breeders Round Table Proceedings 2013, Chiang Mai.

Hutton, S.F., Scott, J.W., Schuster, D.J. (2012). Recessive resistance to tomato yellow leaf curl virus from the tomato cultivar Tyking is located in same region as Ty-5 on chromosome 4. *Journal of American Society of Horticultural Science*, **47**: 324–7.

Jena, K.K. and Mackill, D.J. (2008). Molecular markers and their use in markerassisted selection in rice. Crop Science, **48**: 1266–1276.

Ji, Y., Schuster, D.J., Scott, J.W. (2007b). *Ty-3*, a begomovirus resistance locus near the *Tomato yellow leaf curl virus* resistance locus *Ty-1* on chromosome 6 of tomato. *Molecular Breeding*, **20**: 271-284.

Ji, Y., Scott, J.W., Schuster, D.J., Maxwell, D.P. (2009). Molecular mapping of *Ty-4*, a new Tomato yellow leaf curl virus resistance locus on chromosome 3 of tomato. *Journal of the American Society for Horticultural Science*, **134**: 281-288.

Joseph, M., Gopalakrishnan, S. Sharma, R.K., Singh, A.K., Singh, V.P. Singh, N.K. and Mohapatra, T. (2004). Combining bacterial blight resistance and basmati quality characteristics by phenotypic and molecular marker assisted selection in rice. *Molecular Breeding*, **13**: 377-387.

Kadam S., Singh,K., Shukla, S., Goel, S., Vikram, P., Pawar, V., Gaikwad, K., Renu Khanna, C. and Singh, N. (2012). Genomic assosciations for drought tolerance on the short arm of wheat chromosome 4B. *Funct Integr. Genomics*, **12**: 447-464.

Kumar, K.G. and Hittalmani, S. (2000). Marker assisted backcross gene introgression of major genes for blast resistance in rice-Advances in Rice blast Research, kluwer Publications, pp: 43-45.

Levin, I., Karniel, U., Fogel, D., Reuveni, M., Gelbart, D., Evenor, D., Chen, L., Nahon, S., Shlomo, H., Machbosh, Z. and Lapidot, M. (2013). Cloning and analysis of the tomato yellow leaf curl virus resistance gene Ty-5. In: Tomato breeders round table proceedings 2013, Chiang Mai, Thailand.

Moriones E., Navas-Castillo J. (2000). Tomato yellow leaf curl virus, an emerging virus complex causing epidemics worldwide. *Virus Research*, **71**: 123-134.

Muniyappa, V., Swanson, M.M., Duncan, G.H. and Harrison, B.D. (1991). Particle purification, properties and epitope variability of Indian tomato leaf curl geminivirus. *Annals of Applied Biology*, **118**: 595-604.

Muniyappa, V., Venkatesh, H.M., Ramappa, H.K., Kulkarni, R.S., Zeiden, M., Tarba, C.Y., Ghanim, M. and Czosnek, H. (2000). Tomato leaf curl virus from Bangalore (ToLCV-Ban4): sequence comparison with Indian ToLCV isolates, detection in plants and insects, and vector relationships. *Archives of* Virology, **145**: 1583-1598.

Muthusamy, V., Hossain, F., Thirunavukkarasu, N., Choudhary, M., Saha, S., Bhat, J.S., Prasanna, B.M. and Gupta, H.S. (2014). Development of β-carotene rich maize hybrids through markerassisted introgression of β-carotene hydroxylase allele. *PLoS One*, **9**(12): e113583.

Naika, S., Van Lidt de Jeude, J., de Goffau, M., Hilmi, M. and Van Dam, B. (2005). Cultivation of tomato. Production, processing and marketing. In: B. Van Dam (ed.), Digigrafi, Wageningen, The Netherlands.

Neeraja, C.N., Rodriguez, R.M., Pamplona, A., Heuer, S., Collard, B.C.Y. (2007). A marker-assisted backcrossing approach for developing submergencetolerant rice cultivars. *Theoretical and Applied Genetics*, **115**: 767-776.

Palumbo, J.C., Horowitz, A.R., and Prabhaker, N. (2001). Insecticidal control and resistance management for Bemisia tabaci. *Crop Protection*, **20**(9): 739–765.

Pestsova, E. Ganal, M.W. and Röder M.S. (2000). Isolation and mapping of microsatellite markers specific for the D genome of bread wheat, Genome. **43**: 689-697.

Pico B., Ferriol M., Díez M., Nuez F. (1999). Developing tomato breeding lines resistant to Tomato yellow leaf curl virus. *Plant Breeding*, **118**: 537-542.

Rajpurohit, D., Kumar, R., Kumar, M., Paul, P., Awasthi, A., Basha, P. (2011). Pyramiding of two bacterial blight resistance and a semidwarfing gene in Type 3 Basmati using marker-assisted selection. *Euphytica*, **178**: 111–126.

Roder, M.S., Korzun, V., Wendehake, K., Plaschke, J., Marie-Helene, T., Leroy, P., and Ganal, M.W. (1998). A Microsatellite Map of Wheat. *Genetics*, **149**: 2007–2023.

Saikia, A.K. and Muniyappa, V. (1989). Epidemiology and control of tomato leaf curl virus in southern India. *Tropical Agriculture*, **66**(4): 350- 354.

Seal, S.E., van den Bosch F., Jeger, M.J. (2006). Factors influencing begomovirus evolution and their increasing global significance: implications for sustainable control. Critical Reviews in Plant Sciences **25**: 23-46.

Servin, B., and Hospital, F. (2002). Optimal positioning of markers to control genetic background in marker-assisted backcrossing. *Journal of Heredity*, **93**: 214–217.

Slater, A.T., Cogan, N.O.I. and Forster, J.W. (2013). Cost analysis of the application of marker-assisted selection in potato breeding. *Molecular Breeding*, **32**: 299-310.

Somers, D.J., Isaac, P. and Edwards, K. (2004). A high-density microsatellite consensus map for bread wheat (*Triticum aestivum* L.), *Theoretical and Applied Genetics*, **109**: 1105-1114.

Varma, A., Malathi, V.G. (2003). Emerging geminivirus problems: A serious threat to crop production. *Annals Applied Biology*, **142**:145–164.

Vasudeva, R.S. and Samraj (1948). Leaf curl disease of tomato. *Phytopatholo*gy, **38**: 364-369.

Verlaan, M.G., Szinay, D., Hutton, S.F., de Jong, H., Kormelink, R., Visser, R.G.F. (2011). Chromosomal rearrangements between tomato and *Solanum chilense* hamper mapping and breeding of the TYLCV resistance gene Ty-1. The *Plant Journal*, **68**: 1093-1103.

Yang, X., Caro, M., Hutton, S.F., Scott, J.W., Guo, Y., Wan, X., Rashi, M.H., Szina, D., deJong, H., Visser, R.G.F., Bai, Y. and Du, Y. (2014). Fine mapping of the tomato yellow leaf curl virus resistance gene *Ty-2* on chromosome 11 of tomato. *Molecular Breeding*, **34**: 749-760.

Yang, X., Guo, W., Ma, X., An, Q. and Zhou, X. (2011). Molecular Characterization of *Tomato Leaf Curl China Virus*, infecting tomato plants in china, and functional analyses of its associated betasatellite. *Applied and Environmental microbiology*, **77**(9): 3092-3101.

Yi, M., New, K.T., Vanavichit, A., Chai-arree, W. and Toojinda, T. (2009). Marker assisted backcross breeding to improve cooking quality traits in Myanmar rice cultivar Manawthukha. *Field crops*, **113**: 178-186.

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