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Marker-assisted pyramiding of southern leaf blight resistance QTLs *qSLB3.1* and *qSLB8.1* in maize (*Zea mays*)

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ABSTRACT

Southern leaf blight (SLB) is one of the major diseases that cause substantial yield losses in maize (*Zea mays* L.) worldwide. Stacking broad-spectrum resistance genes/QTLs into prevalent cultivars is the prerequisite for durable disease resistance breeding programme. Therefore, a study was carried at the research farm and molecular biology laboratory of School of Agricultural Biotechnology, Punjab Agricultural University, Ludhiana, Punjab during 2017–21 to introgress SLB resistance QTLs from two donors, viz. CM139 (*qSLB3.1*) and LM5 (*qSLB8.1*) into CM140 inbred using marker-assisted backcross breeding (MABB). Crosses were made between CM139 × CM140 and LM5 × CM140 to generate two separate F_1s. Each F_1 was backcrossed twice to generate BC_2F_1 progenies. Foreground selection was performed at each step using linked flanking markers to each QTL and also evaluated for SLB resistance. The selected heterozygous BC_2F_1 plants from each cross were inter-crossed to combine *qSLB3.1* and *qSLB8.1* in the same genetic background. The F_2 population was also evaluated phenotypically for SLB resistance and other morphological traits. A total of 18 plants were obtained having both the QTLs with homozygous donor alleles. The F_2 plants having both QTLs and singly in the homozygous state were advanced to generate F_3 progenies. The pyramided lines exhibited 29% lesser disease severity than the lines with either QTL. The present results indicated that additive effects of the QTLs for SLB resistance played an important role among these lines. To our best knowledge, this is the first report for the pyramiding of QTLs associated with SLB resistance. The pyramided lines would serve as potential donors in maize breeding programs.

Keywords: Marker-assisted selection, Pyramiding, Quantitative trait loci, Southern leaf blight resistance

Southern leaf blight (SLB) caused by the fungus [(*Bipolaris maydis* (Nishik. and Miyake) Shoemaker, 1959] is a disease of fundamental importance and occurs in different regions of India, including Jammu & Kashmir, Himachal Pradesh, Sikkim, Meghalaya, Punjab, Haryana, Rajasthan, Delhi, Uttar Pradesh, Bihar, Madhya Pradesh, Gujarat, Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu. It can cause yield losses from 9.7–11.7% and even up to 40% depending on the weather conditions (Vasmatkar *et al.* 2019). To date, four physiological races of *Bipolaris maydis*, viz. race O, T (Ullstrup 1972), C (Wei 1988) and S (Sun *et al.* 2020) have been identified. Race O is predominant in India, and not even a single cultivar has been documented as immune against SLB disease though some inbreds impart substantial resistance (Manjunatha *et al.* 2019). Therefore, crop management approaches necessitate developing durable resistance to bypass rapidly evolving virulent strains and resistance breakdown.

SLB resistance is controlled either by a single recessive gene, *rhm* (Smith and Hooker 1973), or two linked recessive genes (Craig and Fajemisin 1969) or two independent recessive genes with complementary effects (Thompson and Berquist 1984). Most of the studies reported that SLB resistance is polygenic in nature and identified QTLs distributed on all maize chromosomes (Balint Kurti *et al.* 2008, Zwonitzer *et al.* 2010, Kump *et al.* 2011, Li *et al.* 2018). We identified two unique SLB resistance QTLs, viz. *qSLB3.1* and *qSLB8.1* from CM139 and LM5 inbreds on chromosomes 3 and 8, respectively (Kaur 2019, Kaur *et al.* 2019). Both QTLs have an additive mode of gene action and are consistent with large effects. Pyramiding QTLs through marker-assisted breeding (MAB) is an efficient strategy for attaining the required phenotypic level of a polygenic trait in various breeding programs (Ashikari and Matsuoka 2006). Since partial resistance exists for SLB so, to enhance resistance, the thought process proceeded to stack the *qSLB3.1* and *qSLB8.1* through stepwise marker-assisted pyramiding in the genetic background of the drought-tolerant CM140 inbred (Kaur *et al.* 2013).

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MATERIALS AND METHODS

The plant materials consisted of two donor parents, viz. LM5 and CM139, one recurrent parent, CM140 and their derived backcross populations, F_2 and F_3 progenies. The experimental materials were raised at the research farm of Punjab Agricultural University, Ludhiana, Punjab during 2017–21. The CM139 and LM5 are resistant to SLB and belong to a semi-exotic and tuxpeno heterotic pool, respectively. CM140 is susceptible to SLB from the indigenous heterotic group. Staggered sowing of parents was done to generate F_1s and backcross-derived populations. The backcross and F_2 populations were grown in a plot with a 4 m row length having 20 cm plant-to-plant and 60 cm row-to-row spacing. Each F_3 progeny of 20 seeds was grown in an ear-to-row manner. The crop was raised following standard agronomical practices.

Marker-assisted breeding scheme for development of F_3 progenies: Crosses were made between CM139 × CM140 and LM5 × CM140 to generate two independent F_1s during rainy (*kharif*) season 2017. The hybridity of F_1 was authenticated through PCR-based co-dominant markers linked to QTLs. The F_1 plants from both crosses were backcrossed with the recurrent parent to generate BC_1F_1 during spring 2018. The BC_1F_1 plants were raised during *kharif* 2019 and screened for targeted QTLs. The confirmed heterozygous plants were again backcrossed to generate BC_2F_1 (Fig 1). The heterozygous BC_2F_1 plants were selected for each QTL based on SLB score as well as morphological attributes towards the recurrent parent. The selected BC_2F_1 plants were intercrossed in combinations to produce F_{1s}. Thirty individuals from each complex F_1 were raised in spring 2020, and selfed to obtain an F_2 population. A total of nine ears were selected from $F_{1\text{-}1}$, $F_{1\text{-}2}$, and $F_{1\text{-}3}$ and raised during *kharif* 2020. The homozygous F_2 plants were identified through foreground selection and advanced to F_3 progenies during *kharif* 2021 for SLB evaluation to see the effect of QTLs in combination and singly.

Genotyping for target QTLs: The genomic DNA of parents, backcross generations and selfed plants was isolated using the CTAB method described by Saghai-Maroof *et al.* (1984) with some minor modifications. The quality and quantity of each DNA sample were ascertained through a nanodrop spectrophotometer and using 0.8% agarose gel. Foreground selection of targeted QTLs was done using flanked linked SSR markers for each QTL. The *qSLB3.1* spanned 1.2 cM bracketed by MSSR-1 (F-5´CGCCAAACTGAAGCTGACAG3´; R-5´ AGCTAACACGTCCAGAAGGC3´) and MSSR-18 (F-5´CTTCGGACTCCTCCCAAAGG3´; R-5´ GCAATCAATAGCCTACCGTTTCTG3´) SSR markers (Kaur 2019), whereas *qSLB8.1* spanned to 7 cM flanked by umc1075 (F-5´GAGAGATGACAGACACATCCTTGG3´; R-5´ ACATTTATGATACCGGGAGTTGGA3´) and umc1858 (F-5´ GTTGTTCTCCTTGCTGACCAGTTT; R-5´ATCAGCAAATTAAAGCAAAGGCAG3´) SSR markers (Kaur *et al.* 2019). During QTLs pyramiding, *qSLB8.1* was fine-mapped to a region of 3 cM linked with pau_52 (F-5´ACACGAAGTACACCTCCTGC3´; R-5´TCATCAGCTCGATCGCTCAC3´) and pau_116 (F-5´ACTGTGCGAGCTTCTGACTC3´; R-5´AGCCGAAGCAGGTTCTACAC3´) SSR markers (Kaur 2020) (Supplementary Table 1). New SSR markers associated with *qSLB8.1* were also employed on the F_2 populations and selection was made considering nested markers. PCR reaction was performed as described by Kaur *et al*. (2019). The amplified products were separated on 8% native PAGE stained with ethidium bromide and visualized on UV light in the AlphImager EC gel documentation system (ProteinSimple, San Jose, CA).

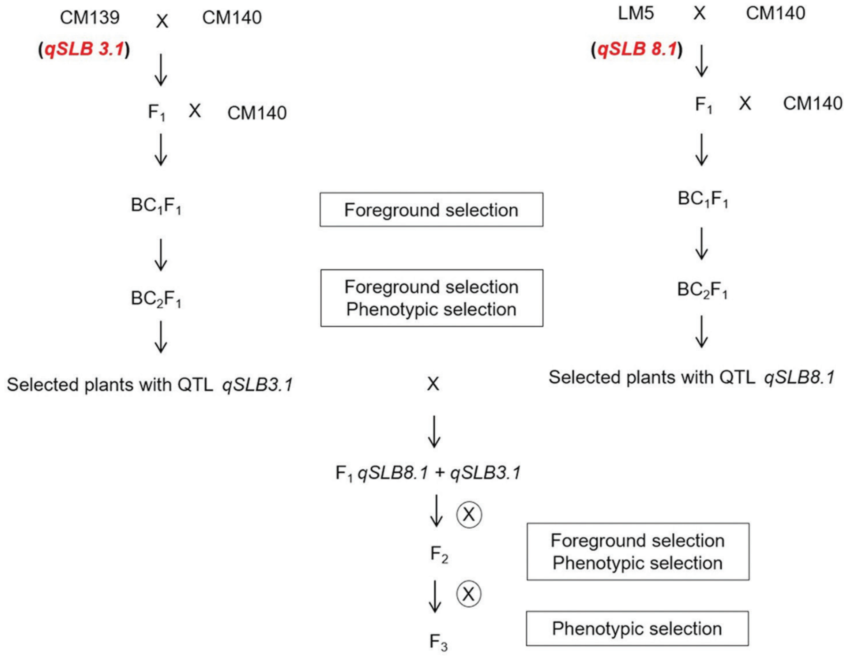

Fig 1 Schematic illustration of marker-assisted pyramiding of southern leaf blight resistance QTLs in the background of CM140.

Evaluation for SLB resistance: The most virulent isolate of *Bipolaris maydis*, *Dm1*, was procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana. The mass inoculum of SLB was prepared by culturing *Dm1* on sorghum (*Sorghum bicolor* L.) seeds following the method of Lim (1975). The inoculations were done by placing 20–30 infected sorghum seeds in whorls of each plant at the seven-leaf stage (V7). The remaining sorghum seeds were meshed by adding 15 gm of carboxymethyl cellulose and dissolved in 5 litres of warm water for a foliar spray with a concentration of 2×10^6 spores/ml. The water solution was sprayed twice on the plants to obtain the desired moisture for appropriate infection development on the same day and the next day of inoculations. The disease score of SLB was recorded after 15, 30, and 45 days of post inoculations (dpi) according to a 1–9 scale (Hooda *et al.* 2018).

Statistical analysis: Mean disease severity from disease score data was calculated over the three ratings of SLB (15 dpi, 30 dpi, and 45 dpi) as:

$$\text{Percent disease severity} = \frac{\text{Sum of class rating}}{\text{Total number of plants} \times \text{Maximum rating}} \times 100$$

The area under disease progress curve (AUDPC) was calculated according to formula given by Shaner and Finney (1977):

$$\text{AUDPC} = \sum_{i=1}^{n} \left[(\text{Xi+1} + \text{Xi}) / 2 \right] \left[\text{ti+1} - \text{ti} \right]$$

Where Xi, the proportion of host tissue damaged on an ith day; ti, the time in days after the appearance of the disease on an ith day; and n, the total number of observations. The unit for sample data is %; the unit for t is time period, and the unit of the AUDPC is %- development stage unit.

RESULTS AND DISCUSSION

Foreground selection of backcross progenies: The use of marker-assisted selection with stringent phenotypic selection enhances the efficiency and precision of the breeding programme for developing multiple disease-resistant varieties (Crossa *et al.* 2017). The F_1s derived from CM139 × CM140 and LM5 × CM140 were confirmed for the presence of the QTLs using QTL-specific flanking SSR markers. Three F_1 plants from each cross were backcrossed to develop the BC_1F_1 progenies. Out of 420 and 630 BC_1F_1 plants, 64 and 95 plants derived from CM139 × CM140 and LM5 × CM140, respectively, had both alleles for *qSLB3.1* and *qSLB8.1* (Table 1). Based on the SLB score and other phenotypic attributes like tassel type, anther and silk colour to recover the maximum recurrent parent background, 15 plants from each cross were backcrossed to generate BC_2F_1 seeds. Three ears from each cross were further selected based on ear shape and kernel colour for raising the BC_2F_1 population. Out of 137 BC_2F_1 plants of cross CM139 × CM140, 40 plants were heterozygous for both markers, while 57 plants were recombinants and 40 plants had recurrent parent alleles (Table 1, Fig 2). Among 266 BC_2F_1 plants derived from cross LM5 × CM140, 76 plants were heterozygous, 100 plants were recombinants, and 90 plants had alleles from a recurrent parent (Fig 2).

Marker-assisted pyramiding of qSLB3.1 and qSLB8.1: Pyramiding of genes/QTLs is required to provide long-term resistance compared to short-term resistance provided by a single gene. A strategy of intercrossing backcross individuals carrying QTLs was followed similarly to Tyagi *et al.* (2014) and Yang *et al.* (2016) to pyramid SLB resistance QTLs. Three BC_2F_1 plants having disease scores of <3 and heterozygous for both the markers (from each cross) were

Table 1 Segregation patterns of flanking markers linked to QTLs, *qSLB3.1* and *qSLB8.1* in different backcross and self-generations

Population	Cross	Total number of plants analyzed	*qSLB3.1* + *qSLB8.1*	*qSLB3.1*	*qSLB8.1*	Recombinants	Recurrent parent type
BC_1F_1	CM139 × CM140	420	-	64	-	198	158
	LM5 × CM140	630	-	-	95	308	227
BC_2F_1	CM139 × CM140	137	-	40	-	57	40
	LM5 × CM140	266	-	-	76	100	90
$F_{2\text{-}1}$	21 × 2	183	29	38	30	-	86
$F_{2\text{-}2}$	64 × 85	84	13	9	30	-	32
$F_{2\text{-}3}$	29 × 3	130	32	24	38	-	36

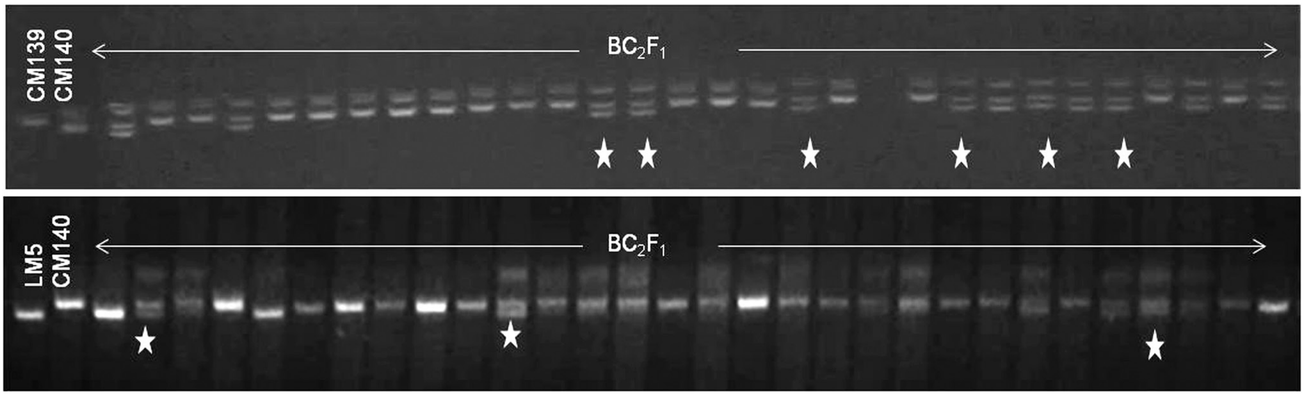

Fig 2 Foreground selection in BC_2F_1 population for southern leaf blight resistance quantitative trait loci using MSSR-18 SSR marker linked to *qSLB3.1* and umc1858 SSR marker linked to *qSLB8.1*. The asterisks represent plants heterozygous for *qSLB3.1* and q*SLB8.1*.

inter-crossed to combine *qSLB3.1* and *qSLB8.1* to generate three F_{1s} from different cross combinations (F_{1-1}, F_{1-2}, and F_{1-3}). The F_{2-1} population developed from F_{1-1} comprised four ears with 94, 89, 109 and 124 seeds, respectively. Likewise, the F_{2-2} population derived from F_{1-2} consisted of three ears having 84, 145, and 105 kernels, respectively. The F_{1-3} was selfed to constitute the F_{2-3} population of two ears (131 and 120 seeds).

A total of 183, 84 and 130 plants from F_{2-1}, F_{2-2}, and F_{2-3} populations, respectively, were subjected to foreground selection for the selection of plants with homozygous alleles for both QTLs. A total of 74 plants from all the three F_2 populations had both QTLs based on foreground selection (Table 1). Out of 74 plants, 18 plants were homozygous for *qSLB3.1* and *qSLB8.1*, 22 plants were homozygous for *qSLB3.1* and heterozygous for *qSLB8.1,* and 34 plants were homozygous for *qSLB8.1* and heterozygous for *qSLB3.1.* 12, 8 and 12 F_2 plants possessing *qSLB3.1* + *qSLB8.1*, *qSLB8.1*, and *qSLB3.1*, respectively, in homozygous condition were selected to generate $F_{2:3}$ lines.

Evaluation for SLB resistance: Several QTLs for SLB resistance have been mapped and most studies focused on identifying and determining the QTL effect within mapping populations (Lenon *et al.* 2017). Generally, QTLs are environment-specific, affected by genetic background, and show epistasis effects (Kump *et al.* 2010). Earlier, no information was available about the QTL × QTL and QTL × genetic background interactions for SLB resistance. To investigate the effects of QTLs singly and in combination in CM140 background, we employed QTLs that had explained phenotypic variance >15% and were consistent over the environments for which host resistance is known to be highly heritable. BC_1F_1 and BC_2F_1 populations displayed a significant variation in SLB resistance. The mean disease score of BC_1F_1 and BC_2F_1 populations derived from the cross CM139 × CM140 increased from 2.6 to 5.1 and 3.3 to 4.8 between 15 and 30 dpi, respectively. However, BC_1F_1 and BC_2F_1 populations from cross LM5 × CM140 exhibited mean disease scores of 3.1 to 5.2 and 3.22 to 5.17 from 15 to 30 dpi, respectively. The resistant parent CM139 had an average disease score of 1.1, 2.2 and 3.5 after 15, 30, and 45 dpi, respectively. On the contrary, LM5 showed an average score of 2.0, 2.9 and 3.4 after 15, 30, and 45 dpi, respectively. The mean score of susceptible parent CM140 after 15, 30 and 45 dpi were 4.0, 7.6, and 8.8, respectively (Table 2).

Out of 412 F_{2-1} plants, 154 had a disease score of 1-3, while 258 plants displayed a 3.1-4.5 SLB score after 15 dpi. The 15, 170 and 227 plants exhibited disease scores of 1-3, 3.1-5, and >5 at 30 dpi. However, 3, 16, 238 and 155 plants had a disease score of 1-3, 3.1-5, 5.1-7 and >7 at 45 dpi, respectively. Out of 325 F_{2-2} plants, 100, 129 and 96 plants showed disease scores of 3.-5, 5.1-7.0 and >7 at 30 dpi, respectively. The average disease score increased from 3.81 to 7.29 from 15 dpi to 45 dpi, respectively, in the F_{2-2} population (Table 2). A total of 116 and 134 F_{2-3} plants had disease scores of 1-3 and 3.1-5 at 15 dpi. However,

Table 2 Southern leaf blight ratings after 15 days interval of post inoculations for parental inbreds and QTLs introgressed F_2 populations and F_3 progenies

Parents/Population	SLB assessment after 15, 30 and 45 dpi												Mean SLB severity (%)	AUDPC[c]
	15 dpi				30 dpi				45 dpi					
	Mean	Range	SD[a]	CV[b]	Mean	Range	SD	CV	Mean	Range	SD	CV		
LM5	2.0	1.5-2.5	0.32	15.81	2.9	2.5-3.5	0.37	12.90	3.4	3.0-3.5	0.21	9.08	30.70	933.33
CM139	1.1	1.0-1.5	0.20	18.18	2.2	2.1-3.0	0.4	18.18	3.5	3.0-4.0	0.32	9.03	25.18	750.00
CM140	4.0	3.5-4.5	0.30	7.90	7.6	7.0-8.0	0.31	4.92	8.8	8.5-9.0	0.20	2.78	75.55	2333.35
F_{2-1}	2.87	1.0-4.5	1.12	39.96	4.63	1.0-8.0	1.33	27.50	6.15	2.0-9.0	1.20	19.30	79.62	2458.33
F_{2-2}	3.81	2.0-5.0	0.92	24.36	5.99	3.0-8.0	1.07	17.88	7.29	4.0-9.0	1.20	16.51	81.47	2499.95
F_{2-3}	2.50	1.0-4.0	0.83	33.31	4.44	1.0-8.0	1.15	25.88	6.20	2.0-9.0	1.28	21.13	77.77	2416.50
F_3 (*qSLB3.1* and qSLB8.1)	1.92	1.25-2.5	0.33	17.51	2.74	2.1-3.2	0.35	12.97	2.9	2.4-3.2	2.24	8.39	29.75	909.37
F_3 (*qSLB3.1*)	2.31	2.05-2.67	0.17	7.48	3.81	2.8-4.7	0.58	15.47	4.63	3.8-5.0	0.31	6.82	42.87	1388.40
F_3 (*qSLB8.1*)	2.46	2.2-2.8	0.21	8.64	3.97	3.1-4.8	0.53	13.50	4.28	3.2-5.0	0.67	15.7	46.02	1279.57

[a]SD, standard deviation; [b]CV, coefficient of variation; [c]Area under disease progress curve is in per cent-day units.

17, 135 and 67 plants had a disease score of 3.1-5, 5.1-7, and >7 at 45 dpi, respectively. The overall percent disease severity ranged from 11-99% in all the F_2 populations. The highest mean AUDPC value was shown by $F_{2\text{-}2}$ (12499.95), followed by $F_{2\text{-}1}$ (2458.33) and $F_{2\text{-}3}$ (2416.50) (Table 2).

Based on phenotypic evaluation for SLB resistance, marker analysis and morphological traits, 32 plants were raised as $F_{2:3}$ lines. 32 $F_{2:3}$ lines had an average disease score of 1-3 at 15 dpi. At 30 dpi, 20 lines exhibited a disease score of 1-3, and 12 had a disease score of 3-5. However, only 12 pyramided lines displayed a disease score of 1-3 at 45 dpi. Improved versions of CM140 with a single QTL showed a decrease in SLB severity. It validates the effectiveness of *qSLB3.1* and *qSLB8.1*. Whereas pyramided F_3 lines exhibited 29.75% decreased disease severity, indicating a synergistic effect of both QTLs. Also, pyramided lines had lower AUDPC than either of the QTL, inferring that additive effects of the QTLs for SLB resistance had played an important role. However, compared to donors, each QTL effect in the CM140 background is not the same due to differences in donor genetic constitutions. It might be due to some modifiers or minor QTLs in the vicinity of introgressed QTL that might have resulted in both positive and negative interactions and QTL × environment interactions.

The results are per previous reports that QTLs in different combinations have a differential response to disease severity (Bai *et al.* 2012). Santa-Cruz *et al.* (2014) studied the effect of individual QTLs across different genetic backgrounds in multi-environment yield trials with and without SLB disease, revealing that introgression 6A was significantly associated with yield reduction. Similarly, the effect of multiple disease resistance QTLs to foliar diseases of maize was studied in a uniform genetic background by Martin *et al.* (2019). Henceforth, the breeding material possessing QTLs with large effects and stability across a wide range of environmental conditions is the key to the successful implementation of QTL introgression (Belcher *et al.* 2012). Pyramid lines developed could serve as valuable genetic resources in hybridization programs and could be evaluated in hotspot regions of SLB in India.

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REFERENCES

Ashikari M and Matsuoka M. 2006. Identification, isolation and pyramiding of quantitative trait loci for rice breeding. *Trends in Plant Sci*ence **11**: 344–50.

Bai B, He Z H, Asad M A, Lan C X, Zhang Y and Xia X C. 2012. Pyramiding adult-plant powdery mildew resistance QTLs in bread wheat. *Crop Pasture Science* **63**: 606–11.

Balint-Kurti P, Zwonitzer J, Pe M, Pea G, Lee M and Cardinal A J. 2008. Identification of quantitative trait loci for resistance to southern leaf blight and days to anthesis in two maize recombinant inbred line populations. *Phytopathology* **96**: 1067–71.

Belcher A, Zwonitzer J, Cruz J, Krakowsky M, Chung C, Nelson R, Arellano C and Balint-Kurti P. 2012. Analysis of quantitative disease resistance to southern leaf blight and of multiple disease resistance in maize, using near-isogenic lines. *Theoretical Applied Genetics* **124**: 433–45.

Craig J and Fajemisin J M. 1969. Inheritance of chlorotic lesion resistance to *Helminthosporium maydis* in maize. *Plant Disease Report* **53**: 742–43.

Crossa J, Pérez-Rodríguez P, Cuevas J, Montesinos-Lopez O, Jarquín D, De Los Campos G, Burgueño J and González-Camacho J M. 2017. Genomic selection in plant breeding: methods, models, and perspectives. *Trends in Plant Science* **22**: 961–75.

Hooda K S, Bagaria P K, Khokhar M, Kaur H and Rakshit S. 2018. *Mass Screening Techniques for Resistance to Maize Diseases*, pp. 93. ICAR-Indian Institute of Maize Research, PAU Campus, Ludhiana.

Kaur K. 2019. 'Fine mapping of QTL *QSlb.pau-3.04* for southern leaf blight resistance in maize (*Zea mays* L.)'. PhD Thesis, Punjab Agricultural University, Ludhiana.

Kaur S. 2020. 'Fine mapping of QTL (*QSLB8.1 and QSLB9.1)* for southern corn leaf blight resistance in maize (*Zea mays* L.)'. MSc Thesis, Punjab Agricultural University, Ludhiana.

Kaur D, Kaur A, Kaur P I, Grewal M S and Vikal Y. 2013. Analysis of molecular diversity in water stress tolerant and susceptible maize inbred lines. *Crop improvement* **40**: 1–10.

Kaur M, Vikal Y, Kaur H, Pal L, Kaur K and Chawla J S. 2019. Mapping quantitative trait loci associated with southern leaf blight resistance in maize (*Zea mays* L.). *Journal of Phytopathology* **167**: 591–600.

Kump K, Holland J, Jung M, Wolters P and Balint-Kurti P. 2010. Joint analysis of near isogenic and recombinant inbred line populations yields precise positional estimates for quantitative trait loci. *Plant Genome* **3**: 142–53.

Kump K L, Bradbury P J, Wisser R J, Buckler E S, Belcher A R, Oropeza-Rosas M A and Holland J B. 2011. Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. *Nature Genetics* **43**: 163–68.

Lennon J R, Krakowsky M, Goodman M, Flint-Garcia S and Balint-Kurti P J. 2017. Identification of teosinte alleles for resistance to southern leaf blight in near isogenic maize lines. *Crop Science* **57**: 1973–83.

Li Y X, Chen L, Li C, Bradbury P J, Shi Y S, Song Y and Wang T. 2018. Increased experimental conditions and marker densities identified more genetic loci associated with southern and northern leaf blight resistance in maize. *Scientific Reports* **8**: 6848.

Lim S M. 1975. Heterotic effects of resistance in maize to *Helminthosporium maydis* race O. *Phytopathology* **65**: 1117–20.

Manjunatha C, Gogoi R, Singh B, Jeevan B, Rai S N and Singh P K. 2019. Phenotypic and physiological characterization of maize inbred lines resistant and susceptible to maydis leaf blight. *Indian Phytopathology* **72**: 217–24.

Martins L B, Rucker E, Thomason W, Wisser R J, Holland J B and Balint-Kurti P. 2019. Validation and characterization of maize multiple disease resistance QTL. G3: *Genes Genome Genetics* **9**: 2905–12.

Saghai-Maroof H A, Soliman K M, Jorgensen A R and Allard R W.1984. Ribosomal DNA spacer length polymorphism in barley: Mendelian inheritance, chromosomal location and population dynamics. *Procceedings of National Academic*

Sciences USA **81**: 8014–18.

Santa-Cruz J H, Kump K L, Arellano C, Goodman M M, Krakowsky M D, Holland J B and Balint-Kurti P J. 2014. Yield effects of two southern leaf blight resistance loci in maize hybrids. *Crop Science* **54**: 882–94.

Shaner G and Finney R E. 1977. The effect of nitrogen fertilization on the expression of slow-mildewing resistance in Knox wheat. *Phytopathology* **67**: 1051–56.

Smith D R and Hooker A L.1973. Monogenic chlorotic-lesion resistance in corn to *Helminthosporium maydis*. *Crop Science* **13**: 330–31.

Sun X, Qi X, Wang W, Liu X, Zhao H, Wu C, Chang X and Zhang M. 2020. Etiology and symptoms of maize leaf spot caused by *Bipolaris* spp. in Sichuan, China. *Pathogen* **9**: 229–46.

Thompson D and Bergquist R. 1984. Inheritance of mature plant resistance to *Helminthosporium maydis* race O in maize. *Crop Science* **24**: 807–11.

Tyagi S, Mir R R, Kaur H, Chhuneja P, Ramesh B, Balyan H S and Gupta P K. 2014. Marker-assisted pyramiding of eight QTLs/genes for seven different traits in common wheat (*Triticum aestivum* L.). *Molecular Breeding* **34**: 167–75.

Ullstrup A J. 1972. The impacts of the southern corn leaf blight epidemis of 1970–71. *Annual Review of Phytopathology* **10**: 37–50.

Vasmatkar P, Kaur K, Pannu P P S, Kaur G and Kaur H. 2019. Unraveling the metabolite signatures of maize genotypes showing differential response towards southern corn leaf blight by 1H-NMR andFTIR spectroscopy. *Physiological and Molecular Plant Pathology* **108**: 101441.

Wei J K, Lui K M, Chen J P, Luo P C and Stadelmann O Y. 1988. Pathological and physiological identification of race C of *Bipolaris maydis* in China. *Phytopathology* **78**: 550–54.

Yang T, Zhang S, Zhao J, Liu Q, Huang Z, Mao X, Dong J and Wang X. 2016. Identification and pyramiding of QTLs for cold tolerance at the bud bursting and the seedling stages by use of single segment substitution lines in rice (*Oryza sativa* L.). *Molecular Breeding* **36**: 96.

Zwonitzer J, Coles N, Krakowsky M, Arellano C, Holland J, McMullen M, Pratt R and Balint-Kurti P. 2010. Mapping resistance quantitative trait loci for three foliar diseases in maize recombinant inbred line population-Evidence for multiple disease resistance. *Phytopathology* **100**: 72–79.