Development of doubled haploid population and QTL mapping for Fusarium stalk rot (FSR) resistance in tropical maize

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Abstract

Fusarium stalk rot disease (FSR) caused by *Fusarium verticilloides* is emerging as the major production constraint in maize across the world. As a prelude to develop maize hybrids resistant to FSR, an attempt was made to identify QTL as the genetics of resistance was found to be quantitative in nature. Two doubled haploid (DH) mapping populations induced from F_2 of crosses VL1043 × CM212 and VL121096 × CM202 were challenged with FSR during two seasons. The FSR response was influenced by significant DHs × season interaction. The DH populations were genotyped employing 164 and 132 polymorphic SNP markers in the DHs induced from the crosses VL1043 × CM212 and VL121096 × CM202, respectively. Inclusive composite interval mapping was performed to detect significant QTL, QTL × QTL, QTL × season interaction effects. Two and one QTL were identified in rabi 2019 and summer 2020, respectively. The QTL identified on the linkage group 10 (qFSR_10_1) was common across two seasons in DHs derived from the cross VL1043 × CM212. Similarly, two QTL each in rabi 2019, summer 2020 and one common QTL (qFSR_6_2) were identified for FSR resistance in DHs derived from F_2 of the cross VL121096 × CM202. The QTL qFSR_10_1 was common in both the crosses. The position and effect of the QTL were varied with the seasons. Seven di-QTL interactions were detected for FSR resistance in both DH populations.

Introduction

Globally, the maize production and productivity are constrained by numerous prevalent and emerging insect pests (Stem borer and Fall armyworm) and diseases (Banded leaf and Sheath blight, Sorghum downy mildew, Turcicum leaf blight and Fusarium stalk rot). Of these, Fusarium stalk rot (FSR) caused by *Fusarium verticillioides* (Saccardo) Nirenberg (formerly called *Fusarium moniliforme* Sheldon) (Seifert et al. 2004), is one of the serious threats to maize cultivation in all continents of the world (CIMMYT, 2004). In India, FSR causes yield loss up to 38% (Singh et al. 2012) in isolated maize growing areas and 100% yield loss was reported in areas where water stress occurs after the flowering stage (Mallikarjun *et al.* 2017). The FSR generally occurs later the flowering stage and before physiological maturity, reducing yields due to premature death of plants with lightweight ears having poorly filled kernels and lodging of infected plants making harvesting difficult thereby, ears are left in the field. Additionally, FSR was also reported to reduce 18.7% cob weight and 11.2% of 1000-grain weight in the infected plants (Cook, 1978).

Genetic intervention is an eco-friendly and cost-saving strategy to reduce the losses caused by diseases including FSR (El-Shafey et al. 1988; Zeller et al. 2000; Jeevan et al., 2020). As a prelude to genetic strategy for FSR management, stable sources of resistance to *F. verticilliodes* (Archana et al. 2019; Ayesha et al. 2020; Showkath *et al.* 2020; Lingaraj et al. 2019) have been reported in tropical maize germplsm. However, the FSR resistance has been reported as complex inheritance with duplicate epistasis (Archana et al. 2019 and Showkath *et al.* 2020). Hence, direct selection for FSR resistance is likely to be less effective. However, the DNA markers could be employed as an effective surrogates of such complex traits in maize, for which identification and validating closely linked molecular markers are essential. QTL mapping has been widely employed to identify the genetic basis of target traits (JiweiYang *et al.* 2020) using various biparental populations *viz*., $F_{2:3}$, backcross, recombinant inbred line (RIL) and doubled haploid (DH) populations. However, no QTLs have been reported for FSR disease resistance so far. Among different mapping populations, RIL and DH populations contain genetically stable families and can be employed to get accurate and effective phenotyping for molecular mapping of target trait. The usual development of RIL populations by continuous self-pollination for more than eight generations is a time-consuming and expensive process. In contrast, DH populations are produced only in two generations and are 100% homozygous. Therefore, presently the DH populations are frequently used for trait mapping in various species (Vanous et al. 2018; JiweiYang *et al.* 2020). Hence, two DH populations induced from F_2 of crosses VL1043 × CM212 and VL121096 × CM202 were used to characterize and decipher the genomic regions controlling FSR resistance in maize.

Material And Methods

Basic genetic material

The basic material for the study consisted of two highly susceptible (VL1043 and VL121096) and two moderately resistant (CM212 and CM202) inbreds to FSR. These inbred lines were procured from the ICRISAT-CIMMYT Asia centre for maize, Hyderabad. The inbred lines were selected based on the previous years disease reaction from artificial disease screening against FSR (Archana et al. 2019) along with high parental polymorphism compared with other inbred lines assayed.

Development of DH lines

The susceptible inbred lines (VL 1043 and VL 121096) were crossed with resistant lines (CM212 and CM202) during summer 2017 to develop two crosses *viz*., VL1043 × CM212 and VL121096 × CM202 and they were selfed to obtain F_2 plants during the rainy season of 2017 at the research farm of College of Agriculture, Mandya, Karnataka, India (12.57°N76.82°E; 695 m AMSL). Without the selection of plants or kernels, the random sample of around 1200 kernels in F_2 generations were planted in 50 rows of 4 m length at the research farm of M/s Corteva Agriscience, Kallinayakanahally, Chikkaballapur District, Karnataka, India (13.46°N, 77.51°E; 684 m AMSL). Each F_2 plant was independently pollinated with male haploid inducer inbred line (Vijay *et al.* 2019). The cob of each cross had kernels with both haploid and diploid genetic contistution. The haploid kernels were identified and selected based on dominant grain purple colour marker gene (*R1-nj* marker). The kernels without any pigmentations were selfed or outcrossed one, the kernels with embryo and endosperm pigmentation were regular diploids, and those with endosperm pigmentation and without embryo pigmentation were haploids.

The selected haploid seeds were germinated on paper towels till the emergence of coleoptiles about 2-cm long. Subsequently, the tip of coleoptiles were cut-off and submerged in colchicine solution with DMSO to allow the uptake of colchicine. Later, the seedlings were washed thoroughly under tap water and planted in biodegradable Jiffy peat pellets (http://www.jiffypot.com), in a shade house for recovery and hardening. Once the seedlings reached three-leaf stage, transplanted to a DH nursery net house. Each plant was selfed, and the harvested cobs (D_0) were doubled haploids. The D_0 seeds were sown and

advanced to D_1 nursery with strict selfing to screen and rogue the haploids, offtypes and false positives. With aforementioned protocol, it was possible to derive 280 and 94 DHF_2 lines (doubled haploid lines derived from F_2 plants) from VL1043 × CM212 and VL121096 × CM202 crosses, respectively.

Characterization of DH lines for FSR resistance

Field layout

The DHF_2s of both crosses and their respective parents as checks were evaluated in the Augmented design (Federer, 1961), and checks were repeated after every 10th row of test entries in two-row plots of 2 m length. All the entries were planted in rows spaced 0.60 m apart with an intra-row spacing of 0.20 m in the artificial disease screening nursery for FSR disease at the College of Agriculture, V. C. Farm, Mandya during the rainy season, 2019 and winter, 2019-20.

Screening for resistance to FSR

Disease screening was done by following the procedure developed by the Indian Institute of Maize Research (IIMR), Ludhiana (2012). To ensure effective inoculation, uniform disease infestation and good disease development, all the plants were inoculated twice, first at 65 DAS and the second inoculation at 75 DAS with a known concentration (1×10^6) of pathogen spores.

Isolation and mass multiplication of F. verticilloides **pathogen**

Maize stalks displaying the typical FSR symptoms were collected from the maize field. The stalk showing infections were cut into small pieces and 4% sodium hypochlorite solution was used for surface sterilization. The surface sterililized pieces were washed twice with sterile distilled water, dried and plated on Potato Dextrose Agar (PDA) medium. To develop pathogen colonies, Petri plates were incubated for five days in a Biological Oxygen Demand (BOD) incubator. Once the pathogen colonies appered which were inspected for typical *F. verticilloides* morphological and fruiting body characteristics. From the confirmed colonies the mycelia were aseptically transferred to sterile Potato Dextrose Broth (PDB) in conical flasks for subsequent mass multiplication. The conical flasks were incubated for 15 days for proper and suffienct development of mycelia. On 15th day, the mycelia of pathogen were grounded and filtered to obtain pathogen spore suspension.

Inoculum Preparation

The pathogen spore suspension was examined under microscope and the haemocytometer was employed to adjust the concentration to 1×10^6 spores per ml. Whenever high spore concentration was observed, the same was diluted with sterile distilled water to retain the required spore concentration.

Artificial inoculation of F. verticilliodes inoculum

At the second internode of all the plants at 65 and 75 DAS, a 2 cm hole was made using jabber. Later the plants were inoculated with 2 ml of pathogen inoculum injected diagonally using the syringe in the hole. After inoculation, irrigation was withheld for four days to enable proper uptake of inoculum by the plants. Further, all the standard package of practices were followed except spray of fungicides after pathogen inoculation.

Sampling and data recording

For disease phenotyping, the stalks were split open before drying, *i.e.*, around 30 days after inoculation. Each of the individual plants of each line was examined for FSR severity and intensity using a 1–9 rating disease scale (Table 1). The FSR scoring pattern was developed based on the spread of inter-node discoloration inside the maize stalks from the point of inoculation (Payak and Sharma 1983). The higher the discoloration, higher was the disease rating.

Table 1
The Fusarium stalk rot disease rating scale in maize (Payak and Sharma 1983)

Disease score	Symptoms	Disease reaction
1	Healthy or slight discolouration at the site of inoculation	Highly resistant
2	Up to 50% of the inoculated internode is discoloured	Resistant
3	51–75% of the inoculated internode is discoloured	Moderately resistant
4	76–100% of the inoculated internode is discoloured	Moderately susceptible
5	Less than 50% discolouration of the adjacent internode	Susceptible
6	More than 50% discolouration of the adjacent internode	Highly susceptible
7	Discolouration of three internodes	Highly susceptible
8	Discolouration of four internodes	Highly susceptible
9	Discolouration of five or more internodes and premature death of a plant	Highly susceptible

Genotyping of DH populations

Parental polymorphism survey

The parents VL1043, VL121096, CM212 and CM202 were genotyped using Corteva Agri-Science Proprietary SNP markers employing Illumina Infinium XT assay. A total of 198 and 199 SNP markers were polymorphic between the parental lines of doubled haploids derived from the F_2 of the cross VL1043 × CM212. While 199 markers were polymorphic between the parental lines of doubled haploids derived from F_2 of the cross VL121096 × CM202. These polymorphic markers were used for genotyping in both the DH populations.

Statistical analysis of FSR response

The disease response data of individual plants in each DH line were averaged in both crosses over the two seasons and were subjected to pooled augmented analysis of variance (ANOVA) to detect the significance of DH line × season interaction. After ascertaining the existence/non-existence of DH line × season interaction, Best Linear Unbiased Predictors (BLUPs) (Schonfeld and Werner 1986) were estimated by considering blocks and DH lines as random effects and seasons as fixed effects with restricted maximum likelihood (REML) estimation mixed model procedure (PROC MIXED) (Patterson and Thompson, 1971; Federer and Wolfinger, 1998) in SAS *ver.* 9.4 software programme (SAS Institute Inc., Cary, NC, USA) to estimate genetic and non-genetic variances across seasons. Based on BLUP scores, the lines were classified as highly resistant (HR), resistant (R), moderately resistant (MR), moderately susceptible (MS), susceptible (S) and highly susceptible (HS).

Detection of QTL controlling resistance to FSR

Linkage map construction of DHF_2s

Two DHF_2 populations derived from crosses VL1043 × CM212 and VL121096 × CM202 were used for linkage map construction using 199 SNPs data on 280 DHF_2s of the cross VL1043 × CM212 and 193 SNPs marker data on 94 DHF_2s of the cross VL121096 × CM202. The linkage analysis was performed using "QTL IciM software version 4.1' programme. A minimum threshold LOD score of 3.0 was set for linkage groups determination. The significance of inter-marker recombination frequencies was converted into map distances using the Kosambi mapping function (Kosambi, 1944).

Identification of QTL controlling resistance to FSR

The initial analysis of QTL controlling FSR resistance was performed by integrating the genotyping and phenotyping data of DH lines using single-marker analysis. The significance of differences among marker classes was tested by F-test (Fisher and Yates, 1949). Significance or non-significance of F-test indicated the presence or absence of association between FSR disease BLUP scores and the test marker.

Subsequently, Inclusive Composite Interval Mapping (ICIM) was used to detect and estimate the size and effects of QTL and QTL × QTL interactions controlling FSR resistance. The positions and effects of QTL and QTL × location interaction conferring FSR resistance were determined at data-driven estimates of threshold LOD scores obtained by 1000 permutations implemented with ICiMapping software version 4.0. Similarly, QTL × QTL interactions controlling FSR resistance were detected and estimated at threshold LOD of 3.0 using QTL ICiMapping software version 4.0 (Wang et al., 2011).

Results

Phenotypic response of DH lines to FSR resistance

The DH lines were developed employing F_2 plants of crosses VL1043 × CM212 and VL121096 × CM202 through *in vivo* haploid induction method and were screened for their response to FSR disease using artificial inoculation technique. The mean disease scores of DH lines in individual seasons and combined over seasons were subjected to ANOVA and the components of variance were computed considering all effects in the statistical model as random. The analysis of variance revealed significant genetic differences among DH lines (Table 2). Best linear unbiased predictors (BLUPs) were estimated since it includes prediction of genetic effects and also estimation of genetic and non-genetic variances simultaneously. The BLUP scores were successfully employed in the classification of DHs into different disease response groups.

In DHF_2s produced from the cross VL1043 × CM212 four lines were resistant in rabi 2019, followed by three lines in summer 2020 and one line in pooled analysis (Table 3). In DHF_2s of cross VL121096 × CM202, one line was highly resistant and another one was resistant in summer 2020 with one line showing resistant reaction when pooled over seasons (Table 3). In both the crosses, most DH lines belonged to a moderately susceptible response group.

Table 2

Analysis of variance of mean FSR disease scores of DH lines induced from F_2 of crosses VL1043 × CM212 and VL121096 × CM202

Source of variation	Degrees of freedom				Mean sum of squares					
	DHF_2 of VL1043 × CM212		DHF_2 of VL121096 × CM202		DHF_2 of VL1043 × CM212			DHF_2 of VL121096 × CM202		
	Rabi 2019 & Summer 2022	Pooled	Rabi 2019 & Summer 2022	Pooled						
					Rabi 2019	Summer 2020	Pooled	Rabi 2019	Summer 2020	Pooled
Block	12	12	5	5	0.021	0.03	0.02	0.07	0.07	0.04
Seasons	-	1	-	1	-	-	2.94***	-	-	0.32
Check	1	1	1	1	103.76***	119.54***	223.02***	42.75***	53.55***	96.00***
Doubled haploids	279	279	93	93	0.60***	0.51***	0.89***	0.53	0.78**	1.15***
Seasons × Doubled haploids	-	280	-	94	-	-	0.22***	-	-	0.18
Error	12	37	5	16	0.05***	0.10***	0.06***	0.15*	0.10***	0.13***

Table 3

Classification of doubled haploid lines into different response groups based on BLUP values of FSR scores

Score	Response	No. of doubled haploid lines					
		DHF_2 of VL1043 × CM212			DHF_2 of VL121096 × CM202		
		Rabi 2019	Summer 2020	Pooled	Rabi 2019	Summer 2020	Pooled
1	Highly resistant	0	0	0	0	1	0
2	Resistant	4	3	1	0	1	1
3	Moderately resistant	76	58	53	25	16	20
4	Moderately susceptible	159	162	189	54	58	58
5	Susceptible	27	48	23	11	11	9
>6 -9	Highly susceptible	14	9	14	4	7	6
Population size		**280**	**280**	**280**	**94**	**94**	**94**

The polymorphism survey between parents of the crosses was carried out using 2000 Corteva AgriScience proprietary SNP markers. These SNPs were distributed evenly across the genome (Figs. 1 and 2). Of the 2000 SNP markers screened, 199 (9.95%) and 193 (9.65%) were polymorphic between the parental lines of doubled haploids derived from F_2 of crosses VL1043 × CM212 and VL121096 × CM202, respectively (Table 4). From here on, the two DHs derived from F_2 of crosses VL1043 × CM212 and VL121096 × CM202 shall be referred to as MP1 and MP2, respectively. Of the 199 and 193 polymorphic SNPs, a linkage map was constructed using the genotyping data of 164 and 132 polymorphic SNPs after excluding 35 and 61 SNPs showing segregation distortion (SD) and those with threshold LOD $\leq$ 2.5 and recombination frequency of 0.3 in MP1 and MP2, respectively. The linkage map length varied from 158.50 cM (LG 6) to 316.26 cM (LG 1) in MP1 (Table 5) and from 151.06 cM (LG 6) to 316.26 cM (LG 1) in MP2 (Table 5). The highest number of markers were mapped on to LG 1 (27 and 24) in MP1 and MP2, while the least number of markers were mapped on to LG 6 (10) in MP1 and LG 7 (10) in MP2. The total length of the linkage map spanned 2156.36 cM and 2100.18 cM of the genome with an average inter-marker distance of 21.56 cM and 21.00 cM in MP1 and MP2, respectively (Table 5).

Table 4

Number of polymorphic SNP markers detected between pair of selected parents in DH populations

Parent 1	Parent 2	Generation	Number of polymorphic SNPs	Number of markers with segregation distortion
VL1043	CM212	DHF_2	199	35
VL121096	CM202	DHF_2	193	61

Table 5

Polymorphic SNP markers assigned to each chromosome and their average distances

Chromosome	No. of SNPs markers		Map length (cM)		Average map distances (cM)	
	DHF_2 of VL1043 × CM212	DHF_2 of VL121096 × CM202	DHF_2 of VL1043 × CM212	DHF_2 of VL121096 × CM202	DHF_2 of VL1043 × CM212	DHF_2 of VL121096 × CM202
Ch 1	27	24	316.26	316.26	31.63	31.63
Ch 2	19	12	249.17	233.46	24.92	23.35
Ch 3	19	16	244.69	239.65	24.47	23.97
Ch 4	13	11	239.72	239.72	23.97	23.97
Ch 5	14	13	219.15	217.72	21.92	21.77
Ch 6	10	11	158.50	151.06	15.85	15.11
Ch 7	14	10	205.52	200.38	20.55	20.04
Ch 8	20	12	186.12	164.70	18.61	16.47
Ch 9	15	11	176.39	176.39	17.64	17.64
Ch 10	13	12	160.84	160.84	16.08	16.08
Total	164	132	2156.36	2100.18	21.56	21.00

Identification of QTL controlling FSR resistance

Detection of main effect QTL

The QTL controlling FSR resistance was detected in MP1 and MP2 by integrating genotyping and phenotyping data following Inclusive Composite Interval Mapping (ICIM) implemented using QTL IciM software version 4.1. Two QTL were detected in MP1 and MP2 during rabi 2019. The size effects of the four QTL ranged from 4.04 to 8.45. Similarly, in summer 2020, one and two QTL were detected in MP1 and MP2, respectively. The size effects of the three QTL ranged from 3.47 to 9.12. A combined QTL analysis was performed upon integrating the genotypic and phenotypic data of both rabi and kharif. The results indicated three QTL each in MP1 and MP2 (Table 6) (Figs. 3 and 4). The *per cent* variation explained by the four QTL ranged from 2.61 to 8.58. However, none of the QTL had a major effect on FSR resistance.

Table 6

QTLs detected for FSR resistance during individual seasons and combined over seasons

VL1043 × CM212 DHF_2								
Season	**Chromosome**	**Flanking markers**		**QTL Position (cM.)**	**Maximum LOD score**	**PVE (%)**	**Additive Genetic effect**	**QTL name**
		Left	**Right**					
Rabi, 2019	1	PHPL_GMT_30	PHPL_GMT_31	316.17	2.39	2.48	-0.16	qFSR_1_1
	5	PHPL_GMT_108	PHPL_GMT_109	216.72	2.47	4.91	-0.25	qFSR_5_1
Summer, 2020	10	PHPL_GMT_193	PHPL_GMT_194	83.60	2.07	3.47	-0.17	qFSR_10_1
Combined	1	PHPL_GMT_30	PHPL_GMT_31	316.17	3.12	2.61	-0.12	qFSR_1_1
	5	PHPL_GMT_93	PHPL_GMT_95	4.02	2.88	2.45	-0.12	qFSR_5_2
	10	PHPL_GMT_193	PHPL_GMT_194	82.80	3.93	3.40	-0.16	qFSR_10_1
VL121096 × CM202 DHF_2								
Rabi, 2019	6	PHPL_GMT_117	PHPL_GMT_119	122.48	1.75	6.35	-0.23	qFSR_6_2
	7	PHPL_GMT_138	PHPL_GMT_139	195.67	1.68	9.09	-0.33	qFSR_7_2
Summer, 2020	6	PHPL_GMT_117	PHPL_GMT_119	122.48	2.64	8.10	-0.32	qFSR_6_2
	7	PHPL_GMT_135	PHPL_GMT_137	165.67	3.12	9.12	0.34	qFSR_7_3
Combined	6	PHPL_GMT_117	PHPL_GMT_119	121.98	4.31	8.06	-0.27	qFSR_6_2
	7	PHPL_GMT_137	PHPL_GMT_138	166.67	4.43	8.58	0.28	qFSR_7_4
	10	PHPL_GMT_198	PHPL_GMT_200	134.00	2.96	5.66	-0.22	qFSR_10_1

Epistasis between FSR QTL regions located on the same chromosome

In the present study, epistatic QTL located on the same as well as those located on different chromosomes were detected. In MP1, two epistatic QTL contributing to FSR resistance were dispersed on chromosomes 3 and 10, positioned at 205.09 cM and 38.00 cM (Table 7 and Fig. 5). The *per cent* phenotypic variation explained by the di-QTL interactions located on the same chromosomes ranged from 2.83 to 4.27. Effects of additive × additive interaction ranged from − 0.44 to 0.26 (Table 7 and Fig. 5). One of the additive × additive interactions had a negative effect which can be fixed by developing inbred lines resistant to FSR. In MP2, one epistatic QTL located on the same chromosomes (LG 10) was detected with *per cent* phenotypic variance of 13.44 and LOD score of 8.32 (Table 8 and Fig. 6).

Table 7

Epistatic QTL located on the same chromosome in F_2 induced DH mapping population derived from VL1043 × CM212

QTL A				QTL B				LOD	Phe var (%)
Chromosome	Position (cM)	Left Marker	Right Marker	Chromosome	Position (cM)	Left Marker	Right Marker		
		On same chromosomes							
3	205.09	PHPL_GMT_70	PHPL_GMT_74	3	220.09	PHPL_GMT_74	PHPL_GMT_75	6.87	4.27
10	38.00	PHPL_GMT_186	PHPL_GMT_188	10	53	PHPL_GMT_189	PHPL_GMT_191	6.27	2.83
		On different chromosomes							
1	182.56	PHPL_GMT_19	PHPL_GMT_20	2	83.24	PHPL_GMT_40	PHPL_GMT_42	5.29	4.71
2	188.24	PHPL_GMT_52	PHPL_GMT_53	3	85.09	PHPL_GMT_65	PHPL_GMT_66	5.79	5.10
3	175.09	PHPL_GMT_70	PHPL_GMT_74	8	181.84	PHPL_GMT_162	PHPL_GMT_163	5.41	4.57
2	188.24	PHPL_GMT_52	PHPL_GMT_53	9	54.13	PHPL_GMT_165	PHPL_GMT_166	5.04	4.49
2	188.24	PHPL_GMT_52	PHPL_GMT_53	10	113	PHPL_GMT_195	PHPL_GMT_197	5.66	4.69

Table 8

Epistatic QTL located on the same chromosome in F_2 induced DH mapping population derived from VL121096 × CM202

QTL A				QTL B				LOD	Phe var (%)
Chromosome	Position (cM)	Left Marker	Right Marker	Chromosome	Position (cM)	Left Marker	Right Marker		
On the same chromosome									
10	23.00	PHPL_GMT_186	PHPL_GMT_187	10	143	PHPL_GMT_198	PHPL_GMT_200	8.32	13.4
On different chromosomes									
6	117.48	PHPL_GMT_117	PHPL_GMT_119	7	140.67	PHPL_GMT_135	PHPL_GMT_137	5.53	11.0
4	232.41	PHPL_GMT_89	PHPL_GMT_91	8	61.84	PHPL_GMT_145	PHPL_GMT_147	5.08	12.9
1	227.56	PHPL_GMT_23	PHPL_GMT_24	10	23	PHPL_GMT_186	PHPL_GMT_187	5.26	10.8
5	100.93	PHPL_GMT_100	PHPL_GMT_101	10	23	PHPL_GMT_186	PHPL_GMT_187	6.64	11.4
2	23.24	PHPL_GMT_34	PHPL_GMT_36	10	143	PHPL_GMT_198	PHPL_GMT_200	7.05	12.6
6	87.48	PHPL_GMT_116	PHPL_GMT_117	10	143	PHPL_GMT_198	PHPL_GMT_200	5.31	10.0

Epistasis between FSR resistance QTL regions located on different chromosomes

A total of five and six epistatic QTL present on different chromosomes were detected in MP1 and MP2, respectively (Tables 7 and 8) (Figs. 5 and 6). The *per cent* phenotypic variation explained by the QTL on different chromosomes ranged from 4.57 to 5.10 and 10.05 to 12.95 in MP1 and MP2, respectively. Effects of additive × additive interaction ranged from − 0.17 to -0.20 in MP1 and from − 0.30 to -0.49 in MP2.

Discussion

In maize breeding, the development and use of doubled haploid (DH) lines offer several advantages over conventionally derived inbred lines. The DH technology delivers 100% homozygous inbred lines in a minimum time period of two generations compared to 6 to 7 selfing generations in single seed descent (SSD) or pedigree methods. The rapid development of DH lines provide more reliable selection and enhanced genetic gains than lines obtained

through consecutive self-pollination because in DH lines, the whole genome has been duplicated and all its genetic loci are in homozygous status (Couto et al. 2019; Prasanna et al. 2012). Further, the faster product cycle times are important for countering emerging diseases and pests.

Four inbred lines with varying FSR response were used to develop two DH populations, which revealed highly significant differences among the progenies for disease reaction (Table 2). Expecting more variation, the F_2s of crosses VL1043 × CM212 and VL121096 × CM202 were subjected to DH production. The differential response of DH lines observed in this study might be due to the interaction of lines with the environment for disease expression. In both crosses, most DH lines belonged to the moderately susceptible response group and only one line was found highly resistant in DHs. This could be due to moderate resistance exhibited by parental genotypes CM212 and CM202. In DHF_2s of the cross VL1043 × CM212 cross, the number of resistant lines was more than DHF_2s of the cross VL121096 × CM202, reflecting on the appearance of transgressive segregants in it. In DHF_2s additional round of recombination must have contributed to an increased genetic variability (Bernardo, 2009; Couto et al., 2019). A priori identification of SNPs polymorphic between parents of the mapping population is essential to develop a linkage map and identify SNPs linked to genomic regions controlling FSR resistance. Though we screened 2000 SNP markers, only 199 and 193 markers were polymorphic between the parents VL1043 and CM212 and VL121096 and CM202, respectively. The level of polymorphism between parents of mapping populations depends on a number of factors such as the type of markers used, the extent of genetic diversity between the parents and the natural and human selection history of the parental lines (Menendez et al., 1997).

Construction of linkage map and QTL detection

The genetic linkage maps constructed will greatly help plant breeders in tagging and introgression of useful traits into different genetic background. Various efforts have been made in the construction of genetic linkage maps in maize employing different marker systems (Senior et al., 1996, Chin et al., 1996, Agrama et al., 1999, George *et al*., 2003, Zwonitzer et al., 2010, Jampatong et al., 2013, Wanlayaporn et al., 2013). However, in our study, the inter-marker distance was comparatively large and it is obviously due to fewer mapped markers possibly driven by low frequency and uneven distribution of recombination events (Sunitha, 2020).

The number, size effects, chromosomal locations, markers flanking the detected QTL differed with the mapping populations in this study. However, major effect QTL were not detected in both the populations and could be attributed to fewer polymorphic SNP markers used in this study. The use of a large number of polymorphic markers is expected to discover a major effect QTL. The QTL detected on a linkage group 10 (qFSR_10_1) was stable across two populations which could be used successfully in transferring resistance to FSR and annotation of this genomic region might result in the identification of useful genes for resistance.

Di-QTL epistasis

Epistasis is an interaction between alleles of two or more genetic loci in the genome (Carlborg and Halley, 2004: Phillips, 2008). The magnitude and direction of additive, additive × additive, additive × dominance and dominance × additive interaction QTL effects significantly influence the phenotype expression based on their dispersion between the parents used in the development of mapping populations. QTL mapping studies have provided more evidence for epistasis controlling yield and other important agronomic traits than classical biometrical genetic studies (Li, 1998). Furthermore, in the lines that have undergone selection, epistasis appears to be contributing to the expression of complex traits (Dudley and Johnson, 2009). Hence, it is important to assess the relative contribution of loci with main gene effects and those with significant epistasis towards the total genetic variation of quantitative traits for exploitation in plant breeding.

Several studies indicated the presence of epistasis for various traits in maize (Lamkey et al., 1995; Wolf and Hallauer, 1997; Lukens and Doebley, 1999). The additive × additive interaction effects were negative which indicated that the two epistatic loci with homozygous alleles from the resistant parents CM212 and CM202 could enhance FSR resistance. The importance of epistatic gene action has been adequately demonstrated in recent QTL mapping studies in the expression of complex traits (Ohno et al., 2000; Yang et al., 2007). However, the *per cent* phenotypic variation and magnitude of additive × additive effects of these QTL were not appreciably high enough for exploitation.

The presence of significant di-QTL interactions detected in MP1 and MP2 revealed that all epistatic interactions were resulted from interactions between loci with non-significant main effects. Rakesh (2018), Sunitha (2020) and Gazala (2021) also reported that epistatic interactions of QTL controlling late wilt resistance in maize were interactions between loci between non-significant main-effects. Similarly, Peng et al. (2011) also reported epistatic interactions of QTL controlling grain yield and kernel-related traits in maize with non-significant main effects.

Declarations

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Author contribution

HCL conceived the study. BMSB and HCL both conducted experiments, analysed and interpreted the results, and wrote the manuscript. MGM assisted in data analysis and manuscript preparation. NM supervised the phenotyping work.

Ethical approval

Not applicable

Consent to participate

Not applicable

Consent to publish

Not applicable

Competing interests

The author declare no competing intersts

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Figures

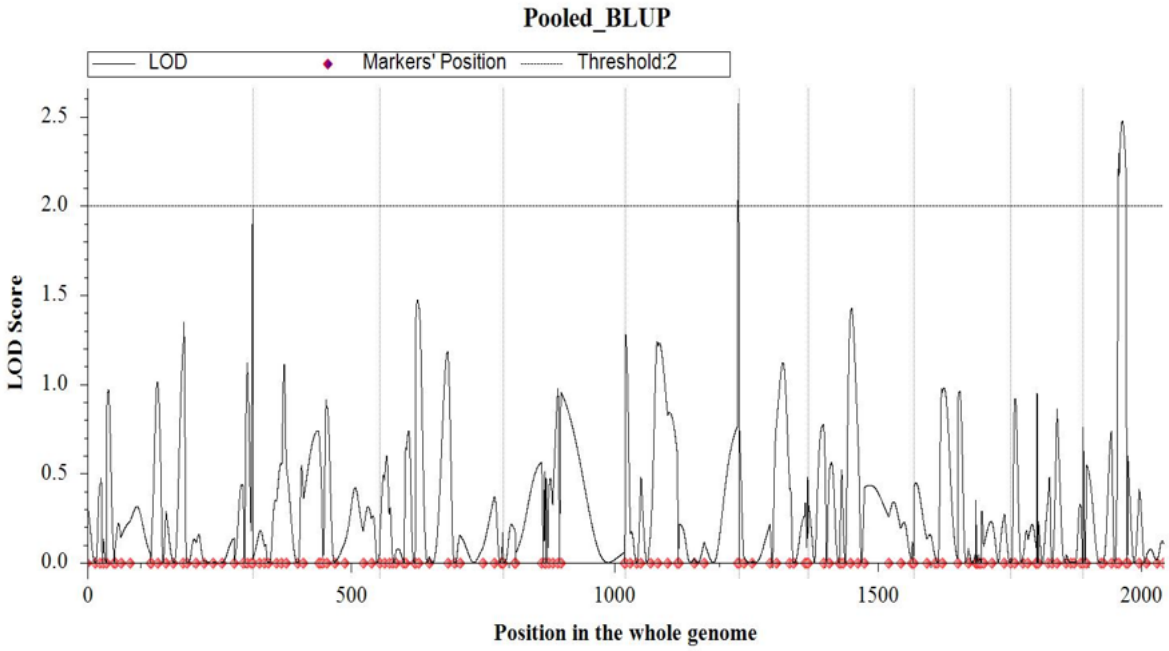

Figure 1

Genome-wide distribution of QTL controlling resistance to FSR disease detected in F_2 induced DH derived from the cross VL1043 × CM212

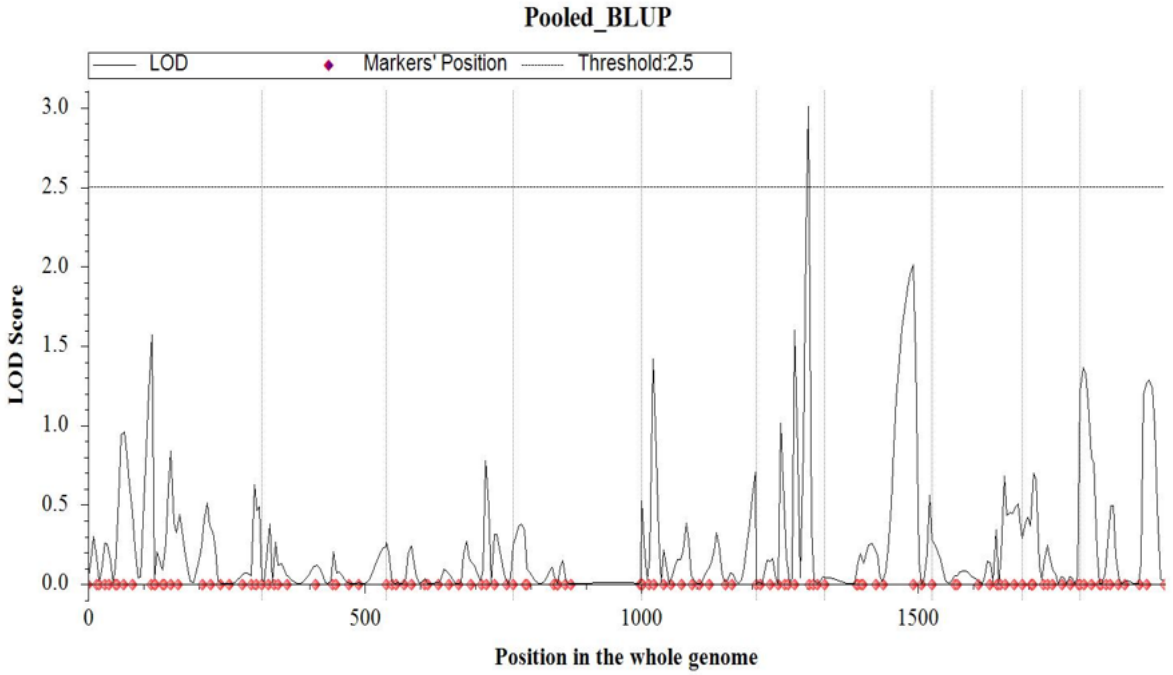

Figure 2

Genome-wide distribution of QTL controlling resistance to FSR disease in F_2 induced DH derived from the cross VL121096 × CM202

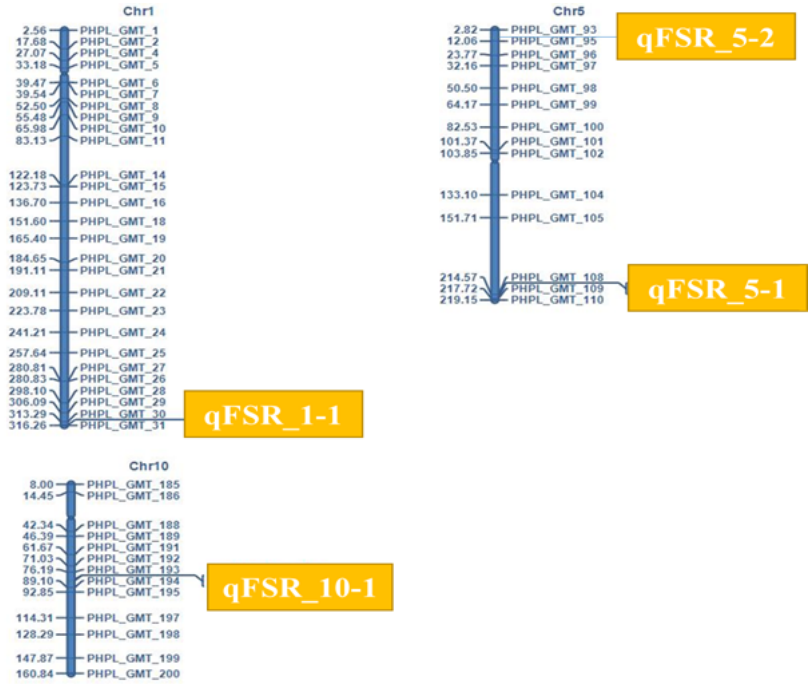

Figure 3

QTL identified in the F_2 induced DH population of cross VL1043 × CM212

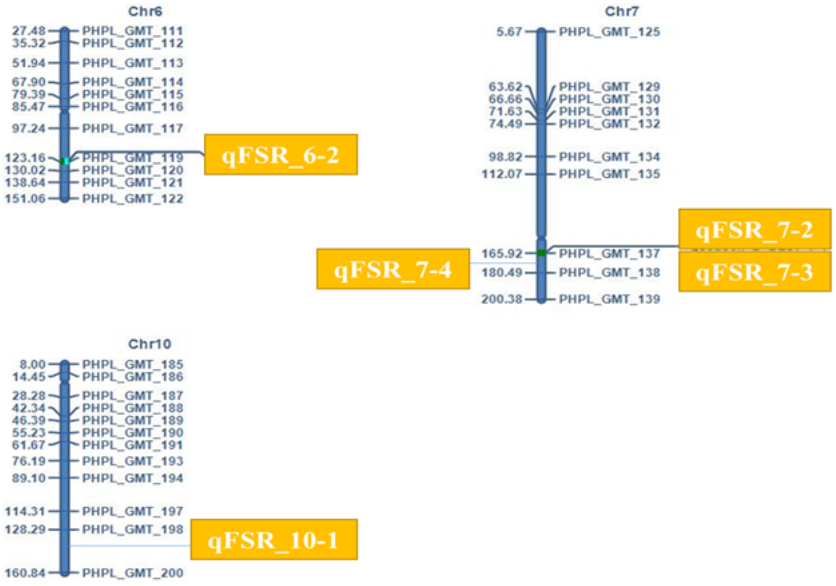

Figure 4

QTL identified in the F_2 induced DH population of cross VL121096 × CM202

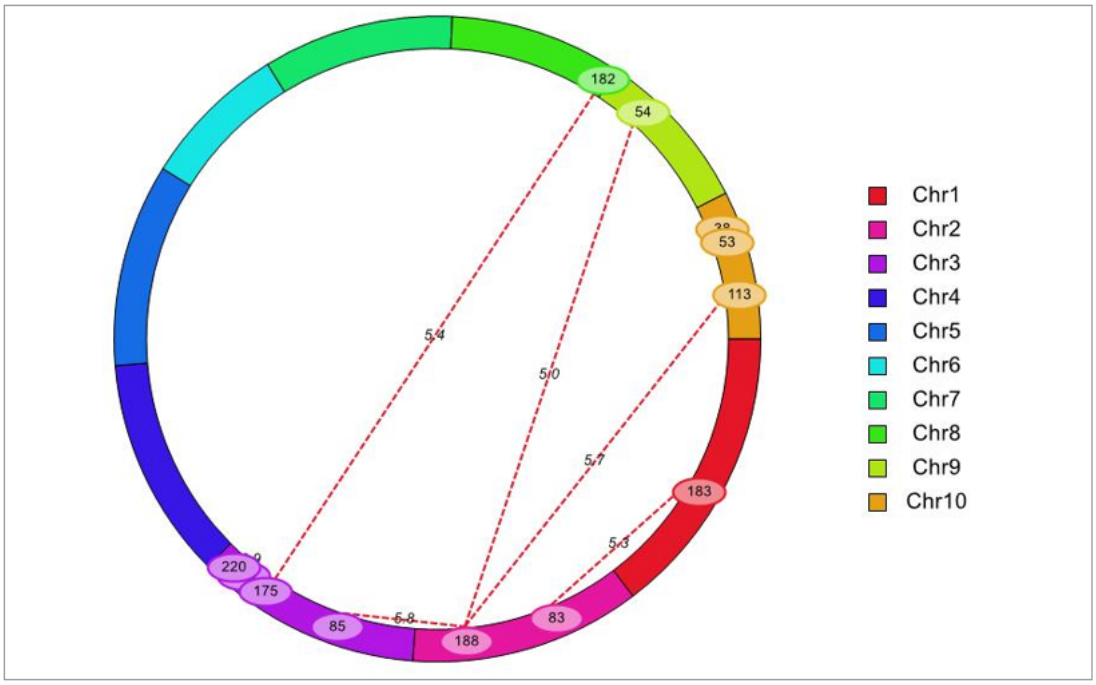

Figure 5

QTL showing epistatic interaction for resistance to FSR disease detected in F_2 induced DH mapping population derived from the cross VL1043 × CM212

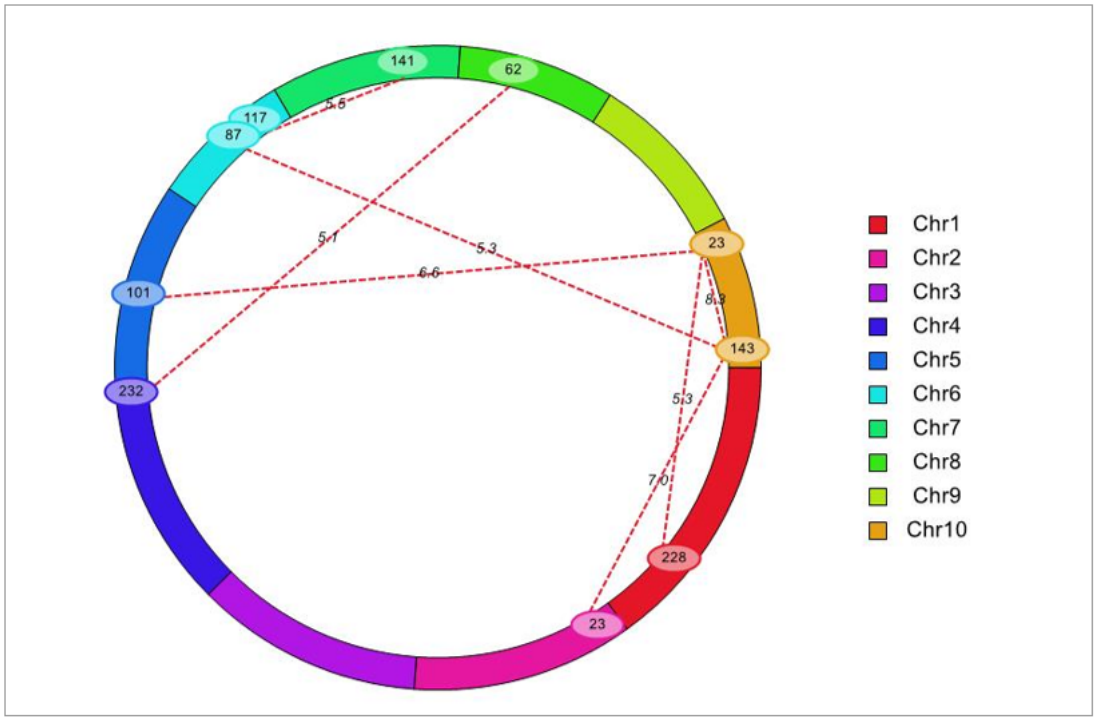

Figure 6

QTL showing epistatic interaction for resistance to FSR disease detected in F_2 induced DH mapping population derived from the cross VL121096 × CM202

Supplementary Files

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- Semanticrepresentation.png