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RESEARCH ARTICLE

Mapping of QTL for resistance to fusarium stalk rot (FSR) in tropical maize (*Zea mays* L.)

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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 developing 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 199 and 193 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 the rainy season of 2019 and winter 2019-20, respectively. The QTL identified in the linkage group 10 (qFSR_10_1) was common across two seasons in DHs derived from the cross VL1043 × CM212. Similarly, two QTL were identified for FSR resistance in DHs derived from the cross VL121096 × CM202 and one QTL (qFSR_6_2) was common. The QTL qFSR_10_1 was common in both the crosses. The position and effect of the QTL varied with the seasons. Seven di-QTL interactions were detected for FSR resistance in both DH populations.

Keywords Maize**,** Fusarium stalk rot, doubled haploid lines, QTL mapping, di-QTL interactions

Introduction

Globally, 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% in isolated maize-growing areas and 100% yield loss was reported in areas where water stress occurs after the flowering stage (Singh et al. 2012). The FSR generally occurs later in 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. Therefore, ears are left in the field. FSR was also reported to reduce 18.7% of cob weight and 11.2% of the 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). Stable sources of resistance to *F. verticilliodes* (Showkath Babu et al. 2020) have been reported in tropical maize germplasm. However, the resistance to FSR has been reported as complex with duplicate epistasis (Showkath Babu et al. 2020). Quantitative Trait Loci (QTL) mapping studies were conducted for tagging resistance to Fusarium ear rot in maize which indicated the quantitative nature of inheritance determined by small effect polygenes (Perez-

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Brito et al. 2001; Robertson Hoyt et al. 2006; Ding et al. 2008; Chen et al. 2012). Hence, direct selection for FSR resistance is likely to be less effective. However, the DNA markers could be employed as effective surrogates of such complex traits in maize, for which identification and validation of closely linked molecular markers are essential. QTL mapping has been widely employed to identify the genetic basis of target traits (Yang et al. 2020) using various biparental populations *viz*., $F_{2:3}$, backcross, recombinant inbred line (RIL) and doubled haploid (DH) populations.QTLs have been detected for Gibberella stalk rot (Pe et al. 1993; Yang et al. 2010; Chen et al. 2012; Zhang et al. 2012; Giomiet al. 2016; Chen et al. 2016), Anthracnose stalk rot (Jung et al. 1994), Fusarium ear rot (Perez-Brito et al. 2001; Ding et al. 2008; Maschietto et al. 2017; Ju et al. 2017), Charcoal ear rot (Rashid et al. 2021) and Fusarium stalk rot (Rashid et al. 2022). However, reports on the identification of QTLs for FSR disease resistance are scanty. Hence, an attempt has been made to identify QTLs for resistance to FSR employing a biparentally derived doubled haploid population.

Materials 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. The inbred CM212 (USA/Acc. No. 2132 (Alm.)-3-2-f-#-13-# -⊗b-####) is early maturing with yellow semi dent whereas CM202 is yellow dent and long duration inbred line and is a selection from C121. These inbred lines were procured from the Indian Institute of Maize Research, Ludhiana. The inbred lines were selected based on previous years' disease reactions from artificial disease screening against FSR. The disease rating of CM202 is 2.0 and that of CM212 is 2.5. These lines displayed high parental polymorphism as compared to other inbred lines assayed.

Development of doubled haploid lines

The susceptible inbred lines (VL 1043 and VL 121096) were crossed with resistant lines (CM212 and CM202) during the summer 2017 to develop two crosses *viz*., VL1043 × CM212 (MP_1) and VL121096 × CM202 (MP_2) 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°N, 76.82°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 by a male haploid inducer inbred line (Chaikam*et al.*2019). Each cross's cob had kernels with haploid and diploid genetic constitutions. The haploid kernels were identified and selected based on the dominant grain purple color marker gene (*R1-nj* marker). The kernels without any pigmentation were selfed or outcrossed ones, 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 the coleoptiles was cut off and submerged in 0.04 to 0.06% colchicine solution with DMSO for 8 to 12 hours to allow the uptake of colchicine. Later, the seedlings were washed thoroughly under tap water and planted in biodegradable Jiffy peat pellets (Jiffy Products S.L. Pvt. Ltd., Mirigama, Sri Lanka; http://www.jiffypot.com), in a shade house for recovery and hardening. Once the seedlings reached the three-leaf stage, they were transplanted to a DH nursery net house. Each plant was selfed, and the harvested cobs (D_0- doubled haploids from the first generation) were doubled haploids. The D_0 seeds were sown and advanced to D1 (doubled haploids from second generation) nursery with strict screening to screen rogue the haploids, off-types, and false positives. With the aforementioned protocol, it was possible to derive 280 and 94 DHF_2 lines (doubled haploid lines derived from F_2 plants) from VL1043 × CM212 (MP1) and VL121096 × CM202(MP2) crosses, respectively.

Field layout for characterization of doubled haploid lines for fusarium stalk rot resistance

The DHF_2s of both crosses and their respective parents *viz*., VL1043 (BLUP value: 7.47), VL121096 (BLUP value: 6.88), CM202 (BLUP value: 2.88) and CM212 (BLUP value: 3.33) as checks were evaluated in the Augmented design (Federer, 1961), and checks were repeated after every10^{th} 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 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 fusarium stalk rot

Disease screening was done 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.

Artificial inoculation

In the second internode of all the plants at 65 and 75 DAS, a 2 cm hole was made using a 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 packages and practices were followed except the spraying of fungicides after pathogen inoculation.

Phenotypic sample and data collection

For disease phenotyping, the stalks were split open before drying, *i.e.,* around 30 days after inoculation. Each of the individual plants in each line was examined for FSR severity and intensity using a 1-9 disease rating 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, the higher was the disease rating.

Genotyping of doubled haploid populations

The parents VL1043, VL121096, CM212 and CM202 were genotyped using Corteva Agri-Science Proprietary SNP markers employing Illumina Infinium XT assay. The polymorphic markers were used for genotyping in both the DH populations.

Statistical analysis of fusarium stalk rot response

The disease response data of individual plants on 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 program (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). The BLUP values were also used to calculate the descriptive statistical parameters like mean, range and standardized range, as per Sunderraj et al. (1972). Genotypic and phenotypic variances were estimated as per Lush (1945). The phenotypic and genotypic coefficient of variation was assessed as per Burton and DeVane (1953) and classified based on Robinson et al. (1949); heritability (broad sense) was calculated based on Lush (1945) and classified based on Robinson et al. (1949); genetic advance and genetic advance as percent of mean were assessed and classified based on Johnson et al. (1955). Skewness, the third-degree statistic and kurtosis, the fourth-degree statistic, were estimated following Snedecor and Cochran (1974). The SPSS software program was employed to study the distribution pattern of DH lines with respect to FSR disease reaction and Spearman's rank correlations for different environments were also calculated.

Linkage map construction and identification of QTL controlling resistance to Fusarium stalk rot

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 the QTL IcIM software program, version 4.1. A minimum threshold LOD score of 3.0 was set for linkage group determination. The significance of inter-marker recombination frequencies was converted into map distances using the Kosambi mapping function (Kosambi 1944). 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 F-test tested the significance of differences among marker classes (Fisher and Yates 1949). Significance or non-significance of the F-test indicates 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 by 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 a threshold LOD of 3.0 using QTL ICiMapping software version 4.0 (Wang et al. 2011).

Results

Phenotypic response of doubled haploid lines to fusarium stalk rot resistance

The parental lines CM212 and CM202 were moderately resistant to the Fusarium stalk rot whereas VL1043 and VL121096 were highly susceptible. 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 to be random. The analysis of variance revealed significant genetic differences among DH lines (Table 1).

The best linear unbiased predictors (BLUPs) were estimated since they simultaneously include the prediction of genetic effects and the estimation of genetic and non-genetic variance. It showed great potential and can be efficiently used in classifying the disease response and analyzing as well as interpreting results. The BLUP scores computed from each DH line were successfully employed in the classification of DHs into different disease response groups. A significantly positive correlation of BLUP scores between the rainy season of 2019 and winter 2019-20 of

Table 1. Analysis of variance of mean Fusarium stalk rot disease scores of doubled haploid 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		
	Rainy season of 2019 and Winter 2019-20		Rainy season of 2019 and Winter 2019-20		Rainy season of 2019	Winter 2019-20	Pooled	Rainy season of 2019	Winter 2019-20	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***
Spearman's Rank correlation					0.40***			0.61***		

*Significant at p = 0.05; **Significant at p = 0.01; ***Significant at p = 0.001

both the crosses indicated that the disease expression was comparatively stable across seasons and hence the data from two seasons were pooled for further analysis (Table 1).

In both crosses (VL1043 × CM212 and VL121096 × CM202), the majority of DH lines belonged to a moderately susceptible response group. The genetic variance (V_G) was higher among DHF_2 lines in VL1043 × CM212 cross, while it was comparable with DHF_{2s} of VL121096 × CM202 (Table 2). Within each population, the lines had similar estimates of residual variance (V_R). In the cross VL121096 × CM202, the estimates of PCV and GCV were comparable with DHF_2 in VL1043 × CM212 cross. In VL1043 × CM212, higher estimates of broad-sense heritability and expected GAM were observed in DHF_2 (Table 2). In the cross VL121096 × CM202, the broad-sense heritability and expected GAM were comparable with VL1043 × CM212. The frequency distribution pattern of DH populations for Fusarium stalk rot was positively skewed and platykurtic in rainy season of 2019, winter 2019-20 and pooled over seasons in F_2 induced DHs of VL121096 × CM202 crosses, respectively (Fig. 2). While in rainy seasonof2019, winter 2019-20 and pooled over seasons DHF_2s of VL1043 × CM212 cross (Fig. 1) exhibited leptokurtic distribution (> 3.0).

The SNP markers were distributed evenly across the genome (Figs. 3 and 4). 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. 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 ≤ 2.5 and recombination frequency of 0.3 in DHs derived from F_2 of crosses VL1043 × CM212 (MP_1) and VL121096 × CM202 (MP_2), respectively. The linkage map length varied from 158.50 cM (LG 6) to 316.26 cM (LG 1) in MP1 and from 151.06 cM (LG 6) to 316.26 cM (LG 1) in MP2. The highest number of markers were mapped onto LG 1 (27 and 24) in MP1 and MP2, while the least number of markers were mapped onto 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.

Detection of main effect QTL controlling Fusarium stalk rot resistance

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 each were detected in MP1 and MP2 during 2019 (Table 3). The per cent phenotypic variation explained by these QTL ranged from 2.48 (qFSR_1_1) to 9.09 (qFSR_7_2). Similarly, in winter 2019-20, one and two QTL were detected in MP1 and MP2, respectively. The range in per cent phenotypic variation explained by these QTL was from 3.47 (qFSR_10_1) to 9.12 (qFSR_7_3). A combined QTL analysis was performed upon integrating the genotypic and phenotypic data of both the rainy and winter seasons. The results indicated the presence of three QTLs each in MP1 and MP2 (Table 4)

Table 2. Estimates of genetic components in maize doubled haploids induced from F_2 of crosses VL1043 × CM212 and VL121096 × CM202 for Fusarium stalk rot resistance

Genetic parameters	DHF_2 of VL1043 × CM212			DHF_2 of VL121096 × CM202		
	Rainy season of 2019	Winter 2019-20	Pooled	Rainy season of 2019	Rainy season of 2019	Pooled
Mean	4.37	4.51	4.44	4.42	4.50	4.46
Range	2.20 - 8.98	2.33 - 7.98	2.79 - 8.48	3.11 - 7.30	1.74 - 7.71	2.42 - 7.51
Standardized range	1.55	1.25	1.28	0.95	1.33	1.14
Genetic variance (V_G)	0.60	0.50	0.47	0.48	0.78	0.59
Residual variance (V_R)	0.04	0.07	0.02	0.11	0.08	0.04
Phenotypic coefficient of variation (PCV)	18.26	16.69	15.70	17.33	20.62	17.84
Genotypic coefficient of variation (GCV)	17.68	15.63	15.38	15.63	19.64	17.27
Heritability (H^2)	0.94	0.88	0.96	0.81	0.91	0.94
Genetic advance (GA)	1.54	1.36	1.38	1.28	1.74	1.54
Predicted genetic advance as % mean at 5% selection intensity (GAM)	35.26	30.15	31.02	29.02	38.54	34.43
Skewness	1.83	1.26	1.97	1.39	0.91	1.35
Kurtosis	5.96	3.28	6.26	2.54	2.50	2.96

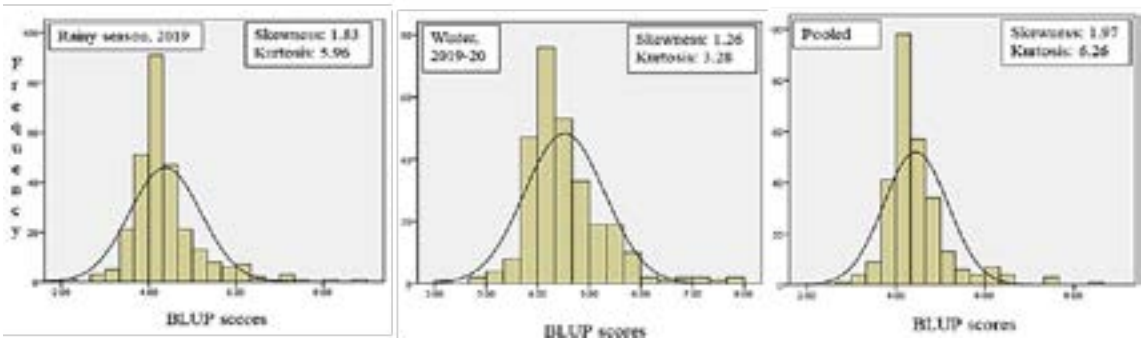

Fig. 1. Frequency distribution for Fusarium stalk rot disease response in double haploids derived from F_2 of VL1043 × CM212

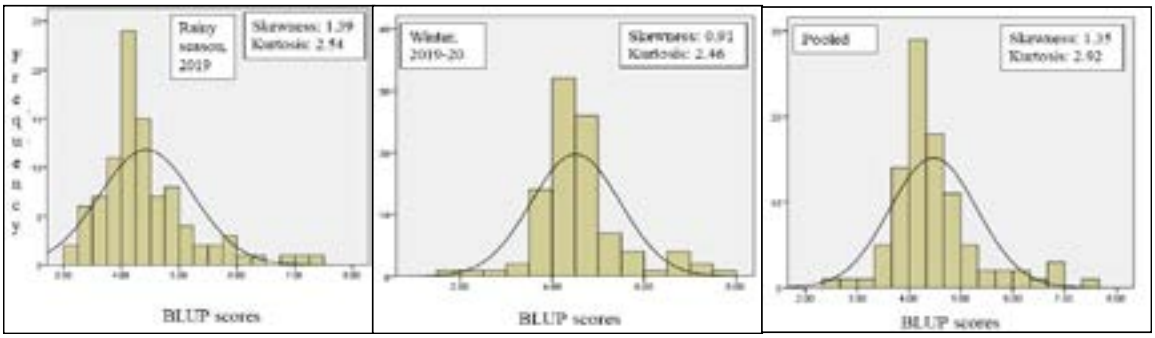

Fig. 2. Frequency distribution for Fusarium stalk rot disease response in doubled haploids derived from F_2 of VL121096 × CM202

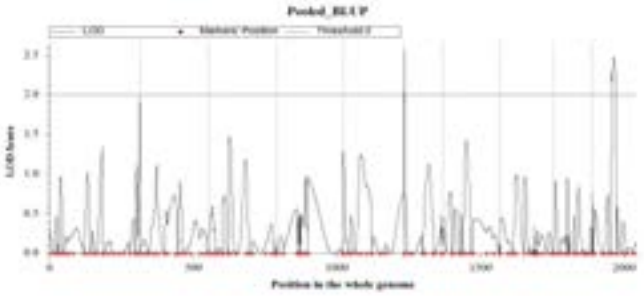

Fig. 3. Genome-wide distribution of QTL controlling resistance to Fusarium stalk rot disease detected in F_2-induced doubled haploids derived from the cross VL1043 × CM212

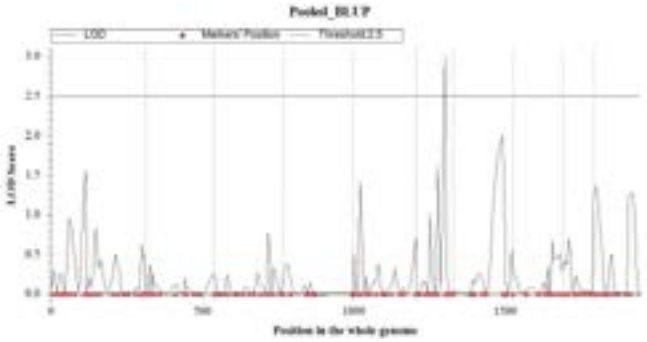

Fig. 4. Genome-wide distribution of QTL controlling resistance to Fusarium stalk rot disease in F_2-induced doubled haploids derived from the cross VL121096 × CM202

(Figs. 3 and 4). The % phenotypic variation explained by the six QTL ranged from 2.45 (qFSR_5_2) to 9.12 (qFSR_7_3). However, the QTLqFSR_7_2 and qFSR_7_3 recorded a near major effect on FSR resistance with phenotypic variation explained as 9.09% and 9.12 %, respectively.

Epistasis between Fusarium stalk rot 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 5 and Fig. 7). The % 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 5 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 chromosome (LG 10) was detected with %phenotypic variance of 13.44 and a LOD score of 8.32 (Table 5 and Fig. 6).

Epistasis between Fusarium stalk rot 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

Table 3. QTLs detected for Fusarium stalk rot resistance during individual seasons and combined over seasons

DHF_{2S} of the cross VL1043 × CM212									
Season	Chromosome	Flanking markers		QTL Position (cM.)	Maximum LOD score	PVE (%)	Additive Genetic effect	Donor FSR allele	QTL name
		Left	Right						
Rainy season, 2019	1	PHPL_GMT_30	PHPL_GMT_31	316.17	2.39	2.48	-0.16	CM 212	qFSR_1_1
	5	PHPL_GMT_108	PHPL_GMT_109	216.72	2.47	4.91	-0.25	CM 212	qFSR_5_1
Winter, 2019-20	10	PHPL_GMT_193	PHPL_GMT_194	83.60	2.07	3.47	-0.17	CM 212	qFSR_10_1
Combined	1	PHPL_GMT_30	PHPL_GMT_31	316.17	3.12	2.61	-0.12	CM 212	qFSR_1_1
	5	PHPL_GMT_93	PHPL_GMT_95	4.02	2.88	2.45	-0.12	CM 212	qFSR_5_2
	10	PHPL_GMT_193	PHPL_GMT_194	82.80	3.93	3.40	-0.16	CM 212	qFSR_10_1
DHF_{2S} of the cross VL121096 × CM202									
Rainy season, 2019	6	PHPL_GMT_117	PHPL_GMT_119	122.48	1.75	6.35	-0.23	CM 202	qFSR_6_2
	7	PHPL_GMT_138	PHPL_GMT_139	195.67	1.68	9.09	-0.33	CM 202	qFSR_7_2
Winter, 2019-20	6	PHPL_GMT_117	PHPL_GMT_119	122.48	2.64	8.10	-0.32	CM 202	qFSR_6_2
	7	PHPL_GMT_135	PHPL_GMT_137	165.67	3.12	9.12	0.34	VL121096	qFSR_7_3
Combined	6	PHPL_GMT_117	PHPL_GMT_119	121.98	4.31	8.06	-0.27	CM 202	qFSR_6_2
	7	PHPL_GMT_137	PHPL_GMT_138	166.67	4.43	8.58	0.28	VL121096	qFSR_7_4
	10	PHPL_GMT_198	PHPL_GMT_200	134.00	2.96	5.66	-0.22	CM 202	qFSR_10_1

Table 4. Epistatic QTL located on the same and different chromosome(s) in F_2 induced doubled haploid mapping population derived from VL1043 × CM212

QTL A				QTL B				LOD	Phenotypic variance (%)	Additive × Additive effect
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	-0.44
10	38.00	PHPL_GMT_186	PHPL_GMT_188	10	53	PHPL_GMT_189	PHPL_GMT_191	6.27	2.83	0.26
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	-0.18
2	188.24	PHPL_GMT_52	PHPL_GMT_53	3	85.09	PHPL_GMT_65	PHPL_GMT_66	5.79	5.10	-0.20
3	175.09	PHPL_GMT_70	PHPL_GMT_74	8	181.84	PHPL_GMT_162	PHPL_GMT_163	5.41	4.57	-0.18
2	188.24	PHPL_GMT_52	PHPL_GMT_53	9	54.13	PHPL_GMT_165	PHPL_GMT_166	5.04	4.49	-0.17
2	188.24	PHPL_GMT_52	PHPL_GMT_53	10	113	PHPL_GMT_195	PHPL_GMT_197	5.66	4.69	-0.18

Table 5. Epistatic QTL located on the same and different chromosome(s) of F_2 induced doubled haploid mapping population derived from VL121096 × CM202

QTL A				QTL B				LOD	Phenotypic variance (%)	Additive × Additive effect
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	13.44	-0.36
On different chromosomes										
6	117.48	PHPL_GMT_117	PHPL_GMT_119	7	140.67	PHPL_GMT_135	PHPL_GMT_137	5.53	11.02	-0.35
4	232.41	PHPL_GMT_89	PHPL_GMT_91	8	61.84	PHPL_GMT_145	PHPL_GMT_147	5.08	12.95	-0.35
1	227.56	PHPL_GMT_23	PHPL_GMT_24	10	23	PHPL_GMT_186	PHPL_GMT_187	5.26	10.82	0.40
5	100.93	PHPL_GMT_100	PHPL_GMT_101	10	23	PHPL_GMT_186	PHPL_GMT_187	6.64	11.46	-0.49
2	23.24	PHPL_GMT_34	PHPL_GMT_36	10	143	PHPL_GMT_198	PHPL_GMT_200	7.05	12.65	-0.35
6	87.48	PHPL_GMT_116	PHPL_GMT_117	10	143	PHPL_GMT_198	PHPL_GMT_200	5.31	10.05	-0.30

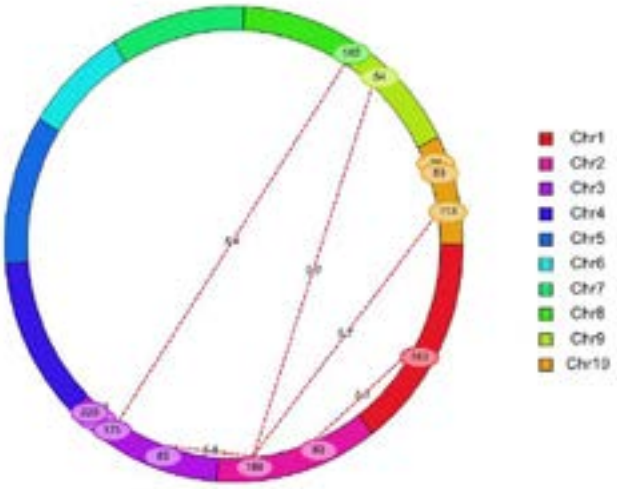

Fig. 5. QTL showing epistatic interaction for resistance to Fusarium stalk rot disease detected inF_2 induced doubled haploid mapping population derived from a cross VL1043 × CM212

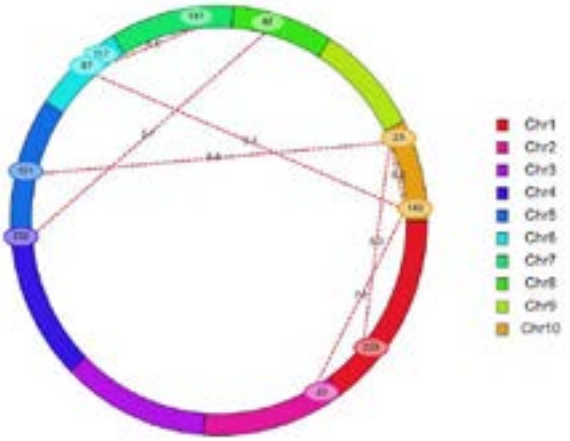

Fig. 6. QTL showing epistatic interaction for resistance to Fusarium stalk rot disease detected in F_2 induced doubled haploid mapping population derived from a cross VL121096 × CM202

(Tables 4 and 5) (Fig. 5). The % phenotypic variation explained by the QTL on different chromosomes ranged from 4.49 to 5.10 and 10.05 to 12.95 in MP1 and MP2, respectively. Effects of the additive × additive interaction ranged from -0.17 to -0.20 in MP1 and from -0.30 to -0.49 in MP2.

Discussion

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. The differential response of DH lines observed in this study might be due to the independent segregaton in the F_2 and also 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 to be 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, the number of resistant lines was more than DHF_2s of the cross VL121096 × CM202, reflecting on its appearance of transgressive segregants. In DHF_2s, an additional round of recombination must have contributed to an increased genetic variability (Bernardo, 2009; Couto et al. 2019). Genetic variation between DH lines was detected since the parents had different alleles controlling FSR resistance. It is assumed that a random sample of gametes during the production of a DH population resulted in this wide genetic variation for disease reaction (Couto et al. 2019). The genetical variation between DH lines gives the estimate of the additive component for FSR. The genetic variance (V_G) was higher between DHF_2 lines in VL1043 × CM212 cross, while it was comparable with DHF_{2s} in VL121096 × CM202. The change in variance is suggestive of linkage disequilibrium. The variances of the DHF_2 were larger, and this reflects higher proportions of extreme genotypes in the DHF_2 at both ends of the phenotypic distribution in that cross. Heritability guides the breeder, indicating the effectiveness and efficiency of selection in plant breeding. Higher heritability and expected GAM together indicated the effectiveness of phenotype-based selection for improving FSR disease resistance. In the rainy season of 2019, winter 2019-20 and pooled over seasons, F_2-induced DHs of VL1043 × CM212 and VL121096 × CM202 crosses exhibited a positively skewed and platykurtic distribution which indicated a larger number of genes having decreasing effects and involvement of dominance based complementary interaction. While, in rainy season of 2019, winter 2019-20 and pooled over seasons DHF_{2s} of the cross VL1043 × CM212 exhibited leptokurtic distribution (> 3.0) which suggested the involvement of fewer number of genes in the FSR disease expression.

A prior 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. The level of polymorphism between parents of mapping populations in this study was a bit less (199 and 193 of 2000 markers used), which might be because the extent of genetic diversity between the parents was less as the parental lines CM212 and CM202 were extensively used in maize breeding programs till recently (Menendez et al. 1997).

Construction of linkage maps and QTL detection

The genetic linkage maps constructed will greatly help plant breeders tag and introduce 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; Giomi et al. 2021). 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 et al. 2022).

The number, size effects, chromosomal locations, and markers flanking the detected QTL differed with the mapping populations in this study. Quantitative trait loci were mapped for FSR on chromosomes 1, 5 and 10 in MP1 but all with minor effects. Similarly, Giomi et al. (2021), Rashid et al. 2021 and Feng et al. (2022) also reported QTLs on these chromosomes.Liu *et al.* (2021) reported QTL in bins 7.02, 7.04, 10.03, and 10.04, which explained variations with a range from 1.39% to 2.04%. These results implied that minor QTL with small effects controlled FSR resistance in maize, and highly influenced by the genetic background of the populations studied (Maschietto et al. 2017; Lanubile et al. 2017; Liu et al. 2021). In DHF_2s of the cross VL121096 × CM202 (MP2), the QTL detected showed near major effects in both the populations (qFSR_7_2, qFSR_6_2, qFSR_7_3, and qFSR_7_4). Several others also detected major effect QTL for resistance to Fusarium ear rot on chromosomes 6 and 7 as in present study (Perez-Brito et al. 2001; Robertson-Hoyt et al. 2006; Ding et al. 2008; Chen et al. 2012; Maschiettoet al. 2017; Giomiet al. 2021; Rashid et al. 2022; Feng et al. 2022). Resistant parent CM212 contributed the favourable alleles for QTLs detected in the cross VL1043 × CM212. While, in VL121096 × CM202 favourable alleles for QTLs were contributed by resistant parent CM202 except for qFSR_7_3 detected in winter, 2019-20 and q_FSR_7_4 detected in combined QTL analysis. 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 (Feng et al. 2022).

Di-QTL epistasis

Epistasis is an interaction between alleles of two or more genetic loci in the genome (Carlborg and Haley 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, assessing 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 is important.

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 mostly 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; Giomi et al. 2021). However, these QTL's % phenotypic variation and magnitude of additive × additive effects were not appreciably high enough for exploitation.

The presence of significant di-QTL interactions detected in MP1 and MP2 revealed that all epistatic interactions resulted from interactions between loci with non-significant main effects. Rakesh et al. (2022) and Sunitha et al. (2022) also reported that epistatic interactions of QTL controlling late wilt resistance in maize were interactions between loci with 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.

Authors' contribution

Conceptualization of research (HCL); Designing of the experiments (HCL, NM, BMSB); Contribution of experimental materials (HCL); Execution of field/lab experiments and data collection (HCL, MBSB, NM); Analysis of data and interpretation (HCL, BMSB, MGM); Preparation of manuscript (HCL, BMSB, MGM).

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