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Discovery of genomic regions associated with resistance to late wilt disease caused by *Harpophora maydis (Samra, Sabet and Hing)* in maize (*Zea mays* L.)

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Abstract

Late wilt disease (LWD) caused by *Harpophora maydis* (Samra, Sabet and Hing) is emerging as major production constraint in maize across the world. As a prelude to develop maize hybrid resistance to LWD, genetic basis of resistance was investigated. Two $F_{2:3}$ mapping populations (derived from CV156670 × 414-33 (P-1) and CV156670 × CV143587 (P-2)) were challenged with LWD at two locations (Kallinayakanahalli and Muppadighatta) during 2017 post-rainy season. A wider range of LWD scores was observed at both locations in both the populations. LWD response was influenced by significant genotype × location interaction. Six and 56 $F_{2:3}$ progeny families showed resistance level better than resistant parent. A total of 150 and 199 polymorphic single nucleotide polymorphism markers were used to genotype P-1 and P-2, respectively. Inclusive composite interval mapping was performed to detect significant Quantitative Trait Loci (QTL), QTL × QTL, QTL × location interaction effects. Three major and four minor QTL controlling LWD resistance were detected on chromosome-1. The position and effect of the QTL varied with the location. Significant di-QTL interactions involving QTL (with significant and/or non-significant effects) located within and between all the ten chromosomes were detected. Five of the seven detected QTL showed significant QTL × location interaction. Though two major QTL (*q-lw-1.5* and *q-lw-1.6*) with lower Q×L interaction effects could be considered as stable, their phenotypic variance is not large enough to deploy them in Marker Assisted Selection (MAS). However, these QTL are of paramount importance in accumulating positive alleles for LWD resistance breeding.

Keywords Late wilt disease · Mapping · QTL × QTL interaction · QTL × Location interaction

Introduction

Maize productivity in India is constrained by several prevalent and emerging insect pests and diseases. Of these, post-flowering stalk rot (PFSR) is a complex disease caused by three pathogens, namely, *Fusarium moniliforme* J. (Sheld), *Macrophomina phaseolina* (Tassi) Goid and *Harpophora maydis* (Samra, Sabet and Hing) (Khokhar et al. 2014).

Stalk rot caused exclusively by seed-borne and soil-borne pathogen *H. maydis* causes premature wilting symptoms at post-flowering stage, a condition known as late wilt disease (LWD). *Harpophora maydis* is similar to *Harpophora* anamorphic states of Gaeumannomyces species in culture. *Harpophora* spp. mycelia are characterized olivaceous brown with radiating hyphae, and cylindrical conidia with curved borne in slimy heads (Gams, 2000). Older hyphae are heavily pigmented, younger hyphae are nearly hyaline and phialides are intermediate in pigmentation (Gams, 2000; Saleh and Leslie, 2004). Typical symptoms of LWD include pre-mature wilting of leaves, discoloration of the stalk followed by stalk tissue disintegration and fibrousness in advanced stages (Samra et al. 1963; Ramana et al. 1997; Johal et al. 2004). Upon severe infection, pathogen colonizes in the kernels causing seed rot and early stage damping-off (El-Shafey and Claflin 1999). Although it is a

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weakly competitive saprophyte, the production of sclerotia in infested host debris ensures its long-term survival (Sabet et al. 1970; Johal et al. 2004). It is also known to infest cotton, lupine, green foxtail and watermelon in addition to corn (Dor and Degani, 2019). The Egyptian, Indian and Hungarian isolates have been reported to differ in their morphology, pathogenicity and route of infection (Warren 1983; Degani et al. 2019).

LWD has been reported in more than 10 maize growing countries (Chalkley, 2016), with significant economic losses in Egypt (Sabet et al. 1961), India (Payak et al. 1970; Sunitha et al. 2020), Spain and Portugal (Molinero-Ruiz et al. 2010) and Israel (Drori et al. 2013; Degani et al. 2019). It is opined that dry environments like India, Egypt and Iberian peninsula maize growing regions are more prone to late wilt infection (Ortiz-Bustos et al. 2019) in the context of increased temperatures due to climate change. Based on survey, economic losses of 40% have been reported in Egypt (Labib et al. 1975; Galal et al. 1979), up to 100% in Israel (Degani et al. 2019) and up to 51% in India (Johal et al. 2004) due to LWD. However, 3.5 to 38.4% loss of grain yield attributable to soil inoculation by *H. maydis* has been reported based on an empirical study using a limited number of genotypes in Egypt (El-Naggarr et al. 2015). Very recently, we have reported 5.8 to 44.2% grain yield losses attributable to LWD by creating artificial epiphytotic condition through injecting *H. maydis* inoculum into the stem internode (Sunitha et al. 2020) in India. It is considered as an endemic disease in maize growing areas (Degani and Cercina, 2014). Despite increasing reports from Israel and Egypt in the last 10 years, there are only a few reports on the incidence of late wilt disease in India. It was identified as threat at Hyderabad, Pantnagar and Rajasthan (Payak et al. 1970; Singh and Siradhana 1986); Johal et al. (2004) reported 70% incidence and economic losses up to 51% in India. Even though this pathogen was identified 30 years back in India, further reports on its incidence and damage were very limited. However, several maize researchers have reported its potential of causing endemic from last one decade (Shekhar et al. 2010; Biradar et al. 2020).

Genetic intervention is regarded as eco-friendly and cost-effective option to mitigate losses caused by LWD (El-Shafey et al. 1988; Zeller et al. 2000). As a prelude to genetic intervention, stable sources of resistance to *H. maydis* (Satyanarayana 1995; Shekhar et al. 2010; Rakesh et al. 2016b; Aruna 2017; Biradar et al. 2020; Kamara et al. 2021) have been identified. The inheritance of resistance to LWD has been reported as complex with significant genotype × environment interaction (Shekhar et al. 2010; Rakesh 2016; Aruna 2017; Biradar 2019). Hence, direct selection for LWD resistance is likely to be less effective. Nevertheless, DNA markers could be used as effective surrogates of such traits in maize for which a priori identification and validation of closely linked markers are essential. As a step towards this, Quantitative Trait Loci (QTL) conferring LWD tolerance (Rakesh 2016) on chromosomes 1, 3, 5, 7 and 10 have been detected. The objectives of our study were to (i) validate the QTL detected by Rakesh (2016), (ii) to detect and estimate size effects of new LWD resistance governing QTL, if any in two different genetic backgrounds, and (iii) detect and estimate size effects of their interaction with two spatial environments.

Material and methods

Basic genetic material and development of mapping populations

The basic genetic material consisting of one LWD-resistant inbred line, CV156670 and two LWD susceptible inbred lines, 414-33 and CV143587, developed and being maintained at Bayer Crop Science (erstwhile Monsanto India Ltd.) were used as parents to develop two connected mapping populations. For intellectual property protection reasons, the pedigree of these inbreds is not included. The two F_1's (available with Bayer Crop Science), CV156670 × 414-33 and CV156670 × CV143587 were selfed during 2017 summer season to derive F_2 populations. Two F_2 populations derived from connected crosses CV156670 × 414-33 and CV156670 × CV143587 were selfed during 2017 rainy season. These $F_{2:3}$ populations (300 progeny families each) constituted inter-connected mapping populations to detect QTL controlling LWD resistance.

Phenotyping mapping populations for responses to LWD

Field experimentation

The mapping populations were evaluated at two locations, Kallinayakanahalli (KNH) 13.464013°N, 77.519178°E and Muppadighatta (MPG) 13.241161°N, 77.483637°E. 300 $F_{2:3}$ progeny families each from two F_2 populations, parental inbred lines and checks (susceptible and resistant check) were sown in single rows of 4-m length with 0.6-m spacing between rows and 0.2-m spacing between plants in two replications following randomized complete block design (RCBD) during 2017 post-rainy season. Recommended package of practices like fertilizer (N and P) application in intervals, earthing-up at knee-height stage and regular weeding were followed to establish good crop stand till artificial inoculation. The $F_{2:3}$ progeny families

were screened for responses to LWD using artificial inoculation. Mass multiplication and inoculum preparation has been followed as per the protocol given by Rakesh et al. (2016a).

Artificial inoculation of H. maydis inoculum

Isolation and mass multiplication of *H. maydis* were done as per Shekhar and Kumar (2012) modified by Rakesh et al. (2016a). Naturally infested LWD stalk specimens collected from commercial maize production fields were used for isolation of pathogens and inoculum preparation. Pure cultured isolate characteristics were compared with reported morphology and fruiting body characteristics of *H. maydis* (Gams, 2000). Similarity with spore characters reported by Rakesh (2016), Aruna (2017) and Biradar et al. (2020) was considered. Pathogenicity test based on Koch's postulates were proved to confirm the pathogen used for inoculation. The inoculum concentration was assessed using hemocytometer and was adjusted to $4 \times 10^6$ spore ml^{-1}. A hole was made at the second internode on the stem of plants using stem jabbers, and 2 ml of the *H. maydis* was injected to the holes using a syringe twice at 45 and 55 days after sowing (DAS). From each progeny family, 20 plants were inoculated. All the recommended production practices were followed except for the application of potassium fertilisers and fungicides to maintain the plants after inoculation.

Sampling and data recording

The stalks of the artificially inoculated plants were split open at 110 DAS to examine the typical symptoms of LWD. Disease symptoms were manifested by discoloration, disintegration and fibrousness at the inoculated internode. Based on the coverage of disease symptom within an internode and its spread across internodes, the data on LWD severity was recorded on all the inoculated plants using modified 1–9 scale given in Supplementary table 1 (Rakesh et al. 2016a).
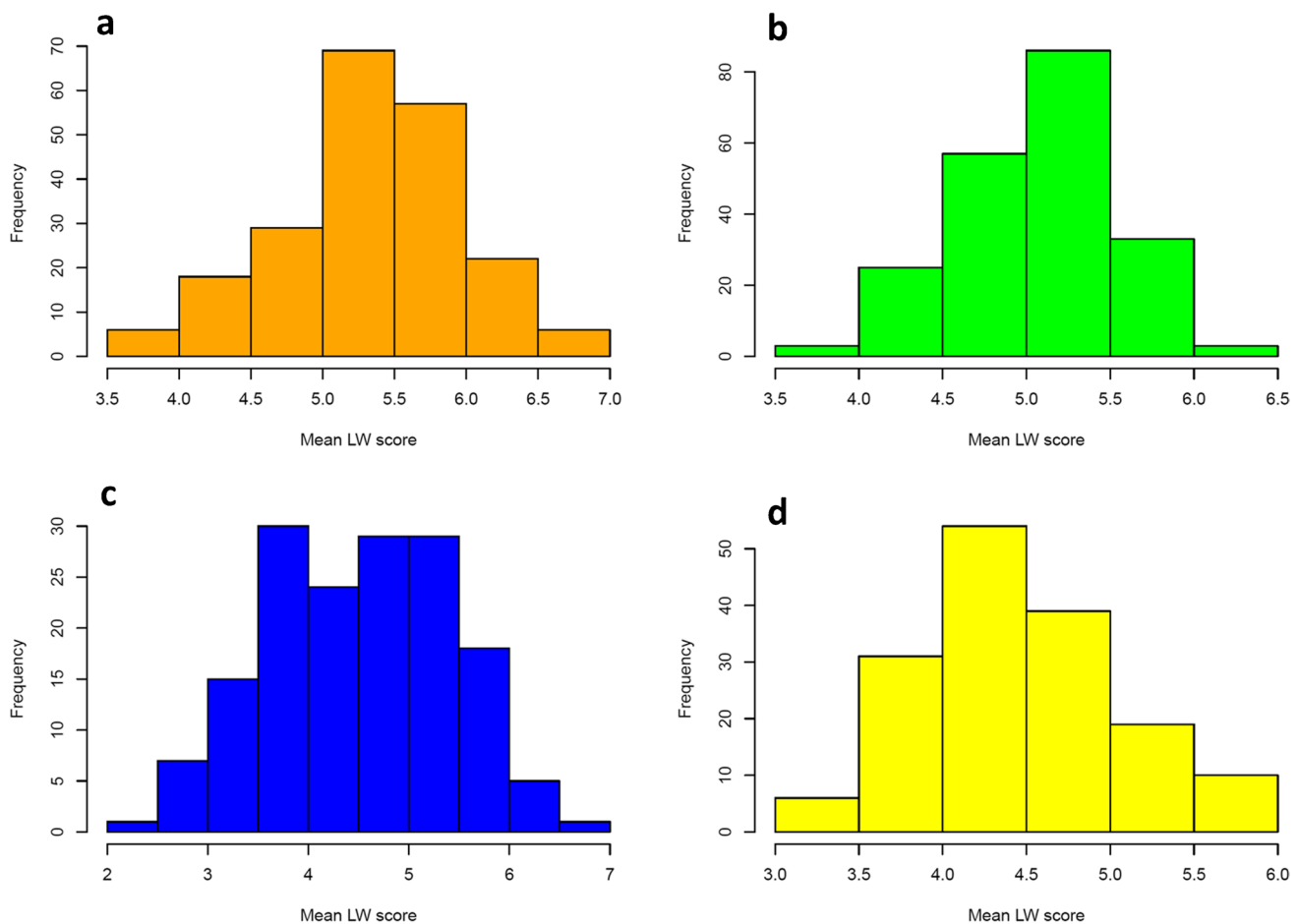
Fig. 1 Frequency distribution for responses to LWD of $F_{2:3}$ populations: **a** P-1 at KNH (Wilk's statistic = 0.988; $P = 0.087$). **b** P-1 at MPG (Wilk's statistic = 0.993; $P = 0.563$). **c** P-2 at KNH (Wilk's statistic = 0.987; $P = 0.177$). **d** P-2 at MPG (Wilk's statistic = 0.984; $P = 0.069$)
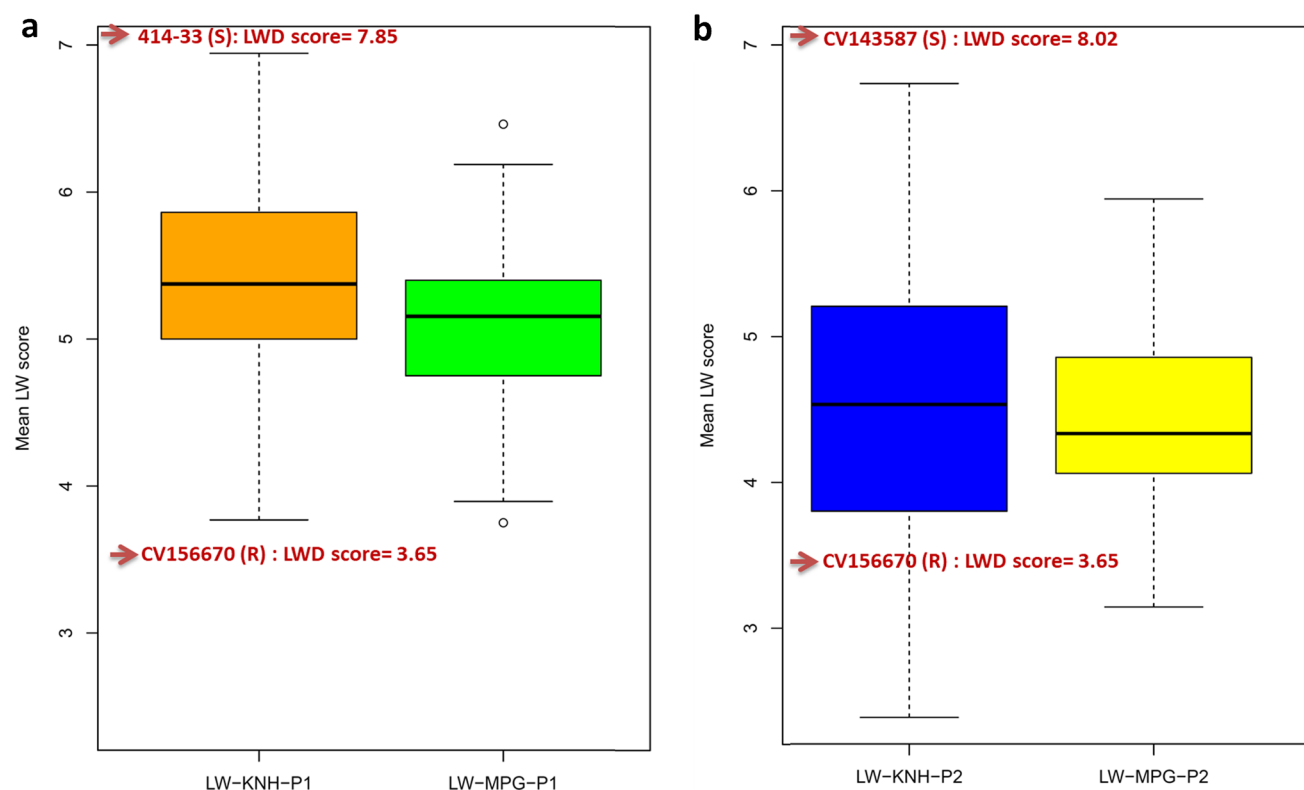
Fig. 2 Patterns of expression of responses to LWD by two $F_{2:3}$ populations (**a** P-1 and **b** P-2) across two locations

Genotyping F$_{2:3}$ families

Five $F_{2:3}$ seeds from each plant of two F_2 populations were pooled and ground to fine powder using liquid nitrogen. DNA isolated from fine powder using CTAB method (Khairallah and Hoisington, 1994) and genotyped using Monsanto proprietary-single nucleotide polymorphism (SNP) markers (Supplementary table 2 and 3) through TaqMan assay (Semagn et al. 2015). A total of 150 and 199 SNP markers polymorphic between the parents of the two mapping populations were selected from proprietary database (unpublished) for genotyping the two mapping populations derived from CV156670 × 414-33 and CV156670 × CV143587, respectively.

Statistical analysis

LWD responses

LWD responses from 20 plants of each $F_{2:3}$ progeny family were recorded (Supplementary figure 1). Data were cured based on missing data points, outliers and correlation between replicates (Mailman et al. 2007; Rakesh 2016). Following data curation, mean LWD scores of 236 and 187 $F_{2:3}$ progeny families out of 300 families of P-1 and P-2, respectively were used for statistical analysis. Analysis of variance (ANOVA) of $F_{2:3}$ progeny families was performed

to partition the total variation in LWD response scores into those attributable to $F_{2:3}$ progeny families and within families. Additionally, pooled ANOVA was performed to test for the consistency of the $F_{2:3}$ progeny families for LWD response across two locations and to quantify the contribution of location and genotype × location interaction. Genotypic data of markers with >15% missing data and markers showing significant segregation distortion were not considered for statistical analysis.

Detection of QTL controlling resistance to LWD

Markers showing segregation distortion were removed (Supplementary table 4 and 5). Information on 115 out of 150 and 137 out of 199 markers were used in P-1 and P-2 respectively after data curation. The genotypic and phenotypic data of $F_{2:3}$ progeny families evaluated in two locations were integrated to detect QTL controlling LWD resistance, initially using single marker analysis. Subsequently, Inclusive Composite Interval Mapping (ICIM) was performed to detect and estimate size effects of QTL and QTL × QTL interactions (Li et al. 2015) controlling LWD resistance at individual locations and across locations (pooling data from both locations). The accuracy of QTL position and significance of size effect of QTL and QTL × location interaction conferring LWD resistance was determined using

Table 1 ANOVA of mean LWD scores in two F$_{2:3}$ populations across two locations

Source of variation	CV156670 × CV143587 (P-1)					CV156670 × CV414-33 (P-2)				
	Degrees of freedom	KNH		MPG		Degrees of freedom	KNH		MPG	
		Mean sum of squares	'F' probability	Mean sum of squares	'F' probability		Mean sum of squares	'F' probability	Mean sum of squares	'F' probability
Between F$_{2:3}$ families	235	0.71**	<0.001	0.47	0.08	186	1.07	<0.05	0.71	<0.001
Within F$_{2:3}$ families	13	0.15		0.17		06	0.32		0.07	

data-driven estimates of threshold LOD scores obtained by 1000 permutations (Churchill and Doerge 1994). Similarly, significant QTL × QTL interactions controlling LWD resistance were detected at threshold LOD value of 5.0, and their size effects were estimated. All these statistical analyses were implemented using QTL ICiMapping software version 4.0 (Wang et al. 2016). The detected di-QTL interactions associated with resistance to LWD, dominance × dominance di-QTL interactions were interpreted based on theoretical investigations of Kearsey and Pooni (1996).

Results

LWD responses of F$_{2:3}$ mapping populations

The LWD response scores of the F$_{2:3}$ progeny families derived from CV156670 × 414-33 (P-1) and CV156670 × CV143587 (P-2) (Supplementary figure 1) recorded at both KNH and MPG locations were normally distributed (Fig. 1a–d). While LWD response scores of F$_{2:3}$ progeny families ranged from 3.17 to 9.00 and from 3.13 to 8.11 at KNH and MPG, respectively in P-1 (Fig. 2a), the responses ranged from 2.38 to 8.38 and from 3.12 to 8.64 at KNH and MPG, respectively in P-2 (Fig. 2b). A wider range of LWD scores at both locations in both the populations indicated sufficient LWD expression. Further, there were considerable numbers of plants towards both resistant and susceptible parents (Fig. 2). Six progeny families including two at KNH and four at MPG in P-1; 56 progeny families including 37 at KNH and 19 at MPG in P-2 showed resistance level better than resistant parent. Similarly, six progeny families including four at KNH and two at MPG in P-1; five progeny families including two at KNH and three at MPG in P-2 showed higher susceptibility than susceptible parent. The F$_{2:3}$ families derived from each of the two mapping populations differed significantly across both the locations except P-1 at MPG (Table 1). Further, LWD responses of F$_{2:3}$ families of both populations interacted significantly with location as evident from significance of genotype× location (G×L) interaction mean squares in pooled ANOVA (Table 2). While mean squares attributable to location was non-significant, those attributable to genotype × location were significant in P-2. This trend was reversed in P-1 (Table 2).

Detection of QTL controlling LWD resistance

QTL main effects

Seven QTL, i.e. two (*q-lw-1.1* and *q-lw-1.2*) in P-1 (Fig. 3) and five (*q-lw-1.3, q-lw-1.4, q-lw-1.5, q-lw-1.6* and *q-lw-1.7*) in P-2 detected on chromosome-1 (Fig. 4) were found associated with LWD resistance with phenotypic variation

Table 2 Pooled ANOVA of mean LWD scores in $F_{2:3}$ populations across two locations

Source of variation	CV156670 × CV143587 (P-1)				CV156670 × CV414-33 (P-2)			
	Degrees of freedom	Mean sum of squares	'F' probability	Percent contribution	Degrees of freedom	Mean sum of squares	'F' probability	Percent contribution
Genotypes	235	1.52	<0.001	46.55	186	3.17	<0.001	77.89
Locations	01	22.41	<0.001	2.92	01	0.01	0.971	0.00
Genotype × location (G × L)	235	0.61	0.08	18.68	186	0.44	<0.001	10.81
Error	470	0.52	-	31.85	372	0.23	-	11.30

explained (PVE) ranging from 4.34 to 13.06% (Table 3). Among these, three major QTL (*q-lw-1.4* and *q-lw-1.6* at MPG; *q-lw-1.5* at KNH) with > 10% PVE were detected in P-2. Among the remaining four minor QTL, two (*q-lw-1.1* and *q-lw-1.2*) were from P-1, and other two (*q-lw-1.3* and *q-lw-1.7*) were from P-2. Further, while five (*q-lw-1.1, q-lw-1.3, q-lw-1.5, q-lw-1.6* and *q-lw-1.7*) of these seven detected QTL showed dominance effects, one (*q-lw-1.4*) showed additive effects in desirable direction (decreasing effects) for LWD resistance (Table 3).

QTL × location interaction

Pooled analysis across locations indicated significant QTL × location (Q×L) interaction. Of the seven detected QTL, five (*q-lw-1.2, q-lw-1.4, q-lw-1.5, q-lw-1.6* and *q-lw-1.7*) interacted significantly with location with their size effects
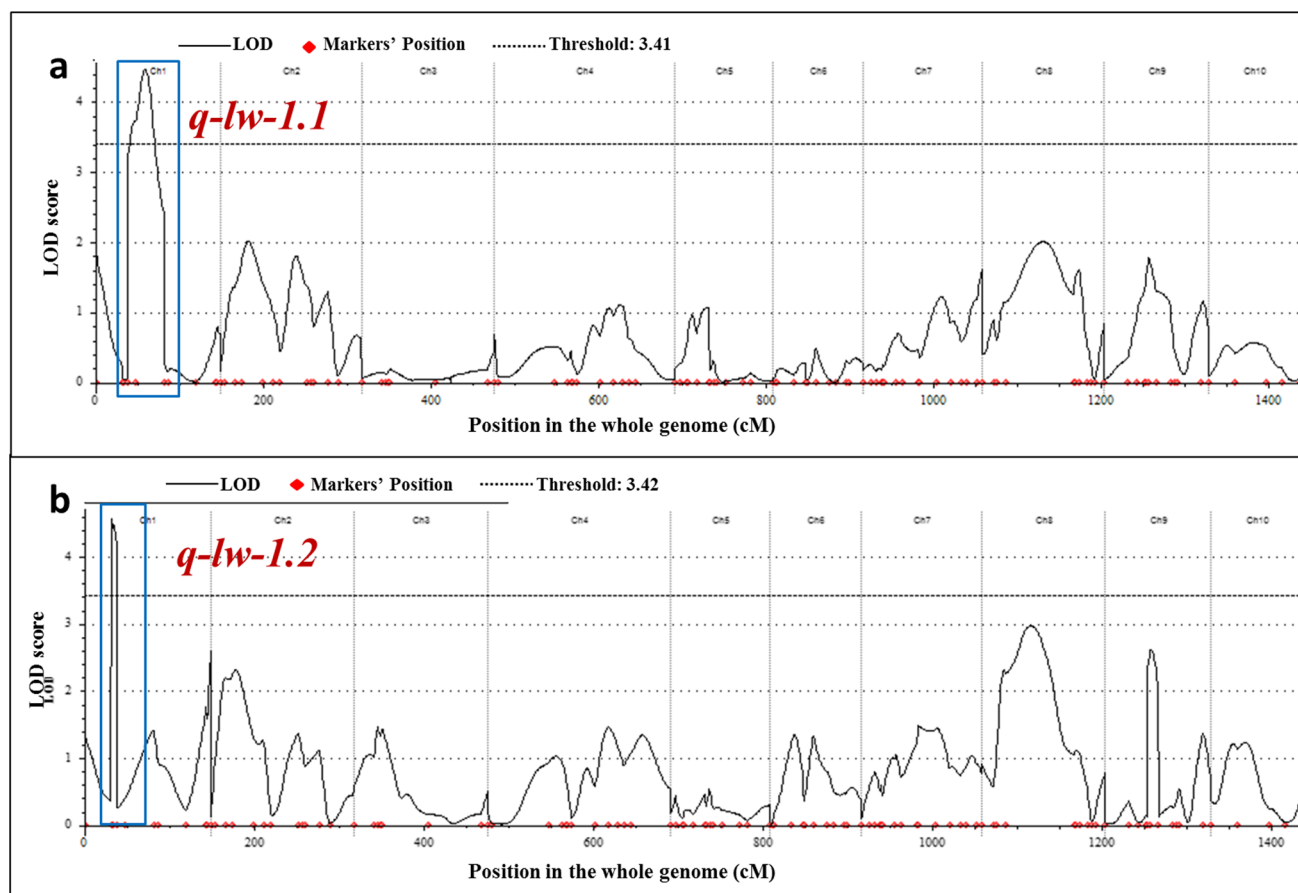
Fig. 3 Genome-wide detection of QTL and their effects controlling LWD response mapped in $F_{2:3}$ of CV156670 (R) × 414-33(S) (P-1) at (a) Kallinayakanahalli and (b) Muppadighatta
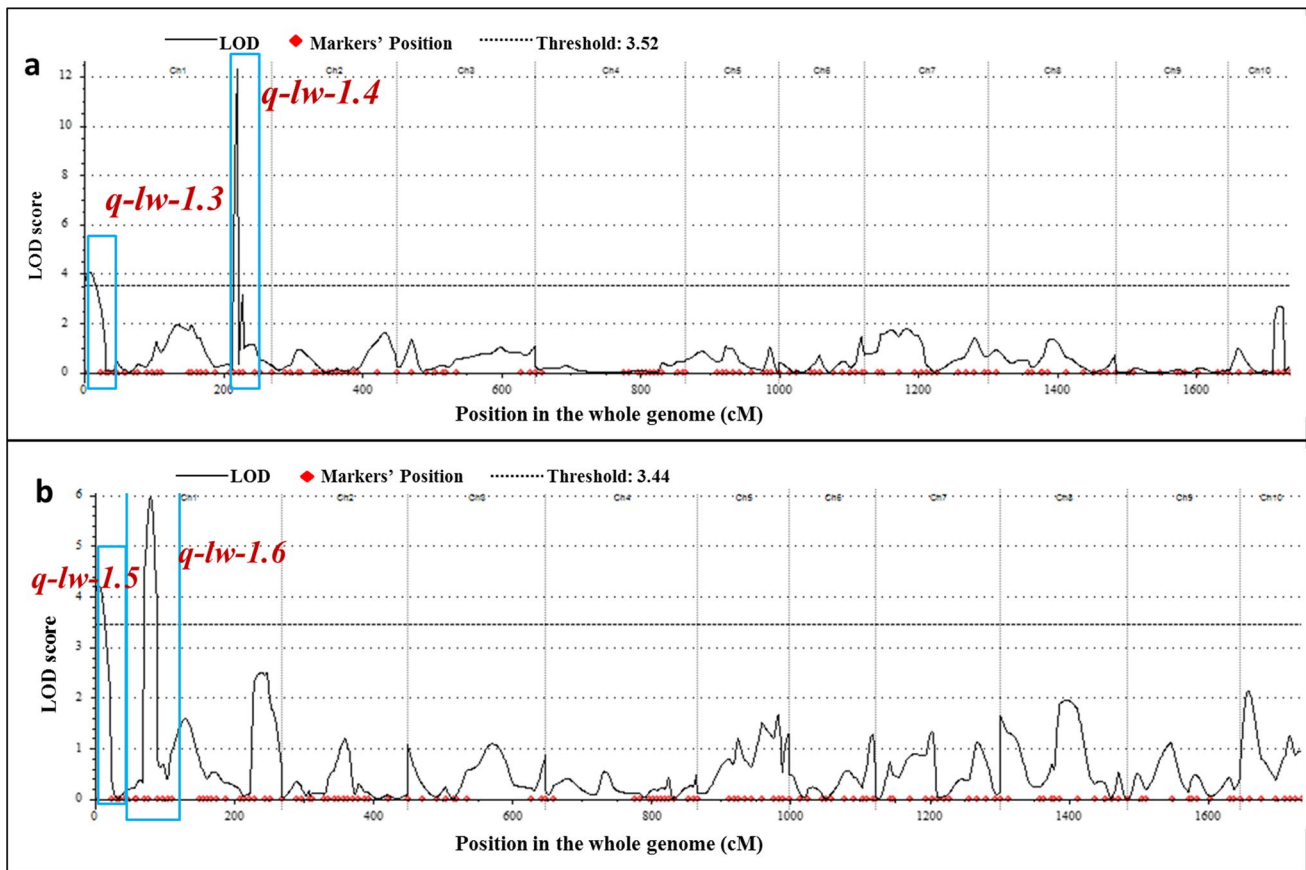
Fig. 4 Genome-wide detection of QTL controlling LWD response and corresponding additive and dominance effects mapped in $F_{2:3}$ of CV156670(R) × CV143587(S) (P-2) at (a) Kallinayakanahalli and (b) Muppadighatta

ranging from 0.04 to 6.80% PVE (Table 3). Of these five interacting QTL, only *q-lw-1.7* was not detected when individual location-wise analysis was performed. This means that, remaining four (*q-lw-1.2, q-lw-1.4, q-lw-1.5 and q-lw-1.6*) of these five QTL detected using pooled analysis were detected only in either of the locations but not in both the locations. These four QTL were also stable for their position but unstable for their effects (decreased size effects) as evident from pooled analysis.

Of the five QTL which showed significant Q × L interaction, one QTL, *q-lw-1.4* (6.80% PVE Q × L), interacted with locations to a greater extent than the other four QTL (Fig. 5). None of the QTL detected through location-wise analysis were common in both locations. Two QTL (*q-lw-1.3* and *q-lw-1.5*) flanked by same pair of markers, namely, Marker-151 and Marker-152 (with inter-marker distance of 23.8 cM) were detected in P-2. While the QTL, *q-lw-1.3* was mapped at 11.40 cM in KNH (in location-wise analysis), *q-lw-1*.5 was mapped at 9.40 cM in MPG (in location-wise as well as pooled analysis). Further, their position and effects differed (Table 3).

Di-QTL epistasis

A total of 48 significant di-QTL interactions with size effects (in % PVE) ranging from 4.00 to 19.03% were detected across both locations in P-1. Of these, ten were between QTL located within the same chromosome (Fig. 6a). Only nine of these 48 interactions involved QTL with significant effects. Similarly, 52 significant di-QTL epistatic interactions including ten within same chromosome were detected in P-2 with their size effects (in % PVE) ranging from 3.18 to 13.83% (Fig. 6b). Only four of these 52 significant di-QTL interactions involved QTL with significant effects. Summation of direction and effects of dominance (h) and dominance × dominance (l) effects associated with all di-QTL interaction effects indicated duplicate di-QTL epistasis in both the populations. Duplicate di-QTL epistasis was in desirable direction (dominant decreasing effects) at KNH and MPG in P-1 and P-2, respectively (Table 4).

Table 3 Position and effect of QTL conferring LWD resistance detected individual location-wise and across locations (pooled)

Trait	QTL	Environment	Population	Genomic position (cM)	Chromosome	Flanking markers Right	Flanking markers Left	LOD	PVE Q (%)	PVE Q × L (%)	Additive effect	Dominance effect
Late wilt disease	q-lw-1.1	Kallinayakanahalli	CV156670 × CV143587 (P-1)	138.60	01	Marker-12	Marker-13	4.45	7.92	-	0.44	**-0.13**
	q-lw-1.2	Muppadighatta		112.60		Marker-9	Marker-10	4.56	9.91	-	0.30	0.05
		Pooled						4.61	8.28	3.80	0.17	0.04
	q-lw-1.3	Kallinayakanahalli	CV156670 × CV414-33 (P-2)	11.40		Marker-151	Marker-152	4.06	4.34	-	0.41	**-0.29**
	q-lw-1.5	Muppadighatta		9.40				4.22	10.10	-	0.33	**-0.31**
		Pooled						8.19	8.85	0.04	0.33	**-0.29**
	q-lw-1.4	Kallinayakanahalli		224.40		Marker-179	Marker-180	12.27	11.72	-	**-0.02**	1.08
		Pooled						12.34	8.73	6.80	**-0.01**	0.56
	q-lw-1.6	Muppadighatta		83.40		Marker-160	Marker-161	5.97	13.06	-	0.40	**-0.28**
		Pooled						6.23	4.35	1.20	0.24	**-0.17**
	q-lw-1.7	Pooled		232.40		Marker-180	Marker-181	5.42	5.60	0.31	0.18	**-0.38**

### Validation of reported QTL conferring resistance to LWD

Rakesh (2016) reported QTL on chromosome-1 at 103.20 cM (IMD- 10.6 cM), flanked by MONIND14101828 and MONIND14262087. This QTL explained only 7.26% of LWD resistance in mapping population derived by CV138811 × CV143587. We identified one of these markers namely, Marker-161 (MONIND14101828) flanking the QTL, q-lw-1.6 could be validated in P-2 (CV156670 × CV143587). However, this QTL flanked by Marker-160 and Marker-161 was detected at position (83.40 cM) and phenotypic variation (13.06% PVE at MPG and 4.35% PVE across locations) different from that reported by Rakesh (2016) (Table 3).

Discussion

LWD is emerging as a major and serious biotic production constraint with a potential to reduce yield and quality of both grain and fodder (Sunitha et al. 2020; El-Naggarr et al. 2015; Drori et al. 2013; Johal et al. 2004). Developing LWD-resistant cultivars is considered as an eco-friendly and sustainable approach to mitigate losses caused by LWD. Precise information on the number and mode of action of genes/QTL controlling resistance to LWD help devise suitable strategies to develop resistant cultivars. The results of our study indicate possible involvement of a large number of genes controlling resistance to LWD as evident from the normal distribution of LWD responses in $F_{2:3}$ populations. Several previous work by Shehata (1976), Nawar and Salem (1985), Abdel-Snbour and Bekhit (1993) and El-Hosary and El-Fiki (2015) have also reported the involvement of a large number of genes controlling LWD resistance in maize. Further, recovery of individuals that surpass the levels of resistant and susceptible parents indicate (i) presence of alleles controlling resistance even in susceptible parent and those controlling susceptibility even in resistant parent and (ii) complementation of parental alleles controlling resistance. Thus, $F_{2:3}$ populations serve as potential source for recovering inbred lines with levels of resistance to LWD better than the resistant parent.

Most of the reports (Wu et al. 2020; Zhang et al. 2012; Yang et al. 2010; Suneetha 2016) related to mapping QTL controlling resistance to stalk rot are predominantly on stalk rot caused by *Fusarium* sp. followed by *Macrophomina phaseolina* but not on the stalk rot caused exclusively by *Harpophora maydis* (Late wilt disease). This could be because of co-existence of *Harpophora* with *Fusarium* sp. and/or *Macrophomina* sp. which form a pathogen complex causing PFSR (Degani et al. 2020; Khokhar et al. 2014). Additionally, soil-borne *Fusarium* sp. and/or *Macrophomina* sp. are known to invade the *H. maydis* infested
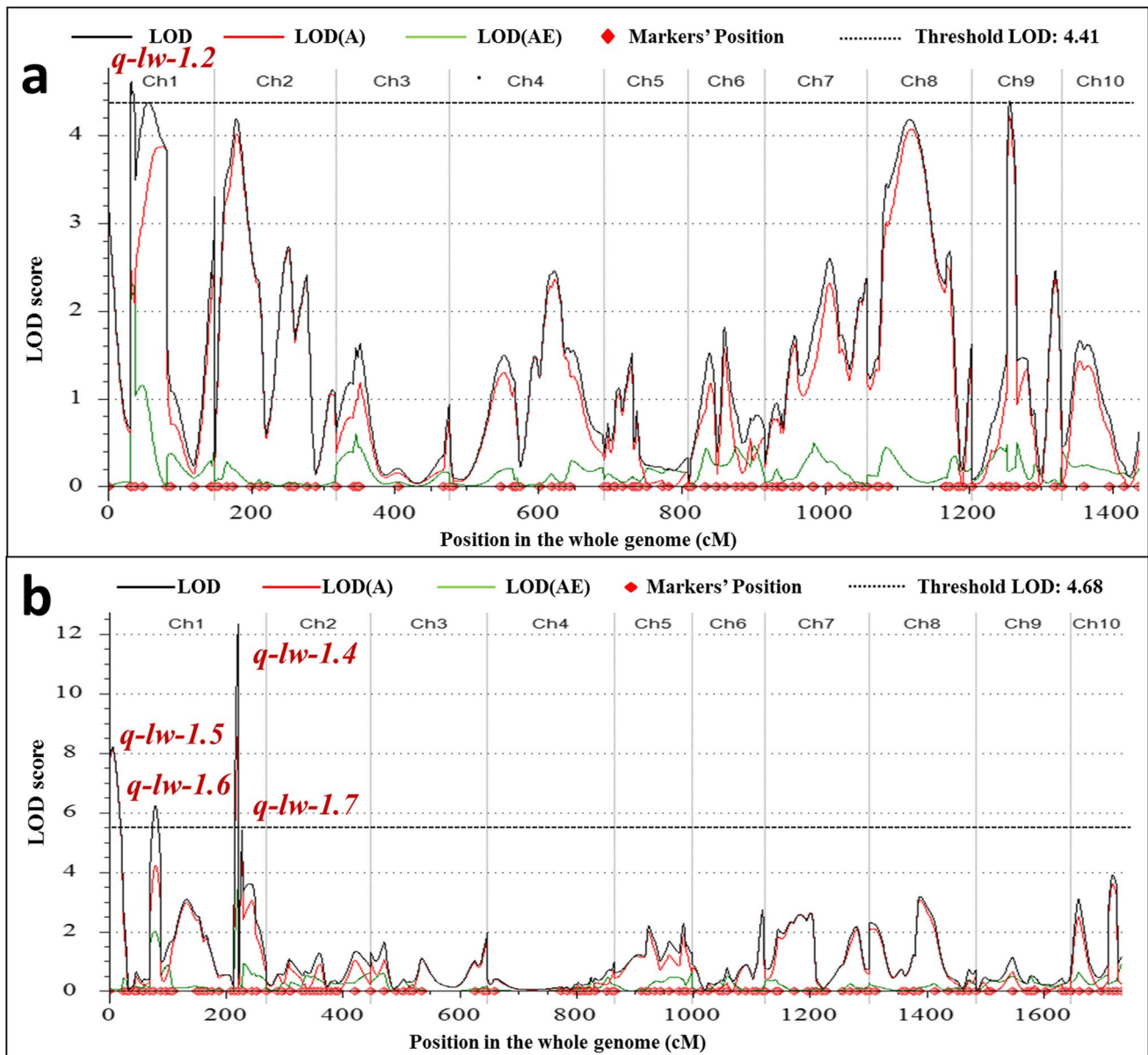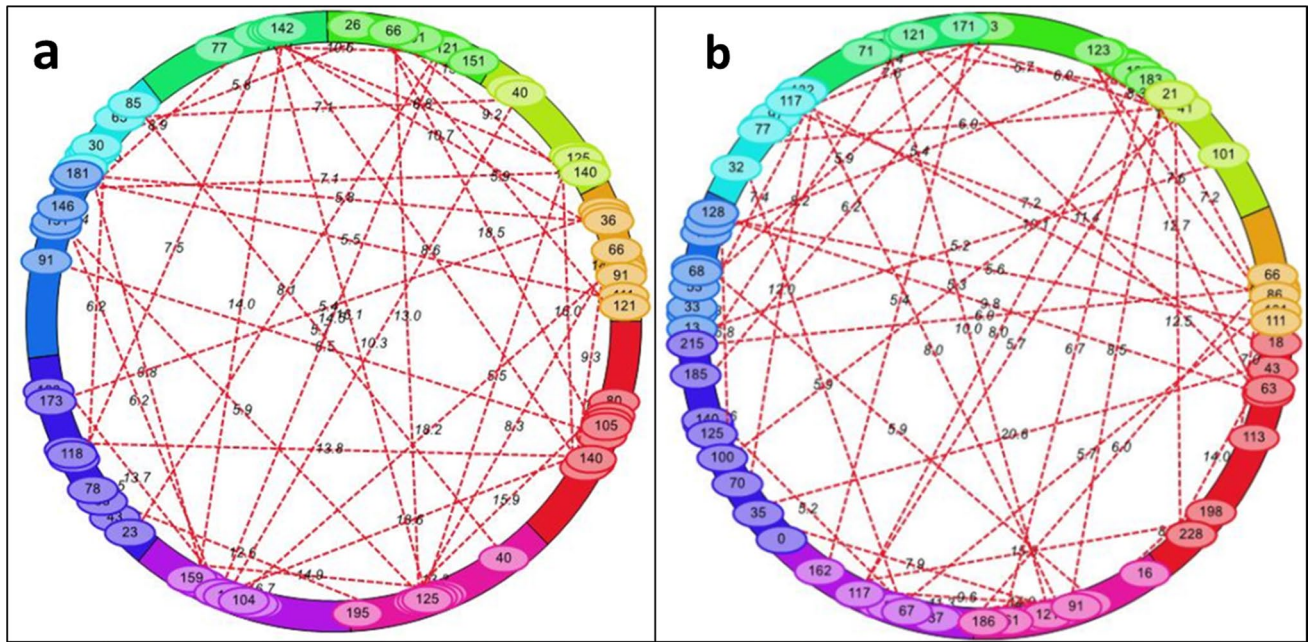
Fig. 5 Genome-wide detection of QTL controlling LWD response and QTL × location interactions in $F_{2:3}$ of (a) CV156670(R) × 414-33(S)/P-1 and (b) CV156670(R) × CV143587(S)/P-2

stalks (Drori et al. 2013). This causes difficulty in phenotyping the symptoms caused exclusively by *H. maydis*. To address the issue of possible confounding effects of pathogens other than *H. maydis*, we used Indian pure isolates of *H. maydis* to inoculate the spores directly into the second internode of the stem (Rakesh et al. 2016a, b) using stem jabbers (specialized injectors) twice at 45 DAS and 55 DAS (Sunitha et al. 2020).

LWD resistance conferring QTL

Difficulty arising due to complexity of the disease and phenotyping could be the potential causes for sporadic reports on genetic basis of LWD resistance. The only report (Rakesh 2016) on mapping genomic regions controlling LWD resistance in two $F_{2:3}$ populations (derived using CV138811 and CV143587 as donor and susceptible parents, respectively) resulted in the identification of three major QTL on chromosomes 3, 5 and 10; and six minor QTL on chromosomes 1, 2, 3, 5, 6 and 7. QTL analysis is not only intended to implement Marker Assisted Selection (MAS) for QTL but

Fig. 6 Genome-wide detection of significant di-QTL interactions controlling LWD response mapped in $F_{2:3}$ of (a) CV156670(R) × 414-33(S)/P-1 (b) CV156670(R) × CV143587(S)/P-2

Table 4 Direction of dominance effects and all possible dominance × dominance di-QTL interactions controlling resistance to LWD without considering their significance or otherwise

Main QTL effect (h)	Dom×Dom effect (l)	CV156670 × CV143587 (P-1)			CV156670 × CV414-33 (P-2)			Interpretation on type of epistasis
		KNH	MPG	Across locations	KNH	MPG	Across locations	
+	+							Complementary epistasis between dominant increasing effect QTLs
-	-							Complementary epistasis between dominant decreasing effect QTLs
+	-		✓		✓			Duplicate epistasis between dominant increasing effect
-	+	✓		✓		✓	✓	Duplicate epistasis between dominant decreasing effect

also for understanding the genetics of the quantitative trait. Hence, all the identified QTL, whether their effects are large or small and with or without environmental sensitivity are informative (Asins 2002). We could identify three major QTL and four minor QTL. In most of the studies, QTL with small effects go undetected due to bias caused by less-stringent threshold levels. To minimize such bias, we used LOD threshold derived from 1000 permutations (Churchill and Doerge 1994).

QTL×location interaction

The position and effect of the QTL associated with LWD resistance varied significantly with the spatial environments represented by two locations. If a quantitative trait exhibits significant genotype by environment interaction, it then follows that underlying QTL should also display significant

interaction with either temporal or spatial environments (Bernardo 2020). F_3 progenies of both the crosses displayed significant interaction with the environment represented by two locations for responses to LWD (Table 2). Hence, detected significant interaction of LWD resistance governing QTL with location environments did not surprise us. Several researchers have also reported significant QTL × location interaction associated with resistance response of maize diseases such as Fusarium ear rot (Wu et al. 2020; Robertson-Hoyt et al. 2006), northern leaf bight (Xia et al. 2020; Chen et al. 2016) and rough dwarf disease (Wang et al. 2019). The major QTL (>10% PVE) detected through location-wise mapping behaved as minor QTL (<10% PVE) when detected through pooled analysis. We could also observe shift in the position of QTL (*q-lw-1.3* and *q-lw-1.5*) within the same

flanking marker interval in different locations. However, such QTL despite showing significant G × E interaction help select genotypes adapted to specific locations (Asins 2002). As expected based on previous reports by Shekhar et al. (2010), we could also observe significant influence of location and genotype × location interaction on the expression of LWD. Significance of G × E interaction for a trait reflects that underlying genomic regions exhibit significant QTL × Location interaction (Bernardo 2020). Among the three major QTL identified by us, although two QTL (*q-lw-1.5* and *q-lw-1.6*) showed significant Q×L interaction, the magnitude of interaction effects was relatively low. These QTL with lower Q×L interaction effects could be considered as stable and deployed to implement MAS for LWD resistance after validation. This is because, the efficiency of MAS may get reduced if detected QTL controlling target trait exhibits significant interaction with either the environment or background genotype used (Asins 2002).

QTL×QTL interaction

The chances of recovering recombinant inbred lines (RILs) with dominant di-QTL interactions are fewer with any population other than F_2. However, even a large F_2 mapping population may contain fewer two-QTL RILs limiting the statistical power of detecting di-QTL interactions (Bernardo, 2020; Pandey et al. 2006). Hence, there are chances that epistatic interactions go undetected. We addressed this limitation by increasing the LOD significance of >5.0 and considering phenotypic data from replicated trials from $F_{2:3}$. Some of the di-QTL interactions which involve QTL with their individual effects being non-significant are also informative, provided their di-QTL interaction effects are of higher magnitude. Under such instance, deployment of QTL alone in MAS without considering its significant di-QTL interaction would be ineffective. Hence, such QTL showing significant epistatic effects of large magnitude need to be introgressed together into recipient genetic background. However, there are chances that the magnitude of the significant di-QTL interaction may get reduced or get enhanced in recipient parent genetic background. The effects of such interacting QTL in recipient genetic background will be known after introgression. The direction of cumulative dominance effects associated with duplicate di-QTL epistasis varied with location. The duplicate di-QTL epistasis with decreasing dominance effects on LWD response is desirable. However, these di-QTL interaction effects need to be confirmed through extensive multi-location evaluation.

QTL genetic background interaction

Genetic background has considerable influence on the position and effect of QTL conferring disease resistance (Asins 2002; Awata et al. 2020). The same was evident in our study, and none of the QTL was common between both populations, P-1 and P-2 (which differed for susceptible parent genome). However, we could validate only one of the reported markers, MONIND14101828 (Marker-161), flanking the reported QTL (Rakesh 2016) in P-2. While the QTL region having this marker, MONIND14101828 (Marker-161), in common is flanked by an inter-marker distance of 11.8 cM in the present study, it is flanked by an inter-marker distance of 10.6 cM in a previous report (Rakesh 2016). Thus, this region needs fine mapping to identify QTL with a narrow inter-marker distance for efficient deployment in MAS.

In summary, three major QTL, *q-lw-1.4, q-lw-1.5* and *q-lw-1.6*, were identified from our study. However, PVE% explained by these three QTL is not large enough for use in MAS, but these are of paramount importance in accumulating positive alleles for LWD resistance breeding. Further, only one of these QTL, *q-lw-1.6*, could be validated. The three major QTL exhibited significant interaction with locations. Considering that the validated QTL reported by us and those reported by previous researcher exhibit significant interactions with locations, further research investigation is essential to identify stable QTL with large PVE for implementing MAS.

Declarations

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