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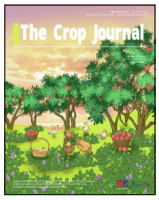

Meta-QTL analysis for mining of candidate genes and constitutive gene network development for fungal disease resistance in maize (*Zea mays* L.)

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ABSTRACT

The development of resistant maize cultivars is the most effective and sustainable approach to combat fungal diseases. Over the last three decades, many quantitative trait loci (QTL) mapping studies reported numerous QTL for fungal disease resistance (FDR) in maize. However, different genetic backgrounds of germplasm and differing QTL analysis algorithms limit the use of identified QTL for comparative studies. The meta-QTL (MQTL) analysis is the meta-analysis of multiple QTL experiments, which entails broader allelic coverage and helps in the combined analysis of diverse QTL mapping studies revealing common genomic regions for target traits. In the present study, 128 (33.59%) out of 381 reported QTL (from 82 studies) for FDR could be projected on the maize genome through MQTL analysis. It revealed 38 MQTL for FDR (12 diseases) on all chromosomes except chromosome 10. Five MQTL namely 1_4, 2_4, 3_2, 3_4, and 5_4 were linked with multiple FDR. Total of 1910 candidate genes were identified for all the MQTL regions, with protein kinase gene families, TFs, pathogenesis-related, and disease-responsive proteins directly or indirectly associated with FDR. The comparison of physical positions of marker-traits association (MTAs) from genome-wide association studies with genes underlying MQTL interval verified the presence of QTL/candidate genes for particular diseases. The linked markers to MQTL and putative candidate genes underlying identified MQTL can be further validated in the germplasm through marker screening and expression studies. The study also attempted to unravel the underlying mechanism for FDR resistance by analyzing the constitutive gene network, which will be a useful resource to understand the molecular mechanism of defense-response of a particular disease and multiple FDR in maize.

1. Introduction

Maize (*Zea mays* L.) is the third most important cereal crop after wheat and rice with annual global production of 1148.48 million tonnes under acreage of 197.20 million hectares [1,2]. It is highly valued for use in the human diet but more as feed and for various industrial uses like starch and bioethanol production [3]. Yearly, there is a 2.24% (2015–2019) increment in global maize production [2] but this trend is insufficient to fulfill the global demand projected for 2050 [4,5]. Over the last decade, maize consumption in India grew at a compounded annual growth rate (CAGR) of 5.6% while production grew at just about 2.9%. Climate change imposes various biotic and abiotic stresses resulting in major economic losses, both in terms of quality and quantity. Biotic stresses include nearly 110 maize diseases caused by fungi, bacteria and viruses prevalent worldwide [6].

In maize, biotic stresses attribute to about 10% of yearly yield loss globally [7]. Several pathogens causing various diseases significantly hamper maize production, intensifying a threat to global food safety and agricultural sustainability [8]. The most predominant fungal diseases of maize are corn leaf blight, leaf spot, downy mildew, smut, rust, ear rot, seedling rot, banded leaf, and sheath blight, leaf spot, stalk rot and aflatoxin contamination. The major diseases of maize excluding viral cause 4% to 14% yield loss of total harvest globally [9]. The use of chemicals for disease control or spread is prevalent but genetic resistance is the most viable,

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cost-effective and eco-friendly method for managing diseases in maize [10]. Breeding for host plant resistance with multiple resistance genes/QTL is a viable option to combat the diseases. With the advancement in genomic technologies, significant progress has been made in the detection of genes/QTL responsible for disease resistance and unraveling the host-pathogen interactions [11]. The development of diverse molecular markers proved crucial to boosting QTL mapping studies [12]. There are two types of resistance associated with diseases, (1) qualitative which is race-specific controlled by a single gene that is dominant or recessive like *R*-gene known as the major gene [13], and (2) quantitative type mechanism is controlled by oligogenic or polygenic, partially dominant genes [14]. Hence, durable resistance can be imparted in maize by combining the major QTL or resistance genes (*R*-genes) for a particular disease against multiple strains of a pathogen or multiple diseases [9]. Fungal Disease Resistance (FDR), which includes gene expression for resistance to particular or multiple fungal diseases, can express itself in two different ways in plant cells – constitutive mode and induced mode, the former being the first line of defense (comprises of physical and chemical barriers to the fungal entrance). The induced mode is the second line of defense, which is initiated when plant cells encounter and sense the presence of a fungal pathogen. Both responses are genetically defined and can have adequate cross-talk.

In maize disease resistance breeding, identification of major QTL *vis-a-vis* their introgression via marker-assisted breeding is an important objective. Previously, a large number of QTL mapping studies have been undertaken for important fungal diseases in maize (Table S1). However, the fine-mapping studies are restricted to a few QTL only [15–19]. Further, results across studies cannot be directly used in any programme due to differences in genetic backgrounds (parents), mapping population type (like F_2, back-cross-BC, recombinant inbred lines- RILs, doubled haploid- DH, etc.) and statistical methods for QTL identification (like single marker analysis, simple/composite/multiple interval mapping) across the studies [20]. Thus, despite the availability of numerous QTL for various diseases, only a few QTL were validated and directly used in MAS programs in maize [21,22]. Validation and fine mapping of the mapped QTL can be potentially utilized to verify and narrow down the genomic regions governing target traits. meta-QTL (MQTL) analysis is a potent approach for the identification of consensus regions using the information on the QTL for different traits across studies [23]. In maize, MQTL analysis has been successfully attempted for various traits like gray leaf spot resistance [24], consensus synthetic QTL for disease resistance [18], root traits [25] and popping traits [26]. Besides maize, MQTL studies have been reported for disease resistance in other crops like rice blast [27], disease resistance in rice [28], tan spot resistance in wheat [29], quality, biotic and abiotic stress-related traits in wheat [30].

The MQTL with a consistent and large effect on the trait of interest, shortest physical distance (smallest confidence interval/CI and the cluster of a large number of initial QTL are the best candidate MQTL for MAS [31], can also be referred as "MAS-friendly MQTL", provided the functionality of flanking markers are validated in related germplasm. Identification of putative candidate genes underlying "MAS-friendly MQTL" with help of the reference map is very important. Hence, the present study aimed at conducting MQTL analysis to identify MQTL for major fungal diseases of maize. The presence of candidate genes for disease resistance in MQTL regions was also verified through comparison with genome-wide association studies (GWAS) for FDR in maize. Identified MQTL/candidate genes (with flanking markers) can be targeted for validation in germplasm followed by introgression through marker-assisted selection (MAS). Further, an attempt has been made to unveil the molecular basis (at gene expression level) of single/multiple FDR in maize. The gene expression data of B73 over the different stages of plant growth was utilized to understand the expression of putative candidate genes and their biological functions in imparting constitutive or induced mode of FDR in maize. The study will help to enhance our understanding of genetic regions and molecular mechanisms of FDR in maize.

2. Materials and methods

2.1. Literature review and QTL database development

The exhaustive literature review was carried out to detect the published QTL mapping studies for major 19 fungal diseases in maize. The diseases were Northern corn leaf blight/NCLB (*Setosphaeria turcica*), Anthracnose leaf blight/ALB (*Colletotrichum graminicola*), Banded leaf and sheath blight/BLSB (*Rhizoctonia solani*), Southern corn leaf blight/SLB (*Bipolaris maydis*), Curvularia leaf spot/CLS (*Curvularia lunata*), Gray leaf spot/GLS (*Cercospora zeae-maydis*), Phaeosphaeria leaf spot/PLS (*Phaeospharia maydis*), Common smut/CS (*Ustilago maydis*), Head smut/HS (*Sporisorium reiliana*), Sorghum downy mildew/SDM (*Peronosclerospora sorghi*), Rajasthan downy mildew/RDM (*Perenosclerospora heteropogoni*), Java downy mildew/JDM (*Peronosclerospora maydis*), Maize stalk rot/MSR (*Fusarium moniliforme*), Gibberella ear rot/GER (*Gibberella zeae*), Fusarium ear rot/FER (*Fusarium verticilliodes*), Fusarium seedling rot/FSR (*Fusarium verticilliodes*), Aspergillus ear rot/AER (*Aspergillus flavus*), Southern rust/SR (*Puccinia polysora*) and Common rust/CR (*Puccinia sorghi*). An extensive systematic literature review was done for the QTL mapping studies on FDR in maize using Google Scholar and Web of Science (Fig. S1). A total of 82 QTL mapping experimental studies from 63 research papers were considered for the study. The information on all essential parameters like QTL name, position, traits, linkage group, LOD values, CI, phenotypic variance explained (R^2), etc. (Table S1) were used for the preparation of QTL files. Sixty-five studies, which did not provide sufficient detailed information regarding QTL, were excluded from the present study. For analysis, the genetic map data was also extracted from the available studies or from the MaizeGDB (https://www.maizegdb.org/) to prepare map files and construct the consensus map with a reference map.

Data on a total of 381 QTL were summarized for fungal diseases for MQTL analysis (Table S1). The QTL with >10% phenotypic variance or 75th percentile of the respective study [32] were used in MQTL analysis as the major effect QTL can be effectively utilized for MAS. The studies used in MQTL analysis had different mapping populations, *viz.*, F_2, F_3, F_5, BC, RILs, DH, etc. derived from diverse parental crosses (Table S2). The QTL for various traits have been considered for these fungal diseases like a weighted mean disease for the environment, best linear unbiased predictors (BLUP) for days to anthesis, lesion length, lesion width, primary diseased leaf area, the incubation period, area under the disease progress curve (AUDPC), days to anthesis for NCLB, AUDPC for ALB, weighted mean diseases (WMD), and early and late rating for GLS as listed in Tables S1 and S2. The prepared QTL and genetic map input files were converted into XML files using MetaQTL software (http//bioinformatics.org/mQTL) that were subsequently utilized as input files in the BioMercator V4.2.3 software for MQTL analysis.

2.2. Map projection and consensus map integration

A high-resolution map i.e., 'ISU Integrated IBM 2009' (https://www.maizegdb.org/data_center/map) consisting of 9073 different markers (RFLP, SSR, SNPs) within 2400.97 cM of chromosome length was used as a reference map. To further enrich the reference map (for the inclusion of SNP-markers-based QTL mapping studies) in the published SNP map [33] was also integrated to develop

a consensus map using BioMercator V4.2.3 software as approach followed in the study [34]. The positions and CI of QTL were used as assigned in the respective studies, except for some QTL (which lacked positions), where positions were allotted based on the flanking markers of the genetic map. For QTL without information on CI, the CI was calculated using the formulas as $530/N \times R^2$ for F_2 and BC while $163/N \times R^2$ for RIL population-based studies, where N denotes the population size and R^2 is the phenotypic variance explained by individual QTL [35]. The major effect QTL from different studies were projected on a consensus map with refined CI and position using BioMercator V4.2.3 software [36].

2.3. QTL meta-analysis

MQTL analysis was performed on independent QTL for a particular trait obtained from different genetic or environmental backgrounds. Based on an integrated consensus map and initial QTL projections, MQTL analysis was carried out on the QTL clusters present on each chromosome using BioMercator V4.2.3. In this method, all the possible QTL combinations were tested based on the QTL model i.e., AIC (Akaike information content), AICc (AIC correction), AIC3 (AIC 3 candidate models), BIC (Bayesian information criterion), and AWE (Average weight of evidence) and the one which maximizes the likelihood was selected. The model with the lowest AIC represents the number of MQTL. Further, the position and CI (95%) of the MQTL were calculated and the flanking markers for MQTL were identified from MaizeGDB.

2.4. Candidate genes identification

The search browser in MaizeGDB (http//maizegdb.org/) was used to determine the physical position of flanking markers for respective MQTL. The obtained physical length of respective MQTL was used to retrieve the candidate genes linked with the particular disease through the 'qTeller' program available on MaizeGDB. Further, the input files were prepared for candidate genes of each MQTL region of fungal diseases, and gene annotation and ontology analysis-related information was extracted using in-house Perl Script. These extracted files contained detailed information regarding identified putative candidate genes like gene transcripts number, their function and description, protein family database (PFAM) IDs, domain description, superfamily IDs and their description, InterPro (IPR) IDs and their description, gene ontology (GO) IDs and their function in cellular, biological and molecular processes. Pathway analysis of candidate genes was done using the Plant Reactome Database [37]. Expression of the putative candidate genes at different stages of maize growth and development was noted in the B73 reference genome, extracting the data from MaizeGDB Atlas [38]. For the display of gene expression information, Morpheus software was used (Morpheus, https//software.broadinstitute.org/morpheus). Pairwise sequence alignment, homology modelling and molecular visualization of *ZmFBL2* and *ZmFBL41* were performed using Emboss Needle service [39], Swiss-model [40] and PyMol molecular graphics, respectively. Protein function classification was performed through the CATH database [41].

2.5. Verification of MQTL with GWAS studies

Information was compiled for marker-trait associations (MTAs) from the available GWAS studies for various fungal diseases in maize. The physical positions of MTAs were compared with the physical positions of the interval of the identified MQTL. GWAS-MTAs that flanked around 5 Mb physical regions to the MQTL were also considered for comparison and considered as part of the MQTL region [42]. RCircos package was used to prepare the Circos plot [43].

3. Results

3.1. QTL distribution on maize genome for various fungal diseases

In the present study, 381 major effect QTL from 82 QTL mapping studies on various fungal diseases were used for analysis (Fig. S1). A total of 128 (33.59%) QTL out of 381 were successfully projected using Biomercator V4.2.3 for 19 fungal diseases (Tables 1, S2) on the consensus map comprising 63,290 markers spanning 2400.4 cM length. The reason for the lower percentage of QTL projection might be the lack of common markers across the studies and the reference map. The MQTL analysis identified 38 MQTL present on all chromosomes (Chr.) of the maize genome except Chr. 10 (Fig. S2). These comprised five MQTL each on Chr. 1, 2, 3, 4, 6, and 8; four MQTL on Chr. 5; and two MQTL each on Chr. 7 and 9 (Table 1). The confidence interval (CI) value of each MQTL was significantly reduced in comparison to the initial CI of respective QTL from a particular study located in that MQTL region (Table 1). Out of 19 fungal diseases, the identified MQTL were associated with the resistance to 12 maize fungal diseases (HS, GLS, FSR, NCLB, GER, SDM, SLB, FER, AER, PLS, CS, BLSB). The projected initial QTL were maximum on Chr. 2 (27 QTL) representing resistance to seven fungal diseases followed by 21 QTL on Chr. 3 (for 8 diseases), 19 QTL each on Chr. 1 (for 7 diseases) and Chr. 4 (for 4 diseases), 10 QTL on Chr. 9 (for 2 diseases), nine QTL each on Chr. 7 (for 2 diseases) and Chr. 8 (for 3 diseases), eight QTL on Chr. 6 (loci for 3 diseases), and minimum six QTL on Chr. 5 (loci for 5 diseases) (Fig. S3; Table 1). The MQTL3_3 region had initial QTL reported to be resistant against the maximum of four diseases (GLS, SDM, SLB, HSR) followed by other four regions, *viz.*, MQTL1_4 (BLSB, SLB, SDM), MQTL2_4 (BLSB, HS, GER), MQTL3_4 (GLS, FER, AER), MQTL5_4 (AER, FER, NCLB) against three diseases, 12 MQTL 1_1 (HS, GLS), 1_2 (FSR, GLS), 1_3 (GLS, PLS), 1_5 (SLB, GLS), 2_1(GLS, NCLB), 2_3 (SLB, GLS), 3_5 (NCLB, GLS), 4_4 (NCLB, GLS), 6_4 (GLS, FSR), 7_1 (GLS, PLS), 8_4 (NCLB, GLS), and 9_2 (SDM, GLS) against two kinds of diseases, and 21 MQTL against single disease *viz.*, 12 MQTL (2_2, 3_1, 4_2, 4_5, 5_1, 5_2, 6_2, 6_3, 7_2, 8_1, 8_3, and 9_2) for GLS, four MQTL (3_2, 4_1, 5_3, and 6_1) for FSR and MQTL 2_5, 4_3, 6_5, 8_2, 8_5 against HS, FER, SDM, NCLB and CS, respectively. Interestingly, out of 38 MQTL, 12 MQTL (MQTL2_2, MQTL3_1, MQTL 4_2, MQTL4_5, MQTL5_1, MQTL5_2, MQTL6_2, MQTL6_3, MQTL7_2, MQTL8_1, MQTL8_3 and MQTL9_2) located on all nine chromosomes except Chr. 1 contained 42 initial QTL of GLS only with average phenotypic variance of 7.7% to 32.49%. In addition to this, MQTL 2_5 and 4_3 were only responsible for resistance against HS and FER, respectively.

In present study, out of 38 MQTL, 26 MQTL including four MQTL (1_1, 1_2, 1_3, 1_5) were localized on Chr. 1; three MQTL (2_1, 2_2, 2_3) on Chr. 2; four MQTL (3_1, 3_3, 3_4, 3_5) on Chr. 3; three MQTL (4_2, 4_4, 4_5) on Chr. 4; two MQTL (5_1, 5_2) on Chr. 5; three MQTL (6_1, 6_3, 6_4) on Chr. 6; two MQTL (7_1, 7_2) on Chr. 7; three MQTL (8_1, 8_3, 8_4) on Chr. 8 and two MQTL (9_1, 9_2) on Chr. 9 were associated with GLS resistance.

The three MQTL 1_3, 3_3 and 3_4 consisted of initial QTL from four different populations, six MQTL 1_2, 2_4, 4_2, 5_4, 8_4, 9_2 contained preliminary QTL from three different populations, 14 and 15 MQTL contained initial QTL from two and one population, respectively as listed in Table 1. These MQTL having a higher number of populations for initial QTL could be considered as unique regions on the genome for FDR.

3.2. Candidate gene identification for FDR

From the identified MQTL a total of 1910 candidate genes were identified in these MQTL regions (Table S3). A maximum of 251

Table 1
Meta-QTL associated with resistance against different fungal diseases and number of putative candidate genes associated with identified MQTL.

No.	Meta-QTL	Position (cM)	Flanking marker	Physical position of flanking markers (bp)	CI (95%)	Average phenotypic variance	Diseases	No. of initial QTL	No. of population	No. of candidate genes
1	MQTL1_1	62.84	PZE.101044686–PZE.101046859	30,280,808–31,870,178	1.44	14.40	HS, GLS	2	2	34
2	MQTL1_2	115.00	PZE.101137683–PZE.101140058	178,347,753–180,722,884	2.21	8.97	FSR, GLS	4	3	36
3	MQTL1_3	175.84	PZE.101193629–SYN25857	241,277,428–241,450,769	1.78	16.65	GLS, PLS	8	4	7
4	MQTL1_4	249.23	PZA00856.2–SYN7984	296,984,345–299,045,202	5.36	19.36	BLSB, SLB, SDM	3	2	59
5	MQTL1_5	344.13	IDP8376–magi24643	282,611,390–286,928,924	8.75	16.50	SLB, GLS	2	2	121
6	MQTL2_1	13.42	PZE.102005877–PZE.102006513	31,09,831–33,94,534	2.98	15.70	GLS, NCLB	6	2	9
7	MQTL2_2	37.83	SYN22129–SYN7603	11,891,593–12,110,007	4.60	17.90	GLS	9	2	10
8	MQTL2_3	112.26	PZE.102136708–PZE.102139664	186,502,150–188,465,323	2.80	19.47	SLB, GLS	4	2	27
9	MQTL2_4	150.28	SYN12108–SYN29886	213,830,471–216,859,153	6.64	11.77	BLSB, HS, GER	3	3	105
10	MQTL2_5	175.95	PZE.102184023–PZE.102187151	227,301,170–231,821,851	0.00	30.98	HS	5	2	97
11	MQTL3_1	19.99	PZE.103006822–SYN15334	38,35,098–39,15,188	0.00	10.50	GLS	3	1	5
12	MQTL3_2	25.19	PZE.103008604–SYN5652	47,10,841–47,22,242	0.40	6.60	FSR	1	1	0
13	MQTL3_3	107.67	PZE.103112740–PZE.103116145	172,489,869–175,942,135	3.43	14.11	GLS, SDM, SLB, HS	7	4	73
14	MQTL3_4	160.53	PZE.103154632–SYN8281	205,769,270–209,218,769	4.33	19.67	GLS, FER, AER	7	4	102
15	MQTL3_5	211.00	IDP5036–TIDP5726	206,225,251–208,615,874	7.48	12.26	NCLB, GLS	3	2	54
16	MQTL4_1	0.020	TIDP5109–csu221	6,93,736–20,60,577	13.72	7.30	FSR	1	1	45
17	MQTL4_2	40.56	PZE.104011437–SYN31962	95,94,061–10,235,511	2.28	12.81	GLS	8	3	6
18	MQTL4_3	117.62	IDP2418–IDP1943	154,287,989–155,404,441	2.67	13.63	FER	2	2	19
19	MQTL4_4	159.46	PZE.104148975–SYN3995	236,268,174–236,710,843	1.57	14.12	NCLB, GLS	5	2	8
20	MQTL4_5	180.74	SYN26001–SYN16460	239,766,096–240,029,515	2.59	11.90	GLS	3	1	14
21	MQTL5_1	27.10	SYN3122–PZE.105015345	59,29,167–65,75,359	6.60	12.40	GLS	1	1	28
22	MQTL5_2	79.08	PZE.105090424–PZE.105100848	127,638,855–151,669,661	3.60	7.70	GLS	1	1	247
23	MQTL5_3	90.60	PZE.105112491–PZE.105113193	169,497,597–170,106,632	0.55	6.70	FSR	1	1	6
24	MQTL5_4	119.59	PZE.105142195–SYN34083	196,601,246–197,996,146	1.39	15.06	AER, FER, NCLB	3	3	40
25	MQTL6_1	17.16	PZE.106033899–PZE.106034370	79,357,043–80,796,527	0.00	6.60	FSR	1	1	13
26	MQTL6_2	35.41	PZE.106053653–PZE.106054182	104,274,311–105,017,386	2.14	10.80	GLS	2	1	9
27	MQTL6_3	45.04	PZE.106057747–PZE.106061581	111,839,865–112,545,401	2.14	10.60	GLS	1	1	16
28	MQTL6_4	55.46	SYN7542–PZE.106070681	124,714,110–125,149,538	0.77	8.53	GLS, FSR	3	2	13
29	MQTL6_5	80.7	SYN22585–SYN26322	151,420,909–152,464,246	0.80	8.10	SDM	1	1	38
30	MQTL7_1	45.92	PZE.107051602–PZE.107064289	101,287,600–121,285,300	11.69	11.21	GLS, PLS	5	2	124
31	MQTL7_2	103.29	PZE.107117132–PZE.107118197	164,496,101–164,988,124	0.24	12.25	GLS	4	2	106
32	MQTL8_1	4.04	SYN10053–PZE.108002818	1,830,837–2,897,539	37.97	10.35	GLS	1	1	21
33	MQTL8_2	37.94	PZE.108010479–PZE.108010837	10,926,467–11,663,404	0.38	12.80	NCLB	1	1	15
34	MQTL8_3	90.92	PZE.108061262–PZE.108083339	109,359,760–139,716,732	23.47	10.97	GLS	2	1	251
35	MQTL8_4	117.62	PZE.1080112595–PZE.108113626	164,067,675–164,450,538	3.14	30.27	NCLB, GLS	4	3	21
36	MQTL8_5	131.25	PZE.108119303–SYN36527	167,004,561–167,538,578	0.65	9.50	CS	1	1	17
37	MQTL9_1	40.55	PZE.109011840–PZE.109013469	12,576,278–13,761,895	6.56	14.90	GLS, SDM	3	2	23

Table 1 (*continued*)

No.	Meta-QTL	Position (cM)	Flanking marker	Physical position of flanking markers (bp)	CI (95%)	Average phenotypic variance	Diseases	No. of initial QTL	No. of population	No. of candidate genes
38	MQTL9_2	69.01	PZE.109051428–PZE.109054974	89,924,624–96,459,804	0.10	32.49	GLS	7	3	91
	Total					13.83		128		1910

HS, head smut; GLS, Gray leaf spot; FER, Fusarium ear rot, FSR, Fusarium seedling rot; PLS, Phaeosphaeria leaf spot; NCLB, Northern corn leaf blight; SLB, Southern corn leaf blight; BLSB, Banded leaf and sheath blight; SDM, Sorghum downy mildew; AER, Aspergillus ear rot; GER, Gibberella ear rot; CS, Common smut.

candidate genes were identified in MQTL 8_3 followed by 247 in MQTL 5_2, while a minimum of five candidate genes were identified in MQTL3_1. Furthermore, >100 candidate genes were present in 6 regions, *viz.*, MQTL 8_3 (251), 5_2 (247), 7_1 (124), 1_5 (121), 2_4 (105), and 3_4 (102), and ≤10 candidate genes were present in 8 regions, *viz.*, MQTL 2_2 (10), 2_1 and 6_2 (9), 4_4 (8), 1_3 (7), 4_2 and 5_3 (6), and 3_1 (5). In MQTL3_4, no candidate genes were identified as being of very low CI, or maybe the region is not well characterized functionally (Tables 1, S3). The average CI of detected MQTL (4.66) and preliminary QTL (15.77) indicates that the CI of each projected MQTL was significantly reduced compared to the CI of preliminary QTL reported in the experimental studies.

Various candidate genes for FDR previously identified in different studies in maize were found to be present in different MQTL regions (for particular diseases in respective MQTL), i.e., *CCoAOMT*, ATP binding protein, Sugar transporter family protein, *Glutathione-S-transferase* for GLS. Various FDR governing putative candidate genes like leucine-rich repeat receptor-like kinase (*LRR-RLK*), pathogenesis and disease-related proteins, *MYB* TFs, *thioredoxin* superfamily protein, *mitogen-activated protein* (*MAP*) *kinases*, *NAC*-domain containing proteins, basic helix-loop-helix (*bHLH*) TFs, etc. were present in MQTL. It supports the significance of each MQTL identified in this study, which has been further discussed, elaborately.

However, some MQTL regions which were associated with a single disease, harboured candidate genes for resistance against other diseases (genes encoding actin-depolymerizing factor in MQTL1_4, WD-40 repeat/transducin family protein in MQTL 1_5, 3_5, 5_2, 7_1, 7_2, 8_2, 8_2, 8_4 and 9_2, lipases in MQTL 5_2, 6_5 and 8_3, alpha/beta-hydrolases superfamily protein in MQTL 3_3, 3_4, 3_5, 5_4, 6_1, 7_1, 8_3, Calmodulin-binding protein in MQTL 1_5, 2_3 and 8_1) as well.

3.3. Verification of MQTL with MTAs of GWAS studies for FDR in maize

The physical positions of MTAs from existing GWAS studies for fungal diseases in maize were compared with the physical positions of the interval of MQTL (Table S4). A total of 14 MTAs from different studies were compared for their co-location with 8 MQTL (Fig. 1). Hence, only 23.68% (9 out of 38) MQTL could be verified with GWAS MTAs. Interestingly, all 14 MTAs for a particular disease matched with the QTL for the same disease in MQTL regions. MQTL 1_2, co-located with maximum MTAs (3) followed by two MTAs in MQTL1_5 (SNP9 and SNP10), in MQTL3_3 (SNP20 and SNP21) and MQTL5_4 (Chr. 5_195185908 and S5_197707198). The positions of three MTAs/SNPs as reported by previous study [44], the underlying MQTL1_2 is very close but differs by a single base pair (S1_179367615, S1_179367616 and S1_179367617) and hence can be considered as hotspot for FSR resistance. In general, the number of the MTAs in the MQTL interval correlated well with the number of diseases contained in the particular MQTL. However, the presence of number of initial QTL and the number of candidate genes in MQTL interval did not correlate well with the number of MTAs co-located in MQTL.

3.4. Elucidating the role of signal transduction factors and constitutive genes in the identified MQTL

Since LRR and MYB proteins are known to be involved in signal transduction, specifically these were analyzed and the expression of 25 LRR proteins and 18 MYB proteins found in the MQTL regions (Fig. S4). Nine genes (*Zm00001d015971*, *Zm00001d015974*, *Zm00001d016000*, *Zm00001d016000*, *Zm00001d016197*, *Zm00001d038224*, *Zm00001d010447*, *Zm00001d010480* and *Zm00001d046488*) were observed to be expressing at relatively higher levels throughout the plant developmental stages (Fig. 2). Out of these, *Zm00001d038224* showed the highest expression levels at the most of plant developmental stages, except embryo and certain stages of whole seed and leaf (Fig. 2). Analysis of protein function showed that all the nine proteins, except *Zm00001d016000,* contain ribonuclease inhibitor domain.

Given the importance of ribonucleases in the development of resistance against the pathogen, efforts were made to search for the presence of RNases in the identified MQTL regions. Six RNases (*Zm00001d053718*, *Zm00001d042612*, *Zm00001d020476*, *Zm00001d043743*, *Zm00001d015999* and *Zm00001d007384*) were found, of which all (except *Zm00001d007384* and *Zm00001d042612*) showed high constitutive expression throughout the stages of development, implicating their importance in imparting FDR in maize (Fig. S5).

The putative genes underlying MQTL regions were assessed for the expression at various stages of plant growth and development, *viz.*, embryo (5 stages; 16, 18, 20, 22 and 24 days after pollination; DAP), endosperm (6 stages; 12, 16, 18, 20, 22 and 24 DAP), whole seed (10 stages; 2, 4, 6, 8, 10, 12, 18, 20, 22 and 24 DAP), crown root (4 stages; Node 1_3, 4, 5 of V7 stage, Node 5 of V13 stage), pollinated internode (2 stages; 24 and 30 DAP), pollinated leaf (5 stages; 0, 12, 18, 24 and 30 DAP), V3 (2 stages; stem and shoot apical meristem/SAM and topmost leaf), V5 (4 stages; bottom of transition leaf, first elongated internode, shoot tip and tip of stage 2 leaf), V7 (2 stages; bottom and tip of transition leaf), V9 (5 stages; 8th leaf, 11th leaf, 4th elongated internode, immature leaf, 13th leaf), V13 (1 stage; immature tassel), V18 (2 stages; immature cob and meiotic tassel) and VT (1 stage; 13th leaf). The gene expression values taken from MaizeGDB Atlas were arranged with a value greater than or equal to 1.0 representing maximal expression. Genes having a constitutive pattern of expression at most or all stages of plant development were noted. A total of 67 constitutively expressed genes were observed (Table S5).

To elucidate the role of these constitutively expressed genes, expression data was analysed with the Plant Reactome Database [37]. Metabolic pathways for five genes (*Zm00001d006667*, *Zm00001d006751*, *Zm00001d017467*, *Zm00001d034629* and *Zm00001d043727)* were observed. Apart from the TCA (tricarboxylic acid) cycle, cofactor synthesis, secondary metabolism and amino acid metabolism were found to be involved (Fig. 3). The methylerythritol phosphate pathway, kievitone and leucine biosynthesis were found to be activated for the above-mentioned genes. Rest of the genes either map to unknown pathways or constitute structural components like cytoskeleton.

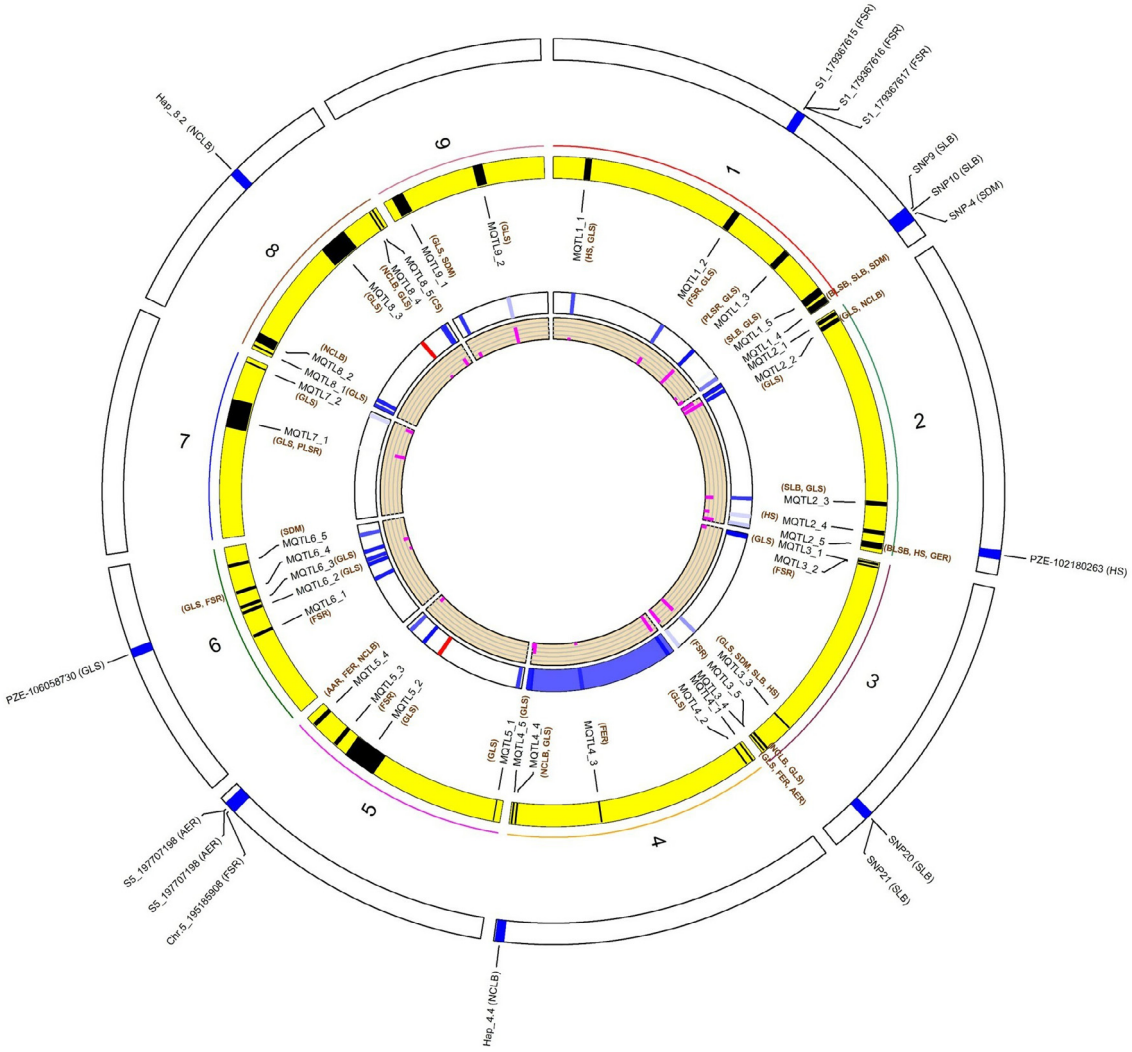

Fig. 1. Circos plot representation for the distribution of MQTL and verification with MTAs for FDR in GWAS studies in maize. The innermost track (histogram) represents the initial number of QTL in the MQTL. The next track (heatmap) shows the number of candidate genes identified in the MQTL interval (Red represents maximum; blue intermediate while light blue the minimum values). The next track represents the chromosomal positions of the MQTL and the underlying disease resistance; The outermost tracks represent the chromosomal positions of the MTAs for particular diseases that overlap the corresponding MQTL interval (verify the genomic regions for disease resistance). In the case of MQTL containing only one initial QTL, the bar is not shown in the innermost track due to limitation of the software. Refer to Table 2 for detailed information.

4. Discussion

Diseases in maize are the major biotic stress which reduces both yield and quality of maize grains [45]. The development of disease-resistant cultivars is the most effective approach to safeguard crops against various kinds of devastating diseases. There are many *R*-genes that have been cloned [46] and proved effective but their resistance potential or power is non-durable particularly with a single gene [47]. Therefore, the combination of *R*-genes with other quantitative resistance genes is considered an efficient approach for building durable resistance (broader specificity) against pathogens [48]. In the past three decades, various QTL were identified in maize against various fungal diseases. Therefore, using previously reported QTL, the present study was aimed to utilize the information on the major QTL for FDR in maize. The purpose was to identify "MAS-friendly MQTL" and putative candidate genes for single or multiple FDR.

4.1. Association of MQTL and candidate gene identification for fungal diseases in maize

The candidate genes reported in the present study have been implicated in other studies as well (Table S6). Among the identified candidate genes, *CCoAOMT* gene (encodes *S-adenosyl-L-methionine-dependent methyltransferases* superfamily protein) has been reported to govern GLS [49] and SLB resistance [49,50]. The role of this enzyme has been elucidated in the phenylpropanoid pathway and lignin production [49]. The transporter genes also play an important role in plant defense mechanisms (pathogens require transporters or channels to move between the cells) as evident in Arabidopsis [51]. In our study, ATP binding protein-encoding genes in MQTL3_3 (*Zm00001d042644*) and MQTL8_3 (*Zm00001d010525*) and sugar transporter family protein gene (*Zm00001d020463*) in MQTL7_2 were identified as key transporter genes. Another candidate gene, *Glutathione-S-transferase* for GLS resistance identified in

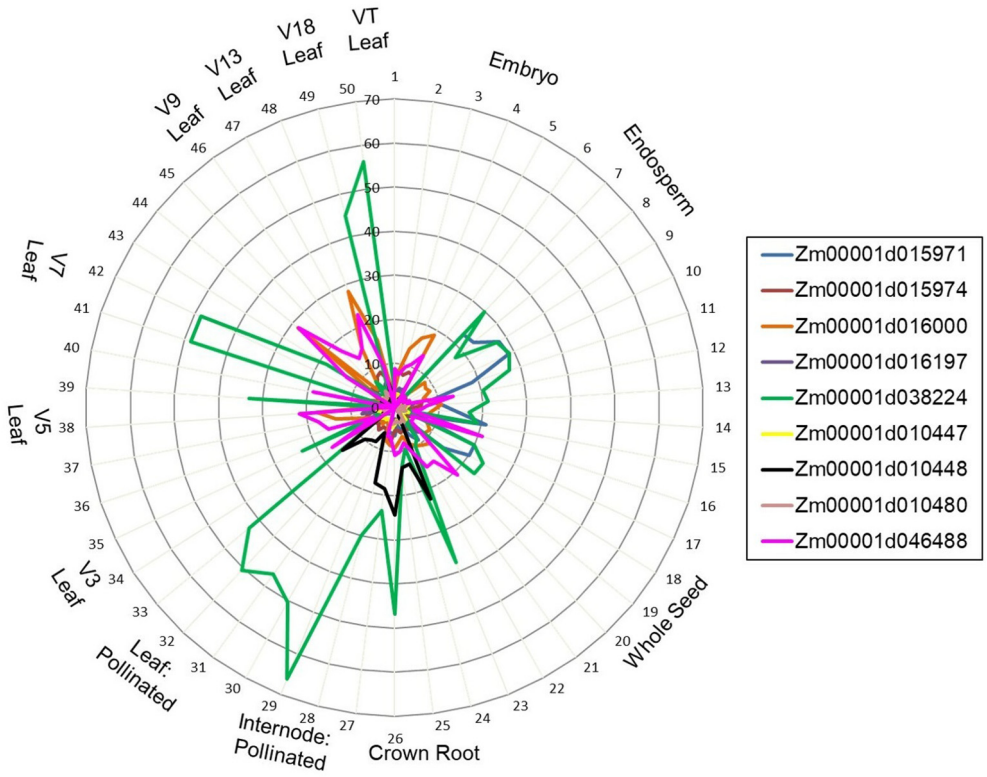

Fig. 2. Radar-plot of nine constitutively expressed proteins with LRR/MYB domain. The developmental stages include embryo (5 stages (1–5); 16, 18, 20, 22 and 24 DAP)); endosperm (6 stages (6–12); 12, 16, 18, 20, 22 and 24 DAP); whole seed (10 stages (13–22); 2, 4, 6, 8, 10, 12, 18, 20, 22 and 24 DAP); crown root (4 stages (23–26); Node 1_3, 4, 5 of V7 stage, Node 5 of V13 stage); Pollinated internode (2 stages (27–28); 24 and 30 DAP); Pollinated leaf (5 stages (29–33); 0, 12, 18, 24 and 30 DAP); V3 (2 stages (34–35); Stem & SAM and Topmost Leaf); V5 (4 stages (36–39); Bottom of transition leaf, First elongated internode, shoot tip and tip of stage 2 leaf); V7 (2 stages (40–41); Bottom and tip of transition leaf); V9 (5 stages (42–46); 8th leaf, 11th leaf, 4th elongated internode, immature leaf, 13th leaf); V13 (1 stage (47); Immature tassel); V18 (2 stages (48–49); Immature cob and meiotic tassel); VT (1 stage (50); 13th leaf). The genes are represented in the figure according to the color legend shown on right.

this study agrees with an earlier report [52]. It is reported to be induced during the early stages of fungal infections and reduces oxidative stress through reactive oxygen species detoxification [53].

The MYB TFs identified in various MQTL play a significant role in plant defense against diseases, i.e., *ATMYB30* induces the hypersensitive response (HR) during pathogen attacks [54]. The MYB domain-containing gene *TaLHY* has been reported effective against ear heading and stripe rust fungus in wheat [55]. The other genes like *chalcone synthase* family protein in MQTL5_2 (*Zm00001d016014*), *carotenoid cleavage dioxygenase* on MQTL1_5 (*Zm00001d034075*), *resistance protein homologs/disease resistance-responsive* family protein in MQTL3_3 (*Zm00001d042633*), NBS-LRR disease resistance protein in MQTL4_1 (*Zm00001d048613*) were also found during gene annotation analysis. *NBS-LRR* are kinds of *R*-genes in plants that work as an immune sensor and their interaction with specific effector proteins of pathogens induces signalling pathways to trigger innate immunity in the plants [56]. The role of *NBS-LRR* encoding different kinds of *Pi*, *Pik*, etc. genes is documented for blast resistance in rice [57,58]. Recent study [59] also reported the role of *NBS-LRR* in imparting powdery mildew resistance in *Vitis vinifera*. Furthermore, the observed genes in various MQTL regions in the present study were previously confirmed as candidate genes for GLS in different studies [24,52].

The presence of candidate gene, *CCoAOMT* in MQTL3_3 and *Glutathione-S-transferase* gene in MQTL1_5 and other genes identified in MQTL regions for SLB resistance are in accordance with candidate genes identified for SLB resistance by another researchers [50].

The remorin, an F-box-like protein that has been reported as a candidate gene for NCLB, plays a significant role in plant-fungal interactions [60]. The F-box proteins found in MQTL3_5 and MQTL5_4 were associated with NCLB resistance. Another candidate gene *ZmWAK-RLK1* has been reported against NCLB [45,61]. These *wall-associated kinase* genes were found in MQTL4_1, MQTL6_2 and MQTL8_3. Additionally, another NCLB resistance-associated candidate genes like protein kinase superfamily in MQTL5_4 and MQTL8_4 were found. The different protein-encoding genes responsible for NCLB resistance identified in the MQTL regions are in agreement of previous reports (Table S6).

The *ZmWAK* for HS was present in MQTL2_4 (*Zm00001d006693*) similar to earlier reported *ZmWAK* gene in *qHSR1* locus for HS resistance [62]. The other associated genes encoding MADS-box transcription factor family protein in MQTL3_3, *NB-ARC* domain-containing disease resistance protein in MQTL2_4, MQTL2_5 and basic leucine zipper (*bZIP*) transcription factor family protein in MQTL2_5 have been reported as candidate genes for HS resistance in previous studies and their role in plant defense response is well documented [63,64]. Therefore, these important MQTL can be further validated in maize germplasm for NCLB and HS resistance.

In addition to HS resistance, the MQTL2_4 also had QTL for BLSB and GER. MQTL2_4 harbors the F-box-like gene (*Zm00001d006761*) like *ZmFBL41*, which has been reported as a resistant gene against BLSB [65]. This region also contained *MAP-kinase* a candidate gene in rice against sheath blight disease [66], and *RECEPTOR-like protein kinase* encoding gene, a candidate gene for sheath blight resistance in rice that recognizes pathogen-associated molecular patterns (PAMPs) and activates the resistance pathways against a wide

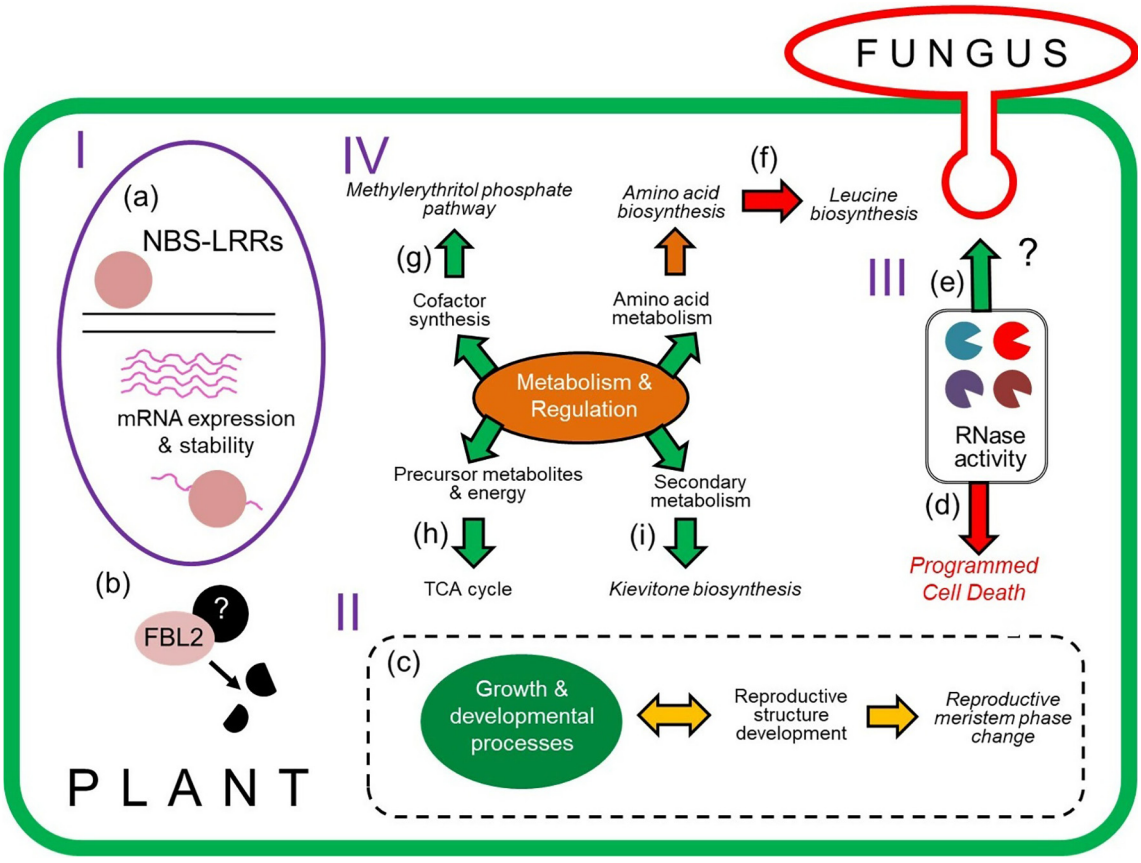

Fig. 3. Schematic representation of the role of maize constitutive gene network in fungal resistance. I. (a) Nuclear expression is initiated majorly by *NBS-LRRs*. (b) FBL2, structurally similar to FBL41, was found to be highly expressed in most of the plant developmental stages. II. (c) Amongst the growth & developmental processes, reproductive structure development is found as a major phenotypic change. III. RNases may play a dual part in inducing programmed cell death to activate systemic plant defenses (d) or in reducing the fungal mRNA pool (e). IV. Metabolic reprogramming leads to both positive and negative regulation of antifungal resistance. (f) Leucine can be potentially utilized by fungus for plant infection. (g) Cofactor synthesis, (h) energy metabolism and (i) kievitone biosynthesis are important in modulating plant response to fungal challenges.

range of pathogens [62,4,66]. The MQTL2_4 also contained gene encoding an ARM-repeat superfamily protein having oxygen transporter activity which plays an important role in various signal transduction pathways under stress conditions [67], zinc finger protein-encoding genes were also previously reported for BLSB [65]. In addition, three genes encoding alpha/beta-hydrolases superfamily protein which exhibit varied catalytic functions for defense mechanism and hormonal regulations [68] were also located in MQTL2_4 as candidate genes for GER [69]. The candidate genes on MQTL2_4 like *Zm00001d006722* belonging to Nucleotide-diphospho-sugar transferases superfamily protein, *Zm00001d006711* gene having Cysteine-rich transmembrane (*CYSTM*) domain have been reported to govern the biotic and abiotic stress response [70].

The *bHLH* DNA-binding superfamily protein gene is involved in plant developmental processes and defensive approaches through interaction between various signaling pathways [71], which was present in MQTL1_2 (*Zm00001d031167*) and MQTL3_4 (*Zm00001d043699*, *Zm00001d043706*). The bHLH TF *GmPIB1* confers resistance against Phytophthora root rot in soybean [72]. The WRKY-DNA binding protein genes in MQTL3_4 (*Zm00001d043663*), QTL4_3 (*Zm00001d051328*) and MQTL6_1 (*Zm00001d036244*) have been reported to be involved in biotic and abiotic stresses as it regulates defense-related genes through signal transduction pathways [73,74]. The overexpressed *WRKY13* gene in rice resulted in enhanced resistance against *Magnaportha grisea* (cause fungal blast disease) through activation of salicylic acid-responsive genes and suppression of jasmonic acid signaling pathways [75]. The ARM-repeat protein encoding gene was also present in MQTL1_2 and MQTL4_1. The MQTL1_2 also had three osmotin protein genes, which are exclusively associated with FSR. The NB-ARC containing disease resistance protein-encoding gene in MQTL4_1 was previously identified as a candidate gene for FSR [76]. The *Zm00001d051340* and *Zm00001d017466* genes of an ethylene-responsive element were present in MQTL4_3 (linked with only FER) and MQTL5_4. Hence, these candidate genes identified in MQTL regions were associated with FSR/FER and corroborated with findings of previous studies (Table S6). In this study, two MQTL regions (3_4 and 5_4) were identified for resistance against AER (cause aflatoxin accumulation). These both regions contained numerous candidate genes for AER and aflatoxin accumulation (Table S6), which have been proved effective against these diseases in earlier studies.

The MQTL3_3 has a candidate gene encoding *2-oxoglutarate* (*2OG*) *Fe(II)-dependent oxygenase* superfamily protein, *LRR-protein kinase* and MQTL1_4 has a gene encoding *P-loop containing nucleoside triphosphate hydrolases* superfamily protein [77] which were observed to be associated with SDM resistance. The MQTL7_1 has *serine/threonine-protein kinase* encoding gene, *LRR-RLK*, *protein kinase* superfamily protein gene, *DNAJ* heat shock protein gene, zinc finger protein, *glycosyl hydrolase* genes, which have been identified in 528 wheat landraces [78] and hard winter wheat [79] for major leaf spot diseases of wheat including Stagonospora nodorum blotch (SNB) caused by *Phaeosphaeria nodorum*. Hence, the

presence of these genes in MQTL7_1 confirms its association with PLS resistance in maize.

The presence of *LRR-transmembrane protein kinase*, zinc finger protein, glycine-rich protein family in MQTL8_5 is in agreement with candidate genes identified for CS resistance [80]. The *RLK*s have been categorized into different kinds based on specific motifs present in their ligand-binding domain. They activate through phosphorylation after ligand attachment and trigger various defense-related signaling pathways like PAMP-immunity pathways (PTI) [81]. Therefore, the role of these genes in plant defense response is well documented.

In the case of MQTL for multiple FDR, MQTL3_3 harbors *Zm00001d042633* gene which is a resistance gene analog (*RGA*) like disease resistance-responsive family protein gene, wound-responsive family protein gene *Zm00001d042615*, *RING/FYVE/PHD* zinc finger superfamily protein gene *Zm00001d042613* [50], two genes *Zm00001d042591*, *Zm00001d042618* for *MADS*-box transcription factor family protein genes [64] and *NB-ARC* domain-containing disease resistance protein gene *Zm00001d042633* [64,82]. The *NAC*-TF was present on MQTL3_3 (*Zm00001d042580*, *Zm00001d042609*) and 1_4 (*Zm00001d034601*). These *NAC* (NAM, ATAF, and CUC) TFs play a very important role in linking various hormonal signalling pathways i.e., abscisic acid, jasmonic acid, salicylic acid, ethylene, and reactive oxygen species to various biotic and abiotic stresses [83]. The phosphatidylinositol transfer family protein was located in MQTL3_3 (*Zm00001d042664*) and 3_4 (*Zm00001d043733*) whose role has been investigated as lipid transfer protein in Arabidopsis for resistance against powdery mildew [84]. The *ABC*-transporter family protein gene (*Zm00001d043722*) in MQTL3_4 plays the role of transporter and channel forming gene in defense response by restricting the movement of the pathogen between the cells [50]. Other genes namely RmlC-like cupins superfamily protein gene (*Zm00001d043710*), and BED zinc finger gene (*Zm00001d043743*), *LRR-RLK* gene (*Zm00001d043648*), and Auxin efflux carrier family protein gene (*Zm00001d043660*) in MQTL3_4 also act as transporter proteins to provide defense response to biotic stresses [85] as demonstrated in wheat against powdery mildew infection [86]. The other genes sphingomyelin synthase-like domain (*Zm00001d034600*) and actin-depolymerizing factor genes (*Zm00001d034643*, *Zm00001d034644*) were found in MQTL1_4. The gene encoding *WD-40* repeat family protein/transducin family protein were identified in MQTL 1_4 (*Zm00001d034592*, *Zm00001d034633*) and MQTL3_4 (*Zm00001d043682*, *Zm00001d043683*). The alpha/beta-hydrolases superfamily protein genes were present in MQTL2_4 (*Zm00001d006720*, *Zm00001d006778*, *Zm00001d006779*), MQTL3_3 (*Zm00001d042596*), MQTL3_4 (*Zm00001d043680*) and MQTL5_4 (*Zm00001d017470*). The MQTL 5_4 has *haloacid dehalogenase-like hydrolase* (*HAD*) superfamily protein gene *Zm00001d017502*. These candidate genes are correlated with the previously described genes and have an association with various economically important fungal diseases [50,52,64,69,87]. Therefore, these five MQTL (governing resistance against three to four diseases) namely 1_4, 2_4, 3_2, 3_4, and 5_4 can be further tested/validated for resistance against other fungal diseases in maize in future studies.

The co-existence of MTAs (identified in GWAS studies) within/around the MQTL region confirms the presence of disease resistance candidate genes in such genomic regions. These MQTL verified with MTAs should be given priority for mining candidate genes governing FDR in maize and subsequent validation through expression studies in related germplasm. The lack of verification for remaining MQTL with GWAS-MTAs can be attributed to the diversity of the genetic material across studies and limited GWAS studies on the diseases for such MQTL. It may also indicate that the QTL identified in mapping studies could be cultivar-specific and not shared across another germplasm.

4.2. Understanding the constitutive gene network in the elucidated MQTL

Constitutive plant defense forms the first-line strategy against a number of pathogens. Various physio-chemical barriers are genetically encoded and play an important role in FDR either as standalone systems or in cross-interaction with an induced mode of defense. In fact, since the induced mode of defense requires a careful selection of plant *R*-genes for the fungal *Avr*-genes, the constant evolution of the fungal proteins may pose a difficulty in keeping a track of the likely evolution of a fungal toxin, thereby resulting in the delayed breeding efforts against the future challenges. On the other hand, constitutive plant defenses are broad-spectrum in nature, involve parameters like permeability of cuticle, the thickness of the cell wall, amount of lignification, etc. [57] and hence can provide durable resistance against particular disease or multiple FDR. The varieties bred for constitutive plant defense, therefore, may be able to protect against multiple biotic stresses, as well as offer adequate resistance for a larger time span than a single, selective *R-Avr* gene-based interaction. To benefit from the vast data generated in the current study, the genes in MQTL were analyzed for their expression profile using MaizeGDB Expression Atlas. Fig. 3 represents a schematic model of the role of maize constitutive gene network in FDR. Of particular interest in this regard are the 25 LRR proteins and 18 MYB domains, which are known to sense outer milieu and facilitate cellular response accordingly [88,89]. Of these 43 genes, nine genes expressed constitutively throughout the various stages of plant development (Figs. 2, 3A, S4). One gene, *Zm00001d038224*, which encodes an *FBL2* ribonuclease inhibitor exhibited the highest expression for most of the stages of plant development (Fig. 2).

Analysis of the nine proteins, *viz.*, *Zm00001d015971*, *Zm00001d015974*, *Zm00001d016000*, *Zm00001d016197*, *Zm00001d038224*, *Zm00001d010447*, *Zm00001d010480*, *Zm00001d010448* and *Zm00001d046488* for the best hits obtained with the CATH family database showed that all, except *Zm00001d016000*, contain a domain of ribonuclease inhibitor. An F-box protein, with ribonuclease inhibitor domain, encoded by *ZmFBL41*, was previously reported to impart resistance against BLSB [65]. *ZmFBL41* shows marked sequence and structural similarity to *ZmFBL2* (*Zm00001d038224*) found in our study, implicating similar mechanisms of action and making *ZmFBL2* a potential target for allele mining (Fig. S6). On the other hand, *Zm00001d038224*, and *Zm00001d046488* have been identified as the two most expressive genes, throughout the plant developmental stages. The similarity of *Zm00001d038224*, *FBL2* gene to *FBL41* previously implicated in BLSB resistance, indicates the utility of allele mining of *Zm00001d038224* for fungal resistance (Fig. 3B).

A total of 67 constitutively expressed genes identified in this study were majorly involved in growth and developmental processes, metabolism and regulation, followed by amino acid metabolism and biosynthesis (Table 2). Amongst the growth and developmental processes, reproductive structure development is found as a major phenotypic change (Fig. 3C). Transition to the reproductive phase may be a strategy by the plant to rush to the next generation following a fungal attack. In addition to this, many ribonucleases are important for biotic stress resistance in plants. The studies [90,91] showed RNase activity to be correlated with FDR. It was observed that the network of constitutively expressed *LRR* genes uncovered in our study encodes the ribonuclease inhibitor domain. This may be important to maintain a critical pool of mRNA molecules for the structural re-programming required to fight off the pathogenic invasion. On the other hand, constitutively expressing maize RNases (observed in MQTL for fungal resistance) may be required for activating programmed cell death (Fig. 3D). In addition, they can potentially degrade fungal mRNA molecules

Table 2
Pathways related to constitutively expressed genes in the elucidated MQTL.

No.	Pathway name	Reactions found	Reactions total	Reactions ratio	Genes involved	MQTL region
1.	Kievitone biosynthesis	1	1	0.001	*Zm00001d043727*	MQTL3_4
2.	Methylerythritol phosphate pathway	1	8	0.01	*Zm00001d006751*	MQTL2_4
3.	Leucine biosynthesis	1	4	0.005	*Zm00001d017467*	MQTL5_4
4.	Reproductive meristem phase change	2	14	0.018	*Zm00001d034629*	MQTL1_4
5.	TCA cycle (plant)	1	9	0.012	*Zm00001d006667*	MQTL2_4
6.	Generation of precursor metabolites and energy	1	10	0.013	*Zm00001d006667*	MQTL2_4
7.	Cofactor biosyntheses	1	72	0.093	*Zm00001d006751*	MQTL2_4
8.	Secondary metabolism	1	77	0.099	*Zm00001d043727*	MQTL3_4
9.	Amino acid biosynthesis	1	98	0.126	*Zm00001d017467*	MQTL5_4
10.	Reproductive structure development	2	84	0.108	*Zm00001d034629*	MQTL1_4
11.	Growth and developmental processes	2	116	0.15	*Zm00001d034629*	MQTL1_4
12.	Amino acid metabolism	1	138	0.178	*Zm00001d017467*	MQTL5_4
13.	Metabolism and regulation	4	599	0.773	*Zm00001d006667*, *Zm00001d006751*, *Zm00001d017467*, *Zm00001d043727*	MQTL2_4, MQTL2_4, MQTL5_4, MQTL3_4

(Fig. 3E). From the pathway analysis, of the constitutively expressed 67 genes, amongst the minor pathways are leucine and kievitone biosynthesis, methylerythritol phosphate pathway and reproductive meristem phase change (Fig. 3F, G, I). Generation of metabolites for energy in the TCA cycle is another pathway (Fig. 3H). Amino acids, influenced by nitrogen nutrition and specifically leucine are known factors in regulating biotic stresses [92]. Isoflavone kievitone is known to be an important determinant of defense against pathogens in legumes. The methylerythritol phosphate pathway generates terpenoids, which are involved in chemical defense against pathogens [93]. On the other hand, leucine biosynthesis is required by pathogenic fungi to adapt to starvation and manifest virulence [94]. The mechanism/receptor through which the plant RNases may enter the fungal milieu is yet unknown. In addition, the genes *viz.*, *Zm00001d015971*, *Zm00001d015974*, *Zm00001d016000*, and the genes *viz.*, *Zm00001d010447*, *Zm00001d010480*, *Zm00001d010448*, are clustered close and can be utilized for introgression in elite germplasm.

On one hand, nuclear expression is modulated largely by *NBS-LRR* proteins, acting as both positive and negative regulators of antifungal resistance. RNases may play a dual part in reducing the fungal mRNA pool or inducing programmed cell death to activate systemic plant defenses. Kieu and co-workers [95] described the utility of loss of susceptible (*S*)-gene function for potato resistance against pathogens. Both *R*- and *S*- genes can be utilized for antifungal resistance. The utilized novel variations on *ZmFBL41* were also utilized to develop resistance against BLSB [65]. This study provided the mechanistic basis of the constitutively expressed gene network of "MAS-friendly MQTL" for FDR in maize. This narrowed dataset can be investigated for the identification of novel alleles for broad-spectrum constitutive defense in diverse maize germplasm.

5. Conclusions

The rising demand for food and feed production demands the development of disease-proof high-yielding maize cultivars to safeguard global food security in the longer run. Hence, it is very important to enhance our understanding of the genomic regions accountable for disease resistance, related candidate genes, expressed proteins, and their action mechanism in imparting disease resistance. The meta-QTL analysis revealed 38 MQTL associated with 12 fungal diseases, of which GLS was linked with 26 MQTL. This indicates the complexity of GLS being governed by polygenes. MQTL governing QTL for multiple diseases or multiple QTL for a particular disease are of prime interest to impart broad-range and durable resistance. Hence, five MQTL namely 1_4, 2_4, 3_3, 3_4 and 5_4 for multiple FDR hold immense importance. The problem of the low rate of projection of initial QTL to MQTL would likely improve in the future with the increasing use of high-density SNP markers. The confirmation of the genomic regions (MQTL) for particular diseases with different GWAS studies and the presence of important putative candidate genes in MQTL further validates the functional relationship of these regions in imparting FDR and their subsequent use in future resistance maize breeding programs. Some MQTL harbouring genes for particular diseases also contained candidate genes for other diseases indicating two possibilities. First, the lack of exploration of such genomic regions for other diseases (FER, HS, GER) or remained undetected due to the absence of nearby markers in related studies and second, the presence of common genes across different diseases (indicates strong evidence for the presence of common mechanism across multiple diseases). Several important candidate genes like kinase families, RLKs, TFs (NAC/MYB/bHLH/bZIP), zinc fingers, pathogenesis-related and disease-responsive proteins underlying were identified in MQTL would prove crucial to understanding the molecular mechanism of multiple FDR. The concerted action and intricate balance of the enzymatic activities of the ribonucleases and the ribonuclease inhibitors are necessary for mediating defense responses. This emphasizes the importance of allele mining of the constitutively expressed pathogenesis-related proteins and their interacting molecules. Co-expression analysis using the bulk-seq approach for disease-resistance and susceptible lines can further reveal molecular insights into disease resistance mechanism. The information generated in this study will be a useful guide for maize breeders to use the "MAS-friendly MQTL" in introgression breeding and likely arouse the interest for gene editing of the novel alleles for single or multiple FDR in maize.

Data availability statement

All data generated or analysed during this study are included in this published article and its supplementary information files.

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No funding was availabe for this work.

CRediT authorship contribution statement

Mamta Gupta: Writing – original draft, Conceptualization, Data curation, Validation, Formal analysis, Investigation, Methodology, Software. **Mukesh Choudhary:** Formal analysis, Software, Valida-

tion, Writing – review & editing. **Alla Singh:** Methodology, Software, Validation, Writing – review & editing. **Seema Sheoran:** Investigation, Methodology. **Deepak Singla:** Software. **Sujay Rakshit:** Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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