RESEARCH ARTICLE

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DIPLODIA EAR ROT RESISTANCE QTL IDENTIFIED IN MAIZE (*Zea mays* L.) USING MULTI-PARENT DOUBLE-HAPLOID POPULATION MAPPING

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SUMMARY

Diplodia ear rot (DER) caused by *Stenocarpella maydis* is one of the most important diseases of corn (*Zea mays* L.) that significantly affects its yield performance. A report from Wise *et al*. (2017) suggests yield loss in maize fields varying from 1–2% to as high as 80%. Symptoms of the disease are characterized generally by white mold in the ears, and small, black pycnidia produced by the fungus. Resistance to *S. maydis* is quantitatively inherited and highly influenced by environment. Studies were conducted in 2018 and 2019 wet seasons in Bukidnon, Philippines to locate QTL and marker positions involved in resistance to DER utilizing multi-parent double-haploid populations (MPDHP). A total of 720 in 2018 and 760 lines in 2019 were planted in augmented RCB design, resistant and susceptible checks were included in the study. Each line was artificially inoculated 15 days after silking. Genotyping was done using 15K Axiom® marker chip developed by Syngenta. QTL associated with DER were detected using composite interval mapping (CIM) with mppR package in R focusing on ancestral and multi-QTL effects (MQEs) model. Heritability estimate observed in 2018 (0.468) was relatively similar in 2019 (0.549). Using 2018 Diplodia Ear Rot Percentage (DLERP) data, QTL in chromosome (chr) 5 (394.1-396.5cM) was detected. MQEs model revealed two QTL located at chr 3 (8.3cM, biall) and chr 5 (395.6cM, par). QTL genetic effects for ancestral and MQEs models were estimated at 7.18% and 10.95%, respectively. In 2019, three QTL were identified on chr. 1 (446.5-551.2cM), chr. 5 (349.7-364.6cM), and chr 10 (174.2-213.0cM). MQEs revealed five QTL located at, chr. 1a (481.0cM, biall), chr. 1b (551.2cM, biall), chr. 2 (83.6cM, biall), chr. 5 (356.8cM, biall), and chr 10 (235.0cM, par) linked to DER. QTL genetic effects for the two models were estimated at 9.39% and 15.84%, respectively. Putative QTL was identified on chromosome 5 (*qder5*) across models and across years. Further implementation of multi-parent population (MPP) approach in different genetic backgrounds is recommended to validate consensus QTL identified in chromosome 5 conferring resistance to DER.

Keywords: *Zea mays* L., diplodia ear rot, augmented RCB design, multi-parent population mapping, ancestral model, multi-QTL effect model

Key findings: Identified QTL on chromosome 5 provides a reference point in understanding genetic resistance to diplodia ear rot. Utilization of double haploid based multi-parent population (MPP) structure gives wider application of discovered QTL with different genetic backgrounds.

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INTRODUCTION

Field corn (*Zea mays*) has dozens of uses, but it is most commonly fed to animals. People don't eat field corn directly but instead, field corn are converted to food products such as corn syrup, corn flakes, yellow corn chips, corn starch or corn flour (Nebraska Corn Board, 2019). In the Philippines, the main agricultural crops include rice, corn, coconut, sugarcane, bananas, pineapple, coffee, mangoes, tobacco, and abaca. In terms of productivity, Isabela was the leading province with 1.18 million metric tons while Bukidnon and South Cotabato followed with 0.81 million metric tons and 0.50 million metric tons of corn produced, respectively (Philippine Statistics Authority, 2015). One of the limitations affecting Philippine maize production was biotic and abiotic constraints. Biotic constraints include insect pests such as Asian corn borer (*Ostrinia furnacalis* Guenee), weeds (e.g. *Rottboellia cochichinensis*), diseases (ear rot, stalk rot); and abiotic constraints such as soil infertility and acidity, and soil erosion (Gerpacio *et al*., 2004). To address some of these challenges, tropical maize breeding has been used as the practical strategy to improve maize performance in terms of development of hybrids with disease and insect resistance, and increased grain yield (Elings *et al.,* 1997).

Diplodia ear rot (DER) caused by *Stenocarpella maydis* is an important ear disease prevalent in Bukidnon province, i.e. North Mindanao. The disease is characterized by a white mold in the ears per se or kernels, resulting in the rotting of the ears. Impacts on the crop include: 1) reduced grain quality and yield due to reduced kernel size and test weight; and 2) ears having no harvestable grains whenever infection occurs at early stage of crop growth. In addition, infected kernels have reduced nutritional value (Flett *et al*., 2000). Unlike some other ear rots, Diplodia does not produce a mycotoxin harmful to livestock. As mentioned in the article by ISAAA published in 2018, uplands of Bukidnon province and North Mindanao (in general) has been identified as key hotspots for disease incidence as experienced by corn growers for more than 10 years, giving them poor harvest. Aside from Philippines, ear rots are also prevalent in Kenya (Kedera *et al*., 1994), South Africa, and United States (Xia *et al*., 2011).

Yield loss in maize fields due to DER can vary from 1–2% to as high as 80% (Wise *et al.,* 2017). DER development favours dry conditions during early vegetative growth stages followed by warm, wet weather within the first 3 weeks after silking and extreme yield loss may occur when rainfall is above average from silking to harvest. Current measures that can manage the development of DER in maize fields includes use of resistant hybrids, reducing inoculum in the field using crop rotation, and application of fungicides to minimized stress caused by foliar diseases that indirectly contribute

for DER development (DEKALB, 2016). Combination of a number of management practices (e.g. proper fertilization, controlling ear feeding insects, early harvesting, and storing harvested ears/grain at the proper moisture) and planting only adapted corn hybrids known to have few ear rot problems are recommended in controlling DER (Cartwright *et al*., 2008).

However, the development of hybrids for increased yield with resistance to diplodia ear rot, bacterial stalk rot, and other leaf diseases is very costly and requires many years of conventional breeding. More efforts focused on field testing/yield trials of new lines in different single cross combinations to identify those lines with general combining ability. Nowadays, new methodologies have been tested, doing away with yield trials, and concentrated in marker assisted selection (MAS) without generating and testing thousands of single crosses. MAS uses molecular markers as cofactors (i.e. marker scores) to improve prediction of individual genetics values in a quantitative trait of interest (Stuber, 1997). To be useful to plant breeders, genetic gains made from MAS must be more cost effective and usable in line development than gains made through traditional breeding.

Quantitative trait locus (QTL) analysis entails finding a relationship between DNA polymorphisms (e.g., SNPs) and phenotypic variation (Doerge, 2002). QTL detection methods highly depends on the genetic properties and the type of the population that is used. Historically, QTL detection has been performed in designed experimental populations involving two parental lines or bi-parental crosses. Multi-parent population (MPP) is an alternative type of population that can improve the chances of QTL detection while broadening its diversity, applicability, and resolution. MPPs can be seen as a compromise between bi-parental crosses and association panels (Garin *et al*., 2018). Currently, the two most popular MPP designs implemented in plants are nested association mapping (NAM) and multi-parent advanced generation inter-cross (MAGIC) populations. Development of NAM population involves a series of crosses between a recurrent 'founder' line and a number of alternative 'founders', i.e. single reference mating design. As proposed by Guo *et al*. (2013), a variant of NAM 'multiple references mating design' (NCD-1) can be implemented where a set of diverse parental lines is divided into several groups and then crossed with one of several reference lines. A key factor of utilization of NAM depends on the numbers of lines screened which in turn capture increased genetic recombination in the populations being evaluated. In contrast, the MAGIC design is more complex where several founders are inter-crossed over multiple generations before selfing to generate inbred lines (Bandillo *et al*., 2013; Scott *et al*., 2020). This study focuses on NCD-1 design where it has been successfully set up for several plant species including oil palm (Billotte *et al*., 2010) and maize (Yu *et al*., 2008)

Several forward genetic approaches were known for the purpose of identifying allelic variants associated with complex traits, understanding the molecular mechanisms of these traits and provide selectable markers for favourable alleles for genetic improvement. At present, these approaches include linkage mapping and association mapping (i.e. linkage disequilibrium mapping). In this study, five elite inbred lines from Syngenta were used as parents and paired combinations crossed to generate MPDHP. Linkage analysis was conducted at Syngenta Philippines, Inc. – Seed Development Unit. The objectives of this study were to identify QTL for diplodia ear rot in MPDHP with different genetic backgrounds and map QTL with higher mapping resolution.

MATERIALS AND METHODS

Plant material

Five maize (*Zea mays* L.) elite inbred lines were used as parents for paired crosses to generate the mapping populations. MPDHPs were developed by Syngenta for DER QTL discovery in 2014 and 2015. The reference/donor line 10MZF-L4LW was crossed to 03MZF-CK83, 07MZF-VDF6, and 07MZF-SCH9 considering genetic similarity of <0.80 between parents. Another cross 04MZF-KQF0 X 03MZF-CK83 was included in the discovery plan to generate NCD-1 design, *see* Figure 1. After generating the F_2 seeds, 500 kernels for each of the populations were sent to Jalapa, Guatemala for DH conversion through embryo rescue methodology. In total, 4 doubled haploid populations were used, *see* Table 1. Same set of entries were used in 2018 and 2019 DER screening experiments. All plant materials used in this study are proprietary to Syngenta Philippines, Inc.

Field experiments

A total of 720 in 2018 (planting date: August 25, 2018) and 760 lines in 2019 (planting date: July 16, 2019) were planted using augmented RCB design (*see* Table 2). Due to the limited number of seeds, only checks are replicated in each of the blocks. The MPDHP developed from five elite inbred lines were screened in Dalwangan, Malaybalay, Bukidnon, a hotspot location for DER. Two check entries in 2018 and 4 check entries in 2019 were randomly assigned to plots in a block to obtain an estimate of the experimental error and for use in adjusting performances of test entries for inter-block soil variation. In the experiments being conducted, plots were limited to 42 (18WEDIDS1), 45 (19WEDISM1), and 35 (19WEDISM2) entries per block to manage blocks effects.

In 2019 Wet Season, two experiments were conducted for DER. The composition of the 1st DER experiment was: (1) total of 410 genotypes; (2) two resistant and 2 susceptible checks were used; (3) block size of 45 entries/block (including the checks); and (4) total number of 10 blocks. The composition of the 2nd DER experiment was: (1) total of 350 genotypes; (2) two resistant and 2 susceptible checks were used; (3) block size of 39 entries/block (including the checks); and (4) total number of 10 blocks.

In addition, supplemental information on rainfall, minimum and maximum temperature, and relative humidity were obtained from August 2018 to January 2019. Data were collected from Bureau of Soils and Water Management located in Dalwangan, Malaybalay, Bukidnon.

Diplodia ear rot artificial inoculation and phenotyping

To ensure that there was homogeneous infection of DER, artificial inoculation through ground infected maize kernel method (Jeffers, 2002) was employed. The DER pathogen was initially isolated from infected maize cobs obtained from Dalwangan, Malaybalay, Bukidnon maize field trials during the main planting season of May to June. Approximately 3 to 5 grams of grounded *S. maydis* infested ears was inoculated in the whorl 3 weeks before tasselling. All plants in each row were rated for DER incidence at harvest. Disease severity was noted, but only DLERP was used in analysis. Disease severity was not analyzed because following initial infection, colonization of maize ears, specifically white mycelial infection progresses from base of ear to tip (Crop Protection Network, 2001).

Statistical analysis of phenotypic data

Data analysis was analysed using PB Tools 1.4 developed by the International Rice Research Institute. All quantitative genetic parameters were estimated based on the progenies of each of the double haploid

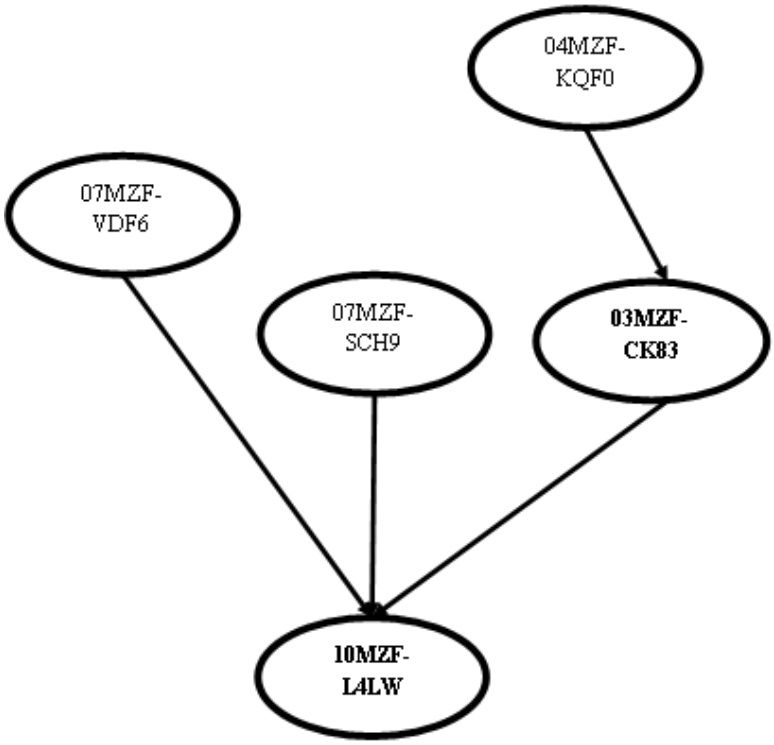

Figure 1. Multiple reference mating design (NCD-1); Male Pool. In this design, a set of diverse parental lines is divided into several groups and then crossed with one of the reference lines (10MZF-L4LW and 03MZF-CK83).

Table 1. List of maize doubled haploid populations used in multi-parent population mapping.

No.	Populations	Parents	DER	Genetic similarity between parents	Population Size
1	15MZF-JRFG	03MZF-CK83/10MZF L4LW	S/R	0.67	210
2	15MZF-JQ94	07MZF-VDF6/10MZF-L4LW	S/R	0.82	232
3	14MZF-PMY6	04MZF-KQF0/03MZF-CK83	R/S	0.65	90
4	15MZF-JQ68	07MZF-SCH9/10MZF-L4LW	S/R	0.71	151

S - Susceptible, R - Resistant

Table 2. Partitioning of the degrees of freedom in an ANOVA table in the analysis of DH populations screened in 2018 and 2019 DER set-up using augmented RCB design.

Source of variation	Degrees of Freedom		
	Trial ID 18WEDS1	Trial ID 19WESM1	Trial ID 19WESM1
Total	756	450	390
Block, B	17	9	9
Entries + Check	721	413	353
Check	1	3	3
Entries	719	409	349
B x check	17	27	27

18WEDS1: 2018 DER Trial set No.1, 19WESM1: 2019 DER Trial set No.1, 19WESM2: 2019 DER Trial set No.2

populations. Least square means were generated for DLERP setting effects to genotype as fixed. Variance components for replication (i.e. checks) and residual were determined by the restricted maximum likelihood (REML) method assuming a random model (Liu *et al*., 2011), see model below.

DLERP = progenies + blocks + error

Heritability on an entry-mean basis was calculated as the ratio of genotypic to phenotypic variance:

$$H^2 = \sigma^2_G / [\sigma^2_G + (\sigma^2_e)],$$

Where, σ_e^2 refers to residual variance.

Genotyping of multi-parent populations

Each of the DH populations and their parental lines were genotyped using modified Affymetrix's GeneChip array. The 15K Axiom® Maize Genotyping Array (designed by Syngenta). The array cross-examines 15,000 selected polymorphic variants representing global diversity in the maize genome (ThermoFisher Scientific, 2018). The array overcomes specific challenges caused by a high content of repetitive elements, high genomic and structural diversity, and low linkage disequilibrium (LD) in maize. The array was validated by using a diverse set of European, US, and tropical/semi-tropical lines from various Dent and Flint gene pools.

Linkage mapping

Analysis for MPDHP linkage mapping was performed using mppR package in R developed by Garin *et al*. (2018). Data processing, marker quality control, QTL detection, QTL effect estimation, and data visualization were performed in R. QTL discovery for DER were done with utilization of 4 different models: i.e. ancestral (anc), parental (par), bi-allelic (biall), and cross-specific (cr). Ancestral model uses relatedness between parents and assumes that parents belonging to the same cluster transmit the same allele. QTL effects were estimated setting to zero the most frequent allele (ancestral/reference) within each connected population. Cross specific model assumes that the QTL alleles that segregate within a particular cross are different from those that segregate in another cross. QTL effects are estimated per cross and all crosses are considered unrelated. Parental model estimates allele effect per parental line, which is considered to be independent of the genetic background. The QTL effect of parent is assumed to be constant in all crosses where this parent has been used. Finally, bi-allelic model assumes that genotypes with the same SNP score transmit the same allele (Blanc *et al*. 2006).

The QTL detection in mppR (Garin *et al*., 2018) was based on the following steps: (a) Optional significance threshold determination by permutation test; (b) cofactors selection by simple interval mapping (SIM); (c) multi-QTL model search using composite interval mapping (CIM); and d) simultaneous evaluation of the selected candidate QTL positions after backward elimination (Zeng, 1994). Optimized threshold value was obtained at 3.97 Permutation test in this study was obtained using the R script below.

```
perm <- mpp_perm (mppData =
mppData, Q.eff = "anc", N = 1000).
```

RESULTS

Response of the populations to DER

Daily minimum temperature mode for 2018 was obtained at 17.7^0C for a period of 6 days compared to 18.5^0C in 2019 for 10 days, *see* Figure 2. Total rainfall was observed in 2018 at a rate of 211.6mm in the month of October, i.e. silking stage compared to 272.1 mm rainfall in 2019 for

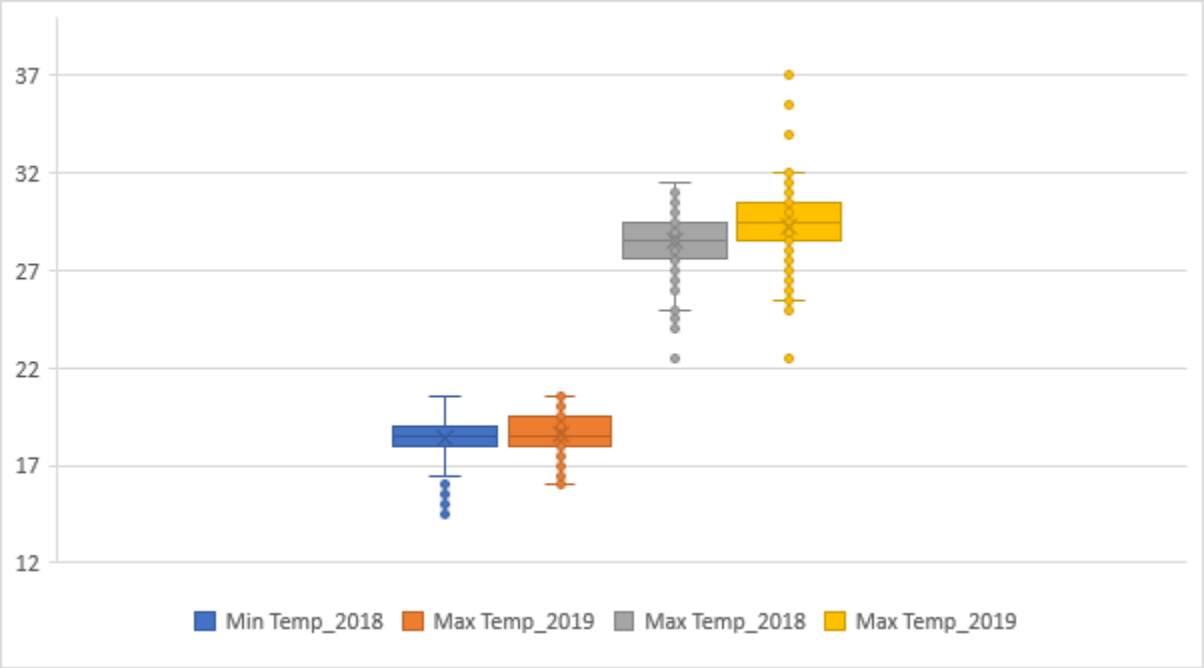

Figure 2. Agroclimatic conditions in Dalwangan, Malaybalay City from August 2018-January 2019 1st season's planting and from July 2019-November 2019 2nd season's planting. Minimum and maximum temperature were observed at 14.5-20.5°C and 22.5-31.5°C. during the 1st season planting while minimum and maximum temperature occurrence in second season's planting were observed at 16.0-20.5°C and 22.5-37.0°C.

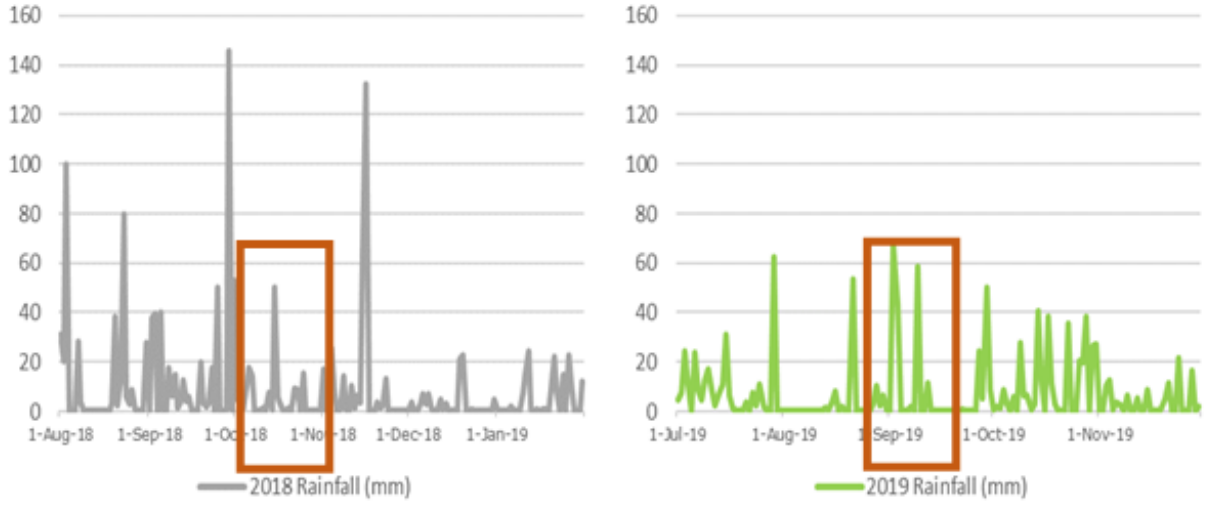

Figure 3. Rainfall conditions in Dalwangan, Malaybalay City from August 2018-January 2019 1st season's planting and from July 2019-November 2019 2nd season's planting. Rainfall occurrence at 0-145.5mm during the 1st season planting while rainfall occurrence in second season's planting was observed at 0-67.5 mm.

Figure 4. Left - Resistant check, Right - susceptible check ear responses to diplodia ear rot.

Table 3. Testing for the significance of genotypic effect and heritability estimates in the analysis of DH populations screened in DER set-up using augmented RCB design.

Trial ID	d.f.	SS	MS	F Value	Residual	Pr (>F)	H est
18WEDS1	460	123339	268.1	2.4	16	0.0216	0.47
19WESM1	403	207738.2	515.5	3.1	26.8	0.0004	0.65
19WESM2	344	190822.6	554.7	1.8	37	0.0168	0.48

18WEDS1: 2018 DER Trial set No.1, 19WESM1: 2019 DER Trial set No.1, 19WESM2: 2019 DER Trial set No.2, H est: Heritability estimate.

the month of September, *see* Figure 3. Relative humidity was comparatively the same in both years at 83.16 and 82.57 for October 2018 and September 2019, respectively. Planting months for the two seasons of screening were different. First season of screening was planted in August and harvested in January 2019. For the second season of screening, trials were planted on July and harvested in November. Data were not combined for the two different seasons, given the differences in planting dates and environmental conditions.

Integrity of screening experiments was validated through DER response of checks. To illustrate, the resistant checks and the susceptible checks were consistent in expressing resistance and susceptible response to DER, see Figure 4. Disease onset was apparent 4 weeks after inoculation and disease response to DER was evaluated at harvest. Analyses of variance in 2018 and 2019 showed significant *p*-value associated with the F statistics of a given effect and test statistics at 0.0216 (18WEDS1), 0.004 (19WESM1), and 0.0168 (19WESM2), *see* Table 3. Heritability estimate observed in 2018 (0.468) was relatively similar in 2019 (0.549). Descriptive statistics including histograms (Figure 5) and qqplots (Figure 6) were obtained for the populations used to determine whether

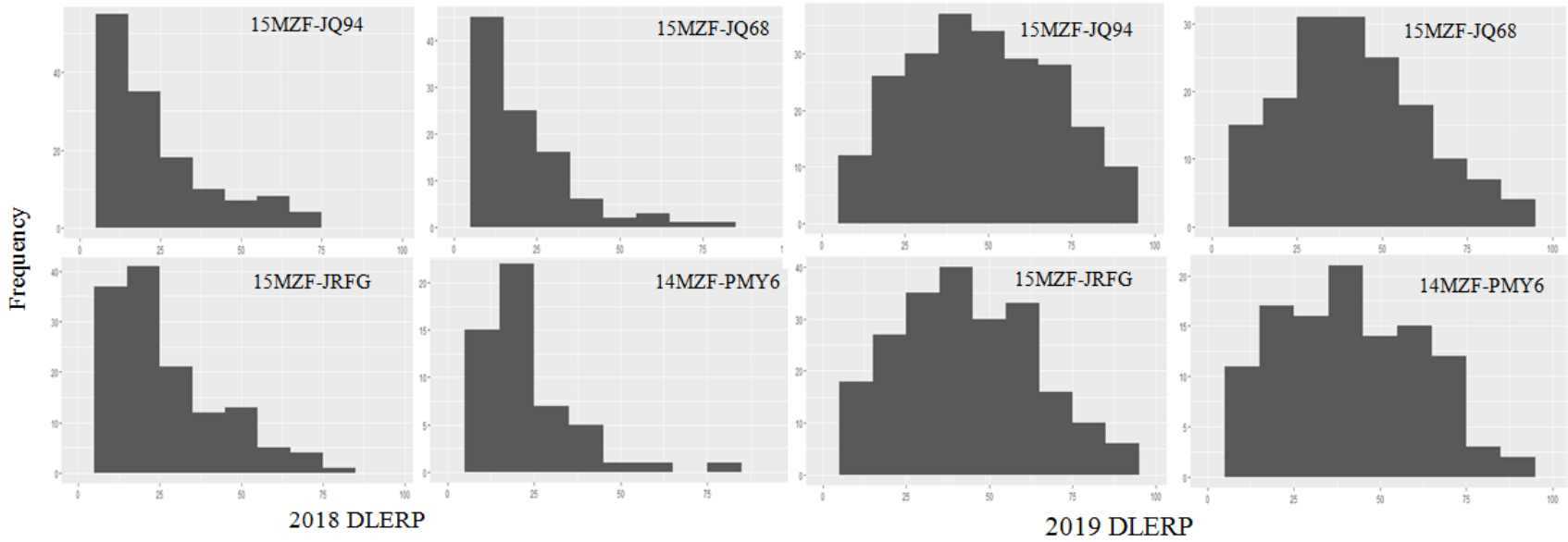

Figure 5. Histogram distributions of MPDHP screened for Diplodia ear rot in 2018 (*left*) and 2019 (*right*).

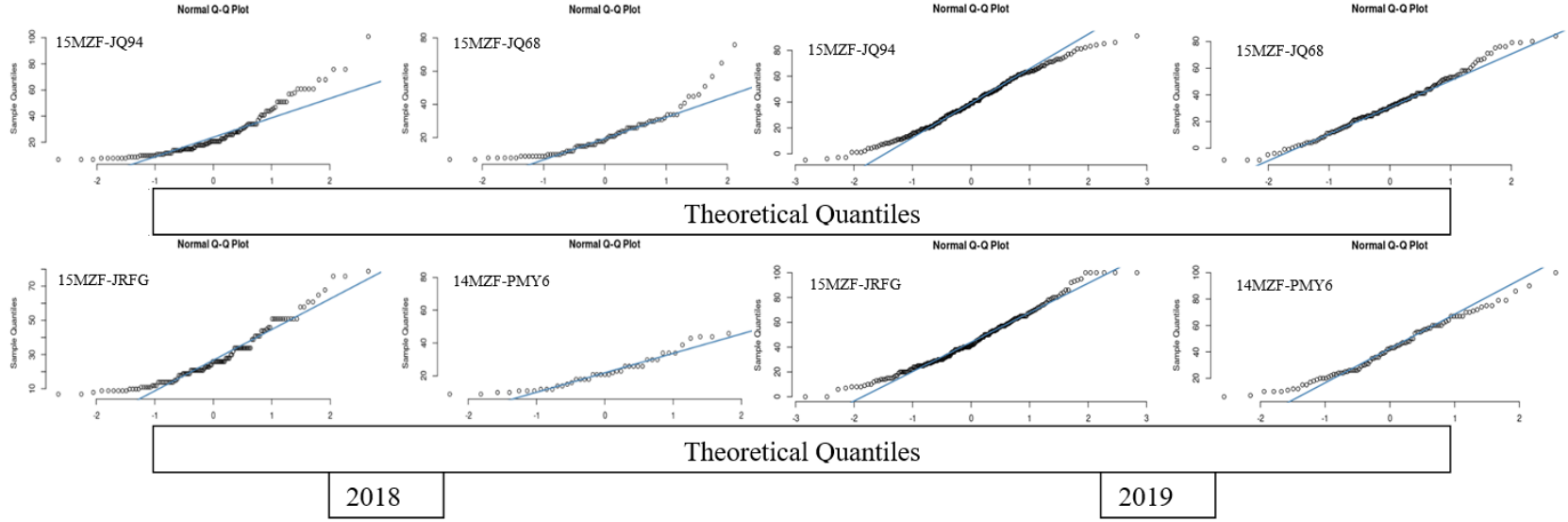

Figure 6. QQ Plots distributions of MPDHP screened for Diplodia Ear Rot in 2018 (*left*) and 2019 (*right*).

the experimental design was effective in obtaining significant differences among DH lines screened for DER. Phenotyping for DER is highly dependent on the weather condition which varies from year to year. In general, DLERP histogram was observed favouring resistance to DER across populations in 2018. QQ plot reveals that the set of data used does not fit from theoretical/normal distribution. In contrast to the data observed in 2019, normal distribution for DLERP histogram and qqplot tests were generally observed for all the mapping populations.

A total of 6 genotypes coming from 3 populations were observed to be resistant to DER (DLERP range from 0 to 10%) in 2018 and 2019 experiments. Specifically, 3 genotypes from 15MZF-JQ94 population (i.e. -39, -251, and -302), 2 genotypes from 15MZF-JQ68 population (i.e. -152 and -184) and 1 genotype in 15MZF-JRFG population (i.e. -29) showed consistent DER resistance.

QTL identified using mppR

Using 2018 DLERP data, QTL on chromosome (chr.) 5 (394.10-396.49cM) was detected with LOD score of 4.93, *see* Figure 7. Multi-QTL effects (MQEs) with cross specific (cr), parental (par), and bi-allelic (biall) models reveal two QTL on chromosome 3 (8.3cM, biall) and chromosome 5 (395.6cM, par; Figure 8. Contribution of each individual QTL were at 7.43% on chr. 5 and 3.77% on chr. 3. QTL genetic effects were observed at 7.18% in ancestral model and 10.95% in MQE model. It is notable that the QTL identified from the susceptible parents (03MZF-CK83, 07MZF-VDF6, 07MZF-SCH9, 04MZF-KQF0) increased the DLERP. The QTL identified on chr. 3 (biall) in 07MZF-VDF6 line has additional increase of 5.53 DLERP effect per cross or parent. Interestingly, QTL on chr. 5 (par) identified in 04MZF-KQF0, 07MZF-VDF6, 03MZF-CK83 lines have additional respective increase of 52.99, 4.02, and 3.87 DLERP effect per cross or parent.

In 2019, three QTL were identified on chromosome 1 (446.5-551.2 cM), 5 (349.7-364.6 cM), and 10 (174.2-213.0 cM), see Figure 7. MQEs reveals five QTL on chromosomes: 1a (481.0 cM, biall), 1b (551.2, biall), 2 (83.6cM, biall), 5 (356.8cM, anc) and 10 (235.0cM, par) linked to DER. QTL genetic effects were at 9.39% and 15.84%, respectively (Figure 8). Summary of identified QTL per chromosome per model is presented in Table 4. QTL identified in chr. 1a (biall) in 07MZF-VDF6 and 04MZF-KQF0 lines have additional increase of 5.60 DLERP effect per cross or parent. QTL identified on chr. 1b (biall) and chr. 2 (biall) in 03MZF-CK83 line has additional increase of 4.25 and 5.01 DLERP effect per cross or parent, respectively. QTL identified on chr. 5 (anc)

Table 4. Summary of QTL identified for DER in 2018 and 2019 per chromosome and across models.

Year	Model	Chromosome	Position (cM)	LOD	R^2
2018	Ancestral	5	394.10-396.49	4.93	7.18%
2018	MQE, biall	3	8.3	-	3.77%
2018	MQE, par	5	395.6	-	7.43%
2019	Ancestral	1	446.5-551.2	3.98	2.89%
2019	Ancestral	5	349.7-364.6	4.08	3.23%
2019	Ancestral	10	174.2-213.0	3.98	3.27%
2019	MQE, biall	1a	481	-	3.62%
2019	MQE, biall	1b	551.2	-	2.14%
2019	MQE, biall	2	83.6	-	3.19%
2019	MQE, anc	5	356.8	-	3.12%
2019	MQE, par	10	235	-	3.77%

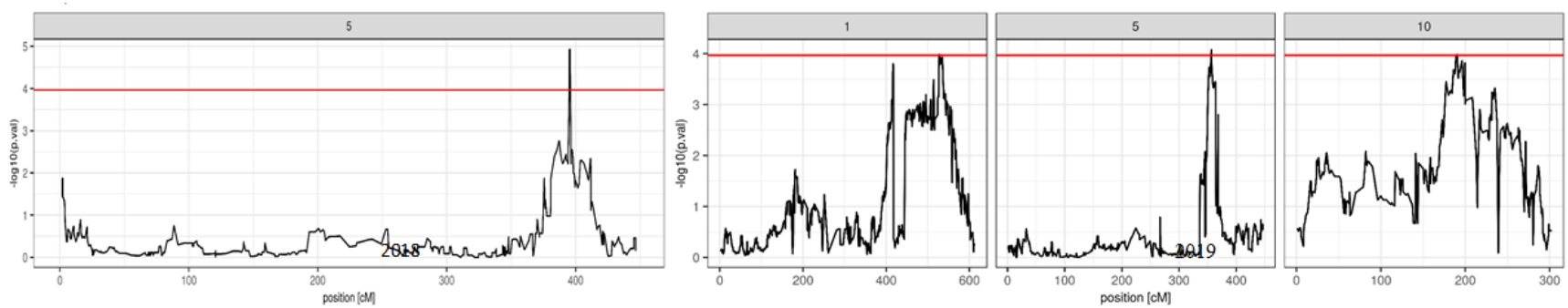

Figure 7. QTL profile identified in chromosome 5 (2018) and chromosomes 1, 5, and 10 (2019) using ancestral model in mppR package in R. LOD Score Threshold (red line) at 3.97 and was obtained using 1000 permutations.

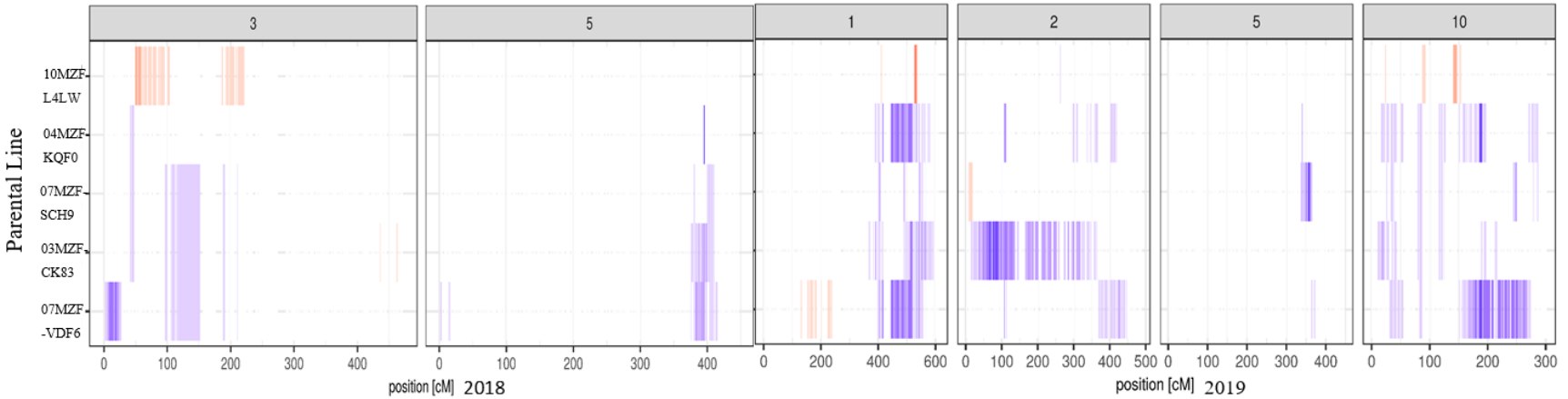

Figure 8. QTL effects among parents using MQE model in mppR package in R. QTL effects on donor line 10MZF-L4LW confirms that it significantly reduces DLERP as indicated by red bars. Susceptible parents had additional DLERP designated by blue bars. The darker the intensity of the color means higher resistance (i.e. red bar) and higher susceptibility (i.e. blue bar) to DER.

in 07MZF-SCH9 and 07MZF-VDF6 lines have additional increase of 7.31 and 3.92 DLERP effect per cross or parent, respectively. Interestingly, QTL on chr. 10 (par) in 04MZF-KQF0, and 07MZF-VDF6 lines have additional increase of 10.64, and 5.76 DLERP effect per cross or parent, respectively.

DISCUSSION

Environmental condition effects on DER response

Resistance to *S. maydis* is quantitatively inherited and highly influenced by environment. It is important to note differences in weather condition across years to determine if ear rot data for both seasons can be combined. Wet weather conditions and moderate temperature allows favourable occurrence of DER infection at the onset of silking stage until two to three weeks after infection (DEKALB, 2016). Less rainfall observed in 2018 at silking stage of maize growth is one of the factors resulting to skewedness towards DER resistance of populations among entries evaluated. Likewise, QTL discovery for DER is highly dependent on environment conditions. As presented in the results, continuous and enough rainfall observed in 2019 lead to better infection (i.e. normal distribution of DER) of DER among the populations evaluated. It is recommended to plant materials for DER screening on May to July to obtain favourable environmental condition (i.e. continuous rainfall) for DER development in hotspot locations. Interestingly, QTL identified on chr 5 was consistently detected in 2018 and 2019 considering low infection rate observed in 2018.

Validation of QTL identified in chromosome 5 -*qder5* and factors affecting mppR

Limited studies on diplodia ear rot resistance in maize has been documented since the study reported by Dorrance and Hinkelmann (1998), and Romero *et al*. in 2012. As far as the author is concerned, this is the 2nd study that looked into genetics of resistance to DER. Similar study from Romero *et al*. (2012) provides results on mapping of genes associated with DER resistance in maize. A total of 7 QTL were identified, wherein QTL on chromosome 6 and 9 were found from the susceptible parent B73 while 2 QTL on chromosome 3 were identified from the resistant parent Mo17. Although these results did not confirmed QTL found in our study, the possible reason could be attributed in the differences in genetic structure (temperate vs. tropical germplasm) and the type of population used (i.e. bi-parental cross vs. MPP). Another consideration in utilization of MPP design resides on the number of crosses in the connected population and number of total genotypes being evaluated. In general, genetic variants which have high minor allele frequencies (close to 0.5) segregate in about half of the families, whereas genetic variants of low minor allele frequencies are found in either very few or a large number of informative families (Allison *et al*., 1999). Theoretical simulations by Muranty (1996): demonstrated the power of QTL detection decreases with increasing number of parents for small effect QTL, given the family size is small. As provided in the results of Billotte *et al*. (2010), success of MPPs in oil palm was driven by large population size providing greater detection power for the QTL of a given parent by several crosses. This supports our data wherein more QTL were identified in 2019 (i.e. 8 QTL at 686 genotypes) compared to 2018 (3 QTL at 384 genotypes).

Preliminary exploration of the genomic regions tagged by QTL identified in this study was performed in the Maize Genetics and Genomics Database (Portwood *et al.,* 2018). Three uncharacterized proteins were found in 178265304 bp – 178394004 bp of chromosome 5. And while it is too early to speculate, it is interesting to note that this region also harbors the protein coding gene Chitinase 1 (Portwood *et al.,* 2018). As reported by Xia *et al*. (2010), chitinase

might be involved in the interaction between host and pathogen. Results from Naumann and Wicklow (2010) study shown LH82 line allele contributing to ear rot resistance caused by *S. maydis*, while B73 line allele might contribute to susceptibility. Furthermore, Naumann *et al.* (2011) described plant class IV chitinases, ChitA and ChitB, as conserved regions playing an important role in plant-fungal interactions specific in maize ear rot (*S. maydis*).

CONCLUSIONS

Overall, this study provides researchers an insight in generating QTL discovery projects in plant diseases utilizing MPDHP. A putative QTL was identified in chromosome 5 (*qder5*) across models and across years given the differences in environmental conditions observed in 2018 and 2019. QTL effects on donor line 10MZF-L4LW confirms that it significantly reduces DLERP, based on 2-year data. Utilization of large population size can provide greater QTL detection power for a given connected populations. Also, QTL identified in a diverse germplasm has wider applicability for future MPP analysis and validation studies. Further implementation of MPP approach in different genetic backgrounds is recommended to validate consensus QTL identified on chromosome 5 conferring resistance to DER.

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