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A Genome-Wide Association Study for Fumonisin Contamination Resistance in Tropical Maize

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Abstract

Native genetic resistance has been considered an effective and environmentally safe alternative to control fungal infections and to reduce mycotoxins accumulation in maize grains. Marker-assisted breeding can be used to accelerate the genetic gains for fumonisin contamination resistance. Thus, the objective of this study was to identify quantitative trait loci (QTLs) and candidate genes for resistance to fumonisin contamination in an Embrapa's tropical maize panel. Two-hundred and five inbreed lines were evaluated in three field trials Brazil in order to quantify fumonisin contamination in maize grains. The lines were genotyped-by-sequencing (GBS), generating 385,654 high-quality polymorphic SNPs. Mycotoxins in the grain samples were isolated using commercial immunoaffinity columns and its concentrations were evaluated by fluorometric technique. Nine quantitative trait loci (QTL) were found associated with fumonisin concentration resistance in maize. Seven candidate genes with annotated functions associated with biosynthetic pathways of pathogen resistance and four genes have not been previously described as related to fumonisins contamination resistance. These findings will be important to better understand the fumonisin contamination resistance and to support the development of SNP markers to accelerate the selection process in tropical maize breeding programs.

Introduction

Maize (*Zea mays* L.) is an important cereal crop grown worldwide for food, feed and for processed industrial products, being the third most consumed cereal after wheat and rice (FAOSTAT 2018). Maize production faces several leaf, ear and kernel diseases that cause grain contamination by hazardous toxins to animal and human health, in addition to grain yield losses. These diseases are caused by fungi species that attack and invade developing ears and kernels. For example, Fusarium ear rot caused by *Fusarium verticillioides* (Sacc.) Nirenberg (syn. F. *moniliforme* Sheldon). This pathogen is the major producer of fumonisins, including fumonisin B1, affecting the grain quality and marketability, besides reducing grain yield by 10 to 30% (Battilan et al. 2008; Mesterházy et al. 2012) due to fumonisin production.

Fumonisins have proven toxicity on animals and possible carcinogenic effects to humans, according to the International Agency for Research on Cancer (IARC 2002). Fumonisin B1, for example, has carcinogenic properties (Gelderblom et al. 1996) and is associated to neural tube birth problems in humans (Missme et al. 1997). In general, tropical countries have more severe infections caused by *Fusarium spp.*, due to its wet and warm weather, even before harvest. Because of the fungi spores can occur on the silks. (Mesterházy et al. 2012) the contamination can happen during or immediately after the flowering time. Moreover, the wounds caused by insects are readily colonized by fungi that boost infections (Lanubile et al. 2014; Chilaka et al. 2016, Wang et al. 2016).

Native genetic resistance has been successfully used to control several diseases in maize. Thus, in order to understand the genetic architecture of fumonisin incidence in maize grains, some QTL studies were performed for the resistance to *Fusarium* and fumonisin contamination (Chen et al. 2016, Zila et al. 2013,

Maschietto et al. 2017, Giomi et al. 2016). For example, Maschietto (2017) identified 15 QTLs for *Fusarium* contamination, 17 QTLs for fumonisin B1 contamination and candidate genes that could accelerate the development of inbred lines showing reduced disease severity and low fumonisin contamination. A genome-wide association study (GWAS) for fumonisin contamination, based on 256 maize inbred lines and 990,000 SNP markers, found 17 QTLs associated to fumonisin contamination resistance (Bolduan et al. 2019). There are currently few results about genomic regions and genes associated with fumonisin contamination resistance, due to the complex genetic architecture of resistance to fumonisin accumulation that appears to be controlled by many quantitative trait loci of small effect (Bolduan et al. 2019). Thus, in the present study, a GWAS was performed to identify genomic regions associated to fumonisin contamination resistance in tropical maize germplasm. Additionally, inbred lines showing low levels of fumonisin contamination were selected as resistance sources for the Embrapa's tropical maize breeding program.

Material And Methods

Genotypes and experimental design

The maize diversity panel was comprised two hundred and five maize inbred lines, from the breeding program of Embrapa (Brazilian Agricultural Research Corporation) Maize and Sorghum, located in Sete Lagoas, state of Minas Gerais, Brazil (Silva et al. 2019). During the 2014/2015 crop season, the 205 lines were evaluated in three field trials conducted in the experimental station of Embrapa Mid-North, located in Teresina, state of Piauí, Brazil. Teresina presents the geographic coordinates of 05°05'S latitude and 42°48'W longitude. The climate, characterized as dry sub-humid, mega-thermal, with moderate water surplus in the summer, is located in a semi-arid area. The soil of the experimental area is a sandy loam textured Dystrophic Yellow Argisol. The lines per se were characterized for the resistance to fumonisin concentration, under natural infection, considering a 9 x 9 lattice design with 81 treatments per trial (75 treatments and 6 common checks), with three replicates. Plots were represented by a 4-m row with 0.8 m spacing between rows and an average density of 60,000 plants per hectare. Fertilization was applied at sowing, with 500 kg/ha of the formulated 08-28-16(N-P-K) 0.3 % of Zn, and in the cover was used 200 kg/ha of 44-0-0 (Urea). Weed, pest control and other agronomical practices were performed as recommendedfor maize crop.

Fumonisin determination

The evaluated trait was the fumonisin concentration in the grain (measured in parts per million, ppm). A sample of 500g of maize kernels was finely grounded and a subsample of 10 g was used to quantify the fumonisin concentration in parts per million (ppm). The analyses were performed in the Laboratory of Food Safety at Embrapa Maize and Sorghum. From this subsample, fumonisins were extracted in a solution of 100 mL of water/methanol mixture (20/80) and 5g of NaCl in a blender for 1 min. Afterwards, it was filtered through Whatman paper and an aliquot of 10 mL of filtered extract was diluted with 40 mL

of 0.1% phosphate Tween-20 solution (phosphate buffer). Then, the solution was filtered again with a 1.0 mm microfiber filter, and 10 mL of this solution was passed through the FumoniTest column. The column was washed with 10 mL of phosphate buffer solution, followed by a second flow of 10 mL of phosphate buffer. The column content was eluted with 1.0 mL of methanol (HPLC grade), collected and mixed with 1 mL of developer. The fumonisin concentration in the grain was quantified in the samples by the FumonitestTM using the Fluorometer VICAM according to the manufacturer's protocols (VICAM, 2015).

GBS-based SNPs

For the 205 inbred lines used in the lattice design, genomic DNA was extracted from young leaves with the CTBA method (Saghai-Maroof et al. 1984). After extraction, the DNA was quantified using a fluorometer, following the manufacturer's instructions, and sent to the Genomic Diversity Facility of the Cornell University (Ithaca, NY, USA) for genotyping-by-sequencing (GBS) (Elshire et al. 2011). The GBS protocol used the restriction enzyme *Ape*KI and sequenced in a multiplex format of 96-plex (Hiseq2500 1 x 100 bp). The sequences were aligned to the B73 reference genome (AGPv3) using the Burrows-Wheeler alignment (BWA) tool (Li et al. 2009) and the SNPs were called using the GBS pipeline available in the software TASSEL-GBS (Glaubitz et al. 2014). Indels and non-biallelic SNPs were removed using the software TASSEL (Bradbury et al. 2007). Missing genotypes were imputed using the default parameters of the Beagle software (Browning et al. 2016), and filtered for MAF higher than 5% and inbreeding (F) higher than 0.8, resulting in 385,654 high-quality polymorphic SNPs.

Estimation of least square means and heritability

First, single trial analyses were performed to verify the quality of the fumonisin concentration dataset. Then, a joint analysis of the three trials were carried out in the statistical package ASReml-R v.3 (Butler et al. 2009) using the following mixed model:

$$y_{ijkl} = \mu + e_j + r_{k(j)} + b_{l(jk)} + g_i + \varepsilon_{ijkl}$$

where y_{ijkl} is the phenotypic value of the i^{th} genotype ($i = 1, \dots, I$) within the block l ($l = 1, \dots, L$) of the replicate k ($k = 1, \dots, K$) and the trial j ($j = 1, \dots, J$); μ is the general mean; e_j is the fixed effect of trial j; $r_{k(j)}$ is the fixed effect of the replicate k within the trial j; $b_{l(jk)}$ is the random effect of the l^{th} block within the replicate k and trial j; g_i is the random effect of the i^{th} genotype, with $g_i \sim N(0, \sigma_G^2)$, in which σ_G^2 is the genetic variance; and ε_{ijkl} is a non-genetic residual effect, with $\varepsilon_{ijkl} \sim N(0, \sigma_\varepsilon^2)$, in which σ_ε^2 is the residual variance. The variance components were estimated by the residual maximum likelihood, using the average information algorithm (Gilmour et al. 1995). Diagnostic plots were used to verify the presence of outliers and the residuals of the fitted models. The heritability (h^2) was estimated using $h^2 = 1 - [\frac{PEV}{2 \times \sigma_g^2}]$, where PEV (prediction error variance) is the mean-variance of the difference between two predicted genetic effects (Cullis et al. 2014). Finally, the effect of genotypes was considered as fixed to obtain the adjusted means of the maize inbred lines.

Population Structure and estimation of Kinship matrix

The kinship matrix (K) was calculated by the identity-by-state (IBS) approximation, as described by Endelman and Jannink (Endelman et al. 2012), using the software TASSEL v.5.2.10 (Bradbury et al. 2007). The population structure was determined via principal component analysis (PCA), using the *pcaMethods* package in R program (RCore Team 2018). Both analyses were performed using 385,654 polymorphic SNPs.

Genome-Wide Association Study

Four genome-wide association models were examined for the Embrapa´s tropical maize panel, comprising 205 inbred lines: (i) the “naïve” model using the general linear model (GLM), with no control for population structure and relatedness; (ii) the GLM corrected for population structure, incorporating the scores for first principal component; (iii) the GLM corrected for population structure, including the scores for the first and the second principal components; (iv) the mixed linear model (MLM) with kinship (K) matrix. GLM and MLM models were fitted using the software TASSEL v5.2.10 (Bradbury et al. 2007). Then, quantile-quantile plots (Q-Q plots) were used to select the best genome-wide association model for controling the detection of false-positive associations. Manhattan plots were drawn using the $-\log_{10}$(*P-value*) of the marker-trait associations and the marker physical positions in Megabasis (Mb). A moderate

stringency threshold of -$\log_{10}$ (*P-value*) equal to 4.0 was adopted to consider SNPs significantly associated to fumonisin contamination in maize.

Linkage Disequilibrium and candidate gene selection

The linkage disequilibrium (LD) decay was estimated using the software TASSEL v.5.2.10 (Bradbury et al. 2007) based on the r^2 pairwise measures. Then, the MaizeGDB genome browser was used to investigate candidate genes in LD with significant SNPs detected by the GWAS, based on the maize B73 genome v3 (RefGen_v3) (Andorf et al. 2010). Finally, the annotations of the candidate genes were performed using the BLAST search, based on the amino acid sequences available in the National Center for Biotechnology Information (NCBI) (Johnson et al. 2008) and in the BLAST2GO (Lanubile et al. 2011) databases.

Results

The GBS sequencing data of 205 sorghum lines resulting in 385,654 high quality SNP markers after imputation and filtering. The highest and lowest marker densities were observed on chromosomes 1 (19.90 markers per Mb) and 4 (15.53 markers per Mb), respectively. The average fumonisin concentration in the Embrapa's tropical maize panel was 3.70 ppm, ranging from 0.03 ppm (line 211_0587_5) to 8.20 ppm (line 313_0578_3) (Table 1). The heritability (h^2 = 0.46) was considered moderate and consistent with other previous GWAS studies of fumonisin contamination resistance in maize (Zila et al. 2013, Chen et al.2016, Maschietto et al. 2017).

Based on the clustering analysis of the kinship matrix, six genetic diversity groups (represented by the colored bars bellow the dendrogram in Fig. 1a) were detected for the 205 Embrapa's maize inbred lines. The lines exhibiting low genetic relationship with the genetic diversity groups were not clustered and represented by the black bar bellow the dendrogram in Fig. 1a. Most of the IBS estimates showed values from 0 to 0.5 in a scale of 0 to 2, in which 2 is the maximum value of genetic similarity (Fig. 1a). The biplot of the first two principal components (PC) is presented in Fig. 1b, in which the colored points represent the maize lines clustered according to the six genetic diversity groups identified by the clustering analysis of the kinship matrix (Fig. 1a). The first (PC1) and the second (PC2) principal components explained 9% and 3% of the genetic variability, respectively.

The selection of the best GWAS model was based on the Q-Q Plot, representing the distribution of the observed by the expected -$\log_{10}$ (*P-values*) under the null hypothesis (Fig. 2). The models corrected by population structure or kinship performed similarly to the naïve model in order to minimize false positives. Thus, the naïve model was selected and used in the association analysis of fumonisin contamination resistance.

Nine quantitative trait loci (QTL) with fumonisin contamination resistance were obtained, considering the threshold of -log10(*P-value*) equals to 4.0 (Fig. 3). QTLs were found in the bins 2.05, 2.08, 3.06, 4.05, 4.06, 5.01, 5.05, 10.03 and 10.04 (Table 2). It should be noted that the QTL in bin 5.01 looks promising due to

the significant association, in addition, it has not been previously identified in other studies. The supporting intervals for the QTLs ranged from thousands to millions of bp and were located according to the B73 genome v3. Therefore, to investigate candidate genes next to associated QTLs with the fumonisin resistance, we considered the genome-wide LD of the Embrapa's tropical maize panel that tended to decay rapidly to $r^2 = 0.1$ within 10 kb (Online Resource 1). Were considered QTL when located in the same LD block (Table 2). Based on the LD blocks of each QTL, seven candidate genes were identified, showing annotated functions likely related to pathogen resistance (Table 2, Online Resource 3).

Next, we explored the genetic constitution of inbreed lines selected for fumonisin resistance using a 10% selection pressure for lines more resistant (top) and the 10% of lines more susceptible bottom (Fig. 4a). This analysis was undertaken with the frequencies of alleles related to fumonisin resistance estimated for nine QTLs (Online Resource 4). The highest frequency of favorable alleles (RR - alleles that increase the resistance) were placed in top lines and, the highest frequency of unfavorable alleles (SS) in bottom lines (Fig. 4b). The mean of favorable alleles for the most resistant inbred lines (top 10%) was 0.573 ppm of fumonisin concentration, while the mean favorable alleles for less resistant inbred lines (bottom 10%) was 6,88 ppm showing the highest fumonisin frequency (Online Resource 2, Fig. 4c). Some lines were a highlight for the classification of resistance and susceptibility in according genotypic makeup. The lines from the top group were derived from founding dent lines (L-228-3x45611x228-3-2-4, L-161x228-3-1-7, and L2280-31612841-102-2-1-4), as well as parents of past commercial Embrapa's hybrids (L20 and L724).

Discussion

Significant differences were observed between the adjusted means of distinct inbred lines, indicating the existance of additive genetic variability for fumonisin contamination resistance in the Embrapa's tropical maize panel. The heritability estimated in this study was moderate and similar to the one reported by Samayoa et al. (2019) using 270 maize inbred lines ($h^2 = 0.46$). Other studies have reported correlations between Fusarium ear rot resistance and fumonisin contamination ranging from 0.87 to 0.92 (Bolduan et al. 2009, Robertson-Hoyt et al. 2006), suggesting that indirect selection could be an alternative to reduce the fumonisin contamination in maize grains. However, Eller et al. (2008) showed that the selection for Fusarium ear rot resistance is not always successful to reduce fumonisin contamination, requiring more QTL studies to better understand its genetic basis.

It was not possible to conduct out the study of Genotype x Environment (GxE). Giomi et al. (2016)

looked at Genotype x Year (G xY) for maize inbreds Fusarium ear rot in two years trials and found G x Y to be small. The GxY variance components were minor compared to those of principal effects (results not shown) indicating that the ranking of genotypes for disease, severity tended to be stable across years and fungal species. Because of that, the three-field trials conducted were considered similar in the Giomi et al. (Giomi et al. 2016) study. According to Samayoa et al. (2015) in study genome-wide association analysis for fumonisin content in maize kernels the phenotypic mean across environments would finely

correspond to genotype performance because genotype x environment significant effects have been rather attributed to the heterogeneity of genotypic variances than to the lack of correlation of genotype performance in different environments. Hence, in a condition such as that, as our phenotypic trait was assessed with reasonable precisions based on our heritability estimates, we do not expect dramatic impacts of additional trials, particularly for QTL.

Linkage disequilibrium (LD) measures are usually specific to the target germplasm panel, varying considerably between distinct genomic regions (Romay et al. 2013) Thus, different values of linkage disequilibrium (LD) decay were reported by Romay et al. (2013) (r^2 = 0.1, 1 kb), Zila et al. (2013) (r^2 = 0.2, 10 kb), and Zila et al. (2014) (r^2 = 0.2, 1 kb) in maize. In the present study, the average LD over chromosomes dropped below r^2 = 0.1 within approximately 10 kb (Fig. A.1). Lu et al. (2010) have also reported an average LD extension of 10 kb, using haplotype-based analysis of 2,052 SNP markers and 305 inbred lines and obtained 28,791 high-quality SNP markers among the 45,868 SNPs. Similar results of the LD were observed by Wang at al. (2017). Using 45,868 SNPs with an average LD decay of r^2 = 0.1, the GWAS of head smut resistance in a panel of 144 inbred lines allowed identified eighteen candidate genes. In relation to other studies, we achieved greater coverage of the genome with 385,654 high-quality SNPs, on average, 38,706 SNPs were obtained per chromosome, ranging from 59,939 for chromosome 1 to 27,910 SNPs for chromosome 10. This measure of LD decay was valuable to identify candidate genes associated to the QTL detected for the fumonisin contamination resistance in the Embrapa's tropical maize panel.

Nine high-resolution QTLs were significantly associated with fumonisin contamination resistance. Genes located within the genomic interval of 10 kb were considered in LD with the detected QTLs. These candidate genes were classified according to the MaizeGDB genome browser. The QTLs in bins 2.05, 2.08, 3.06, 5.01 and 10.03 presented seven genes (Fig. 3, Table 2), were also associated to fumonisin contamination in other studies (Zila et al. 2014, Zila et al. 2013, Maschietto et al. 2017, Coan et al. 2018, Samayoa et al. 2015) (Online Resource 5). Unveiled four genes in the regiões 2.05, 4,05, and 5.01 (Table 2) i.e. have not been previously described as related to fumonisins contamination resistance (Fig. 3). Some of these candidate genes colocalized with QTLs shown in Table 2, like GRMZM2G013200 (Bin 4.01), GRMZM2G051270 (Bin 5.05), and GRMZM2G083347 (Bin 10.03), were simultaneously detected in the Gene Ontology analysis with annotated functions to resistance to pathogens (Online Resource 5).

There are different immune strategies for defense against pathogens in plants (Wit 2007). Pathogen-associated molecular patterns (PAMPs), for example, are patterns recognized by receptors (pattern-recognition receptors - PRRs), which induce the immune response (pattern triggered immunity - PTI). PRRs can be categorized as receptor kinases localized on plasma membrane (RKs) or receptor-like proteins (RLPs) (Boutrot and Zipfel, 2017; Zhang et al. 2017), which reinforces the host defenses. The effector-triggered immunity (ETI), mediated by resistance proteins (RPs), is a secondary immune response that when activated allows the plant to stop the pathogen development. During the induction of local immune responses, a systemic acquired resistance (SAR) can become activated. The maize, for

example, when infected by *Fusarium verticillioides*, expresses a set of defense genes (Lanubile et al. 2014; Wang et al. 2016). This response seems to play a primary role in the resistance of maize to *Fusarium verticillioides*, where salicylic acid and jasmonic acid signaling pathways can be involved (Wang et al. 2016). Hence, genes directly involving in the immune response in plants are more suitable as candidate genes for the associations found for fumonisin resistance.

GRMZM2G060216 (176 Mbp, bin 3.06) and GRMZM2G083347 (14 Mbp, bin 10.03) genes were described as involved in response to jasmonic acid (JA) (Online Resource 3). The signaling pathway of JA promotes downstream activation of defense genes responsive at PR (pathogenesis-related) proteins, such as chitinases (Lanubile et al. 2012). Hormonal signalizing via salicylic acid, auxin, abscisic acid, ethylene, and by own jasmonic acid, are orchestrated until they reach the nucleus (Berens et al. 2017; Lanubile et al. 2014; Wang et al. 2016). Besides this, the GRMZM2G060216 gene refers to the transcription factor LG2, which is related to systemic acquired resistance by the salicylic acid-mediated signaling pathway (Chen et al. 2012). Galić et al. (2019) in a study to assess the factors affecting Fusarium ear rot and fumonisin contamination of maize, identified genes that can confer resistance, and found genes that code for NAC transcription factor.

GRMZM2G036708 gene was described as a cysteine synthase (bin 2.05, 107 Mbp). The largest class of resistance proteins involved in ETI response consists of nucleotide-binding-leucine rich repeat (NB-LRR) proteins (Samayoa et al. 2019). Ormancey et al. (2018) demonstrated that the protein acts as a negative regulator of fumonisin B1 induced cell death in Arabidopsis. The cysteine synthase that like other several PRRs, has leucine-rich receptor-like kinases, that were also identified in studies of associated with Fusarium ear rots are one of the greatest challenges for maize consumption chain. So resistance to contamination by fumonisins is a major challenge maize (Lanubile et al. 2014; Wang et al. 2016).

The gene GRMZM2G154156 (107 Mbp, bin 2.05) was described as a protein from the Ubiquitin ligase complex. The regulatory process in ubiquitylation specifically resides in the E3 ligase and the cognate substrate. A number of abiotic stresses are mediated by protein ubiquitylation processes processes (Haak et al. 2017). Members from this family are involved in the regulation of some biological processes, including vegetative growth, plant reproduction, biotic and abiotic stresses tolerance. Furthermore, the ubiquitin ligases ring domain ligase 3 (gene RGLG3) and gene RGLG4 coordinately and positively regulate fumonisin B1 triggered programmed cell death by modulating the Jasmonic acid signaling pathway in a coronatine insensitive 1 (COI1)- and the gene MYC2-dependent manner in Arabidopsis (Zhang et al. 2015).

Coan et al. (2018) also reported SNP significantly associated with Fusarium ear rot in bin 10.03, in SNP physical position 234 Mbp. Wisser et al. (2006) found bin 10.03 to contain a large QTL conditioning resistance to several maize diseases. Therefore is important for resistance since common rust resistance genes rp1 and rp5 were found in this bin (Chen et al. 2016, Coan et al. 2018). Several SNPs associated with the candidate genes presented protein domains that have high similarity to the pathogenesis-related proteins and were reported to improve disease resistance.

The gene GRMZM2G022213 (208 Mbp, bin 2.08 - Table 2) annotated as zinc finger protein MAGPIE, regulates tissue boundaries cell division and asymmetric cell division (Welch et al. 2007). GRMZM2G051270 gene located in the bin 5.05, 7 Mbp, correspond to a sulfate adenylyltransferase cysteine (Table 2). ATP-S could be involved in plant-tolerance to several abiotic stresses via different S-compounds pathogen responses (Álvarez et al. 2012). S-containing compounds is directly or indirectly modulated/regulated by ATP-S and are involved in plant tolerance to both biotic and abiotic stresses (Anjum et al. 2015). There is a high correlation between fumonisin contamination, linoleic acid content and masking action in maize hybrids with higher oleic to linoleic ratio (Dall'Asta et al. 2012). This masking phenomenon consists of the formation of covalent bonds between the tricarballylic groups of fumonisins and sulfhydryl groups of the side chains of amino acids in proteins. The gene GRMZM2G051270 present the sulfate groups and might be related to the increase of fatty acid composition on fumonisin contamination and the occurrence of hidden fumonisins in maize.

The SNPs linked to candidate genes significantly associated with fumonisin resistance could be used as molecular markers to select resistent genotypes and decrease mycotoxin contamination. The unknown genes or not directly involved genes have the potential to be investigated, since they might be involved in resistance, as biochemical and genetic pathways leading to resistance to fumonisin are complex and, for the most part, unknown (Zila et al. 2014). Thus, the QTLs significative associated with fumonisin resistance in this study are promising to be useful for whole-genome selection in tropical maize.

The Lines (410399_19_1, 371056_1, 211_0587_5, 552697_F, 2841, L724, L_228_3x45611_x228_3__2_4_x228_3__1_1, 3821095_5) had a higher frequency of favorable alleles for the resistance to fumonisin concentration (70% RR - alleles that increase the resistance) and on average provided the best resistance (Online Resource 4), also suggesting a predominance of the dominance effects in the resistance to fumonisin contamination in tropical maize.

The complex nature of resistance challenged maize breeders to effectively incorporate novel resistance alleles into adapted breeding pools; as a result, most commercial maize hybrids have lower levels of resistance than desired (Bush et al. 2004). Therefore, inbred lines that presented a higher frequency of favorable alleles and lower fumonisin content could be used in future crosses for the generation of resistant hybrids, supporting advances in plant breeding.

Conclusions

Fumonisin contamination in grains of the Embrapa's tropical maize panel showed substantial additive polygenic variation. GWAS identified 9 QTLs associated to the fumonisin contamination resistance. Seven candidate genes with annotated functions associated with biosynthetic pathways of pathogen resistance were identified within the LD blocks of these QTLs. Furthermore, four genes have not been previously reported in other studies for fumonisin contamination resistance were colocalized with significant QTLs. SNP markers located within these candidate genes should be validated and potentially used for introgressing favorable alleles in Embrapa's tropical maize elite lines.

Declarations

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Author contributions KJS methodology, formal analysis, writing original draft; CTG validation, data curation, review, editing, funding acquisition; SMST validation, data curation, review, editing; KCB formal analysis, writing, editing; RST investigation, resources, data curation, funding acquisition; VAVQ methodology, investigation, resources, data curation, funding acquisition; RRPC investigation, formal analysis; JHSG formal analysis; NTO formal analysis; CMBD validation, data curation, funding acquisition; LASD editing and supervision; LJMG conceptualization, resources, data curation, funding acquisition; JOM conceptualization, validation, review, supervision; MMP conceptualization, methodology, validation, resources, data curation, supervision, project administration, funding acquisition.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Álvarez C, Bermúdez MA, Romero LC, Gotor C (2012) Cysteine homeostasis plays na essential role in plantimmu- nity. New Phytol. 193 165–177. https//doi:10.1111/j.1469-8137.2011. 03889.x
2. Anjum NA, Gill R, Kaushik M, Hasanuzzaman M, Pereira E, Ahmad I, Gill SS (2015) ATP-sulfurylase, sulfur-compounds, and plant stress tolerance. Frontiers in plant science 6 210. https//doi:103389/fpls2015.00210
3. Andorf CM, Lawrence CJ, Harper LC, Schaeffer ML, Campbell DA et al (2010) The Locus Lookup tool at MaizeGDB: identification of genomic regions in maize by integrating sequence information with physical and genetic maps. Bioinformatics 26 434–436. https//doi: 10.1093/bioinformatics/btp556
4. Battilani P, Pietri A, Barbano C, Scandolara A, Bertuzzi T, Marocco A (2008) Logistic regression modeling of cropping systems to predict fumonisin contamination in maize. J. Agric. Food Chem. 56 10433–10438. https://doi.org/10.1021/jf801809d
5. Berens M, Berry HM, Mine A, Arguesco CT, Tsuda K (2017) Evolution of hormone signaling networks in plant defense. Annu. Rev.Phytopathol. 55 401–425. https//doi: 10.1146/annurev-phyto-080516-

035544.

6. Bolduan C, Miedaner T, Schipprack W, Dhillon BS, Melchinger AE (2009) Genetic variation for resistance to ear rots and mycotoxin contamination in earlyEuropean maize inbred lines. Crop Sci. 49 2019–2028. https://doi.org/10.2135/cropsci2008.12.0701
7. Bolduan yoa LF, Cao R, Santiago RA, Malvar A, and Butrón A (2019) Genome-wide association analysis for fumonisin content in maize kernels. BMC Plant Biol. 19: 166. https://doi.org/10.1186/s12870-019-1759-1
8. Boutrot F, and Zipfel C (2017) Function, discovery, and exploitation of plant pattern recognition receptors for broad-spectrum disease resistance. Annu. Rev. Phytopathol. 55 257–286. https: doi: 10.1146/annurev-phyto-080614-120106
9. Bradbury PJ, Zhang Z, Kroon DE,. Casstevens TM, Ramdoss Y, Buckler ESL (2007) TASSEL: software for association mapping of complex traits in diverse samples. Bioinformatics Applications Note 23 2633–2635. https://doi.org/10.1093/bioinformatics/btm308
10. Browning BL, Browning SR (2016) Genotype imputation with millions of reference samples. The American Journal of Human Genetics 98(1):116–126. https://doi.org/10.1016/j.ajhg.2015.11.020
11. Butler DG, Cullis BR, Gilmour AR, and Gogel BJ (2009) ASReml-R reference manual Release 3. Tech. Rep. Queensland Dep. Primary Ind., Brisbane, QLD.
12. Bush BJ, Carson ML, Cubeta MA, Hagler WM, Payne GA (2004) Infection and fumonisin production by Fusarium verticillioides in developing maize kernels. Phytopatholog. 94 88–93. https://doi.org/10.1094/PHYTO.2004.94.1.88
13. Chen J, Shrestha R, Ding J et al (2016) Genome-wide association study and QTL mapping reveal genomic loci associated with Fusarium ear rot resistance in tropical maize germplasm. G3:genes. Genomes Genet 6 3803–3815. https ://doi. org/10.1534/g3.116.03456 1
14. Chilaka AC, De Boevre M, Atanda OO, De Saeger S (2016) Occurrence of Fusarium mycotoxins in cereal crops and processed products (Ogi) from Nigeria. Toxins (Basel) 8 1–18. https://doi.org/10.3390/toxin s8110 342
15. Coan MMD, Senhorinho HJC, Pinto RJB et al (2018) Genome-wide association study of resistance to ear rot by Fusarium verticillioides in a tropical field maize and popcorn core collection. Crop Sci (58:564–578. https://doi.org/10.2135/crops ci201 7.05.0322.
16. Cullis B, Jefferson P, Thompson R, Smith AB (2014) Factor analytic and reduced animal models for the investigation of additive genotype-by-environment interaction in outcross¬ing plant species with application to a Pinus radiata breeding program. Theor. Appl. Genet. 127 2193–2210. https://doi:10.1007/s00122-014-2373-0.
17. Dall'Asta C, Falavigna C, Galaverna G (2012) Battilani P Role of maize hybrids and their chemical composition in Fusarium infection and fumonisin production. Journal of Agricultural and Food Chemistry 60 3800-3808. https://doi.org/10.1021/jf300250z
18. Eller MS, Holland JB (2008) Breeding for improved resistance to fumonisin contamination in maize. Toxin Rev. 27(3–4):371–89. https://doi.org/10.1080/15569540802450326

19. Endelman JB, and Jannink JK (2012) Shrinkage estimation of the realized relationship matrix. G3 (Bethesda) 2 1405–1413. https://doi:10.1534/g3.112.004259
20. Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, Mitchell SE (2011) A robust, simple genotyping-bysequencing (GBS) approach for high diversity species. PLoS One. 6 1–10. https://doi.org/10.1371/journal.pone.0019379
21. FAOSTAT (2018) UN Food and Agriculture Organization, Corporate Statistical Database (FAOSTAT). Retrieved October 10, 2020.
22. Galić V, Šimić D et al (2019) Analysis of Fusarium ear rot and fumonisin contamination in testcrosses of a maize biparental population. Crop Breed. Appl. Biotechnol (19). https://doi.org/10.1590/1984-70332019v19n1a06
23. Gelderblom WCA, Snyman SD, Smuts CM, Van der L, et al (1996) "Hepatotoxicity and carcinogenicity of the fumonisins in rats. A review regarding mechanistic implications for establishing risk in humans," in Fumonisins in Food, eds L. S. Jackson, J. W. De Vries, and L. B. Bullerman (New York, NY: Plenum Press) 279–296. https://doi:10.1007/978-1- 4899-1379-1_24
24. Gilmour AR, Thompson R, and Cullis BR (1995) Efficient algorithm for REML estimation in linear mixed models. Biometrics 51 1440–1450. https://doi: 10.2307/2533274
25. Giomi GM, Kreff ED, Iglesias J et al (2016) Quantitative trait loci for Fusarium and Gibberella ear rot resistance in Argentinian maize germplasm. Euphytica 211:287. https://doi.org/10.1007/s1068 1-016-1725-z
26. Glaubitz JC, Casstevens TM, Lu F, Harriman J, Elshire RJ, Sun Q et al (2014) A high capacity genotyping by sequencing analysis pipeline. PLoS ONE 9 903–916. https://doi.org/10.1371/journal.pone.0090346
27. Haak DC, Fukao T, Grene R, Hua Z, Ivanov R, Perrella G, Li S (2017) Multilevel Regulation of Abiotic Stress Responses in Plants. Frontiers in Plant Science 8, 1564. https://doi:10.3389/fpls.2017.01564
28. Hashimoto M, Negi J, Young J, Israelsson M, Schroeder JI, Iba K (2006) Arabidopsis HT1 kinase controls stomatal movements in response to CO2. Nat Cell Biol 8 391–397. https://doi:10.1038/ncb1387IARC: Fumonisin B. Sometraditional herbalmedicines, somemycotoxins, naphthalene and styrene (2002) In: 82 Monograph of the International Agency for Research of Cancer on the evaluation of carcinogenic risks to humans. Lyon France 301–306
29. Johnson M, Zaretskaya I, Raytselis Y, Merezhuk Y, McGinnis S Madden T (2008) NCBI BLAST: a better web interface. Nucleic Acids Res 36(Web Server issue):W5-9. https//doi: 10.1093/nar/gkn201
30. Lanubile A, Pasini L, Lo Pinto M, Battilani P, Prandini A, Marocco A (2011) Evaluation of broad spectrum sources of resistance to *Fusarium verticillioides* and advanced maize breeding lines. World Mycotoxin J. 1 43–51. https://doi: 10.3920/WMJ2010.1206
31. Lanubile A, Ferrarini A, Maschietto V, Delledonne M, Marocco A, and Bellin D (2014) Functional genomic analysis of constitutive and inducible defense responses to Fusarium verticillioides infection in maize genotypes with contrasting ear rot resistance. BMC Genomics 15:710. https://doi: 10.1186/1471-2164- 15-710

32. Lanubile A, Maschietto V, Borrelli VM et al (2017) Molecular basis of resistance to Fusarium ear rot in maize. Front Plant Sci 8 1–13. https://doi.org/10.3389/fpls.2017.01774.
33. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows–Wheeler transform. Bioinformatics 25 1754–1760. https://doi:10.1093/bioinformatics/btp324
34. Lu Y, Zhang S, Shah T, Xie C, Hao Z, Li X, Farkhari M, Ribaut J, Cao M, Rong T, Xu Y (2010) Joint linkage–linkage disequilibrium mapping is a powerful approach to detecting quantitative trait loci underlying drought tolerance in maize. Proc. Natl. Acad. Sci. U.S.A. 107 19585–19590. https ://doi: 10.1073/pnas.1006105107
35. Maschietto V, Colombi C, Pirona R et al (2017) QTL mapping and candidate genes for resistance to Fusarium ear rot and fumonisin contamination in maize. BMC Plant Biol 17–21. https://doi.org/10.1186/s1287 0-017-0970-1
36. Mesterházy Á, Lemmens M,. Reid LM, Breeding for resistance to ear rots caused by Fusarium spp in maize - A review (2012) Plant Breed 131 1–19. https ://doi.org/10.1111/j.1439-0523.2011.01936 .x
37. Missmer SA, Suarez L, Felkner M, Wang E, Merrill Jr AH, Rothman GP, Desjardins AE (1997) Fumonisins in maize: Can we reduce their occurrence? Plant Dis 81 556–565 https://doiorg/10.1094/PDIS.1997.81.6.556
38. Ormancey M, Thuleau P, Van Der Hoorn RAL, Grat S, Testard A, Kamal KY, et al (2018) Sphingolipid-induced cell death in Arabidopsis is negatively regulated by the papain-like cysteine protease RD21. Plant Science, https://doi.org/10.1016/j.plantsci.2018.10.028
39. Poland JA, Bradbury PJ, Buckler ES, Nelson RJ (2011) Genome-wide nested association mapping of quantitative resistance to northern leaf blight in maize. Proc. Natl. Acad. Sci. 108:6893–6898. https://doi:10.1073/pnas.1010894108
40. RCore Team (2018) R: A language and environment for statistical computing. R Foundation for Statistical Computing Vienna Austria. URL http://www.R-project.org.
41. Robertson-Hoyt LA, Jines MP, Balint-Kurti PJ, Kleinschmidt CE, White DG, Payne GA, Marago CM, Molnar TL, Holland JB (2006) QTL mapping for Fusarium ear rot and fumonisin contamination resistance in two maize populations. Crop Sci. 46(4)1734–43. https://doi.org/10.2135/cropsci2005.12-0450.
42. Romay MC, Millard MJ, Glaubitz JC, Peiffer JA, Swarts KL, Casstevens TM, et al (2013) Comprehensive genotyping of the USA national maize inbred seed bank. Genome Biol. 14 R55. https://doi:10.1186/gb-2013-14-6-r55
43. Saghai-Maroof MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. ProcNatlAcadSci USA 81 8014–8018. https://doi: 10.1073/pnas.81.24.8014
44. Samayoa LF, Malvar RA, Olukolu BA, Holland JB (2015) A Genome-wide association study reveals a set of genes associated with resistance to the Mediterranean corn borer (*Sesamia nonagrioides* L.) in a maize diversity panel. BMC Plant Biol. 15(1):35. https://doi.org/10.1186/s12870-014-0403-3

45. Silva KJ, Guimarães CT, Guilhen JHS et al (2019) High-density SNP-based genetic diversity and heterotic patterns of tropical maize breeding lines. Crop Science 60 779–787. https://doi.org/10.1002/csc2.20018
46. Wang Y, Zhou Z, Gao J et al (2016) The mechanisms of maize resistance to Fusarium verticillioides by comprehensive analysis of RNA-seq data. Front. Plant Sci. https://doi.org/10.3389/fpls.2016.01654
47. Wang Y, Bouwmeester (2017) K L-type lectin receptor kinases: New forces in plant immunity. PLoS Pathog. 13(8):1164. https://doi.org/10.1371/journal.ppat.1006433
48. Welch D, Hassan H, Blilou I, Immink R, Heidstra R, Scheres B (2007) Arabidopsis JACKDAW and MAGPIE zinc finger proteins delimit asymmetric cell division and stabilize tissue boundaries by restricting SHORTROOT action. Genes Dev. 21 2196-2204. https://doi: 10.1101/gad.440307
49. Wisser RJ, Balint-Kurti PJ, Nelson RJ (2006) The genetic architecture of disease resistance in maize: a synthesis of published studies. Phytopathology 96 120–129. https://doi:10.1094/PHYTO-96-0120
50. Wit PJ (2007) How plants recognize pathogens and defend themselves. Cellular and Molecular Life Science In Press. https://doi.org/10.1007/s00018-007-7284-7.
51. Zhang X, Wu Q, Cui, S, Ren J, Qian W, Yang Y et al (2015) Hijacking of the jasmonate pathway by the mycotoxin fumonisin B1 (FB1) to initiate programmed cell death in Arabidopsis is modulated by RGLG3 and RGLG4. J. Exp. Bot. 66, 2709–2721. https://doi: 10.1093/jxb/erv068
52. Zhang X,Valdés-López O, Arellano C, Stacey G, Balint-Kurti P (2017) Genetic dissection of the maize (*Zea mays* L.) MAMP response. Theor. Appl. Genet. 130 1155–1168. https://doi: 10.1007/s00122-017-2876-6
53. Zila CT, Samayoa LF, Santiago R et al (2013) A genome-wide association study reveals genes associated with Fusarium ear rot resistance in a maize core diversity panel. G3: genes. Genom Genet 3 2095–2104. https ://doi.org/10.1534/g3.113.00732 8
54. Zila CT, Ogut F, Romay MC et al (2014) Genome-wide association study of Fusarium ear rot disease in the U.S.A. Maize inbred line collection. BMC Plant Biol 14 1–15. https ://doi.org/10.1186/ s1287 0-014-0372-6.

Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures

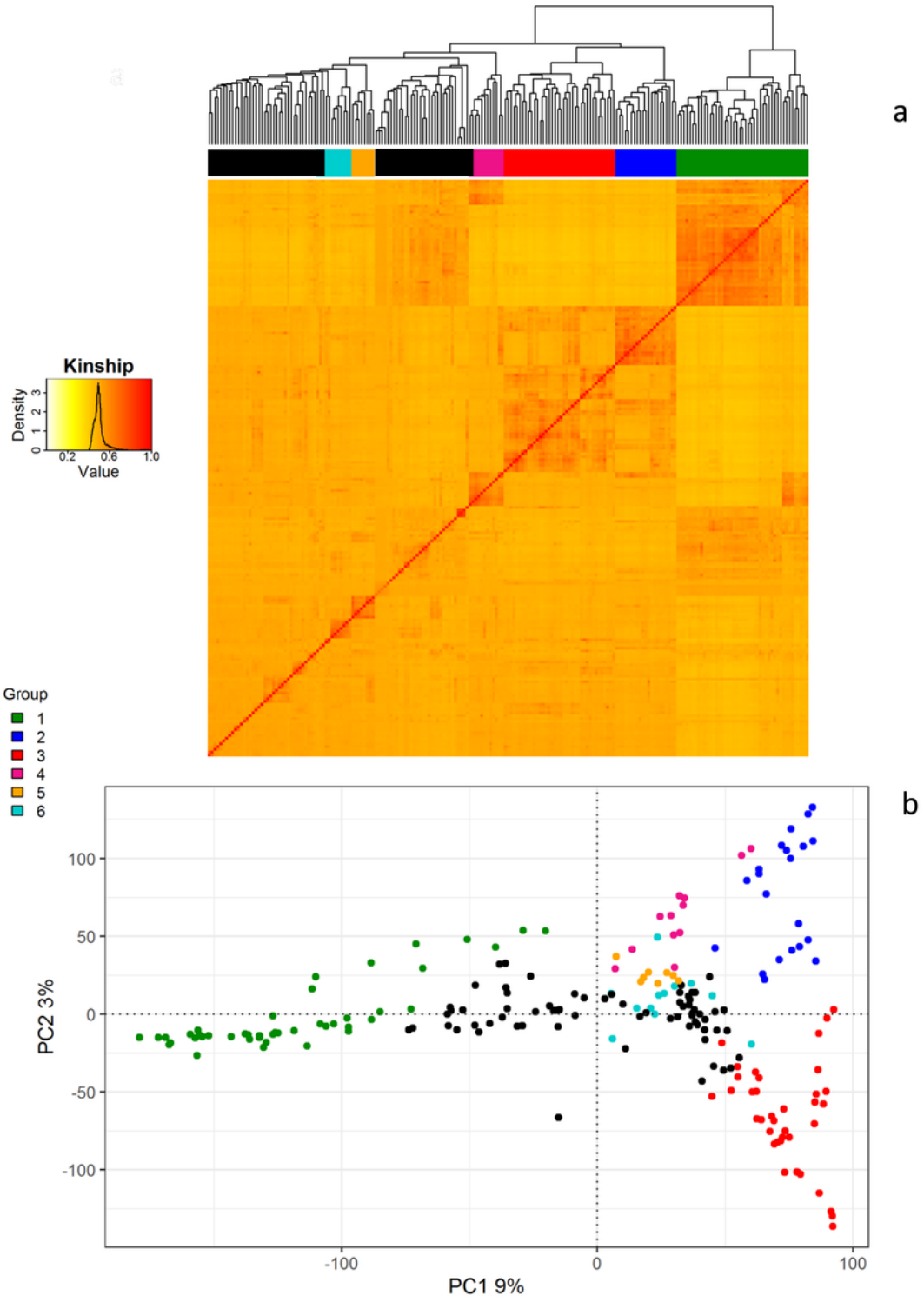

Figure 1

Heatmap to kinship values for 205 lines and dendrogram based on 385,654 SNPs. (a) The color histogram (kinship) shows the distribution of values for coefficients of coancestry in the kinship matrix, the stronger red bigger the genetic relationship between individuals. *Kinship centered in IBS. (b) Scatter plots of the 205 maize inbred lines according to the first two principal components (PC1 and PC2). Colors represent the genetic diversity groups identified through the histogram (kinship)

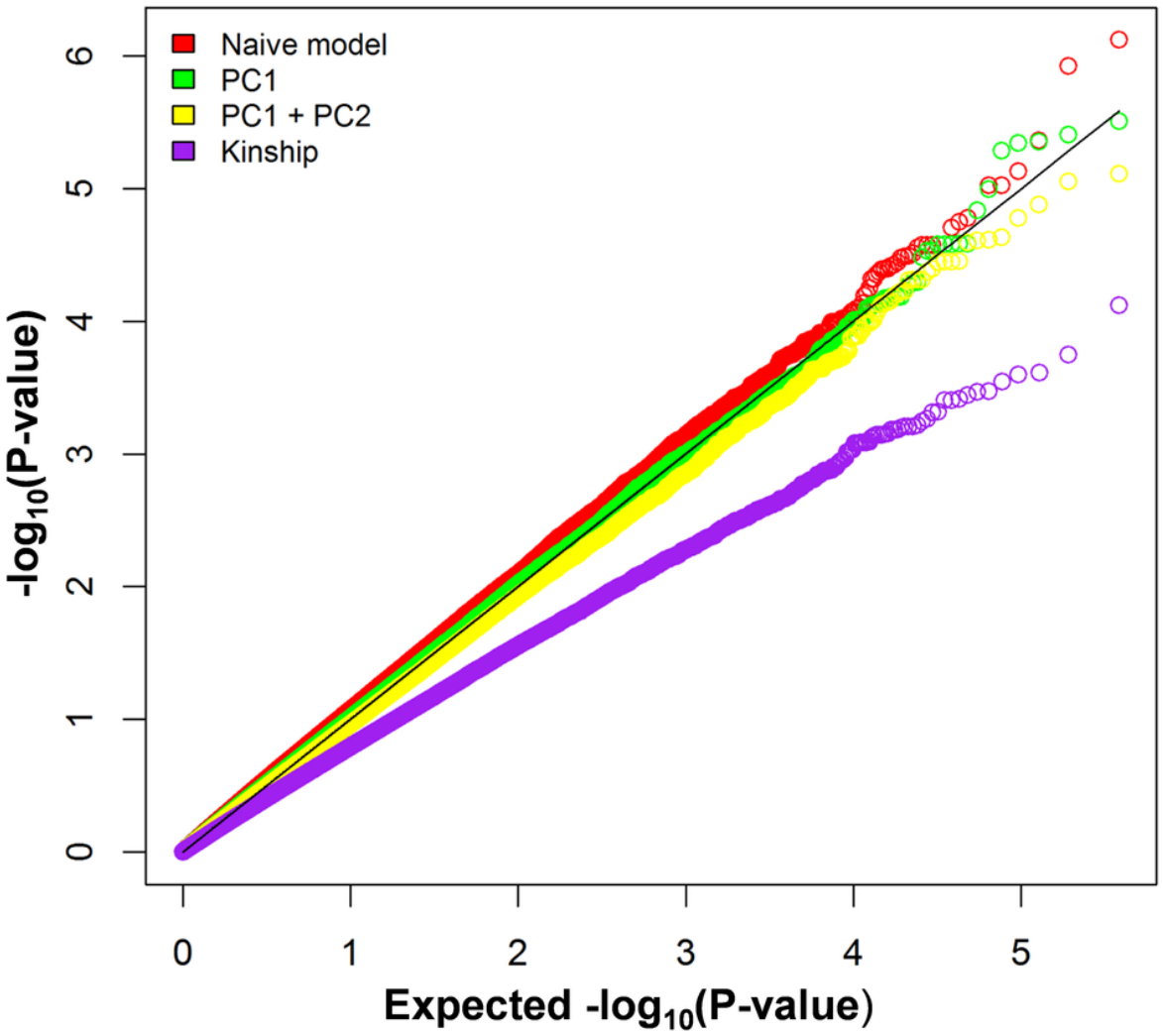

Figure 2

Quantile-quantile plots (Q-Q plots) for fumonisin association analysis using the “naïve”, PC1, PC1 + PC2, and kinship models. The black line is the expected distribution under the nullity hypothesis. Assuming there are few true associations is expected that the observed P values will almost follow the expected P value

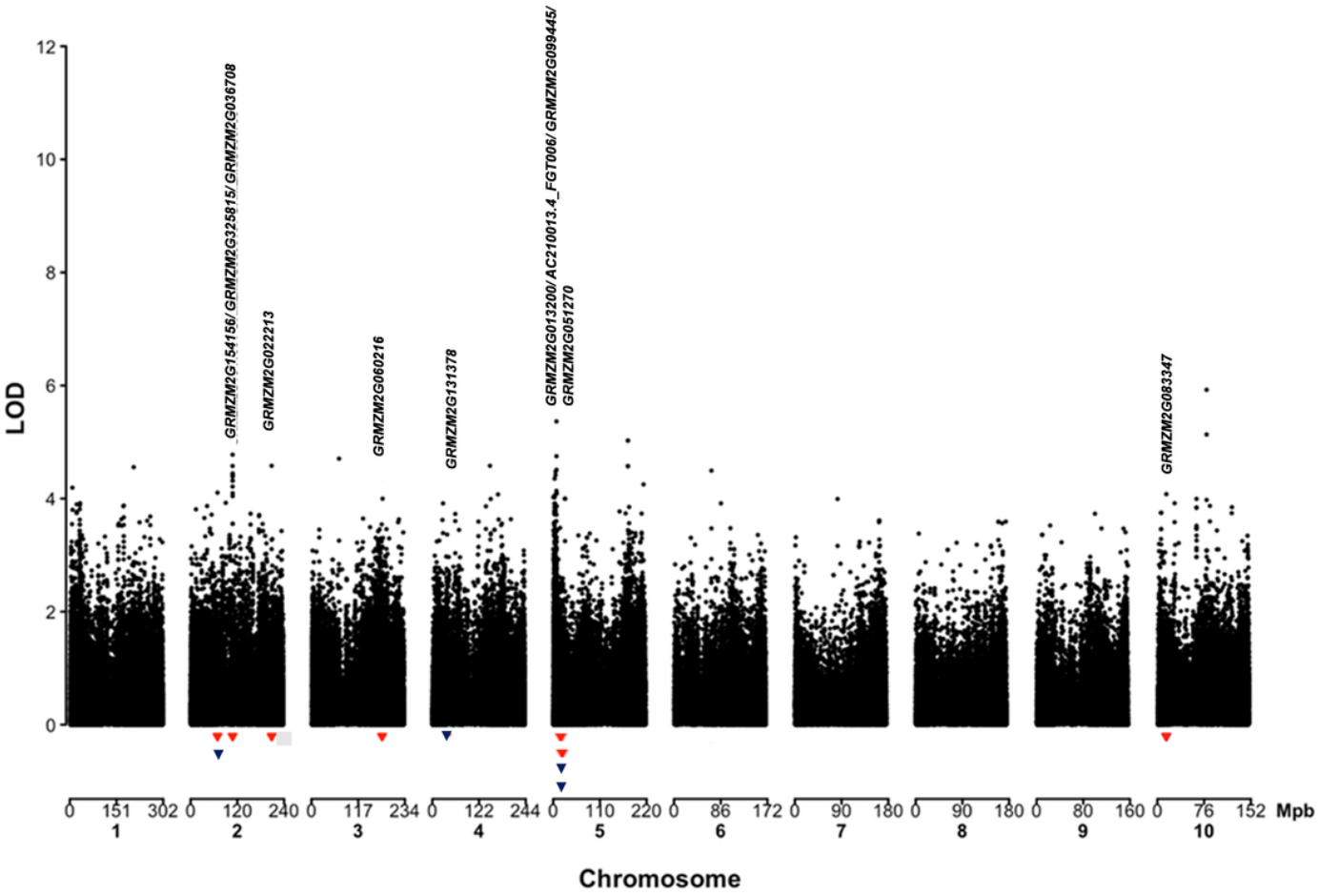

Figure 3

GWAS for fumonisins resistence in a panel of tropical maize inbred lines. Manhattan plot of association results from naïve models. Axes: the −log10 p-values (y axis) plotted against the position on each chromosome (x axis). Horizontal line represents the genome-wide significance -log10(P) equal to 4.0 as considered by Samayoa et al. (2019). Red and blue inverted triangles depict the positions of QTLs for harbored candidate genes and genes have not been previously reported related to fumonisin contamination in other studies respectively

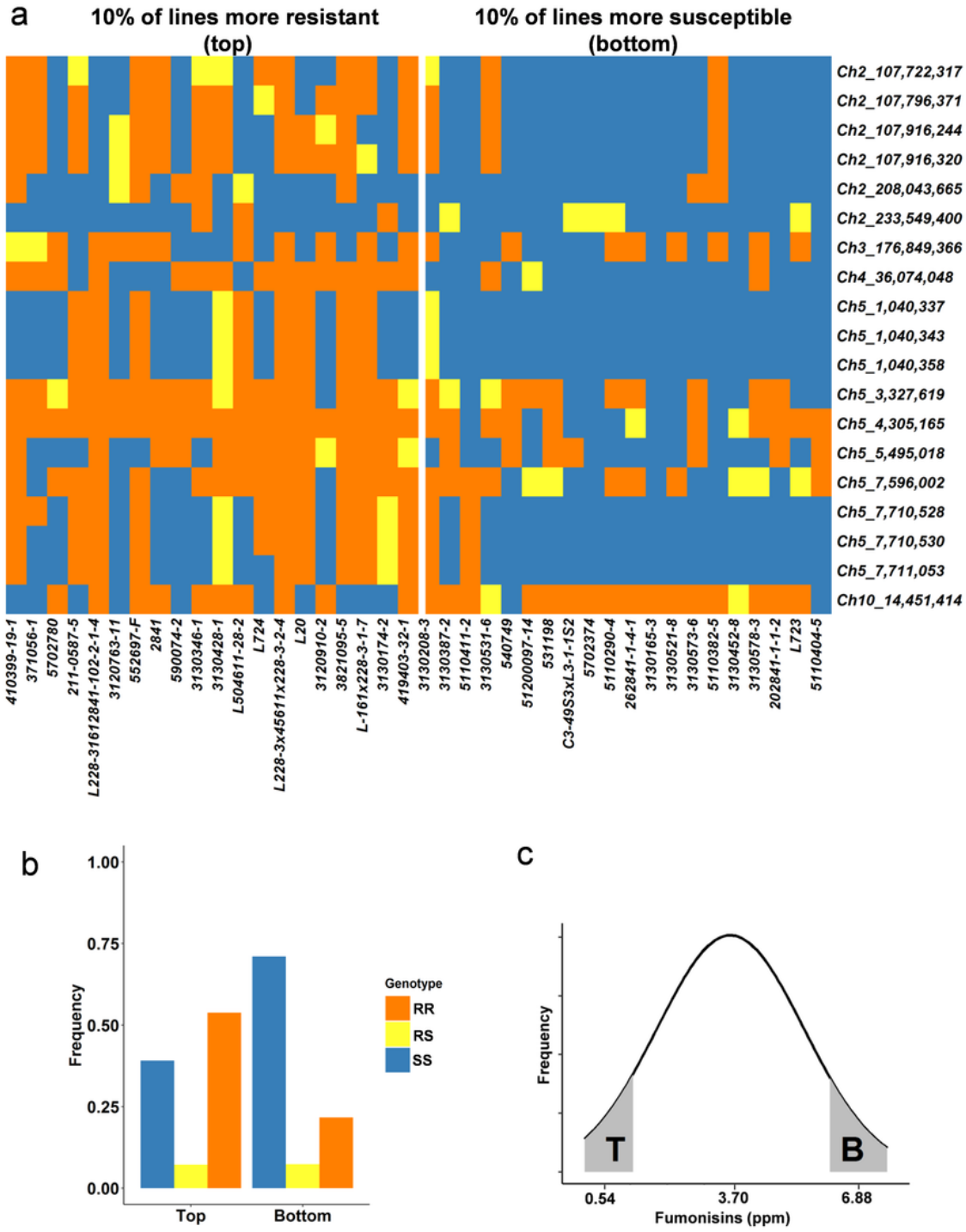

Figure 4

a Heatmap with single-nucleotide polymorphisms (SNPs) significantly associated with fumonisin concentration and the respective maize lines that are resistant (top) and susceptible (bottom). b Frequency of favorable alleles and corresponding genotypes. c Dispersion of fumonisin data (T: Top and B: bottom)

Supplementary Files

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