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Genome-wide association analysis of ear rot resistance caused by *Fusarium verticillioides* in maize

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ABSTRACT

The identification of causal regions associated with resistance to *Fusarium verticillioides* can be useful to understand resistance mechanisms and further be used in breeding programs. In this study, a genome-wide association study (GWAS) was conducted to identify candidate markers associated with resistance to the ear rot caused by the fungus *F. verticillioides*. A total of 242 maize inbred lines were genotyped with 23,153 DArT-seq markers. A total of 12 DArTs were associated with ear rot resistance. Some DArTs were localized close to genes with functions directly related to ear rot resistance, such as a gene responsible for the innate immune response that belongs to the class of NBS-LRR receptors. Some markers were also found to be closely associated with genes that synthesize transcription factors (nactf11 and nactf61), genes responsible for the oxidation-reduction process and peroxidase activity. These results are encouraging since some candidate markers can present functional relationship with ear rot resistance in maize.

1. Introduction

Maize (*Zea mays* L.) is one of the most widely cultivated cereals in the world, and its economic importance is characterized by its diverse uses. The global demand for maize has been increasing, stimulated mainly by the growth of Asian countries and the use of maize for ethanol production in the United States. Additionally, growth in the meat sector, especially poultry and pork, has also increased demand [1,2].

The development of high-performance cultivars, improvement of cropping systems, soil fertility and others factors have contributed to increase the maize grain yield. However, the occurrence of successive growth without crop rotation has contributed to changes in the population dynamics of pathogens, causing an increase in the incidence and severity of diseases in crops [3,4]. The increased incidence and severity of diseases in maize crops has caused great concern among farmers since diseases are the main factor underlying the reduction in grain yield and sanitary quality.

Among the main maize diseases found in Brazil and worldwide, it is worth highlighting *Fusarium* ear rot caused by *F. verticillioides* [5]. In addition to reducing production and grain quality, this pathogen also produces secondary toxic metabolites called mycotoxins, such as fumonisins [6,7], which exert toxic effects including the development of cancer in both humans and animals [7–9].

Some agronomic practices are recommended to reduce the pathogen inoculum in the crop area, such as crop rotation, use of healthy seeds, use of transgenic hybrids for pest control, weed control, use of resistant cultivars, and growth at the recommended density, among others [5,10,11]. These practices are often insufficient to control the pathogen because it has several hosts and survives in crop debris. Thus, the best technique for controlling the pathogen might include the use of resistant cultivars [12].

It is known that resistance to ear rot caused by *F. verticillioides* is under polygenic control and is highly influenced by the environment [13–16]. Despite the advantage of using resistant genotypes, a few resistant cultivars remain on the market due to the complexity of the genetic architecture of ear rot resistance [17–19].

When breeding for ear rot resistance, indirect selection is usually performed, wherein resistance is evaluated based on auxiliary traits such as the number of contaminated ears or the percentage of kernels presenting rot [20]. Considering the low efficacy of phenotypic selection for resistant genotypes, the use of marker-assisted selection (MAS) has emerged as a strategy to improve selection efficiency [15,21].

The use of genetic selection via MAS has been widely used for the

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detection of genes with major effects and in introgression to improve certain traits. However, the use of assisted selection and QTL mapping has shown some limitations due to long selection cycles and the search for significant marker-QTL associations, resulting in an inability to capture the effects of smaller genes [22,23].

Association studies are a good alternative to overcoming some of these limitations of QTL mapping. In general, association studies utilize the linkage disequilibrium among markers and genes or target QTL to identify associations; this is possible through linkage disequilibrium analysis using all recombination events that would have occurred between the gene and the marker in the past in the population to be used in the association study [24].

Few studies have identified causal regions associated with resistance to *F. verticillioides*. Zila et al. [25] used an association study to identify candidate genes related to ear rot resistance and identified three SNPs related to resistance to this disease. In a further study, Zila et al. [26], using a panel of inbred lines and a set of markers larger than the previous study, identified seven SNPs associated with ear rot resistance caused by *F. verticillioides*. Chen et al. [27], using a large number of tropical inbred lines, identified 45 SNPs and 15 haplotypes associated with ear rot resistance.

Thus, the aims of this study were i) to use an association study to identify markers associated with ear rot resistance caused by *F. verticillioides*; ii) to identify possible genes related to resistance to disease, and iii) to verify the association ability using multiple markers via BSSV.

2. Results

2.1. Clustering the lines according to Dart-seq information

To characterize the inbred lines, a phylogenetic tree was constructed using the neighbor-joining method (Fig. 1). Through this graph, it was possible to observe that the inbred lines used in the association study were grouped in different clusters that confirmed their different origins. In the top part of the phylogenetic tree, it is possible to observe a cluster of inbred lines derived from the group Stiff Stalk Synthetic (SSS-B73-B17) and inbred lines belonging to the group UNR. In the center part of the tree, there is an overlap of the clusters that includes the inbred lines belonging to the subgroups FDK-Arg, Non Stiff Stalk Synthetic (NSSS-PG84, TAT), Lancaster, MNK-ARG and Iodent. At the inferior part of the graph are the lines from the groups Tropical Dent, Tropical Flint, Suwan, and Amarilo Dent, which belong to the tropical subgroup.

2.2. Association analysis of ear rot resistance caused by F. verticillioides

The proportion of the total variance explained by the genetic variance for the joint analysis was 46.74%. For the individual analysis of Lavras, this proportion was 51.21% and for the Uberlandia analysis, it was 36.85% (Table 1).

Based on the corrected means, the association study was performed using the BSSV method and mixed models.

2.2.1. Association analysis using the BSSV method

In the joint analysis using the BSSV method, five DArTs were significantly associated with ear rot resistance. These DArTs were identified on chromosomes 7 (position 121,027,885 bp and position 158,258,060 bp), 10 (position 78,145,475 bp) and in the unidentified group (Ni) (Table 2 and Fig. 2).

For the Lavras individual analysis, five DArTs were significantly associated with chromosomes 1 (position 53,413,365 bp), 7 (position 124,256,014 bp), 8 (position 135,672,358 bp), and the unidentified group (Ni). In Uberlandia, two DArTs were significantly associated with resistance; one was identified on chromosome 4 (position 36,911,488 bp) and another on chromosome 5 (position 55,253,902 bp) (Table 2 and Fig. 2). From a total of 12 DArTs that were significantly associated with resistance using the BSSV method, two DArTs showed significance in both the Lavras and joint analyses – none of the markers displayed a significant association in both environments.

Heatmap graphs were drawn based on genomic windows containing markers that were significantly associated with ear rot resistance (Supplementary material, Figs. S1–S10).

Considering the DArTs that were significantly associated with ear rot resistance, one was located inside an encoding region of the gene (exon) in the physical map of the maize. Three DArTs were located within a non-coding region (introns), and the remainder were identified in regions close to some genes (intergenic regions) (Table 2).

In the joint analysis, the DArT (4582295) identified on chromosome 10 (position 78,145,475 bp) was located within the gene GRMZM2G003715 (position 78,142,498–78,146,839 bp). This gene synthesizes nactf 61 (NAC-transcription factor 61), which is responsible for the regulation of transcription. In addition, this same DArT was also located close to genes related to the oxidation-reduction process (GRMZM2G078906; position 78,074,125–78,075,875 bp and AC216369.3; position 78,109,504–78,113,483 bp), which might also present some relationship with the resistance. The DArT (4592049) identified on chromosome 7 (position 121,027,885 bp) was located in an intergenic region but rather close to the genes (GRMZM2G147966; position 121,043,853–121,048,231 bp and GRMZM2G169201; position 121,052,889–121,056,040 bp). These genes are responsible for the oxidation-reduction process and the peroxidase activity, respectively. The DArT (4579702) identified on chromosome 7 (position 158,258,060 bp) was located close to the GRMZM2G394261 gene (position 158,297,463–158,301,857 bp), which is directly linked to the pathogen immune response. This gene belongs to the class of the nucleotide-binding site leucine-rich repeat (NBS-LRR) and is responsible for signal transduction and the innate immune response, which are important for the recognition of pathogen effectors (Table 2).

In an individual analysis of Lavras, one DArT was identified within the non-coding region (intron) of the gene. This DArT (4772985; position 135,672,358 bp) identified on chromosome 8 was located within a gene with an unknown function (GRMZM2G100229, position 135,668,261–135,672,867 bp). Its marker was also located close to the gene GRMZM2G100146 (position 135,663,882–135,666,488 bp) encoding histone deacetylase 2 (HDT2) (Zm-HD2b), which plays roles in plant development and the stress response. The DArT (4767436; position 53,413,365 bp) located on chromosome 1 was located in an intergenic region very close (6 kbp) to a gene (GRMZM2G031001, position 53,419,144–53,421,347 bp) that synthesizes nactf 11 (NAC-transcription factor 11), which is responsible for regulating transcription and may play important roles in regulating transcriptional reprogramming associated with stress responses in plants. The DArT (2518496; position 124,256,014 bp) identified on chromosome 7 was located close (7 kbp and 10 kbp) to genes (GRMZM2G121649; position 124,249,378–124,254,808 bp and GRMZM2G151299; position 124,246,426–124,248,089 bp) with no known function. This DArT was also located close (57 kbp) to the GRMZM2G007249 gene (position 124,313,854–124,315,851 bp), which is responsible for the synthesis of ACCO 1 (1-aminocyclopropane-1-carboxylate oxidase2), a protein that participates in ethylene biosynthesis (Table 2).

In the individual analysis of Uberlandia, two DArTs were significantly associated with disease resistance. One marker was located in a non-coding region (intron) and another in the exon of the gene. The DArT (4772929; position 36,911,488 bp) was identified on chromosome 4 and was located within the GRMZM2G046743 gene (position 36,901,205–36,913,985 bp), which is responsible for the transport of amino acids. The other DArT (4592572; position 55,253,902 bp) identified on chromosome 5 was located within the GRMZM2G162184 gene (position 55,243,566–55,261,337 bp) responsible for S-adenosyl-L-homocysteine activity, protein binding and zinc ion bonds (Table 2).