RESUMEN

La Fusariosis de la Espiga de Trigo (FET) causada por *Fusarium graminearum* genera pérdidas en rendimiento y contaminación de granos con micotoxinas. Existe escasa variabilidad genética a la resistencia en el germoplasma de trigo candeal. La línea recombinante cromosómica endocriada LDN(Dic-3A), presenta promisorios niveles de resistencia. Los objetivos de esta Tesis comprenden: *i)*- identificar genes implicados en la resistencia a FET en LDN(Dic-3A); *ii)*- transferir el QTL de resistencia de LDN(Dic-3A) a variedades susceptibles de trigo candeal; *iii*)- desarrollar un ensayo *in vitro* en plántula para identificar genotipos resistentes y su relación con la severidad de la enfermedad.

La identificación de la expresión diferencial de genes inducida en diferentes tiempos post-inoculación con *F. graminearum* entre LDN(Dic-3A) y el parental susceptible LDN se basó principalmente en la técnica de cDNA-AFLP. De ~500 fragmentos derivados de transcripción (TDF) identificados con las distintas combinaciones de cebadores utilizados, 85 mostraron expresión diferencial: el 36% y el 19% fueron identificados en LDN(Dic-3A) y LDN, respectivamente, mientras que el 45% se indujeron en ambos genotipos. Los patrones de TDFs obtenidos mediante cDNA-AFLP demostraron ser reproducibles mediante la técnica de RT-PCR, dando validez a nuestro sistema experimental. La comparación con secuencias depositadas en bases de datos mostró que entre los TDFs identificados se hallan proteínas asociadas a la respuesta temprana a la infección, receptores NBS-LRR y receptores quinasa involucrados en el reconocimiento específico del determinante de avirulencia del patógeno. Fueron identificados además TDFs que, aunque no pudo asignárseles una proteína o función, resultaron específicos de la respuesta a la inoculación. La identidad de TDFs con ESTs de genotecas de espiga de materiales de *T. aestivum* inoculadas con *F. graminearum* constituye un sustento adicional para esta afirmación. El mapeo *in silico* permitió localizar 28 TDFs en el genoma de *T. aestivum*, siendo el brazo cromosómico 5BL el más representado, además de obtener las regiones genómicas y regulatorias de varios genes. A partir de estas regiones, pudo determinarse la existencia de mecanismos de regulación de la transcripción en común entre algunos genes asociados a los TDFs, entre ellas las proteínas WRKY implicadas en la regulación de los genes asociados con la defensa ante patógenos. La integración de la información obtenida sugiere que la interacción trigo - *F. graminearum* no sería una interacción compatible como generalmente se

cree sino que se trataría de una interacción "gen a gen" que finalmente lleva a la expresión de genes asociados a la defensa.

Hemos asumido además el desafío de desarrollar cultivares de trigo candeal resistentes. La compleja herencia de la resistencia y los efectos ambientales, son los responsables del escaso éxito obtenido hasta el momento por los mejoradores en la incorporación al mercado de cultivares resistentes. En este trabajo, por medio de cruzamientos se incorporó el QTL de resistencia *Qfhs.ndsu-3AS* de LDN(Dic-3A) en los cultivares BESM y BCAN. El microsatélite *Xgwm2*, ligado al QTL de resistencia permitió reducir la cantidad de individuos que continuaron en el programa de mejoramiento. En la generación F3, se seleccionaron los individuos homocigotos para el alelo de resistencia, y en F4, se evaluó la severidad en espiga identificando individuos con niveles de resistencia similares o mejores que el parental resistente. El programa de mejoramiento continuará con autofecundaciones de genotipos resistentes hasta alcanzar estabilidad en la resistencia junto con la presencia de caracteres agronómicos de interés.

La evaluación del comportamiento ante *F. graminearum* de nuevos materiales requiere de la existencia de ensayos rápidos y confiables. Hemos desarrollado un ensayo *in vitro* a través de la evaluación de las variables *Germinación*, *Largo de coleoptilo*, *Peso de coleoptilo*, *Peso de raíces*, utilizando siete variedades comerciales de trigo candeal, el trigo candeal LDN y las líneas resistentes LDN(Dic-3A) y LDN-DGE1 y dos genotipos de trigo pan A601 y A601S3. Las variables de plántula explicaron entre el 51 y el 74% de la severidad de la enfermedad, siendo *Largo y Peso de coleoptilo* las más eficaces para predecir la resistencia a FET. Los genotipos introgresados mostraron un buen comportamiento en el ensayo en plántula y menor daño en espiga comparados con los susceptibles, sugiriendo que la prueba *in vitro* es efectiva para la determinación de la resistencia a FET en diferentes fondos genéticos. Entonces, se propone un ensayo *in vitro* basado en las variables de coleoptilo para evaluar de manera eficaz, rápida y económica el nivel de resistencia a FET, definiendo el *Índice de Resistencia en Plántula*, altamente correlacionado con severidad, para cada genotipo.

Los principales hallazgos de esta Tesis pueden compendiarse indicando que se ha establecido que la interacción trigo - *F. graminearum* sería una interacción "gen a gen" que lleva a la expresión de genes asociados a la defensa, la obtención de genotipos de trigo candeal resistente a

fusariosis de la espiga y el dearrollo de un ensayo *in vitro* predictor del comportamiento de los genotipos ante la infección.

ABSTRACT

Fusarium head blight (FHB) caused by *Fusarium graminearum* produce yield losses and contamination of grain with mycotoxins. Scarce genetic variability for resistance exists in durum wheat germplasm. The LDN(Dic-3A) recombinant inbred chromosome line showed to be resistant to FBH. The goals of this Thesis include: *i*)- identification of genes involved in FHB resistance in LDN(Dic-3A); *ii*)- transference from LDN(Dic-3A) to susceptible durum varieties of the resistance QTL; *iii*)- development of an *in vitro* seedling assay to identify wheat resistant genotypes and their relationship with disease severity.

Analysis of differential gene expression induced at different time points post- inoculation with *F. graminearum* between LDN(Dic-3A) and the susceptible parental LDN was performed by cDNA-AFLP technique. A total 85 out of the ~500 transcript-derived fragments (TDFs) identified with the diverse primer combination used showed to be differentially expressed: 36% and 19% were identified in LDN(Dic-3A) and LDN, respectively, whereas 45% were induced in both genotypes. The TDF patterns obtained though cDNA-AFLP showed to be reproducible by RT-PCR, supporting the reliability of our experimental system to identify differentially expressed transcripts. Comparison with protein databases revealed that among the cloned TDFs, several showed identity to proteins associated with early response to infection, to NBS-LRR and kinases receptors involved in specific recognition of avirulence pathogen determinant. However, there was a group of TDFs that, in spite of being specific of the inoculation response, could not be assigned to characterized proteins. The identity of these TDFs with ESTs from libraries from *T. aestivum* inoculated with *Fusarium graminearum* additionally supports this affirmation.

The availability of *T. aestivum* genome sequences allowed the *in silico* mapping of 28 TDFs and the identification of several genes and regulatory regions, being the 5BL chromosome arm where most TDFs were located. The analysis of the regulatory regions revealed the existence of transcription regulation mechanisms shared by some TDFs associated genes, such as WRKY proteins, implied in the regulation of genes associated to pathogen defence. The present results suggest that wheat – *F. graminearum* interaction is governed by gene-for-gene relationships.

The development of resistant cultivars has been a difficult task due to the complex inheritance of resistance and the influence of environmental factors. The resistance QTL *Qfhs.ndsu-3AS* from

LDN(Dic-3A) was incorporated in the cultivars Buck Emeralda and Buck Candisur through crosses. F3 homozygous individuals for the resistance allele were subjected to marker assisted selection using the *Xgwm2* microsatellite, linked to the mentioned QTL, allowing a reduction in the number of individuals included in the following steps of the breeding program. In F4, there were selected the individuals that showed equal or better resistance performances compared to the resistant parent, evaluated through the spike severity at 21 days post-inoculation. The breeding program will continue by selfing resistant genotypes to obtain plant materials that possess both stable resistance and suitable agronomic traits.

Rapid and trustable assays are required for the evaluation of germoplasm response to *F. graminearum* infection. In this Thesis, it was developed an *in vitro* assay through the evaluation of the variables *Germination*, *Coleoptile length*, *Coleoptile weight* and *Root weight* using seven varieties of commercial durum, durum wheat cv. LDN and the derived resistant lines LDN(Dic-3A) and LDN-DGE1 and two common wheat genotypes, A601 and A601S3. The seedling variables explained between 51 and 74% of the disease severity, being *Coleoptile length and weight* the ones that more effectively predicted the resistance to FHB. The introgressed genotypes showed better performance in the seedling assay and relatively lower damage in the spikes in relation to susceptible ones, suggesting that this *in vitro* test can detect FHB resistance in different genetic backgrouds. Thus, we propose an *in vitro* assay based on coleoptile variables to perform quick, qualified and cost-effective evaluation of the FHB resistance level, defining the *Seedling Resistance Index*, highly related to severity, for each genotype.

Thus, the present Thesis allowed us to postulate that the interaction wheat – *F. graminearum* could be classified as "gen-for-gen" leading to the expression of defense-related genes. Concurrently, there were obtained genotypes resistant to *F. graminearum* and it was developed an *in vitro* assay that predicts the genotypes reponse to infection.

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