

Identification of differentially expressed genes from Spanish *Ulmus minor* genotypes with contrasted tolerance to *Ophiostoma novo-ulmi*

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Background

Dutch elm disease (DED) is a vascular wilt disease caused by two fungi, *Ophiostoma ulmi* and the more pathogenic *Ophiostoma novo-ulmi*. The spores of DED fungi germinate within branches where the fungus grows and the hyphae spread through xylem vessels inducing their blockage and cavitation, resulting in foliar wilting and the subsequent tree death. The outbreak of two pandemics during the last century severely affected North American and European elm populations. Nowadays, numerous trees are still dying due to the difficulties to control this highly virulent disease. *Ulmus minor* and *U. americana* are the species most drastically affected by DED. Since 1928, in an effort to conserve the genetic resources of native *Ulmus* species and to obtain DED tolerant genotypes several breeding programmes have been carried out in the United States and Europe. Initially, some Asian elms that present a high degree of tolerance to *O. novo-ulmi* were used as source of genetic resistance by crossing them with native species. Recently, the Spanish elm breeding programme obtained seven highly tolerant native *U. minor* genotypes that are registered as forest reproductive material [1]. Using three *U. minor* genotypes with contrasted tolerance to *O. novo-ulmi* (susceptible, moderate and highly tolerant) a massive transcriptome in response to abiotic and biotic stress was constructed. Different replicates of these genotypes were inoculated with *O. novo-ulmi*, *O. ulmi* or the endophyte fungi *Daldinia concentrica* as biotic stress factors and other plants were subjected to a water stress treatment as an abiotic stress factor. A sample containing RNAs pooled from all genotypes and treatments were 454 pirosequenced obtaining a final transcriptome with 58,395 unigenes.

Methods

A selection of unigenes was included in the microarray design (Agilent 8 x 60K, Agilent Technologies, CA, USA). Four Spanish elms with remarkable differences in tolerance to DED were selected for this study. Their degree of tolerance to *O. novo-ulmi* was evaluated by assessing the percentage of wilting leaves at 28, 60 and 120 days post inoculation (dpi) with the pathogen. Two genotypes, M-DV1 (Dehesa de la Villa, Madrid) and VA-AP38 (Arrabal del Portillo, Valladolid), showed high degree of susceptibility to *O. novo-ulmi* whereas the other two genotypes, AB-AM2.4 (Almansa, Albacete) and M-DV2.3 (Dehesa de la Villa, Madrid, registered genotype), were highly tolerant. Five years old plants were inoculated with a highly pathogenic local strain of *O. novo-ulmi* (Z-BU1). The inoculation was carried out about 15-30 days after full leaf development following the protocol described by Solla *et al.* [2]. Control plants were inoculated with distilled water. Two-year-old twigs were collected from a height of 2 meters at 1, 3, 7, 14 and 21 dpi from both control and infected trees. Leaves were removed from the branches, keeping only the stem, immediately frozen in liquid nitrogen and stored at -80° C. RNA from the tissue sampled was hybridised to the microarrays

Results and Conclusions

Tolerance of specific *U. minor* genotypes to *O. novo-ulmi* may be determined, in a great extent, by induced plant defence mechanisms. The activation of a molecular response during fungal colonization should result in the activation of chemical and/or anatomical defence mechanisms; for instance, the accumulation of fungitoxic phenolics or the formation of suberized barriers of parenchyma cells that prevent the spread of the pathogen. In the present work, a total of 2,279 unigenes significantly modified their level of transcripts for any sample point or genotype during the treatment. A set of 236 genes out of the previously mentioned group were identified in the four studied genotypes which is indicative of an expression regulation during infection. An enrichment analysis of this group showed an increase of Gene Ontology (GO) terms related to “response to biotic stimulus”, “cell communication” or “extracellular region”. Genes usually related to tolerance to *O. novo-ulmi* as *PAL* (phenylalanine ammonia lyase) or *CHT* (chitinases) showed significant upregulation. However these genes would not be directly related with tolerance since they changed their expression in both, susceptible and resistant genotypes. In contrast, a consistent group of genes significantly modified the level of transcripts exclusively in tolerant genotypes; 157 genes were identified in AB-AM2.4, 88 genes in M-DV2.3 and 18 genes in both genotypes. In both cases, time course analysis highlighted two main patterns: genes differentially expressed at 1 dpi and genes differentially expressed from 14 to 21 dpi. Notable increases in level of transcripts of *LRR* (Leucine rich repeat), *MYB* and *BHLH* transcription factors among others were identified. These results suggest that *U. minor* tolerance to *O. novo-ulmi* is related to the expression of these differential genes.

Competing interests

The authors declare that they have no competing interests.

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