UNIVERSIDAD POLITECNICA DE MADRID
Departamento de Biotecnologia, E.T.S.I. Agronomos, C. Universitaria, 28040, Madrid, Spain.

A. Delibes, I. Lopez-Brana, S. Moreno-Vazquez, and E. Simonetti.

UNIVERSIDAD DE LLEIDA
Departamento de Produccion Vegetal y Ciencia Forestal, Institut de Recerca i Tecnologia Agroalimentaries (UdL-IRTA), Rovira Roure, 191-25198 Lleida, Spain.

J. A. Martin-Sanchez, E. Sin, C. Martinez, G. Briceno-Felix, A., Michelena, and L. Torres.

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTIFICAS
Departamento de Proteccion Vegetal, Centro de Ciencias Medioambientales, Serrano, 115, 28006, Madrid, Spain.

ME. Andres and M.D. Romero.

Peroxidase expression in a cereal cyst nematode (Heterodera avenae) resistant hexaploid wheat line.

The incompatible interaction between plant and pathogen is often determined by the hypersensitive reaction (HR). This response is associated with accumulation of reactive oxygen species (ROS), which results in adverse growth condi-

tions for pathogens. Two major mechanisms involving either NADPH oxidases or peroxidases have been proposed for generation of ROS. Peroxidases (PER, EC 1.11.1.7), present in all land plants, are members of a large multigenic family with high number of isoforms involved in a broad range of physiological processes.

PER genes, which are expressed in nematode feeding sites, have been identifed in several plant species (Zacheo et al. 1997). A strong correlation between HR and PER activities at four and seven days post nematode infection, was detected in roots of wheat lines carrying *Cre2, Cre5* (from *Ae. ventricosa*) or *Cre7* (from *Ae. triuncialis*) *Heterodera avenae* resistance genes (Andrés et al. 2001; Montes et al. 2003, 2004).

We have studied changes in root of peroxidase mRNAs levels after infection by *H. avenae* of a wheat/*Ae. ventricosa* introgression line (H-93-8) carrying *Cre2* (Delibes et al. 1993). We also report and classify the predicted protein sequences derived from complete peroxidase transcripts.

materials and methods. Seedlings from the resistant line (H-93-8), obtained from the cross [(*T. turgidum* cv. Rubroatum, H-1-1/*Ae. ventricosa*, AP-1)//*T. aestivum* cv. Almatense, H-10-15] (Delibes et al. 1993) were inoculated with pathotype Ha71 of *H. avenae*. Root sections and leaves were harvested 4 and 7 days-after-infection; uninoculated tissues served as controls. Total RNA was extracted using the method of Båga et al. (1995). PER cDNAz were synthesized using 3´RACE a Superscript™ one-step RT-PCR kit (Invitrogen Life Technologies, San Diego, CA) and a 5´RACE SMART™ RACE cDNA Amplifcation Kit (Clontech Laboratories, Inc, Mountain View, CA) kit according to the manufacturer's recommendations. Primers from conserved regions of plant peroxidase genes were used for second cDNA synthesis and PCR. Preferential amplifcation of different PER sequences was obtained with primers designed from low-sequence-homology areas. Amino acid sequences were derived from the coding regions and aligned using MultAlign program (Corpet 1988). A distance-based tree was constructed by NEIGHBOR Joining with MEGA version 3.1 (Kumar et al. 2004).

The expression levels of each PER group in inoculated roots and uninoculated controls were determined by qRT-PCR. Primers for each peroxidase cluster were designed using Primer Express 2.0 software (PE Applied Biosystems, Foster City, CA). PCRs were performed using Power SYBR® Green PCR Master Mix (PE Applied Biosystems, Foster City, CA) according to the manufacturer's instructions in a ABIPRISM 7300 Detection System and software (PE Applied Biosystems, Foster City, CA).

results and conclusions. Comparative analysis of the amino acid sequences predicted from cDNAs revealed that they contain conserved structural features and activity sites of typical class III peroxidases. The distance tree of wheat line H-93-8 peroxidases was organized in fve major clusters of homologous genes (*Pox1*, *Pox2*, *Pox3*, *Pox4*, and *Prec1*; Fig. 1), strongly supported by Bootstrap values. Interestingly, two members from rice peroxidase group IV (BAC79531.1, BAC83103.1, Passardi et al. 2004), which resulted equivalent to pathogen inducible proteins (Chittoor et al. 1997), were closely related to *Pox1*, *Pox2*, and *Pox3*.

Both with and without attack, all PER groups showed weak expression profles in leaves. PER classifed as *Pox1*, *Pox2*, and *Pox3* exhibited enhanced expression in infected roots when compared to noninoculated controls. Nematode infection apparently did not alter the expression pattern of *Pox4*, *Prec1*, and *Putper* in roots. The *Pox3* cluster showed the highest levels of transcription, independently of attack.

Acknowledgement. Financial support for this work was from Grant AGL2004-06791-CO4 from the Ministerio de Ciencia y Tecnología of Spain.

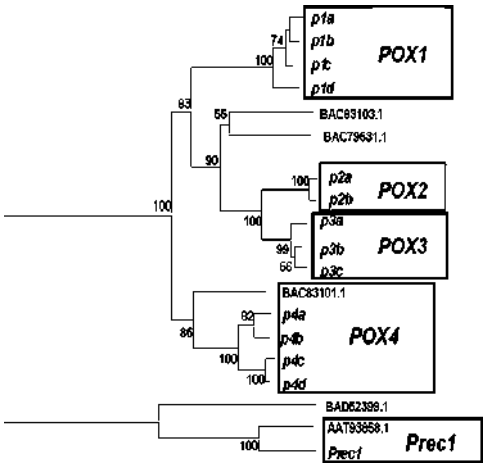

fig. 1. Unrooted phylogenetic tree of wheat line H-93-8 PER based on predicted protein sequences and its relationships with representative rice peroxidases (GenBank accession numbers: AAT93858.1, BAD62399.1, BAC83101.1, BAC79531.1, BAC83103.1). The tree was constructed by the NEIGHBOR joining method. Values at nodes indicate Bootstrap supports greater than 50 %.

references.

Andrés MF, Melillo MT, Delibes A, Romero MD, and Bleve-Zacheo T. 2001. Changes in wheat root enzymes correlated with resistance to cereal cyst nematodes. New Phytol 152:343-354.

Båga M, Chibbar RN, and Kartha KK. 1995. Molecular cloning and expression analysis of peroxidase genes from wheat. Plant Mol Biol 29:647-662.

BOE. 2008. ORDEN APA/303/2008, de 28 de enero, por la que se dispone la inclusión de diversas variedades de distintas especies en el Registro de Variedades Comerciales. Boletín Ofcial del Estado No. 37, 12/02/2008.

Chittoor JM, Leach JE, and White FF. 1997. Differential induction of a peroxidase gene family during infection of rice by *Xanthomonas oryzae* pv *oryzae*. Mol Plant-Microbe Interact 10:861-871.

Corpet F. 1988. Multiple sequence alignment with hierarchical clustering. Nucleic Acids Res 16:10881-90

Delibes A, Romero D, Aguaded S, Duce A, Mena M, López Braña I, Andrés MF, Martín Sánchez JA, and García Olmedo F. 1993. Resistance to the cereal cyst nematode (*Heterodera avenae* Woll.) transferred from the wild grass *Aegilops ventricosa* to hexaploid wheat by a "stepping stone" procedure. Theor Appl Genet 87:402-408.

Kumar S, Tamura K, and Nei M. 2004. MEGA3: Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. Briefngs in Bioinformatics 5:150-163.

Montes MJ, López Braña I, Romero MD, Sin E, Andrés MF, Martín Sánchez JA, and Delibes A. 2003. Comparative study of two *Heterodera avenae* resistance genes of *Aegilops ventricosa*. Differences in defence enzymes induction and chromosomal location in wheat/*Aegilops ventricosa* introgression lines. Theor Appl Genet 107:611-618.

Montes MJ, López Braña I, and Delibes A. 2004. Root enzyme activities associated with resistance to *Heterodera avenae* conferred by gene *Cre7* in a wheat/*Aegilops triuncialis* introgression lines. J Plant Physiol 161:493-495.

Passardi F, Longet D, Penel C, and Dunand C. 2004. The class III peroxidase multigenic family in rice and its evolution in land plants. Phytochem 65:1879-1893.

Romero MD, Montes MJ, Sin E, Lopez-Braña I, Duce A, Martín-Sánchez JA, Andrés MF, and Delibes A. 1998. A cereal cyst nematode (*Heterodera avenae* Woll.) resistance gene transferred from *Aegilops triuncialis* to hexaploid wheat. Theor Appl Genet 96:1135-1140.

Zacheo G, Bleve-Zacheo T, and Melillo MT. 1997. Biochemistry of plant defence responses to nematode infection. *In:* Cellular and molecular aspects of plant-nematode interactions (Fenoll C, Grundler FMW, and Ohl SA, Eds). Kluver, Dordrecht, The Netherlands. Pp. 201-213.

Release of Mapeña spring bread wheat.

mapeña is a spring bread cultivar released in 2007 carrying the *Cre*7 resistance gene to *H. avenae* transferred from *Ae. triuncialis* (Romero et al. 1998). The cultivar was developed from the cross 'TR-353/Betres//Alcotán/3/Rinconada/4/3*Betres' under the designation ID-2181. Mapeña is a high-yielding, medium maturing, semidwarf cultivar with moderate resistance to leaf rust, yellow rust, powdery mildew, and Septoria. This cultivar is better adapted to the southern and northeastern wheat growing regions of Spain. Mapeña has good quality properties for baking industry and is registered in the Spanish Catalogue of Commercial Plant Varieties (BOE, 2008).

Coöperation with other institutions.

We are coöperating with Agrosa Semillas Selectas SA.

Personnel.

Dr. Guillermo Briceño-Felix left the bread wheat program in the UdL-IRTA Center. Dra. María Dolores Romero has just retired from Consejo Superior de Investigaciones Cientifcas.

publications.

Acreche MM, Briceño-Felix G, Martín-Sánchez JA, and Sláfer GA. 2008. Physiological bases of genetic gains in Mediterranean bread wheat yield in Spain. Eur J Agron 28:162-170.

Andrés MF, Simonetti E, González-Belinchón CM, Moreno S, López-Braña I, Romero MD, Martín-Sánchez JA, and Delibes A. 2006. Peroxidase expression in a cereal cyst nematode (*Heterodera avenae*) resistant hexaploid wheat line. *In:* Proc 12th Cong Mediterranean Phytopathogical Union, Rodas, Greece, 11–15 June. Book of Abstracts, pp. 536-537.

Andrés MF, Delibes A, and López-Braña I. 2008. Utilización de marcadores moleculares en el estudio de nematodos ftoparásitos. *In:* Herramientas biotecnológicas en Fitopatología (Sociedad Española de Fitopatología, Pallás V, Escobar C, Rodríguez Palenzuela P and Marcos JF, Eds). Ediciones Mundi Prensa, Madrid. Pp. 189-204 (In Spanish).

Delibes A, López-Braña I, Moreno-Vázquez S, and González-Belinchón CM. 2005. Selección y caracterización molecular y agronómica de trigos hexaploides portadores de genes de resistencia a *Heterodera avenae* y/o *Mayetiola destructor* transferidos desde *Aegilops*. PHYTOMA-España 169:72-75 (In Spanish).

Delibes A, López-Braña I, Moreno-Vázquez S, Gonzalez-Belinchón CM, Romero MD, Andres MF, Martín-Sánchez JA, Briceño G, Sin E, Martínez C, Michelena A, Del Moral J, Pérez Rojas F, and Senero M. 2005. Resistance of bread wheat advanced lines to nematodes and Hessian fy. Progress update. Ann Wheat Newslet 51:161-163.

Delibes A, López-Braña I, Simonetti E, Romero MD, Andres MF, Martín-Sánchez JA, Briceño G, Sin E, Martínez C, Michelena A, and Torres L. 2006. Characterization of resistance to cereal cyst nematodo (*Heterodera avenae*) in *Triticum aestivum*/*Aegilops* introgression lines. Ann Wheat Newslet 52:123-125.

Delibes A, López-Braña I, Martín-Sánchez JA, Sin E, Del Moral J, and Pérez Rojas F. 2007. Characterization of the Hessian fy biotype present in the south-western of Spain. Ann Wheat Newslet 53:87-89.

Delibes A, López-Braña I, Moreno-Vázquez S, Simonetti E, Romero MD, Andres MF, Martín-Sánchez JA, Briceño-Félix G, Sin E, Martínez C, Michelena A, and Torres L. 2007. New advanced lines releases. Ann Wheat Newslet 53:90-91.

Delibes A, López-Braña I, Moreno-Vázquez S, and Martín-Sánchez JÁ. 2008. Review. Characterization and selection of hexaploid wheats containing resistance to *Heterodera avenae* or *Mayetiola destructor* introgressed from *Aegilops*. Spanish J Agric Res 6 (in press).

Martín-Sánchez JA, Sin E, Delibes A, López-Braña I, Romero MD, Andres MF, Del Moral J, Torres L, and Briceño-Felix G. 2005. Advanced bread wheat lines with Hessian fy and cereal cyst nematode resistance genes transferred from *Ae. ventricosa* and *Ae. triuncialis*. *In:* Proc 7th Internat Wheat Conf, Mar del Plata, Argentina. Book of Abstracts, p. 146.

Montes MJ, Andrés MF, Sin E, López-Braña I, Martín-Sánchez JA, Romero MD, and Delibes A. 2008. Cereal cyst nematode resistance conferred by *Cre7* gene from *Aegilops triuncialis* and their relationship with *Cre* genes from Australian wheat cultivars. Genome (in press).

Moreno-Vázquez S, Ning J, and Meyers BC. 2005. hATpin, a family of MITE-like hAT mobile elements conserved in diverse plant species that forms highly stable secondary structures. Plant Mol Biol 58:869-886.

Moreno S, López-Braña I, González-Belinchón CM, Simonetti E, Delibes A, Romero MD, Andrés MF, and Martín-Sánchez JA. 2005. Peroxidase induction in resistant hexaploid wheat in response to cereal cyst nematode (*Heterodera avenae*) infection. *In:* Plant Genomics and Environment (abiotic and biotic). Plant Genomics European Meeting Amsterdam, The Netherlands. Book of Abstracts, p. 212, resume P 8-030.

Simonetti E, González-Belinchón CM, Andrés MF, Moreno S, López-Braña I, Romero MD, Martín-Sánchez JA, and Delibes A. 2006. Análisis de la expresión de peroxidasas en una línea de introgresión *Aegilops*/trigo resistente a *Heterodera avenae*. *In:* Proc XIII Congreso de la Sociedad Española de Fitopatología, 18–22 September, Murcia, Spain. Book of Abstracts, p. 381, resume N-11.

Simonetti E, López-Braña I, Andrés MF, Sin E, Moreno S, Martín-Sánchez JA, and Delibes A. 2007. Peroxidase expression in a cereal cyst nematode (*Heterodera avenae*) resistant hexaploid wheat line. *In:* Proc Plant Genomics European Meeting, Tenerife, Spain, 3–6 October. Book of Abstracts, p. 173, resume P07.4.

Sin E, Martín-Sánchez JA, López-Braña I, Simonetti E, Andrés MF, Del Moral J, Moreno S, Torres i Ruíz L, Briceño G, and Delibes A. 2006. Advanced bread wheat lines carrying *Cre2*, *Cre7*, *H27* and *H30* resistance genes transferred from *Ae. ventricosa* and *Ae. triuncialis*. *In:* Proc 5th Plant Genomics European Meetings, Venice, Italy, 11–14 October. Book of Abstracts, p. 368, resume P 10.24.

Sin E, Martín-Sánchez JA, Delibes A, López-Braña I, Pérez-Rojas F, and Del Moral J. 2006. Evaluation of Hessian fy population (*Mayetiola destructor* Say) in the south-western of Spain. *In:* EUCARPIA Cereal Section Meeting, Cereal Science and Technology for Feeding ten Billion People: Genomics Era and Beyond, Lleida, Spain, 13–17 November. Book of Abstracts, p. 168.