CIHEAM
Options Méditerranéennes

Diversity and evolution in the barley pathogen Pyrenophora teres

Rau D., Attene G., Brown A.H.D., Nanni L., Maier F.J., Balmas V., Saba E., Schafer W., Papa R.

in

Molina-Cano J.L. (ed.), Christou P. (ed.), Graner A. (ed.), Hammer K. (ed.), Jouve N. (ed.), Keller B. (ed.), Lasa J.M. (ed.), Powell W. (ed.), Royo C. (ed.), Shewry P. (ed.), Stanca A.M. (ed.).
Cereal science and technology for feeding ten billion people: genomics era and beyond

Zaragoza : CIHEAM / IRTA
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 81

2008
pages 147-150

Article available on line / Article disponible en ligne à l'adresse :

---

http://om.ciheam.org/article.php?IDPDF=800825

To cite this article / Pour citer cet article

Rau D., Attene G., Brown A.H.D., Nanni L., Maier F.J., Balmas V., Saba E., Schafer W., Papa R. **Diversity and evolution in the barley pathogen Pyrenophora teres.** In : Molina-Cano J.L. (ed.), Christou P. (ed.), Graner A. (ed.), Hammer K. (ed.), Jouve N. (ed.), Keller B. (ed.), Lasa J.M. (ed.), Powell W. (ed.), Royo C. (ed.), Shewry P. (ed.), Stanca A.M. (ed.). *Cereal science and technology for feeding ten billion people: genomics era and beyond.* Zaragoza : CIHEAM / IRTA, 2008. p. 147-150 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 81)

CIHEAM

http://www.ciheam.org/
http://om.ciheam.org/

Diversity and evolution in the barley pathogen *Pyrenophora teres*

D. Rau*, G. Attene, A.H.D. Brown***, L. Nanni*, F.J. Maier****, V. Balmas*****, E. Saba**, W. Schafer**** and R. Papa***

*Dipartimento di Scienze degli Alimenti, Facoltà di Agraria, Università Politecnica delle Marche, Via Brecce Bianche, 60131, Ancona, Italy

**Dipartimento di Scienze Agronomiche e Genetica Vegetale Agraria, Università degli Studi di Sassari, Center for Biotechnology Development and Biodiversity Research Via E. de Nicola, 07100 Sassari, Italy

***Centre for Plant Biodiversity Research, CSIRO Plant Industry, Canberra, ACT 2601, Australia

****Department of Applied Molecular Biology of Plants III (AMPIII), Institute of General Botany, Universität Hamburg, Ohnhorststrasse 18, 22609 Hamburg, Germany

*****Dipartimento di Protezione delle Piante, Università degli Studi di Sassari, Center for Biotechnology Development and Biodiversity Reaserch, Via E. de Nicola, 07100 Sassari, Italy

Net blotch is a foliar disease of barley that has a worldwide distribution and can cause substantial yield losses (Jordan *et al.,* 1985; Steffenson *et al.,* 1991). The casual agent is the heterothallic ascomycetes *Pyrenophora teres* Drechsler (anamorph: *Drechslera teres* [Sacc.] Shoemaker). Two morphologically similar intraspecific *formae speciales* of the net blotch pathogen (that produce different symptoms) are known: the net form (NF) (*P. teres* f. sp. *teres*) and the spot form (SF) (*P. teres* f. sp. *maculata*) (Smedegard-Petersen, 1971). A better understanding of the genetic relationships between these two forms and of the evolutionary potential of the pathogen populations would be useful to improve strategies of resistance breeding and to determine the correct management of the resistance genes for this pathogen (McDonald and Linde, 2002). Here, we present and summarize the results of a study aimed at improving our knowledge of the biological and pathological status of *P. teres*.

Population genetic structure of *P. teres* Drechs. collected from landraces of cultivated barley (*Hordeum vulgare* L.), as revealed by AFLP markers

Monoconidial cultures of *P. teres* were isolated from leaves collected from six populations of the barley landrace 'S'orgiu sardu' (Attene *et al*., 1996; Papa *et al*., 1998) that were growing in five agro-ecological areas of Sardinia, Italy. They were genotyped using AFLPs (Rau *et al*., 2003). One hundred and fifty isolates were from either NF or SF lesions.

Of 121 AFLP markers, 42%, were polymorphic. Cluster analysis resolved the isolates into two strongly divergent groups (F_{ST} = 0.79), corresponding to the NF and SF forms of the pathogen. The absence of intermediate genotypes and the low number of shared markers between the two groups indicated that hybridization between the two *formae* is rare or absent in the field in Sardinia. Our observations are in line with those of Williams *et al*. (2001), who used the same marker system, while the RAPD similarity between one isolate obtained from NF x SF artificial crosses with some field isolates has led some authors to the opposite conclusion (Campbell *et al*., 2002); however, the power of this approach in the detection of hybrids is questionable.

Five out of the six barley populations hosted both forms of the pathogen, but in different proportions. The SF populations were similar in overall polymorphism to the NF populations. However, compared to SF, NF occurred in all of the fields sampled and showed a higher population divergence (F_{ST} = 0.43 *versus* F_{ST} = 0.09 with all isolates; F_{ST} = 0.37 *versus* F_{ST} = 0.06 with clone corrected samples). While the potential role of selection cannot be ruled out *a priori*, this difference in divergence is most probably due to a lower migration rate of NF as compared to SF (Rau *et al*., 2003).

As *P. teres* is know to have a sexual stage, recombination is likely to have a crucial role in shaping

the population structure of this organism. Tybarenc *et al*. (1981) pointed out that with multilocus genotypes, associations among alleles at different loci and over-represented genotypes provide indirect genetic evidence of clonality. AFLP fingerprints resolved 117 distinct genotypes among the 150 isolates sampled (78%), 87% in the SF and 68% in the NF isolates. Although the absolute numbers may be a function of the number of AFLP markers assayed, the relative difference suggests that clonality is more prevalent among the NF isolates (with 11 of 46 haplotypes observed more than once), compared with the SF isolates (seven of 71 haplotypes). Digenic and multilocus (I_A test; Brown, 1980) analyses revealed a variable level of linkage disequilibrium across the populations of both NF and SF, with populations displaying non-significant to highly significant levels of linkage disequilibrium (Rau *et al*., 2003). This suggests that sexual reproduction occurs at significant levels within the NF and SF populations, and that the relative frequencies of sexual and asexual reproduction vary across the different environments.

Isolation and characterization of the mating-type locus of the barley pathogen *P. teres* and frequencies of mating-type idiomorphs within and among fungal populations collected from barley landraces

In heterothallic ascomycetes such as *P. teres*, a single regulatory locus, referred to as the mating type (*MAT*) locus (Kronstad and Staben, 1997; Turgeon, 1998) determines cross compatibility. At this locus, two unorthodox alleles (idiomorphs) are present (*MAT*-1 and *MAT*-2). The sexual cycle is initiated only when two fungal strains carrying different idiomorphs interact (Kronstad and Staben, 1997).

We have isolated and characterised both the *MAT*-1 and *MAT*-2 idiomorphs of *P. teres* f. sp. *teres* mating-type genes (*MAT*-1: 1190 bp; *MAT*-2: 1055 bp) (Rau *et al*., 2005), with the predicted *MAT* proteins of 379 and 333 aminoacids, respectively. The molecular organisation of *MAT*-1 of *P. teres* is similar to that of other Loculoascomycetes belonging to Pleosporales, such as *Cochliobolus heterostrophus* (Wirsel *et al*., 1998), *Alternaria alternata* (Arie *et al*., 2000), *Leptosphaeria maculans* (Cozijnsen and Howlett 2003) and *Phaeosphaeria nodorum* (Bennet *et al*., 2003), where also only an alpha-containing protein is encoded. In all the ascomycetes studied so far, all of the *MAT*-2 genes contain only a single gene encoding a protein with the HMG box as the DNA-binding motif (Turgeon, 1998). This is also the case with *MAT*-2 of *P. teres*, where its size (2028 bp) is intermediate between *C. heterostrophus* (1171 bp) and *P. nodorum* (4505 bp), and closer to *A. alternata* (2256 bp). Phylogenetic analysis has revealed that *P. teres* fits into the Pleosporales group, and despite being well separated, is positioned between *P. nodorum* (Phaeospaheriaceae) and *L. maculans* (Leptosphaeriaceae). Moreover, the clear-cut separation between *P. teres* and the *Cochliobolus* subsp. observed in this study is consistent with other observations based on ITS and GPD DNA sequences (Zhang and Berbee, 2001).

A mating-type PCR assay has also been developed to allow easy mating-type detection in both NF and SF (Rau *et al*., 2005). Using this system, we analysed 150 isolates (68 NF, 82 SF) that were collected from six Sardinian barley landrace populations and previously characterised by AFLP (Rau *et al*., 2003). Indeed, because the mating-type frequencies determine the probability that two random isolates are compatible, the study of the relative frequencies and distributions of mating-types provides an indicator of the likely prevalence of sexual reproduction within and among populations (*e.g.* for ascomycetes barley pathogens: Zhong and Steffenson, 2001; Linde *et al*., 2003; Goodwin *et al*., 2003). The two mating types were present in both the NF and SF populations at the field level, indicating that they have all maintained the potential for sexual reproduction. Despite the two forms being sympatric in five out of the six barley fields (Rau *et al*., 2005), no intermediate isolates were detected by AFLP analysis (Rau *et al*., 2003). These results again suggest that the two forms are likely to be genetically isolated under field conditions.

In all of the samples of *P. teres*, the ratio of the two mating types was consistently in agreement with the 1:1 null hypothesis. This ratio is expected when segregation distortion and clonal selection among mating types are absent or asexual reproduction is rare; if sexual reproduction is operating, the approximate 1:1 ratio between mating types can be maintained by negative-frequency-dependent selection, a kind of balancing selection (Richman, 2000). Overall, despite it not being possible to completely rule out the role of gene flow in the spread the two mating types over the regional territory,

sexual reproduction appears to be the major process that has equalised the frequencies of the two mating types within these populations.

Phylogeny and evolution of mating-type genes from *P. teres*, the causal agent of barley "net blotch" disease

The *MAT* genes appear to be evolving at a faster rate than other sequence regions, such as ITS and GPD that are often used for phylogenetic studies (Turgeon, 1998). Moreover, as the *MAT* gene variation is high among species and low within species, it has been hypothesized that the *MAT* genes "have the potential to mark species boundaries" (Yun *et al*., 2000). Our main aim was thus to test the patterns of sequence divergence within and between the two *MAT* loci and among the *Pyrenophora* taxa (Rau *et al*., unpublished results). The *MAT*-1 and *MAT*-2 mating-type genes were sequenced from 22 NF isolates (12 *MAT*-1 and 10 *MAT*-2 sequences) and 17 SF isolates (10 *MAT*-1 and seven *MAT*-2 sequences) that were collected from the above-detailed Sardinian barley landrace populations, and worldwide (Canada, USA, Germany, Italian peninsula, China and Australia). On the basis of a parsimony network analysis, NF and SF of *P. teres* are phylogenetically separate and reciprocally monophyletic both for *MAT*-1 and *MAT*-2. This resolution perfectly concurred with groups based on disease symptoms and AFLP analysis. Moreover, more than 85% of the total nucleotide variation was found between *formae speciales*. The two forms do not share any polymorphisms. Six diagnostic nucleotide polymorphisms were found, as would be expected in an advanced state of biological speciation and after a long history of reproductive isolation (Koufopanou *et al*., 2001; Geiser *et al*., 1998; Kasuga *et al*., 1999; Taylor *et al*., 2000). When the putative peptides were compared, three diagnostic non-synonymous mutations were found, one in *MAT*-1 and two in *MAT*-2.

In this study we also aimed to evaluate the hypothesis regarding the nature of selective pressures acting on the *MAT* genes of *P. teres*. Neutrality tests suggested that the patterns of variations could probably be due to enforced isolation between NF and SF: selection within forms tends to be purifying, and between forms, diversifying.

Finally, for comparison with *P. teres* sequence data, the mating-type genes from *Pyrenophora graminea* were also isolated and sequenced. Their structures and the predicted protein products of *MAT*-1 and *MAT*-2 were highly similar to those of *P. teres*. Interestingly, divergence between *P. graminea* and *P. teres* is of a similar magnitude to that between NF and SF of *P. teres*. The *MAT* genes of *P. graminea* were closer to those of SF than of NF, with the *MAT*-2 SF peptide not different from the *MAT*-2 peptide of *P. graminea*. Taylor *et al*. (2004) inferred that gene flow takes place between *P. graminea* and *P. teres* from evidence from retrotransposon-based markers (S-SAP).

Overall, these data suggest a long genetic isolation between the two forms of *P. teres* and that hybridization is rare or absent under field conditions, with each form having its own particular niche specialization.

Overall conclusions

Overall, the data suggest that the migration rate is lower in NF than in SF, although it would be interesting to confirm this observation on a higher geographical scale prior to testing for differential dispersal systems or dispersal attitude between these two forms. Moreover, several lines of evidence indicate that sexual reproduction has a significant role in both of these forms, indicating a high potential with both forms for the arising of new pathogenic variants. Finally, it appears that there has been a long history of genetic isolation between the two forms, and that the introgression rate in the field is insufficient to oppose differentiation by genetic drift, as revealed by the reciprocal monophyly of the two forms. Thus, the *P. teres* - *H. vulgare* plant-pathosystem can probably be better considered as a system comprising two closely related pathogen species infecting the same host. This is consistent with the observation of the occurrence of (at least partially) independent QTLs for disease resistance to the two forms in barley (Richter *et al*., 1998; Williams *et al*., 1999). Thus barley breeding programmes aimed at improving the resistance to net blotch should focus on the identification of different sources of resistance for the two pathogens.

References

Arie, T., Kaneko, I., Yoshida, T., Noguchi, M., Nomura, Y. and Yamaguchi, I. (2000). *Molecular Plant-Microbe Interactions,* 13: 1330-1339.

Attene, G., Ceccarelli, S. and Papa, R. (1996). *Genetic Resources and Crop Evolution,* 43: 385-393.

Bennet, R.S., Yun, S.H., Lee, T.Y., Turgeon, B.J., Arseniuk, E., Cunfer, B.M. and Bergstrom, G.C. (2003). *Fungal Genetics and Biology,* 40: 25-37.

Brown, A.H.D., Feldman, M.W. and Nevo, E. (1980). *Genetics,* 96: 523-536.

Campbell, G.F., Lucas, J.A. and Crous, P.W. (2002). *Mycological Research,* 106: 602-608.

Cozijnsen, A.J. and Howlett, B.J. (2003). *Current Genetics,* 43: 351-357.

Geiser, D.M., Pitt, J.I. and Taylor, J.W. (1998). *Proc. Natl. Acad. Sci., USA,* 95: 388-393.

Goodwin, S.B., Waalwijk, C., Kema, G.H., Cavaletto, J.R. and Zhang, G. (2003). *Molecular Genetics and Genomics,* 269: 1-12.

Jordan, J.W.L., Best, G.R. and Allen, E.C. (1985). *Plant Pathology,* 34: 200-206.

Kasuga, T., Taylor, J.W. and White, T.J. (1999). *Journal of Clinical Microbiology,* 37: 653-663.

Koufopanou, V., Burt, A., Szaro, T. and Taylor, J.W. (2001). *Molecular Biology and Evolution,* 18: 1246-1258.

Kronstad, J.W. and Staben, C. (1997). *Annual Review of Genetics,* 31: 245-276.

Linde, C.C., Zala, M., Ceccarelli, S. and McDonald, B.A. (2003). *Fungal Genetics and Biology,* 40: 115-125.

McDonald, B.A. and Linde, C. (2002). *Annual Review of Phytopathology,* 40: 349-379.

Papa, R., Attene, G., Barcaccia, G., Ohgata, A. and Konishi, T. (1998). *Plant Breeding,* 117: 523-530.

Rau, D., Brown, A.H.D., Brubaker, C.L., Attene, G., Balmas, V., Saba, E. and Papa, R. (2003). *Theoretical and Applied Genetics,* 106: 947-959.

Rau, D., Maier, F.J., Papa, R., Brown, A.H.D., Balmas, V., Saba, E., Schafer, W. and Attene, G. (2005). *Genome,* 48: 855-869.

Richman, A. (2000). *Molecular Ecology,* 9: 1953-1963.

Richter, K. Shondelmaier, J. and Jung, C. (1998). *Theoretical and Applied Genetics,* 97: 1125-1234.

Smedegård-Petersen, V. (1971). *Royal Veterinary and Agricultural University, Copenhagen*, pp. 124-144.

Steffenson, B.J., Webster, R.K. and Jackson, L.F. (1991). *Plant Disease,* 75: 96-100.

Taylor, E.J., Kostantinova, P., Leigh, F., Bates, J.A. and Lee, D. (2004). *Genome,* 47: 519-525.

Taylor, J.W., Jacobson, D.J., Kroken, S., Kasuga, T., Geiser, D.M., Hibbet, D.S. and Fisher, M.C. (2000). *Fungal Genetics and Biology,* 31: 21-32.

Turgeon, B.G. (1998). *Annual Review of Phytopathology,* 36: 115-137.

Tybarenc, M., Kjellberg, F., Arnaud, J., Oury, B. and Frederique Breniere, S. (1991). *Proceedings of National Academy of Science of USA,* 88: 5129-5133.

Williams, K.J., Lichon, A., Gianquitto, P., Kretschmer, J.M., Karakousis, A., Manning, S., Langridge, P. and Wallwork, H. (1999). *Theoretical and Applied Genetics,* 99: 323-327.

Williams, K.J., Smyl, C., Lichon, A., Wong, K.J. and Wallwork, H. (2001). *Australasian Plant Pathology,* 30: 37-44.

Wirsel, S., Horwitz, B., Yamaguchi, K., Yoder, O.C. and Turgeon, B.G. (1998). *Molecular General Genetics,* 259: 272-281.

Yun, S.H., Arie, T., Kaneko, I., Yoder, O.C. and Turgeon, B.G. (2000). *Fungal Genetics and Biology,* 31: 7-20.

Zhang, G. and Berbee, M.L. (2001). *Mycologia,* 93: 1048-1063.

Zhong, S. and Steffenson, B.J. (2001). *Mycologia,* 93: 852-863.