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Genetic variation, occurrence of mating types and different forms of *Pyrenophora teres* causing net blotch of barley in Finland

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The amplified fragment length polymorphism (AFLP) was used to study genetic variation in *Pyrenophora teres* causing net blotch of barley in Finland. The mean similarity was 93% between all isolates and a bit higher within two distinct populations based on 175 AFLP markers. Despite the high genetic similarity, 70 unique AFLP genotypes were identified among 72 isolates. Most of the genetic variation (68.5%) was observed within a field population and a smaller portion (30.3%) between them. Significant genetic differentiation (Fst = 0.308, $P < 0.001$) was identified between field populations. However only 1.2% of the variation was observed between mating types within a field and a lack of genetic differentiation (Fsc = 0.017, $P = 0.087$) was observed. The occurrence of the form of blotch (spot type, f. sp. *maculata*, or net type, *P. teres* f. sp. *teres*) was identified with specific PCR. All isolates were found to be of the net type. The existence of both mating types (MAT1 and MAT2) was identified for the first time in Finland and the ratio of the two mating types was almost 1:1 in both locations. The evolutionary potential and the possibility of sexual reproduction of *P. teres* occurring in Finland are discussed.

INTRODUCTION

Pyrenophora teres (anamorph *Drechslera teres*; syn. *Helminthosporium teres*) is an economically important pathogen of cultivated barley, *Hordeum vulgare*, worldwide. *Pyrenophora teres* causes net blotch disease on barley, which reduces grain yield and malting quality by reducing the green leaf area and grain size. The disease symptoms are seen during the growing season on barley leaves and leaf sheaths as net or spot type blotches. The different types of symptoms are caused by two different special forms of the fungus, f. sp. *teres* (net type symptoms) and f. sp. *maculata* (spot type) (Smedegård-Petersen 1971). The two forms of the fungi do not differ morphologically from each other. *Pyrenophora teres* can survive and overwinter on infested stubble or on seed and is capable of asexual reproduction during the growing season (Duczek *et al.* 1999). For sexual reproduction, two mating types are needed because the fungus is heterothallic and self-sterile (McDonald 1963).

Net blotch disease can be controlled either with fungicides or through the use of genetically resistant cultivars of barley. Resistance genes against net and spot type of *P. teres* have been identified and localised on different chromosomes on barley (Bockelman, Sharp & Eslick 1977, Williams *et al.* 1999, Manninen *et al.* 2000, Raman *et al.* 2003). However, the durability of disease resistance depends on the ability of the pathogen population to evolve so that it can overcome resistance. Pathogens with a high evolutionary potential are more likely to overcome the resistance (McDonald & Linde 2002a, b). For many agricultural pathogens, their biology and genetic structure is known well enough to predict their evolutionary potential (McDonald & Linde 2002b) but information on *P. teres* is limited. Rau *et al.* (2003) studied the genetic variation in *P. teres* populations in Italy, which differs greatly from North European conditions. A preliminary study has examined the genetic variation of Finnish *P. teres* populations (Peltonen *et al.* 1996). However, isolates were collected from different years and sites and no genetic structure of field populations, or the genetic differentiation between, or among populations were studied. Information on the reproduction system and the genetic structure of a pathogen population helps in planning of plant breeding or fungicide control strategies.

Amplified fragment length polymorphism (AFLP) originally described by Vos *et al.* (1995) has proven to

be a very reproducible and useful method in analysing genetic variation in fungal populations (e.g. Majer *et al.* 1996, Rau *et al.* 2003). AFLP can distinguish closely related isolates better than other DNA-based techniques such as RFLP, microsatellites, sequencing of the ITS region, and RAPD analysis (Purwantara *et al.* 2000). It can be used to detect clonality in field populations of pathogenic fungi and to show whether disease spread is due to asexual or sexual spores (Purwantara *et al.* 2000). AFLP has been used to demonstrate recombination (Campbell, Crous & Lucas 1999) and genetic differentiation (Rau *et al.* 2003) in *P. teres*.

The objective of the present study was to determine the genetic variation between and within two Finnish field populations of *P. teres*. The distribution of genetic variation between mating types was also examined. Moreover, the genetic differentiation between populations and its significance were assessed. The existence of different mating types and net/spot types of *P. teres* in Finnish field populations was investigated using specific PCR.

MATERIALS AND METHODS

Population samples

Four upper leaves of main shoots of naturally infected barley tillers showing typical net blotch symptoms were collected from two fields (Fig. 1). In the Jokioinen area, leaves were collected from 36 plants at 20 m intervals from cv. 'Inari' at the beginning of Aug. 2001. In the Ylistaro area, leaves were collected similarly from cv. 'Arve' at the end of July 2001. Jokioinen and Ylistaro are 400 km apart. The area of each field location was approx. 1 ha. The dry leaves collected were kept in paper bags for 6 months in $+20\,^{\circ}$C in dry conditions until the fungus was cultured. Monoconidial isolates (36 per collection site) were obtained from dry leaf material according to McDonald (1967). The isolates collected from two distinct fields were considered as separate populations; representative isolates have been deposited in the collection of fungus culture at Agrifood Research, Jokioinen (MTT).

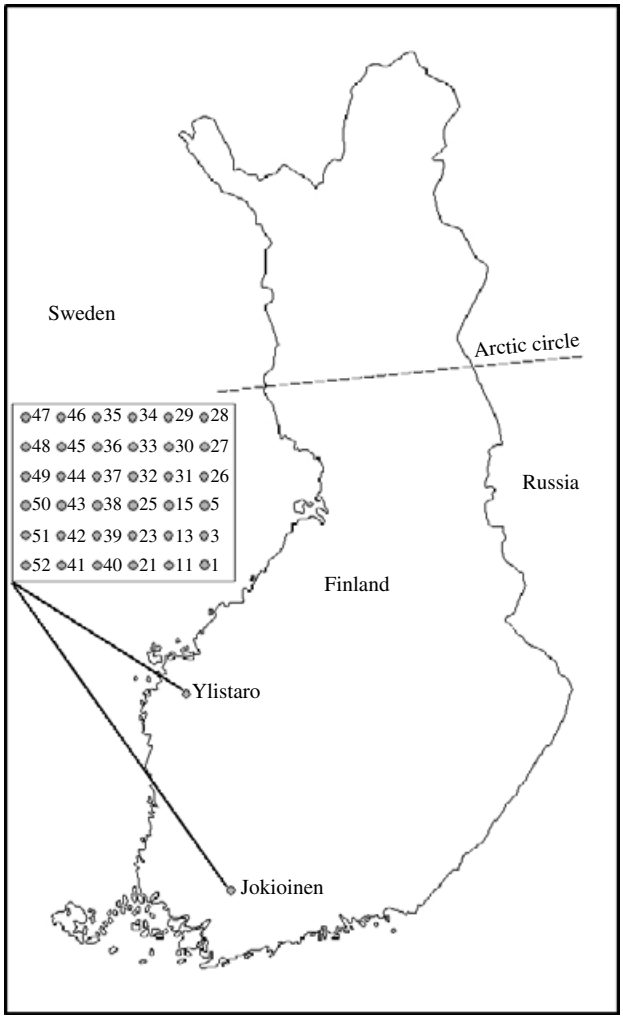

Fig. 1. Location of the collection sites in Finland separated by 400 km, and the position of collection of infected barley leaves from 36 plants located 20 m apart in a 1 ha field (small figure).

Fungal culture and DNA isolation

After monoconidial isolates were obtained, they were maintained on 2.5% V8 agar under nuv light at $18\,^{\circ}$. Mycelia were transferred with a sterile needle from 14 d-old cultures onto 10% (w/v) yeast extract (Hispanlab) and 10% (w/v) saccharose (BDH Laboratory Supplies, Poole) liquid media containing 50 mg l^{-1} kanamycin (Sigma, Steinheim). The cultures were incubated for 10 d on a rotating shaker at 100 rpm at room temperature. Media was poured away before the mycelium was transferred to plastic centrifuge tube (35 ml) containing distilled water and centrifuged at 3670 ***g*** (r_{av} 9 cm) for 5 min at $4\,^{\circ}$. After removal of supernatant, the tube was filled again with distilled water and the centrifugation was repeated as above. The mycelium was lyophilised (Gamma 1–20), kept at $-20\,^{\circ}$, and for long term storage at $-70\,^{\circ}$. The lyophilised mycelium was ground with sand and a ceramic cylinder in a tube (40 s at volume 5 in FastPrep, Q-biogene, Hilden). DNA was isolated from 20–30 μg lyophilised mycelium with DNeasy plant mini kit (Qiagen, Hilden). Prior to AFLP and PCR analyses DNA was diluted to concentration 25 ng μl^{-1}.

AFLP analysis

The AFLP analysis was performed according to Vos *et al.* (1995) and Williams *et al.* (2001) with a few modifications. *Pst*I (a rare cutter) and *Mse*I (a frequent cutter) restriction enzymes, adaptors matching the restriction sites and primers with selective nucleotides were used. The sequences of primers and adaptors used are presented in Table 1.