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Molecular evidence of *Erysiphe pisi* on pea and *E. trifoliorum* on white clover in northeast India

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Abstract Phylogenetic analysis was conducted using the ITS region of nrDNA for confirmation of the identity of anamorphic powdery mildew pathogens on pea and white clover. Maximum likelihood and bayesian analysis clearly indicated that the pathogen responsible for powdery mildew on pea in northeast India is *Erysiphe pisi* rather than *E. trifoliorum* (syn. *E. trifolii*) which has been reported from north and central India. The pathogen *E. trifoliorum* was found associated with white clover. Reliable identification should be the first step in any breeding program.

Keywords Pea · ITS · Erysiphe pisi · Erysiphe trifoliorum

Powdery mildew of pea is an important disease causing heavy yield losses (25–51 %) (Ram and Prasad 1994). Both *Erysiphe pisi* and *E. trifoliorum* (syn. *E. trifolii*) have been reported causing powdery mildew on pea. These species can be distinguished from each other using appendage type on chasmothecia (Braun et al. 2010). However, in tropical climates, chasmothecia formation is rare, hence there is a dependence on anamorphic characters for identification. For *E. pisi* and *E. trifoliorum*, anamorph characters such as conidial size and foot cell size overlap considerably making identification using anamorphic characters almost impossible (Attanayake et al. 2010). Recently, *E. trifoliorum* has been reported from north and central India (Fondevilla et al. 2013).

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Three genes for resistance against *E. pisi* have been described; *er1, er2* and *Er3* (Fondevilla et al. 2013 and references therein). Recently, it was demonstrated that *E. trifoliorum* can overcome *er1* and *Er3* in India as well as in Spain (Fondevilla et al. 2013) whereas all three genes are highly effective against *E. pisi*. These studies demonstrate that pathogen identity is most important for resistance breeding and reliable identification of the powdery mildew pathogen responsible in north east India is necessary.

Samples of pea powdery mildew were collected for molecular analysis. Voucher specimens have been deposited in Agharkar Research Institute Herbarium (AMH-9657), India and in the Herbarium of Martin-Luther- University, Institute of Biology, Dept. of Geobotany, Germany (HAL 2244 F.). DNA isolation was done using a Qiagen kit. PCR was done using universal ITS primers 1 and 4 (White et al. 1990). Nested PCR was also performed using ITS 5–4 then ITS 1-PM 6 combinations. Cycling conditions were initial denaturation 5 min (94 °C), denaturation 30s (94 °C), annealing 40s (52–50 °C, stepdown approach −1 °C in each cycle), extension 50s (72 °C) and final extension 10 min (72 °C). Sequencing was done using primers ITS 1, ITS 4 or PM 6. Sequences have been deposited in Genbank under accession numbers KF156939 and KF946096. Similarity searches were performed using Blastn at NCBI. Both the sequences had 100 % similarity with *E. pisi* sequences. Powdery mildew on white clover (weed sample collected from Shillong, Meghalaya, India) was also identified using the same protocol and sequences have been deposited in Genbank under accession numbers KM095756 and KM485681. Scanning electron microscopy (SEM) (JEOL JSM 6360, JEOL, Tokyo, Japan) was also conducted for both the powdery mildews. Suitable areas were selected using a dissecting microscope and placed on double-sided cellotape then sputter-coated with gold under vacuum using Fine Coat Ion Sputter JFC–1100. Gold-coated

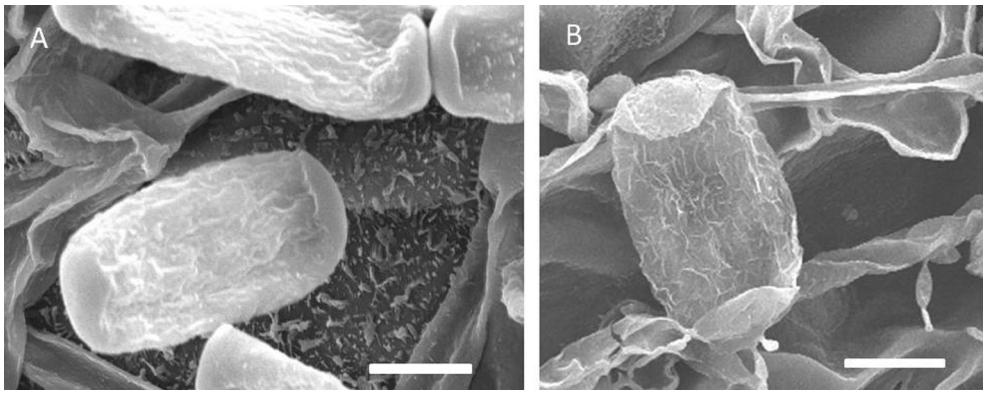

Fig. 1 **a** Conidium of pea powdery mildew (*Erysiphe pisi*) and **b** Conidium of white clover powdery mildew pathogen (*Erysiphe trifoliorum*) using Scanning Electron Microscopy

samples were then placed on aluminium stubs for SEM. Both the powdery mildews were found to be morphologically indistinguishable (Fig. 1).

Sequences for the powdery mildew pathogens reported on legumes (*E. diffusa*, *E. glycines*, *E. astragali*, *E. bremeri* and *E. trifoliorum*) were also obtained from NCBI and used in phylogenetic analysis along with four sequences generated in this study. These pathogens were selected on the basis of the information provided in Braun et al. (2010). Alignment was done using ClustalW implemented in MEGA 6.0 (Tamura et al. 2013). Evolutionary model was also inferred using MEGA 6.0. Phylogenetic analysis was done using Maximum likelihood method with Kimura 2-parameter model (Bootstrap=1000 replicates). A discrete Gamma distribution was used to model evolutionary rate differences among sites. Tree optimisation was done using an extensive subtree pruning and regrafting method (SPR-5). Phylogenetic analysis was also done using BEAST v1.6.1 (Drummond et al. 2010). The parameters set for using BEAUti were generations=10000000 and site heterogeneity=gamma. The starting tree was generated randomly. Two independent runs were done and then combined using LogCombiner v1.6.1 (Rambaut and Drummond 2010a). The program Tracer was used for convergence analysis (Rambaut and Drummond 2007). Trees were summarised using TreeAnnotator v1.6.1 with burnin=2000 (Rambaut and Drummond 2010b). FigTree was used for visualising the tree (Rambaut 2012). The sequence AB015934 (*E. glycines* var *glycines*) was used for rooting the tree. Maximum likelihood as well as bayesian analysis both clustered pea powdery mildew sequences (KF156939 and KF946096) with *E. pisi* (FJ378879 and FJ378872) and sequences of white clover powdery mildew (KM095756 and KM485681) clustered within the clade containing *E. trifoliorum* (Bootstrap=99 % and posterior probability of 1.0) (Fig. 2).

This study provides convincing evidence for the presence of *E. pisi* on pea and *E. trifoliorum* on white clover in this part of the country. This information is useful to breeders and pathologists for devising better management strategies. Since both the pathogens are present, extensive surveys are being conducted to gather comprehensive information related to distribution of these pathogens on these plant species as well as on other related species in this region.

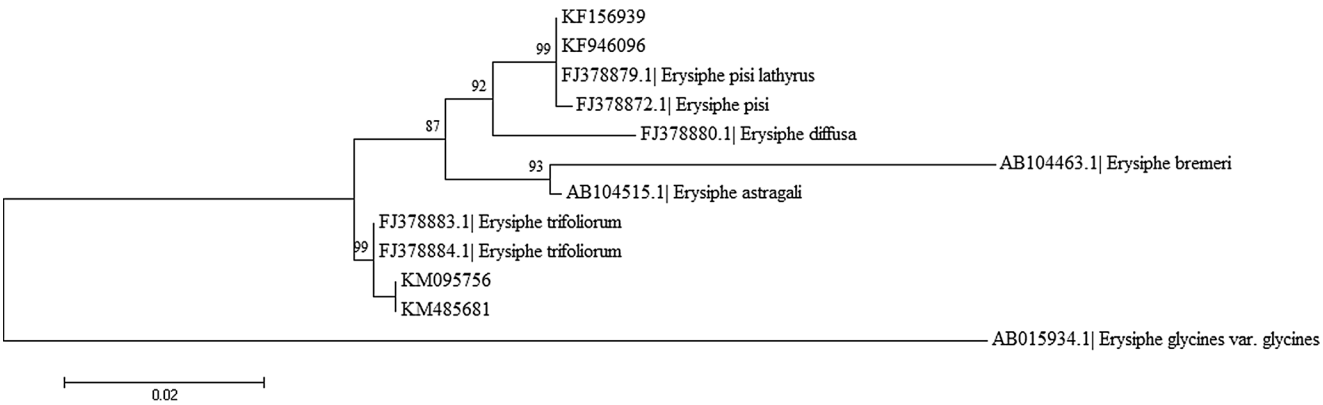

Fig. 2 Phylogenetic tree inferred using maximum likelihood method and bootstrap values are depicted above the branches

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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