Erysiphe quercicola, a powdery mildew fungus on Khasi mandarin in North East India

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Abstract Powdery mildew on Khasi mandarin (*Citrus reticulata*) is considered as one of the factors responsible for citrus decline in this region. Molecular identification was done for powdery mildew pathogen (anamorph) on *Citrus reticulata* based on nrDNA ITS region (ITS 1-5.8s and ITS2). Maximum likelihood analysis clustered the sequences from this study with *Erysiphe quercicola*.

Keywords *Citrus reticulata* · *Erysiphe quercicola* · *Erysiphe alphitoides* · ITS

North East India has very rich diversity of *Citrus* spp. (Patel et al. 2008). Khasi mandarin (*Citrus reticulata*) is the main species cultivated in this region. However, in the past few years, citrus decline has become a major problem reducing the yield of previously highly productive plants. Many abiotic and biotic factors are considered responsible for the decline and powdery mildew is among one of the major causes severely affecting new flushes and seedlings (Singh 2006; Sharma et al. 2001).

In the absence of chasmothecia and reliable anamorphic characters, nuclear rDNA region can be used successfully to link a species anamorph to its corresponding teleomorph and to investigate phylogenetic relationships between different powdery mildew species (Braun and Takamatsu 2000;

Cunnington 2002; Cunnington et al. 2003; Takamatsu 2004; Takamatsu et al. 2006).

Khasi mandarin leaves with abundant greyish-white powdery mildew colonies (Fig. 1) were collected for morphological analysis using scanning electron microscopy (SEM) and molecular identification. The voucher specimen was deposited at the Agharkar Research Institute Herbarium, Pune, India (AMH- 9665).

Scanning electron microscopy (JEOL JSM 6360, JEOL, Tokyo, Japan) was conducted on pre-prepared specimens. Powdery mildew colonies were selected using a dissecting microscope and placed on double-sided cello tape then sputter-coated with gold under vacuum using Fine Coat Ion Sputter JFC-1100. Gold-coated samples were then placed on aluminium stubs for SEM. Conidia were ellipsoid- cylindrical (LM) and had longitudinal ‘angular’ wrinkling patterns (using SEM) on the conidial surface and lobed appressoria on hyphae were observed which are considered typical for *Erysiphe* sp. (*Pseudoidium* anamorph) (Figs. 2 & 3) (Braun and Cook 2011).

The genomic DNA of isolates Pmcr-01 and Pmcr-02 was extracted using the Qiagen Stool Kit. Nested PCR was performed using ITS 5-4 then ITS 1- PM 6 combinations. The PCR was performed in a total volume of 50 µl with the following reagents; ready to use Thermo Scientific DreamTaq PCR Master Mix (×2) (Thermo Fisher Scientific)- 25 µl containing DNA polymerase, PCR buffer, MgCl₂ and dNTPs, 0.4 µl of each primer and ~10 ng of template DNA. 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). These PCR conditions were

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Fig. 1 Powdery mildew symptoms on the leaves of Khasi mandarin

according to Takamatsu and Kano (2001) with minor modifications. Sequencing was done using primers ITS 1 and PM 6.

Consensus sequences were generated, deposited in GenBank (KP797974 and KR131403) and compared with those of reference strains in the GenBank database (Limkaisang et al. 2006; Holford et al. 2010; Takamatsu et al. 2007). Alignment was done using Muscle implemented in MEGA 6.0 (Tamura et al. 2013). Evolutionary model was inferred using jModeltest (Posada 2008). Phylogenetic analysis was done using the Maximum likelihood method with Tamura-3- parameter model. Clade stability was assessed by 1000 bootstrap replicates with random sequence addition. 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).

The ITS sequences (KP797974 and KR131403) of powdery mildew isolates from Khasi mandarin were 99 % similar to *E. quercicola* (AB237791, AB237792, AB237793) and

Fig. 2 Longitudinal 'angular' wrinkling pattern on the conidium of *Erysiphe quercicola* (Bar = 5 μ m)

Fig. 3 Appressoria on hyphae of the anamorph of *Erysiphe quercicola* (Bar = 10 μ m)

Oidium citri (HM164136) sequences in nBlast searches. The maximum likelihood analysis clustered KP797974 and KR131403 with the rest of *E. quercicola* reference sequences with a bootstrap value of 99 % (Fig. 4). The tree was rooted with the sequence of *E. abbreviata* (AB271785). Based on these results, it is clear that the anamorph of the powdery mildew pathogen on *Citrus reticulata* belongs to *E. quercicola*.

Two species that cause powdery mildew are recorded on *Citrus* spp., *Fibroidium tingitaninum* (= *Oidium tingitaninum*) and *Pseudoidium anacardi* (= *Oidium citri*), with the last species having non-catenate conidia (Braun and Cook 2011). The causal organism of powdery mildew on Khasi mandarin has previously been identified as *Oidium tingitaninum* (= *Acrosporium tingitaninum*) in some reports from different parts of North East India (Gupta and Srivastava 1994). The anamorph of *E. quercicola* also has non-catenate conidia.

Takamatsu et al. (2007) named the fungus on *Quercus phillyraeoides*, as *Erysiphe quercicola*. This fungus was distributed in warmer regions in southern Japan, was genetically separated from *E. alphitoides* sensu stricto, and had chasmothecia with appendages shorter than the chasmothecial diameter (in case of *E. alphitoides* appendages are 0.75–2 times as long as the chasmothecial dia.) (Takamatsu et al. 2007). *Erysiphe quercicola* is able to infect other oak species and shows a high level of genetic similarity to anamorphs of powdery mildew species that colonise hosts such as *Citrus* spp., *Anacardium occidentale* (cashew), *Mangifera indica* (mango) and *Acacia* spp. from tropical and subtropical regions of Asia, Africa and South America (Takamatsu et al. 2007). In the absence of chasmothecia it is very difficult to distinguish *E. quercicola* from *E. alphitoides* as the anamorph characters are not clearly distinguishable (Takamatsu et al. 2007).

This appears to be the first correct record of powdery mildew pathogen on *C. reticulata* from North East India based on molecular identification. The present findings are important

Fig. 4 Phylogenetic tree inferred using the maximum likelihood method and bootstrap values are depicted above the branches. Scale bar represents number of substitutions per site

since Khasi mandarin is the main crop grown by farmers in the North East and powdery mildew is a devastating disease causing huge losses especially on new growth and in seedling stage.

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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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