First report of powdery mildew caused by *Golovinomyces bolayi* on okra (*Abelmoschus esculentus*)

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Abstract

Powdery mildew collected from *Abelmoschus esculentus* (Malvaceae) in Karaj (Iran) was identified as *Golovinomyces bolayi*, a species that was recently delimited from the *G. orontii* species complex. The fungus was identified based on morphological characteristics of its asexual and sexual morphs, and rDNA ITS sequence analyses. This is the first identification of *Golovinomyces bolayi* as the causal agent of powdery mildew on *Abelmoschus esculentus* globally. Earlier, closely related taxa from the *G. orontii* species complex were reported from okra from different parts of the world.

Keywords Erysiphaceae · Okra · Plant disease · rDNA ITS

Okra (*Abelmoschus esculentus*, syn. *Hibiscus esculentus*) is an annual vegetable crop with lots of health benefits. Okra plant is infected by different powdery mildew species viz. *Fibroidium abelmoschi* (anamorphic *Podosphaera* sp.), *Podosphaera xanthii*, *Leveillula taurica* s.l., *Golovinomyces orontii* (Braun and Cook 2012) and *Golovinomyces spadiceus* (Moparthi et al. 2018). *Podosphaera* species are common powdery mildew on *Abelmoschus esculentus* and occur worldwide. Recently, *Golovinomyces* spp. were also recorded on okra in Europe, Israel and the United States (Braun 1995; Moparthi et al. 2018; Voytyuk et al. 2006).

During summer and fall of 2019 powdery mildew infections were observed on okra germplasms grown for regeneration of seeds on research fields of Seed and Plant Improvement Institute, Alborz Province, Karaj, Iran. Samples of infected parts were collected in separate paper bags and transferred to the laboratory. The fungal structures were studied by means of a standard light microscope (BH2, Olympus, Tokyo, Japan).

Total DNA was isolated from fungal specimens by the Chelex method (Hirata and Takamatsu 1996).

For PCR, rDNA ITS powdery mildews specific primers PMITS1 (5'-TCGGACTGGCC(T/C)AGGGAGA-3') and PMITS2 (5'-TCACTCGCCGTTACTGAGGT-3') were used for amplification and sequencing of the internal transcribed spacers (Cunnington et al. 2003).

The nucleotide sequences of the polymerase chain reaction (PCR) products were obtained using direct sequencing in a Genetic Analyzer 3130XL (Applied Biosystems, USA) in Rooyan Zista Gene company (Iran, Tehran). Sequences were analyzed and edited using MEGA 7.0 (Kumar et al. 2016). Sequences were compared with the sequences available in the NCBI GenBank nucleotide database using a BLASTN search method. Several sequences (preferably type or reference sequences, if available) from GenBank were selected for phylogenetic analyses. Phylogenetic trees were obtained using the Minimum

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Fig. 1 Powdery mildew infection on okra plants

Fig. 2 *Golovinomyces bolayi*, a ascoma; b appendages; c asci; d ascospores; Scale bar for a = 100 μm , b, c = 50 μm , d = 20 μm

Evolution method in MEGA 7.0 (Kumar et al. 2016). The ITS sequence generated in this study was deposited in GenBank under accession number MT250933.

All okra germplasm plants grown in the field showed severe powdery mildew infections. Disease symptoms appeared as irregular patches on both side of leaves and then extended to whole leaves and other parts such as pods and fruits (Fig. 1).

Mycelium thin, effuse, on both sides of leaves (amphigenous), and on stems; hyphae straight to sinuous, branched, thin-walled, smooth, hyaline; hyphal appressoria nipple-shaped, solitary; conidiophores solitary, arising from the upper surface of hyphal mother cells or lateral, 75–250 μm long, basal septum close to the junction of the supporting hypha or up to 8 μm farther; foot-cells cylindrical or subcylindrical, usually straight or slightly curved to sinuous, 33–105 (–220) $\times$ 7.5–11 (–15) μm , followed by 1–3(–4) shorter cells; conidia in chains, ellipsoid, doliiiform to cylindrical, 22–42 (–52) $\times$ 12–16 (–18.5) μm (dried conidia in 50% lactic acid), conidial germination belonging to the Euoidium type (on natural plant leaves). Chasmothecia amphigenous, mostly hypophilous, scattered to $\pm$ gregarious, 92–120 (–160) μm diam; appendages numerous, arising from the lower half, mycelioid, simple, rarely branched, interwoven with each other and with the mycelium, up to 2.5 times as long as the

Fig. 3 *Golovinomyces bolayi*, a, b conidiophores; c conidia; d conidium germination (on plant leaves); Scale bar for a,b = 50 μ m, b, c = 20 μ m

chasmothecial diam., 4–9 μ m wide, septate, asci broad ellipsoid, saccate, subclavate, 60–88 $\times$ 35–47 μ m, subsessile to stalked, stalk sometimes elongated, up to 23 μ m, 2-spored, ascospores ellipsoid-ovoid, 25–30 $\times$ 16–20 μ m (Figs. 2, 3, 4). Specimen was deposited in Fungarium of University of Guilan under the accession number GUM 1576.

According to BLAST search, full rDNA ITS sequence (including 5.8S) gene showed 99.8% (Identity: 505/506) similarity to accession numbers LC417106 (isolate OLM:35939, holotype for *G. bolayi*, Braun et al. 2019). Moreover, we found 19 rDNA ITS sequences in GenBank that showed 100% similarity to our isolates. Most were identified as *G. bolayi* by submitters such as LC417096, AB769464, AB769463, AB769456, AB769453 and AB427188. Interestingly our sequence was 100% similar to accession number MK601682 which recently was

reported on *Veronica persica* as *G. bolayi* from Iran (Golmohammadi et al. 2019). Several reliable sequences were selected for phylogenetic analysis. These sequences belong to *G. ambrosiae*, *G. bolayi*, *G. circumfusus*, *G. latisporus*, *G. orontii* and *G. tabaci*. *Golovinomyces* on *Abelmoschus esculentus* from Iran clustered with *G. bolayi* isolates and was separate from closely related species (Fig. 5).

Nearly all records of *Golovinomyces* on okra plants assign to the plurivorous and taxonomically complicated powdery mildew species *G. cichoracearum*. (including *G. orontii*) and *Oidium* sp. Recently, taxonomy of *G. cichoracearum*/*G. orontii* complex was revised based on morphology and phylogenetic analyses and several species have segregated from this complex such as *G. ambrosiae*, *G. bolayi*, *G. circumfusus*, *G. latisporus* (Braun and Cook 2012; Braun et al. 2019; Qiu et al. 2020). Considering the above mentioned

Fig. 4 Drawing from *Golovinomyces bolayi*, a conidiophores; b conidia; c conidium germination; Scale bar for a = 50 μm , b, c = 20 μm

evidence, the powdery mildew on okra was belonging to *G. bolayi*.

In this study we retrieved two recently published sequences from *G. orontii* on *Abelmoschus manihot* (accession numbers MH414544, MH414543) from Italy (Garibaldi et al. 2019). Interestingly these sequences had 100% similarity to the Iranian fungus. As shown in Fig. 5, these sequences are in the same clade as the Iranian fungus. Hence, we suggest *Abelmoschus manihot* must be infected by *G. bolayi* rather than *G. orontii* in Italy. A recent record of *G. spadiceus* from Georgia, US (Moparthi et al. 2018) on okra plant is interesting because this was the first record of a segregated member of *G. cichoracearum/orontii* complex which has been identified based on morphology and rDNA ITS

sequence on this plant. However, Qiu et al. (2020) have shown that the older name *G. ambrosiae* (= *E. ambrosiae*) has priority over *G. spadiceus*, and this name is now the correct name for *G. spadiceus*. Hence, we suggest (as shown in Fig. 5) report from Georgia (US), should be assigned to *G. ambrosiae*.

During the pathogenicity test, seven days after inoculation, symptoms started to develop on leaves, as on the originally diseased leaves. No symptoms were observed on control plants. The asexual morphological characters of inoculated leaves were same as originally diseased leaves isolates. To our knowledge, this study provides evidence for the first identification of *G. bolayi* as the causal agent of powdery mildew on okra in Iran, and also globally.

Fig. 5 A Minimum Evolution tree based on ITS sequences for 39 specimens of *Golovinomyces orontii* complex species. The percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. All positions containing gaps and missing data were eliminated. *Golovinomyces asterum* var. *asterum* (AB769416) was used as outgroup taxon. Evolutionary analyses were conducted in MEGA 7

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