

## Molecular Evidence Reveals Presence of *Albugo candida* on *Brassica juncea* var *rugosa* in Northeast India

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Received 20 November 2015; Accepted 30 December 2015; Published online 29 January 2016

**Abstract** White rust was observed on plants of *lai-patta* (*Brassica juncea* var *rugosa*) during December 2013. Host plant (leaves) is used for vegetable purpose in this region. Morphological and molecular evidence based on ITS and *cox2* region confirmed the presence of *Albugo candida* on this host. To our knowledge, this is the first confirmed record of this pathogen on this host. This information will be useful to breeders and also help in devising management strategies in future.

**Keywords** *Brassica juncea* var *rugosa*, *Albugo candida*, ITS, *Cox2* region.

### Introduction

*Brassica juncea* var *rugosa* known as cabbage leaf mustard or more commonly as *lai-patta* in this region is cultivated for vegetable purpose. This cruciferous plant is considered related to cabbage, cauliflower,

turnip, radish and brussels sprout. It is commonly grown in backyard, cultivated land and *Jhum* areas for its leaves. Leaves are also used in dried form and are considered rich source of antioxidants (flavonoids, sulforaphane and indoles) [1].

White rust was noticed on the plants of *lai-patta* during December 2013. On Brassicaceae several species of *Albugo* i.e. *Albugo hesleri*, *A. leimonios*, *A. koreana*, *A. voglmayrii*, *A. laibachii* and *A. hohenheimia*, apart from the generalist species *A. candida*, are reported [2]. Hence, molecular and morphological characterisation was done for ascertaining the species responsible for causing white rust of *B. juncea* var *rugosa*.

(This work was done under the Institute project (IXX05432). Thanks to Animal Production division of our Institute for providing facilities for molecular work. The microscope used in this study has been procured under National Initiative on Climate Resilient Agriculture project -PI, Dr DJ Rajkhowa).

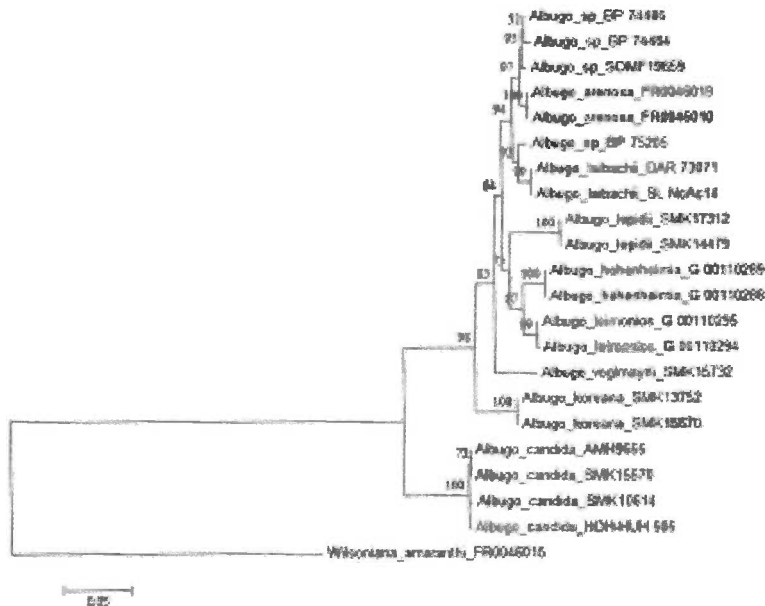
### Materials and Methods

Light microscopy was conducted using Olympus BX 53 microscope equipped with camera DP 72 (Olympus), and image analysis software-cell Sens Standard 1.5 (Olympus) and observations were made using 3% potassium hydroxide as mounting medium. A voucher specimen has been deposited in Agharkar Research Institute Herbarium (AMH-9655), Pune, India.

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**Fig. 1.** Phylogenetic tree inferred using maximum likelihood method and bootstrap values are depicted above the branches. Scale bar represents number of substitutions per site.

Molecular analysis was also conducted for confirmation of the identity. DNA isolation was done using a Qiagen kit. Amplification of *Cytochrome oxidase* subunit II (*cox 2*) region was done using primers designed by Hudspeth et al. [3]. PCR conditions were initial denaturation for 5 minutes, denaturation 30s (94°C), annealing 40s (54–50°C, touchdown with 1°C decrement in every cycle), extension 50s (depending on the primer combination (72°C) and final extension 10 min (72°C) (38 cycles). The ITS region was amplified using ITS1-0 and LR0 [2]. Sequencing was done using the same primers. Similarity searches were performed using Blastn at NCBI. Closely related sequences were included in the analysis. Alignment was done using Muscle as implemented in MEGA 6.0 [4]. Partition homogeneity test was conducted for testing the combinability of the *cox2* and ITS datasets using PAUP\* [5]. Alignments of *cox2* and ITS were concatenated using Sequence matrix program [6]. The program jModel test was used for model selection [7]. Phylogenetic analysis using Maximum likelihood criteria with model HKY+G was conducted on concatenated *cox2* and ITS alignments using MEGA 5.03.

The concatenated sequence from *Wilsoniana amaranthi* (FR 0046015) was used to root the tree [8]. Clade stability was assessed using 1000 bootstrap replicates.

## Results and Discussion

Morphological examination revealed that sori were hypophyllous, distinct, irregular and whitish, sporangiohores hyaline, cylindrical, straight to slightly curved, 25–32 µm long and width 13.6–16.5 µm. Sporangia in basipital chains, hyaline, primary sporangia measured 12.5–15.7 µm (av 13.2 µm) (wall thickness ~1.1 µm) and secondary sporangia 16.5 to 19.5 µm (av 17.3 µm).

The sequences were deposited in Gen Bank with accession numbers (*cox2* :KJ700640 and ITS: KJ700641). Phylogenetic analysis placed our sequences in the clade containing *A. candida* sequences with 100% bootstrap support confirming the identity of the fungus as *A. candida* (Fig. 1).

Earlier, *Albugo candida* was believed to infect the members of Brassicaceae (more than 200 species of several genera) with very broad host range [9, 10]. But molecular evidence and morphological evidence based on oospore ornamentation has revealed presence of high genetic diversity in genus *Albugo* as well as in *A. candida* [2, 11–14]. Many species of *Albugo* have been shown to be host specific like *A. koreana* [15], *A. voglmayrii* [11], *A. laibachii* [13], *A. hohenheimia*, *A. hesleri* and *A. leimonios* [2], and *A. rorippae* [12]. *Albugo lepidii* like *A. candida* has also been known to be infecting native hosts on different continents (widespread). Species *A. hohenheimia*, *A. laibachii* and *A. rorippae* are known from limited geographical range only [8]. *Albugo arenosa* is also considered widespread species, which was created on the basis of phylogeny and oospore ornamentation [8]. It has also been proved by Ploch and Thines [16] that *A. candida* can also survive as a asymptomatic endophyte. In the pathosystem (*Albugo*-Brassica), it is still not clear whether host jumps or co-divergence have shaped the diversification of *Albugo* [8, 17].

To our knowledge, this is the first concrete record of this pathogen based on molecular and morphological evidence. Exact identity of the pathogen is important for taking management decisions as well as for breeding programs.

## Conclusion

Our results clearly proved that the pathogen causing white rust on *B. juncea* var *rugosa* is *A. candida*.

## References

1. Singh BK, Verma VK, Ramakrishna Y (2014) Leafy vegetables of Mizoram. In : Peter KV (ed). Climate resilient crops for the future. NIPA, New Delhi, India, pp 383–404.
2. Ploch S, Choi YJ, Rost C, Shin HD, Schilling E, Thines M (2010) Evolution of diversity in *Albugo* is driven by high host specificity and multiple speciation events on closely related Brassicaceae. *Mol Phylogenet Evol* 57 : 812–820.
3. Hudspeth DS, Stenger DC, Hudspeth ME (2003) A *cox2* phylogenetic hypothesis for the downy mildews and white rusts. *Fungal Divers* 13 : 47–57.
4. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6. Molecular evolutionary genetics Analysis version 6.0. *Mol Biol Evol* 30 : 2725–2729.
5. Swofford DL (2001) PAUP\* : Phylogenetic analysis using parsimony (\*and other methods) Version 4.0b10. Sinauer, Sunderland, MA.
6. Vaidya G, Lohman DJ, Meier R (2011) Sequence matrix: Concatenation software for the fast assembly of multigene datasets with character set and codon information. *Cladistics* 27 : 171–180.
7. Posada D (2008) jModel test 0.1 package available at <http://darwin.uvigo.es>.
8. Mirzaee MR, Ploch S, Runge F, Telle S, Nigrelli L, Thines M (2013) A new presumably widespread species of *Albugo* parasitic to *Strigosella* spp. (Brassicaceae). *Mycol Progress* 12 : 45–52.
9. Choi D, Priest MJ (1995) A key to the genus *Albugo*. *Mycotaxon* 53 : 261–272.
10. Saharan GS, Verma PR (1992) White rusts : A review of economically important species. Int Develop Res Center, Ottawa, Canada.
11. Choi YJ, Shin HD, Ploch S, Thines M (2008) Evidence for uncharted biodiversity in the *Albugo candida* complex, with the description of a new species. *Mycol Res* 112 : 1327–1334.
12. Choi YJ, Thines M (2011) Morphological and molecular confirmation of *Albugo resedae* (Albuginales, Oomycota) as a distinct species from *A. candida*. *Mycol Prog* 10 : 143–148.
13. Thines M, Choi YJ, Kemen E, Ploch S, Holub EB, Shin HD, Jones JDG (2009) A new species of *Albugo* parasitic to *Arabidopsis thaliana* reveals new evolutionary patterns in white blister rusts (Albuginaceae). *Persoonia* 22 : 123–128.
14. Voglmayr H, Riethmüller A (2006) Phylogenetic relationships of *Albugo* species (white blister rusts) based on LSU rDNA sequence and oospore data. *Mycol Res* 110 : 75–85.
15. Choi YJ, Shin HD, Hong SB, Thines M (2007) Morphological and molecular discrimination among *Albugo candida* materials infecting *Capsella bursa-pastoris* worldwide. *Fungal Divers* 27 : 11–34.
16. Ploch S, Thines M (2011) Obligate biotrophic pathogens of the genus *Albugo* are widespread as asymptomatic endophytes in natural populations of Brassicaceae. *Mol Ecol* 20 : 3692–3699.
17. Thines M, Voglmayr H (2009) An introduction to the white blister rusts (Albuginaceae) In : Lamour K, Kamoun S (eds). Oomycete genetics and genomics : Biology, interactions with plants and animals, and toolbox. Wiley-Blackwell, pp 77–92.