

# Short Communication: Genomic Screening of 341 *Macrophomina phaseolina* Genomes Reveals Near-Universal Conservation of CYP51A and SDHB Fungicide Target-Site Hotspots

MD. WALID HASAN JOY

[mdwalidhasanjoy@gmail.com](mailto:mdwalidhasanjoy@gmail.com)

Patuakhali Science and Technology University <https://orcid.org/0009-0005-7067-3512>

MD. MONOAR HOSSAIN MUNNA

Bangladesh Agricultural University

MD. RUBEL MAHMUD

Patuakhali Science and Technology University

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## Short Report

**Keywords:** *Macrophomina phaseolina*, fungicide resistance, CYP51A, SDHB, target-site mutation

**Posted Date:** September 9th, 2026

**DOI:** <https://doi.org/10.21203/rs.3.rs-10831095/v1>

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# Abstract

Fungicide resistance in fungal plant pathogens frequently arises from point mutations at conserved target-site residues, most notably in CYP51 (azole target) and SDH (succinate dehydrogenase inhibitor, SDHI, target) genes. Whether resistance-associated variants already segregate within natural populations of *Macrophomina phaseolina*, the causal agent of charcoal rot on jute and numerous other crops, has not previously been examined at the genomic level. We screened a total of 341 publicly available *M. phaseolina* genome assemblies for amino acid identity at three CYP51A azole-resistance hotspots (G54, L98, M220) and three SDHB SDHI-resistance hotspots (P225, N230, H272), using homology-based mapping against reference sequences from *Aspergillus fumigatus* and *Botrytis cinerea*, the species in which these hotspots were originally characterized. The findings reveal that 99.4% of genomes carried the wild-type, sensitivity-associated residue combination at CYP51A, and 100% at SDHB. Across the sequenced global population, these results indicate that resistance-associated variants at both loci are currently rare to absent in *M. phaseolina*, providing a low-cost genomic baseline for future fungicide-resistance surveillance.

## Full Text

*Macrophomina phaseolina* is a soilborne, necrotrophic ascomycete that causes charcoal rot across more than 500 plant species. Jute (*Corchorus olitorius* and *C. capsularis*) is Bangladesh's principal fibre crop, on which the pathogen causes fibre yield losses of up to 30% (Roy et al. 2008; Islam et al. 2012). Fungicides remain the primary management tool against charcoal rot, and among available fungicide classes, the azoles (demethylation inhibitors, targeting CYP51) and succinate dehydrogenase inhibitors (SDHIs, targeting the SdhB subunit) are of particular resistance-risk concern because each acts on a single, well-characterized protein target. In *Aspergillus fumigatus*, azole resistance arises from substitutions at CYP51A residue G54 (Diaz-Guerra et al. 2003), M220 (Mellado et al. 2004), and L98 (Mellado et al. 2007). In *Botrytis cinerea*, SDHI resistance arises from substitutions at SDHB residues P225, N230, and H272, with different substitutions producing distinct cross-resistance profiles across the SDHI class (Lalève et al. 2014; Veloukas et al. 2013). Although *M. phaseolina*'s phenotypic fungicide sensitivity is known to vary – for example, Egyptian cotton-root isolates ranged from highly sensitive to comparatively tolerant against the SDHI fungicide flutolanil (Mahmoud et al. 2006) – no study has examined whether these specific, previously characterized resistance-associated substitutions are already present within the species at the genomic level.

We retrieved all publicly available *M. phaseolina* genome assemblies at scaffold, chromosome, or complete assembly level from NCBI using the NCBI datasets tool; after quality filtering, 341 assemblies were retained. Because CYP51A and SDHB hotspots have not previously been characterized in *M. phaseolina* itself, we used *tblastn* (BLAST+; Camacho et al. 2009) to search each genome against reference sequences from the species in which each hotspot was originally established: *Aspergillus fumigatus* CYP51A (UniProt Q4WNT5) for the G54/L98/M220 hotspots, and *Botrytis cinerea* (teleomorph *Botryotinia fuckeliana*) SDHB for the P225/N230/H272 hotspots. For each genome, the single highest-

scoring alignment (by bitscore) was retained per gene, and the residue occupying each hotspot position was extracted by mapping alignment coordinates back to the reference sequence's numbering; reference-sequence sanity checks confirmed correct recovery of the expected wild-type residue at each position prior to genome-level scoring. A third candidate locus, beta-tubulin (the benzimidazole fungicide target), was also screened, but the observed pattern – a divergence from the classical wild-type residues across nearly the entire population – could not be reliably distinguished, using a single cross-species reference, from a genuine resistance signal versus an ancestral, species-level sequence difference. This locus is therefore not reported here and is left for dedicated follow-up work using *M. phaseolina*'s own annotated tubulin sequences.

Of the 341 genomes screened, 339 (99.4%) carried the wild-type residue combination at all three CYP51A hotspots; the remaining two genomes (0.6%) carried an alternate combination at positions 98 and 220, with no substitution detected at position 54 in any genome. At SDHB, all 341 genomes (100%) carried the wild-type combination at all three hotspots, with no substitution detected at any position in any genome (Table 1; Fig. 1).

**Table 1** Fungicide target-site hotspot conservation across 341

*Macrophomina phaseolina* genome assemblies

| Gene   | n genomes | Wild-type (n) | % wild-type | % variant |
|--------|-----------|---------------|-------------|-----------|
| CYP51A | 341       | 339           | 99.4        | 0.6       |
| SDHB   | 341       | 341           | 100.0       | 0.0       |

Across the sequenced global *M. phaseolina* population, resistance-associated substitutions are rare at CYP51A and absent at SDHB, with the wild-type residue combination occupying both loci in nearly all genomes. This genomic picture is broadly consistent with the phenotypic findings of Mahmoud et al. (2006), whose Egyptian cotton-root isolates showed reduced but not qualitatively resistant sensitivity to the SDHI fungicide flutolanil; the near-total loss of sensitivity typically associated with an SDHB target-site mutation was not reported, consistent with the absence of any SDHB hotspot variant found here. The two genomes carrying an alternate CYP51A combination are candidates for future targeted phenotypic follow-up, where corresponding isolates are available, rather than for genomic over-interpretation alone. Several limitations apply: this is a genomic prediction, not a phenotypic confirmation, and a wild-type genotype does not rule out resistance through uncharacterized mechanisms; the reference sequences used to define hotspot positions were drawn from other fungal species rather than from *M. phaseolina* itself, following standard practice in the absence of within-species characterization, but direct experimental confirmation of these residues' functional role in *M. phaseolina* has not yet been performed; the sequenced genome pool available in public databases may not fully represent field populations under active fungicide selection pressure; and, as noted above, the beta-tubulin locus was screened but is not reported here pending resolution of an ambiguous

population-wide pattern. Given the low cost of this screening approach using only public data and standard bioinformatic tools, we propose CYP51A and SDHB genotyping as a low-cost, scalable baseline for ongoing fungicide-resistance surveillance in *M. phaseolina*, particularly relevant to jute-growing regions such as Bangladesh, where azole- and SDHI-class fungicides may see increasing use.

## Declarations

### Author contributions

Md. Walid Hasan Joy conceived the study and performed the bioinformatic analyses. Md. Monoar Hossain Munna conducted the literature review and contributed to manuscript writing. Dr. Md. Rubel Mahmud reviewed the work and provided overall direction and supervision. All authors read and approved the final manuscript.

### Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. Computational costs associated with this study were personally funded by the authors.

Conflict of interest: The authors declare no conflict of interest.

Data availability: All genome assemblies analysed in this study are publicly available through NCBI GenBank under the accessions listed in Supplementary Table S1. Analysis scripts are available from the corresponding author upon reasonable request.

Artificial Intelligence disclosure: The authors acknowledge the use of Claude (Anthropic, Claude Sonnet 5) solely for language refinement and manuscript formatting assistance during preparation of this manuscript. The research question, study design, bioinformatic analyses, and scientific conclusions presented in this manuscript are entirely the authors' own.

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## Figures

## Fungicide target-site hotspot conservation across *M. phaseolina*

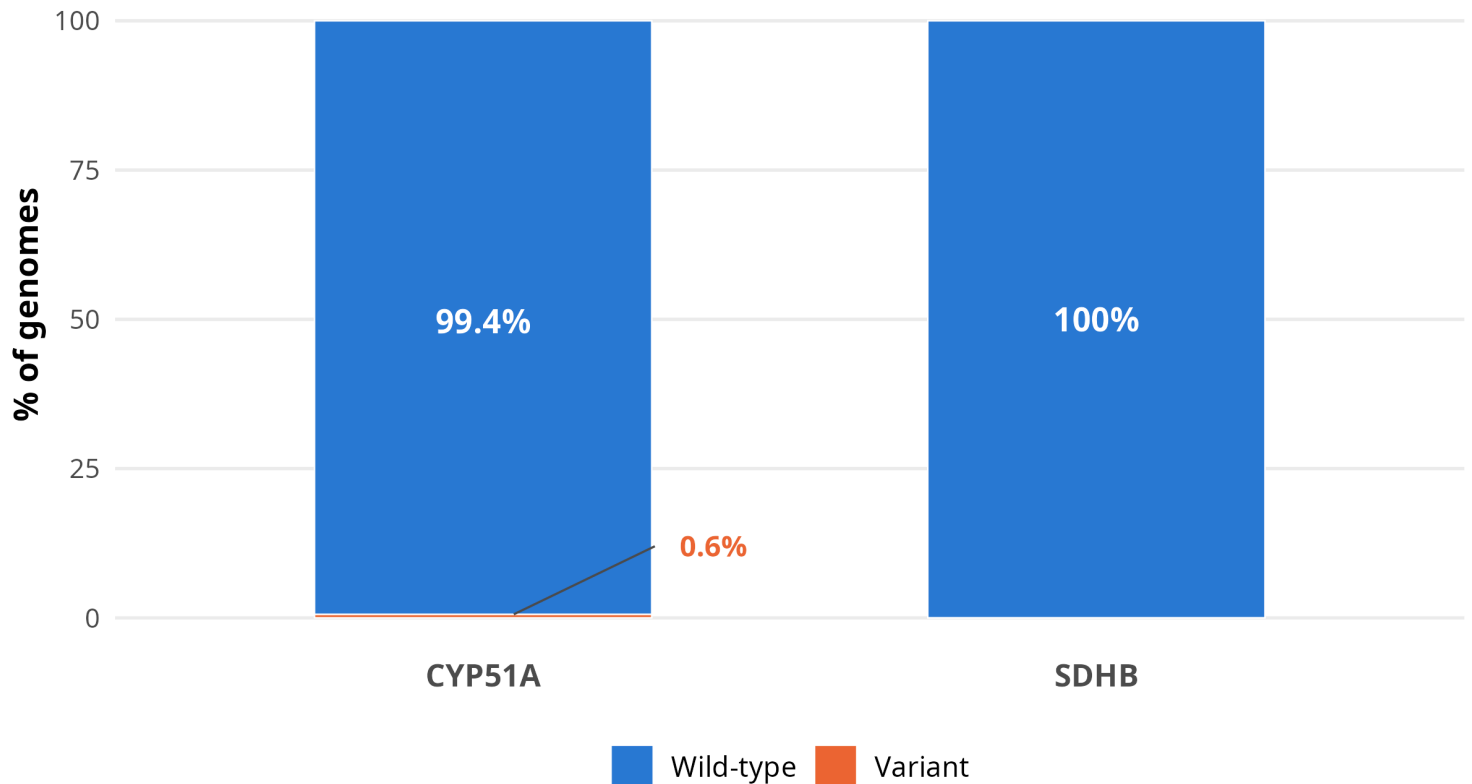


**Figure 1**

Percentage of *Macrophomina phaseolina* genomes (n = 341) carrying the wild-type versus variant residue combination at CYP51A (G54, L98, M220) and SDHB (P225, N230, H272) fungicide target-site hotspots.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTableS1.xlsx](#)