Spray-induced gene silencing targeting the transcription factor *Bcmrr1*: A sustainable RNAi-based strategy to control *Botrytis cinerea* and overcome multidrug resistance

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Abstract

Botrytis cinerea, the causal agent of gray mold, represents a serious threat to horticultural crops worldwide and is considered a high-risk pathogen for fungicide resistance. Among the mechanisms conferring fungicide resistance in B. cinerea, the most prominent are point mutations in fungicide target genes and the overexpression of drug efflux transporters, which result in multidrug resistance (MDR). In this study, spray-induced gene silencing (SIGS) targeting Bcmrr1, a transcriptional regulator of the ABC transporter BcatrB, was evaluated as a novel strategy to control fungicide-sensitive and MDR B. cinerea isolates. Double-stranded RNA (dsRNA) targeting Bcmrr1 and BcatrB were initially tested in vitro and in detached-leaves assays. Both dsRNAs effectively inhibited spore germination and reduced lesion development, fungal biomass, and target gene expression. However, Bcmrr1-dsRNA consistently provided superior and more stable protection and was therefore selected for further analyses. Foliar application of Bcmrr1-dsRNA conferred effective disease suppression for up to 14 days post-treatment, associated with decreased fungal colonization and transient Bcmrr1 repression. Comparable protective effects were observed in apple fruit, confirming cross-host efficacy. Notably, co-application of Bcmrr1-dsRNA with sublethal doses of fludioxonil enhanced disease control and partially restored fungicide sensitivity in MDR isolates, demonstrating a synergistic interaction between RNAi-based and chemical control strategies. Cross-species analyses revealed high sequence conservation of Bcmrr1 within the Botrytis genus and significant inhibition of B. fragariae and B. pseudocinerea, highlighting the broad-spectrum potential of this approach. Collectively, these findings identify Bcmrr1 as a promising RNAi target for overcoming multidrug resistance in and establish SIGS as a selective and environmentally sustainable tool for gray mold management in horticultural systems.