

Supplemental Materials and Methods

Volcano plot for RNA-seq. From the 12,008 genes identified in the transcriptomic analysis, 9,499 genes were found to have FPKM values equal to or above 0.05. These 9,499 genes were plotted using csVolcano from cummerbund, significant genes defined by $\alpha = 0.05$.

Heterologous expression of ABC transporters. The galactose-inducible expression vector pYES2 (Invitrogen) containing the full-length cDNA sequences of *ShatrD* (TCONS_00024506) was transformed into a drug-hypersensitive *Saccharomyces cerevisiae* mutant (AD12345678:AD1-8) (1) to generate two independent mutants (AD1-8:atrD-1 and -2). The ShPDR1 expressing yeast mutants (AD1-8:PDR1-1 and -2) and the control yeast mutant containing the empty pYES2 vector (AD1-8-pYES2) generated by Sang *et al.* (2) were used in this study. The yeast spot assay of the ShPDR1 and ShatrD expressing yeast mutants and empty vector mutant was conducted by the method described in Sang *et al.* (2). Briefly, 5 μ L of cell suspension from each yeast mutant culture at 0.5 OD₆₀₀ were spotted onto bacto-yeast nitrogen base (YNB) agar medium lacking uracil, containing 2% galactose and amended without and with fungicides and plant growth regulators (PGRs). The name of chemicals and their concentrations are as follows: propiconazole (0.005 μ g mL⁻¹), iprodione (500 μ g mL⁻¹), boscalid (200, 500, and 750 μ g mL⁻¹), fluxapyroxad (500 μ g mL⁻¹), flurprimidol (25 μ g mL⁻¹), paclobutrazol (0.1 μ g mL⁻¹), and fludioxonil (250, 500, and 750 μ g mL⁻¹). The sensitivity of yeast mutants to respective fungicides and PGRs was qualitatively assessed after incubation at 30°C for 3 days (iprodione and fludioxonil), 4 days (flurprimidol), and 6 days (untreatment, propiconazole, fluxapyroxad, paclobutrazol, and boscalid). Two biological replicates and three technical replicates per biological replicate were performed for each mutant and treatment.

Probabilistic variant detection. To identify variants between 78 putative fungal specific Zn₂Cys₆ transcription factors from the MDR strain HRI11 and sensitive strain HRS10, Map Reads to Reference and Probabilistic Variant Detection on the CLC Genomics Workbench 6 software (Qiagen CLC Bio) were conducted. 78 transcripts from HRI11 were mapped to 78 transcripts from HRS10 and the mapped result was used for Probabilistic Variant Detection analysis. The filtering resulted in variants with following characteristics: i) no ignore non-specific matches, ii) minimum coverage = 1, variant probability = 10, required variant count = 1, and iii) maximum expected variants = 1 and genetic code = 1 standard. These filter criteria resulted in identifying 4 variants, TCONS_00003992 (T2558C), TCONS_00016457 (T891G, C2268G), TCONS_00003660 (G272C).

Phylogenetic Analysis. The amino acid sequences of *S. homoeocarpa* CYP561, CYP65, and CYP68, its putative orthologs in select fungal species from Basic Local Alignment Search Tool (BLAST) in Fungal Cytochrome P450 Database (FCPD: <http://p450.riceblast.snu.ac.kr>) and National Center for Biotechnology Institution (NCBI), CYP561, CYP65, and CYP68 in select plant and animal from FCPD, and reported CYP65 and CYP68 genes involved in biosynthesis of secondary metabolites (3-7) obtained from GenBank were used for the phylogenetic analysis. MAFFT v7.110 was used to align amino acid sequences with automated strategy selection and BLOSUM62 scoring matrix parameters (8). Neighbor-joining phylogenetic analysis was conducted using MEGA v5 with the aligned amino acid sequences with the Poisson model, gamma distributed rates among sites, complete deletion of missing data, and 1,000 bootstrap

replicates (9). For phylogenetic analysis of ShXDR1 orthologs, the amino acid sequences of ShXDR1, its putative orthologs in select fungal species identified from the BLAST in NCBI, and the reported transcription factors involved in multidrug resistance (10-13) obtained from GenBank were used for the phylogenetic analysis. The alignment and neighbor-joining phylogenetic tree were performed followed by the aforementioned methods.

Silencing of *ShXDR1* by expression of double stranded RNA. Each sense and antisense fragment (nucleotides 71-270) of *ShXDR1* amplified from cDNA of strain HRI11 was inserted into the plasmid pFGC1008 (Arabidopsis Biological Resource Center, Columbus, OH, USA). The sense and antisense fragment from plasmid pFGC1008 was inserted into plasmid pYHN3-ptpC to generate the plasmid pYHN3-ptpC-dsRNAXDR1 that constitutively overexpresses hairpin double stranded RNA (dsRNA) of target sequences of *ShXDR1*. The plasmid DNA (8 μg) was transformed into protoplasts from strain HRI11 using a PEG-mediated transformation system (14). *In vitro* sensitivity tests of two selected transformants (HRI11(dsRNA-XDR1)-1 and -2), HRS10, and HRI11 to propiconazole (1 $\mu\text{g ml}^{-1}$), iprodione (3 $\mu\text{g ml}^{-1}$), boscalid (1000 $\mu\text{g ml}^{-1}$), and flurprimidol (15 $\mu\text{g ml}^{-1}$) were conducted. The expression of *CYP56I*, *CYP65*, *CYP68*, *ShPDR1*, *ShatrD*, and *ShXDR1* was quantified in the aforementioned four strains before and after treatment of propiconazole (1 $\mu\text{g ml}^{-1}$) for 40 min.

Knock-out of ShXDR1 by a CRISPR-Cas9 system. Two vectors p415-PtpC-Cas9-TtpC-CYC1t (Addgene Plasmid #68059) and p426-SNR52p-gRNA.csr-1.Y-SUP4t (Addgene Plasmid #68060) were used for a CRISPR-Cas9 system (15). The vector p426-SNR52p-gRNA.csr-1.Y-SUP4t was modified by insertion of target sequences (5'-GCGAACAGACCTGGGTATGTGG-3') from *ShXDR1* gene sequences using two-round PCR. Two fragments were amplified from the vector p426-SNR52p-gRNA.csr-1.Y-SUP4t using two primer sets (F_NheI_SNR52p and R_gRNA_XDR1; F_gRNA_XDR1 and R_KpnI_CYC1t). Two fragments that are overlapped the target sequences were used as PCR templates to amplify one fragment using F_NheI_SNR52p and R_KpnI_CYC1t. The resultant fragment and the vector p426-SNR52p-gRNA.csr-1.Y-SUP4t were digested by restriction enzymes NheI and KpnI and the purified insert and linearized vector were ligated to generate p426-SNR52p-gRNA.ShXDR1.Y-SUP4t. For the split-marker approach, two constructs (One with 1 kb of upstream region of the *ShXDR1* and 731 bp of hph and one with 1 kb of downstream region of the *ShXDR1* and 1126 bp of PtpC-hph) were amplified using two primer sets F_upXDR1/R_YG and F_HY/R_downXDR1 from the vector Topo- Δ ShXDR1 (donor vector DNA). Before *S. homoeocarpa* transformation, four different ways of DNA constructs were prepared: donor vector DNA only (5 μg), donor vector DNA (5 μg) + p415-PtpC-Cas9-TtpC-CYC1t (5 μg) + p426-SNR52p-gRNA.ShXDR1.Y-SUP4t (5 μg), split marker constructs only (each 2.5 μg), split marker constructs (each 2.5 μg) + p415-PtpC-Cas9-TtpC-CYC1t (5 μg) + p426-SNR52p-gRNA.ShXDR1.Y-SUP4t (5 μg). Hygromycin-resistant transformants were obtained 4-10 days after incubation. The hyphal-tip of transformants was transferred to PDA containing hygromycin B (100 $\mu\text{g ml}^{-1}$). *In vitro* sensitivity test of the transferred transformants to the DMI fungicide (propiconazole) was conducted. The transformants and strain HRI11 were grown on PDA for 3 days and an agar plug (5 mm) of each strain from the edge of colonies was placed on PDA and propiconazole (1 $\mu\text{g ml}^{-1}$) amended PDA. After 2 days of incubation at 25°C, the number of transformants grown or not grown on propiconazole amended PDA was counted. The expression of *ShatrD* was quantified in strains HRI11, Trans1, and Trans 2 before and after treatment of propiconazole (1 $\mu\text{g ml}^{-1}$) for 40 min.

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Sequence information

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