Molecular Mechanisms of Succinate Dehydrogenase Inhibitor Resistance in Phytopathogenic Fungi

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The succinate dehydrogenase inhibitor (SDHI) is a class of fungicides, which is widely and rapidly used to manage fungal pathogens in the agriculture field. Currently, fungicide resistance to SDHIs has been developed in many different plant pathogenic fungi, causing diseases on crops, fruits, vegetables, and turf. Understanding the molecular mechanisms of fungicide resistance is important for effective prevention and resistance management strategies. Two different mechanisms have currently been known in SDHI resistance. The SDHI target genes, SdhB, SdhC, and SdhD, mutation(s) confer resistance to SDHIs. In addition, overexpression of ABC transporters is involved in reduced sensitivity to SDHI fungicides. In this review, the current status of SDHI resistance mechanisms in phytopathogenic fungi is discussed.

Keywords: ABC transporters, Fungicide resistance, Succinate dehydrogenase inhibitors, Target gene mutation

Introduction

The current broad-spectrum fungicides for control of fungal diseases include sterol demethylation inhibitors (DMIs), quinone-outside inhibitors (QoIs), and succinate dehydrogenase inhibitors (SDHIs). DMIs and QoIs have been used at least 3–4 decades, and resistance to DMIs and QoIs has been reported in many pathogenic fungi. SDHIs are relatively new fungicide classes than DMIs and QoIs and have been rapidly adopted by the fungicide market in the agricultural field. However, repeated applications of SDHIs have led to develop resistance to SDHIs in economically important fungal pathogens, such as Botrytis cinerea and Zymoseptoria tritici. The recent review paper of SDHI resistance was written by Sierotzki and Scalliet (2013), which described the mode of action of SDHIs and different aspects of SDHI resistance. Although the Fungicide Resistance Action Committee reports the status of SDHI resistance every year, the lists of reported fungal pathogens with SDHI resistance are not all included. This review describes the current knowledge of SDHI resistance and the resistance mechanisms in plant pathogenic fungi.

SDHI Fungicides

The mode of action of SDHIs is the inhibition of fungal respiration by blocking the ubiquinone-binding (Qp) site. The Qp pocket is structurally defined by the interface between the succinate dehydrogenase (SDH) enzymes (succinate ubiquinone oxidoreductase [EC 1.3.5.1]), SdhB, SdhC, and SdhD subunits (Avenot and Michailides, 2010; Sierotzki and Scalliet, 2013). The first generation of SDHIs is carboxin and oxycarboxin and has been applied to control...
basidiomycete plant pathogens (e.g., *Rhizoctonia* sp. and rusts) (Avenot and Michailides, 2010; Von Schmeling and Kulka, 1966). The newer generation SDHIs such as boscalid, pentiopyrad, fluopyram, and fluxapyroxad are the broad-spectrum fungicides against fungal pathogens of various crops, fruits, vegetables and turf (Avenot and Michailides, 2010; Sierotzki and Scalliet, 2013). Currently, 23 different compounds (benodanil, flutolanil, mepronil, isofetamid, fluopyram, fenfuram, carboxin, oxycarboxin, thifluzamide, benzovindiflupyr, bixafen, fluindapyr, fluxapyroxad, furametpyr, inpyrflozinam, isopyrazam, penfufen, pentiopyrad, sedaxane, isofluYPyram, pydifulmetofen, boscalid, and pyraziflumid) in the 11 different chemical group of SDHIs have been listed in Fungicide Resistance Action Committee (2020). Chemical group of fungicides such as pentiopyrad and bixafen have exerted high selection pressure to fungal pathogens on different plant hosts, but some SDHIs like fluopyram displays higher activity against pathogens on broad leaved than graminaceous hosts (Sierotzki and Scalliet, 2013). The main molecular mechanism of SDHI resistance in fungi is the mutation(s) in the SDHI target genes, *SdhB*, *SdhC*, and *SdhD*, resulting in a decreased in binding affinity for SDHI fungicides. Also, overexpression of efflux transporter(s) in fungal species causes a reduced intracellular accumulation of SDHI fungicides, which confers reduced sensitivity to SDHI fungicides (Fig. 1).

**Fig. 1.** Molecular mechanisms of succinate dehydrogenase inhibitor (SDHI) resistance in plant pathogenic fungi. (A) Mutation(s) in the *SdhB*, *SdhC*, and *SdhD* results in a decreased in binding affinity for SDHI fungicides. (B) Overexpression of efflux transporters causes a reduced intracellular accumulation of SDHI fungicides. The illustration was created using a web app, BioRender (http://www.biorender.com).

### Mechanisms of SDHI Resistance: Mutations in the SDHI Target Genes (*SdhB*, *SdhC*, and *SdhD*)

**Resistance in *Alternaria alternata* and *A. solani.*** In *Alternaria* species, field resistance of *A. alternata* and *A. solani* to SDHIs have been mainly reported and mutations in the SDHI target genes (*SdhB*, *SdhC*, and *SdhD*) in the field resistant isolates were detected (Avenot et al., 2019, 2015; Fan et al., 2015; Sierotzki et al., 2013; Yang et al., 2015). SDHI fungicides have been applied to pistachio and peach orchards to control *Alternaria* late blight and *Alternaria* brown spot caused by *A. alternata*, respectively. The first report of SDHI resistance in *A. alternata* was from pistachio orchards after only 2 years of use of the boscalid-containing product Pristine (Avenot and Michailides, 2007). A mutation (H277Y or H277R) in *SdhB* found in *A. alternata* field strains from pistachio was correlated with boscalid resistance (Avenot et al., 2008). Following the first mutation detection, Avenot et al. (2009) found various mutations in *SdhB* (H277Y/R/L, P230A/R/I/F/D, and N235D/T/E/G), *SdhC* (H133R, H134R, and S135R), and *SdhD* (D123E and H133P/R) from the SDHI fungicide resistant *A. alternata* field isolates (Avenot et al., 2009, 2014, 2019). In peach orchards, *A. alternata* SDHI-resistant isolates harbored mutations in *SdhB* (H277Y/R/L), *SdhC* (G79R and H134R), and *SdhD* (A47T and D123E). The sensitivity assay of the resistant isolates revealed that mutations in *SdhB* (H277Y/R) and *SdhC* (H134R) were consistently correlated with the phenotype exhibiting the high level of resistance to boscalid. Mutations *SdhC* (H134R) and *SdhD* (D123E) were associated with high resistance to pentiopyrad and the isolates containing *SdhB* (H277L) or *SdhC* (H134R) showed the highest resistance level to boscalid, fluopyram, pentiopyrad, and fluxapyroxad among the strains from peach orchards (Yang et al., 2015). The study of fitness of *A. alternata* field isolates indicated that genotypes of *SdhB* (H277Y) and *SdhC* (H134R) do not suffer the fitness penalties on growth, spore production, osmotic and oxidative sensitivity, and pathogenicity but the *SdhD* (D123E) genotype displayed hypersensitive to oxidative stress and lower production of spores (Fan et al., 2015).

Resistance of *A. solani* to boscalid was reported from various potato production regions (Florida, Idaho, Minnesota, Nebraska, North Dakota, Texas, and Wisconsin) in the United States in 2010 and 2011 (Gudmestad et al., 2013). The same research group investigated the sequences of *SdhB*, *SdhC*, and *SdhD* of boscalid resistant *A. solani* isolates and detected
the mutations in \textit{SdhB} (H278Y/R), \textit{SdhC} (H134R), and \textit{SdhD} (D123E and H133R) (Mallik et al., 2014). Bauske et al. (2018) described the temporal and spatial distribution of these \textit{Sdh} gene mutations. The \textit{SdhB} (H278Y/R) were the most prevalent distributed mutations from 2010 to 2011 in the sampled states, but the \textit{SdhC} (H134R) was the most prevalent mutation from 2013 to 2015. The strains with \textit{SdhB} (H278Y/R) were highly collected in North Dakota, Minnesota, and Wisconsin, but the strains with \textit{SdhC} (H134R) were prevalently distributed in Colorado, Texas, North Dakota, and Minnesota (Bauske et al., 2018). In addition, the parasitic fitness study of \textit{A. solani} strains revealed that the five \textit{Sdh} gene mutations do not confer fitness penalties, and only the SDHI-resistant strain containing the D123E mutation exhibited more aggressiveness on tomato leaves than the sensitive strain (Bauske and Gudmestad, 2018). Interestingly, the strains containing the D123E mutation were highly resistant to boscalid but sensitive to fluopyram (Bauske et al., 2018; Mallik et al., 2014), which supports that a lack of cross-resistance between boscalid and fluopyram in other studies (Fairchild et al., 2013; Gudmestad et al., 2013; Mallik et al., 2014; Miles et al., 2014).

**Resistance in \textit{Botrytis cinerea}**. Gray mold caused by \textit{Botrytis cinerea} is the devastating disease on more than 230 host plants including economically important fruits, such as apples, grapes, and strawberries (Jarvis, 1977). The SDHI resistance in \textit{B. cinerea} has been mostly reported in apple orchards, vineyard, and strawberry farms (Kim and Xiao, 2010; Leroch et al., 2011; Leroux et al., 2010; Veloukas et al., 2011). Also, \textit{B. cinerea} isolates exhibiting resistance to multiple fungicides including boscalid were reported from kiwifruits (Bardas et al., 2010). The molecular mechanism of SDHI resistance in the \textit{B. cinerea} field isolates was investigated; mutations (\textit{SdhB}: N230I, H272Y/R/L, and P225F) in the isolates from strawberry farms (Veloukas et al., 2011), mutations (\textit{SdhB}: P225L, N230I, and H272Y/R/L) in isolates from the vineyards (Leroux et al., 2010) and mutations in the isolates from apple orchards (Yin et al., 2011) were discovered in the \textit{B. cinerea} field resistant isolates. Since the initial detection of mutations in \textit{Sdh} genes of \textit{B. cinerea}, the \textit{SdhB} (P225F/H/L/T), \textit{SdhB} (N230I), and \textit{SdhB} (H272Y/L/R) mutation mutants exhibiting resistance to SDHIs have been found in China, Greece, Italy, Spain, and the United States (Amiri et al., 2014; De Miccolis Angelini et al., 2014; Fan et al., 2015; Fernández-Ortuño et al., 2017; Grabke and Stammier, 2015; Hu et al., 2016; Li et al., 2014; Veloukas et al., 2013). In the study of Veloukas et al. (2013), the sensitivity of \textit{B. cinerea} strains harboring five different mutations in \textit{SdhB} were assayed to eight SDHI fungicides (boscalid, isopyrazam, fenfuram, carboxin, fluopyram, bixafen, fluxapyroxad, and benodaniil). The mutation \textit{SdhB} (P225F) conferred resistance to all eight fungicides, but mutation \textit{SdhB} (N230I) conferred moderate resistance to boscalid, fluopyram, fluxapyroxad and low resistance to isopyrazam, bixafen, fenfuram, benodanil, and carboxin. The strains with different mutations of \textit{SdhB} (H272L/R/Y) in the same codon exhibited different patterns of sensitivity to SDHI fungicides. Especially, the \textit{SdhB} (H272Y) mutants showing resistance to boscalid and low resistance to isopyrazam, biafen, fenfuram, and carboxin displayed increased sensitivity to benodanil and fluopyram. Velouskas et al. (2013) suggested that the benzamide derivatives benodanil and fluopyram might bind better in the Q-pocket of strains containing a tyrosine at the codon 272.

**Resistance in \textit{Zymoseptoria tritici}**. Septoria tritici blotch (STB), caused by \textit{Zymoseptoria tritici} (synonym, \textit{Mycosphaerella graminicola, Septoria tritici}), is an economically important wheat disease worldwide (Eyal et al., 1987). SDHI fungicides have been applied to wheat fields for controlling STB several years, but the fungal populations resistant to SDHIs have been detected in Europe. The SDHI-resistant strains collected from Europe contained several mutations in \textit{SdhB} (N225T and T268I) and \textit{SdhC} (T79N, W80S, N86S, H152R, and V166M). The microtitre and curative greenhouse tests of mutants indicated that the \textit{SdhC} (H152R) mutants showed the highest resistance levels to all SDHIs tested (fluxapyroxad, fluopyram, isopyrazam, bixafen, ben佐indiflupyr, and penthiopyrad) and lower efficacy to SDHIs than other mutants (Rehfus et al., 2018). Yamashita and Fraaije (2018) investigated cross-resistance of lab mutants and field strains of \textit{Z. tritici} to SDHIs. The lab mutants harboring \textit{SdhC} (A84V) exhibited resistance both fluopyram and isofetamid. Interestingly, the field resistant strains showed the similar phenotype as the lab mutants but did not contain any mutations in \textit{SdhB}, \textit{SdhC}, and \textit{SdhD}. They also found these field resistant strains in Europe and New Zealand before applications of SDHIs widely. This study is the first report of non-target site SDHI resistance (Yamashita and Fraaije, 2018).

**Resistance in other phytopathogenic fungi**. Due to wide applications of SDHIs to various hosts, many other
fungal pathogens have developed resistance to SDHIs. In cucurbits, the SDHI resistance mechanisms of three different plant pathogens (*Corynespora cassicola*, *Podosphaera xanthii*, and *Didymella bryoniae*) have been reported (Avenot et al., 2012; Miyamoto et al., 2010a, 2010b). Resistance of *Corynespora cassicola*, the causal agent of corynespora leaf spot, to boscalid was firstly reported in Japan, and mutations in *SdhB* (H278Y/R) and *SdhC* (S73P) were detected by sequencing analysis of Sdh genes (Miyamoto et al., 2009, 2010a). Cucumber powdery mildew fungus (*Podosphaera xanthii*) showing resistance to boscalid also contained an amino acid substitution (histidine to tyrosine) in a third cysteine-rich center in *SdhB* (homologous to H272 in *B. cinerea*) (Miyamoto et al., 2010b).

| Table 1. SDHI resistance and molecular mechanisms reported in phytopathogenic fungi |
|---------------------------------|-------------------------------------------------|-------------------------------------------------|-------------|
| Species                        | Host               | Resistance mechanism                                                                 | Reference                      |
|--------------------------------|--------------------|-------------------------------------------------------------------------------------|------------------------------|
| *Alternaria alternata*         | Pistachio          | *SdhB*: H277Y/R, P230A/R/I/F/D, N235D/T/E/G *SdhC*: H133R, H134R, S135R *SdhD*: D123E, H133P/R | Avenot et al. (2008)          |
|                               |                    | *SdhB*: H277Y/R/L *SdhC*: G79R, H134R *SdhD*: A47T, D123E                           | Avenot et al. (2009)          |
| *Alternaria alternata*         | Peach              | *SdhB*: H277Y/R/L *SdhC*: H134R *SdhD*: D123E, H133R                              | Avenot et al. (2014)          |
| *Alternaria solani*            | Potato             | *SdhB*: H278Y/R *SdhC*: H134R *SdhD*: D123E, H133R                              | Avenot et al. (2019)          |
| *Botrytis cinerea*             | Apple              | *SdhB*: H272Y/L/R *SdhC*: G79R, H134R *SdhD*: A47T, D123E                         | Yang et al. (2015)            |
|                               | Grape              | *SdhB*: P225L, H272Y/R/L *SdhC*: G79R, H134R *SdhD*: A47T                         | Fan et al. (2015)             |
| *Botrytis cinerea*             | Strawberry         | *SdhB*: P225F/H/L/T *SdhC*: G79R, H134R *SdhD*: A47T                             | Amiri et al. (2014)           |
|                               |                    | *SdhB*: H267Y *SdhC*: G91R *SdhD*: A47T, D123E                                   | Hu et al. (2016)              |
| *Botrytis elliptica*           | Lily               | *SdhB*: 272Y/R *SdhC*: G79R, H134R *SdhD*: A47T                                 | Fernández-Ortuño et al. (2017)|
| *Blumeriella jaapii*           | Cherry             | *SdhB*: H260R                                                                     | Veloukas et al. (2011)        |
| *Clarireedia homoeocarpa*      | Turf               | *SdhB*: H272Y/L/R                                                                |                             |
| *Corynespora cassicola*        | Cucurbits          | *SdhB*: H278Y/R                                                                   |                             |
|                               |                    | *SdhC*: S73P                                                                     |                             |
|                                |                    | *SdhD*: S89P, G109V                                                              |                             |
| *Didymella bryoniae*           | Cucurbits          | *SdhB*: H277Y/R                                                                   |                             |
| *Podosphaera xanthii*          | Cucurbits          | *SdhB*: H277Y/R                                                                   |                             |
| *Pyrenophora teres*            | Barley             | *SdhB*: H277Y                                                                     |                             |
|                                |                    | *SdhC*: H146R                                                                     |                             |
|                                |                    | *SdhD*: H132R                                                                     |                             |
| *Sclerotinia sclerotiorum*     | Oilseed rape       | *SdhB*: H273Y                                                                     |                             |
|                                |                    | *SdhC*: H146R                                                                     |                             |
|                                |                    | *SdhD*: H132R                                                                     |                             |
| *Stemphylium vesicarium*       | Asparagus          | *SdhB*: P225L                                                                     |                             |
| *Venturia inaequalis*          | Apple              | *SdhB*: H272Y/R                                                                   |                             |
| *Zymoseptoria tritici*         | Wheat              | *SdhB*: N225T, T268I, T268; *SdhC*: T79N, W80S, N86S, V166M, H152R              |                             |

SDHI, succinate dehydrogenase inhibitor.
and H277R) were found in the field SDHI-resistant isolates of *Didymella bryoniae*, causing gummy stem blight of cucurbits, in the United States (Avenot et al., 2012).

Recently, mechanisms of SDHI resistance have been reported in *Pyrenophora teres* (the causal agent of barley net blotch), *Clarireedia homoeocarpa* (the causal agent of dollar spot) and *Blumeriella jaapii* (the causal agent of cherry leaf spot) (Outwater et al., 2019; Popko et al., 2018; Rehfus et al., 2016). Several mutations (*SdhB*: H277Y; *SdhC*: N75S, G79R, H134R, and S135R; *SdhD*: D124N/E, H134R, and D145G) were associated with SDHI resistance in *Pyrenophora teres* (Rehfus et al., 2016), and a single mutation (*SdhB*: H260R) was correlated with boscalid resistance in Michigan populations of *Blumeriella jaapii* (Outwater et al., 2019). In *Clarireedia homoeocarpa* (previously *Sclerotinia homoeocarpa*), three different mutations (*SdhB*: H267Y, *SdhC*: G91R, and *SdhC*: G150R) were detected in the resistant isolates collected from golf courses in the United States and Japan. The involvement of three mutations in SDHI resistance was genetically confirmed through the fungal transformation system (Popko et al., 2018). The Fungicide Resistance Action Committee also listed the reported SDHI resistance and mutations in the *Sdh* genes in phytopathogenic fungi including *Botrytis elliptica*, *Sclerotinia sclerotiorum*, *Stemphylium vesicarium*, and *Venturia inaequalis* (Fungicide Resistance Action Committee, 2015). The fungal species and *Sdh* gene mutations described in this study are listed in Table 1.

**Mechanisms of SDHI Resistance: Overexpression of Efflux Pump Transporters**

During monitoring and mechanism studies of fungicide resistance in *C. homoeocarpa*, the field isolates exhibiting reduced sensitivity to propiconazole, iprodione, boscalid and flurprimidol were found (Popko et al., 2012; Sang et al., 2015, 2018). These multidrug-resistant isolates overexpressed two ABC efflux transporters (*ShatrD* and *ShPDR1*), which were involved in reduced sensitivity to various fungicides including SDHIs (Fig. 1). A mutation (M853T) in the transcription factor *ShXDR1* in these resistant isolates conferred overexpression of CYP450s and ABC transporters, resulting in multidrug resistance. This is the first study of SDHI resistance mechanism by efflux pump transporters (Sang et al., 2018) (Table 1). In *B. cinerea*, the multidrug-resistant strains overexpressing efflux transporters were prevalent in vineyards of Germany (Kretschmer et al., 2009). There were three types of multidrug-resistant strains, which are MDR1 type strains (overexpression of ABC transporters *atrB*), MDR2 type strains (overexpression of MFS transporters *MFS2*) and MDR3 type strains (overexpressing both transporters) (Kretschmer et al., 2009; Lerch et al., 2011). The MDR1 type strains were not significantly associated with reduced sensitivity to boscalid, and the MDR 2 and 3 types showed slightly reduced sensitivity to boscalid (Kretschmer et al., 2009). The differences of SDHI resistance by multidrug resistance mechanisms between the ABC transporters in *C. homoeocarpa* and *B. cinerea* might be that each transporter has different substrate specificity.

**Conclusion**

This diversity of target gene mutations and multidrug resistance mechanisms makes resistance management difficult. Since each mutation has a different effect on cross-resistance patterns to SDHIs, the rapid detection of mutation(s) in the fungal populations is necessary to manage SDHI resistance. Currently, allele-specific real-time PCR assay has been developed for the quantitative detection of different SDHI-resistant genotypes (De Miccolis Angelini et al., 2014). Also, several *SdhB* mutations in *B. cinerea* were accurately and rapidly detected using high resolution melting analysis (Samaras et al., 2016). Together with such molecular characterization and rapid detection of SDHI mutations, use of a limited number of fungicides and strategic applications of chemical rotation and combination based on mode of action are required to prolong the field efficacy of SDHIs.

**Conflicts of Interest**

No potential conflict of interest relevant to this article was reported.

**Acknowledgments**

This work was supported by two grants from the Rural Development Administration of Korea (PJ01483603) and National Research Foundation of Korea (2020R1C1C1010108).
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