

**Type of the Paper (Article)**

**Characterization of the molecular mechanisms of resistance against DMI fungicides in *Cercospora beticola* populations from the Czech Republic**

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**Supplementary Table S1.** Fungicide sensitivity of *Cercospora beticola* against three DMIs fungicides (propiconazole, prochloraz, and epoxiconazole). The EC<sub>50</sub> values are in (µg/mL).

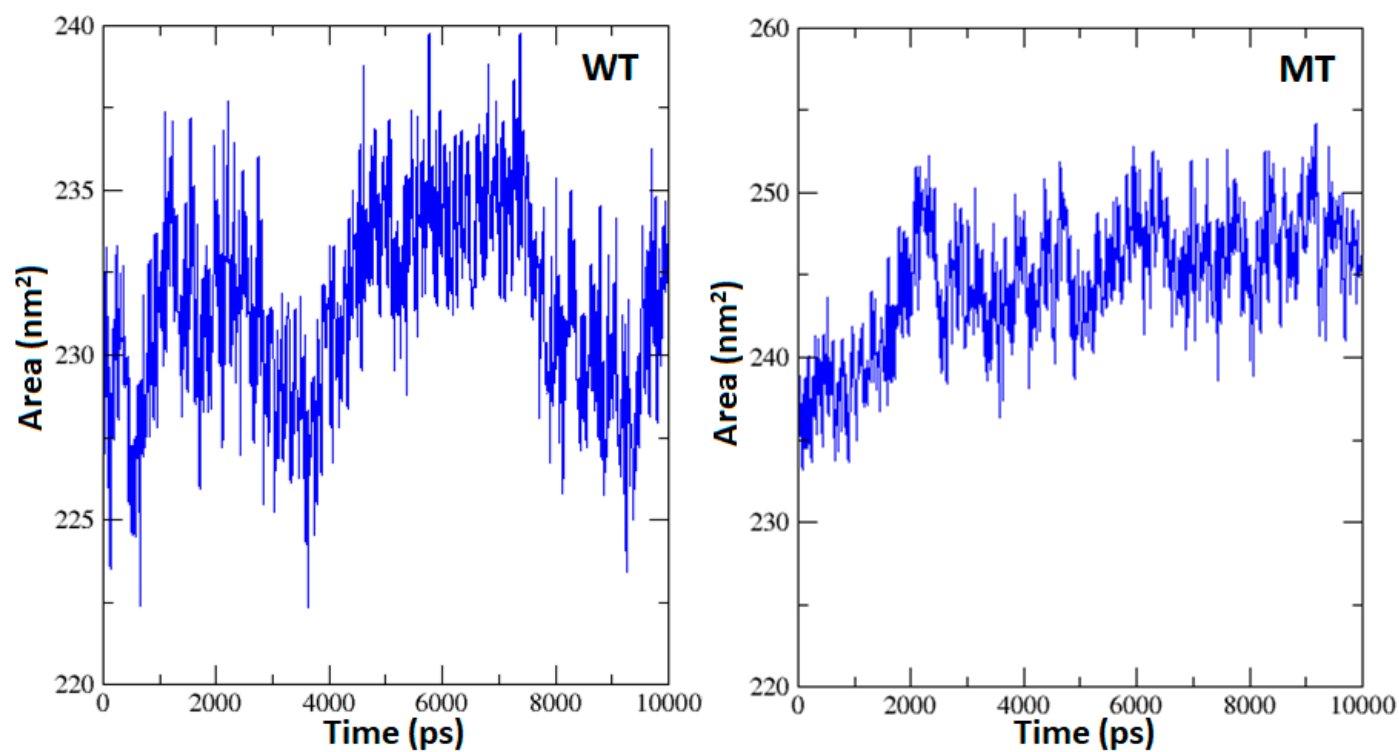
Isolates	Propiconazole		Prochloraz		Epoxiconazole	
	Average	Standard deviation	Average	Standard deviation	Average	Standard deviation
7B_1 (S3)	0.17	0.02	0.19	0.02	0.05	0.01
1_6 (S4)	0.05	0.01	0.32	0.22	0.45	0.32
ST_8 (R2)	14.27	2.94	2.29	0.23	2.19	0.17
36_2 (R5)	5.43	0.54	44.01	5.85	2.78	0.13
M61_5 (R10)	3.11	0.14	4.36	0.42	39.53	6.23
7A_2	1.36	1.05	1.98	1.21	2.06	0.64
7A_3	1.22	0.04	2.06	0.56	1.14	0.93
7A_4	1.81	1.52	1.53	1.09	1.52	0.51
7A_5	1.29	0.59	ND	ND	1.61	0.35
3_2	0.90	0.54	1.96	0.65	5.72	8.22
3_3	1.24	1.00	1.17	0.25	1.06	0.70
3_4	2.71	1.54	1.10	0.80	ND	ND
3_5	0.62	0.54	0.47	0.37	1.18	0.34
4_2	1.84	0.92	1.48	0.22	ND	ND
4_3	1.13	0.19	1.53	1.24	0.84	0.40
4_4	0.24	0.08	0.61	0.53	1.29	0.35
5_2	1.22	0.62	2.05	0.94	1.49	1.19
5_3	1.47	0.66	1.48	0.38	1.06	0.51
5_4	0.95	0.68	0.98	0.90	1.47	0.20
5_5	0.35	0.25	0.75	0.34	1.22	0.50
vs_1	1.04	0.15	0.87	0.22	7.60	11.16
vs_2	0.97	0.27	ND	ND	3.99	3.46
vs_3	ND	ND	1.17	0.51	4.92	5.94
8_1	ND	ND	2.39	1.29	2.37	1.06
8_2	1.06	0.37	2.68	1.51	1.24	0.37
8_3	1.04	0.12	1.45	0.96	1.20	0.79
9_1	2.03	0.90	0.98	0.57	ND	ND
9_2	1.32	0.27	1.15	1.01	1.11	0.79
9_3	1.12	0.82	1.14	0.95	1.15	0.59
11_1	1.29	0.39	0.92	0.70	1.06	0.41
11_2	ND	ND	2.80	1.44	1.43	0.73
11_3	1.30	0.40	1.50	1.32	1.88	0.15

17_1	1.46	0.91	1.42	1.03	1.41	1.13
17_2	1.25	0.54	1.30	0.98	1.50	0.71
17_3	1.49	0.70	0.31	0.29	1.72	1.26
20_1	ND	ND	0.97	0.46	1.19	0.30
23_1	1.12	0.69	0.50	0.57	1.42	0.91
24_1	1.22	0.68	1.02	0.66	0.54	0.45
25_1	2.54	0.95	0.77	0.78	1.23	0.85
70_5	1.21	0.50	0.53	0.44	1.41	0.57
70_1	1.86	0.23	0.57	0.41	1.47	0.95
70_2	0.71	0.46	ND	ND	1.12	0.42
72_1	1.29	1.07	1.79	1.26	1.30	0.42
73_1	0.91	0.83	0.62	0.49	0.93	0.73
E_1	1.91	0.77	1.47	1.98	0.50	0.38
E_2	1.01	0.34	0.68	0.45	0.95	0.47
E_3	1.02	0.26	0.80	0.91	0.57	0.46
E_4	1.01	0.26	1.75	0.52	0.96	0.83
E_5	0.89	0.92	1.35	0.49	0.91	0.64
M61_1	0.97	0.56	0.83	0.95	1.13	0.72
M61_2	0.88	0.49	1.48	0.43	ND	ND

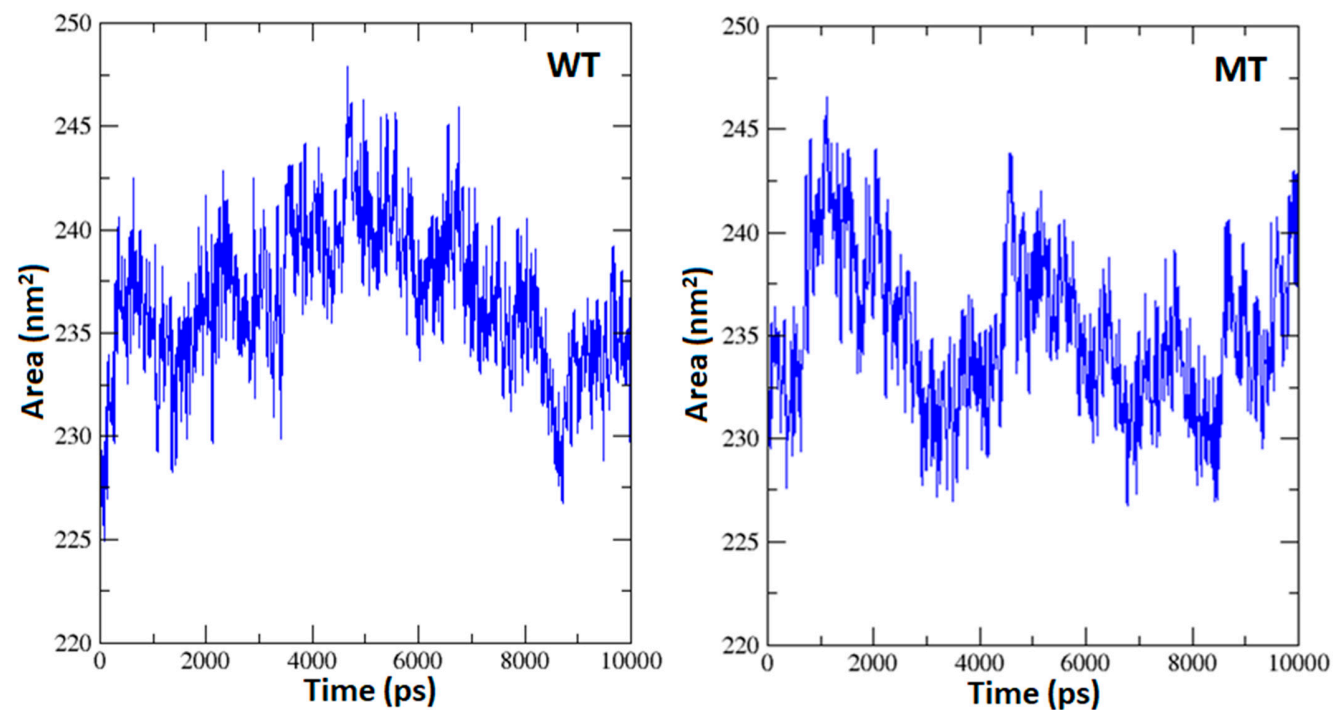
#ND: “not done”.

#S: sensitive biotype

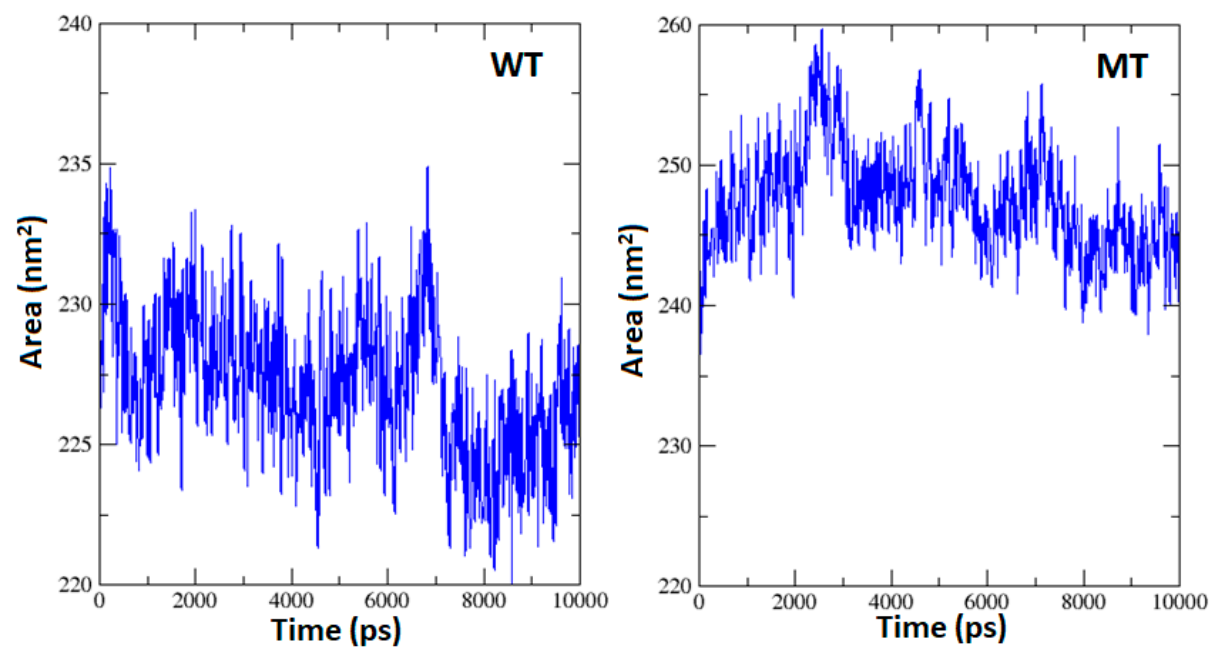
#R: resistant biotype



**Supplementary Figure S1.** Solvent accessible surface area analysis during propiconazole-*Cyp51* interaction between wild type (WT) genotype and mutant type (MT) genotype of *Cercospora beticola*.



**Supplementary Figure S2.** Solvent accessible surface area analysis during prochloraz-*Cyp51* interaction between wild type (WT) genotype and mutant type (MT) genotype of *Cercospora beticola*.



**Supplementary Figure S3.** Solvent accessible surface area analysis during epoxiconazole *Cyp51* interaction between wild type (WT) genotype and mutant type (MT) genotype of *Cercospora beticola*.

**Supplementary Table S2.** Lennard-Jones energies short-range (LJ-SR) and Coulombic potential within R-coulomb (Coul-SR) between *Cyp51* protein of *Cercospora beticola* and fungicide molecule, during MD simulations.

<b>Propiconazole</b>				
	<b>Wild Type</b>		<b>Mutant Type</b>	
<b>Energy type</b>	<b>Interaction energy (kJ/mol)</b>	<b>Estimated error</b>	<b>Interaction energy (kJ/mol)</b>	<b>Estimated error</b>
Coul-SR	-18.392	1.6	-23.0891	2.7
LJ-SR	-162.367	2.4	-144.432	2.2
<b>Prochloraz</b>				
	<b>Wild Type</b>		<b>Mutant Type</b>	
<b>Energy type</b>	<b>Interaction energy (kJ/mol)</b>	<b>Estimated error</b>	<b>Interaction energy (kJ/mol)</b>	<b>Estimated error</b>
Coul-SR	-18.5526	1.5	-13.4189	3.2
LJ-SR	-140.738	1	-121.644	1.8
<b>Epoxiconazole</b>				
	<b>Wild Type</b>		<b>Mutant Type</b>	
<b>Energy type</b>	<b>Interaction energy (kJ/mol)</b>	<b>Estimated error</b>	<b>Interaction energy (kJ/mol)</b>	<b>Estimated error</b>
Coul-SR	-16.2723	3.7	-7.09995	1
LJ-SR	-123.091	3.1	-119.641	1.3

**Supplementary Table S3.** Analysis of gene expression variation of *Cyp51* gene of *Cercospora beticola*. The relative gene expression values are in terms of  $2^{-\Delta\Delta C_t}$  values. “\*” denotes significant at 5% significant level.

Isolates	Propiconazole			
	Constitutive expression	Fungicide-induced expression	Significant at 5% significance level	Fold change
S3	0.96 ( $\pm$ 0.03)	0.96 ( $\pm$ 0.34)	NS	NA
S4	1.28 ( $\pm$ 0.25)	1.23 ( $\pm$ 0.11)	NS	NA
R2	1.04 ( $\pm$ 0.11)	3.35( $\pm$ 0.38)	*	3.21
Isolates	Prochloraz			
	Constitutive expression	Fungicide-induced expression	Significant at 5% significance level	Fold change
S3	0.96 ( $\pm$ 0.03)	1.17 ( $\pm$ 0.32)	NS	NA
S4	1.28 ( $\pm$ 0.25)	1.84 ( $\pm$ 0.36)	NS	NA
R5	1.07 ( $\pm$ 0.17)	4.98 ( $\pm$ 0.64)	*	4.67
Isolates	Epoiconazole			
	Constitutive expression	Fungicide-induced expression	Significant at 5% significance level	Fold change
S3	0.96 ( $\pm$ 0.03)	1.56 ( $\pm$ 0.56)	NS	NA
S4	1.28 ( $\pm$ 0.25)	1.49 ( $\pm$ 0.08)	NS	NA
R10	0.95 ( $\pm$ 0.09)	3.25 ( $\pm$ 0.67)	*	3.43

#NS: “non-significant”

#NA: “not applicable”

#S: sensitive biotype

#R: resistant biotype