

Supplementary Data

Supplementary Figure 1 Sequence consistency analysis of 30 DQD/SDH proteins from plants.

Supplementary Figure 2 Comparison of the partial DQD/SDH sequences from the tea plant and other species (*Arabidopsis* and *Vitis vinifera*) using DNAMAN 6.0. A. DQD unit; B. SDH unit. Amino acid numeration is based on the protein sequence of *A. thaliana*. The key amino acids are marked with red dots; the G-motif with a green box; and the NRT motif with a blue box. Conserved amino acids are marked with black (similarity = 100%), dark gray (similarity ≥ 75%), and light gray (similarity ≥ 50%) colors.

Supplementary Figure 3 HPLC analysis of recombinant *CsDQD/SDHa* enzyme reaction products. (A) HPLC chromatograms from top to bottom are for the standard of SA (top), boiled enzyme reaction with 3-DHS and NADPH (middle), and enzyme production of SA (bottom); (B) HPLC chromatograms from top to bottom are for the standard of 3-DHS (top), boiled enzyme reaction with SA and NADP⁺ (middle), and enzyme production of 3-DHS (bottom); (C) MS/MS molecular ion characteristics of the enzyme products of 3-DHS (171.0) and SA (173.0).

Supplementary Figure 4 Selection of the optimum pH value for the *CsDQD/SDHs* reaction. A, B, and C showed the production accumulation profile at various pH values from *CsDQD/SDHa*, *CsDQD/SDHc*, and *CsDQD/SDHd*, respectively. The top line shows the 3-DHS reduction reaction, the bottom line shows the SA oxidation reaction. ▲: represents the citric acid buffer; ●: represents the BTP-HCl buffer; ■: represents the sodium carbonate buffer.

Supplementary Table S1 The primer sequences used in this study.

Supplementary Table S2 ID of multiple plant DQD/SDH proteins used in this study.

Purpose	Name	5'-3' sequence	Restriction site
Protein expression	<i>CsDQD/SDHa-pMAL-F</i>	<u>GGATCC</u> ATGGAGTTGGTTCACCT	BamHI
	<i>CsDQD/SDHa-pMAL-R</i>	<u>CTGCAG</u> TCAATTTGCCATGAT	PstI
	<i>CsDQD/SDHb-pMAL-F</i>	<u>GGATCC</u> ATGGGTAGTGTGGGGTG	BamHI
	<i>CsDQD/SDHb-pMAL-R</i>	<u>CTGCAG</u> TCAGAACGTTGCGAAAAT	PstI
	<i>CsDQD/SDHc-pMAL-F</i>	<u>GGATCC</u> CATGGCCTCTGGAAGCTTCG	BamHI
	<i>CsDQD/SDHc-pMAL-R</i>	<u>CTGCAG</u> CATGCGTGTCTGACATG	PstI

	<i>Cs</i> DQD/SDHd-pMAL-F	<u>GGATCC</u> ATGACTCTCAGCAGCAT	BamHI
	<i>Cs</i> DQD/SDHd-pMAL-R	<u>CTGCAG</u> TTATGTGTTCTTGCTA	PstI
qPCR	<i>Cs</i> DQD/SDHa-F	ACAACCGCCTGGACATCAC	
	<i>Cs</i> DQD/SDHa-R	CCATCAAACCCCTTCACCC	
	<i>Cs</i> DQD/SDHb-F	ATCACTCCCATCTCCGCTCAC	
	<i>Cs</i> DQD/SDHb-R	GACAACCCTCGCTCCTCTG	
	<i>Cs</i> DQD/SDHc-F	GTAGTAGCGGAGTTAGGAGCG	
	<i>Cs</i> DQD/SDHc-R	GAAGATCAGGGCGTGGATTG	
	<i>Cs</i> DQD/SDHd-F	GACAGGCTGCATTGCGTCAG	
	<i>Cs</i> DQD/SDHd-R	GGCCCCTTCACCCATAACA	
	GAPDH-F	TTGGCATCGTTGAGGGTCT	
	GAPDH-R	CAGTGGAACACGGAAAGC	
MTC <i>S</i> DQD D/SDHb	<i>Cs</i> DQD/SDHb-338G→S -F	GTGGGCCACAGCAAAAGTCCCCTCTC CATAAT	
	<i>Cs</i> DQD/SDHb-338G→S -R	ATTATGGAGAACAGGGACTTTGCTGTG GCCCAC	
	<i>Cs</i> DQD/SDHb-381G→T F	GCAGGTTTCAGTGTAAACATTCCATACA AGGAA	
	<i>Cs</i> DQD/SDHb-381G→T R	TTCCTTGTATGGAAATGTTACACTGAAA CCTGC	
	<i>Cs</i> DQD/SDHb-483D→N -F	AGGGTTGTCATTTAACATTGATTTG ACAGA	
	<i>Cs</i> DQD/SDHb-483D→N -R	TCTGTCAAAATCAATGTTAAAATGACA ACCCT	
	<i>Cs</i> DQD/SDHb-484I→R- F	GTTGTCATTTGACCGTGATTTGACA GAGCA	
	<i>Cs</i> DQD/SDHb-484I→R-	TGCTCTGTCAAAATCACGGTCAAAAT	

	R	GACAAC
	<i>CsDQD/SDHb-485D→T-</i>	GTCATTTGACATTACTTTGACAGAG
	F	CAAAG
	<i>CsDQD/SDHb-485D→T-</i>	CTTGCTCTGTCAAAAGTAATGTAAA
	R	AATGAC
<i>CsDQDa</i>	<i>CsDQDa-F</i>	<u>GGATCCAAGAACTCAACCCTAATTGT</u>
	<i>CsDQDa-R</i>	<u>CTGCAGGATATTGTACAAGTTCACCAA</u>
<i>CsSDHa</i>	<i>CsSDHa-F</i>	<u>GGATCCATTATCGGGAAGCCTGTTGGCC</u>
	<i>CsSDHa-R</i>	<u>CTGCAGTCCAGTGAACCTCTCGAATTG</u>

Species	Designation	Accession number
<i>Arabidopsis thaliana</i>	AtSDH	AAF08579
<i>Vitis vinifera</i>	<i>VvSDH1</i>	KU163040
	<i>VvSDH2</i>	KU163041
	<i>VvSDH3</i>	KU163042
	<i>VvSDH4</i>	KU163043
<i>Nicotiana tabacum</i>	NtSDH1	AAS90325
	NtSDH2	AAS90324
<i>Solanum lycopersicum</i>	SISDH1	AAC17991
	SISDH2	XP_010327280
	SISDH3	XP_004242317
<i>Diospyros kaki</i>	DkSDH	BAI40147
<i>Eucalyptus grandis</i>	EgSDH1	HO1214.1
	EgSDH2	HO4428.1

	EgSDH3	HO4427.1
	EgSDH4	BO1770.2
	EgSDH5	J00263.6
<i>Fragaria vesca</i> subsp. <i>vesca</i>	FvSDH1	XP_004302480
	FvSDH2	XP_004302479
	FvSDH3	XP_004289250
	FvSDH4	XP_004288087
<i>Populus trichocarpa</i>	PoptrSDH1	010G019000
	PoptrSDH2	013G029900
	PoptrSDH3	005G043400
	PoptrSDH4	014G135500
	PoptrSDH5	013G029800
<i>Juglans regia</i>	JrSDH	AAW65140
