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(54) **AROGENATE DEHYDROGENASE POLYNUCLEOTIDES, POLYPEPTIDES AND METHODS OF USING THE SAME**

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(73) Assignee: **Wisconsin Alumni Research Foundation**, Madison, WI (US)

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C12N 9/02	(2006.01)
C12P 19/60	(2006.01)

(52) **U.S. Cl.**

CPC ***C12N 15/825*** (2013.01); ***C12N 9/001*** (2013.01); ***C12P 19/60*** (2013.01); ***C12N 15/8222*** (2013.01); ***C12Y 103/01043*** (2013.01)

(57) **ABSTRACT**

The invention generally relates to arogenate dehydrogenase polynucleotides and methods of using the same. More specifically, the invention relates in part to compositions including arogenate dehydrogenase polynucleotides from beet varieties and other Caryophyllales species and methods of using the same.

Specification includes a Sequence Listing.

Fig. 1A

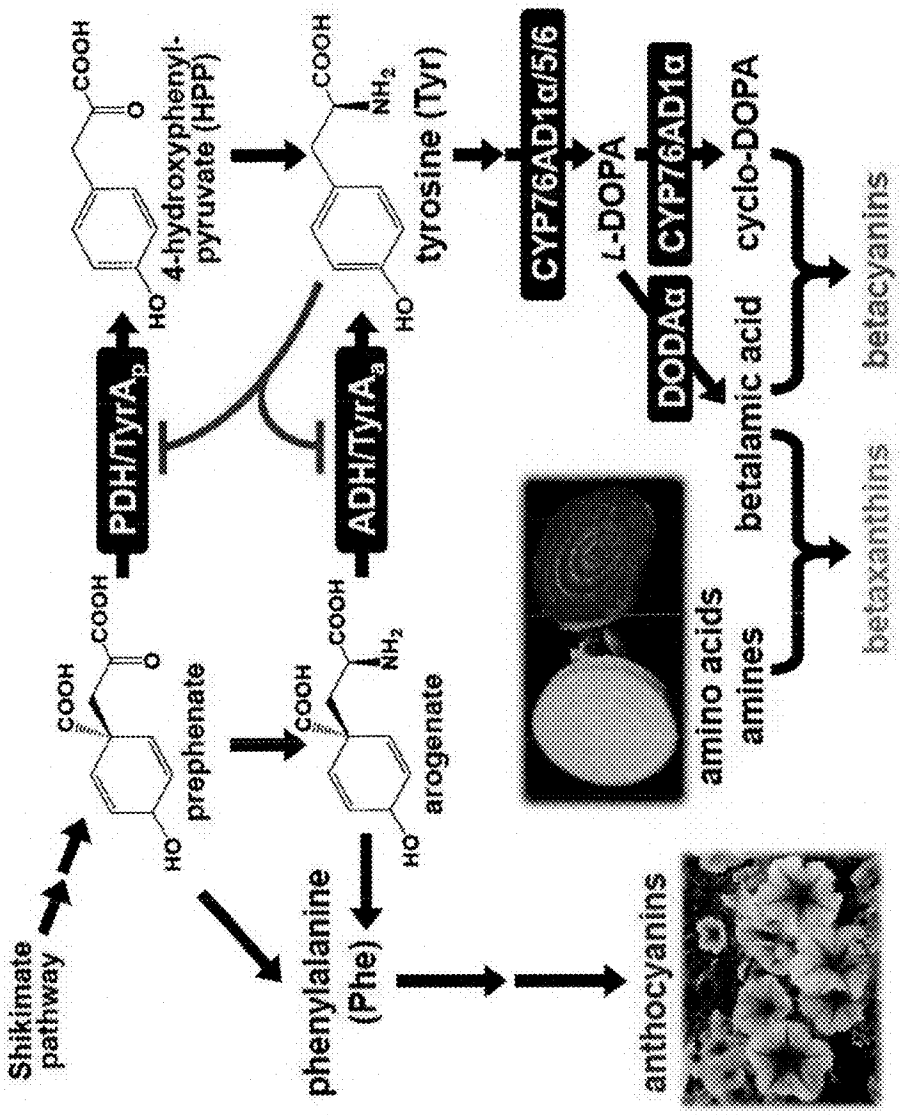

Shikimate pathway
prephenate
arogenate
PDH/TyrA$_2$
ADH/TyrA$_3$
4-hydroxyphenyl-pyruvate (HPP)
tyrosine (Tyr)
phenylalanine (Phe)
CYP76AD1α/5/6
L-DOPA
DODAα
CYP76AD1α
cyclo-DOPA
betalamic acid
amino acids
amines
betaxanthins
betacyanins
anthocyanins

Fig. 1B

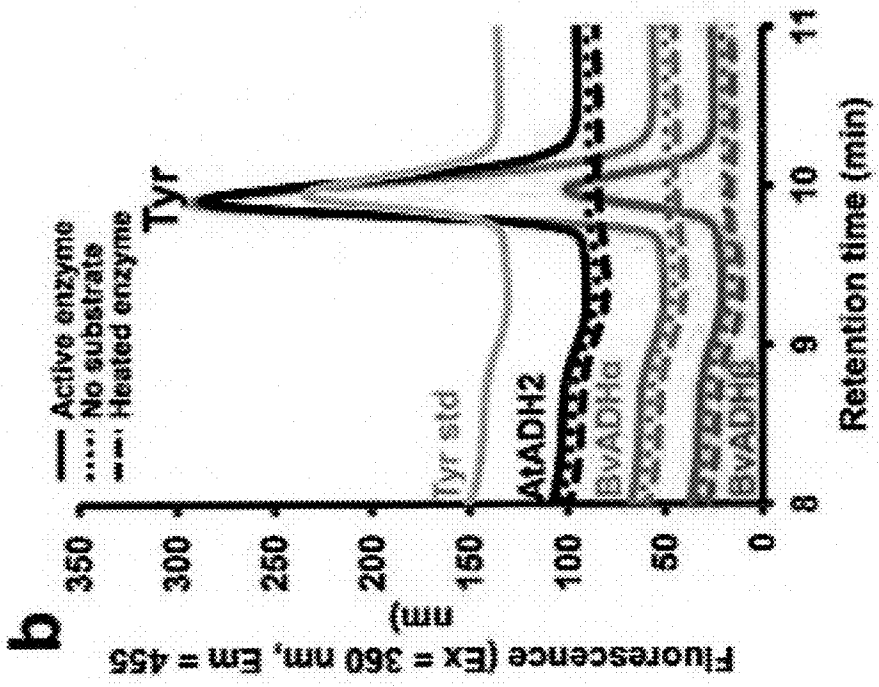

b
Fluorescence (Ex = 360 nm, Em = 455 nm)
350
300
250
200
150
100
50
0
8
9
10
11
Retention time (min)
Active enzyme
No substrate
Heated enzyme
Tyr
Tyr std
AtADH2
BvADH8
BvADH7

Fig. 1C

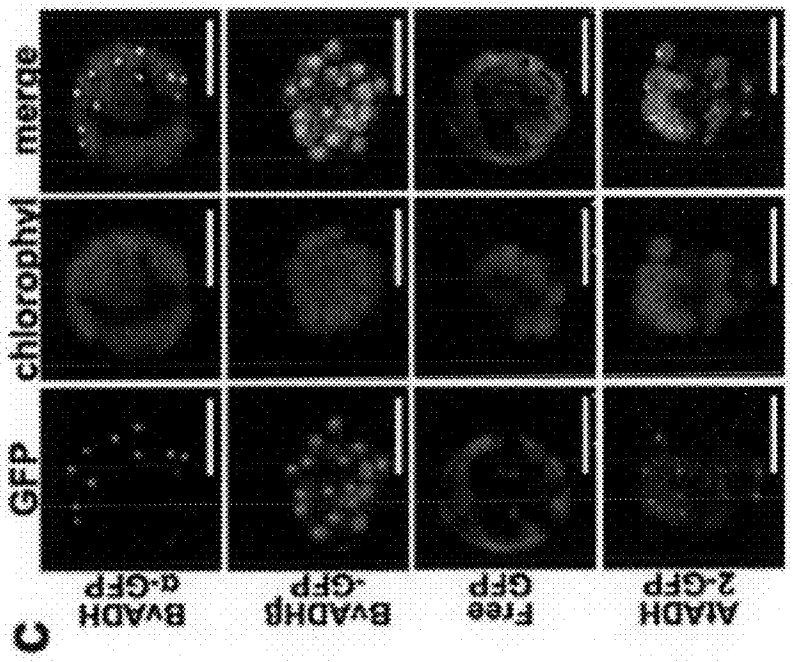

C
GFP
chlorophyl
merge
AtADH 2-GFP
Free GFP
BvADHβ -GFP
BvADH α-GFP

Fig. 1D

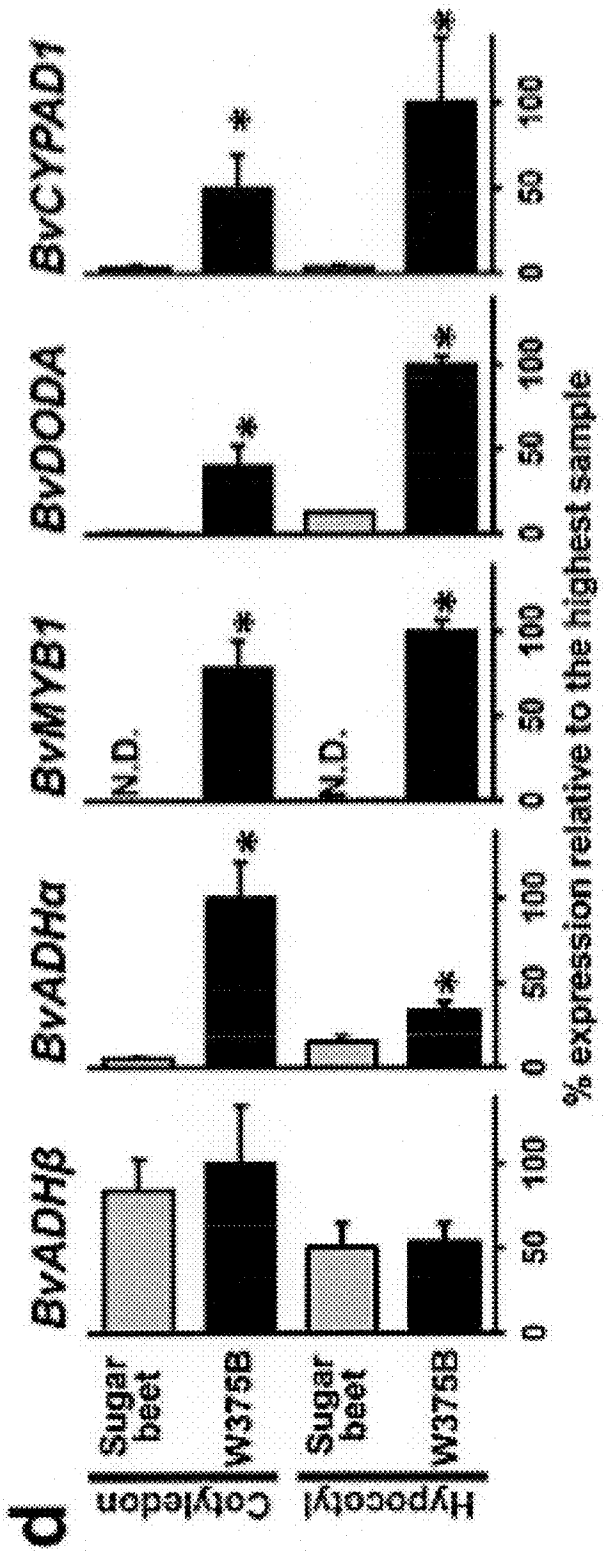

d
Cotyledon
Hypocotyl
Sugar beet
W375B
Sugar beet
W375B
BvADHβ
BvADHα
BvMYB1
BvDODA
BvCYPAD1
N.D.
N.D.
0
50
100
% expression relative to the highest sample

Fig. 2A

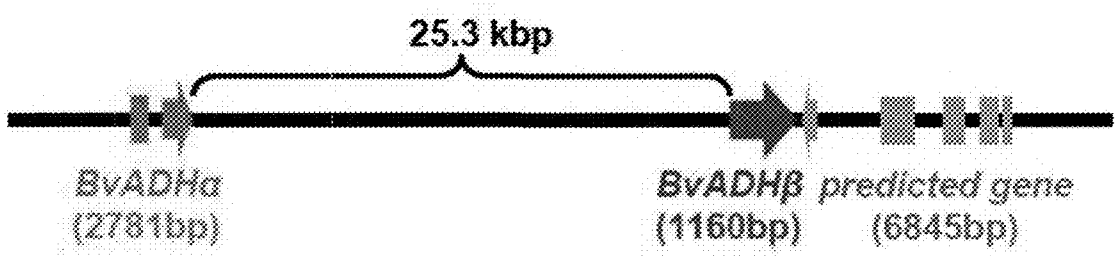


Fig. 2B

b

	BvADHα	BvADHβ	AtADH1	AtADH2	GmPDH1	AaPDH	EcPDH	SyADH
BvADHα	100	66	66	61	52	18	28	24
BvADHβ	66	100	72	59	54	24	26	25
AtADH1	66	72	100	61	56	22	25	34
AtADH2	61	59	61	100	52	23	23	32
GmPDH1	52	54	56	52	100	23	23	29
AaPDH	18	24	22	23	23	100	21	28
EcPDH	28	26	25	23	23	21	100	23
SyADH	24	25	34	32	29	28	23	100

Fig. 3A

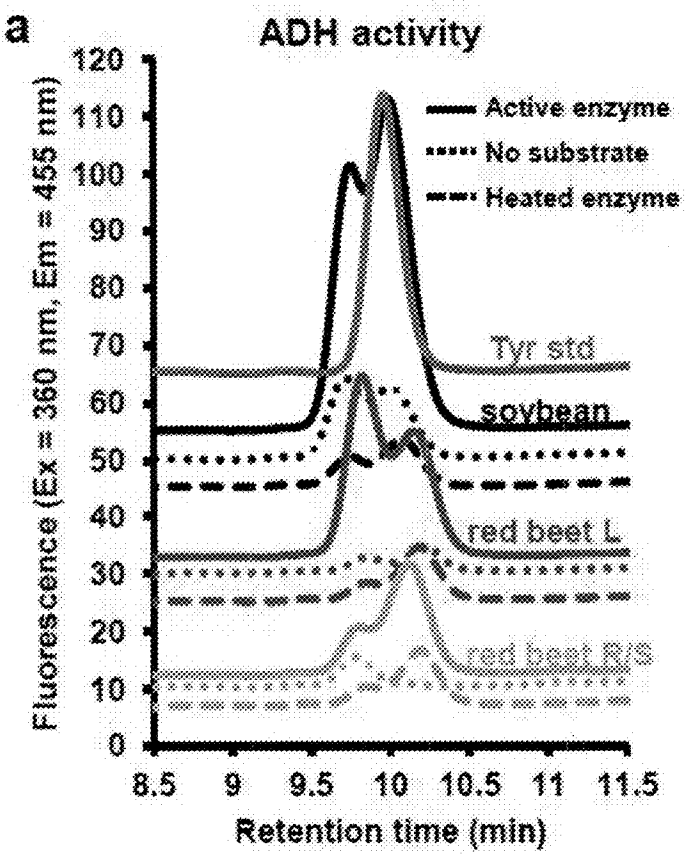

a
ADH activity
Active enzyme
No substrate
Heated enzyme
Tyr std
soybean
red beet L
red beet R/S
Fluorescence (Ex = 360 nm, Em = 455 nm)
Retention time (min)
0
10
20
30
40
50
60
70
80
90
100
110
120
8.5
9
9.5
10
10.5
11
11.5

Fig. 3B

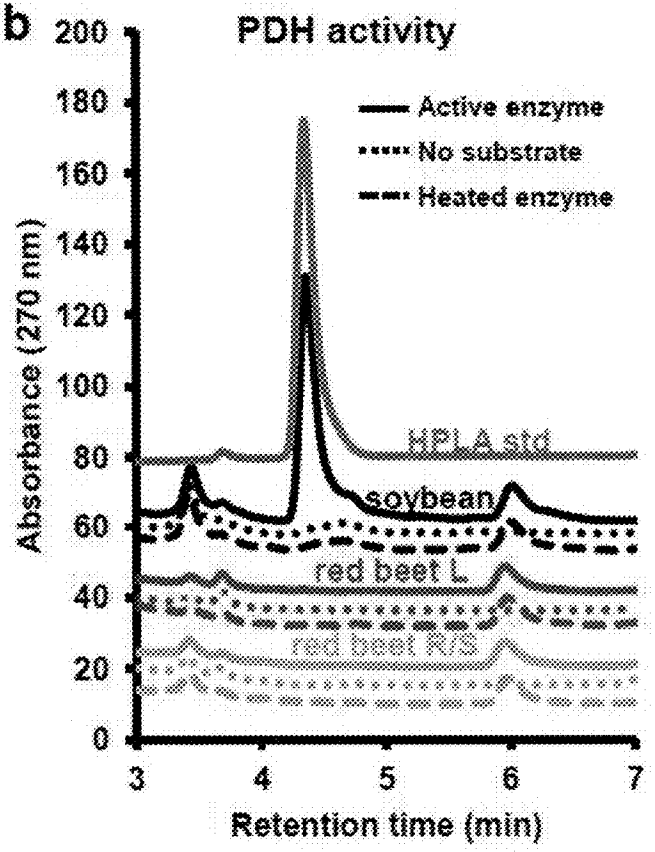

b
PDH activity
Active enzyme
No substrate
Heated enzyme
HPLA std
soybean
red beet L
red beet R/S
Absorbance (270 nm)
Retention time (min)
0
20
40
60
80
100
120
140
160
180
200
3
4
5
6
7

Fig. 3C

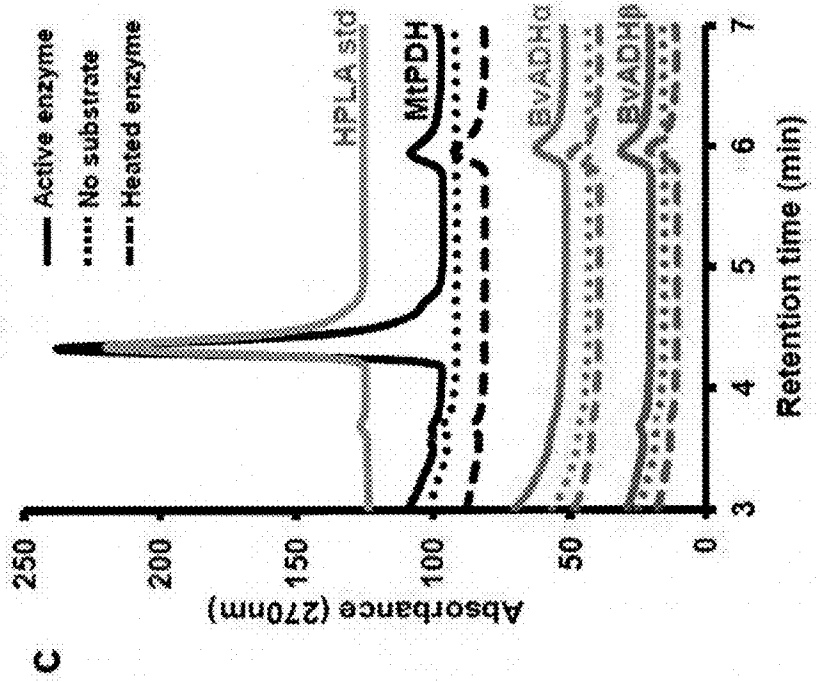

C
PDH activity
Active enzyme
No substrate
Heated enzyme
HPLA std
MtPDH
BvADHα
BvADHβ
Absorbance (270nm)
250
200
150
100
50
0
3
4
5
6
7
Retention time (min)

Fig. 4

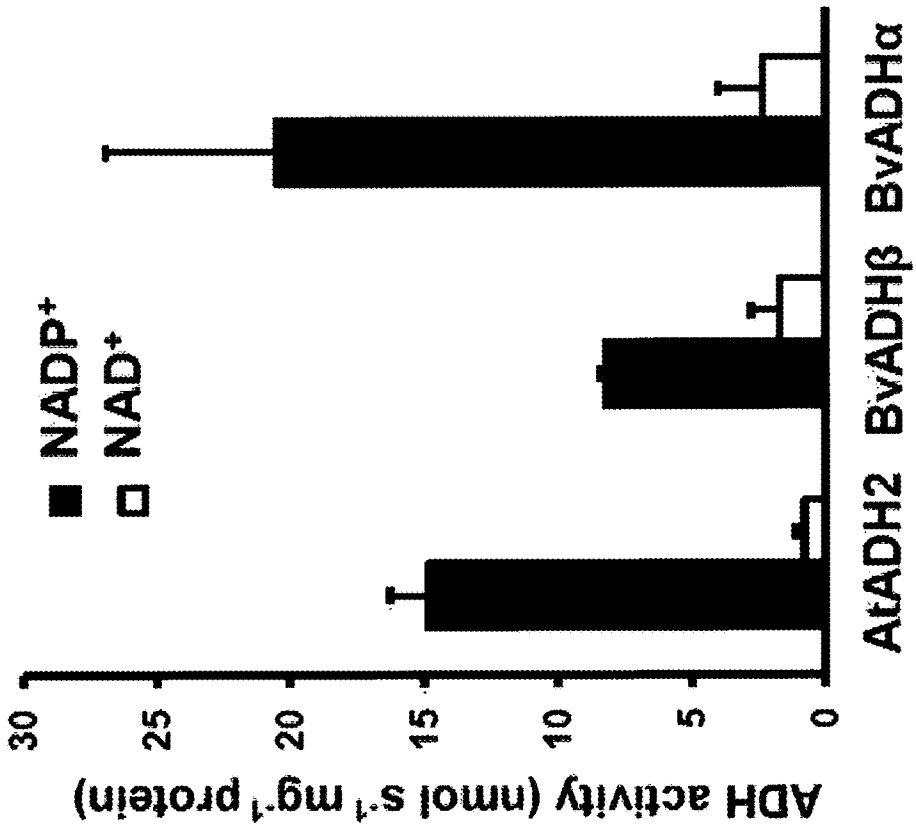

NADP+
NAD+
ADH activity (nmol s-1 mg-1 protein)
0
5
10
15
20
25
30
AtADH2
BvADHβ
BvADHα

Fig. 5A

Fig. 5A cont.

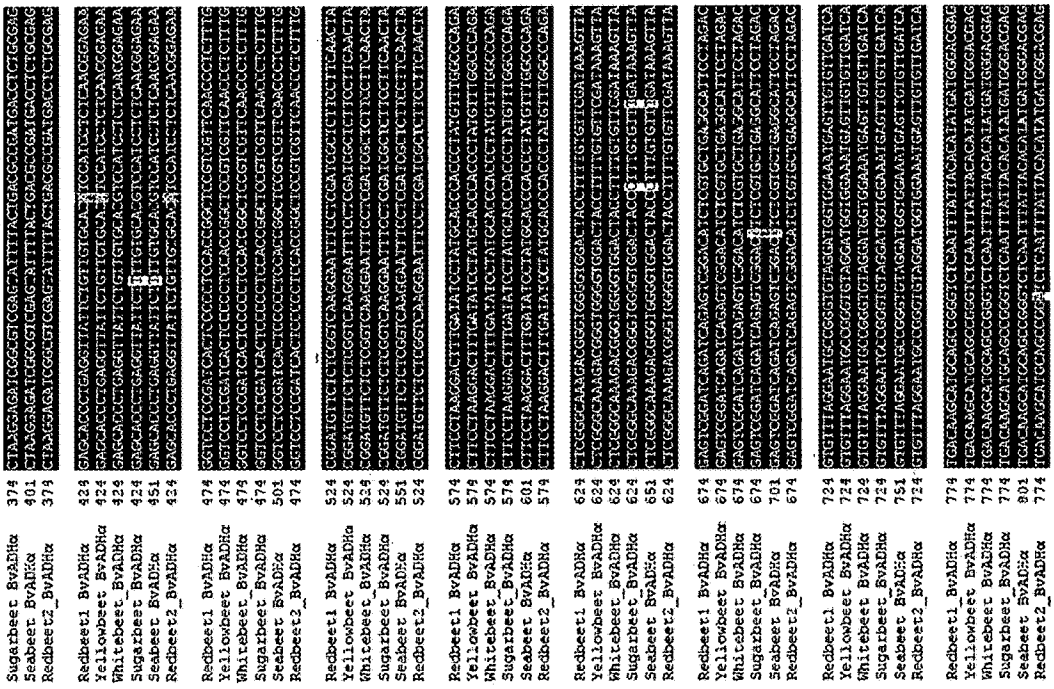


Fig. 5A cont.

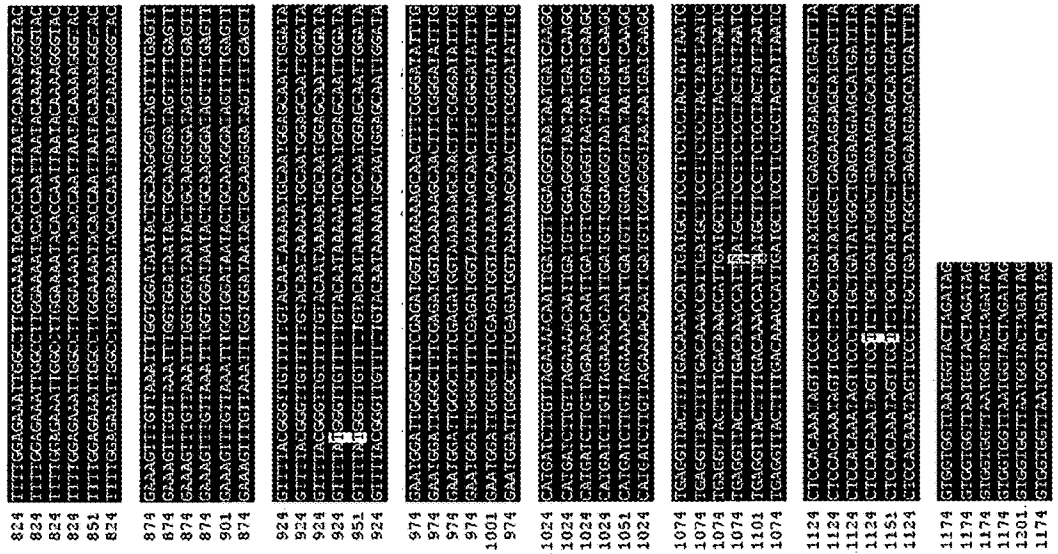


Fig. 5B

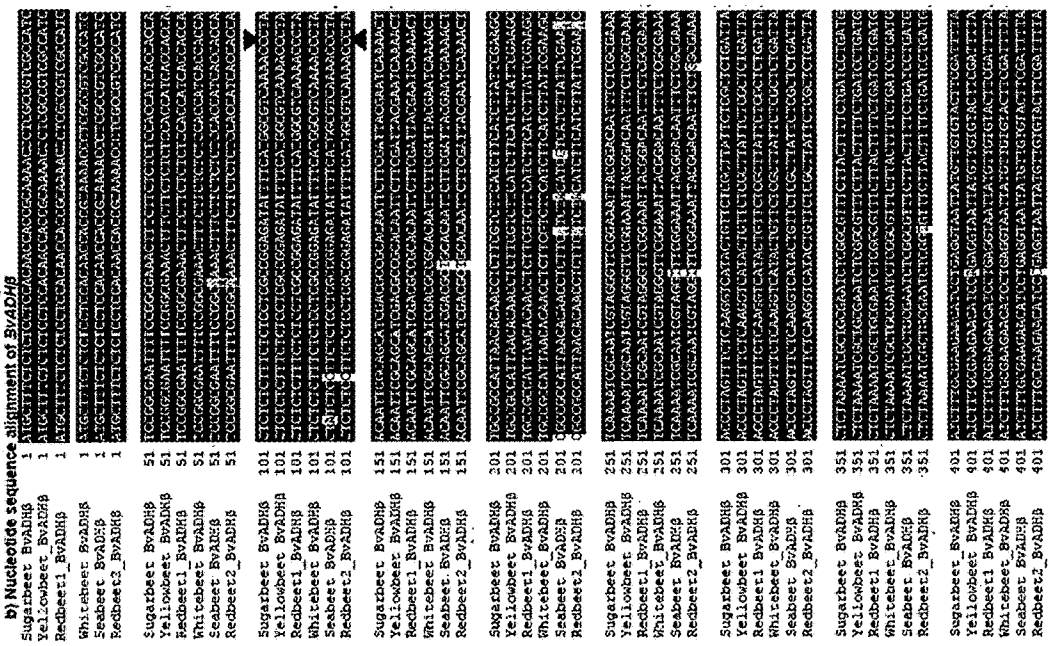
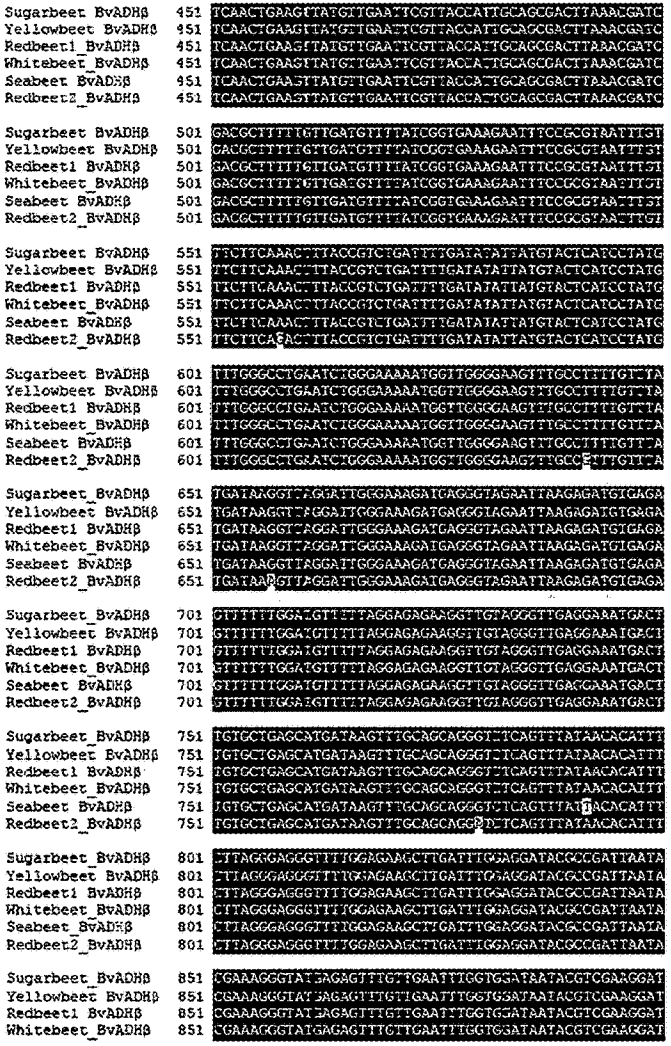


Fig. 5B cont.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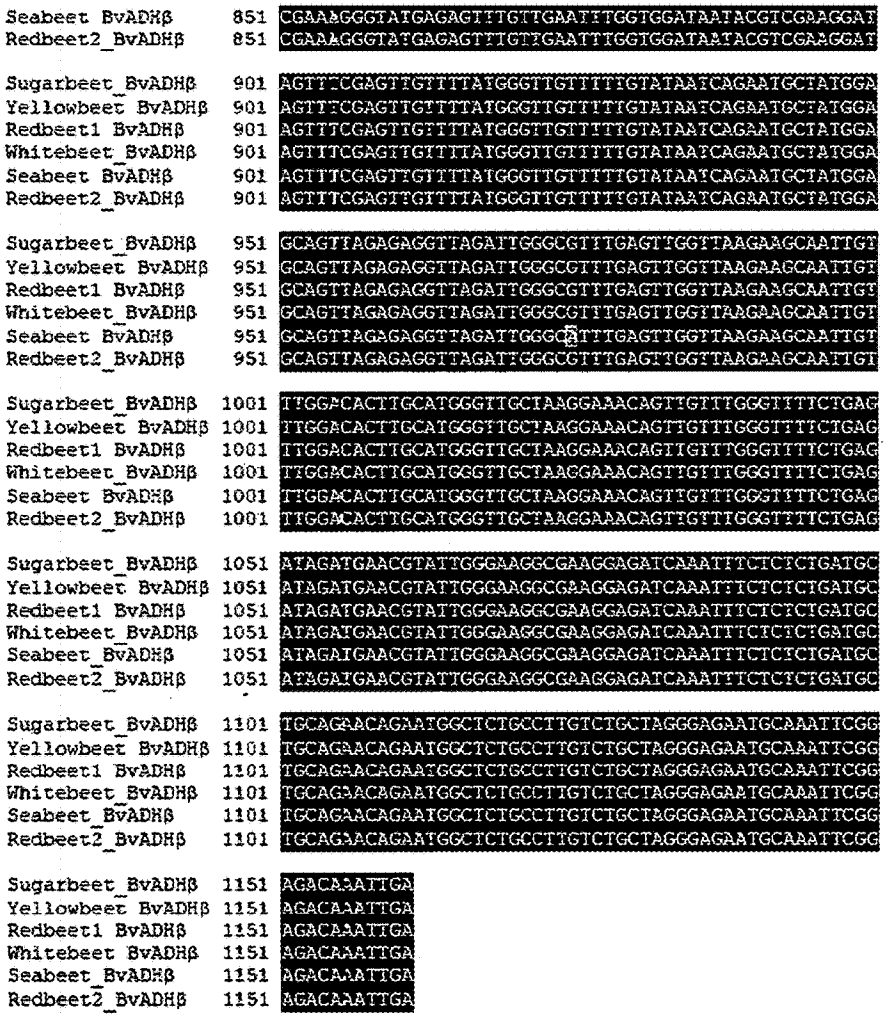
Yellowbeet BvADHβ 451 TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC
Redbeet1_BvADHβ 451 TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC
Whitebeet_BvADHβ 451 TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC
Seabeet BvADHβ 451 TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC
Redbeet2_BvADHβ 451 TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC
Sugarbeet BvADHβ 501 GACGCTTTTTGTTGATGTTTTATCGGTGAAAGAATTTCCGCGTAATTTGT
Yellowbeet BvADHβ 501 GACGCTTTTTGTTGATGTTTTATCGGTGAAAGAATTTCCGCGTAATTTGT
Redbeet1 BvADHβ 501 GACGCTTTTTGTTGATGTTTTATCGGTGAAAGAATTTCCGCGTAATTTGT
Whitebeet_BvADHβ 501 GACGCTTTTTGTTGATGTTTTATCGGTGAAAGAATTTCCGCGTAATTTGT
Seabeet BvADHβ 501 GACGCTTTTTGTTGATGTTTTATCGGTGAAAGAATTTCCGCGTAACTTGT
Redbeet2_BvADHβ 501 GACGCTTTTTGTTGATGTTTTATCGGTGAAAGAATTTCCGCGTAACTTGT
Sugarbeet BvADHβ 551 TTCTTCAAACTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG
Yellowbeet BvADHβ 551 TTCTTCAAACTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG
Redbeet1 BvADHβ 551 TTCTTCAAACTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG
Whitebeet_BvADHβ 551 TTCTTCAAACTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG
Seabeet BvADHβ 551 TTCTTCAAACTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG
Redbeet2_BvADHβ 551 TTCTTCAGACTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG
Sugarbeet BvADHβ 601 TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA
Yellowbeet BvADHβ 601 TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA
Redbeet1 BvADHβ 601 TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA
Whitebeet_BvADHβ 601 TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA
Seabeet BvADHβ 601 TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA
Redbeet2_BvADHβ 601 TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCRTTTGTTTA
Sugarbeet_BvADHβ 651 TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA
Yellowbeet BvADHβ 651 TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA
Redbeet1 BvADHβ 651 TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA
Whitebeet_BvADHβ 651 TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA
Seabeet BvADHβ 651 TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA
Redbeet2_BvADHβ 651 TGATAARGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA
Sugarbeet_BvADHβ 701 GTTTTTTGGATGTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT
Yellowbeet BvADHβ 701 GTTTTTTGGATGTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT
Redbeet1 BvADHβ 701 GTTTTTTGGATGTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT
Whitebeet_BvADHβ 701 GTTTTTTGGATGTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT
Seabeet BvADHβ 701 GTTTTTTGGATGTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT
Redbeet2_BvADHβ 701 GTTTTTTGGATGTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT
Sugarbeet_BvADHβ 751 TGTGCTGAGCATGATAAGTTTGCAGCAGGGTTTCAGTTTATAACACATTT
Yellowbeet BvADHβ 751 TGTGCTGAGCATGATAAGTTTGCAGCAGGGTTTCAGTTTATAACACATTT
Redbeet1 BvADHβ 751 TGTGCTGAGCATGATAAGTTTGCAGCAGGGTTTCAGTTTATAACACATTT
Whitebeet_BvADHβ 751 TGTGCTGAGCATGATAAGTTTGCAGCAGGGTTTCAGTTTATAACACATTT
Seabeet BvADHβ 751 TGTGCTGAGCATGATAAGTTTGCAGCAGGGTTTCAGTTTATTACACATTT
Redbeet2_BvADHβ 751 TGTGCTGAGCATGATAAGTTTGCAGCAGGRTTTCAGTTTATAACACATTT
Sugarbeet_BvADHβ 801 CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA
Yellowbeet BvADHβ 801 CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA
Redbeet1 BvADHβ 801 CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA
Whitebeet_BvADHβ 801 CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA
Seabeet BvADHβ 801 CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA
Redbeet2_BvADHβ 801 CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA
Sugarbeet_BvADHβ 851 CGAAAGGGTATGAGAGTTTGTTGAATTTGGTGGATAATACGTCGAAGGAT
Yellowbeet BvADHβ 851 CGAAAGGGTATGAGAGTTTGTTGAATTTGGTGGATAATACGTCGAAGGAT
Redbeet1 BvADHβ 851 CGAAAGGGTATGAGAGTTTGTTGAATTTGGTGGATAATACGTCGAAGGAT
Whitebeet_BvADHβ 851 CGAAAGGGTATGAGAGTTTGTTGAATTTGGTGGATAATACGTCGAAGGAT

Fig. 5B cont.


Seabeet BvADHβ 851 CGAAAGGGTATGAGAGTTTGTTGAATTTGGTGGATAATACGTCGAAGGAT
Redbeet2_BvADHβ 851 CGAAAGGGTATGAGAGTTTGTTGAATTTGGTGGATAATACGTCGAAGGAT
Sugarbeet_BvADHβ 901 AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA
Yellowbeet BvADHβ 901 AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA
Redbeet1 BvADHβ 901 AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA
Whitebeet_BvADHβ 901 AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA
Seabeet BvADHβ 901 AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA
Redbeet2_BvADHβ 901 AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA
Sugarbeet_BvADHβ 951 GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT
Yellowbeet BvADHβ 951 GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT
Redbeet1 BvADHβ 951 GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT
Whitebeet_BvADHβ 951 GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT
Seabeet BvADHβ 951 GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT
Redbeet2_BvADHβ 951 GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT
Sugarbeet_BvADHβ 1001 TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG
Yellowbeet BvADHβ 1001 TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG
Redbeet1 BvADHβ 1001 TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG
Whitebeet_BvADHβ 1001 TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG
Seabeet BvADHβ 1001 TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG
Redbeet2_BvADHβ 1001 TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG
Sugarbeet_BvADHβ 1051 ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC
Yellowbeet BvADHβ 1051 ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC
Redbeet1 BvADHβ 1051 ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC
Whitebeet_BvADHβ 1051 ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC
Seabeet_BvADHβ 1051 ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC
Redbeet2_BvADHβ 1051 ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC
Sugarbeet_BvADHβ 1101 TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG
Yellowbeet BvADHβ 1101 TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG
Redbeet1 BvADHβ 1101 TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG
Whitebeet_BvADHβ 1101 TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG
Seabeet_BvADHβ 1101 TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG
Redbeet2_BvADHβ 1101 TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG
Sugarbeet_BvADHβ 1151 AGACAAATTGA
Yellowbeet BvADHβ 1151 AGACAAATTGA
Redbeet1 BvADHβ 1151 AGACAAATTGA
Whitebeet_BvADHβ 1151 AGACAAATTGA
Seabeet_BvADHβ 1151 AGACAAATTGA
Redbeet2_BvADHβ 1151 AGACAAATTGA

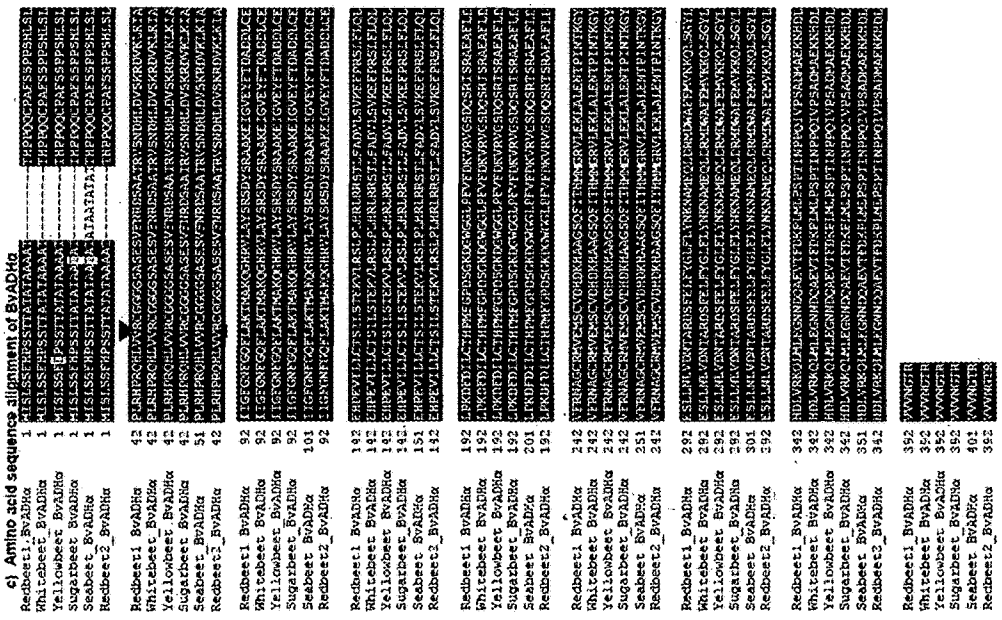


Fig. 5C

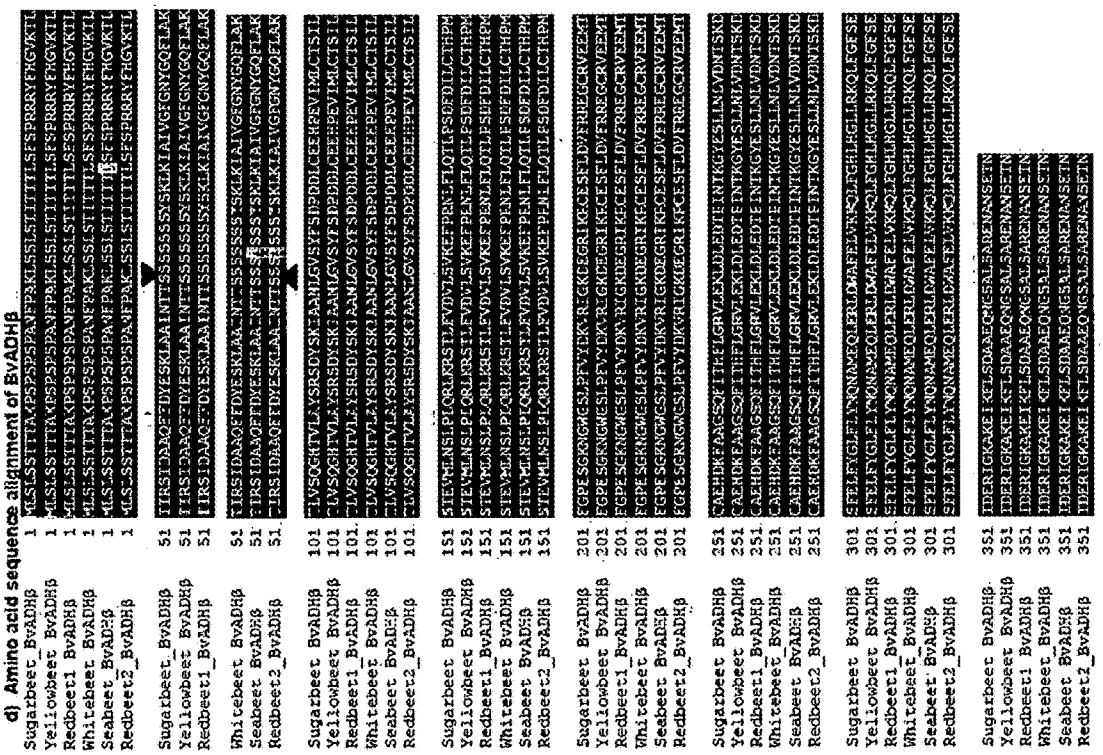


Fig. 5D

Fig. 6

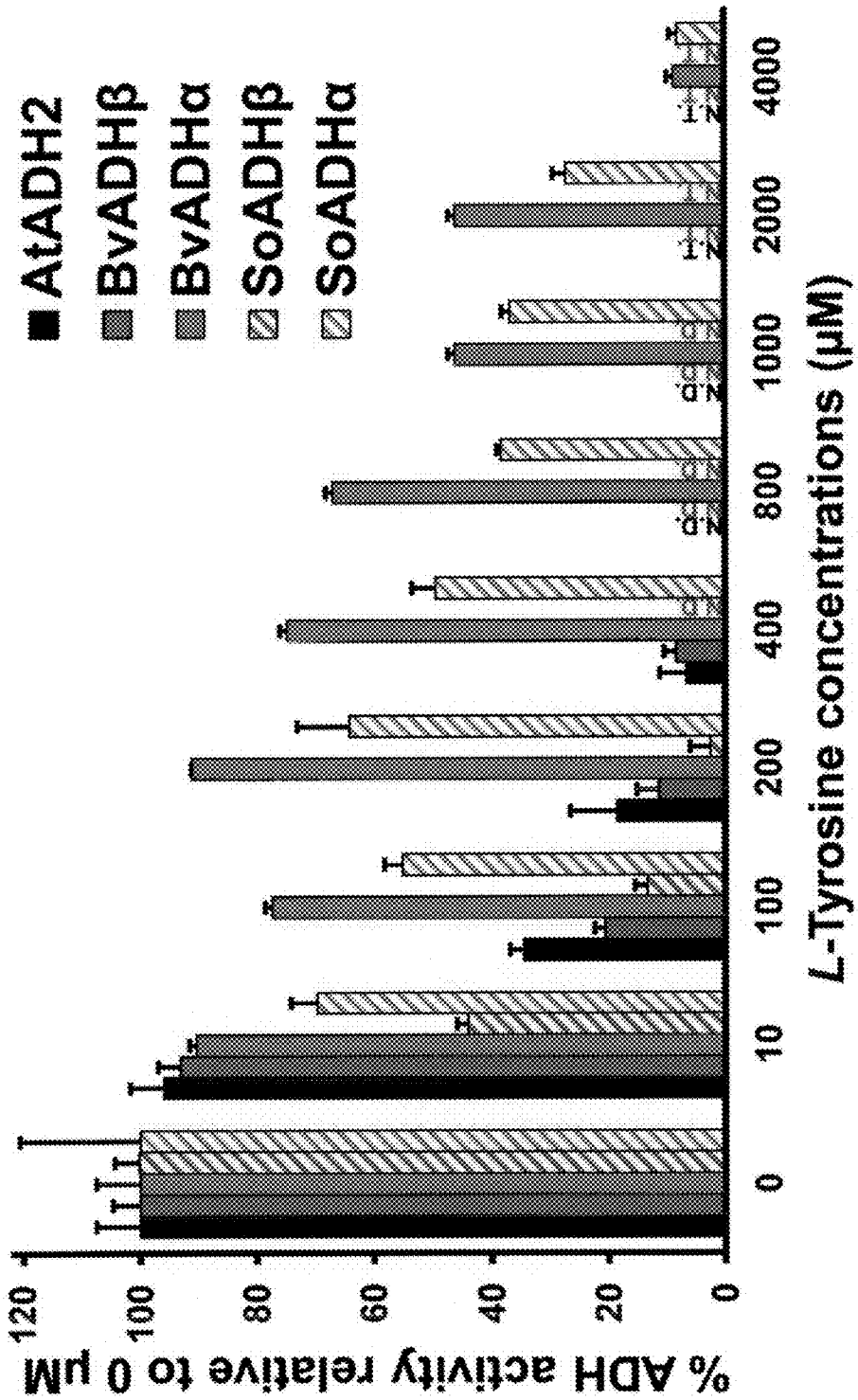

AtADH2
BvADHβ
BvADHα
SoADHβ
SoADHα
% ADH activity relative to 0 μM
120
100
80
60
40
20
0
0
10
100
200
400
800
1000
2000
4000
L-Tyrosine concentrations (μM)

Fig. 7

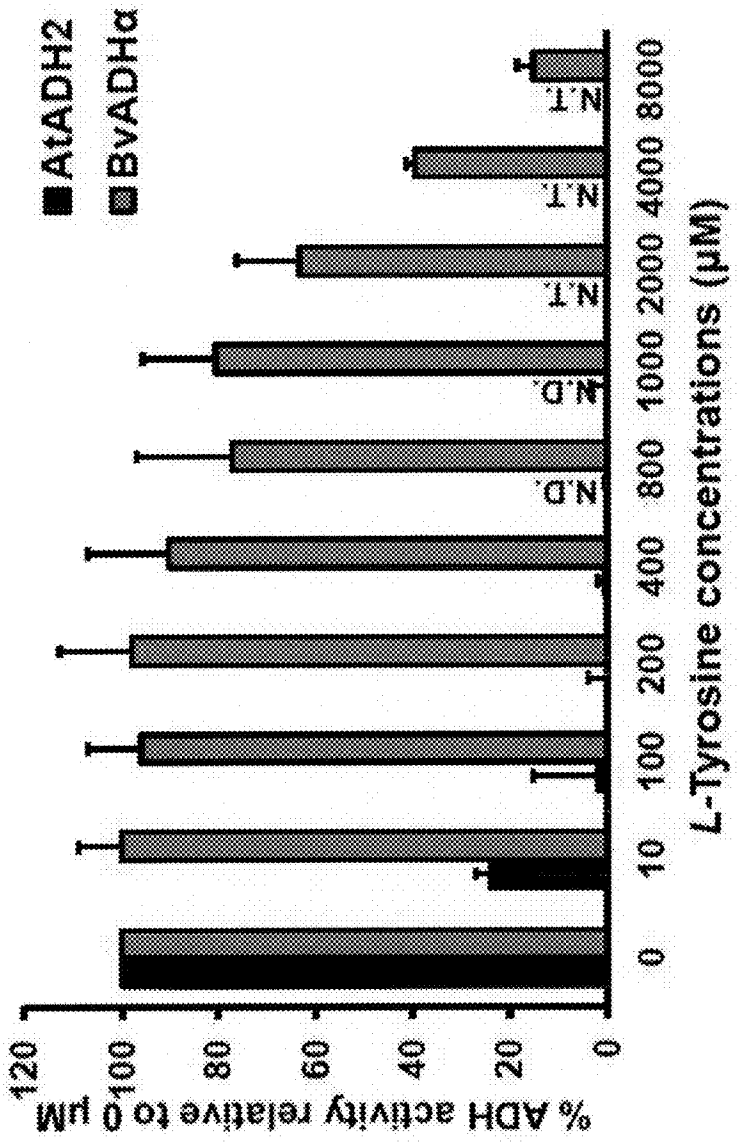
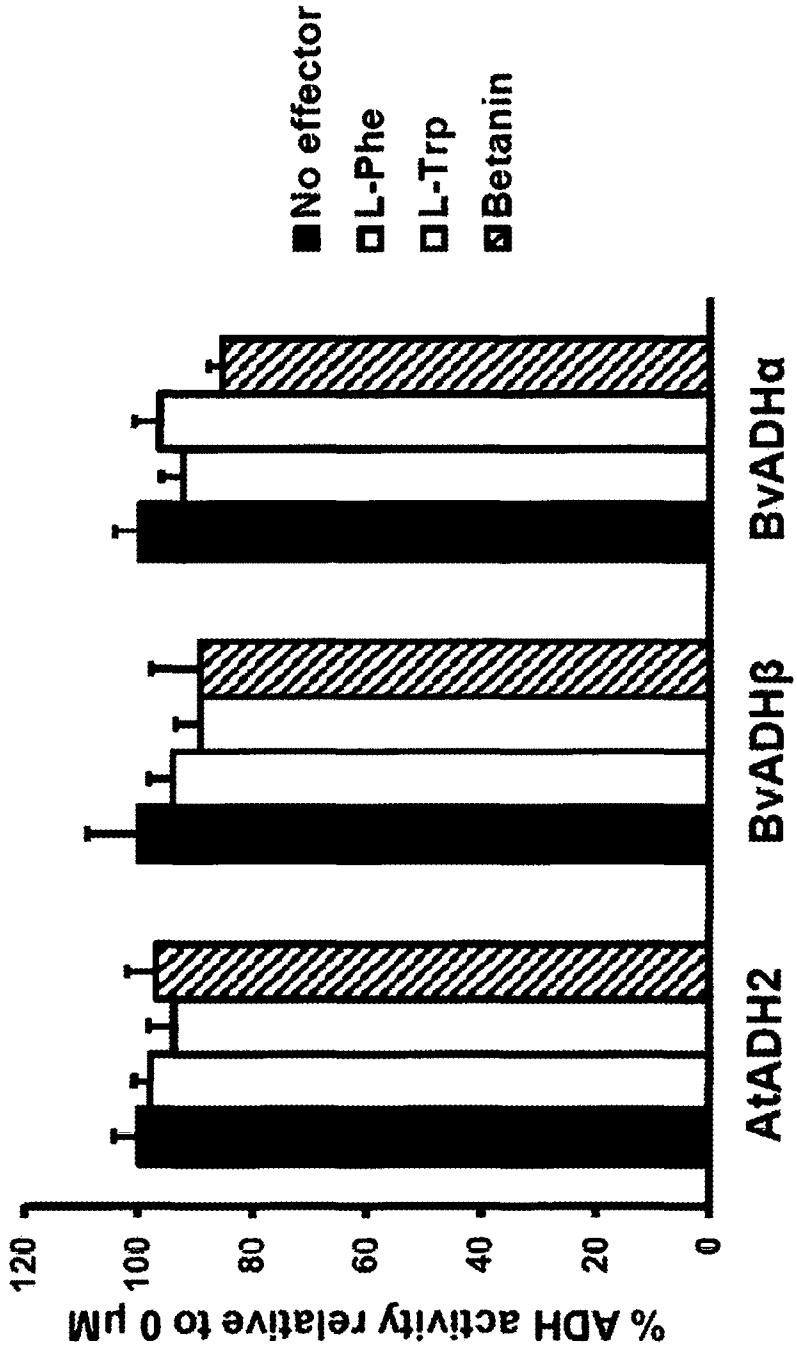

AtADH2
BvADHα
% ADH activity relative to 0 μM
120
100
80
60
40
20
0
0
10
100
200
400
800
1000
2000
4000
8000
L-Tyrosine concentrations (μM)
N.D.
N.D.
N.T.
N.T.
N.T.

Fig. 8


No effector
L-Phe
L-Trp
Betanin
% ADH activity relative to 0 μM
120
100
80
60
40
20
0
AtADH2
BvADHβ
BvADHα

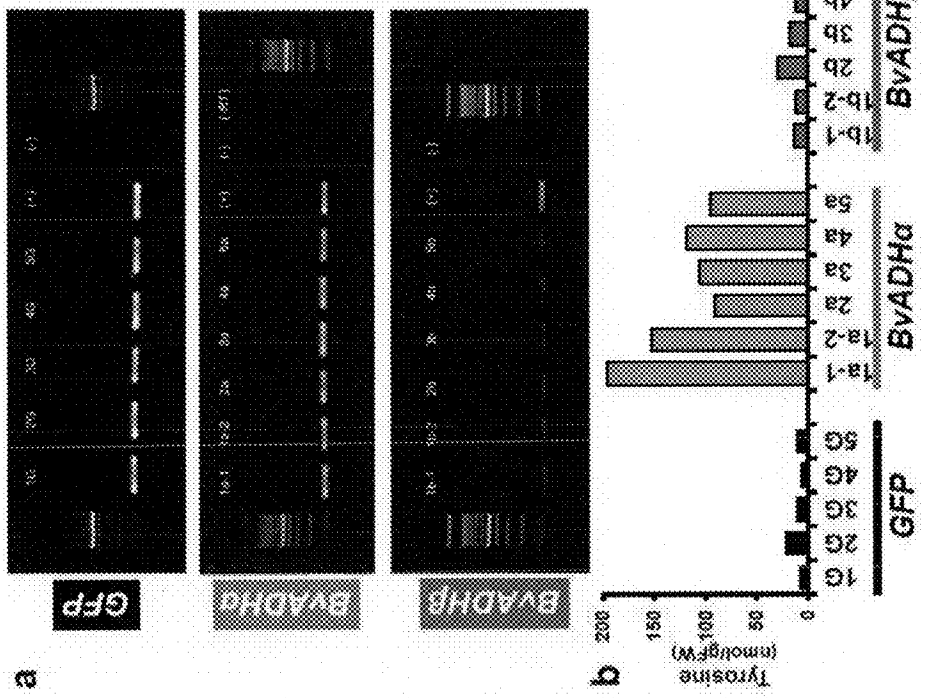

a
GFP
BvADHα
BvADHβ
b
Tyrosine (nmol/gFW)
200
150
100
50
0
1G
2G
3G
4G
5G
1a-1
1a-2
2a
3a
4a
5a
1b-1
1b-2
2b
3b
4b
5b
GFP
BvADHα
BvADHβ

Fig. 9A

Fig. 9B

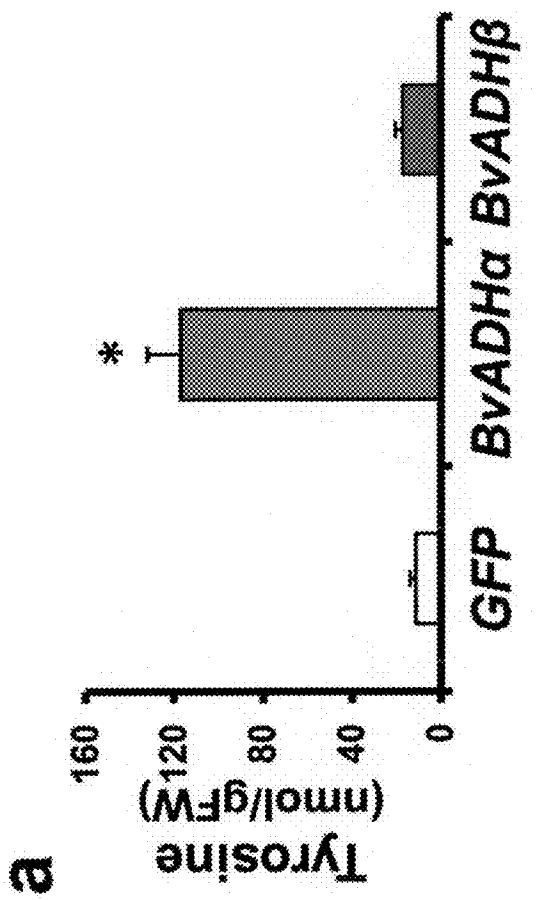


Fig. 10A

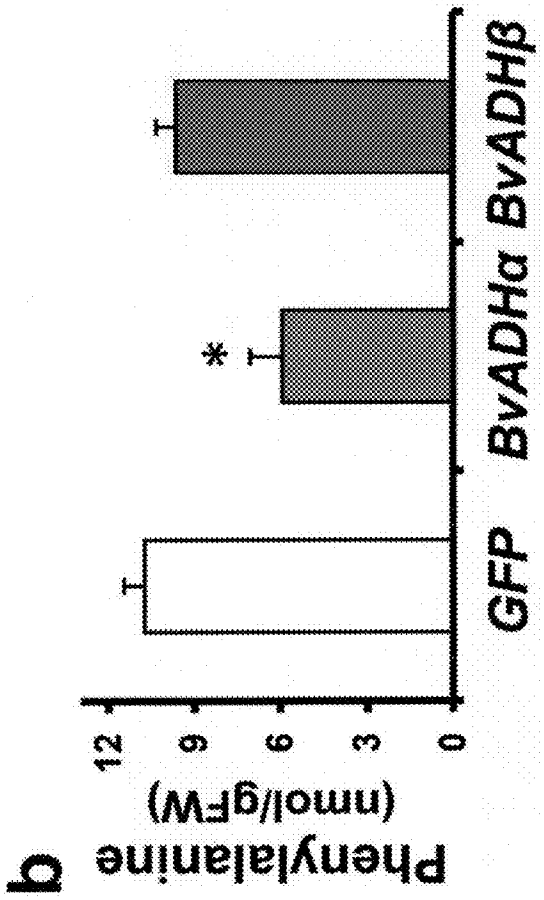


Fig. 10B

Fig. 11A

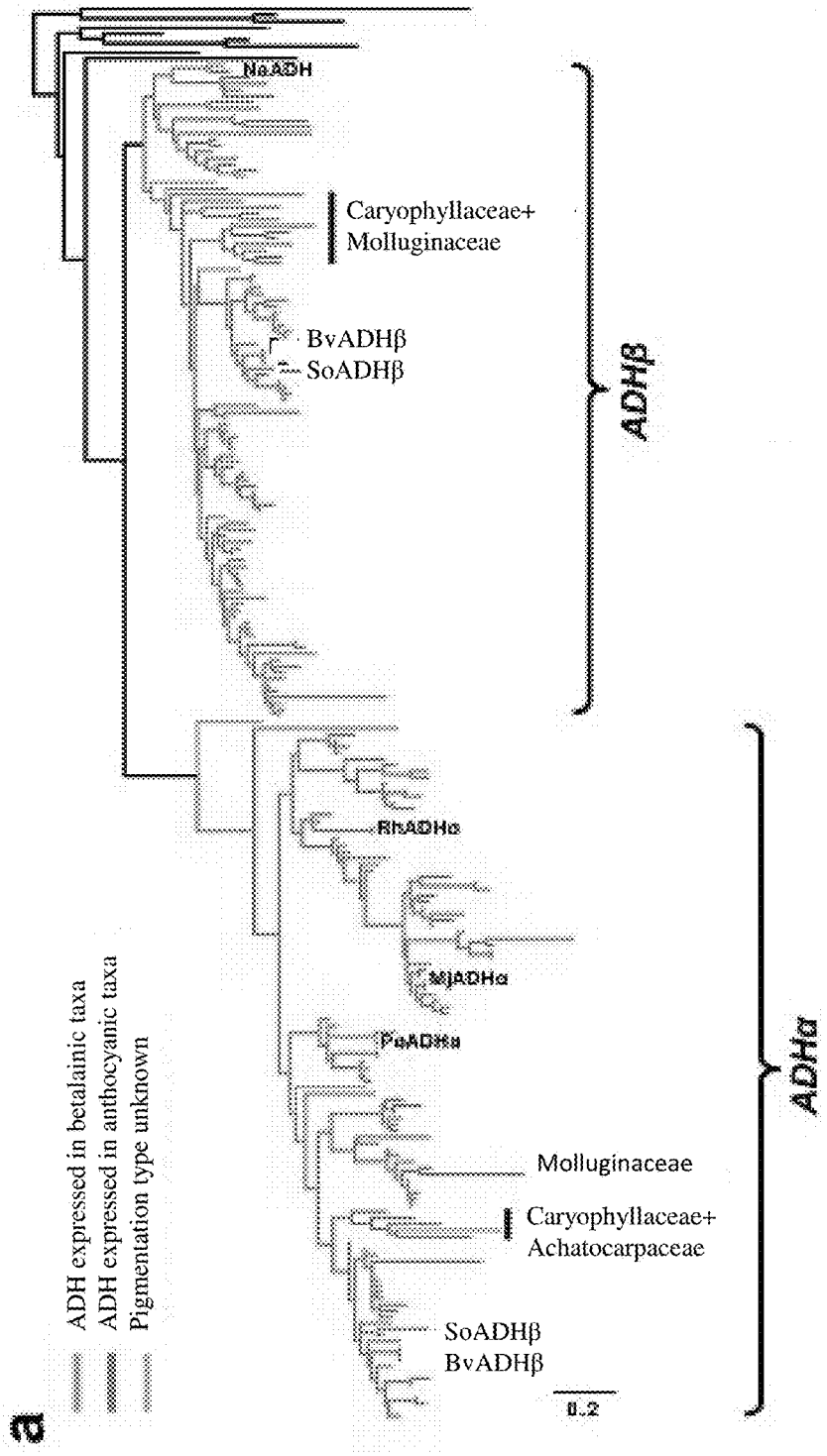

a
ADH expressed in betalainic taxa
ADH expressed in anthocyanic taxa
Pigmentation type unknown
NaADH
Caryophyllaceae+
Molluginaceae
BvADHβ
SoADHβ
ADHβ
RhADHα
MjADHα
PeADHα
Molluginaceae
Caryophyllaceae+
Achatocarpaceae
SoADHβ
BvADHβ
0.2
ADHα

Fig. 11B

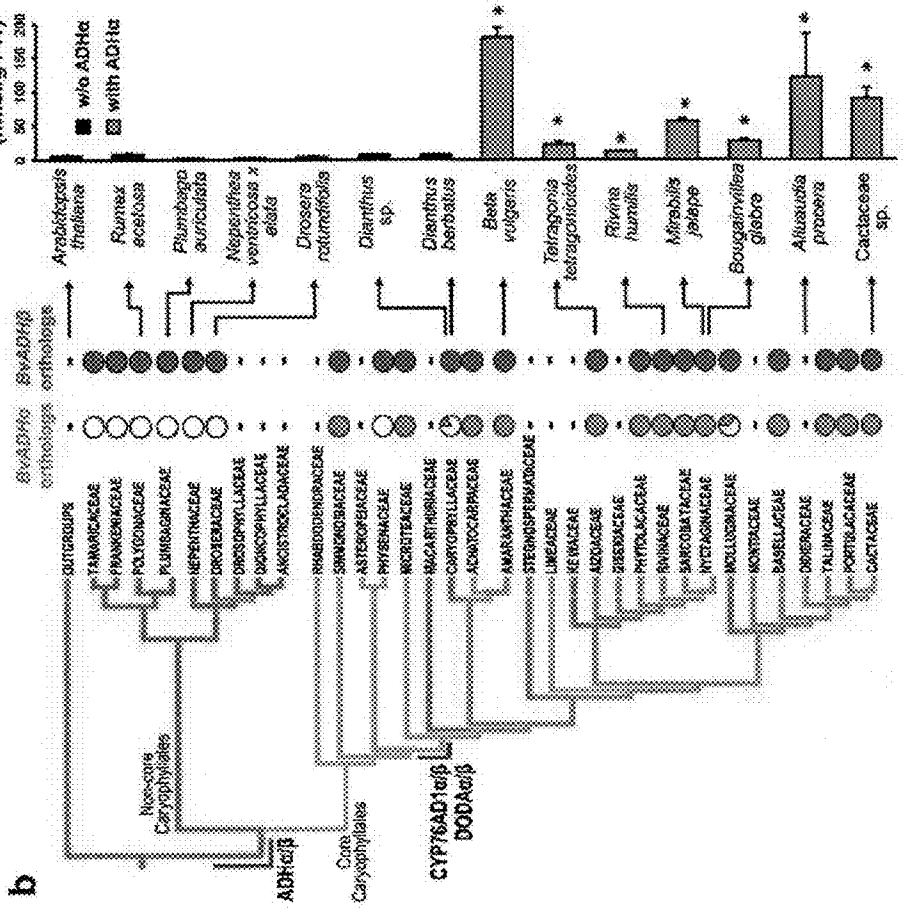
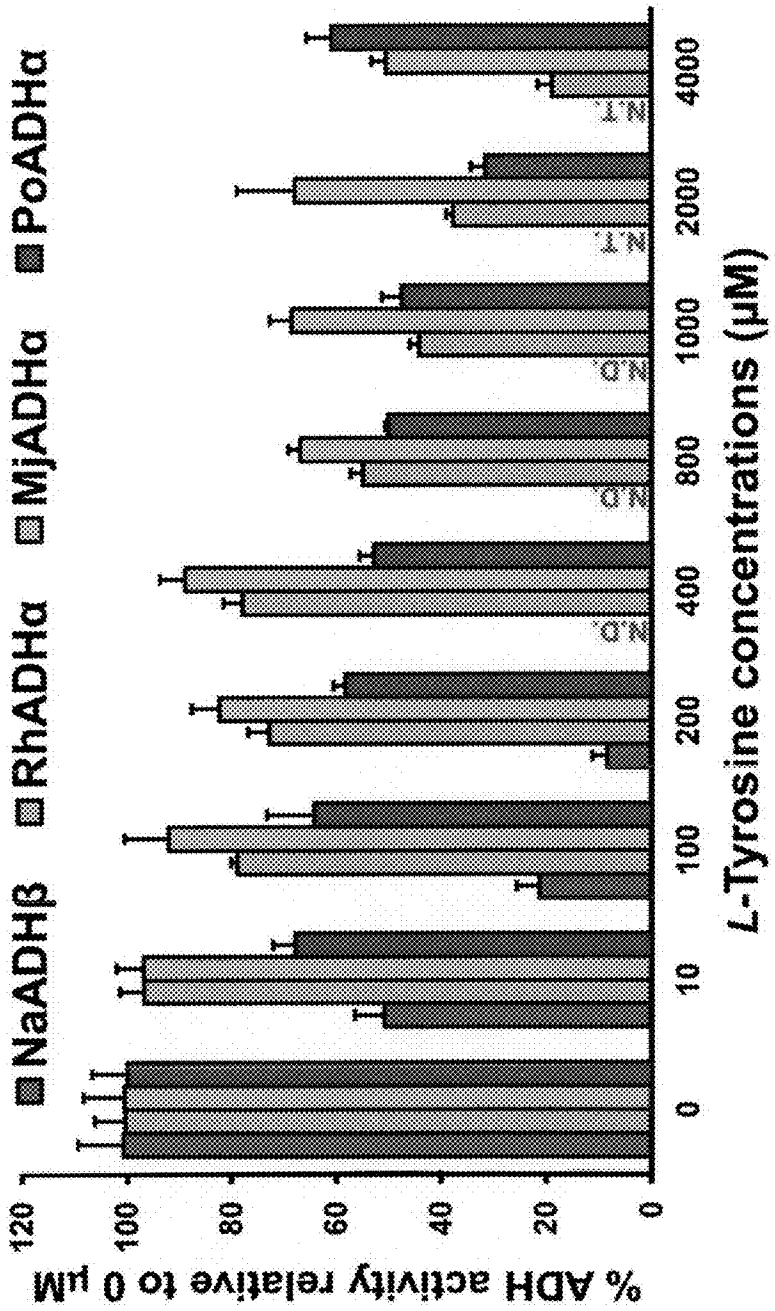


Fig. 11C

Fig. 12


NaADHβ
RhADHα
MjADHα
PoADHα
% ADH activity relative to 0 μM
120
100
80
60
40
20
0
0
10
100
200
400
800
1000
2000
4000
N.D.
N.T.
L-Tyrosine concentrations (μM)

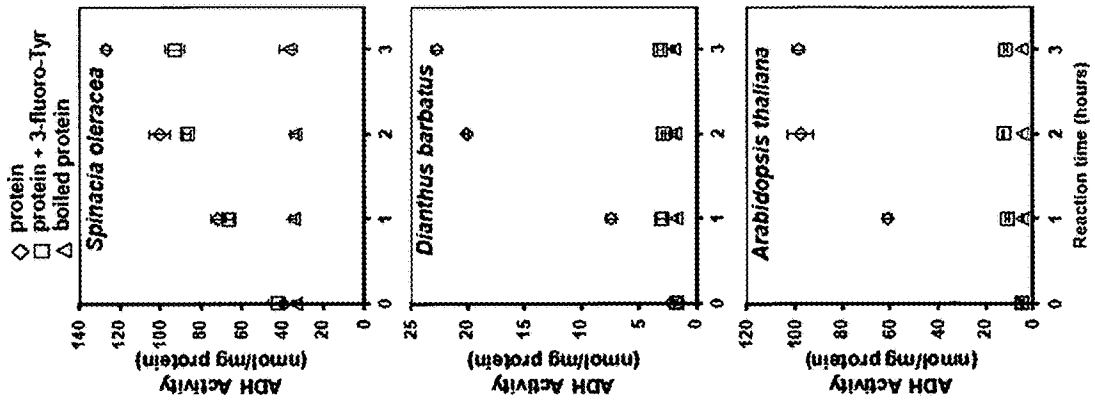

◇ protein
□ protein + 3-fluoro-Tyr
△ boiled protein
Spinacia oleracea
Dianthus barbatus
Arabidopsis thaliana
ADH Activity
(nmol/mg protein)
Reaction time (hours)

Fig. 13

Fig. 14A

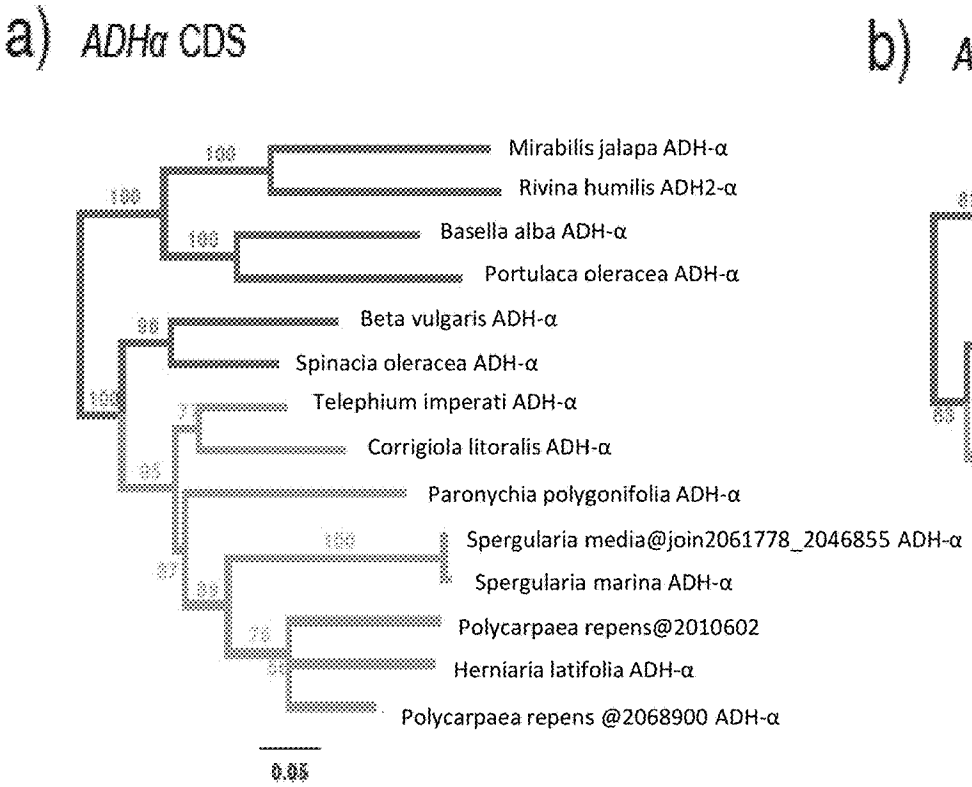


Fig. 14B

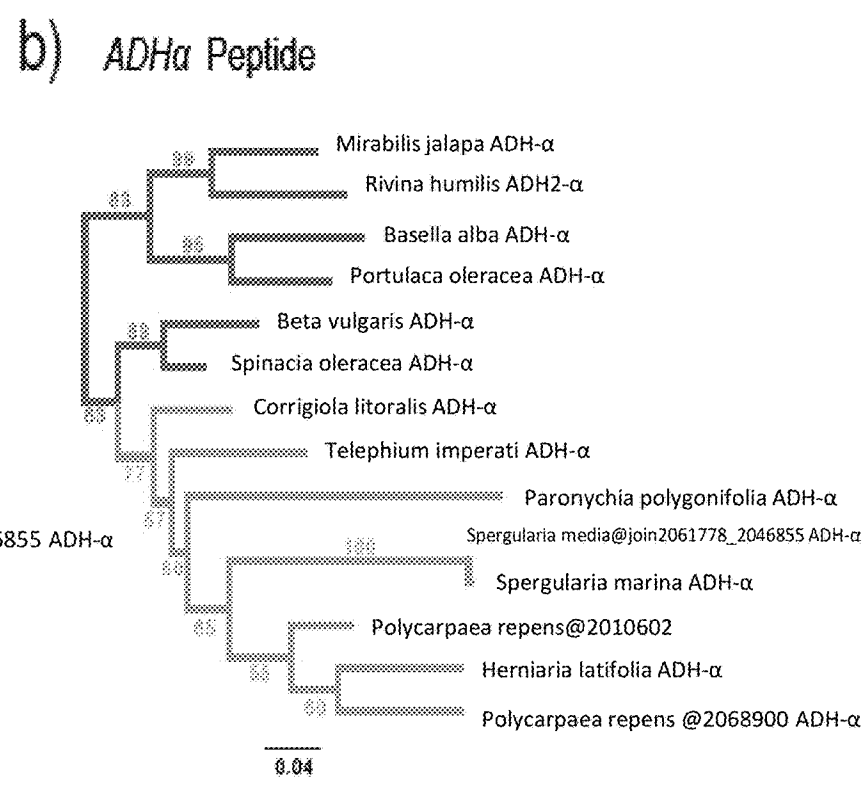


Fig. 15

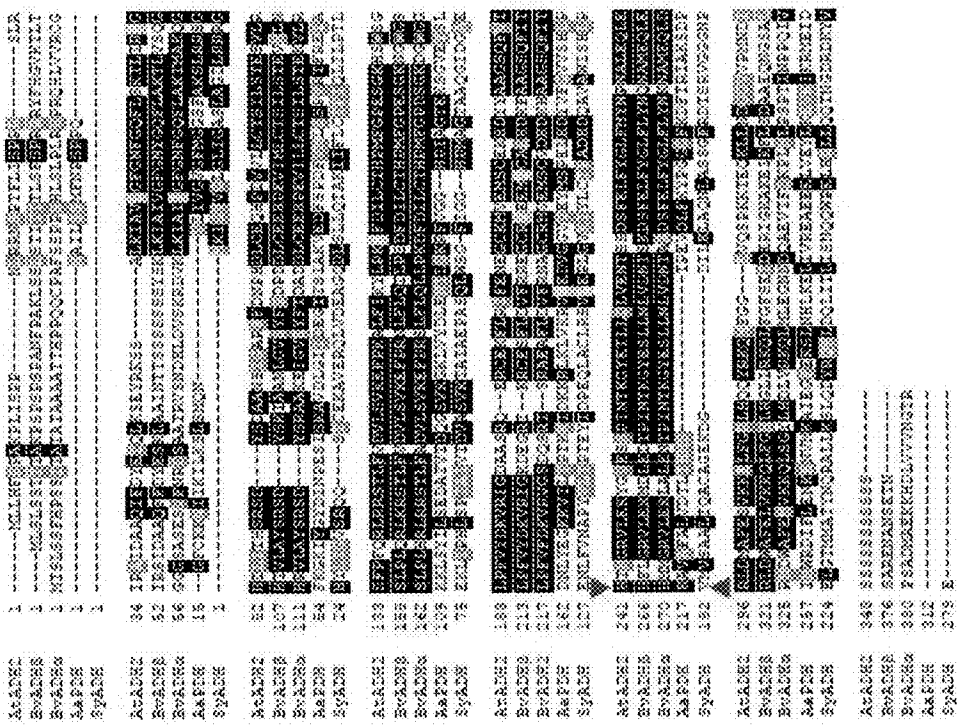


Fig. 16

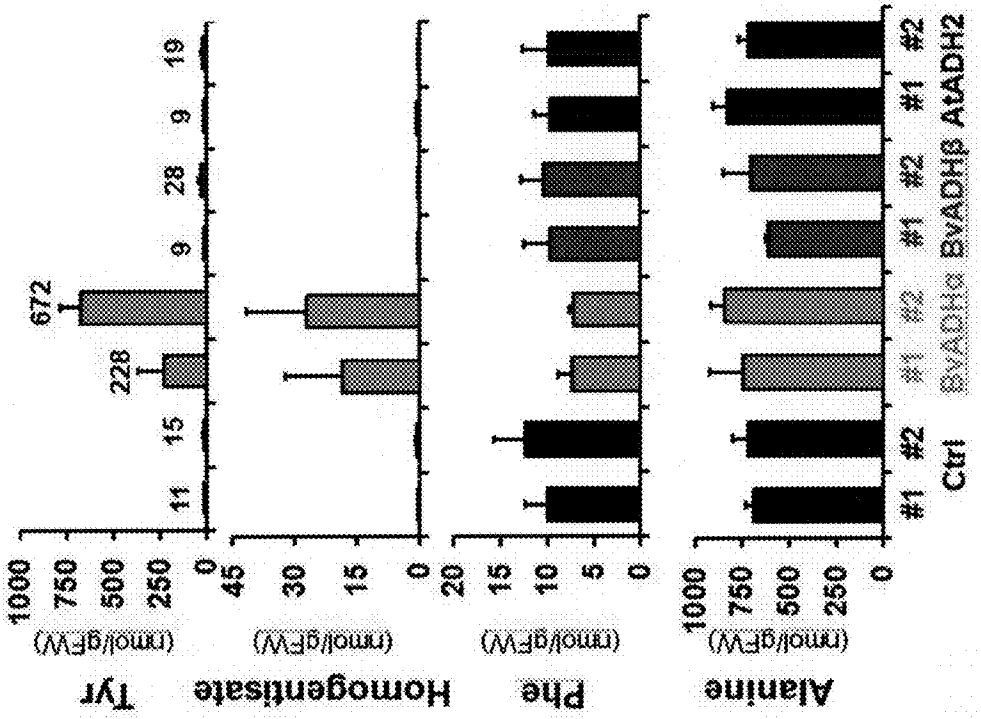

Alanine
Phe
Homogentisate
Tyr
(nmol/gFW)
1000
750
500
250
0
20
15
10
5
45
30
15
672
228
11
15
9
28
9
19
Ctrl
#1 #2
BvADHα #1 #2
BvADHβ #1 #2
AtADH2 #1 #2

Fig. 17

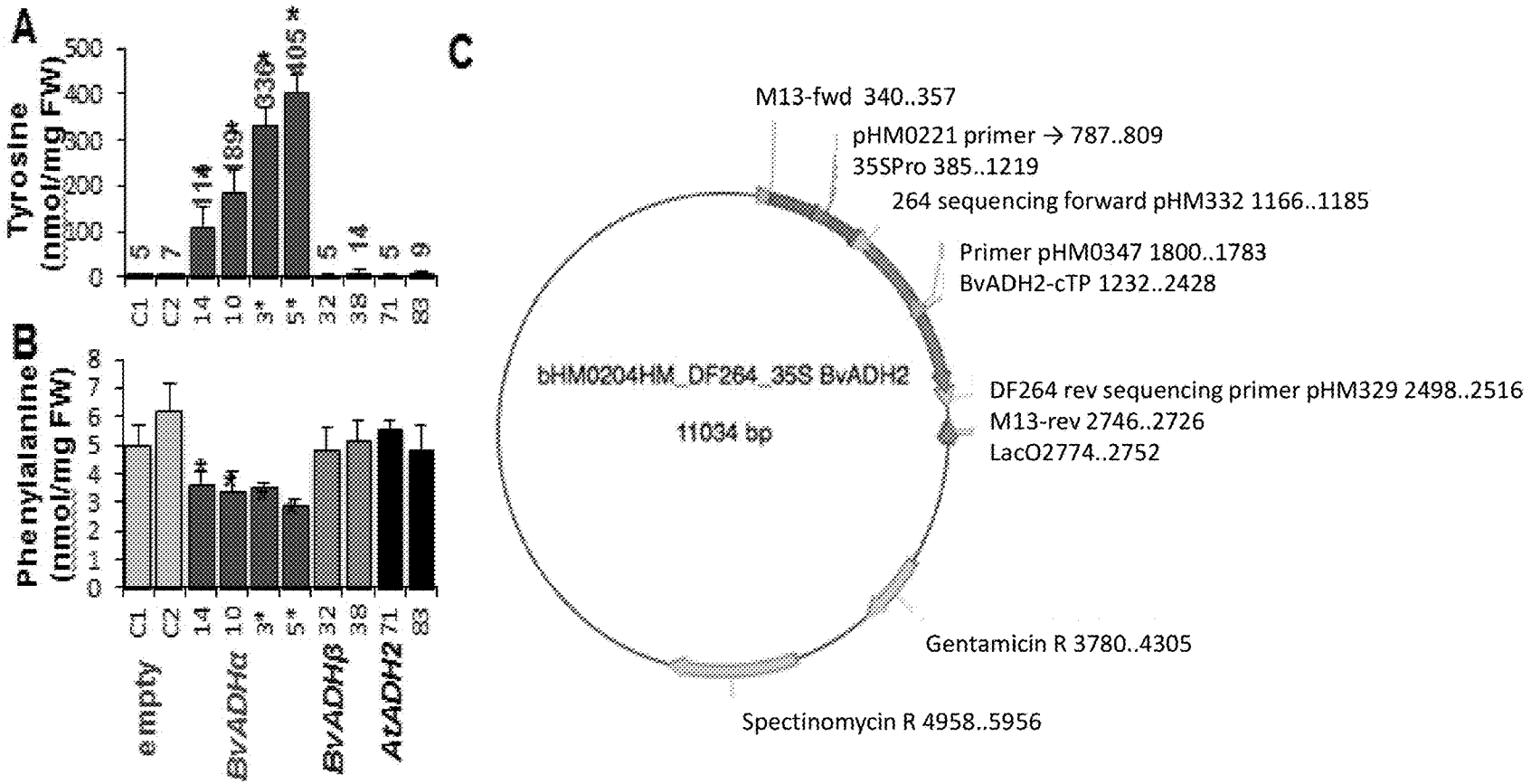
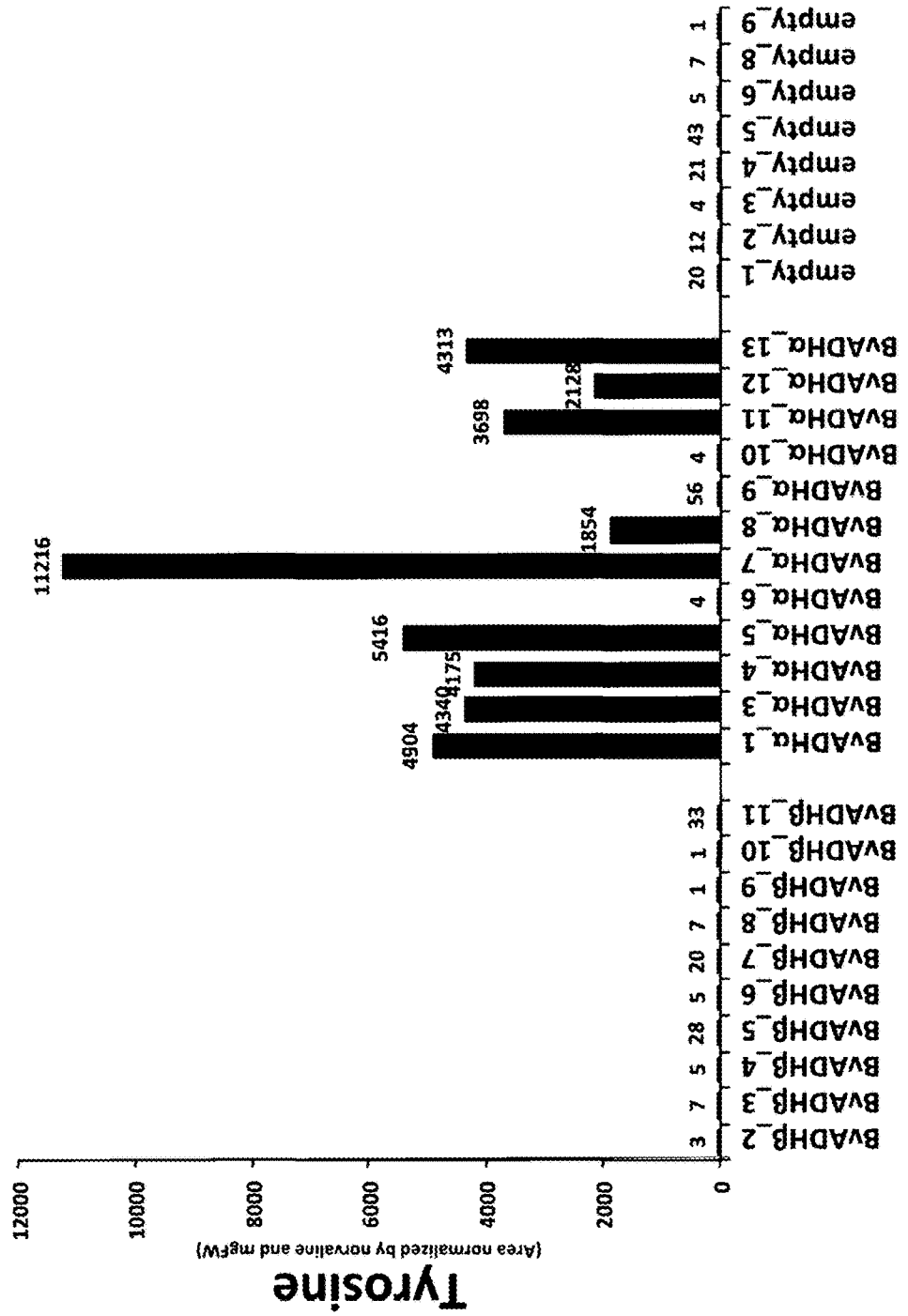

A
Tyrosine (nmol/mg FW)
5
7
114
189*
330*
405*
5
14
5
9
C1
C2
14
10
3*
5*
32
38
71
83
B
Phenylalanine (nmol/mg FW)
empty
BvADHα
BvADHβ
AtADH2
C
M13-fwd 340..357
pHM0221 primer → 787..809
35SPro 385..1219
264 sequencing forward pHM332 1166..1185
Primer pHM0347 1800..1783
BvADH2-cTP 1232..2428
DF264 rev sequencing primer pHM329 2498..2516
M13-rev 2746..2726
LacO2774..2752
Gentamicin R 3780..4305
Spectinomycin R 4958..5956
bHM0204HM_DF264_35S BvADH2
11034 bp

Fig. 18


Tyrosine
(Area normalized by norvaline and mgFW)
12000
10000
8000
6000
4000
2000
0
BvADHβ_2
BvADHβ_3
BvADHβ_4
BvADHβ_5
BvADHβ_6
BvADHβ_7
BvADHβ_8
BvADHβ_9
BvADHβ_10
BvADHβ_11
BvADHα_1
BvADHα_3
BvADHα_4
BvADHα_5
BvADHα_6
BvADHα_7
BvADHα_8
BvADHα_9
BvADHα_10
BvADHα_11
BvADHα_12
BvADHα_13
empty_1
empty_2
empty_3
empty_4
empty_5
empty_6
empty_8
empty_9
3 7 28 5 20 1 33
4904 4340 4175 5416 4 11216 1854 56 4 3698 2128 4313
20 12 4 21 43 5 7 1

AROGENATE DEHYDROGENASE POLYNUCLEOTIDES, POLYPEPTIDES AND METHODS OF USING THE SAME

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] The present application claims the benefit of priority to U.S. Provisional Patent Application No. 62/459,798, filed on Feb. 16, 2017, the content of which is incorporated herein by reference in its entirety.

STATEMENT REGARDING FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with United States government support under grant number 2015-67013-22955 awarded by the US Department of Agriculture, National Institute of Food and Agriculture. The government has certain rights in this invention.

SEQUENCE LISTING

[0003] This application is being filed electronically via EFS-Web and includes an electronically submitted Sequence Listing in .txt format. The .txt file contains a sequence listing entitled "2018-02-16_5671-00079_ST25.txt" created on Feb. 16, 2018 and is 126,668 bytes in size. The Sequence Listing contained in this .txt file is part of the specification and is hereby incorporated by reference herein in its entirety.

INTRODUCTION

[0004] Plants synthesize numerous specialized metabolites (also known as secondary metabolites), which play crucial roles in plant adaptation. In contrast to well-documented diversification of plant enzymes directly involved in specialized metabolism, relatively little is known about the evolution of primary metabolic enzymes that provide precursors to the production of various specialized metabolites.

[0005] L-Tyrosine (Tyr) is an aromatic amino acid required for protein biosynthesis in all organisms; however, it is synthesized de novo only in bacteria, fungi and plants, but not in animals. Consequently, animals have to consume Tyr, or L-phenylalanine (Phe) that can be hydroxylated to Tyr. Besides protein biosynthesis, plants also use Tyr to produce a diverse array of specialized metabolites that are important for defense (e.g. dhurrin), antioxidants (e.g. tocopherols), and pollinator attraction (e.g., betalains). Notably, humans have a long history of utilizing Tyr-derived specialized metabolites, such as the psychedelic alkaloid mescaline derived from the cactus *Lophophora williamsii* and the analgesic morphine derived from *Papaver somniferum* (oppium poppy).

[0006] Tyr is synthesized from prephenate, which is converted from the final product of the shikimate pathway, chorismate. In most bacteria and fungi, prephenate is oxidatively decarboxylated by prephenate dehydrogenase ($TyrA_p$/PDH, hereafter referred only as PDH; EC 1.3.1.12) to produce 4-hydroxyphenylpyruvate (HPP), which is subsequently transaminated to Tyr (See, e.g., FIG. **1**). On the other hand, most plants first transaminate prephenate into arogenate and subsequently decarboxylate into Tyr by arogenate dehydrogenase ($TyrA_a$/ADH, hereafter referred only as ADH; EC 1.3.1.78), both steps occurring in the plastids. The Tyr pathway is usually highly regulated at PDH and ADH. These homologous enzymes are strongly feedback inhibited by Tyr and control carbon flow between the two competing Tyr and Phe pathways. A recent report showed that, in addition to plastidic ADH enzymes, some plants possess a PDH enzyme(s) that is not inhibited by Tyr and is localized to the cytosol. Clearly, there is evolutionary variation in the Tyr pathway(s) in different plant lineages that warrants investigation. In addition, the contribution of Tyr biosynthesis and its regulation to the generation of Tyr-derived plant natural products is currently unknown.

[0007] Betalains are a class of pigments that, within the flowering plants, occur exclusively in the order Caryophyllales where they replace the otherwise ubiquitous anthocyanins. Within Caryophyllales, the majority of families are betalain pigmented. In two families, Molluginaceae and Caryophyllaceae, however, evolutionary reversions from betalain to anthocyanin pigmentation have occurred, highlighting the fact that these two classes of water-soluble pigments have never been found in the same organism. Betalains and anthocyanins are synthesized from Tyr and Phe, respectively, but have similar chemical properties and physiological functions in pollinator attraction and stress tolerance. Betalains are also used as a natural food dye (E162) and have anticancer and antidiabetic properties. Furthermore, intermediates in the betalain pathway are important pharmaceuticals [e.g. L-dihydroxyphenylalanine (L-DOPA) for the treatment of Parkinson's disease) or are substrates for other pharmaceutical agents (e.g. the production of dopamine and isoquinoline alkaloids such as morphine). Consequently, understanding the coordinated regulation of Tyr and betalain biosynthesis has the potential to enhance the production of Tyr, and the yield of Tyr-derived plant natural products important for human health and nutrition.

SUMMARY

[0008] In one aspect, ADH polynucleotides encoding ADH polypeptides are provided. The polynucleotides may encode a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to any one of the polypeptides of SEQ ID NOS: 1-20, 43, 45, or 47. SEQ ID NOS: 1-20, 43, 45, or 47 are polypeptide sequences of ADHα and ADHβ polypeptides identified in W357B red beet variety, Big Buck sugar beet variety, Touch Stone yellow beet variety, Blankoma white beet variety, Sea beet PI562585 variety, and other Caryophyllales species.

[0009] In another aspect, constructs are provided. The constructs may include a heterologous promoter operably linked to any one of the polynucleotides described herein.

[0010] In a further aspect, vectors including any of the constructs or polynucleotides described herein are provided.

[0011] In another aspect, cells including any of the polynucleotides, constructs, or vectors described herein are provided.

[0012] In a further aspect, plants including any of the polynucleotides, constructs, vectors, or cells described herein are also provided.

[0013] In a still further aspect, methods for increasing production of at least one product of the tyrosine or HPP pathways in a cell are provided. The methods may include introducing any of the polynucleotides, constructs, or vectors described herein into the cell. Optionally, the methods may further include purifying the product of the tyrosine or HPP pathways from the cells.

BRIEF DESCRIPTION OF DRAWINGS

[0014] This patent or application contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawings will be provided by the Office upon request and the payment of the necessary fee.

[0015] FIGS. **1**A-**1**D shows *Beta vulgaris* have two ADH enzymes localized in the plastids. FIG. **1**A shows tyrosine and betalain biosynthetic pathways in plants. L-Tyrosine (Tyr) can be synthesized from prephenate via arogenate dehydrogenase (ADH/TyrA$_a$) or prephenate dehydrogenase (PDH/TyrA$_p$). Tyr is exported from the plastid to cytosol and then converted to L-dihydroxyphenylalanine (L-DOPA) by CYP76AD1α, CYP76AD5, and CYP76AD6 (CYP76AD1α/5/6). L-DOPA is then eventually converted to betalains, red betacyanins and yellow betaxanthins. Biosynthesis of Tyr competes for arogenate or prephenate substrate with that of L-phenylalanine (Phe), the precursor of anthocyanins. Blue lines denote feedback regulation by Tyr. DODA, L-DOPA dioxygenase. FIG. **1**B is a graph showing arogenate substrate was incubated with the purified recombinant enzymes of BvADHα or BvADHβ together with $NADP^+$ cofactor and the production of Tyr was analyzed. The High Pressure Liquid Chromatography (HPLC) traces were offset for presentation. *Arabidopsis thaliana* ADH2 (AtADH2) was used as a control for the ADH assay. In FIG. **1**C green fluorescence protein (GFP) was fused at the C-terminal of BvADHα and BvADHβ and transiently expressed in *Arabidopsis* protoplasts. Free GFP and GFP-fused *Arabidopsis* ADH2 (AtADH2) were used as controls for cytosolic and plastidic localization, respectively. Representative images show GFP fluorescence and chlorophyll autofluorescence in green and magenta, respectively. Scale bars, 10 μm. FIG. **1**D is a set of graphs showing expression levels of BvADHα and BvADHβ were compared with those of betalain pathway genes in the cotyledon and hypocotyl of 7 day-old sugar beet and red beet (W357B). Asterisks indicate significant differences between the two genotypes ($p<0.05$, Student's t-test). Bars represent percent expression relative to the sample with the highest expression. Data are means of three biological replicates±s.e.m. N.D., not detectable.

[0016] FIGS. **2**A-**2**B show physical location, homology, and phylogeny of BvADHα and BvADHβ. FIG. **2**A shows the location and physical distance of BvADHα and BvADHβ on chromosome 8 of the *B. vulgaris* genome. A nearby gene is indicated in gray. FIG. **2**B shows amino acid identity of ADH and PDH proteins from different plants and bacteria. AaPDH, *Aquifexaeolicus*; AtADH1 and AtADH2, *Arabidopsis thaliana*; GmPDH1, *Glycine max*; EcPDH, *Escherichia coli*; and SyADH, *Synechocystis* sp. PCC6803.

[0017] FIGS. **3**A-**3**C shows ADH but not PDH activity detected from *B. vulgaris* tissues (FIGS. **3**A, **3**B) or recombinant enzyme (FIG. **3**C). Arogenate (FIG. **3**A) or prephenate (FIGS. **3**B, **3**C) substrates were incubated with $NADP^+$ cofactor and desalted protein crude extract (FIGS. **3**A, **3**B) of beet leaf (L), root/stem (R/S) tissues or recombinant enzyme of BvADHα or BvADHβ together with $NADP^+$ cofactor (FIG. **3**C). The production of Tyr (FIG. **3**A) or HPP (which was converted to 4-hydroxyphyenyllactic acid, HPLA) FIGS. **3**B, **3**C were analyzed by HPLC. The HPLC traces were offset for presentation. *Arabidopsis thaliana* ADH2 (AtADH2) [17,18] and *Medicago truncatula* PDH (MtPDH) [22] were used as a control for the ADH and PDH assay, respectively.

[0018] FIG. **4** shows BvADHs prefer $NADP^+$ over NAD^+ as cofactor. ADH activity was analyzed using $NADP^+$ or $NADP^+$ cofactor, which is expressed as the mean of three independent experiments±s.e.m. in nmols-1 mg-1 of protein.

[0019] FIGS. **5**A-**5**D show no amino acid changes were found in the mature protein coding region of BvADHα among different *B. vulgaris* varieties. The BvADHα and BvADHβ genes were sequenced from five different varieties of domesticated (red 1 [W357B], red 2 [Bohardy], sugar, yellow, and white) and a wild beet (sea beet ascension number PI562585). In nucleotide sequence comparisons of BvADHα (FIG. **5**A, SEQ ID NOs: 21-25, 44) and BvADHβ (FIG. **5**B SEQ ID NOs: 34-38, 48), several single nucleotide polymorphisms (SNPs) were found among varieties. Amino acid sequence alignments of BvADHα (FIG. **5**C, SEQ ID NOs: 1-5, 43) and BvADHβ (FIG. **5**D, SEQ ID NOs: 14-18, 47), however, showed that these SNPs were mostly synonymous (no changes in amino acid), with two exceptions found in the N-terminal predicted chloroplast transit peptide, which was eliminated for recombinant enzyme expression. The predicted chloroplast transit peptide cleavage sites are denoted by green triangles.

[0020] FIG. **6** shows beet and *spinach* ADHα but not ADMβ have reduced sensitivity to Tyr. ADH activity was measured at different Tyr concentrations using $NADP^+$ cofactor and purified recombinant ADH enzymes of beet (BvADHα, BvADHβ), *spinach* (SoADHα, SoADHβ), and *Arabidopsis* (AtADH2). Data are expressed as the percentage of respective control activity without Tyr (0 μM) and means of three independent experiments±s.e.m. N.D., not detectable; N.T., not tested.

[0021] FIG. **7** shows recombinant His-tagged BvADHα also exhibits reduced sensitivity to Tyr relative to AtADH2. BvADHα and AtADH2 recombinant enzymes were also generated as 6×His-tag proteins to determine if GST-tag affects Tyr sensitivity of BvADHα. The His-BvADHα recombinant enzyme still exhibited relaxed sensitive to Tyr inhibition. Data are expressed as the percentage of respective control activity without Tyr (0 μM) and the means of three independent experiments±s.e.m. N.D., not detectable; N.T., not tested.

[0022] FIG. **8** shows BvADHs are not inhibited by phenylalanine, tryptophan, and betanin. ADH activity of BvADHα, BvADHβ and AtADH2 was measured in the presence and absence of 1 mM final concentration of L-phenylalanine (L-Phe), L-tryptophan (L-Trp), and betanins as an effector. Data are expressed as the percentage of respective control activity without effector and the mean of three independent experiments±s.m.e. No significant reduction was observed by any effector treatment relative to respective no effector control ($P<0.05$, student t test).

[0023] FIGS. **9**A-**9**B show transgene expression and tyrosine levels of individual leaf samples of infiltrated *Nicotiana benthamiana. Agrobacterium tumefaciens* carrying the construct of 35S::GFP, 35S::BvADHα, or 35S::BvADHβ was infiltrated to *Nicotiana benthamiana* leaves (sample names ending with G, a, and b, respectively). 1a-1 and 1a-2 are technical replicates of the same leaf infiltrated with 35S::BvADHα, so do 1b-1 and 1b-2 for 35S::BvADHβ. FIG. **9**A shows expression of respective transgenes shown by RTPCR. (+) denotes a positive control using the original

plasmid as a template, while (−) indicates a negative control cDNA from a leaf area without infiltration. (−RT) is an additional negative control without reverse transcriptase to detect genomic DNA contamination. FIG. **9**B shows tyrosine contents of individual samples. Two technical replicates showed very similar results. Means±s.e.m. of Tyr and other amino acids analysis are shown in FIGS. **10**A-**10**B and Table 2.

[0024] FIGS. **10**A-**10**B shows heterologous expression of BvADHα but not BvADHβ increases tyrosine levels in *Nicotiana benthamiana. Agrobacterium tumefaciens* carrying the construct of 35S::GFP, 35S::BvADHα, or 35S::BvADHβ was infiltrated to *N. benthamiana* leaves, which were analyzed for amino acid contents using GC-MS. The levels of tyrosine (FIG. **10**A) and phenylalanine (FIG. **10**B) are shown. Asterisks indicate significant differences from the 35S::GFP control ($p<0.05$, Student's t-test). Data are means±S.E.M. (n=5).

[0025] FIGS. **11**A-**11**C show phylogenetic distribution of ADHα in Caryophyllales. The blue and pink branches represent anthocyanin and betalain-producing families, respectively, while families with unclear/unidentified pigmentation are shown in gray. FIG. **11**A shows maximum-likelihood phylogeny of ADH genes in Caryophyllales. Scale bar indicates inferred number of amino acid substitution per site. ADH enzymes characterized in this study are indicated at the end of each branch. FIG. **11**B shows presence and absence of BvADHα and BvADHβ orthologs detected from genome or transcriptome data was mapped to the family-level phylogenetic tree of the Caryophyllales order. Filled circles denote that corresponding orthologs were detected in all species within the family, whereas partially filled circles indicate that the filled portion of the species within each family had corresponding orthologs. Open circles denote no corresponding orthologs were detected. Red lines indicate estimating timings of duplication events of ADH and betalain pathway genes (CYP76AD1 and DODA). Dash lines (−) represent families with no available transcriptomic or genomic data. FIG. **11**C shows Tyr contents analyzed in various Caryophyllales species. *Arabidopsis thaliana* was used as outgroup. Orange bars indicate species having ADHα orthologs. Young leaf tissues were used for all samples except a Cactaceae species, in which flowers were used to avoid succulent tissues. Asterisks denote significant difference from *Arabidopsis* ($p<0.05$) based on fixed effect model (see method). Also, a statistical analysis based on the mixed effect model showed significant differences between two groups, plants with and without ADHα ($p<0.0001$). Bars represent means±s.e.m. (n=four biological replicates).

[0026] FIG. **12** shows ADHα from various species of core Caryophyllales also exhibit relaxed sensitivity. ADH activity was measured under different Tyr concentrations using purified recombinant ADH enzymes of *Nepenthes ventricosa*× *alata* (NaADHβ), *Rivina humilis* (RhADHα), *Mirabilis jalapa* (MjADHα), and *Portulaca oleracea* (PoADHα) ADH. Data are expressed as the percentage of respective control activity without Tyr (0 μM) and the mean of three independent experiments±s.e.m. N.D., not detectable; N.T., not tested.

[0027] FIG. **13** shows Tyr sensitivity of ADH activity from plant tissues. The plastid extracts of *spinach* (Spinaciaoleracea), and the crude extracts of *Dianthus barbatus* and *Arabidopsis thaliana* were incubated with 1 m Marogenate substrate and 1 mM NADP+ cofactor for indicated times. Plastids were isolated for *spinach* ADH assays to eliminate strong polyphenoloxidase activity present in the crude extracts. Data are means±s.e.m. (n=4). Activity increased linearly during the first two hours, which were used to calculate ADH activity presented in Table 4.

[0028] FIGS. **14**A-**14**B shows ADHα sequences used for texting relax selection. FIGS. **14**A and **14**B show ADHα orthologs of Caryophyllaceae (blue, designated as test branches in RELAX analysis, Table 5), as compared to those betalain-producing Caryophyllales species (pink, designated as reference branches in RELAX analysis, Table 5). Blue branches showed no obvious acceleration of substitution in their coding sequences (CDS, FIG. **14**A), whereas there was apparent acceleration in their peptide sequences (FIG. **14**B). Tips marked with '@' are from assembled transcriptomes. The rest of the sequences are from PCR and Sanger sequencing from DNA (*H. latifolia, S. marina*, and *P. polygonifolia*) or RNA.

[0029] FIG. **15** shows the Histidine 217 residue responsible for Tyr sensitivity of *Aquifex aeolicus* PDH (AaPDH) is still present in BvADHα. Previous studies showed that the H217 residue of AaPDH (denoted by red triangles) is absent in Tyr-insensitive ADH of *Synechocystis* sp. PCC6803 (SyADH) and confers Tyr sensitivity of AaPDH (Sun et al., 2009, Legrand, P. et al. 2008). The amino acid alignment of AaPDH, SyADH together with BvADHα, BvADHβ, and *Arabidopsis* ADH (AtADH2) (SEQ ID NOs: 1, 14, and 92-94) showed that corresponding His residues are present in all plant ADHs. This result suggests that yet to be identified novel residues and mechanism are involved in the relaxed Tyr sensitivity of BvADHα.

[0030] FIG. **16** shows expression of BvADHα in *Arabidopsis* leads to hyper-accumulation of tyrosine. Overexpression of tyrosine-insensitive BvADHα, but not BvADHβ or AtADH2, in *Arabidopsis* drastically enhanced accumulation of tyrosine and homogentisate, the downstream product of tyrosine and precursor of tocopherols and plastoquinone. Four-week old *Arabidopsis* leaf tissue was submitted to chemical analysis by GC-MS. Two representative homozygous lines for each construct were selected. Control plants (Ctrl) are lines transformed with the empty vector. The content of tyrosine (Tyr), homogentisate, phenylalanine (Phe), and alanine (Ala) are shown as nmol/g of fresh weight. Samples were normalized by the internal recovery standard, norvaline. Values are mean of 3 biological replicates±SD (standard deviation). The above experiments were repeated at least 3 times with similar results.

[0031] FIG. **17** shows in planta expression of de-regulated BvADHα leads to enhanced accumulation of Tyr in *Arabidopsis*.

[0032] FIG. **18** shows heterologous expression of de-regulated BvADHα leads to hyper-accumulation of Tyr in *Glycine max* (soybean).

DETAILED DESCRIPTION

[0033] The present inventors investigated the Tyr biosynthetic pathway and its regulation in table beet (*Beta vulgaris* L.), which produces high levels of betalains. Using comparative genomics, biochemical, and cellular analyses, they found that *B. vulgaris* possesses two paralogous genes encoding two ADH enzymes, which they named ADHα and ADHβ. Interestingly, ADHα but not ADHβ exhibited relaxed sensitivity to Tyr inhibition. Although the present

inventors recently reported that legume PDH enzymes are also Tyr insensitive, BvADHα and legume PDHs have two major differences. First, legume PDHs are localized in the cytosol, whereas BvADHα (and BvADHβ) was targeted to the plastids. Second, legume PDHs completely lost Tyr sensitivity but BvADHα was still inhibited by Tyr at higher concentrations.

[0034] Other insensitive ADH/PDH enzymes have been previously found in microorganisms and the structural analyses of Tyr sensitive and insensitive enzymes identified histidine 217 as a possible residue responsible for its Tyr sensitivity. However, the corresponding histidine residue was still present in BvADHα, suggesting that different mechanisms, and as yet unidentified residues, are involved in the relaxed Tyr sensitivity of BvADHα. The identified BvADHα and other Caryophyllales ADHα enzymes may be introduced into various types of cells to deregulate Tyr biosynthesis and redirect carbon flow from Phe to Tyr, to improve the production of Tyr-derived products (e.g., vitamin E, isoquinoline alkaloids including morphine).

[0035] ADH polynucleotides encoding ADH polypeptides are provided. The polynucleotides may encode a polypeptide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to any one of the polypeptides of SEQ ID NOS: 1-20, 43, 45, or 47. SEQ ID NOS: 1-20, 43, 45, or 47 are polypeptide sequences of ADHα and ADHβ polypeptides identified in W357B red beet variety, Big Buck sugar beet variety, Touch Stone yellow beet variety, Blankoma white beet variety, Sea beet PI562585 variety, and other Caryophyllales species.

[0036] As used herein, the terms "polynucleotide," "polynucleotide sequence," "nucleic acid" and "nucleic acid sequence" refer to a nucleotide, oligonucleotide, polynucleotide (which terms may be used interchangeably), or any fragment thereof. These phrases also refer to DNA or RNA of natural or synthetic origin (which may be single-stranded or double-stranded and may represent the sense or the antisense strand). The polynucleotides may be cDNA or genomic DNA.

[0037] In some embodiments, the polynucleotides of the present invention may include any one of the polynucleotide sequences of SEQ ID NOS: 21-40, 44, 46, or 48 or a polynucleotide having at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% sequence identity to any one of the polynucleotide sequences of SEQ ID NOs: 21-40, 44, 46, or 48. SEQ ID NOS: 21-40, 44, 46, or 48 are polynucleotide sequences of ADHα and ADHβ polynucleotides that encode the ADHα and ADHβ polypeptides of SEQ ID NOS: 1-20, 43, 45, or 47 and identified in W357B red beet variety, Big Buck sugar beet variety, Touch Stone yellow beet variety, Blankoma white beet variety, Sea beet PI562585 variety, and other plant species. The polynucleotide sequences of SEQ ID NO: 21-40, 44, 46, or 48 are cDNA sequences.

[0038] Polynucleotides homologous to the polynucleotides described herein are also provided. Those of skill in the art understand the degeneracy of the genetic code and that a variety of polynucleotides can encode the same polypeptide. In some embodiments, the polynucleotides (i.e., polynucleotides encoding the ADH polypeptides) may be codon-optimized for expression in a particular cell including, without limitation, a plant cell, bacterial cell, or fungal cell. While particular polynucleotide sequences which are found in plants are disclosed herein any polynucleotide sequences may be used which encode a desired form of the polypeptides described herein. Thus, non-naturally occurring sequences may be used. These may be desirable, for example, to enhance expression in heterologous expression systems of polypeptides or proteins. Computer programs for generating degenerate coding sequences are available and can be used for this purpose. Pencil, paper, the genetic code, and a human hand can also be used to generate degenerate coding sequences.

[0039] Regarding ADH polypeptides, the phrases "% sequence identity," "percent identity," or "% identity" refer to the percentage of residue matches between at least two amino acid sequences aligned using a standardized algorithm. Methods of amino acid sequence alignment are well-known. Some alignment methods take into account conservative amino acid substitutions. Such conservative substitutions, explained in more detail below, generally preserve the charge and hydrophobicity at the site of substitution, thus preserving the structure (and therefore function) of the polypeptide. Percent identity for amino acid sequences may be determined as understood in the art. (See, e.g., U.S. Pat. No. 7,396,664, which is incorporated herein by reference in its entirety). A suite of commonly used and freely available sequence comparison algorithms is provided by the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST), which is available from several sources, including the NCBI, Bethesda, Md., at its website. The BLAST software suite includes various sequence analysis programs including "blastp," that is used to align a known amino acid sequence with other amino acids sequences from a variety of databases.

[0040] Polypeptide sequence identity may be measured over the length of an entire defined polypeptide sequence, for example, as defined by a particular SEQ ID number, or may be measured over a shorter length, for example, over the length of a fragment taken from a larger, defined polypeptide sequence, for instance, a fragment of at least 15, at least 20, at least 30, at least 40, at least 50, at least 70 or at least 150 contiguous residues. Such lengths are exemplary only, and it is understood that any fragment length supported by the sequences shown herein, in the tables, figures or Sequence Listing, may be used to describe a length over which percentage identity may be measured.

[0041] Suitably, the polypeptides encoded by the polynucleotides provided herein are not sensitive to tyrosine inhibition. The polypeptide is considered to not be sensitive, i.e. to lack sensitivity to tyrosine feedback inhibition, if at least 50% of the activity in the absence of tyrosine is maintained in the presence of 1-100 μM (or any range therein) tyrosine. The polypeptide is considered to lack tyrosine feedback sensitivity if at least 40% of the activity in the absence of tyrosine is maintained in the presence of 1 mM tyrosine.

[0042] The ADH polypeptides disclosed herein may include "variant" polypeptides, "mutants," and "derivatives thereof." As used herein the term "wild-type" is a term of the art understood by skilled persons and means the typical form of a polypeptide as it occurs in nature as distinguished from variant or mutant forms. As used herein, a "variant, "mutant," or "derivative" refers to a polypeptide molecule having an amino acid sequence that differs from a reference protein or polypeptide molecule. A variant or mutant may have one or more insertions, deletions, or substitutions of an

amino acid residue relative to a reference molecule. For example, a ADH polypeptide mutant or variant may have one or more insertions, deletions, or substitution of at least one amino acid residue relative to the ADH "wild-type" polypeptides disclosed herein. The polypeptide sequences of the "wild-type" ADH polypeptides from beets and other plant species are presented in SEQ ID NOS: 1-20, 43, 45, or 47. These sequences may be used as reference sequences.

[0043] The ADH polypeptides provided herein may be full-length polypeptides or may be fragments of the full-length polypeptide. As used herein, a "fragment" is a portion of an amino acid sequence which is identical in sequence to but shorter in length than a reference sequence. A fragment may comprise up to the entire length of the reference sequence, minus at least one amino acid residue. For example, a fragment may comprise from 5 to 1000 contiguous amino acid residues of a reference polypeptide, respectively. In some embodiments, a fragment may comprise at least 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 150, 250, or 500 contiguous amino acid residues of a reference polypeptide. Fragments may be preferentially selected from certain regions of a molecule. The term "at least a fragment" encompasses the full length polypeptide. A fragment of an ADH polypeptide may comprise or consist essentially of a contiguous portion of an amino acid sequence of the full-length ADH polypeptide (See SEQ ID NOS: 1-20, 43, 45, or 47). A fragment may include an N-terminal truncation, a C-terminal truncation, or both truncations relative to the full-length ADH polypeptide.

[0044] A "deletion" in an ADH polypeptide refers to a change in the amino acid sequence resulting in the absence of one or more amino acid residues. A deletion may remove at least 1, 2, 3, 4, 5, 10, 20, 50, 100, 200, or more amino acids residues. A deletion may include an internal deletion and/or a terminal deletion (e.g., an N-terminal truncation, a C-terminal truncation or both of a reference polypeptide).

[0045] "Insertions" and "additions" in an ADH polypeptide refer to changes in an amino acid sequence resulting in the addition of one or more amino acid residues. An insertion or addition may refer to 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, or more amino acid residues. A variant of an ADH polypeptide may have N-terminal insertions, C-terminal insertions, internal insertions, or any combination of N-terminal insertions, C-terminal insertions, and internal insertions.

[0046] The amino acid sequences of the ADH polypeptide variants, mutants, derivatives, or fragments as contemplated herein may include conservative amino acid substitutions relative to a reference amino acid sequence. For example, a variant, mutant, derivative, or fragment polypeptide may include conservative amino acid substitutions relative to a reference molecule. "Conservative amino acid substitutions" are those substitutions that are a substitution of an amino acid for a different amino acid where the substitution is predicted to interfere least with the properties of the reference polypeptide. In other words, conservative amino acid substitutions substantially conserve the structure and the function of the reference polypeptide. Conservative amino acid substitutions generally maintain (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a beta sheet or alpha helical conformation, (b) the charge or hydrophobicity of the molecule at the site of the substitution, and/or (c) the bulk of the side chain.

[0047] The disclosed variant and fragment ADH polypeptides described herein may have one or more functional or biological activities exhibited by a reference polypeptide (e.g., one or more functional or biological activities exhibited by wild-type ADH polypeptides (i.e, SEQ ID NOS: 1-20, 43, 45, or 47). Suitably, the disclosed variant or fragment ADH polypeptides retain at least 20%, 40%, 60%, 80%, or 100% of the arogenate dehydrogenase activity of the reference polypeptide (i.e., SEQ ID NOS: 1-20, 43, 45, or 47). As used herein, a "functional fragment" of an ADH polypeptide is a fragment of, for example, one of the polypeptides of SEQ ID NOS: 1-20 that retains at least 20%, 40%, 60%, 80%, or 100% of the arogenate dehydrogenase activity of the full-length ADH polypeptide. Exemplary functional fragments of the ADH polypeptides disclosed herein may include, for example, fragment ADH polypeptides of the polypeptides of SEQ ID NOS: 1-20 that lack the N-terminal plastid transit peptide within these sequences. The N-terminal plastid transit peptide (identified by SEQ ID NO: 41 for BvADHα and SEQ ID NO: 42 for BvADHβ) functions to localize the ADH polypeptides of SEQ ID NOS: 1-20, 43, 45, or 47 to the plastid in plant cells. This function is not necessarily required for the ADH polypeptides arogenate dehydrogenase activity and thus may be removed from SEQ ID NOS: 1-20, 43, 45, or 47.

[0048] FIGS. **5** and **15** show sequence alignments including some of the ADH polypeptides disclosed as SEQ ID NOs: 1-20. Based on these alignments it becomes immediately apparent to a person of ordinary skill in the art that various amino acid residues may be altered (i.e. substituted, deleted, etc.) without substantially affecting the arogenate dehydrogenase activity of the polypeptide. For example, a person of ordinary skill in the art would appreciate that substitutions in a reference ADH polypeptide could be based on alternative amino acid residues that occur at the corresponding position in other ADH polypeptides from other species. SEQ ID NOS: 1-20, 43, 45, or 47 may also include ADH polypeptides that are not shown in FIGS. **5** and **15**. A person of ordinary skill in the art, however, could easily align these polypeptide sequences with the polypeptide sequences shown in FIGS. **5** and **15** to determine what additional variants could be made to the ADH polypeptides.

[0049] In another aspect of the present invention, constructs are provided. As used herein, the term "construct" refers to recombinant polynucleotides including, without limitation, DNA and RNA, which may be single-stranded or double-stranded and may represent the sense or the antisense strand. Recombinant polynucleotides are polynucleotides formed by laboratory methods that include polynucleotide sequences derived from at least two different natural sources or they may be synthetic. Constructs thus may include new modifications to endogenous genes introduced by, for example, genome editing technologies. Constructs may also include recombinant polynucleotides created using, for example, recombinant DNA methodologies.

[0050] The constructs provided herein may be prepared by methods available to those of skill in the art. Notably each of the constructs claimed are recombinant molecules and as such do not occur in nature. Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, and recombinant DNA techniques that are well known and commonly employed in the art. Standard techniques available to those skilled in the art may be used for cloning, DNA

and RNA isolation, amplification and purification. Such techniques are thoroughly explained in the literature.

[0051] The constructs provided herein may include a heterologous promoter operably linked to any one of the polynucleotides described herein. As used herein, the terms "heterologous promoter," "promoter," "promoter region," or "promoter sequence" refer generally to transcriptional regulatory regions of a gene, which may be found at the 5' or 3' side of the ADH polynucleotides described herein, or within the coding region of the ADH polynucleotides, or within introns in the ADH polynucleotides. Typically, a promoter is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. The typical 5' promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence is a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase.

[0052] In some embodiments, the disclosed ADH polynucleotides are operably connected to the heterologous promoter. As used herein, a polynucleotide is "operably connected" or "operably linked" when it is placed into a functional relationship with a second polynucleotide sequence. For instance, a promoter is operably linked to an ADH polynucleotide if the promoter is connected to the ADH polynucleotide such that it may affect transcription of the ADH polynucleotides. In various embodiments, the ADH polynucleotides may be operably linked to at least 1, at least 2, at least 3, at least 4, at least 5, or at least 10 promoters.

[0053] Heterologous promoters useful in the practice of the present invention include, but are not limited to, constitutive, inducible, temporally-regulated, developmentally regulated, chemically regulated, tissue-preferred and tissue-specific promoters. The heterologous promoter may be a plant, animal, bacterial, fungal, or synthetic promoter. Suitable promoters for expression in plants include, without limitation, the 35S promoter of the cauliflower mosaic virus, ubiquitine, tCUP cryptic constitutive promoter, the Rsyn7 promoter, pathogen-inducible promoters, the maize In2-2 promoter, the tobacco PR-1a promoter, glucocorticoid-inducible promoters, estrogen-inducible promoters, tetracycline-inducible promoters, tetracycline-repressible promoters, and promoters for monocots like actin. Other promoters include the T3, T7 and SP6 promoter sequences, which are often used for in vitro transcription of RNA. In mammalian cells, typical promoters include, without limitation, promoters for Rous sarcoma virus (RSV), human immunodeficiency virus (HIV-1), cytomegalovirus (CMV), SV40 virus, and the like as well as the translational elongation factor EF-1α promoter or ubiquitin promoter. Those of skill in the art are familiar with a wide variety of additional promoters for use in various cell types. In some embodiments, the heterologous promoter includes a plant promoter, either endogenous to the plant host or heterologous.

[0054] Vectors including any of the constructs or polynucleotides described herein are provided. The term "vector" is intended to refer to a polynucleotide capable of transporting another polynucleotide to which it has been linked. In some embodiments, the vector may be a "plasmid," which refers to a circular double-stranded DNA loop into which additional DNA segments may be ligated. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome, such as some viral vectors or transposons. Plant mini-chromosomes are also included as vectors. Vectors may carry genetic elements, such as those that confer resistance to certain drugs or chemicals.

[0055] Cells including any of the polynucleotides, constructs, or vectors described herein are provided. Suitable "cells" that may be used in accordance with the present invention include eukaryotic or prokaryotic cells. Suitable eukaryotic cells include, without limitation, plant cells, fungal cells, and animal cells. Suitable prokaryotic cells include, without limitation, gram-negative and gram-positive bacterial species. In some embodiments, the cell is a plant cell such as, without limitation, a soybean plant cell, a mung bean plant cell, an opium poppy plant cell, a *quinoa* plant cell, an alfalfa plant cell, a rice plant cell, a wheat plant cell, a corn plant cell, a sorghum plant cell, a barley plant cell, a millet plant cell, an oat plant cell, a rye plant cell, a rapeseed plant cell, a beet plant cell, and a *miscanthus* plant cell. In some embodiments, the cell is a bacterial or fungal cell.

[0056] Plants including any of the polynucleotides, constructs, vectors, or cells described herein are also provided. Suitable plants may include, without limitation, a beet plant, a soybean plant, a mung bean plant, an opium poppy plant, a *quinoa* plant, an alfalfa plant, a rice plant, a wheat plant, a corn plant, a sorghum plant, a barley plant, a millet plant, an oat plant, a rye plant, and a rapeseed plant as well as perennial grasses such as a *miscanthus* plant. For example, ADH polynucleotides encoding any one of the ADH polypeptides of SEQ ID NOS: 1-20, 43, 45, or 47 may be used to generate transgenic plants.

[0057] Portions or parts of these plants are also useful and provided. Portions and parts of plants includes, without limitation, plant cells, plant tissue, plant progeny, plant asexual propagates, plant seeds. The plant may be grown from a seed comprising transgenic cells or may be grown by any other means available to those of skill in the art. Chimeric plants comprising transgenic cells are also provided and encompassed.

[0058] As used herein, a "plant" includes any portion of the plant including, without limitation, a whole plant, a portion of a plant such as a part of a root, leaf, stem, seed, pod, flower, cell, tissue plant germplasm, asexual propagate, or any progeny thereof. Germplasm refers to genetic material from an individual or group of individuals or a clone derived from a line, cultivar, variety or culture. Plant refers to whole plants or portions thereof including, without limitation, plant cells, plant protoplasts, plant tissue culture cells or calli. For example, a beet plant refers to whole beet plant or portions thereof including, without limitation, beet plant cells, beet plant protoplasts, beet plant tissue culture cells or calli. A plant cell refers to cells harvested or derived from any portion of the plant or plant tissue culture cells or calli.

[0059] Methods for increasing production of at least one product of the tyrosine or HPP pathways in a cell are provided. The methods may include introducing any of the

polynucleotides, constructs, or vectors described herein into the cell. Suitable products of the tyrosine or HPP pathways include, without limitation, vitamin E, plastoquinone, a cyanogenic glycoside, a benzylisoquinoline alkaloid, rosmarinic acid, betalains, suberin, mescaline, morphine, salidroside, a phenylpropanoid compound, dhurrin, a tocochromanol, ubiquinone, lignin, a catecholamine such as epinephrine (adrenaline) or dopamine (i.e., L-dihydroxyphenylalanine (L-DOPA)), melanin, an isoquinoline alkaloid, hydroxycinnamic acid amide (HCAA), an amaryllidaceae alkaloid, hordenine, hydroxycinnamate, hydroxylstyrene, or tyrosine. Phenylpropanoid compounds (i.e., lignin, tannins, flavonoids, stilbene) may be produced from tyrosine, for example, by combining the polypeptides disclosed herein with a tyrosine-ammonia lyase (TAL) or by using cells that naturally have a TAL such as grass cells.

[0060] As used herein, "introducing" describes a process by which exogenous polynucleotides (e.g., DNA or RNA) are introduced into a recipient cell. Methods of introducing polynucleotides into a cell are known in the art and may include, without limitation, microinjection, transformation, and transfection methods. Transformation or transfection may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a host cell. The method for transformation or transfection is selected based on the type of host cell being transformed and may include, but is not limited to, the floral dip method, *Agrobacterium*-mediated transformation, bacteriophage or viral infection, electroporation, heat shock, lipofection, and particle bombardment. Microinjection of polynucleotides may also be used to introduce polynucleotides into cells.

[0061] In some embodiments, the present methods may further include purifying the product of the tyrosine or HPP pathways from the cells. As used herein, the term "purifying" is used to refer to the process of ensuring that the product of the tyrosine or HPP pathways is substantially or essentially free from cellular components and other impurities. Purification of products of the tyrosine or HPP pathways is typically performed using analytical chemistry techniques such as high performance liquid chromatography (HPLC) and other chromatographic techniques. Methods of purifying such products are well known to those skilled in the art. A "purified" product of the tyrosine or HPP pathways means that the product is at least 85% pure, more preferably at least 95% pure, and most preferably at least 99% pure.

[0062] The present disclosure is not limited to the specific details of construction, arrangement of components, or method steps set forth herein. The compositions and methods disclosed herein are capable of being made, practiced, used, carried out and/or formed in various ways that will be apparent to one of skill in the art in light of the disclosure that follows. The phraseology and terminology used herein is for the purpose of description only and should not be regarded as limiting to the scope of the claims. Ordinal indicators, such as first, second, and third, as used in the description and the claims to refer to various structures or method steps, are not meant to be construed to indicate any specific structures or steps, or any particular order or configuration to such structures or steps. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as") provided herein, is intended merely to facilitate the disclosure and does not imply any limitation on the scope of the disclosure unless otherwise claimed. No language in the specification, and no structures shown in the drawings, should be construed as indicating that any non-claimed element is essential to the practice of the disclosed subject matter. The use herein of the terms "including," "comprising," or "having," and variations thereof, is meant to encompass the elements listed thereafter and equivalents thereof, as well as additional elements. Embodiments recited as "including," "comprising," or "having" certain elements are also contemplated as "consisting essentially of" and "consisting of" those certain elements.

[0063] Recitation of ranges of values herein are merely intended to serve as a shorthand method of referring individually to each separate value falling within the range, unless otherwise indicated herein, and each separate value is incorporated into the specification as if it were individually recited herein. For example, if a concentration range is stated as 1% to 50%, it is intended that values such as 2% to 40%, 10% to 30%, or 1% to 3%, etc., are expressly enumerated in this specification. These are only examples of what is specifically intended, and all possible combinations of numerical values between and including the lowest value and the highest value enumerated are to be considered to be expressly stated in this disclosure. Use of the word "about" to describe a particular recited amount or range of amounts is meant to indicate that values very near to the recited amount are included in that amount, such as values that could or naturally would be accounted for due to manufacturing tolerances, instrument and human error in forming measurements, and the like. All percentages referring to amounts are by weight unless indicated otherwise.

[0064] No admission is made that any reference, including any non-patent or patent document cited in this specification, constitutes prior art. In particular, it will be understood that, unless otherwise stated, reference to any document herein does not constitute an admission that any of these documents forms part of the common general knowledge in the art in the United States or in any other country. Any discussion of the references states what their authors assert, and the applicant reserves the right to challenge the accuracy and pertinence of any of the documents cited herein. All references cited herein are fully incorporated by reference in their entirety, unless explicitly indicated otherwise. The present disclosure shall control in the event there are any disparities between any definitions and/or description found in the cited references.

[0065] Unless otherwise specified or indicated by context, the terms "a", "an", and "the" mean "one or more." For example, "a protein" or "an RNA" should be interpreted to mean "one or more proteins" or "one or more RNAs," respectively.

[0066] The following examples are meant only to be illustrative and are not meant as limitations on the scope of the invention or of the appended claims.

EXAMPLES

Example 1—Relaxation of Tyrosine Pathway Regulation Underlies the Evolution of Betalain Pigmentation in Caryophyllales

[0067] This Example is based on data reported in Lopez-Nieves et al., "Relaxation of Tyrosine Pathway Regulation

Underlies the Evolution of Betalain Pigmentation in Caryophyllales," *New Phytologist,* 217(2):896-908 (2018), the contents of which (including all supplemental data, figures, and associated materials) is incorporated herein by reference.

SUMMARY

[0068] Diverse natural products are synthesized in plants by specialized metabolic enzymes, which are often lineage-specific and derived from gene duplication followed by functional divergence. However, little is known about the contribution of primary metabolism to the evolution of specialized metabolic pathways.

[0069] Betalain pigments, uniquely found in the plant order Caryophyllales, are synthesized from the aromatic amino acid L-tyrosine (Tyr) and replaced the otherwise ubiquitous phenylalanine-derived anthocyanins. This study combined biochemical, molecular and phylogenetic analyses and uncovered coordinated evolution of Tyr and betalain biosynthetic pathways in Caryophyllales.

[0070] We found that *Beta vulgaris*, which produces high levels of betalains, synthesizes Tyr via plastidic arogenate dehydrogenases ($TyrA_a$/ADH) encoded by two ADH genes (BvADHα and BvADHβ). Unlike BvADHβ and other plant ADHs that are strongly inhibited by Tyr, BvADHα exhibited relaxed sensitivity to Tyr. Also, Tyr-insensitive BvADHα orthologs arose during the evolution of betalain pigmentation in the core Caryophyllales and later experienced relaxed selection and gene loss in lineages that reverted from betalain to anthocyanin pigmentation, such as Caryophyllaceae.

[0071] These results suggest that relaxation of Tyr pathway regulation increased Tyr production and contributed to the evolution of betalain pigmentation, highlighting the significance of upstream primary metabolic regulation for the diversification of specialized plant metabolism.

INTRODUCTION

[0072] Plants synthesize numerous specialized metabolites (also known as secondary metabolites), which play crucial roles in plant adaptation. In contrast to well-documented diversification of plant enzymes directly involved in specialized metabolism (Chen et al., 2011; Mizutani & Ohta, 2010; Moghe & Last, 2015; Pichersky & Lewinsohn, 2011; Weng, 2014), relatively little is known about the evolution of primary metabolic enzymes that provide precursors to the production of various specialized metabolites.

[0073] L-Tyrosine (Tyr) is an essential aromatic amino acid required for protein biosynthesis in all organisms; however, it is synthesized de novo only in bacteria, fungi, and plants, but not in animals. Consequently, animals have to consume Tyr or L-phenylalanine (Phe) that can be hydroxylated to Tyr (Pencharz et al., 2007). Besides protein biosynthesis, plants also use Tyr to produce a diverse array of specialized metabolites that are important for defense (e.g. dhurrin, Gleadow & Møller, 2014), stress tolerance (e.g. tocopherols, Mene-Saffrane et al., 2010), and pollinator attraction (e.g., betalains, Tanaka et al., 2008). Notably, humans have a long history of utilizing Tyr-derived specialized metabolites, such as the psychedelic alkaloid mescaline derived from the cactus *Lophophora williamsii* (Ibarra-Laclette et al., 2015) and the analgesic morphine derived from *Papaver somniferum* (opium poppy, Beaudoin & Facchini, 2014; Millgate et al., 2004).

[0074] Tyr is synthesized from prephenate, which is converted from the final product of the shikimate pathway, chorismate (Maeda & Dudareva, 2012; Siehl, 1999; Tzin, V. & Galili, 2010). In most bacteria and fungi, prephenate is oxidatively decarboxylated by prephenate dehydrogenase ($TyrA_p$/PDH, hereafter referred only as PDH; EC 1.3.1.12) to 4-hydroxyphenylpyruvate (HPP), which is transaminated to Tyr (Bentley, 1990, FIG. **1**A). On the other hand, most plants first transaminate prephenate into arogenate and subsequently decarboxylate into Tyr by arogenate dehydrogenase ($TyrA_a$/ADH, hereafter referred only as ADH; EC 1.3.1.78, Rippert & Matringe, 2002a,b), both steps occurring in the plastids (Dal Cin et al., 2011; Rippert et al., 2009; FIG. **1**A). The Tyr pathway is usually highly regulated at PDH and ADH. These homologous enzymes are strongly feedback inhibited by Tyr and control carbon flow between the two competing Tyr and Phe pathways (Gaines et al., 1982; Bentley, 1990; Rippert & Matringe, 2002a,b; FIG. **1**B). A recent report showed that, in addition to plastidic ADH enzymes, some plants possess a PDH enzyme(s) that is not inhibited by Tyr and is localized to the cytosol (Rubin & Jensen, 1979; Schenck et al., 2015; 2017; Siehl, 1999). Clearly, there is evolutionary variation in the Tyr pathway(s) in different plant lineages that warrants investigation.

[0075] Betalains are a class of Tyr-derived pigments that, within the flowering plants, occur exclusively in the order Caryophyllales where they replace the otherwise ubiquitous anthocyanins (Mabry, 1964; Tanaka et al., 2008). Within Caryophyllales, the majority of families are betalain pigmented. In two families, Molluginaceae and Caryophyllaceae, however, evolutionary reversions from betalain to anthocyanin pigmentation have occurred (Brockington et al., 2015), highlighting the fact that these two classes of water-soluble pigments have never been found in the same organism (Bate-Smith, 1962; Brockington et al., 2011; Clement & Mabry, 1996; Mabry, 1964). Betalains and anthocyanins are synthesized from Tyr and Phe, respectively, but have similar physiological functions in pollinator attraction and stress tolerance (Tanaka et al., 2008). Betalains are also used as a natural food dye (E162) and have anticancer and antidiabetic properties (Khan, 2015; Lee et al., 2014; Neelwarne & Halagur, 2012). Furthermore, intermediates in the betalain pathway are important pharmaceuticals [e.g. L-dihydroxyphenylalanine (L-DOPA) for the treatment of Parkinson's disease] or are substrates for other pharmaceutical agents (e.g. the production of dopamine and isoquinoline alkaloids such as morphine). Consequently, understanding the coordinated regulation of Tyr and betalain biosynthesis has the potential to enhance the production of Tyr, and the yield of Tyr-derived plant natural products important for human health and nutrition.

[0076] Betalain biosynthesis starts with hydroxylation of Tyr to L-DOPA by at least three closely related cytochrome P450 enzymes (CYP76AD1, CYP76AD5, and CYP76AD6, FIG. **1**A) (Polturak et al., 2016; Sunnadeniya et al., 2016). L-DOPA is further converted into betalamic acid or cyclo-DOPA by L-DOPA dioxygenases (DODA, Christinet et al., 2004; Gandía-Herrero & García-Carmona, 2012) or CYP76AD1 (Hatlestad et al., 2012), respectively (FIG. **1**A). Betalamic acid then spontaneously reacts with cyclo-DOPA

or amines to produce various forms of betacyanins or betaxanthins, respectively, which are usually further glycosylated. Recent studies found that the two key enzymes within the betalain pathway, DODA, and CYP76AD1, duplicated just prior to the emergence of betalain pigmentation (Brockington et al., 2015). Subsequently, one of the duplicated copies (DODAα and CYP76AD1α) in both genes became specialized for betalain biosynthesis and were lost or downregulated in the anthocyanin-producing families such as Molluginaceae and Caryophyllaceae (Brockington et al., 2015). Despite recent and rapid progress in understanding the betalain pathway enzymes and their evolution, little is known about the regulation of primary Tyr metabolism in relation to the evolution of this novel Tyr-dependent betalain pathway.

[0077] Here we first investigated the Tyr biosynthetic pathway and its regulation in table beet (*Beta vulgaris* L.), which produces high levels of betalains (Goldman, 1996). Using comparative genomics, biochemical, and cellular analyses, we found plastidic ADH enzymes from *B. vulgaris* that exhibit relaxed sensitivity to Tyr inhibition in vitro and in vivo. Phylogenetic analysis combined with recombinant enzyme characterization further demonstrated that de-regulated ADH enzymes emerged during the evolution of betalain pigmentations in the core Caryophyllales, and were lost or downregulated following disappearance of betalains. Furthermore, transient expression of the de-regulated ADH in *Nicotiana benthamiana* led to high accumulation of Tyr in planta. The results revealed the important contribution of primary Tyr pathway regulation to the unique evolution of a plant specialized metabolic pathway, betalain biosynthesis.

Materials and Methods

[0078] Plant Source and Growth Conditions *B. vulgaris* varieties, red beet (W357B), yellow beet (Touch Stone), and white beet (Blankoma), were provided by Dr. Irwin Goldman from the University of Wisconsin-Madison, Department of Horticulture (Goldman, 1996), whereas sugar beet (Big Buck) and sea beet (PI 562585) were commercial sugar beets obtained from the Heirloom Seeds (West Finley, Pa., USA) and the National Plant Germplasm System (NPGS), respectively. *Spinach* (*Spinacia oleraceae*), Pigeonberry (*Rivina humilis*), four o'clock (*Mirabilis jalapa*), and common purslane (*Portulaca oleracea*) were grown from seed with a growing mix soil (Fafard®, Agawam, Mass., USA) in a growth chamber under 12 hr light (100 μE), 22° C. and 60% humidity. After one month of growth, their leaves were harvested for RNA extraction.

Identification and Cloning of ADH Homologs from Caryophyllales

[0079] BLASTP searches were performed using the protein sequences of ADH and PDH enzymes from *A. thaliana* (AtADH1/At5g34930, NP_173023; AtADH2/At1g15710, NP_198343), *Glycine max* (GmPDH, KM507071), *Synechocystis* sp. PCC6803 (SyADH, WP_010872597), *Escherichia coli* (EcPDH, WP_052912694), *Aquifex aeolicus* (AaPDH, WP_010881139) as queries against the sugar beet genome (*Beta vulgaris* http://bvseq.molgen.mpg.de/) (FIG. **2**B). Potential ADH candidates were identified based on a broad phylogenetic analysis that included various plant ADH and PDH sequences.

[0080] Genomic DNA was extracted using Tris-sodium chloride-EDTA/sodium dodecyl sulfate buffer and precipitated with isopropanol and 200 mM ammonium acetate. For RNA isolation, the method described by Wang et al (2011) was used with some modifications. The tissues were ground in a mortar with liquid nitrogen and powder polyvinylpyrrolidone (PVP). After addition of 700 μL fresh pre-warmed lysis buffer (2% CTAB, 2 M NaCl, 100 mM Tris-HCl pH 8, 25 mM EDTA and 5% β-mercaptoethanol), the samples were shaken vigorously for 2 min and incubated in a water bath at 65° C. for 5 min. The RNA was converted into complementary DNA (cDNA) using the High-Capacity cDNA Reverse Transcription Kit (Applied Biotechnology, USA) and SuperScript IV Reverse Transcriptase with oligo dT_{20} primer or random primers (Invitrogen, USA).

[0081] Cloning primers were designed with the Invitrogen primer design (http://tools.lifetechnologies.com/content.cfm?pageid=9716) and the PCR In-Fusion® primers designing program (http://bioinfo.clontech.com/infusion/convertPcrPrimersInit.do, Clontech, Mount View, Calif.). All ADH candidate genes, except for PoADHα (see below), were PCR amplified from cDNA using gene-specific primers (Table 1) and Phusion DNA polymerase (Thermo, Waltham, Mass.) with the following conditions: initial denaturation at 95° C. for 5 min, 35 cycles of amplification at 95° C. for 30 s, 58° C. for 30 s, 72° C. for 30 s, with a final extension at 72° C. for 10 min. The PCR fragments were purified using QIAquick gel extraction kit (Qiagen, Valencia, Calif.) and were inserted into the pGEX-2T vector (GE Healthcare) at EcoRI and BamHI sites using the In-Fusion cloning method (Clontech). PoADHα was gene synthesized (Biomatik, Cambridge, Ontario, Canada) and directly cloned into the same pGEX-2T vector. For generation of His-tagged proteins, the cloned PCR fragments were inserted into the pET28a vector (Novagen, Madison Wis., USA) at NdeI and EcoRI site.

TABLE 1

Primers used as indicated in the description and methods

Species (gene)	Purpose	Primer name	Primer sequence 5' to 3'
Beta vulgaris (BvADHβ)	RT-PCR	pHM0290SLN BvADHβF	GGTTCCGCGTGGATCCCTAACAATTC GCAGCAT (SEQ ID NO: 49)
Beta vulgaris (BvADHβ)	RT-PCR	pHM0291SLN RBvADHβR	AATTCGGAGACAAATTGAGAATTCAT CGTGACTG (SEQ ID NO: 50)
Beta vulgaris (BvADHα)	RT-PCR	pHM0372SLN BvADHαF	CTGGTTCCGCGTGGATCCTGCGGTGG AGGTGGTTCG (SEQ ID NO: 51)

TABLE 1 -continued

Primers used as indicated in the description and methods

Species (gene)	Purpose	Primer name	Primer sequence 5' to 3'
Beta vulgaris (BvADHα)	RT-PCR	pHM0373SLN BvADHαR	GTTAATGGTACTAGATAGGAATTCAT CGTGACTGA (SEQ ID NO: 52)
Arabidopsis thaliana (AtADH2)	Cloning	pHM0384SLN AtADHαF	CTGGTTCCGCGTGGATCCGCAATCGA CGCCGCCCAA (SEQ ID NO: 53)
Arabidopsis thaliana (AtADH2)	Cloning	pHM0385SLN AtADHαR	TCATCATCATCATCTTAAGAATTCATC GTGACTGA (SEQ ID NO: 54)
Spinacea oleracea (SoADHβ)	Cloning	pHM0582SoA DHβF	CTGGTTCCGCGTGGATCCGCCGCTAC CAATACCTCC (SEQ ID NO: 55)
Spinacea oleracea (SoADHβ)	Cloning	pHM0583SoA DHβR	AATTCAGAGATCAATTGAGAATTCAT CGTGACTGA (SEQ ID NO: 56)
Spinacea oleracea (SoADHα)	Cloning	pHM0584SoA DHαF	CTGGTTCCGCGTGGATCCTGCGCCGC CTCTGACTCC (SEQ ID NO: 57)
Spinacea oleracea (SoADHα)	Cloning	pHM0585SoA DHαR	TGGTAATAATTCTAGATAGGAATTCA TCGTGACTGA (SEQ ID NO: 58)
Nepenthes alata (NaADHβ)	Cloning	pHM0603SLN NaADHF	CTGGTTCCGCGTGGATCCGCCGCGCT GCCAAACGACT (SEQ ID NO: 59)
Nepenthes alata (NaADHβ)	Cloning	pHM0604SLN NaADHR	AAATGTTGAGAGAAATTGAGAATTCA TCGTGACTGA (SEQ ID NO: 60)
Portulaca oleracea(PoADHα)	RT-PCR	pHM0609SLN PoADHαAF	CTGGTTCCGCGTGGATCCTGCTCATCA TCATCATCAT (SEQ ID NO: 61)
Portulaca oleracea(PoADHα)	RT-PCR	pHM0610SLN PoADHαAR	CGTCAACGATAGATCATAGGAATTCA TCGTGACTGA (SEQ ID NO: 62)
Mirabilis jalapa(MjADHα)	Cloning	pHM0624SLN MjADHαAF	CTGGTTCCGCGTGGATCCATAGCGAT AGTTGGGTTTG (SEQ ID NO: 63)
Mirabilis jalapa(MjADHα)	Cloning	pHM0625SLN MjADHαAR	TATCAATGGTCGTCGATAGGAATTCA TCGTGACTGA (SEQ ID NO: 64)
Rivina hurndis(RhADHα)	Cloning	pHM0647SLN RhADHαF	CTGGTTCCGCGTGGATCCTGCACGGC CTTCACTAAAAC (SEQ ID NO: 65)
Rivina humilis(RhADHα)	Cloning	pHM0648SLN RhADHαR	TCAATGGATCAAAGCGGTAGGAATTC ATCGTGACTGA (SEQ ID NO: 66)
Beta vulgaris (BvADHα)	RT-PCR	BvADHα_q_F	TCAAGCTGAGGTTACTTTTGACA (SEQ ID NO: 67)
Beta vulgaris (BvADHα)	RT-PCR	BvADHα_q_R	AAGAAGCATGATTTAGTGGTGGT (SEQ ID NO: 68)
Beta vulgaris (BvADHβ)	RT-PCR	BvADHα_q_F	TGCAGCGACTTAAACGATCG (SEQ ID NO: 69)
Beta vulgaris (BvADHβ)	RT-PCR	BvADHα_q_R	TTGGGGAAGTTTGCCGTTTG (SEQ ID NO: 70)
Beta vulgaris (BvADHα)	RT-PCR	pHM0793SLN BvADHαF	AGTTCCCTCTGCTGATATG (SEQ ID NO: 71)
Beta vulgaris (BvADHα)	RT-PCR	pHM0794SLN BvADHαR	GTGGTTAATGGTACTAGATAG (SEQ ID NO: 72)
Beta vulgaris (BvADHβ)	qPCR	pHM0791SLN BvADHβF	GCGAAGGAGATCAAATTTCT (SEQ ID NO: 73)
Beta vulgaris (BvADHβ)	qPCR	pHM0792SLN BvADHβR	TCAATTTGTCTCCGAATTTGC (SEQ ID NO: 74)
Beta vulgaris (BvADHα)	qPCR	BvADHα_F	ATGATTTCACTCTCTTCTTTTCATCC (SEQ ID NO: 75)

TABLE 1 -continued

Primers used as indicated in the description and methods

Species (gene)	Purpose	Primer name	Primer sequence 5' to 3'
Beta vulgaris (BvADHα)	qPCR	BvADHα_R	GATTTAGTGGTGGTTAATGGTACTAG ATAG (SEQ ID NO: 76)
Beta vulgaris (BvADHβ)	qPCR	BvADHβ_F	ATGCTTTCTCTCTCCTCCAC (SEQ ID NO: 77)
Beta vulgaris (BvADHβ)	qPCR	BvADHβ_R	CAAATTCGGAGACAAATTGA (SEQ ID NO: 78)
Beta vulgaris (BvActin)	qPCR	pHM0001HM BvACT	TCTATCCTTGCATCTCTCAG (SEQ ID NO: 79)
Beta vulgaris (BvActin)	qPCR	pHM0002HM BvACT	TCTCCAAGGGCGAGTATGAT (SEQ ID NO: 80)
Beta vulgaris (BvDODA)	qPCR	pHM0003HM BvDODA	CATTGGTTCAGGAAGTGCAA (SEQ ID NO: 81)
Beta vulgaris (BvDODA)	qPCR	pHM0004HM BvDODA	CCTTTGATTCATGGCTTCGT (SEQ ID NO: 82)
Beta vulgaris (BvMYB1)	qPCR	pHM057613vM YB1F	TATCAAACGAGGGCACTTC (SEQ ID NO: 83)
Beta vulgaris (BvMYB1)	qPCR	pHM0577BvM YB1R	GATGGTCTTTGATAGCAGC (SEQ ID NO: 84)
Beta vulgaris (BvCYP76AD1)	qPCR	pHM0005HM BvCYP76AD1	CTTTTCAGTGGAATTAGCCCACC (SEQ ID NO: 85)
Beta vulgaris (BvCYP76AD1)	qPCR	pHM0006HM BvCYP76AD1	TGGAACATTATGGAAGATATTGGG (SEQ ID NO: 86)
GFP	qPCR	tGFP_q_F	GGCTGGAAGAGTGATCGGAG (SEQ ID NO: 87)
GFP	qPCR	tGFP_q_R	ACGCTACTGTTGAGCATCTTCA (SEQ ID NO: 88)
Gene Racer oligoT	RT-PCR	GeneRacer OligoT	GCTGTCAACGATACGCTACGTAACGGCA TGACAGTG(T)20 (SEQ ID NO: 89)
Eukaryotic translational elongation factor 1α	qPCR	EF1α_q_F	AGCTTTACCTCCCAAGTCATC (SEQ ID NO: 90)
Eukaryotic translational elongation factor 1α	qPCR	EF1α_q_R	CCAAGATTGACAGGCGTTCT (SEQ ID NO: 91)

Recombinant Enzyme Expression and Purification

[0082] The His-tagged recombinant protein expression was carried out as we described previously (Dornfeld et al., 2014). For GST-tagged recombinant protein expression, the cloned pGEX-2T vectors were introduced into Rosetta-2 *E. coli* competent cells (Novagen, Madison Wis., USA) and cultured overnight at 37° C., 200 r.p.m. in 10 mL LB medium containing Ampicillin (100 μg/mL). The ten milliliters of the overnight culture were transferred to 1 L LB medium with Ampicillin (100 μg/mL and further incubated at 37° C. and 200 r.p.m. until the OD_{600} reached 0.3. The temperature was then changed to 18° C. and, after 1 hr, isopropyl 13-D-1-thiogalactopyranoside (IPTG, 400 mM final concentration) was added to induce recombinant protein expression. After overnight incubation at 18° C. under constant shaking at 200 r.p.m., cultures were harvested by centrifugation at 2,000 g for 10 min at 4° C., and the pellet was washed with 0.9% NaCl solution. The samples were harvested and resuspended in 25 mL of lysis buffer [phosphate-buffered saline (PBS) pH 7.4, 1 mM phenylmethylsulfonyl fluoride (PMSF), 1 mM dithiothreitol (DTT) and plant proteases inhibitor cocktail (Amresco, Solon, Ohio, USA)]. The resuspended cells were sonicated for periods of 20 s for 5 min. The cell lysate was centrifuged at 10,000 g for 30 min at 4° C., and the supernatant was applied to Fast Protein Liquid Chromatography (FPLC, AKTApure25 FPLC system, GE Healthcare) equipped with GSTtrap™FF (GE Healthcare, USA). Prior and after injection, the column was washed with five times bed volume wash buffer A (PBS, pH 7.6) followed by five times bed volume of wash buffer B (10 mM glutathione, 1.54 g of reduced glutathione dissolved in 500 mL of 50 mM Tris-HCl, pH 8). The recom-

binant enzymes containing GST-tag were eluted with ten-bed volumes of the elution buffer B and collected into Eppendorf tubes containing 500 Recombinant enzymes eluted in the fraction five and six, which were combined and desalted using a gel filtration column (Sephadex G50-80 resin, Sigma-Aldrich, St Louis, Mo., USA) in the reaction buffer [200 mM HEPES (pH 7.6), 50 mM KCl, 10% ethylene glycol]. Enzyme concentrations were measured using Bradford assay (Bio-Rad, Des Plaines, Ill., USA) and the enzyme purity was estimated by running on SDS-PAGE gel and analyzing with ImageJ (http://imagej.nih.gov/ij/).

ADH and PDH Activity Assays In Vitro

[0083] ADH and PDH activity from beet tissues (FIGS. **3**A, **3**B) were analyzed by using the leaves and stem/root crude protein extract of red beet (W357B). The beets were grown in a greenhouse for 12 weeks with a temperature of 22-25° C. and 16 hr of ambient and supplemented lights. Protein extraction was performed by grinding 1 g of tissues in liquid nitrogen and resuspending the powder in the extraction buffer [200 mM HEPES (pH 7.6), 50 mM KCl, 10% ethylene glycol, 1 mM PMSF, 1 mM DTT and plant proteases inhibitor cocktail (Ameresco)]. The extracts were desalted using the gel filtration column (Sephadex G50-80 resin, Sigma-Aldrich St. Louis, Mo., USA) into the reaction buffer. The ADH or PDH assays were performed by mixing the desalted protein extract with 1 mM $NADP^+$ and 1 mM L-arogenate or prephenate in a total volume of 10 μL or 25 μL, respectively. L-Arogenate was prepared by enzymatic conversion from prephenate (Sigma-Aldrich, St. Louis, Mo., USA), as previously described (Schenck et al., 2015). The reactions were started by adding the enzyme (crude extract or recombinant enzyme) and incubated at 37° C. for 45 min. The reaction was stopped with two times volume of methanol. The same ADH and PDH assay protocols were used for initial characterization of purified recombinant BvADH enzymes

[0084] For detection of Tyr product from the ADH assays, 10 μL of the reaction mixture was first derivatized with the equal volume of the 40.26 mM OPA solution [5.4 mg OPA (Sigma-Aldrich, St. Louis, Mo., USA) mixed in 100 μL methanol, 5 μL 2-mercaptoethanol and 900 μL 0.4M boric acid) for 3 min, injected to high pressure liquid chromatography (HPLC, Agilent 1260) equipped with the Eclipse XDH-C18 column (5 μm, 3.0×150 mm, Agilent, USA), and separated by a 30 min linear gradient from 20-45% methanol in 0.1% ammonium acetate at a flow rate of 0.8 ml/min. The substrate and product of ADH assays (Tyr and arogenate, respectively) were detected by a fluorescence detector (Agilent, USA) with excitation at 360 nm and emission of 455 nm. For PDH assays, the reactions were stopped by addition of $NaBH_4$, which converts the reaction product HPP into hydroxyphenyllactic acid (HPLA), followed by neutralization with 100 μl of 6 N HCl as described by Schenck et al. 2015. The HPLC was equipped with ZORBAX SB-C18 column (Agilent, USA) using a 6 min isocratic elution at 25% methanol in 0.1% phosphoric acid, followed by a 20 min linear gradient of 25-60% methanol at a flow rate of 1.0 mL/min. The HPLA were monitored by absorption at 270 nm.

[0085] To test the electron donor and substrate preferences of purified recombinant enzymes, the ADH and PDH reactions were performed as described above, except for 12 min with 400 μM L-arogenate and 1 mM cofactor (NAD^+ or $NADP^+$). The reaction was stopped by placing the tubes on ice and immediately measured for the production of the reduced cofactor, NAD(P)H, at 340 nm by spectrophotometer (NanoDrop 2000, Thermo Scientific, USA). The quantification was based on the standard curve of authentic NADPH.

[0086] To examine Tyr sensitivity of the purified recombinant enzymes, ADH assay was performed as described previously (Schenck et al., 2015) but in the presence or absence of different concentrations of L-Tyr. Tyr was first dissolved in 0.025 N NaOH at 100 mM (as the water solubility of Tyr is very low, <2 mM), which was diluted to 4 mM to 10 μM final concentration in 0.0025 N NaOH. The reactions contained 500 mM HEPES (pH 7.6) to maintain the final pH at 7.6. The production of reduced cofactor (NADPH) was monitored at 340 nm using a spectrophotometer every two minutes for 10 min. In addition, other effectors (L-Phe, L-Trp, and betanin) were used to test possible inhibition of the enzyme ADH activity at a final concentration of 1 mM. All of the reactions were performed under non-saturated condition, where activity increased linearly depending on reaction times and enzyme concentrations.

Transient Expression of BvADHα and BvADHβ in *Nicotiana benthamiana*

[0087] ADHα and ADHβ sequences used for *N. benthamiana* agroinfiltration were amplified from *Beta vulgaris* var. *vulgaris* variety "Boltardy" (Chiltern Seeds, UK) swollen hypocotyl and leaf tissue cDNA libraries respectively, which were prepared using BioScript Reverse Transcriptase (Bioline Reagents, London, UK). Transcripts were amplified by PCR using gene specific primers (Table S1) and Phusion High-Fidelity DNA polymerase (Thermo Fisher Scientific, Waltham, Mass., USA). Vectors for transient transformation were constructed with Golden Gate cloning using the MoClo Tool Kit (Weber et al., 2011; Addgene, Cambridge, Mass., USA), with the BpiI and BsaI restriction sites eliminated after cloning. The turboGFP sequence used in this assay was a variant codon-optimized for plants contained in the MoClo Plant Parts Kit (Engler et al., 2014; Addgene, Cambridge, Mass., USA). BvADHα, BvADHβ, and turboGFP sequences were ultimately cloned into the pICH86988 binary vector under control of the Cauliflower Mosaic Virus 35S promoter and the *Agrobacterium tumefaciens* octopine synthase (OCS) terminator.

[0088] Transient gene expression assays in *N. benthamiana* were performed according to the previously described agroinfiltration method with some modifications (Sparkes et al., 2006). All constructs were transformed into the *Agrobacterium tumefaciens* GV3101 strain, and grown in LB media supplemented with kanamycin (50 mg/L), gentamycin (25 mg/L) and rifampicin (50 mg/L) until reaching an OD_{600} of 1.5. Cultures were then brought to a final OD_{600} of 0.5 in infiltration media (10 mM $MgCl_2$, 0.1 mM acetosyringone, 10 mM MES at pH 5.6) for three hours prior to infiltration. Infiltration spots corresponding to 35S::BvADHα, 35S::BvADHβ, and 35S::turboGFP were performed in the same leaves of 6-week old *N. benthamiana* plants alternating the position of the spots between plants in a clockwise manner to account for intra-leaf variation (Barshandy et al., 2015). Infiltrated tissue was sampled three days post-infiltration from five biological replicates for tyrosine quantification and qRT-PCR analysis.

[0089] For quantification of tyrosine and other amino acids, ~40 mg fresh weight tissues were harvested, lyophilized, sent from the University of Cambridge (UK) to the University of Wisconsin-Madison (USA), and analyzed exactly as described. Tyrosine and other amino acids were extracted and measured as described previously (Wang et al., 2017). Amino acid standards (Sigma-Aldrich, St. Louis, Mo., USA) of 4 to 1000 μM were prepared the same way to make standard curves.

Phylogenetic Analysis

[0090] Amino acid sequences from genomes (full open reading frame) and transcriptomes (full or partial open reading frame) of Brockington et al. (2015) were used for phylogenetic analysis following methods described in Brockington et al. (2015) with minor modifications. In addition, we carried out analysis of dN/dS ratio in ADHα to test for relaxed selection in anthocyanic lineages (Table 2).

TABLE 2

Sequences of Caryophyllales (ingroups) and non-Caryophyllales (outgroups) used in this Example.

Taxon	Source	Accession code	Citation
Ingroups			
Achatocarpaceae_Phaulothamnus_spinescens	Smith Lab	MJM1677	(Brockington et al., 2015)
Aizoaceae_Cypselea_humifusum	1KP	GJNX	(Matasci et al., 2014)
Aizoaceae_Delosperma_echinatum	1KP	BJKT	(Matasci et al., 2014)
Aizoaceae_Sesuvium_porfulacastrum	1KP	HZTS	(Matasci et al., 2014)
Aizoaceae_Sesuvium_verrucosum	1KP	EDIT	(Matasci et al., 2014)
Aizoaceae_Trianthemum_porfulacastrum	1KP	OMYK	(Matasci et al., 2014)
Aizoaceae_Zaleya_penfandra	1KP	BERS	(Matasci et al., 2014)
Amaranthaceae_Aerva_javanica	1KP	HDSY	(Matasci et al., 2014)
Amaranthaceae_Aerva_lanata	1KP	PDQH	(Matasci et al., 2014)
Amaranthaceae_Alternanthera_brasiliana	1KP	ZBPY	(Matasci et al., 2014)
Amaranthaceae_Alternanthera_caracasana	1KP	OHKC	(Matasci et al., 2014)
Amaranthaceae_Alternanthera_sessilis	1KP	BWRK	(Matasci et al., 2014)
Amaranthaceae_Alternanthera_fenella	1KP	EYRD	(Matasci et al., 2014)
Amaranthaceae_Amaranthus_cruentus	1KP	XSSD	(Matasci et al., 2014)
Amaranthaceae_Amaranthus_retroflexus	1KP	WMLW	(Matasci et al., 2014)
Amaranthaceae_Atriplex_hortensis	1KP	ONLQ	(Matasci et al., 2014)
Amaranthaceae_Atriplex_prostrata	1KP	AAXJ	(Matasci et al., 2014)
Amaranthaceae_Atriplex_rosea	1KP	CBJR	(Matasci et al., 2014)
Amaranthaceae_Bassia_scoparia	1KP	WGET	(Matasci et al., 2014)
AmaranthEceae_Beta_maritima	1KP	FVXD	(Matasci et al., 2014)
Amaranthaceae_Beta_vulgaris	Genome	v1.1	(Dohm et al., 2014)
Amaranthaceae_Blutaparon_vermiculare	1KP	CUTE	(Matasci et al., 2014)
Amaranthaceae_Chenopodium_amaranticolor	SRA	SRX151423	(Zhang et al., 2012)
Amaranthaceae_Chenopodium_quinoa	1KP	SMMC	(Matasci et al., 2014)
Amaranthaceae_Froelichia_floridana	Smith Lab	MJM1665	(Brockington et al., 2015)
Amaranthaceae_Salicornia_europaea	SRA	SRX302090	(Fan et al., 2013)
Basellaceae_Basella_alba	1KP	CTYH	(Matasci et al., 2014)
Cactaceae_Lophophora_williamsii	1KP	CPKP	(Matasci et al., 2014)
Cactaceae_Pereskia_aculeata	1KP	JLOV	(Matasci et al., 2014)
Caryophyllaceae_Cerastium_arvense	Smith Lab	MJM1767	(Brockington et al., 2015)
Caryophyllaceae_Dianthus_caryophyllus	Genome	v1.0	(Yagi et al., 2014)
Caryophyllaceae_Drymaria_cordata	Smith Lab	LCMsn	(Brockington et al., 2015)
Caryophyllaceae_Polycarpaea_repens	1KP	RXEN	(Matasci et al., 2014)
Caryophyllaceae_Saponaria_officinalis	1KP	SKNL	(Matasci et al., 2014)
Caryophyllaceae_Schiedea_membranacea	1KP	OLES	(Matasci et al., 2014)
Caryophyllaceae_Silene_latifolia	1KP	FZQN	(Matasci et al., 2014)
Caryophyllaceae_Silene_latifoliaSRA	SRA	SRX118777-SRX118782	(Muyle et al., 2012)
Caryophyllaceae_Silene_vulgaris	SRA	SRX096120	N/A[1]
Caryophyllaceae_Spergulana_media	1KP	TJES	(Matasci et al., 2014)
Droseraceae_Aldrovanda_vesiculosa	Smith Lab	MJM1652	(Brockington et al., 2015)
Droseraceae_Dionaea_muscipula	SRA	SRX312294	(Jensen et al., 2015)
Frankeniaceae_Frankenia_laevis	1KP	WPYJ	(Matasci et al., 2014)
Microteaceae_Microtea_debilis	1KP	YNFJ	(Matasci et al., 2014)
Molluginaceae_Mollugo_cerviana	1KP	RNBN	(Matasci et al., 2014)
Molluginaceae_Mollugo_nudicaulis	1KP	SCAO	(Matasci et al., 2014)
Molluginaceae_Mollugo_verticillata	1KP	NXTS	(Matasci et al., 2014)
Nepenthaceae_Nepenthes_alata	1KP	WQUF	(Matasci et al., 2014)
Nyctaginaceae_Abronia_carletonii	Smith Lab	MJM1751	(Brockington et al., 2015)
Nyctaginaceae_Acleisanthes_lanceolata	Smith Lab	MJM1741	(Brockington et al., 2015)
Nyctaginaceae_Acleisanthes_obtusa	Smith Lab	MJM1697	(Brockington et al., 2015)
Nyctaginaceae_Anulocaulis_leiosolenus	Smith Lab	SRX717838	(Yang et al., 2015)
Nyctaginaceae_Boerhavia_burbidgeana	1KP	VJPU	(Matasci et al., 2014)
Nyctaginaceae_Boerhavia_coccinea	1KP	ZBTA	(Matasci et al., 2014)
Nyctaginaceae_Bougainvillea_spectabilis	1KP	JAFJ	(Matasci et al., 2014)
Nyctaginaceae_Bougainvillea_stipitata	Smith Lab	SRX718672	(Yang et al., 2015)
Nyctaginaceae_Cyphomeris_gypsophiloides	Smith Lab	MJM1714	(Brockington et al., 2015)
Nyctaginaceae_Guapira_obtusata	Smith Lab	SRX718384	(Yang et al., 2015)
Nyctaginaceae_Mirabilis_jalapa	1KP	JGAB	(Matasci et al., 2014)

TABLE 2-continued

Sequences of Caryophyllales (ingroups) and non-Caryophyllales (outgroups) used in this Example.

Taxon	Source	Accession code	Citation
Nyctaginaceae_Mirabilis_multiflora	Smith Lab	MJM1771	(Brockington et al., 2015)
Nyctaginaceae_Pisonia_aculeata	Smith Lab	SRX718389	(Yang et al., 2015)
Nyctaginaceae_Pisonia_umbellifera	Smith Lab	SFB29	(Brockington et al., 2015)
Physenaceae_Physena_madagascariensis	1KP	RUUB	(Matasci et al., 2014)
Phytolaccaceae_Ercilla_volubilis	Smith Lab	MJM1649	(Brockington et al., 2015)
Phytolaccaceae_Hilleria_latifolia	1KP	SFKQ	(Matasci et al., 2014)
Phytolaccaceae_Petiveria_alliacea	1KP	AZBL	(Matasci et al., 2014)
Phytolaccaceae_Phytolacca_americana	1KP	BKQU	(Matasci et al., 2014)
Phytolaccaceae_Phytolacca_bogotensis	1KP	MRKX	(Matasci et al., 2014)
Phytolaccaceae_Phytolacca_diuica	Smith Lab	SFB31	(Brockington et al., 2015)
Phytolaccaceae_Rivina_humilis	Smith Lab	SRX718277	(Yang et al., 2015)
Phytolaccaceae_Seguieria_aculeata	Smith Lab	SRX718486	(Yang et al., 2015)
Plumbaginaceae_Limonium_spectabile	1KP	WOBD	(Matasci et al., 2014)
Polygonaceae_Antigonon_leptopus	Smith Lab	MJM1811	(Brockington et al., 2015)
Polygonaceae_Fagopyrum_esculentum	SRA	SRX112838	N/A[1]
Polygonaceae_Polygonum_convolvulus	1KP	FYSJ	(Matasci et al., 2014)
Polygonaceae_Polygonum_cuspidatum	SRA	SRX079484	(Hao et al., 2012)
Polygonaceae_Rheum_nobile	SRA	SRX621187	N/A[1]
Polygonaceae_Rheum_rhabarbarum	SRA	SRX286365	N/A[1]
Polygonaceae_Rumes_acetosa	SRA	ERX190940	N/A[1]
Polygonaceae_Rumex_palustris	SRA	ERX190941, ERX190942	N/A[1]
Portulacaceae_Portulaca_amilis	1KP	LDEL	(Matasci et al., 2014)
Portulacaceae_Portulaca_cryptopetala	1KP	LLQV	(Matasci et al., 2014)
Portulacaceae_Portulaca_grandiflora	1KP	CPLT	(Matasci et al., 2014)
Portulacaceae_Portulaca_molokiniensis	1KP	UQCB	(Matasci et al., 2014)
Portulacaceae_Portulaca_oleracea	1KP	EZGR	(Matasci et al., 2014)
Portulacaceae_Portulaca_pilosa	1KP	IWLS	(Matasci et al., 2014)
Portulacaceae_Portulaca_suffruticosa	1KP	GCYL	(Matasci et al., 2014)
Sarcobataceae_Sarcobatus_vermiculatus	1KP	GIWN	(Matasci et al., 2014)
Summondsiaceae_Simmondsia_chinensis	1KP	CVDF	(Matasci et al., 2014)
Talinaceae_Talinum_sp	1KP	LKKX	(Matasci et al., 2014)
Tamaricaceae_Reaumuria_trigyna	SRA	SRX099851, SRX105466	N/A[1]
Tamaricaceae_Tamarix_hispida	SRA	All 8 runs in PRJNA170420	(Wang et al., 2014)
Outgroups			
Arabidopsis_thaliana	Genome	Accessed May 28, 2014	(Goodstein et al., 2012)
Oryza_sativa	Genome	Accessed Apr. 21, 2015	(Goodstein et al., 2012)
Solanum_lycopersicum	Genome	Accessed May 28, 2014	(Goodstein et al., 2012)
Vitis_vinifera	Genome	Accessed Apr. 21, 2015	(Goodstein et al., 2012)

[1]N/A, not available.

Subcellular Localization of GFP-Fused ADH Enzymes

[0091] The subcellular localization experiments of GFP-fused ADH enzymes were conducted as we described previously (Schenck et al., 2015).

Accession Numbers

[0092] The Genbank accession numbers for the sequences mentioned in this article are: BvADHβ W357B red beet variety (KY207366), BvADHβ Boltardy red beet variety (MF346292), BvADHβ Big Buck sugar beet variety (KY207367), BvADHβ Touch Stone yellow beet variety (KY207368), BvADHβ Blankoma white beet variety (KY207369), BvADHβ Sea beet P1562585 variety (KY207370), BvADHα Big Buck sugar beet variety (KY207371), BvADHα W357B red beet variety (KY207372), BvADHα Boltardy red beet variety (MF346291), BvADHα Blankoma white beet variety (KY207373), BvADHα Touch Stone yellow beet variety (KY207374), BvADHα Sea beet P1562585 variety (KY207375), SoADHβ (KY207376), SoADHα (KY207378), NaADHβ (KY207377), MjADHα (KU881770), RhADHα (KY207379), PoADHα (KY207380), SmADHα (KY274179), PpADHα (KY274180), and H1ADHα (KY274181).

Results

[0093] *B. vulgaris* has two ADH enzymes.

[0094] To first investigate how *B. vulgaris* synthesizes Tyr, protein crude extracts of red beet leaf and root/stem tissues were analyzed for ADH and PDH activity, the production of Tyr or HPP from arogenate or prephenate, respectively. Tyr was produced from arogenate in the red beet extracts of both leaves and roots/stems (FIG. **3**A) similar to soybean leaf extract, which was previously shown to have both ADH and PDH activity (Schenck et al., 2015). On the other hand, unlike the soybean leaf extract, HPP production was not

detected in the leaf and root/stem extracts of red beet (FIG. **3**B). These results showed that red beet has ADH but not PDH activity.

[0095] To identify the gene(s) responsible for the ADH activity in *B. vulgaris*, previously reported plant and microbial ADH and PDH genes (Bonvin et al., 2006; Hudson et al., 1984; Legrand et al., 2006; Rippert & Matringe, 2002a, b; Schenck et al., 2015, FIG. **2**B) were used to BLAST against the genome of sugar beet, another cultivar of *B. vulgaris* (Dohm et al., 2014) (assembly v.1.2 http://bvseq.molgen.mpg.de). Two *B. vulgaris* sequences homologous to these ADH and PDH genes were found on chromosome 8 of the *B. vulgaris* genome 25.3 kbp apart (FIG. **2**A). They were more similar to plant ADHs and PDHs (59 to 61% similarity at amino acid levels) than bacterial ones (24 to 40% similarity, FIG. **2**B). Within plants, the two ADH candidate genes from *B. vulgaris* both belong to the canonical ADH clade containing *Arabidopsis* ADHs (Rippert & Matringe, 2002a, b), rather than the non-canonical clade containing legume PDHs (Schenck et al., 2015; 2017), and appear to be derived from a recent duplication within the order Caryophyllales.

[0096] For biochemical characterization, these two putative BvADHs were expressed in *E. coli* as recombinant enzymes, which were further purified using affinity chromatography and subjected to ADH and PDH assays. Both of the beet recombinant enzymes showed ADH activity (i.e. the production of Tyr from arogenate, FIG. **1**B) and strongly preferred $NADP^+$ over NAD^+ (FIG. **4**) similar to other plant ADH enzymes and activities (Gaines et al., 1986; Rippert & Matringe, 2002a,b). On the other hand, neither of the beet enzymes exhibited detectable PDH activity (FIG. **3**C), which is consistent with the lack of PDH activity in beet tissues (FIG. **3**B) and also confirmed the absence of *E. coli* PDH contamination (Hudson et al., 1984). Therefore, these two genes were designated as *B. vulgaris* arogenate dehydrogenases (BvADHα and BvADHβ).

Both BvADHs are Plastid Localized but Only BvADHα Expression is Correlated with Betalain Pathway Genes.

[0097] Most plant enzymes involved in the aromatic amino acid pathways are localized within the plastids (Dal Cin et al., 2011; Maeda & Dudareva, 2012; Rippert et al., 2009), and both BvADH proteins also have a predicted N-terminal plastid transit peptide (FIGS. **5**A-**5**D). To experimentally determine the subcellular localization of BvADHs, a green fluorescent protein (GFP) was fused to the C-terminal of BvADHs, expressed in *Arabidopsis* protoplasts, and analyzed for their localization using confocal microscopy. The fluorescence signal of GFP fused with BvADHα or BvADHβ overlapped with chlorophyll autofluorescence, which was different from the free GFP control and similar to GFP fused with plastidic *Arabidopsis* ADH (Rippert et al., 2009) (AtADH2, FIG. **1**C). These results suggest that both BvADHs are targeted to the plastids and that Tyr is mainly produced by the plastidic arogenate pathway in *B. vulgaris*.

[0098] To examine expression patterns of BvADHs, especially in comparison to the betalain pathway genes, expression levels of BvADHα and BvADHβ were analyzed and compared with those of DODAα, CYP76AD1α, and BvMYB1 in cotyledon and hypocotyl tissues of sugar and red beets (FIG. **1**D). Consistent with previous studies (Hatlestad et al., 2012; 2015), DODAα and CYP76AD1α, as well as BvMYB1 transcription factor, were much more highly expressed in red than sugar beet. Interestingly, BvADHα expression showed similar trends and was significantly higher in red than sugar beet in both cotyledon and hypocotyl tissues. On the other hand, BvADHβ expression levels were very similar between genotypes in both tissue types (FIG. **1**D). These results showed that expression of BvADHα, but not BvADHβ, is correlated with those of betalain pathway genes in *B. vulgaris*.

BvADHα but not BvADHβ Exhibits Relaxed Sensitivity to Tyr

[0099] Both ADH and PDH enzymes are usually inhibited by Tyr in most organisms (Bentley, 1990; Connelly & Conn, 1986; Gaines et al., 1982; Rippert & Matringe, 2002a,b; Sun, 2009). To determine if the BvADHs are also feedback regulated by Tyr, ADH activity of the recombinant BvADH enzymes were analyzed in the presence and absence of Tyr as an effector molecule. The ADH activity of glutathione S-transferase (GST)-tagged BvADHβ was inhibited by 80% and 100% in the presence of 100 μM and 1 mM Tyr, respectively (FIG. **6**), similar to the Tyr-sensitive *Arabidopsis* AtADH2 (Rippert & Matringe, 2002a,b). In contrast, ADH activity of BvADHα was reduced only by half at 1 mM Tyr (FIG. **6**). Similar results were obtained for histidine (His)-tagged ADH enzymes, where BvADHα showed much less sensitivity to Tyr than AtADH2 (FIG. **7**), though the expression of His-tagged BvADHβ was not successful. Other aromatic amino acids (Phe and tryptophan) as well as betanin, the major betacyanin accumulated in red beet, did not significantly reduce the ADH activity of BvADHα, BvADHβ, or AtADH2 at 1 mM (FIG. **8**). These results revealed that BvADHα, but not BvADHβ, has relaxed sensitivity to Tyr inhibition.

Heterologous Expression of BvADHα but not BvADHβ Increase Tyr Accumulation in Plants.

[0100] To test if BvADHα having relaxed sensitivity to Tyr can enhance the production of Tyr in planta, BvADHα and BvADHβ were transiently expressed in *N. benthamiana* through Agrobacteria infiltration (FIG. **9**A, Sparkes et al., 2006) and their impacts on Tyr production were analyzed. A control vector expressing GFP was also infiltrated as a negative control (FIG. **9**A). BvADHα expression resulted in >10-fold increase in Tyr levels relative to the GFP control, while the increase of Tyr due to BvADHβ expression was not significantly different (FIGS. **10**A & **9**B, Table 3). Interestingly, phenylalanine (Phe) levels were decreased significantly under BvADHα, but not BvADHβ expression (FIG. **10**B). Other amino acid levels were largely unaffected by BvADHα or BvADHβ expression (Table 3). These results demonstrate that BvADHα expression leads to elevated accumulation of Tyr in planta.

TABLE 3

Amino Acid levels of *Nicotiana benthamiana* leaves expressing GFP, BvADHα, BvADHβ. Agrobacteria carrying the 35S::GFP, 35S::BvADHα, or 35S::BvADHβ construct were infiltrated to *Nicotiana benthamiana* leaves and the levels of amino acids were analyzed after three days post-infiltration. Data are mean ± s.e.m. (nmol/gFW, n = 5 biological replications). Asterisks denote values significantly different from the control 35S::GFP sample (Student t-test, $p < 0.01$). Tryptophan, lysine, cysteine, and histidine levels were below quantification threshold.

Amino Acids	35S::GFP	35S::BvADHα	35S::BvADHβ
alanine	99.8 ± 15.5	93.0 ± 14.8	88.1 ± 20.0
glycine	15.5 ± 1	17.5 ± 2.1	13.6 ± 0.2

TABLE 3-continued

Amino Acid levels of *Nicotiana benthamiana* leaves expressing GFP, BvADHα, BvADHβ. Agrobacteria carrying the 35S::GFP, 35S::BvADHα, or 35S::BvADHβ construct were infiltrated to *Nicotiana benthamiana* leaves and the levels of amino acids were analyzed after three days post-infiltration. Data are mean ± s.e.m. (nmol/gFW, n = 5 biological replications). Asterisks denote values significantly different from the control 35S::GFP sample (Student t-test, $p < 0.01$). Tryptophan, lysine, cysteine, and histidine levels were below quantification threshold.

Amino Acids	35S::GFP	35S::BvADHα	35S::BvADHβ
valine	23.9 ± 9.7	23.8 ± 8.3	22.1 ± 8.4
leucine	21.3 ± 10.4	21.8 ± 9.2	18.8 ± 8.3
isoleucine	13.8 ± 7	13.3 ± 5.7	13.3 ± 6.7
proline	154.8 ± 67.4	126.7 ± 56.3	137.3 ± 75.4
methionine	2.8 ± 0.4	3.1 ± 0.4	2.6 ± 0.2
serine	57.4 ± 8	58.6 ± 11.7	43.9 ± 3.9
threonine	69.4 ± 7.5	67.8 ± 8.6	58.1 ± 6.5
phenylalanine	10.8 ± 0.7	5.9 ± 1.2*	9.7 ± 0.7
aspartic acid	173.5 ± 45.5	176.8 ± 40.6	132.7 ± 41.5
glutamic acid	941.6 ± 45.8	968.1 ± 91.6	746.4 ± 111.4
ornithine[a]	54.9 ± 1.6	56.2 ± 2.4	48.4 ± 2.9
asparagine	6.8 ± 1.2	6.9 ± 1.5	4.9 ± 1.0
glutamine	345.2 ± 116.1	348.7 ± 138.4	291.3 ± 107.7
tyrosine	11.2 ± 2.8	116.8 ± 15.1*	17.2 ± 3.2

[a]Arginine was quantified as its non-enzymatic degradation product ornithine.

BvADHα Orthologs Emerged During the Evolution of Betalain Pigmentation in Caryophyllales.

[0101] Domestication has modified metabolic traits in various crops (Hanson et al., 1996; Rapp et al., 2010; Rong et al., 2014). Thus, we hypothesized that the BvADHα enzyme with relaxed Tyr regulation was selected during domestication and intensification of color in table beets, that have been used at least since the Roman times (Biancardi et al., 2012; Dohm et al., 2014). To test this hypothesis, the nucleotide and protein sequences of BvADHα (and BvADHβ) were compared among different domesticated beets, red beet (W357B), sugar beet (Big Buck), yellow beet (Touch Stone), and white beet (Blankoma), as well as their wild relative, sea beet (Biancardi et al., 2012) (*Beta vulgaris* subsp. *maritima*). Several single nucleotide polymorphisms (SNPs) were detected among different lines in both BvADHα and BvADHβ (FIGS. **5**A, **5**B). However, only a few of them affected the amino acid sequences and were within and near the N-terminal signal peptide of BvADHα and BvADHβ, respectively (FIGS. **5**C, **5**D). Thus, the mature enzyme regions of BvADHα were unaltered during domestication.

[0102] To further test if the ADHα enzymes with reduced Tyr sensitivity are restricted to the species *B. vulgaris*, the corresponding genes for BvADHα and BvADHβ were cloned from a closely related species within the same Amaranthaceae family, *spinach* (*Spinacia oleracea*), whose draft genome is available (http://bvseq.molgen.mpg.de). *Spinach* ADHα and ADHβ orthologs (SoADHα and SoADHβ) had 77 and 83% identity at amino acid levels to the corresponding BvADHs in the mature enzymatic regions. The recombinant enzymes of *spinach* ADHs showed similar Tyr sensitivity to beet ADHs: SoADHα, but not SoADHβ, exhibited reduced Tyr sensitivity (FIG. **6**). These results suggest that the reduced Tyr sensitivity of BvADHα at least at the enzyme level was not the result of selection during domestication of beet cultivars, but was already present in the common ancestor of the beet and *spinach* ADHα enzymes.

[0103] To determine the origin and molecular evolution of BvADHα, we mined genome and transcriptomic data across the Caryophyllales for ADH orthologs and performed a phylogenetic analysis (FIG. **11**A). The results indicate that a gene duplication event on the branch leading to stem Caryophyllales produced ADHα and ADHβ lineages. While ADHβ orthologs were expressed across the entire Caryophyllales, expression of ADHα closely parallels betalain production in Caryophyllales. ADHα expression is undetectable from the anthocyanic clade that diverged prior to the earliest inferred origin of betalain synthesis (hereafter referred to as non-core Caryophyllales; Brockington et al., 2009). Two families in the Caryophyllales, Molluginaceae and Caryophyllaceae have reverted from betalain to anthocyanin pigmentation (Brockington et al., 2011, 2015). Presence of the ADHα orthologs in the transcriptomes of Molluginaceae and Caryophyllaceae was much less common than the presence of BvADHβ (FIGS. **11**A, **11**B). Thus the presence of ADHα, but not ADHβ, closely mirrors the distribution of betalain pigmentation across Caryophyllales, similar to the pattern in two other genes of the betalain pathway, CYP76AD1α and DODAα (Brockington et al., 2015).

Betalain-Producing Species have Deregulated BvADHα Enzyme and Elevated Tyr Levels.

[0104] To further test experimentally if ADHα orthologs across Caryophyllales share the unique property of reduced Tyr inhibition, ADH genes from representative members of Caryophyllales (Brockington et al., 2011) were cloned and the Tyr sensitivity of encoded enzymes was evaluated. An ADHβ enzyme from the anthocyanin-producing non-core Caryophyllales, *Nepenthes ventricosa×alata* (NaADHβ, Nepenthaceae, FIG. **11**B), was strongly inhibited by Tyr (FIG. **12**) similar to beet and *spinach* ADHβ (FIG. **6**). On the other hand, ADHα orthologs from betalain-producing families, *Rivina humilis* (RhADHα, Rivinaceae), *Mirabilis jalapa* (MjADHα, Nyctaginaceae), and *Portulaca oleracea* (PoADHα, Portulacaceae), all shared relaxed Tyr inhibition and retained 42% to 68% of ADH activity even at 1 mM Tyr (FIG. **12**).

[0105] To test if Tyr-insensitivity of the recombinant ADHα enzyme is also detectable in vivo, Tyr sensitivity of leaf ADH activity was analyzed from species containing ADHα (i.e. *spinach*) and ones lacking ADHα [i.e. *Arabidopsis thaliana; Dianthus barbatus*, Caryophyllaceae]. *Spinach* rather than beet was used due to its cleaner background during HPLC-based enzyme assay. As shown in Table 4 and FIG. **13**, ADH activity of *Arabidopsis* and *Dianthus barbatus* tissues was strongly inhibited (92-95%) by 0.5 mM of Tyr effector, whereas that of *spinach* was much more resistant to Tyr inhibition (only ~21% inhibited), consistent with the presence of SoADHα with relaxed sensitivity to Tyr (FIG. **6**).

TABLE 4

Tyr sensitivity of ADH activity from plant tissue extracts. Total protein extracts of spinach, *Dianthus barbatus*, and *Arabidopsis* leaf tissues were used to analyze ADH activity in the presence and absence of 0.5 mM Tyr analog (3-fluoro-Tyr), which were used to calculate percent inhibition. ADH activity was measured with 1 mM arogenate substrate and 1 mM $NADP^+$ cofactor during 2 hr incubation (see FIG. 13). Data are means ± s.e.m. (n = 4).

	ADH activity (nmol/mg protein)		
species	0 mM 3-fluoro-Tyr	0.5 mM 3-fluoro-Tyr	inhibition (%)
Spinach oleracea	66.4 ± 5.0	52.7 ± 1.9	20.7%
Dianthus barbatus	18.1 ± 0.3	0.9 ± 0.2	95.0%
Arabidopsis thaliana	93.5 ± 5.2	7.8 ± 0.5	91.6%

[0106] To further test if the presence of deregulated ADHα leads to increased Tyr accumulation in betalain-producing species, Tyr levels were quantified in young leaves of a variety of Caryophyllales species with or without ADHα and also in *Arabidopsis thaliana* as a comparison. Anthocyanin-producing species from non-core Caryophyllales (e.g. *Nepenthes ventricosa×alata*) and Caryophyllaceae (e.g. *Dianthus barbatus*) had Tyr levels (2.1 to 8.8 nmol/gFW) comparable to that of *Arabidopsis* (5.3 nmol/gFW). On the other hand, while large variations were observed, betalain-producing ADHα-containing species all had significantly higher Tyr levels (from 12 to 180 nmol/gFW) than *Arabidopsis* (FIG. **11**C). These results demonstrate that betalain-producing species have ADHα with relaxed sensitivity to Tyr inhibition and accumulate elevated levels of Tyr.

ADHα Orthologs Underwent Relaxed Selection and Gene Loss in Lineages that have Reverted from Betalain to Anthocyanin Pigmentation

[0107] Interestingly, when ADHα orthologs were recovered from Caryophyllaceae or Molluginaceae transcriptomic data, they were often recovered in partial sequences, indicating general low abundance. Within the Caryophyllaceae, ADHα orthologs was only detected in the subfamily Paronychioideae (Greenberg & Donoghue, 2011), which forms a grade paraphyletic to the rest of the family. To test for relaxed selection in anthocyanic lineages we further examined a subset of ADHα orthologs with sequences either verified by Sanger sequencing or by transcriptome read mapping and manual inspection of read coverage. Although no obvious acceleration of substitution was observed in Caryophyllaceae from nucleotide coding sequences (CDS, FIG. **14**A), there was apparent acceleration in their amino acid sequences (FIG. **14**B). Furthermore, the dN/dS ratio in Caryophyllaceae ADHα (0.166) was elevated compared to the rate among betalain-producing ADHα (0.0743) under the Partitioned MG94×REV Model, assuming homogenous synonymous and nonsynonymous rates across sites. In addition, we found evidence of relaxed selection (as opposed to intensification of positive selection) that contributes to the increase in nonsynonymous rate in Caryophyllaceae under the RELAX framework (p=5.6E-8, Table 5) (Wertheim et al., 2014). Moreover, the genome assembly of the anthocyanic carnation (*Dianthus caryophyllus*, Caryophyllaceae subfamily Caryophylloideae that nested within subfamily Paronychioideae, Greenberg & Donoghue, 2011; Yagi et al., 2014) lacked ADHα ortholog and only contained ADHβ ortholog, suggesting complete gene loss of ADHα in the subfamily Caryophylloideae (Greenberg & Donoghue, 2011). Species within the anthocyanic Caryophyllaceae, therefore, exhibit the transition from relaxed selection to gene loss of ADHα orthologs, which associates with the loss of betalain pigmentation in Caryophyllaceae.

TABLE 5

RELAX analysis support the acceleration in amino acid substitution in Caryophyllales is due to relaxed purifying selection, instead of intensified positive selection

Model	log L	# par.	AICc	Ltree	Branch set	ω1 (purifying selection)	ω2 (nearly neutral)	ω3 (positive selection)
Partitioned MG94xREV	−5484.8	38	11046.5	2.23	Reference	0.0743 (100%)		
					Test	0.166 (100%)		
Null	−5374.3	41	10831.7	11.9	Reference	0.00 (83%)	0.550 (15%)	30.9 (1.4%)
					Test	0.00 (83%)	0.550 (15%)	30.9 (1.4%)
Alternative	−5359.6	42	10804.2	84.5	Reference	0.00598 (91%)	0.650 (7.9%)	540 (1.5%)
					Test	0.0646 (91%)	0.794 (7.9%)	29.0 (1.5%)

K = 0.54. Test for selection relaxation (K < 1) was significant (p = 5.6e−8, LR = 29.48)

Discussion

[0108] This study found that *B. vulgaris* has ADH but no PDH enzymes or activity (FIG. **1**B, FIGS. **3**, **4**). This is similar to most plants (Connelly & Conn, 1986; Gaines et al., 1982; Rippert & Matringe, 2002a,b) but different from legumes that have both ADH and PDH (Rubin & Jensen, 1979; Schenck et al., 2015; 2017; Siehl, 1999). Thus, *B. vulgaris* synthesizes Tyr via the ADH pathway that occurs within the plastids (Rippert et al., 2009) (FIG. **1**C). We also found that *B. vulgaris* possesses two paralogous genes encoding the ADH enzymes, namely ADHα and ADHβ. Interestingly, ADHα but not ADHβ exhibited relaxed sensitivity to Tyr inhibition (FIG. **6**). Although recent studies reported that the legume PDH enzymes are also Tyr insensitive (Schenck et al., 2015; 2017), BvADHα and legume PDHs have two major differences. First, legume PDHs are localized in the cytosol (Schenck et al., 2015), whereas BvADHα (and BvADHβ) was targeted to the plastids (FIG. **1**C). Second, legume PDHs completely lost Tyr sensitivity (Schenck et al., 2015) but BvADHα was still inhibited by Tyr at higher concentrations (FIG. **6**, FIG. **7**). The maintenance of inhibition at higher concentration is likely necessary because Phe biosynthesis is also localized within the plastids, and thus BvADHα is directly competing for the

arogenate substrate with Phe biosynthesis (FIG. **1**A). Complete loss of ADH regulation by Tyr would, therefore, deplete Phe and essential Phe-derived compounds (e.g., proteins, lignin).

[0109] Other insensitive ADH/PDH enzymes have been previously found in microorganisms (Legrand et al., 2006) and the structural analyses of Tyr sensitive and insensitive enzymes identified histidine 217 as a possible residue responsible for its Tyr sensitivity (Legrand et al., 2006; Sun et al., 2009). Also, phylogeny-guided structure-function analysis revealed that converting a single active site aspartate 222 residue into a non-acidic residue played a key role in the evolution of the legume PDH enzymes and simultaneously introduced prephenate substrate specificity and Tyr insensitivity (Schenck et al., 2017). However, the corresponding histidine and aspartate residues are still present in BvADHα (FIG. **15**), suggesting that different mechanisms, and as yet unidentified residues are involved in the relaxed Tyr sensitivity of BvADHα.

[0110] Previous analyses of molecular evolution of DODAα and CYP76AD1α, two enzymes which convert Tyr into betalains (Christinet et al., 2004; Gandía-Herrero & García-Carmona, 2012; Hatlestad et al., 2012), revealed that both of these genes arose through gene duplication, just prior to the origin of betalain pigmentation in Caryophyllales (Brockington et al., 2015). Similarly, this study found that ADHα orthologs arose by gene duplication, prior to the emergence of DODAα and CYP76AD1α (FIGS. **11**A and **11**B), intimately associated with the origin of betalain pigmentation. One of the duplicated copies, ADHα, underwent neofunctionalization and became much less sensitive to Tyr inhibition, which is the key regulatory mechanism of Tyr biosynthesis (Maeda & Dudareva, 2012; Rippert & Matringe, 2002a,b). ADHα enzymes with relaxed Tyr sensitivity are maintained in all betalain-producing species of Caryophyllales, at least the ones that we analyzed (FIGS. **6** and **12**). Furthermore, the expression pattern of BvADHα is distinct from that of BvADHβ and similar to those of the betalain biosynthetic genes (DODAα and CYP76AD1α) and MYB1 transcription factor (FIG. **1**D), suggesting that the alteration of ADHα enzyme property was accompanied by changes in its expression profile. Although similar examples of biochemical and transcriptional changes during the evolution of plant specialized metabolic enzymes/genes have been reported (Kajikawa et al., 2017; Moghe & Last, 2015; Panchy et al., 2016; Weng et al., 2012; Xu et al., 2017), here we revealed a unique example of coordinated evolution of primary amino acid pathway (i.e. Tyr biosynthesis) and its downstream specialized metabolism (i.e. betalain biosynthesis).

[0111] In the anthocyanic Caryophyllaceae, the transition of betalain pigmentation to anthocyanin pigmentation was associated with down-regulation, relaxed natural selection, and deletion of ADHα (FIGS. **11**, and **14**, Table 5). Similar down-regulation and deletion of genes were also observed during the loss of flower petals (Zhang et al., 2013) and arbuscular mycorrhizal symbiosis (Delaux et al., 2014) in various plant lineages. Together these lines of evidence suggest that maintenance of the ADHα is superfluous, following loss of betalain pigmentation. The ultimate cause of reversion of betalain to anthocyanin pigmentation in multiple lineages within the core Caryophyllales is currently unknown. It may be due to a number of factors, including: i) metabolic cost of nitrogen-containing alkaloid betalain pigments, ii) shift in pollinator populations that are attracted by unique spectra (e.g. blue) of some anthocyanins, iii) increased demand for other Phe-derived compounds (e.g. tannins, flavonoids), or iv) simple genetic drift enabled by the presence of still intact Phe, phenylpropanoid, core flavonoid pathways in betalain-producing plants (Brockington et al, 2011; Shimada et al., 2005; Xu et al., 2016).

[0112] A mechanism underlying the mutually exclusive distribution of betalain and anthocyanin pigments has long fascinated evolutionary biologists (Brockington et al, 2011; Des Marais, 2015). Our analyses now provide one possible explanation. The relaxation of the Tyr-mediated feedback inhibition may direct more carbon flow towards Tyr, and away from Phe biosynthesis (FIG. **1**A), as demonstrated by increased Tyr and decreased Phe levels upon transient expression of ADHα (FIG. **10**). This may create a surplus of Tyr at the expense of Phe-derived products such as anthocyanins. Furthermore, betalain-producing, ADHα-containing core Caryophyllales species accumulated more Tyr than plants not possessing ADHα (FIG. **11**C). The involvement of other factors such as transcriptional regulation of betalain, anthocyanin, and Tyr/Phe pathway genes remain to be examined (Hatlestad et al., 2015; Ambawat et al, 2013), however our data provide a fascinating insight into the contribution of Tyr biosynthesis regulation to the evolution of a novel betalain pigment biosynthesis.

[0113] Prior heterologous reconstructions of specialized metabolic pathways resulted in significant accumulations of Tyr-derived plant natural products, such as a cyanogenic glycoside, dhurrin, in *Arabidopsis* (~4% per dry weight, Tattersall et al., 2001; Kristensen et al., 2005) and betalains in tobacco (330 mg kg^{-1} approaching red beet extract of 760 mg kg^{-1}, Polturak et al., 2016). In other cases, however, DODA and CYP76AD1 expression in *Arabidopsis* still required feeding of Tyr for betalain production (Harris et al., 2012; Sunnadeniya et al., 2016). Therefore, "pulling" a precursor (e.g. Tyr) may not be always enough to efficiently produce its downstream product, and "pushing" the precursor supply may be also important. Indeed, in red beets, increased Tyr levels have a strong positive correlation with enhanced accumulation of betalains (Wang et al., 2017), suggesting that elevated production of Tyr plays important role in overall production of betalains. Over 100-fold increase in Tyr accumulation observed in *N. benthamiana* leaves expressing ADHα (FIG. **10**) further demonstrates an exciting opportunity to introduce Caryophyllales ADHα enzymes into other plants and microbes, deregulate Tyr biosynthesis, and boost the availability of Tyr and the production of Tyr-derived products (e.g., vitamin E, isoquinoline alkaloids including morphine).

Additional Materials and Methods

[0114] ADH Activity from Plant Tissue Extracts

[0115] *Spinach oleracea* seeds (HighMowing, Wolcott, Vt.) and pink *Dianthus barbatus* (BloomIQ, Lansing, Mich.) seedlings were purchased from a nursery and were grown together with *Arabidopsis thaliana* (ecotype Columbia) in 22° C., 60% humidity, and 12/12 h light cycle growth chamber. Leaves of *spinach* and *Arabidopsis* seedlings were harvested at 3-week-old, and *Dianthus barbatus* leaves were harvested at 6-week-old. The crude extracts of *Arabidopsis* or *Dianthus barbatus* were prepared from ~1 g leaf tissues according to Aryal et al. (2014). For *spinach,* ~10 g leaf tissues were used to isolate the plastids according to Aryal

et al. (2014) in order to avoid the undesired cytosolic polyphenol oxidase activity. Crude or plastid fractions were desalted by Sephadex G50 column to obtain protein extracts, and protein concentration of all biological replicates were adjusted to 0.06, 0.85, and 0.6 mg/mL for *spinach, Dianthus barbatus*, and *Arabidopsis* extracts, respectively. Time course ADH activity assays at 0, 1, 2, and 3 hr were performed in the presence and absence of 500 μM Tyr analog, 3-fluoro-Tyr, in 10 μL reaction containing 50 mM sodium phosphate (pH 8.0), 1 mM arogenate, 1 mM $NADP^+$, 10 μg/mL tetracycline (to inhibit prokaryotic-type protein synthesis of plastids or bacterial contamination), and 0.3, 4.25, and 3 μg of *spinach, Dianthus*, and *Arabidopsis* protein, respectively. The reaction was stopped by adding 20 μL methanol containing 10 μM norvaline as an internal standard. Respective boiled protein extracts were used as negative controls. ADH activity was quantified by the formation of tyrosine according to (Schenck et al., 2015), except that tyrosine was detected as o-phthalaldehyde derivative with excitation/emission wavelength of 360/455 nm by fluorescence detector, and o-phthalaldehyde derivative of the norvaline internal standard was quantified at 336 nm by DAD detector.

Analysis of Tyr Contents from Caryophyllales Tissues

[0116] Metabolite extracts of thirteen Caryophyllales species were prepared from ~70 mg of youngest leaves, except for flowers of a Cactaceae species to avoid succulent tissues. All plants were grown and harvested at Botany Greenhouse of the University of Wisconsin-Madison. Young leaf tissues of ~4 weeks-old *Arabidopsis* Columbia ecotype were used as a control. Harvested tissues were extracted by adding 400 μL extraction buffer containing methanol:chloroform (2:1, v/v) and 100 μM 4-chlorobenzoic acid (an internal standard). After adding 300 μL water and 125 μL chloroform, the mixture was vigorously mixed by a vortex mixer for 5 min and centrifuged at 20,000 g for 5 min for phase separation. The upper polar phase of 400 μL was transferred to a new centrifuge tube and dried down in a benchtop speed vacuum (Labconco, Kansas City, Mo., USA). The dried polar phase was resuspended in 200 μL methanol. After centrifugation at 20,000 g for 5 min, 20 μL was injected into the Agilent 1260 HPLC equipped with Atlantis T3 C-18 column (3 μm, 2.1×150 mm, Waters, Milford, Mass.), and separated by the following gradient of acetonitrile (B) in 0.1% formic acid (A): 1% B for the first 5 min, followed by a linear increase to 76% B at 10 min, an isocratic elution at 76% B until 16 min, followed by re-equilibration at 1% B. Tyr was monitored with the fluorescence detector at 274 and 303 nm for excitation and emission, respectively. The internal standard was monitored by photodiode array detector at 270 nm. Statistical analyses were conducted by the Statistica Analysis Software (SAS) based on the "mixed" effect model (Pinheiro, 2000) to compare between the two groups having and not-having ADHα and using the "fixed" effect model (Milliken, 2009) to compare individual samples against *Arabidopsis* control.

Reverse Transcription PCR (RT-PCR) Analysis

[0117] RT-PCR was carried out on five biological replicates for each infiltrated vector (FIG. **9**B). Two technical replicates were additionally analyzed for one sample each for BvADHα and BvADHβ infiltrations. RNA was extracted and DNAse treated using the RNeasy Plant Mini Kit and the RNAse-free DNAse set (Qiagen, Hilden, Germany). cDNA was prepared using BioScript Reverse Transcriptase (Bioline Reagents, London, UK) and an oligo$(dT)_{18}$ primer according to the manufacturer's recommendations. A control with no reverse transcription was included to test the presence of genomic DNA. RT-PCR was performed on a 1:10 cDNA dilution with the KAPA 2G Fast DNA Polymerase kit (KAPA Biosystems, Wilmington, Mass., USA) and an Eppendorf Mastercycler Nexus (Eppendorf, Hamburg, Germany). Amplification conditions were as follow: initial step of 1 min at 95° C. followed by 30 cycles of 10 s at 95° C., 10 s at 60° C. and 2 s at 72° C., and a final step of 5 min at 72° C. Amplicons were visualised on 2% agarose gel electrophoresis using ethidium bromide (0.1 μg/ml) and run at 120V for 20 min. The expected size for the reactions is 140, 90 and 111 bp for BvADHα, BvADHβ, and tGFP, respectively. Primers used are described in Table 1.

Quantitative Real-Time PCR (qRT-PCR) Analysis

[0118] For quantification of endogenous expression of BvACTIN (internal control), BvADHα, BvADHβ, BvDODA, BvMYB1 and BvCYP76AD1, red beet (W357B) and sugar beet (Big Buck) plants were grown in 22° C., 60% humidity, and 12/12 hr light cycle in a growth chamber. The seedlings were harvested at 7-days after germination and the tissue was divided into cotyledon and hypocotyl. RNA was extracted (Oñate-Sánchez and Vicente-Carbajosa, 2008) and DNAse treated (Ambion, Austin Tex., USA) following by cDNA preparation using MLV Reverse Transcriptase (Promega, Madison, Wis., USA). qRT-PCR was performed using the GoTaq qPCR Master Mix (Promega, Madison, Wis., USA), and the Stratagene Mx3000P qPCR System (Agilent Technologies, Stratagene, La Jolla, Calif., USA). Amplification conditions were as follow: an initial step of 1 min at 95° C. followed by 45 cycles of 15 s at 95° C., 30 s at 60° C. and 30 s at 72° C. The gene expression of BvADH was normalized using BvACTIN as an internal control and analyzed by using the relative expression of the genes. The results are shown in % expression relative to the highest sample (FIG. **1**D). Primers used in all qPCR analysis are listed in Table 1.

Phylogenetic Analysis

[0119] Amino acids from genomes (full open reading frame) and transcriptomes (full or partial open reading frame) of Brockington et al. (2015) were used in this analysis with minor modifications in species included (Table 2). The final taxon sampling in this study consisted of 95 species, with 91 ingroup species (89 transcriptomes and 2 genomes) representing 26 of the 39 families in Caryophyllales (Hernández-Ledesma et al., 2015) and four outgroup genomes from eudicots and monocots (Table 2). Amino acid sequences of the 11 functionally characterized ADH genes were used as baits to search against each of the 95 species. To maximize the sensitivity of homology searches in order to identify short and incomplete sequences from de novo assembled transcriptomes, we used SWIPE v2.0.11 (Rognes, 2011) with a high E-value cutoff of 10 and low minimal bitscore cutoff of 30. Hits from all 11 query sequences against each species were ranked from high to low by bitscore, and the top 10 hits from each species were pooled and used for the initial phylogenetic analysis.

[0120] The pooled top hits from each of the 95 species, together with the 11 baits were used as the starting sequence file (948 sequences). An initial phylogenetic analysis was conducted using MAFFT v7.215 with "--genafpair--maxit-

erate 1000" (Katoh & Standley, 2013). Columns with more than 90% missing data in the resulting alignment were trimmed using Phyutility v2.2.6 with "-clean 0.1" (Smith & Dunn, 2008) and a phylogeny was estimated using RAxML v8.1.5 with the model "PROTCATWAG" (Stamatakis, 2014). After visually examining the alignment and tree, tips with branch lengths that were outliers were removed (any terminal branches that had on average more than two substitutions for each amino acid site; or more than ten times longer than its sister group and on average had more than one substitution per site; Yang and Smith, 2014). Monophyletic or paraphyletic tips that belonged to the same species from transcriptome data most often resulted from isoforms produced during de novo assembly. These were masked, leaving only the tip with the highest number of aligned characters (Yang and Smith, 2014). Internal branches with molecular branch lengths longer than 1 were likely due to distantly related paralogs or assembly artifacts and were pruned. A large number of distantly related genes, isoforms, and assembly errors were removed during the tip trimming and long branch removing process, with 251 sequences left. A new fasta file was written from remaining tips, and this alignment, tree building, and tree trimming procedure was repeated once, with 229 sequences left. Following the homology search and filtering, we extracted the Caryophyllales ADH gene lineage rooted by outgroup genomes (Yang and Smith, 2014). While visually examining alignment and tree we found the sequence Cham@c36044_g1_i2_242_1480_minus that belonged to *Chenopodium giganteum*, but were placed in between ADHα and ADHβ, outside of Chenopodiaceae. Further examination of the alignment showed that the half of the sequence was closely related to ADHα, and the other half closely related to ADHβ. Although this can be real, it is most likely an assembly error and was removed from the analysis. Indeed, *Chenopodium giganteum* had additional, correctly assembled ADHα and ADHβ copies nested in respective Chenopodiaceae clades. Therefore this putative chimeric sequence was removed.

[0121] Remaining sequences belonged to the Caryophyllales ADH lineage were aligned with MAFFT with "--genafpair --maxiterate 1000" and trimmed by Phyutility with "-clean 0.3". An alternative alignment was constructed with PRANK v140603 using default settings (Löytynoja & Goldman, 2008; 2010), poorly aligned sequences were manually removed, and trimmed by Phyutility with "-clean 0.1". We used two alternative alignment methods because MAFFT tends to force regions to align even when they are highly divergent whereas PRANK tends to introduce lots of gaps in highly divergent regions. On the other hand, PRANK is an iterative alignment, tree building, and refinement pipeline that we run five iterations before obtaining the final alignment. For both trimmed alignments, a phylogenetic tree was constructed using RAxML with "-m PROTCATAUTO" and 200 rapid bootstrap replicates to evaluate support. Given that the resulting tree topologies and support values using both alignments were very similar we are presenting the results from MAFFT. The code used in the phylogenetic analysis is available from https://bitbucket.org/yangya/adh_2016.

Testing for Relaxed Section in Caryophyllaceae

[0122] To test for shift in selection pressure in ADHα associated with loss of betalain, we carried out selection analysis on a reduced data set that included representative sequences across ADHα that were either verified by Sanger sequencing or by mapping reads back to the de novo assembled contigs and carefully examining read coverages visually.

[0123] Within the family Caryophyllaceae, ADHα expression was detected in the transcriptome of only the subfamily Paronychioideae. Those ADHα transcripts from *Corrigiola litoralis* and *Telephium imperati* were both confirmed by PCR and Sanger sequencing. Two *Spergularia media* fragments from transcriptome assembly were both belonged to ADHα and are non-overlapping in the alignment. These two fragments could be from two loci or from a single locus. To distinguish between these two scenarios, we first extended the two fragments separately using Assembly by Reduced Complexity (Hunter et al., 2015, ARC v.1.1.3) with maximum 10 cycles, Bowtie 2 v2.2.8 (Langmead & Salzberg, 2012) for read mapping and Newbler v2.9 (454 Life Sciences, downloaded Mar. 17, 2015) for assembly. After extending the original assembly and aligning it with other ADHα sequences, the two extended fragments were still 22 base pairs apart. To evaluate whether these two fragments were supported by raw reads we concatenated the two fragments by fixing the direction and adding 22 Ns to the middle, and mapped raw reads to the concatenated reference using Bowtie 2 with the setting "--phred64 --very-fast-local". The 22 bp gap was highly supported by read pairs and the joined read were kept for subsequent dN/dS analysis. We carried out the same procedure for *Polycarpaea repens* but were unable to join the reads nor confirm they are paralogs due to low read coverage and a longer gap between the two fragments. Therefore, the two fragments were kept in the alignments for phylogenetic analysis but were removed for dN/dS analysis.

[0124] To obtain ADHα sequences from additional species of Caryophyllaceae, primers were designed to the conserved portion of the *Spergularia media* contig, and were used to amplify ADHα sequences from the closely related *Spergularia marina*. Inverse PCR was used to obtain ADHα sequences from *Spergularia marina*, Paronychia polygonifolia and *Herniaria latifolia*. For inverse PCR, genomic DNA was digested with restriction enzymes EcoRI and MfeI, and fragments were circularised with T4 ligase (Biolabs, New England). Nested primers were used to amplify the fragment containing the ADHα ortholog. Amplified products were sanger sequenced to acquire the 5' and 3' terminals of the locus. In summary, a total of six well-supported ADHα sequences were then taken forward for the dN/dS selection analyses.

[0125] Our final alignment for selection analysis included eight ADHα sequences in Caryophyllaceae and six additional sequences from representative betalain-producing species across rest of the ADHα lineage. We first trimmed the alignment to remove signal peptide and poorly aligned ends, leaving the region from BvADHα amino acid no. 79 to 354 that covered the enzyme active domain. We then carried out phylogenetic analyses for both alignments in RAxML, with the model "GTRCAT" for the codon alignment and "PROTCATAUTO" for the amino acids alignment, and 200 rapid bootstrap replicates to evaluate node support (FIG. **14**A, **14**B). To quantify the rate shift, we carried out RELAX analysis (Wertheim et al., 2014) as implemented in the online portal Datamonkey (Kosakovsky Pond & Frost, 2005, accessed Mar. 19, 2016), using the trimmed CDS matrix with *Polycarpaea repens* removed.

RELAX has the advantage of distinguishing between increased positive selection vs. reduced purifying selection, both of which would result in accelerated average dN/dS values. We designated all crown branches in Caryophyllaceae as the testing branches and the rest branches as the background. We fitted the partitioned MG94×REV model that assumes all sites having unified dN and dS value, allowing the rate to vary between the test and background branches. We also fitted the RELAX model that takes site heterogeneity into account. The RELAX null model assumes all background and test branches share the same rate in each rate category, whereas the RELAX alternative model allows substitution rate to vary between the test and background branches in each rate category, and sites can move among rate categories.

Example 2: Overexpression of BvADHα but not BvADHβ Leads to High Accumulation of Tyrosine in *Arabidopsis thaliana*

[0126] *Beta vulgaris* accumulates high amounts of endogenous tyrosine as well as its derived metabolites betalains due to the presence of the tyrosine-insensitive BvADHα enzyme. To further test if the lack of BvADHα feedback regulation is a critical factor for high tyrosine accumulation in plant tissues, BvADHα, BvADHβ, and *Arabidopsis* ADH2 (AtADH2) were individually overexpressed by the 35S promoter of the cauliflower mosaic virus (CaMV) in *A. thaliana* Col-0 background. The empty vector containing no gene was also introduced as a negative control. Gas chromatography-mass spectrometry (GC-MS) based metabolite analysis showed that overexpression of BvADHα but not BvADHβ or AtADH2 leads to much higher accumulation of tyrosine than the empty vector control (nearly 50-fold increase, FIG. **16**). In addition, BvADHα expression resulted in a slightly reduction of an aromatic amino acid phenylalanine and drastic increase in homogentisate, the downstream product of tyrosine and precursor of tocopherols (vitamin E). No differences were observed for most amino acids, including alanine. These results provide proof-of-concept demonstration that the production of tyrosine can be substantially enhanced by the expression of a tyrosine-insensitive ADH enzyme (i.e. BvADHα) in plant tissues. In addition, the observed increase of homogentisate as a consequence of high levels of tyrosine suggests that Tyr availability is a limit-step for the production of Tyr-derived secondary metabolites in plants such as tocopherols or betalains.

Material and Methods

[0127] Cloning of BvADHα, BvADHβ and AtADH2 cDNAs into Overexpression Binary Vector

[0128] Total RNA isolated from *Beta vulgaris* and *Arabidopsis thaliana* leaf tissues were used to synthesize cDNA using random primers and the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Specific oligonucleotides to amplify each of the desired cDNAs were designed using In-Fusion® Primer design tool (Clontech). PCR fragments were obtained using Phusion High-Fidelity DNA polymerase and cloned into the binary vector DF_264 vector, downstream of the 35S CaMV promoter, using the In-Fusion® HD cloning kit. Plasmid was linearized with the restriction enzymes XbaI and BamHI (FastDigest, Thermo Scientific) and the enzymes sites were preserved after cloning. XbaI site is upstream of ATG start codon and BamHI is downstream of TAA stop codon. All reactions were performed accordingly with the instructions of the manufacter. In-Fusion cloning reactions were transformed into *E. coli* Stellar™ Competent cells (Clontech) and positive colonies were selected on LB agar plates containing 50 μg/mL Spectinomycin. Antibiotic resistance colonies were confirmed for the presence of the cDNA insert by colony PCR and submitted to plasmid isolation. cDNA inserts were checked for possible point mutations by SANGER sequencing the obtained plasmids using primers annealing at the 35S CaMV promoter and NOS terminator. Confirmed vectors were transformed into *Agrobacterium tumefaciens* GV3101 by freeze-thaw method.

Arabidopsis Transformation and Transgenic Selection.

[0129] Flowering *A. thaliana* Col-0, 5-6 weeks old, were used to plant transformation by floral dip (Bent A (2006) *Arabidopsis thaliana* floral dip transformation method. *Methods Mol Biol.* 343: 87-103). Briefly, flower buds were submerged into *Agrobacterium* GV3101 solution. The excess of solution was removed using absorbent paper. Plants were transfer to a close container to preserve humidity and kept in a dark environment for 16 hours after transformation. After this period of time, plants were acclimated back to the growth chamber. The transformation process was repeated after 5 days of the first transformation and plants were kept in the growth chamber until harvesting. T_0 seeds were chlorine sterilized and germinated on ½ Force Murashige and Skoog (MS) agar plates supplemented with 1% Sucrose and 100 μg/mL of Gentamycin. 10 positive T_1 seedlings for each construct were transferred to soil and seeds were harvested for each individual plant. Transgenic lines were then checked for the number of insertions based on the segregation ratio of antibiotic resistant T_2 seedlings. Single-insertion homozygous T_2 lines were then germinated on soil and 4-weeks old plants were analyzed for Tyr and other organic acids contents by gas chromatography-mass spectrometry analysis (GC-MS).

GC-MS Analysis

[0130] Four-week old *Arabidopsis* plants overexpressing BvADHα, BvADHβ, AtADH2 or empty vector were submitted to GC-MS analysis. Briefly, approximately 30 mg of fresh leaf tissue was excised from at least 3 plants of each transgenic line to compound one biological replicate. Tissue sample was transferred to a 1.5 mL microcentrifuge tube and 400 μL of solvent extraction solution [Methanol:Chloroform (2:1) with 100 μM norvaline]. Three 3 mm glass beads were added to each tube and samples were submitted to GenoGrindr (1500 strokes/min) for 5 min. After a brief spin 300 μL of water, followed by 125 μL of Chloroform were added to each sample. Samples were vortex on high for 30 seconds and centrifuged at 21000×g for 5 minutes to achieve phase separation. The aqueous phase was carefully transferred to a new 1.5 mL tube and transfer to speedvac system at room temperature until completely dry. After dry, the polar phase compounds were resuspended in 210 μL of methanol containing 100 μM 4-chlorobenzoic acid. Samples were sonicated for 10 min and insoluble remaining debris was removed by centrifugation at 21000×g for 5 min. at room temperature. 100 μL of supernatant was transferred into a glass vial and the methanol was dry out in the speed

vac. After dry, the inserts were transferred to a glass vial and the pellets were ressuspended in 40 μL pyridine. Samples were submitted to sonication for 10 min and 40 μL of N-methyl-N-(tert-butyldimethylsilyl) trifluoroacetamide with 1% tertbutyldimetheylchlorosilane (MTBSTFA+1% t-BDMCS) was added to each sample. Samples were incubated at 80° C. for 1 hour and transferred to analysis on GC-MS. The GC-MS was stablished as Hold at 70° C. for 2 min, increased to 250° C. by 5° C. per min., then hold at 300° C. for 10 min. Amino acid standard (Sigma, #AAS18) was used to stablish the standard curve of each amino acid. Peak areas were normalized by the internal standard norvaline and by fresh tissue weight (g).

Example 3—In Planta Expression of Tyr-Insensitive BvADHα Leads to Enhanced Accumulation of Tyr in *Arabidopsis*

[0131] BvADHα was heterologously expressed in *Arabidopsis*, which only has Tyr-inhibited ADH enzymes (Rippert and Matringe, 2002a; Rippert and Matringe, 2002b; Schenck et al., 2015). Overexpression of BvADHα, but not Tyr-inhibited BvADHβ or AtADH2, resulted in elevated Tyr accumulation by up to 60-fold compared to empty vector controls in T_3 single insertion homozygous lines (FIG. **17**). Also, the BvADHα lines reduced levels of Phe. Thus, expression of de-regulated BvADHα can increase the carbon flow through the shikimate pathway and direct away from Phe biosynthesis to drastically enhance availability of Tyr.

Example 4—Heterologous Expression of Tyr-Insensitive BvADHα Leads to Hyper-Accumulation of Tyr in *Glycine max* (Soybean)

[0132] BvADHα or BvADHβ was also heterologously expressed in *Glycine max* (soybean), which has both Tyr-inhibited ADH and Tyr-insensitive PDH enzymes (Schenck et al., 2015). When Tyr levels were analyzed in the leaves of antibiotic resisitant T_1 transgenic lines, nine out of twelve BvADHα overexpression lines showed nearly 1,000 fold increase in Tyr relative to empty vector control (FIG. **18**). All of BvADHβ transgenic lines showed basal levels of Tyr similar to empty vector controls. Three BvADHα lines with low Tyr were likely unsuccessful transformants.

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SEQUENCE LISTING

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305                 310                 315                 320

Glu Gln Leu Asp Arg Met Asp Trp Ala Phe Glu Met Val Lys Lys Gln
                325                 330                 335

Leu Ser Gly Tyr Leu His Asp Leu Val Arg Lys Gln Leu Met Leu Glu
            340                 345                 350

Gly Asn Asn Asp Gln Ala Glu Val Thr Phe Asp Lys Pro Leu Met Leu
        355                 360                 365

Pro Ser Pro Thr Ile Asn Pro Pro Gln Ile Val Pro Ser Ala Asp Met
    370                 375                 380

Ala Glu Lys Lys His Asp Leu Val Val Val Asn Gly Thr Arg
385                 390                 395


<210> SEQ ID NO 5
<211> LENGTH: 407
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
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<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(407)
<223> OTHER INFORMATION: BvADH-alpha Sea beet PI562585 variety

<400> SEQUENCE: 5

Met Ile Ser Leu Ser Ser Phe His Pro Ser Ser Thr Thr Ala Thr Ala
1 5 10 15

Thr Ala Ala Thr Ala Thr Ala Thr Ala Ala Thr Ala Thr Ala Thr Thr
20 25 30

His Pro Pro Gln Gln Cys Pro Ala Phe Ser Ser Pro Pro Ser His Leu
35 40 45

Ser Leu Pro Leu Arg His Pro Arg Gln His Leu Val Val Arg Cys Gly
50 55 60

Gly Gly Gly Ser Ala Ser Glu Ser Val Phe Asn Arg Asp Ser Ala Ala
65 70 75 80

Thr Arg Val Ser Asn Asp His Leu Asp Val Ser Lys Arg Asp Val Lys
85 90 95

Leu Lys Ile Ala Ile Ile Gly Phe Gly Asn Phe Gly Gln Phe Leu Ala
100 105 110

Lys Thr Met Ala Lys Gln Gly His Arg Val Leu Ala Tyr Ser Arg Ser
115 120 125

Asp Tyr Ser Arg Ala Ala Lys Glu Ile Gly Val Glu Tyr Phe Thr Asp
130 135 140

Ala Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Leu Leu Cys Thr
145 150 155 160

Ser Ile Leu Ser Thr Glu Lys Val Leu Arg Ser Leu Pro Leu His Arg
165 170 175

Leu Arg Arg Ser Thr Leu Phe Ala Asp Val Leu Ser Val Lys Glu Phe
180 185 190

Pro Arg Ser Leu Phe Leu Gln Leu Leu Pro Lys Asp Phe Asp Ile Leu
195 200 205

Cys Thr His Pro Met Phe Gly Pro Asp Ser Gly Lys Asp Gly Trp Gly
210 215 220

Gly Leu Pro Phe Val Phe Asp Lys Val Arg Val Gly Ser Asp Gln Ser
225 230 235 240

Arg Thr Ser Arg Ala Glu Ala Phe Leu Asp Val Phe Arg Asn Ala Gly
245 250 255

Cys Arg Met Val Glu Met Ser Cys Val Asp His Asp Lys His Ala Ala
260 265 270

Gly Ser Gln Phe Ile Thr His Met Met Gly Arg Val Leu Glu Lys Leu
275 280 285

Ala Leu Glu Asn Thr Pro Ile Asn Thr Lys Gly Tyr Glu Ser Leu Leu
290 295 300

Asn Leu Val Asp Asn Thr Ala Arg Asp Ser Phe Glu Leu Phe Tyr Gly
305 310 315 320

Leu Phe Leu Tyr Asn Lys Asn Ala Met Glu Gln Leu Asp Arg Met Asp
325 330 335

Trp Ala Phe Glu Met Val Lys Lys Gln Leu Ser Gly Tyr Leu His Asp
340 345 350

Leu Val Arg Lys Gln Leu Met Leu Glu Gly Asn Asn Asp Gln Ala Glu
355 360 365

Val Thr Phe Asp Lys Pro Leu Met Leu Pro Ser Pro Thr Ile Asn Pro

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    370                 375                 380

Pro Gln Ile Val Pro Ser Ala Asp Met Ala Glu Lys Lys His Asp Leu
385                 390                 395                 400

Val Val Val Asn Gly Thr Arg
                405


<210> SEQ ID NO 6
<211> LENGTH: 346
<212> TYPE: PRT
<213> ORGANISM: Spinacea oleracea
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(346)
<223> OTHER INFORMATION: SoADH-alpha

<400> SEQUENCE: 6

Cys Ala Ala Ser Asp Ser Val Phe Asn His Asp Ile Gly Val Pro Phe
1               5                   10                  15

Val Ser Thr Arg Ala Ser Gly Glu Val Pro Glu Val Asn Ser Arg Asp
            20                  25                  30

Ile Lys Leu Lys Ile Ala Ile Ile Gly Phe Gly Asn Phe Gly Gln Phe
        35                  40                  45

Leu Ala Lys Thr Ile Thr Lys Gln Gly His Arg Val Leu Ala Tyr Ser
    50                  55                  60

Arg Ser Asp Tyr Ser Arg Ala Ala Lys Glu Ile Gly Val Glu Tyr Phe
65                  70                  75                  80

Ser Asp Ala Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Leu Leu
                85                  90                  95

Cys Thr Ser Ile Leu Ser Thr Glu Lys Val Leu Arg Ser Leu Pro Leu
            100                 105                 110

His Arg Leu Arg Arg Ser Thr Leu Phe Val Asp Val Leu Ser Val Lys
       115                 120                 125

Glu Phe Pro Arg Ser Leu Phe Leu Gln Val Leu Pro Lys Asp Phe Asp
    130                 135                 140

Ile Leu Cys Thr His Pro Met Phe Gly Pro Asp Ser Gly Lys Ser Gly
145                 150                 155                 160

Trp Gly Gly Leu Pro Phe Val Phe Asp Lys Val Arg Val Gly Ser Asp
                165                 170                 175

Pro Thr Arg Ala Ala Arg Thr Glu Ala Phe Leu Asp Ile Tyr Arg Asn
            180                 185                 190

Ala Gly Cys Arg Met Val Glu Met Thr Cys Ala Asp His Asp Lys His
        195                 200                 205

Ala Ala Gly Ser Gln Phe Ile Thr His Met Met Gly Arg Val Leu Glu
    210                 215                 220

Lys Leu Ala Leu Glu Asn Thr Pro Ile Asn Thr Lys Gly Tyr Glu Ser
225                 230                 235                 240

Leu Leu Asn Leu Val Asp Asn Thr Ala Arg Asp Ser Phe Glu Leu Phe
                245                 250                 255

Tyr Gly Leu Phe Leu Tyr Asn Lys Asn Ala Met Glu Gln Leu Asp Arg
            260                 265                 270

Met Asp Trp Ala Phe Glu Met Val Lys Lys Gln Leu Ser Gly Tyr Leu
        275                 280                 285

His Asp Leu Val Arg Lys Gln Leu Met Leu Glu Thr Thr Asn Glu Gln
    290                 295                 300
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Val Gly Phe Asp Gln Thr Phe Met Leu Pro Ser Pro Ala Asp Asn Pro
305                 310                 315                 320

Arg Gln Thr Pro Pro Ser Ala Ala Val Ser Glu Asn Ser Lys Pro Asp
                325                 330                 335

Phe Val Val Val Asn Gly Asn Asn Ser Arg
            340                 345


<210> SEQ ID NO 7
<211> LENGTH: 396
<212> TYPE: PRT
<213> ORGANISM: Spergularia marina
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(396)
<223> OTHER INFORMATION: Spergularia marina ADH-alpha

<400> SEQUENCE: 7

Met Met Asn Ser Ile Ser Phe Val Asn Ser Ser Ser Thr Thr Thr Ala
1               5                   10                  15

Asp Ile Ile Tyr Leu Asn His Gln Phe Ser Arg His Lys Cys Phe Ser
            20                  25                  30

Arg Leu Pro Arg Asp Ala Thr Pro Arg Asp Arg Arg Lys Ile Ser Leu
        35                  40                  45

Ala Arg Ala Ile Asn Gly Ser Pro Thr Cys Ser His Val Glu Ile Asp
    50                  55                  60

Gln Thr Leu Val Ser Ser Ser Gln Ala Thr Thr Arg Ala Cys Ser Asn
65                  70                  75                  80

Glu Gln Lys Lys Leu Lys Ile Ala Val Val Gly Phe Gly Asn Phe Gly
                85                  90                  95

Gln Phe Leu Ala Arg Glu Met Val Lys Gln Gly His Gln Val Leu Ala
            100                 105                 110

Tyr Ser Arg Ser Asp Tyr Ser Lys Val Ala Lys Glu Ile Gly Val Gln
       115                 120                 125

Phe Phe Arg Asp Pro Asp Asp Leu Cys Glu Glu His Pro Gln Val Val
    130                 135                 140

Leu Leu Cys Thr Ser Ile Leu Ser Thr Glu Lys Val Leu Arg Ser Leu
145                 150                 155                 160

Pro Val Asp Arg Leu Arg Arg Ser Thr Leu Ile Val Asp Val Leu Ser
                165                 170                 175

Val Lys Glu Phe Pro Arg Thr Leu Phe Leu Arg His Leu Pro Glu Asp
            180                 185                 190

Leu Asp Ile Leu Cys Thr His Pro Met Phe Gly Pro Asp Ser Gly Lys
        195                 200                 205

Ser Gly Trp Asp Gly Leu Pro Phe Val Phe Asp Lys Val Arg Val Gly
    210                 215                 220

Ser Asp Pro Thr Arg Thr His Arg Val Asn Thr Phe Leu Asp Ile Phe
225                 230                 235                 240

Lys His Ala Gly Cys Arg Met Val Glu Met Thr Cys Met Asp His Asp
                245                 250                 255

Lys His Ala Ala Gly Ser Gln Phe Ile Thr His Met Met Gly Arg Val
            260                 265                 270

Leu Glu Lys Val Gly Leu Ser Asn Thr Pro Ile Asn Thr Lys Gly Tyr
        275                 280                 285
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Glu Ser Leu Leu Asn Leu Val Asp Asn Thr Ala Arg Asp Ser Phe Glu
    290                 295                 300

Leu Phe Tyr Gly Leu Phe Leu Tyr Asn Lys Asn Ala Met Glu Glu Leu
305                 310                 315                 320

Asp Arg Leu Asp Trp Ala Phe Asp Thr Val Lys Met Gln Leu Ser Gly
                325                 330                 335

Tyr Leu His Asp Phe Ala Ser Lys Lys Leu Met Leu Glu Thr Gly Asn
            340                 345                 350

Glu Leu Ala Gly Ile Val Ser Gly Lys Ile Gly Asp Asp Asn His Asn
        355                 360                 365

Asn Lys Arg Leu Met Leu Ser Pro Pro Thr Asn Ser Tyr Lys Asn Val
    370                 375                 380

Thr Phe Thr Asp Thr Lys Val Ser Glu Lys Met Met
385                 390                 395


<210> SEQ ID NO 8
<211> LENGTH: 375
<212> TYPE: PRT
<213> ORGANISM: Rivina humilis
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(375)
<223> OTHER INFORMATION: RhADH-alpha

<400> SEQUENCE: 8

Cys Thr Ala Phe Thr Lys Thr Asn Asn Asn Asn Ala Leu Gly Tyr Gly
1               5                   10                  15

Tyr Gly Tyr Gly Tyr Gly Tyr Gly Tyr Asp Lys Asn Lys Val Ser Ser
            20                  25                  30

Thr Glu Gln Gly Asp Glu Val Ser Gly Ser Ser Ser Asn Ser Lys Lys
        35                  40                  45

Leu Lys Ile Gly Ile Ile Gly Phe Gly Asn Phe Gly Gln Phe Met Ala
    50                  55                  60

Lys Thr Met Val Lys His Gly His Thr Val Leu Ala Tyr Ser Arg Ser
65                  70                  75                  80

Asp Tyr Ser Arg Ala Ala His Thr Ile Gly Val Arg Tyr Phe Ser Asp
                85                  90                  95

Pro Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Leu Leu Cys Thr
            100                 105                 110

Ser Ile Leu Ser Thr Glu Arg Val Leu Arg Ser Leu Pro Leu His Arg
       115                 120                 125

Leu Arg Arg Ser Thr Leu Val Ala Asp Val Leu Ser Val Lys Glu Phe
    130                 135                 140

Pro Arg Ser Leu Phe Leu Gln Leu Leu Pro Ser Asp Phe Asp Ile Leu
145                 150                 155                 160

Cys Thr His Pro Met Phe Gly Pro Asp Ser Gly Lys Ala Gly Trp Gly
                165                 170                 175

Gly Leu Pro Phe Val Phe Asp Lys Val Arg Val Gly Ser Gln Pro Glu
            180                 185                 190

Arg Leu Thr Arg Val Glu Ala Phe Leu Asp Ile Phe Arg Asp Ala Gly
        195                 200                 205

Cys Arg Met Val Glu Met Ser Cys Ala Glu His Asp Arg His Ala Ala
    210                 215                 220

Gly Ser Gln Phe Ile Thr His Met Met Gly Arg Val Leu Glu Lys Leu
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225                 230                 235                 240

Ala Leu Glu Asp Thr Pro Ile Asn Thr Lys Gly Tyr Glu Ser Leu Leu
                245                 250                 255

Asn Leu Val Asp Asn Thr Ala Arg Asp Ser Phe Glu Leu Phe Tyr Gly
            260                 265                 270

Leu Phe Leu Tyr Asn Lys Asn Ala Met Glu Gln Leu Asp Arg Met His
        275                 280                 285

Trp Ala Phe Glu Thr Val Lys Gln Gln Leu Ser Gly Tyr Leu His Val
    290                 295                 300

Leu Val Arg Lys Gln Leu Met Leu Glu Thr Ser Ser Gly Asn Asp Asn
305                 310                 315                 320

Asn Asn Thr Asn Asn Ile Asn Ile Ser Ser Gly Asp Asn Ile Asn Asn
                325                 330                 335

Lys Asp Thr Asn Asn Lys Leu Met Leu Pro Ser Pro Gly Ile Ser Ser
            340                 345                 350

Ala Lys Ile Val Pro Pro Val Gln Glu Lys Glu Lys His Asp Leu Val
        355                 360                 365

Met Leu Asn Gly Ser Lys Arg
    370                 375


<210> SEQ ID NO 9
<211> LENGTH: 341
<212> TYPE: PRT
<213> ORGANISM: Portulaca oleracea
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(341)
<223> OTHER INFORMATION: PoADH-alpha

<400> SEQUENCE: 9

Cys Ser Ser Ser Ser Ser Ser Ser Ala Ser Ile Ile Ile Asn Gly Ser
1               5                   10                  15

Gly Ser Ser Thr Thr Asn Ser Ser Val Phe Asp Ala Ser Ser Ser Ser
            20                  25                  30

Asp Ser Asp Val Lys Lys Arg Ser Glu Val Lys Leu Lys Ile Gly Ile
        35                  40                  45

Ile Gly Phe Gly Lys Phe Gly Gln Phe Leu Ala Lys Arg Ile Val Ser
    50                  55                  60

Gln Gly His Asp Val Leu Ala Tyr Ser Arg Ser Asp Tyr Ser Arg Val
65                  70                  75                  80

Ala Ser Glu Ile Gly Val Arg Phe Phe Ser Asp Ala Asp Asp Leu Cys
                85                  90                  95

Glu Glu His Pro Gln Val Ile Leu Leu Cys Thr Ser Ile Leu Ser Thr
            100                 105                 110

Glu Arg Val Leu Arg Ser Leu Pro Leu His Arg Leu Arg Arg Ser Thr
       115                 120                 125

Leu Phe Ala Asp Val Leu Ser Val Lys Glu Phe Pro Arg Ser Leu Phe
    130                 135                 140

Leu Gln Leu Leu Pro Ser Asp Phe Asp Ile Leu Cys Thr His Pro Met
145                 150                 155                 160

Phe Gly Pro Asp Ser Gly Lys Ser Gly Trp Asp Ser Leu Pro Phe Val
                165                 170                 175

Phe Asp Lys Val Arg Val Gly Ser Thr Pro Thr Arg Val Thr Arg Ser
            180                 185                 190
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Glu Ala Phe Leu Asp Ile Phe Arg Thr Ala Gly Cys Arg Met Val Glu
        195                 200                 205

Met Ser Cys Ala Glu His Asp Lys His Ala Ala Gly Ser Gln Phe Ile
    210                 215                 220

Thr His Met Met Gly Arg Val Leu Glu Lys Leu Asp Leu Glu Asn Thr
225                 230                 235                 240

Pro Ile Asn Thr Arg Gly Tyr Glu Ser Leu Arg Asn Leu Val Asp Asn
                245                 250                 255

Thr Ala Arg Asp Ser Phe Glu Leu Phe Tyr Gly Leu Phe Leu Tyr Asn
            260                 265                 270

Lys Asn Ala Thr Glu Gln Leu Asp Arg Met Asp Trp Ala Phe Glu Met
        275                 280                 285

Val Lys Lys Gln Leu Ser Gly Tyr Leu His His Leu Val Arg Lys Gln
    290                 295                 300

Leu Met Leu Glu Ser Ser Asn Thr His Glu Asn His Val Asp Asn Lys
305                 310                 315                 320

Leu Leu Leu Pro Glu Asn Lys Gln Lys Gln His Asp Leu Val Val Val
                325                 330                 335

Val Asn Asp Arg Ser
            340


<210> SEQ ID NO 10
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Paronychia polygonifolia
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: Paronychia polygonifolia ADHa

<400> SEQUENCE: 10

Met Asn Ser Ile Ser Ile Val Ser Ser Thr Lys Ser Thr Tyr Tyr Lys
1               5                   10                  15

Val Tyr Gln Phe Pro Ser Pro Lys Ile Cys Phe Phe His Pro Ser Lys
            20                  25                  30

Leu Ser Ile Pro Ser Cys His Leu Lys Phe Gln Asn Phe Ala Val Arg
        35                  40                  45

Cys Asn Ser Ser Asn Asn Pro Lys Asn Val Ser Asn
    50                  55                  60


<210> SEQ ID NO 11
<211> LENGTH: 239
<212> TYPE: PRT
<213> ORGANISM: Herniaria latifolia
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(239)
<223> OTHER INFORMATION: Herniaria latifolia ADHa

<400> SEQUENCE: 11

Val Gly Phe Gly Asn Phe Gly Gln Phe Leu Ala Lys Glu Met Val Lys
1               5                   10                  15

Gln Gly His Gln Val Leu Ala Tyr Ser Arg Ser Asp Tyr Ser Arg Val
            20                  25                  30

Ala Gln Glu Ile Gly Val Gln Tyr Phe Ser Asn Pro Asp Asp Leu Cys
        35                  40                  45
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Lys Glu His Pro Glu Val Ile Leu Leu Cys Thr Ser Ile Leu Ser Thr
    50                  55                  60

Glu Lys Val Leu Asn Thr Leu Pro Leu Asp Arg Leu Arg Pro Ser Thr
65                  70                  75                  80

Leu Phe Ser Asp Val Leu Ser Val Lys Glu Phe Pro Arg Thr Leu Phe
                85                  90                  95

Leu Gln Gln Leu Pro Glu Asp Phe Asp Ile Ile Cys Thr His Pro Met
            100                 105                 110

Phe Gly Pro Asp Ser Gly Lys His Gly Trp Ala Gly Leu Pro Tyr Val
        115                 120                 125

Tyr Asp Lys Val Arg Val Gly Leu Asp Pro Thr Arg Ile Arg Arg Ala
    130                 135                 140

Glu Ala Phe Leu Asn Ile Phe Glu Arg Ala Gly Cys Arg Met Val Glu
145                 150                 155                 160

Met Thr Cys Ala Glu His Asp Lys His Ala Ala Gly Ser Gln Phe Ile
                165                 170                 175

Thr His Met Leu Gly Arg Val Leu Glu Lys Val Gly Leu Leu Asn Thr
            180                 185                 190

Pro Ile Asn Thr Lys Gly Tyr Glu Ser Leu Leu Ser Leu Val Asp Asn
        195                 200                 205

Thr Ala Arg Asp Ser Phe Glu Leu Phe Tyr Gly Leu Phe Leu Tyr Asn
    210                 215                 220

Lys Asn Ala Met Glu Gln Leu Asp Arg Leu Asp Trp Ala Phe Asp
225                 230                 235


<210> SEQ ID NO 12
<211> LENGTH: 351
<212> TYPE: PRT
<213> ORGANISM: Corrigiola litoralis
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(351)
<223> OTHER INFORMATION: Corrigiola litoralis ADHa

<400> SEQUENCE: 12

Met Asn Gly Ser Ala Asp His Phe His Pro Asn Ile Lys Val Asn Gly
1               5                   10                  15

Glu Val Leu Asn Pro Met Val Gly Ser Ser Asp Val Ala Glu Asp Val
            20                  25                  30

Lys Leu Lys Ile Ala Ile Val Gly Phe Gly Asn Phe Gly Gln Phe Leu
        35                  40                  45

Ala Lys Glu Ile Val Lys Gln Gly His Lys Val Leu Ala Tyr Ser Arg
    50                  55                  60

Ser Asp Tyr Ser Lys Ala Ala Lys Glu Ile Gly Val Gln Tyr Phe Ser
65                  70                  75                  80

Asp Ala Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Leu Leu Cys
                85                  90                  95

Thr Ser Ile Leu Ser Thr Glu Lys Val Met Arg Ala Leu Pro Ile His
            100                 105                 110

Arg Leu Arg Arg Ser Thr Leu Phe Val Asp Val Leu Ser Val Lys Glu
        115                 120                 125

Phe Pro Arg Ser Leu Phe Leu Gln Val Leu Pro Lys Asp Phe Asp Ile
    130                 135                 140

Leu Cys Thr His Pro Met Phe Gly Pro Asp Ser Gly Lys Ala Gly Trp
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145                 150                 155                 160

Gly Gly Leu Pro Phe Val Phe Asp Lys Val Arg Val Ala Pro Asp Ser
                165                 170                 175

Thr Arg Ala Thr Arg Ala Glu Ala Phe Leu Asp Ile Phe Arg Arg Ala
            180                 185                 190

Gly Cys Arg Met Val Glu Met Thr Cys Ala Asp His Asp Lys His Ala
        195                 200                 205

Ala Gly Ser Gln Phe Ile Thr His Met Met Gly Arg Val Leu Glu Lys
    210                 215                 220

Ile Gly Leu Glu Asn Thr Pro Ile Asn Thr Lys Gly Tyr Glu Ser Leu
225                 230                 235                 240

Leu Asn Leu Val Asp Asn Thr Ala Arg Asp Ser Phe Glu Leu Phe Tyr
                245                 250                 255

Gly Leu Phe Leu Tyr Asn Lys Asn Ala Met Glu Gln Leu Asp Arg Met
            260                 265                 270

Asp Trp Ala Phe Glu Met Ile Lys Lys Arg Leu Ser Gly Tyr Leu His
        275                 280                 285

Asp Leu Val Arg Lys Gln Leu Met Leu Glu Thr Thr Gly Asn Asp Gln
    290                 295                 300

Ala Gly Leu Thr Asn Gly Ala Lys Asn Asn His Asp Lys Lys Leu Met
305                 310                 315                 320

Leu Pro Pro Pro Ala Ala Asn Pro Ser Met Ile Val Pro Ser Ala Ala
                325                 330                 335

Thr His Glu Lys Lys His Asp Leu Val His Val Asn Gly Ser Arg
            340                 345                 350


<210> SEQ ID NO 13
<211> LENGTH: 325
<212> TYPE: PRT
<213> ORGANISM: Telephium imperati
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(325)
<223> OTHER INFORMATION: Telephium imperati ADHa

<400> SEQUENCE: 13

Met Val Gly Pro Ser Glu Ser Gly Lys Asp Val Lys Leu Glu Ile Ala
1               5                   10                  15

Val Val Gly Phe Gly Asn Phe Gly Gln Phe Leu Gly Arg Glu Ile Val
            20                  25                  30

Lys Gln Gly His Glu Val Leu Ala Tyr Ser Arg Ser Asp Tyr Ser Lys
        35                  40                  45

Val Ala Lys Glu Ile Gly Val Arg Tyr Phe Ser Asp Ala His Asp Leu
    50                  55                  60

Cys Glu Glu His Pro Glu Val Ile Leu Leu Cys Thr Ser Ile Leu Ser
65                  70                  75                  80

Thr Glu Arg Val Leu His Ser Leu Pro Leu Asn Arg Leu Arg Arg Ser
                85                  90                  95

Thr Leu Phe Val Asp Val Leu Ser Val Lys Glu Phe Pro Arg Asn Leu
            100                 105                 110

Phe Leu Gln Asn Leu Pro Asn Asp Phe Asp Ile Leu Cys Thr His Pro
       115                 120                 125

Met Phe Gly Pro Asp Ser Gly Lys Ala Gly Trp Asp Gly Leu Pro Phe
    130                 135                 140
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Val Phe Asp Lys Val Arg Val Gly Ser Asp Pro Ala Arg Thr Thr Arg
145                 150                 155                 160

Ala Asp Thr Phe Leu Asp Ile Phe Arg Asn Ala Gly Cys Arg Met Val
                165                 170                 175

Glu Met Ser Cys Ala Glu His Asp Arg His Ala Ala Gly Ser Gln Phe
            180                 185                 190

Ile Thr His Met Met Gly Arg Val Leu Glu Lys Ile Gly Leu Glu Asn
        195                 200                 205

Thr Pro Ile Asn Thr Lys Gly Tyr Glu Ser Leu Leu Asn Leu Val Asp
    210                 215                 220

Asn Thr Ala Arg Asp Ser Phe Glu Leu Phe Leu Tyr Tyr Lys Asn Ala
225                 230                 235                 240

Met Glu Gln Leu Asp Arg Met Asp Trp Ala Phe Glu Met Ile Lys Lys
                245                 250                 255

Gln Leu Ser Gly Tyr Leu His Glu Leu Val Arg Lys Gln Leu Met Leu
            260                 265                 270

Glu Thr Asn Asn Asp Gln Ser Gly Ile Ile Asn Gly Lys Thr Asn Cys
        275                 280                 285

Asp Lys Arg Leu Met Leu Pro Pro Pro Ala Ala Asn Pro Ser Val Ile
    290                 295                 300

Val Pro Asp Pro Val Pro Ala Val Lys Lys Lys His Asp Leu Val His
305                 310                 315                 320

Val Asn Gly Ser Arg
                325


<210> SEQ ID NO 14
<211> LENGTH: 360
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(360)
<223> OTHER INFORMATION: BvADH-beta Big Buck sugar beet variety

<400> SEQUENCE: 14

Met Leu Ser Leu Ser Ser Thr Thr Thr Ala Lys Pro Ser Pro Ser Pro
1               5                   10                  15

Ser Pro Ala Asn Phe Pro Ala Lys Leu Ser Ser Leu Ser Thr Ile Thr
            20                  25                  30

Thr Thr Leu Ser Phe Ser Pro Arg Arg Arg Tyr Phe His Gly Val Lys
        35                  40                  45

Thr Leu Thr Ile Arg Ser Ile Asp Ala Ala Gln Phe Phe Asp Tyr Glu
    50                  55                  60

Ser Lys Leu Ala Ala Ile Asn Thr Thr Ser Ser Ser Ser Ser Ser Ser
65                  70                  75                  80

Tyr Ser Lys Leu Lys Ile Ala Ile Val Gly Phe Gly Asn Tyr Gly Gln
                85                  90                  95

Phe Leu Ala Lys Thr Leu Val Ser Gln Gly His Thr Val Leu Ala Tyr
            100                 105                 110

Ser Arg Ser Asp Tyr Ser Lys Ile Ala Ala Asn Leu Gly Val Ser Tyr
        115                 120                 125

Phe Ser Asp Pro Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Met
    130                 135                 140
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Leu Cys Thr Ser Ile Leu Ser Thr Glu Val Met Leu Asn Ser Leu Pro
145                 150                 155                 160

Leu Gln Arg Leu Lys Arg Ser Thr Leu Phe Val Asp Val Leu Ser Val
                165                 170                 175

Lys Glu Phe Pro Arg Asn Leu Phe Leu Gln Thr Leu Pro Ser Asp Phe
            180                 185                 190

Asp Ile Leu Cys Thr His Pro Met Phe Gly Pro Glu Ser Gly Lys Asn
        195                 200                 205

Gly Trp Gly Ser Leu Pro Phe Val Tyr Asp Lys Val Arg Ile Gly Lys
    210                 215                 220

Asp Glu Gly Arg Ile Lys Arg Cys Glu Ser Phe Leu Asp Val Phe Arg
225                 230                 235                 240

Arg Glu Gly Cys Arg Val Glu Glu Met Thr Cys Ala Glu His Asp Lys
                245                 250                 255

Phe Ala Ala Gly Ser Gln Phe Ile Thr His Phe Leu Gly Arg Val Leu
            260                 265                 270

Glu Lys Leu Asp Leu Glu Asp Thr Pro Ile Asn Thr Lys Gly Tyr Glu
        275                 280                 285

Ser Leu Leu Asn Leu Val Asp Asn Thr Ser Lys Asp Ser Phe Glu Leu
    290                 295                 300

Phe Tyr Gly Leu Phe Leu Tyr Asn Gln Asn Ala Met Glu Gln Leu Glu
305                 310                 315                 320

Arg Leu Asp Trp Ala Phe Glu Leu Val Lys Lys Gln Leu Phe Gly His
                325                 330                 335

Leu His Gly Leu Leu Arg Lys Gln Leu Phe Gly Phe Ser Glu Ile Asp
            340                 345                 350

Glu Arg Ile Gly Lys Ala Lys Glu
        355                 360


<210> SEQ ID NO 15
<211> LENGTH: 60
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(60)
<223> OTHER INFORMATION: BvADH-beta W357B red beet variety

<400> SEQUENCE: 15

Met Leu Ser Leu Ser Ser Thr Thr Thr Ala Lys Pro Ser Pro Ser Pro
1               5                   10                  15

Ser Pro Ala Asn Phe Pro Ala Lys Leu Ser Ser Leu Ser Thr Ile Thr
            20                  25                  30

Thr Thr Leu Ser Phe Ser Pro Arg Arg Arg Tyr Phe His Gly Val Lys
        35                  40                  45

Thr Leu Thr Ile Arg Ser Ile Asp Ala Ala Gln Phe
    50                  55                  60


<210> SEQ ID NO 16
<211> LENGTH: 386
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(386)
<223> OTHER INFORMATION: BvADH-beta Touch Stone yellow beet variety
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<400> SEQUENCE: 16

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Met Leu Ser Leu Ser Ser Thr Thr Thr Ala Lys Pro Ser Pro Ser Pro
1               5                  10                  15

Ser Pro Ala Asn Phe Pro Ala Lys Leu Ser Ser Leu Ser Thr Ile Thr
            20                  25                  30

Thr Thr Leu Ser Phe Ser Pro Arg Arg Arg Tyr Phe His Gly Val Lys
        35                  40                  45

Thr Leu Thr Ile Arg Ser Ile Asp Ala Ala Gln Phe Phe Asp Tyr Glu
    50                  55                  60

Ser Lys Leu Ala Ala Ile Asn Thr Thr Ser Ser Ser Ser Ser Ser Ser
65                  70                  75                  80

Tyr Ser Lys Leu Lys Ile Ala Ile Val Gly Phe Gly Asn Tyr Gly Gln
                85                  90                  95

Phe Leu Ala Lys Thr Leu Val Ser Gln Gly His Thr Val Leu Ala Tyr
            100                 105                 110

Ser Arg Ser Asp Tyr Ser Lys Ile Ala Ala Asn Leu Gly Val Ser Tyr
       115                 120                 125

Phe Ser Asp Pro Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Met
    130                 135                 140

Leu Cys Thr Ser Ile Leu Ser Thr Glu Val Met Leu Asn Ser Leu Pro
145                 150                 155                 160

Leu Gln Arg Leu Lys Arg Ser Thr Leu Phe Val Asp Val Leu Ser Val
                165                 170                 175

Lys Glu Phe Pro Arg Asn Leu Phe Leu Gln Thr Leu Pro Ser Asp Phe
            180                 185                 190

Asp Ile Leu Cys Thr His Pro Met Phe Gly Pro Glu Ser Gly Lys Asn
        195                 200                 205

Gly Trp Gly Ser Leu Pro Phe Val Tyr Asp Lys Val Arg Ile Gly Lys
    210                 215                 220

Asp Glu Gly Arg Ile Lys Arg Cys Glu Ser Phe Leu Asp Val Phe Arg
225                 230                 235                 240

Arg Glu Gly Cys Arg Val Glu Glu Met Thr Cys Ala Glu His Asp Lys
                245                 250                 255

Phe Ala Ala Gly Ser Gln Phe Ile Thr His Phe Leu Gly Arg Val Leu
            260                 265                 270

Glu Lys Leu Asp Leu Glu Asp Thr Pro Ile Asn Thr Lys Gly Tyr Glu
        275                 280                 285

Ser Leu Leu Asn Leu Val Asp Asn Thr Ser Lys Asp Ser Phe Glu Leu
    290                 295                 300

Phe Tyr Gly Leu Phe Leu Tyr Asn Gln Asn Ala Met Glu Gln Leu Glu
305                 310                 315                 320

Arg Leu Asp Trp Ala Phe Glu Leu Val Lys Lys Gln Leu Phe Gly His
                325                 330                 335

Leu His Gly Leu Leu Arg Lys Gln Leu Phe Gly Phe Ser Glu Ile Asp
            340                 345                 350

Glu Arg Ile Gly Lys Ala Lys Glu Ile Lys Phe Leu Ser Asp Ala Ala
        355                 360                 365

Glu Gln Asn Gly Ser Ala Leu Ser Ala Arg Glu Asn Ala Asn Ser Glu
    370                 375                 380

Thr Asn
385
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<210> SEQ ID NO 17
<211> LENGTH: 386
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(386)
<223> OTHER INFORMATION: BvADH-beta Blankoma white beet variety

<400> SEQUENCE: 17

Met Leu Ser Leu Ser Ser Thr Thr Thr Ala Lys Pro Ser Pro Ser Pro
1 5 10 15

Ser Pro Ala Asn Phe Pro Ala Lys Leu Ser Ser Leu Ser Thr Ile Thr
20 25 30

Thr Thr Leu Ser Phe Ser Pro Arg Arg Arg Tyr Phe His Gly Val Lys
35 40 45

Thr Leu Thr Ile Arg Ser Ile Asp Ala Ala Gln Phe Phe Asp Tyr Glu
50 55 60

Ser Lys Leu Ala Ala Ile Asn Thr Thr Ser Ser Ser Ser Ser Ser Ser
65 70 75 80

Tyr Ser Lys Leu Lys Ile Ala Ile Val Gly Phe Gly Asn Tyr Gly Gln
85 90 95

Phe Leu Ala Lys Thr Leu Val Ser Gln Gly His Thr Val Leu Ala Tyr
100 105 110

Ser Arg Ser Asp Tyr Ser Lys Ile Ala Ala Asn Leu Gly Val Ser Tyr
115 120 125

Phe Ser Asp Pro Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Met
130 135 140

Leu Cys Thr Ser Ile Leu Ser Thr Glu Val Met Leu Asn Ser Leu Pro
145 150 155 160

Leu Gln Arg Leu Lys Arg Ser Thr Leu Phe Val Asp Val Leu Ser Val
165 170 175

Lys Glu Phe Pro Arg Asn Leu Phe Leu Gln Thr Leu Pro Ser Asp Phe
180 185 190

Asp Ile Leu Cys Thr His Pro Met Phe Gly Pro Glu Ser Gly Lys Asn
195 200 205

Gly Trp Gly Ser Leu Pro Phe Val Tyr Asp Lys Val Arg Ile Gly Lys
210 215 220

Asp Glu Gly Arg Ile Lys Arg Cys Glu Ser Phe Leu Asp Val Phe Arg
225 230 235 240

Arg Glu Gly Cys Arg Val Glu Glu Met Thr Cys Ala Glu His Asp Lys
245 250 255

Phe Ala Ala Gly Ser Gln Phe Ile Thr His Phe Leu Gly Arg Val Leu
260 265 270

Glu Lys Leu Asp Leu Glu Asp Thr Pro Ile Asn Thr Lys Gly Tyr Glu
275 280 285

Ser Leu Leu Asn Leu Val Asp Asn Thr Ser Lys Asp Ser Phe Glu Leu
290 295 300

Phe Tyr Gly Leu Phe Leu Tyr Asn Gln Asn Ala Met Glu Gln Leu Glu
305 310 315 320

Arg Leu Asp Trp Ala Phe Glu Leu Val Lys Lys Gln Leu Phe Gly His
325 330 335

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Leu His Gly Leu Leu Arg Lys Gln Leu Phe Gly Phe Ser Glu Ile Asp
            340                 345                 350

Glu Arg Ile Gly Lys Ala Lys Glu Ile Lys Phe Leu Ser Asp Ala Ala
        355                 360                 365

Glu Gln Asn Gly Ser Ala Leu Ser Ala Arg Glu Asn Ala Asn Ser Glu
    370                 375                 380

Thr Asn
385
```

<210> SEQ ID NO 18
<211> LENGTH: 386
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(386)
<223> OTHER INFORMATION: BvADH-beta Sea beet PI562585 variety

<400> SEQUENCE: 18

```
Met Leu Ser Leu Ser Ser Thr Thr Thr Ala Lys Pro Ser Pro Ser Pro
1               5                   10                  15

Ser Pro Ala Asn Phe Pro Ala Lys Leu Ser Ser Leu Ser Thr Ile Thr
            20                  25                  30

Thr Thr Ile Ser Phe Ser Pro Arg Arg Arg Tyr Phe His Gly Val Lys
        35                  40                  45

Thr Leu Thr Ile Arg Ser Ile Asp Ala Ala Gln Phe Phe Asp Tyr Glu
    50                  55                  60

Ser Lys Leu Ala Ala Ile Asn Thr Thr Ser Ser Ser Thr Ser Ser Ser
65                  70                  75                  80

Tyr Ser Lys Leu Lys Ile Ala Ile Val Gly Phe Gly Asn Tyr Gly Gln
                85                  90                  95

Phe Leu Ala Lys Thr Leu Val Ser Gln Gly His Thr Val Leu Ala Tyr
            100                 105                 110

Ser Arg Ser Asp Tyr Ser Lys Ile Ala Ala Asn Leu Gly Val Ser Tyr
        115                 120                 125

Phe Ser Asp Pro Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Met
    130                 135                 140

Leu Cys Thr Ser Ile Leu Ser Thr Glu Val Met Leu Asn Ser Leu Pro
145                 150                 155                 160

Leu Gln Arg Leu Lys Arg Ser Thr Leu Phe Val Asp Val Leu Ser Val
                165                 170                 175

Lys Glu Phe Pro Arg Asn Leu Phe Leu Gln Thr Leu Pro Ser Asp Phe
            180                 185                 190

Asp Ile Leu Cys Thr His Pro Met Phe Gly Pro Glu Ser Gly Lys Asn
        195                 200                 205

Gly Trp Gly Ser Leu Pro Phe Val Tyr Asp Lys Val Arg Ile Gly Lys
    210                 215                 220

Asp Glu Gly Arg Ile Lys Arg Cys Glu Ser Phe Leu Asp Val Phe Arg
225                 230                 235                 240

Arg Glu Gly Cys Arg Val Glu Glu Met Thr Cys Ala Glu His Asp Lys
                245                 250                 255

Phe Ala Ala Gly Ser Gln Phe Ile Thr His Phe Leu Gly Arg Val Leu
            260                 265                 270

Glu Lys Leu Asp Leu Glu Asp Thr Pro Ile Asn Thr Lys Gly Tyr Glu
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        275                 280                 285

Ser Leu Leu Asn Leu Val Asp Asn Thr Ser Lys Asp Ser Phe Glu Leu
    290                 295                 300

Phe Tyr Gly Leu Phe Leu Tyr Asn Gln Asn Ala Met Glu Gln Leu Glu
305                 310                 315                 320

Arg Leu Asp Trp Ala Phe Glu Leu Val Lys Lys Gln Leu Phe Gly His
                325                 330                 335

Leu His Gly Leu Leu Arg Lys Gln Leu Phe Gly Phe Ser Glu Ile Asp
            340                 345                 350

Glu Arg Ile Gly Lys Ala Lys Glu Ile Lys Phe Leu Ser Asp Ala Ala
        355                 360                 365

Glu Gln Asn Gly Ser Ala Leu Ser Ala Arg Glu Asn Ala Asn Ser Glu
    370                 375                 380

Thr Asn
385


<210> SEQ ID NO 19
<211> LENGTH: 321
<212> TYPE: PRT
<213> ORGANISM: Spinacea oleracea
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(321)
<223> OTHER INFORMATION: SoADH-beta

<400> SEQUENCE: 19

Ala Ala Thr Asn Thr Ser Thr Ala Thr Ser Ser Ser Gln Ser Ser Tyr
1               5                   10                  15

Ser Lys Leu Lys Val Ala Ile Val Gly Phe Gly Asn Tyr Gly Gln Phe
            20                  25                  30

Leu Ala Lys Thr Met Val Ser Gln Gly His Thr Val Leu Ala Tyr Ser
        35                  40                  45

Arg Ser Asp Tyr Ser Lys Ile Ala Pro Asn Leu Gly Val Ser Phe Phe
    50                  55                  60

Ser Asp Pro Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Leu Leu
65                  70                  75                  80

Cys Thr Ser Ile Leu Ser Thr Glu Phe Met Leu Asn Ser Leu Pro Leu
                85                  90                  95

Gln Arg Leu Lys Arg Ser Thr Leu Phe Val Asp Val Leu Ser Val Lys
            100                 105                 110

Glu Phe Pro Arg Asn Leu Phe Leu Gln Thr Leu Pro Pro Asp Phe Asp
       115                 120                 125

Ile Leu Cys Thr His Pro Met Phe Gly Pro Glu Ser Gly Lys Asn Gly
    130                 135                 140

Trp Gly Gly Leu Pro Phe Val Tyr Asp Lys Val Arg Ile Gly Lys Ala
145                 150                 155                 160

Glu Arg Arg Ile Arg Arg Cys Glu Asn Phe Leu Asp Val Phe Arg Arg
                165                 170                 175

Ala Gly Cys Arg Val Glu Glu Met Thr Cys Ala Glu His Asp Lys Tyr
            180                 185                 190

Ala Ala Gly Ser Gln Phe Ile Thr His Phe Leu Gly Arg Val Leu Glu
        195                 200                 205

Lys Leu Asp Leu Glu Asp Thr Pro Ile Asn Thr Lys Gly Tyr Glu Ser
    210                 215                 220
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Leu Leu Asn Leu Val Asp Asn Thr Ser Lys Asp Ser Phe Glu Leu Phe
225                 230                 235                 240

Tyr Gly Leu Phe Leu Tyr Asn Gln Asn Ala Met Glu Gln Leu Glu Arg
                245                 250                 255

Leu Asp Trp Ala Phe Glu Leu Val Lys Lys Gln Leu Phe Gly His Leu
            260                 265                 270

His Gly Leu Leu Arg Gly Gln Leu Phe Gly Cys Thr Glu Ile Asp Glu
        275                 280                 285

Arg Leu Glu Lys Ala Lys Glu Leu Lys Phe Leu Ser Asp Ala Thr Thr
    290                 295                 300

Gln Asn Gly Ser Ala Ser Ala Pro Arg Glu Asn Ala Asn Ser Glu Ile
305                 310                 315                 320

Asn
```

<210> SEQ ID NO 20
<211> LENGTH: 321
<212> TYPE: PRT
<213> ORGANISM: Nepenthes alata
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(321)
<223> OTHER INFORMATION: NaADH-beta

<400> SEQUENCE: 20

```
Ala Ala Leu Pro Asn Asp Tyr Glu Thr Lys Leu Ser His Leu Pro Ser
1               5                   10                  15

Ser Phe Ala Lys Leu Lys Val Gly Ile Ile Gly Phe Gly Asn Tyr Gly
            20                  25                  30

Gln Phe Leu Ala Lys Thr Leu Val Arg Gln Gly His Thr Val Leu Ala
        35                  40                  45

His Ser Arg Ser Asn Tyr Ser Gln Asn Ala Ala Lys Leu Gly Val Ser
    50                  55                  60

Phe Phe Tyr Asp Pro Asn Asp Leu Cys Glu Glu His Pro Glu Val Ile
65                  70                  75                  80

Leu Leu Cys Thr Ser Ile Leu Ser Thr Glu Ser Val Leu Arg Ser Leu
                85                  90                  95

Pro Leu Gln Arg Leu Lys Arg Ser Thr Leu Phe Val Asp Val Leu Ser
            100                 105                 110

Val Lys Glu Phe Pro Arg Ser Leu Leu Leu Gln Ile Leu Pro Pro Asp
       115                 120                 125

Leu Asp Ile Leu Cys Thr His Pro Met Phe Gly Pro Glu Ser Gly Lys
    130                 135                 140

Asn Gly Trp Ser Gly Leu Pro Phe Val Tyr Asp Lys Val Arg Ile Gly
145                 150                 155                 160

Glu His Glu Ile Arg Val Asn Arg Cys Asp Asn Phe Ile Glu Val Phe
                165                 170                 175

Arg Arg Glu Gly Cys Arg Met Val Gln Met Ser Cys Ala Glu His Asp
            180                 185                 190

Arg His Ala Ala Gly Ser Gln Phe Ile Thr His Met Met Gly Arg Val
        195                 200                 205

Leu Glu Lys Leu Lys Leu Glu Asp Thr Pro Ile Asn Thr Lys Gly Tyr
    210                 215                 220

Glu Ser Leu Leu Asn Leu Val Glu Asn Thr Ala Arg Asp Ser Phe Glu
225                 230                 235                 240
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Leu Phe Tyr Gly Leu Phe Leu Tyr Asn Lys Asn Val Met Glu Gln Leu
245 250 255

Glu Arg Met Asp Leu Ala Phe Glu Met Val Lys Lys Gln Leu Phe Gly
260 265 270

His Leu His Gly Leu Leu Arg Ser Gln Leu Phe Asp Gly Ser Glu Met
275 280 285

Glu Val Arg Val Glu Glu Glu Arg Lys Leu Leu Ser Asp Gly Ser Gln
290 295 300

Asn Gly His Val Phe Ser Ser Phe Ser Asp Ser Lys Asn Val Glu Arg
305 310 315 320

Asn

<210> SEQ ID NO 21
<211> LENGTH: 1197
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1197)
<223> OTHER INFORMATION: Beta vulgaris Big Buck arogenate dehydrogenase alpha, complet CDS

<400> SEQUENCE: 21

atgatttcac tctcttcttt tcatccttcc tccaccaccg ccaccgccac cgccgccacc 60

gccaccaccc acccaccaca acaatgtccc gctttttcct ctcctccatc gcatctctcg 120

cttcctttac gccaccctcg ccaacacctt gtagttcggt gcggtggagg tggttcggcc 180

tccgaatcgg tatttaaccg tgatagtgct gctactcgtg tttctaatga tcatcttgac 240

gttagtaaaa gagatgttaa gcttaagatt gctattattg ggtttggtaa ctttggccag 300

tttttggcta agacaatggc taagcaaggt catagagtgt tggcttactc acgctcggac 360

tactcccgcg ctgctaagga gatcggcgtc gagtatttta ctgacgccga tgacctctgc 420

gaggagcacc ctgaggttat tcttttgtgc acgtccatcc tctcaacgga gaaggtcctc 480

cgatcactcc ccctccaccg gctccgtcgt tcaaccctct ttgcggatgt tctctcggtc 540

aaggaatttc ctcgatcgct cttccttcaa ctacttccta aggactttga tatcctatgc 600

acccacccta tgtttggccc agactcgggc aaagacgggt ggggtggact acccttgtg 660

tttgataaag ttagagtcgg atcagatcag agtcggacgt ctcgtgctga ggcattccta 720

gacgtgttta ggaatgccgg gtgtaggatg gtggaaatga gttgtgttga tcatgacaag 780

catgcagccg ggtctcaatt tattacacat atgatgggac gagttttgga gaaattggcc 840

ttggaaaata caccaattaa tacaaaaggg tacgaaagtt tgttaaattt ggtggataat 900

actgcaaggg atagttttga gttgttttat gggttgtttt tgtacaataa aaatgcaatg 960

gagcaattgg atagaatgga ttgggctttc gagatggtaa aaaagcaact ttcgggatat 1020

ttgcatgatc ttgttagaaa acaattgatg ttggagggta ataatgatca agctgaggtt 1080

acttttgaca aaccattaat gcttcctttct cctactatta atcctccaca aatagttcct 1140

tctgctgata tggctgagaa gaagcatgat ttagtggtgg ttaatggtac tagatag 1197

<210> SEQ ID NO 22
<211> LENGTH: 1197
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:

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<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1197)
<223> OTHER INFORMATION: Beta vulgaris W357B arogenate dehydrogenase alpha, complete CDS

<400> SEQUENCE: 22

atgatttcac tctcttcttt tcatccttcc tccaccaccg ccaccgccac cgccgccgcc 60

gccaccaccc acccacctca acaatgtccc gctttttcct ctcctccgtc gcatctctcg 120

cttcctttac gccaccctcg ccaacacctt gtagttcggt gcggtggagg tggttcggcc 180

tccgaatcgg tatttaaccg tgatagtgct gctactcgtg tttctaatga tcatcttgac 240

gttagtaaaa gagatgttaa gcttaagatt gctattattg ggtttggtaa ctttggccag 300

tttttggcta agacaatggc taagcaaggt catagagtgt tggcttactc acgctcggac 360

tactcccgcg ctgctaagga gatcggcgtc gagtatttta ctgacgccga tgacctctgc 420

gaggagcacc ctgaggttat tctgttgtgc acatccatcc tctcaacgga gaaggtcctc 480

cgatcactcc ccctccaccg gctccgtcgt tcaaccctct ttgcggatgt tctctcggtc 540

aaggaatttc ctcgatcgct cttccttcaa ctacttccta aggactttga tatcctatgc 600

acccacccta tgtttggccc agactcgggc aaagacgggt ggggtggact acctttttgtg 660

ttcgataaag ttagagtcgg atcagatcag agtcggacat ctcgtgctga ggcattccta 720

gacgtgttta ggaatgccgg gtgtaggatg gtggaaatga gttgtgttga tcatgacaag 780

catgcagccg ggtctcaatt tattacacat atgatgggac gagttttgga gaaattggcc 840

ttggaaaata caccaattaa tacaaaaggg tacgaaagtt tgttaaattt ggtggataat 900

actgcaaggg atagttttga gttgttttac gggttgtttt tgtacaataa aaatgcaatg 960

gagcaattgg atagaatgga ttgggctttc gagatggtaa aaaagcaact ttcgggatat 1020

ttgcatgatc ttgttagaaa acaattgatg ttggagggta ataatgatca agctgaggtt 1080

acttttgaca aaccattgat gcttccttct cctactatta atcctccaca aatagttccc 1140

tctgctgata tggctgagaa gaagcatgat ttagtggtgg ttaatggtac tagatag 1197

<210> SEQ ID NO 23
<211> LENGTH: 1197
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1197)
<223> OTHER INFORMATION: Beta vulgaris Blankoma arogenate dehydrogenase alpha, complete CDS

<400> SEQUENCE: 23

atgatttcac tctcttcttt tcatccttcc tccaccaccg ccaccgccac cgccgccgcc 60

gccaccaccc acccaccaca acaatgtccc gctttttcct ctcctccgtc gcatctctcg 120

cttcctttac gccaccctcg ccaacacctt gtagttcggt gcggtggagg tggttcggcc 180

tccgaatcgg tatttaaccg tgatagtgct gctactcgtg tttctaatga tcatcttgac 240

gttagtaaaa gagatgttaa gcttaagatt gctattattg ggtttggtaa ctttggccag 300

tttttggcta agacaatggc taagcaaggt catagagtgt tggcttactc acgctcggac 360

tactcccgcg ctgctaagga gatcggcgtc gagtatttta ctgacgccga tgacctctgc 420

gaggagcacc ctgaggttat tctgttgtgc acgtccatcc tctcaacgga gaaggtcctc 480

-continued

cgatcactcc ccctccaccg gctccgtcgt tcaaccctct ttgcggatgt tctctcggtc 540

aaggaatttc ctcgatcgct cttccttcaa ctacttccta aggactttga tatcctatgc 600

acccacccta tgtttggccc agactcgggc aaagacgggt ggggtggact acctttttgtg 660

ttcgataaag ttagagtcgg atcagatcag agtcggacat ctcgtgctga ggcattccta 720

gacgtgttta ggaatgccgg gtgtaggatg gtggaaatga gttgtgttga tcatgacaag 780

catgcagccg ggtctcaatt tattacacat atgatgggac gagttttgga gaaattggcc 840

ttggaaaata caccaattaa tacaaaaggg tacgaaagtt tgttaaattt ggtggataat 900

actgcaaggg atagttttga gttgttttac gggttgtttt tgtacaataa aaatgcaatg 960

gagcaattgg atagaatgga ttgggctttc gagatggtaa aaaagcaact ttcgggatat 1020

ttgcatgatc ttgttagaaa acaattgatg ttggagggta ataatgatca agctgaggtt 1080

acttttgaca aaccattgat gcttcttctt cctactatta atcctccaca aatagttccc 1140

tctgctgata tggctgagaa gaagcatgat ttagtggtgg ttaatggtac tagatag 1197

<210> SEQ ID NO 24
<211> LENGTH: 1197
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1197)
<223> OTHER INFORMATION: Beta vulgaris Touch Stone arogenate dehydrogenase alpha, complete CDS

<400> SEQUENCE: 24

atgatttcac tctcttcttt tcatccttcc tccaccaccg ccaccgccac cgccgccgcc 60

gccaccaccc acccacctca acaatgtccc gctttttcct ctcctccgtc gcatctctcg 120

cttcctttac gccaccctcg ccaacacctt gtagttcggt gcggtggagg tggttcggcc 180

tccgaatcgg tatttaaccg tgatagtgct gctactcgtg tttctaatga tcatcttgac 240

gttagtaaaa gagatgttaa gcttaagatt gctattattg ggtttggtaa ctttggccag 300

tttttggcta agacaatggc taagcaaggt catagagtgt tggcttactc acgctcggac 360

tactcccgcg ctgctaagga gatcggcgtc gagtatttta ctgacgccga tgacctctgc 420

gaggagcacc ctgaggttat tctgttgtgc acatccatcc tctcaacgga gaaggtcctc 480

cgatcactcc ccctccaccg gctccgtcgt tcaaccctct ttgcggatgt tctctcggtc 540

aaggaatttc ctcgatcgct cttccttcaa ctacttccta aggactttga tatcctatgc 600

acccacccta tgtttggccc agactcgggc aaagacgggt ggggtggact acctttttgtg 660

ttcgataaag ttagagtcgg atcagatcag agtcggacat ctcgtgctga ggcattccta 720

gacgtgttta ggaatgccgg gtgtaggatg gtggaaatga gttgtgttga tcatgacaag 780

catgcagccg ggtctcaatt tattacacat atgatgggac gagttttgga gaaattggcc 840

ttggaaaata caccaattaa tacaaaaggg tacgaaagtt tgttaaattt ggtggataat 900

actgcaaggg atagttttga gttgttttac gggttgtttt tgtacaataa aaatgcaatg 960

gagcaattgg atagaatgga ttgggctttc gagatggtaa aaaagcaact ttcgggatat 1020

ttgcatgatc ttgttagaaa acaattgatg ttggagggta ataatgatca agctgaggtt 1080

acttttgaca aaccattgat gcttcttctt cctactatta atcctccaca aatagttccc 1140

tctgctgata tggctgagaa gaagcatgat ttagtggtgg ttaatggtac tagatag 1197

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<210> SEQ ID NO 25
<211> LENGTH: 1224
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1224)
<223> OTHER INFORMATION: Beta vulgaris subsp.maritima PI562585 arogenate dehydrogenase alpha, complete CDS

<400> SEQUENCE: 25

atgatttcac tctcttcttt tcatccttcc tccaccaccg ccaccgccac cgccgccacc 60

gccaccgcca ccgccgccac cgccaccgcc accacccacc caccacaaca atgtcccgct 120

ttttcctctc ctccatcgca tctctcgctt cctttacgcc accctcgcca acaccttgta 180

gttcggtgcg gtggaggtgg ttcggcctcc gaatcggtat ttaaccgtga tagtgctgct 240

actcgtgttt ctaatgatca tcttgacgtt agtaaaagag atgttaagct taagattgct 300

attattgggt ttggtaactt tggccagttt ttggctaaga caatggctaa gcaaggtcat 360

agagtgttgg cttactcacg ctcggactac tcccgcgctg ctaaggagat cggcgtcgag 420

tattttactg acgccgatga cctctgcgag gagcaccctg aggttattct tttgtgcacg 480

tccatcctct caacggagaa ggtcctccga tcactccccc tccaccggct ccgtcgttca 540

accctctttg cggatgttct ctcggtcaag gaatttcctc gatcgctctt ccttcaacta 600

cttcctaagg actttgatat cctatgcacc caccctatgt ttggcccaga ctcgggcaaa 660

gacgggtggg gtggactacc ctttgtgttt gataaagtta gagtcggatc agatcagagt 720

cggacgtctc gtgctgaggc attcctagac gtgtttagga atgccgggtg taggatggtg 780

gaaatgagtt gtgttgatca tgacaagcat gcagccgggt ctcaatttat tacacatatg 840

atgggacgag ttttggagaa attggccttg gaaaatacac caattaatac aaaagggtac 900

gaaagtttgt taaatttggt ggataatact gcaagggata gttttgagtt gttttatggg 960

ttgtttttgt acaataaaaa tgcaatggag caattggata gaatggattg ggctttcgag 1020

atggtaaaaa agcaactttc gggatatttg catgatcttg ttagaaaaca attgatgttg 1080

gagggtaata atgatcaagc tgaggttact tttgacaaac cattaatgct tccttctcct 1140

actattaatc ctccacaaat agttccttct gctgatatgg ctgagaagaa gcatgattta 1200

gtggtggtta atggtactag atag 1224

<210> SEQ ID NO 26
<211> LENGTH: 1041
<212> TYPE: DNA
<213> ORGANISM: Spinacia oleracea
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1041)
<223> OTHER INFORMATION: Spinacia oleracea arogenate dehydrogenase alpha, partial CDS

<400> SEQUENCE: 26

tgcgccgcct ctgactccgt gttcaaccac gatattggtg tgccttttgt ctcaacacgc 60

gcttccggcg aggtgccgga ggttaacagt agagatatta agcttaagat cgcgatcatt 120

gggttcggga actttggtca gtttttggct aagactatta ctaagcaagg tcacagagtt 180

ttggcttact cccggtcaga ttactcccgt gctgctaagg agatcggcgt cgagtatttc 240

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tccgacgccg atgatctttg cgaagagcat cccgaggtga tactcctatg cacttcaatc 300

ctctcaacag agaaggtcct ccgttcgctc cccctccacc gccttcgccg gtccaccctc 360

ttcgtggacg tcctctcggt gaaggagttc ccgcgttcac ttttcctcca agtccttcct 420

aaagactttg acatcctttg cacccacccc atgttcggcc cagactcagg caaaagcgga 480

tggggtgggc tccccttttgt cttcgacaaa gtccgagtcg ggtcggaccc aacccgggcg 540

gctcggactg aggcgttcct agacatttat aggaacgccg ggtgtaggat ggtggaaatg 600

acatgcgcgg accacgacaa gcacgcggct gggtcgcaat tcataaccca catgatgggc 660

cgggttttgg agaaattagc cctcgaaaac acaccgatta acacgaaagg gtacgagagt 720

ttgttgaact tggtggataa tacggcccgg gacagctttg agttgtttta cggactgttt 780

ttgtacaaca agaacgcgat ggaacaattg gatagaatgg attgggcttt cgagatggta 840

aagaagcaac tttcgggtta tttgcatgat cttgttagga aacaattgat gctagagact 900

accaatgaac aagttgggtt tgatcagacg ttcatgcttc cttctcctgc cgataatcct 960

cgtcaaacac caccctcggc tgccgtttcc gagaattcga aacccgattt tgtggtggta 1020

aatggtaata attctagata g 1041

<210> SEQ ID NO 27
<211> LENGTH: 1128
<212> TYPE: DNA
<213> ORGANISM: Rivina humilis
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1128)
<223> OTHER INFORMATION: Rivina humilis arogenate dehydrogenase alpha, partial CDS

<400> SEQUENCE: 27

tgcacggcct tcactaaaac taataataat aatgccttgg gttatggtta cggttatggt 60

tatggttatg gctatgacaa aaacaaggtg tctagtactg aacagggtga tgaggtttcg 120

ggttcgagtt cgaattcgaa gaagctgaag attggtataa ttgggttcgg gaactttggt 180

cagtttatgg caaagacgat ggtgaaacat ggtcacactg tgcttgctta ttctcgttcc 240

gattactcac gtgctgctca taccatcggt gttcgctact tctctgatcc tgatgacttg 300

tgcgaagagc accctgaggt gattctactg tgcacctcca tcttatccac tgaaagggtg 360

cttcggtcac taccgcttca tcgcctacgc cgctcaacac tcgttgcgga tgtgctgtcg 420

gtcaaggaat tcccacgttc actcttccta caactcctcc cttctgactt tgacatcctt 480

tgcactcatc ctatgttcgg accggactcc ggcaaggccg ggtggggcgg tcttcctttc 540

gtctttgaca aagtccgggt tggatcccaa cccgaacgcc tcacccgtgt tgaggccttc 600

ctggacattt tccgggatgc cgggtgccgg atggtggaga tgagttgtgc tgagcatgac 660

aggcatgctg ctgggtcaca attcataaca cacatgatgg gacgtgtgtt agagaagctt 720

gcacttgagg acacaccaat taacaccaaa gggtatgaga gtttgttgaa cttggttgat 780

aacactgcta gggacagttt tgagctgttt tatggactct ttttatacaa caagaatgca 840

atggaacagc ttgatagaat gcattgggca tttgagacag tgaagcaaca gctctctggt 900

tatttgcatg tccttgttag gaagcagttg atgttggaga cttcttccgg taatgacaat 960

aataatacta ataatattaa tattagcagt ggtgataata ttaataataa ggacacaaat 1020

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aataaattaa tgttaccttc tcctgggatt agttctgcta aaattgttcc accagtacag 1080

gagaaggaga aacatgactt ggtgatgctc aatggatcaa agcggtag 1128

<210> SEQ ID NO 28
<211> LENGTH: 1026
<212> TYPE: DNA
<213> ORGANISM: Portulaca oleracea
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1026)
<223> OTHER INFORMATION: Portulaca oleracea arogenate dehydrogenase alpha, partial CDS

<400> SEQUENCE: 28

tgctcatcat catcatcatc cagtgccagc atcatcatca atggttccgg tagctccacg 60

acaaactcga gcgtcttcga tgctagttct tcctccgatt cagacgtaaa aaaaaggtca 120

gaagtgaagc tgaaaatcgg gatcattgga tttgggaagt ttggacagtt tctagcgaag 180

agaattgtga gtcagggtca tgatgtcttg gcgtattctc ggtcggatta ctcacgggtg 240

gcatcggaga ttggcgtacg gttcttctct gacgccgatg acctctgcga ggagcaccct 300

caggtgatcc tgttatgcac atcaatcctg tcaaccgagc gcgttctgcg ctcgcttcca 360

ctacacaggc tccgtcgatc caccctgttc gcggatgtcc tgtccgtaaa agagttcccg 420

cggtcactct tcttacaatt actcccctcc gacttcgaca ttctatgcac acaccccatg 480

ttcggacccg actcaggcaa gtccgggtgg gacagtcttc cctttgtctt cgacaaggtc 540

cgggtcggat ccacccctac tcgggtcacc cggtccgagg ccttcctaga catcttccgg 600

accgccgggt gtaggatggt ggaaatgagc tgcgccgagc acgacaaaca cgcagccggg 660

tcccagttca taacccatat gatgggccgg gttctcgaga agttagactt ggaaaacaca 720

cccataaaca ccagaggata tgagagtttg agaaacctgg tggacaacac ggcaagggac 780

agctttgagc tgttttatgg attgtttttg tacaacaaaa acgcgacgga gcagcttgac 840

aggatggatt gggcattcga gatggttaag aaacaacttt ctgggtatct tcatcatcta 900

gttaggaaac agttgatgtt agagagtagt aatacacatg aaaatcatgt tgacaacaaa 960

ttgttgcttc cagagaataa gcagaagcaa catgacttgg tcgtcgtcgt caacgataga 1020

tcatag 1026

<210> SEQ ID NO 29
<211> LENGTH: 1191
<212> TYPE: DNA
<213> ORGANISM: Spergularia marina
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1191)
<223> OTHER INFORMATION: Spergularia marina arogenate dehydrogenase alpha mRNA, complet CDS

<400> SEQUENCE: 29

atgatgaatt ctatctcctt tgtcaactct tcctcaacaa caaccgcgga tattatctac 60

ttaaaccacc aattttcgcg tcacaagtgt ttttctcgtc ttcctcggga cgcaactcct 120

agggaccgtc ggaagatttc cttggctaga gccatcaacg gctcacctac gtgtagccat 180

gttgaaatcg accaaacgtt ggttagctct agccaagcta ctactagagc ttgtagtaat 240

gagcaaaaga agcttaaaat cgcggtcgta gggttcggga attttggaca gtttttggct 300

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agagaaatgg ttaagcaagg acatcaagtg ttggcttact ctcgctctga ttactcaaag 360

gttgctaaag agattggtgt ccaattcttt agggaccctg atgacctttg cgaggaacat 420

cctcaggtgg ttcttttatg cacctctatt ctctcaacgg agaaggtcct tcgctccctc 480

ccggttgacc gccttcgccg ttccaccctc attgttgacg tcctctcggt taaggagttt 540

ccgcgcaccc ttttcctccg gcacttgcct gaggacttgg acatcctttg cacccatcca 600

atgtttggcc cggactctgg caagtccggg tgggatgggc taccctttgt atttgataaa 660

gtccgagttg gatcagaccc aacccggacc cacagagtca acacattctt ggatatattt 720

aaacacgcag ggtgtagaat ggttgagatg acgtgtatgg accatgacaa gcatgcagcc 780

ggttcccagt ttataaccca catgatgggt cgggtcttag agaaagtggg cctttcaaat 840

acacccatta atacaaaagg gtatgagagt ttgttgaatt tggtggataa tacagcaaga 900

gatagctttg agttgtttta tggactgttt ttgtacaaca aaaatgcaat ggaggagttg 960

gatagattgg actgggcctt tgatacggta aaaatgcagc tttctgggta tttgcatgat 1020

tttgctagta aaaagttgat gttggagact ggtaatgaac tagctgggat tgttagtggt 1080

aaaattggcg acgacaatca taataacaag aggttaatgc tctcccctcc tacaaattct 1140

tacaagaatg ttacttttac tgatacgaaa gtttcggaga aaatgatgtg a 1191

<210> SEQ ID NO 30
<211> LENGTH: 1158
<212> TYPE: DNA
<213> ORGANISM: Paronychia polygonifolia
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1158)
<223> OTHER INFORMATION: Paronychia polygonifolia dehydrogenase alpha mRNA, complete CDS

<400> SEQUENCE: 30

atgaattcta tctctattgt aagctctact aagtctactt attacaaagt ctaccaattt 60

ccatcaccta agatatgttt cttccaccct tctaagctct ctattccttc ttgccacctt 120

aagtttcaaa attttgccgt acgttgcaat agtagtaaca acccaaaaaa tgtttcaaac 180

tctaaggata ataaatggaa gcctagtgaa attaacaagg gaattaagct taaaatcgcg 240

gtagtggggt tcggcaactt tgggcagttc ttggctaagg aaatggttaa gcaaggccat 300

caagtggtgg cgtactctcg tactgattat actaaggttg ctcaagatat gggtgttcgc 360

ttcttttctg atgcttgtga aatgttcatt gagcaacccg aggtgattct aatgtgcacc 420

tctatcctct ctacggagaa ggtgttgcgc tccctccctc tccaccgtct ccggccagcc 480

accatcttcg tggacgtcct ctccgtgaag gagttccccc ggtccctctt cctccaacac 540

ctccccaagg acttcggcat cctttgcact cacccaatgt ttgggccaaa ctcagccaag 600

gccgggtggg ccgggctccc cttcgttcta gacagggttc gggtcagtat tgacccgacc 660

caagccaccc ggacagaggc attcctagac atattccgaa atgcagggtg taggatggtg 720

gaaatgactt gtgaagacca tgacaagcat gcagccgggt cacagttcat aacccacatg 780

atgggtcggg ttcttgagaa agtggggctc cgaaatacac ccattaatac aaaagggtac 840

gaaagtttgt tgaatttggt ggagaataca ggaagagata gctttgagtt gttttatggg 900

ttgttcttgt acaatgaaaa tgcaatggtg caattagaga ggttggactg ggcttttaag 960

aaggttaaga gtcaactttc tgcatgtatg catgatcatg ttagggagag ccttatgttt 1020

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gagtctcatg gagatcaaaa taagattatg aaaaaggcga gttacaagtc actcctatca 1080

gcctatacag aaaaaagtaa taagattgtc aaagatacaa agattaagaa ggacttggtg 1140

attagtgggc aacaataa 1158

<210> SEQ ID NO 31
<211> LENGTH: 720
<212> TYPE: DNA
<213> ORGANISM: Herniaria latifolia
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(720)
<223> OTHER INFORMATION: Herniaria latifolia arogenate dehydrogenase alpha mRNA, partial CDS

<400> SEQUENCE: 31

agtcgggttt gggaactttg ggcagttctt agccaaagaa atggtgaagc aaggtcatca 60

agtgttggct tattctcgct ctgattattc aagggttgct caggagattg gcgtacagta 120

tttctcaaat cccgacgacc tttgcaaaga gcatcctgag gttatcctcc tgtgcacatc 180

catcctctcc actgaaaaag tcctaaatac ccttcccctc gaccgcctcc gaccatcaac 240

tctcttctcc gatgtgctct ccgtcaagga attccctcgt acacttttcc tccagcaact 300

acccgaggac tttgacatca tctgtaccca tccaatgttc ggcccggact cgggcaaaca 360

cgggtgggca gggctcccct acgtctacga caaagtacgt gtcgggttgg atccgacccg 420

gatccgccga gcggaggcat ttcttaacat tttcgaaagg gcagggtgta ggatggtgga 480

gatgacgtgt gcagagcatg acaagcatgc agctgggtcc cagttcataa cccacatgtt 540

gggccgagtt ttggagaaag tgggcctttt aaatacgccc attaacacaa aagggtacga 600

gagtttgttg agcttggtgg ataatacagc aagagacagc tttgagttgt tttatgggct 660

ttttttgtac aacaaaaatg caatggagca gttggatcga ttggattggg cctttgacat 720

<210> SEQ ID NO 32
<211> LENGTH: 1077
<212> TYPE: DNA
<213> ORGANISM: Corrigiola litoralis
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1077)
<223> OTHER INFORMATION: Corrigiola litoralis arogenate dehydrogenase alpha, partial CDS

<400> SEQUENCE: 32

tgcagcaagg gtgtgcatgg catgaatggc tcagctgatc attttcatcc taacattaag 60

gttaatggtg aggttttgaa ccctatggtt ggctctagtg atgtagccga ggatgttaag 120

ctaaaaatcg ccatagttgg gtttggaaac ttcggacaat tcttggctaa ggaaattgtt 180

aagcagggtc ataaggtgtt ggcttactct cggtctgatt actctaaggc tgctaaggag 240

attggtgtgc agtatttttc cgatgctgat gacctgtgtg aggagcatcc tgaggtgatc 300

ctcctttgca cctctatcct ctcaacggag aaggtgatgc gcgccctccc tatccaccgc 360

cttcgccggt ccaccctctt cgtcgatgtt ctctcagtga aggagttccc ccgctcactc 420

ttcctccaag ttctccctaa ggactttgac atcctctgca cccacccaat gttcggccct 480

gactccggca aagccgggtg gggtggactc ccttttgtct ttgacaaagt tcgggttgcg 540

ccagactcca cccgggctac tagggccgag gcatttctag acatcttcag aagagcaggg 600

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tgccgaatgg tagaaatgac ttgtgcagac cacgacaagc atgcagcagg atcgcagttc 660

atcacacaca tgatgggtcg ggtgctagag aaaatagggc ttgaaaatac tcccatcaac 720

acaaaagggt acgagagttt gctcaatttg gtggacaata cggcgagaga cagctttgag 780

ttgttttatg ggttgttttt gtataataag aacgcaatgg agcagttaga tagaatggac 840

tgggcttttg agatgataaa gaagcgactt tcaggatact tgcatgatct tgttaggaag 900

cagttgatgc tagaaactac tggtaatgat caagctggtc taactaacgg tgcaaaaaat 960

aatcatgaca agaagctcat gcttcctcct cctgctgcca atccttctat gattgttcct 1020

tctgctgcta ctcatgagaa gaagcatgat ttggtgcatg tcaatggaag cagatga 1077

<210> SEQ ID NO 33
<211> LENGTH: 990
<212> TYPE: DNA
<213> ORGANISM: Telephium imperati
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(990)
<223> OTHER INFORMATION: Telephium imperati arogenate dehydrogenase alpha, partial CDS

<400> SEQUENCE: 33

gtattgggga gtatggttgg tcctagtgag agtgggaagg atgttaagct tgaaatcgcg 60

gtagtcgggt tcgggaactt tgggcagttt ttgggtaggg aaattgttaa gcaggggcat 120

gaggtgttgg cttattctcg gtctgattac tccaaagttg ctaaggagat tggtgtacgt 180

tatttttccg acgctcatga cttgtgtgag gagcatcctg aggtgatcct cctatgcaca 240

tccatcctct caacagagag ggtcctccac tccctccctc taaaccgcct ccgccgctcc 300

accctcttcg tcgacgtcct ctccgtgaag gagttccccc gaaacctctt cctccaaaac 360

ctccccaacg acttcgacat cctctgcacc cacccaatgt tcggcccgga ctccggcaaa 420

gccggctggg acgggctccc cttcgtgttc gacaaggtcc gggtcgggtc agacccggcc 480

cggaccaccc gggccgacac attcctagac atattcagga atgcagggtg caggatggtg 540

gaaatgtcct gtgcagagca tgacaggcac gcagccgggt cacaattcat aacccacatg 600

atgggtcggg ttttggagaa aatcgggctc gaaaacacac ccattaacac aaaagggtac 660

gagagtttgt tgaatttggt ggataataca gcaagggata gctttgaatt gtttttgtat 720

tataagaatg caatggagca attagatagg atggattggg cttttgagat gattaagaag 780

cagctttctg ggtatttgca tgagcttgtt aggaagcaat tgatgctaga gactaataat 840

gatcaatccg ggataattaa tggtaaaact aattgtgata aacgactaat gcttcctcct 900

ccggccgcta atccgtctgt aattgttcct gatcctgttc ctgctgtgaa gaagaagcat 960

gatttggtgc atgtcaatgg aagtagatga 990

<210> SEQ ID NO 34
<211> LENGTH: 1161
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1161)
<223> OTHER INFORMATION: Beta vulgaris W357B arogenate dehydrogenase betta mRNA, complete CDS

<400> SEQUENCE: 34

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atgctttctc tctcctccac aaccaccgca aaaccctcgc cgtcgccatc tccggcgaat 60

tttccggcga aactttcttc tctctccacc atcaccacca ctctctcttt ctctcctcgc 120

cggagatatt ttcatggcgt caaaacccta acaattcgca gcatcgacgc cgcacaattc 180

ttcgattacg aatcaaaact tgccgccatt aacacaacct cttcgtcttc atcttcatct 240

tattcgaagc tcaaaatcgc aatcgtaggg ttcggaaatt acggacaatt tctcgcgaaa 300

accctagttt ctcaaggtca tactgttctc gcttattctc gctctgatta ctctaaaatc 360

gctgcgaatc tcggcgtttc ttacttttct gatcctgatg atctttgcga agaacatcct 420

gaggtaatta tgttgtgtac ttcgatttta tcaactgaag ttatgttgaa ttcgttacca 480

ttgcagcgac ttaaacgatc gacgcttttt gttgatgttt tatcggtgaa agaatttccg 540

cgtaatttgt ttcttcaaac tttaccgtct gattttgata tattatgtac tcatcctatg 600

tttgggcctg aatctgggaa aaatggttgg ggaagtttgc cttttgttta tgataaggtt 660

aggattggga aagatgaggg tagaattaag agatgtgaga gttttttgga tgtttttagg 720

agagaaggtt gtagggttga ggaaatgact tgtgctgagc atgataagtt tgcagcaggg 780

tctcagttta taacacattt cttagggagg gttttggaga agcttgattt ggaggatacg 840

ccgattaata cgaaagggta tgagagtttg ttgaatttgg tggataatac gtcgaaggat 900

agtttcgagt tgttttatgg gttgtttttg tataatcaga atgctatgga gcagttagag 960

aggttagatt gggcgtttga gttggttaag aagcaattgt ttggacactt gcatgggttg 1020

ctaaggaaac agttgtttgg gttttctgag atagatgaac gtattgggaa ggcgaaggag 1080

atcaaatttc tctctgatgc tgcagaacag aatggctctg ccttgtctgc tagggagaat 1140

gcaaattcgg agacaaattg a 1161

<210> SEQ ID NO 35
<211> LENGTH: 1161
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1161)
<223> OTHER INFORMATION: Beta vulgaris Big Buck arogenate dehydrogenase
betta mRNA, complete CDS

<400> SEQUENCE: 35

atgctttctc tctcctccac aaccaccgca aaaccctcgc cgtcgccatc tccggcgaat 60

tttccggcga aactttcttc tctctccacc atcaccacca ctctctcttt ctctcctcgc 120

cggagatatt ttcatggcgt caaaacccta acaattcgca gcatcgacgc cgcacaattc 180

ttcgattacg aatcaaaact tgccgccatt aacacaacct cttcgtcttc atcttcatct 240

tattcgaagc tcaaaatcgc aatcgtaggg ttcggaaatt acggacaatt tctcgcgaaa 300

accctagttt ctcaaggtca tactgttctc gcttattctc gctctgatta ctctaaaatc 360

gctgcgaatc tcggcgtttc ttacttttct gatcctgatg atctttgcga agaacatcct 420

gaggtaatta tgttgtgtac ttcgatttta tcaactgaag ttatgttgaa ttcgttacca 480

ttgcagcgac ttaaacgatc gacgcttttt gttgatgttt tatcggtgaa agaatttccg 540

cgtaatttgt ttcttcaaac tttaccgtct gattttgata tattatgtac tcatcctatg 600

tttgggcctg aatctgggaa aaatggttgg ggaagtttgc cttttgttta tgataaggtt 660

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aggattggga aagatgaggg tagaattaag agatgtgaga gttttttgga tgtttttagg 720

agagaaggtt gtagggttga ggaaatgact tgtgctgagc atgataagtt tgcagcaggg 780

tctcagttta ttacacattt cttagggagg gttttggaga agcttgattt ggaggatacg 840

ccgattaata cgaaagggta tgagagtttg ttgaatttgg tggataatac gtcgaaggat 900

agtttcgagt tgttttatgg gttgtttttg tataatcaga atgctatgga gcagttagag 960

aggttagatt gggcgtttga gttggttaag aagcaattgt ttggacactt gcatgggttg 1020

ctaaggaaac agttgtttgg gttttctgag atagatgaac gtattgggaa ggcgaaggag 1080

atcaaatttc tctctgatgc tgcagaacag aatggctctg ccttgtctgc tagggagaat 1140

gcaaattcgg agacaaattg a 1161

<210> SEQ ID NO 36
<211> LENGTH: 1161
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1161)
<223> OTHER INFORMATION: Beta vulgaris Touch Stone arogenate dehydrogenase betta mRNA, complete CDS

<400> SEQUENCE: 36

atgctttctc tctcctccac aaccaccgca aaaccctcgc cgtcgccatc tccggcgaat 60

tttccggcga aactttcttc tctctccacc atcaccacca ctctctcttt ctctcctcgc 120

cggagatatt ttcatggcgt caaaacccta acaattcgca gcatcgacgc cgcacaattc 180

ttcgattacg aatcaaaact tgccgccatt aacacaacct cttcgtcttc atcttcatct 240

tattcgaagc tcaaaatcgc aatcgtaggg ttcggaaatt acggacaatt tctcgcgaaa 300

accctagttt ctcaaggtca tactgttctc gcttattctc gctctgatta ctctaaaatc 360

gctgcgaatc tcggcgtttc ttacttttct gatcctgatg atctttgcga agaacatcca 420

gaggtaatta tgttgtgtac ttcgatttta tcaactgaag ttatgttgaa ttcgttacca 480

ttgcagcgac ttaaacgatc gacgcttttt gttgatgttt tatcggtgaa agaatttccg 540

cgtaatttgt ttcttcaaac tttaccgtct gattttgata tattatgtac tcatcctatg 600

tttgggcctg aatctgggaa aaatggttgg ggaagtttgc cttttgttta tgataaggtt 660

aggattggga aagatgaggg tagaattaag agatgtgaga gttttttgga tgtttttagg 720

agagaaggtt gtagggttga ggaaatgact tgtgctgagc atgataagtt tgcagcaggg 780

tctcagttta taacacattt cttagggagg gttttggaga agcttgattt ggaggatacg 840

ccgattaata cgaaagggta tgagagtttg ttgaatttgg tggataatac gtcgaaggat 900

agtttcgagt tgttttatgg gttgtttttg tataatcaga atgctatgga gcagttagag 960

aggttagatt gggcgtttga gttggttaag aagcaattgt ttggacactt gcatgggttg 1020

ctaaggaaac agttgtttgg gttttctgag atagatgaac gtattgggaa ggcgaaggag 1080

atcaaatttc tctctgatgc tgcagaacag aatggctctg ccttgtctgc tagggagaat 1140

gcaaattcgg agacaaattg a 1161

<210> SEQ ID NO 37
<211> LENGTH: 1161
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris

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<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1161)
<223> OTHER INFORMATION: Beta vulgaris Blankoma arogenate dehydrogenase betta mRNA, complete CDS

<400> SEQUENCE: 37

atgctttctc tctcctccac aaccaccgca aaaccctcgc cgtcgccatc tccggcgaat 60

tttccggcga aactttcttc tctctccacc atcaccacca ctctctcttt ctctcctcgc 120

cggagatatt ttcatggcgt caaaacccta acaattcgca gcatcgacgc cgcacaattc 180

ttcgattacg aatcaaaact tgccgccatt aacacaacct cttcgtcttc atcttcatct 240

tattcgaagc tcaaaatcgc aatcgtaggg ttcggaaatt acggacaatt tctcgcgaaa 300

accctagttt ctcaaggtca tactgttctc gcttattctc gctctgatta ctctaaaatc 360

gctgcgaatc tcggcgtttc ttacttttct gatcctgatg atctttgcga agaacatcct 420

gaggtaatta tgttgtgtac ttcgatttta tcaactgaag ttatgttgaa ttcgttacca 480

ttgcagcgac ttaaacgatc gacgcttttt gttgatgttt tatcggtgaa agaatttccg 540

cgtaatttgt ttcttcaaac tttaccgtct gattttgata tattatgtac tcatcctatg 600

tttgggcctg aatctgggaa aaatggttgg ggaagtttgc cttttgttta tgataaggtt 660

aggattggga aagatgaggg tagaattaag agatgtgaga gttttttgga tgtttttagg 720

agagaaggtt gtagggttga ggaaatgact tgtgctgagc atgataagtt tgcagcaggg 780

tctcagttta taacacattt cttagggagg gttttggaga agcttgattt ggaggatacg 840

ccgattaata cgaaagggta tgagagtttg ttgaatttgg tggataatac gtcgaaggat 900

agtttcgagt tgttttatgg gttgtttttg tataatcaga atgctatgga gcagttagag 960

aggttagatt gggcgtttga gttggttaag aagcaattgt ttggacactt gcatgggttg 1020

ctaaggaaac agttgtttgg gttttctgag atagatgaac gtattgggaa ggcgaaggag 1080

atcaaatttc tctctgatgc tgcagaacag aatggctctg ccttgtctgc tagggagaat 1140

gcaaattcgg agacaaattg a 1161

<210> SEQ ID NO 38
<211> LENGTH: 1161
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1161)
<223> OTHER INFORMATION: Beta vulgaris subsp.maritima PI562585 arogenate dehydrogenase betta, complete CDS

<400> SEQUENCE: 38

atgctttctc tctcctccac aaccaccgca aaaccctcgc cgtcgccatc tccggcgaat 60

tttccggcaa aactttcttc tctctccacc atcaccacca ctatctcctt ctctcctcgc 120

cggagatatt ttcatggcgt caaaacccta acaattcgca gcatcgacgc tgcacaattc 180

ttcgattacg aatcaaaact cgccgccatt aacacaacct cttcatctac atcgtcatct 240

tattcgaaac tcaaaatcgc aatcgtaggt ttcggaaatt acggacaatt tctcgcgaaa 300

accctagttt ctcaaggtca tactgttctc gcttattctc gctctgatta ctctaaaatc 360

gctgcgaatc tcggcgtttc ttacttttct gatcctgatg atctttgcga agaacatcct 420

gaggtaatta tgttgtgtac ttcgatttta tcaactgaag ttatgttgaa ttcgttacca 480

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ttgcagcgac ttaaacgatc gacgcttttt gttgatgttt tatcggtgaa agaatttccg 540

cgtaatttgt ttcttcaaac tttaccgtct gattttgata tattatgtac tcatcctatg 600

tttgggcctg aatctgggaa aaatggttgg ggaagtttgc cttttgttta tgataaggtt 660

aggattggga aagatgaggg tagaattaag agatgtgaga gttttttgga tgtttttagg 720

agagaaggtt gtagggttga ggaaatgact tgtgctgagc atgataagtt tgcagcaggg 780

tctcagttta ttacacattt cttagggagg gttttggaga agcttgattt ggaggatacg 840

ccgattaata cgaaagggta tgagagtttg ttgaatttgg tggataatac gtcgaaggat 900

agtttcgagt tgttttatgg gttgtttttg tataatcaga atgctatgga gcagttagag 960

aggttagatt gggcatttga gttggttaag aagcaattgt ttggacactt gcatgggttg 1020

ctaaggaaac agttgtttgg gttttctgag atagatgaac gtattgggaa ggcgaaggag 1080

atcaaatttc tctctgatgc tgcagaacag aatggctctg ccttgtctgc tagggagaat 1140

gcaaattcgg agacaaattg a 1161

<210> SEQ ID NO 39
<211> LENGTH: 966
<212> TYPE: DNA
<213> ORGANISM: Spinacia oleracea
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(966)
<223> OTHER INFORMATION: Spinacia oleracea arogenate dehydrogenase
betta, partial CDS

<400> SEQUENCE: 39

gccgctacca atacctccac cgccacctct tcctcacagt cgtcgtactc gaagctcaag 60

gtggcaatcg ttgggttcgg aaactatgga caatttctcg caaaaactat ggtttctcaa 120

ggtcatactg ttcttgcata ttctcggtct gattactcga aaatagctcc aaatctgggc 180

gtttcgttct tttccgatcc tgatgattta tgtgaagaac atccggaggt aattttgctg 240

tgcacttcga ttttatcaac tgaatttatg ttgaattcac taccattgca acgtcttaag 300

aggtcgacgc tttttgttga tgttttatcg gttaaggagt ttccccgtaa cttgtttctt 360

cagactttgc cgcctgattt tgatatttta tgcactcatc ctatgtttgg tcctgaatct 420

gggaaaaatg gatggggagg tttgccgttt gtttatgata aggttaggat tgggaaagca 480

gagcgtagaa ttaggaggtg tgagaatttt ttggatgttt ttaggagagc agggtgtagg 540

gttgaggaga tgacttgtgc agagcatgat aaatacgcgg cgggttcaca gtttattacg 600

catttcctgg ggagggtttt ggagaagctt gatttggagg atacaccgat taacacgaaa 660

gggtacgaga gtttgttgaa tttggtggat aatacgtcga aggatagttt cgagttgttt 720

tatgggttgt ttttgtacaa ccagaatgct atggagcagt tggagaggtt agattgggca 780

ttcgagttgg ttaagaagca gttgtttggg catttgcatg gtttgttaag gggtcagttg 840

tttgggtgta ctgagattga tgaacgtctt gagaaggcaa aggagttgaa gtttctttct 900

gatgccacga cacaaaatgg ctctgcctcc gctcctagag aaaatgcaaa ttcagagatc 960

aattga 966

<210> SEQ ID NO 40
<211> LENGTH: 966
<212> TYPE: DNA

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<213> ORGANISM: Nepenthes ventricosa
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(966)
<223> OTHER INFORMATION: Nepenthes ventricosa x Nepenthes alata
arogenate dehydrogenase betta, partial CDS

<400> SEQUENCE: 40

gccgcgctgc caaacgacta cgaaacgaag ctttcccatc tccctagttc tttcgcgaaa 60

ctcaaggtcg ggatcattgg gttcggcaat tacgggcagt tccttgccaa aaccctagtc 120

cggcaaggcc acaccgttct cgctcattct cgctccaatt actcccaaaa cgccgcgaag 180

ctcggcgtct ctttcttcta tgatcccaat gacctatgcg aggaacaccc ggaagttatc 240

ctcctctgca cctcgattct gtcgacggaa tctgtcctcc ggagcctgcc attgcagcgg 300

ctcaagcggt ctactctctt cgtcgacgtt ttgtcggtga aggagtttcc tcgatcgctt 360

ttgctccaaa ttctgccccc tgacttagac attctctgca ctcaccccat gttcgggccg 420

gaatccggca agaacggctg gagcgggctg ccgttcgttt acgataaggt tagaatcggc 480

gaacatgaga ttagggttaa caggtgtgat aattttatcg aagtgttcag gagggaaggg 540

tgtaggatgg tacagatgag ctgtgcggag cacgatcggc atgcggctgg ctctcagttt 600

ataactcata tgatggggag agttttggag aagttgaaat tagaggatac gcccattaat 660

acgaaaggct atgagagttt gttgaatttg gtggagaaca ctgcgaggga tagtttcgag 720

ttgttttatg ggctgtttct gtataataag aacgttatgg agcagctgga gaggatggat 780

ttagcgttcg agatggttaa aaagcagttg tttggccatt tacatgggtt gttgaggagc 840

cagttgtttg atggttccga aatggaagtt agagtggagg aggagagaaa attgttgtcc 900

gatgggtctc agaatgggca cgtttttttct tcttttttcag atagtaaaaa tgttgagaga 960

aattga 966

<210> SEQ ID NO 41
<211> LENGTH: 53
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(53)
<223> OTHER INFORMATION: BvADHa N-terminal plastid transit peptide

<400> SEQUENCE: 41

Met Ile Ser Leu Ser Ser Phe His Pro Ser Ser Thr Thr Ala Thr Ala
1 5 10 15

Thr Ala Ala Ala Ala Thr Thr His Pro Pro Gln Gln Cys Pro Ala Phe
20 25 30

Ser Ser Pro Pro Ser His Leu Ser Leu Pro Leu Arg His Pro Arg Gln
35 40 45

His Leu Val Val Arg
50

<210> SEQ ID NO 42
<211> LENGTH: 74
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(74)
<223> OTHER INFORMATION: BvADHb N-terminal plastid transit peptide

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<400> SEQUENCE: 42

Met Leu Ser Leu Ser Ser Thr Thr Thr Ala Lys Pro Ser Pro Ser Pro
1 5 10 15

Ser Pro Ala Asn Phe Pro Ala Lys Leu Ser Ser Leu Ser Thr Ile Thr
20 25 30

Thr Thr Leu Ser Phe Ser Pro Arg Arg Arg Tyr Phe His Gly Val Lys
35 40 45

Thr Leu Thr Ile Arg Ser Ile Asp Ala Ala Gln Phe Phe Asp Tyr Glu
50 55 60

Ser Lys Leu Ala Ala Ile Asn Thr Thr Ser
65 70

<210> SEQ ID NO 43
<211> LENGTH: 398
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(398)
<223> OTHER INFORMATION: BvADH-alpha Boltardy red beet variety

<400> SEQUENCE: 43

Met Ile Ser Leu Ser Ser Phe His Pro Ser Ser Thr Thr Ala Thr Ala
1 5 10 15

Thr Ala Ala Ala Ala Thr Thr His Pro Pro Gln Gln Cys Pro Ala Phe
20 25 30

Ser Ser Pro Pro Ser His Leu Ser Leu Pro Leu Arg His Pro Arg Gln
35 40 45

His Leu Val Val Arg Cys Gly Gly Gly Gly Ser Ala Ser Glu Ser Val
50 55 60

Phe Asn Arg Asp Ser Ala Ala Thr Arg Val Ser Asn Asp His Leu Asp
65 70 75 80

Val Ser Lys Arg Asp Val Lys Leu Lys Ile Ala Ile Ile Gly Phe Gly
85 90 95

Asn Phe Gly Gln Phe Leu Ala Lys Thr Met Ala Lys Gln Gly His Arg
100 105 110

Val Leu Ala Tyr Ser Arg Ser Asp Tyr Ser Arg Ala Ala Lys Glu Ile
115 120 125

Gly Val Glu Tyr Phe Thr Asp Ala Asp Asp Leu Cys Glu Glu His Pro
130 135 140

Glu Val Ile Leu Leu Cys Thr Ser Ile Leu Ser Thr Glu Lys Val Leu
145 150 155 160

Arg Ser Leu Pro Leu His Arg Leu Arg Arg Ser Thr Leu Phe Ala Asp
165 170 175

Val Leu Ser Val Lys Glu Phe Pro Arg Ser Leu Phe Leu Gln Leu Leu
180 185 190

Pro Lys Asp Phe Asp Ile Leu Cys Thr His Pro Met Phe Gly Pro Asp
195 200 205

Ser Gly Lys Asp Gly Trp Gly Gly Leu Pro Phe Val Phe Asp Lys Val
210 215 220

Arg Val Gly Ser Asp Gln Ser Arg Thr Ser Arg Ala Glu Ala Phe Leu
225 230 235 240

Asp Val Phe Arg Asn Ala Gly Cys Arg Met Val Glu Met Ser Cys Val

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               245                 250                 255

Asp His Asp Lys His Ala Ala Gly Ser Gln Phe Ile Thr His Met Met
            260                 265                 270

Gly Arg Val Leu Glu Lys Leu Ala Leu Glu Asn Thr Pro Ile Asn Thr
        275                 280                 285

Lys Gly Tyr Glu Ser Leu Leu Asn Leu Val Asp Asn Thr Ala Arg Asp
    290                 295                 300

Ser Phe Glu Leu Phe Tyr Gly Leu Phe Leu Tyr Asn Lys Asn Ala Met
305                 310                 315                 320

Glu Gln Leu Asp Arg Met Asp Trp Ala Phe Glu Met Val Lys Lys Gln
                325                 330                 335

Leu Ser Gly Tyr Leu His Asp Leu Val Arg Lys Gln Leu Met Leu Glu
            340                 345                 350

Gly Asn Asn Asp Gln Ala Glu Val Thr Phe Asp Lys Pro Leu Met Leu
        355                 360                 365

Pro Ser Pro Thr Ile Asn Pro Pro Gln Ile Val Pro Ser Ala Asp Met
    370                 375                 380

Ala Glu Lys Lys His Asp Leu Val Val Val Asn Gly Thr Arg
385                 390                 395


<210> SEQ ID NO 44
<211> LENGTH: 1197
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1197)
<223> OTHER INFORMATION: BvADH-alpha Boltardy red beet variety

<400> SEQUENCE: 44

atgatttcac tctcttcttt tcatccttcc tccaccaccg ccaccgccac cgccgccgcc     60

gccaccaccc acccacctca acaatgtccc gctttttcct ctcctccgtc gcatctctcg    120

cttcctttac gccaccctcg ccaacacctt gtagttcggt gcggtggagg tggttcggcc    180

tccgaatcgg tatttaaccg tgatagtgct gctactcgtg tttctaatga tcatcttgac    240

gttagtaaaa gagatgttaa gcttaagatt gctattattg ggtttggtaa ctttggccag    300

tttttggcta agacaatggc taagcaaggt catagagtgt tggcttactc acgctcggac    360

tactcccgcg ctgctaagga gatcggcgtc gagtatttta ctgacgccga tgacctctgc    420

gaggagcacc ctgaggttat tctgttgtgc acatccatcc tctcaacgga gaaggtcctc    480

cgatcactcc ccctccaccg gctccgtcgt tcaaccctct ttgcggatgt tctctcggtc    540

aaggaatttc ctcgatcgct cttccttcaa ctacttccta aggactttga tatcctatgc    600

acccacccta tgtttggccc agactcgggc aaagacgggt ggggtggact acctttttgtg    660

ttcgataaag ttagagtcgg atcagatcag agtcggacat ctcgtgctga ggcattccta    720

gacgtgttta ggaatgccgg gtgtaggatg gtggaaatga gttgtgttga tcatgacaag    780

catgcagccg gatctcaatt tattacacat atgatgggac gagttttgga gaaattggcc    840

ttggaaaata caccaattaa tacaaaaggg tacgaaagtt tgttaaattt ggtggataat    900

actgcaaggg atagttttga gttgttttac gggttgtttt tgtacaataa aaatgcaatg    960

gagcaattgg atagaatgga ttgggctttc gagatggtaa aaaagcaact ttcgggatat   1020

ttgcatgatc ttgttagaaa acaattgatg ttggagggta ataatgatca agctgaggtt   1080
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acttttgaca aaccattgat gcttccttct cctactatta atcctccaca aatagttccc   1140

tctgctgata tggctgagaa gaagcatgat ttagtggtgg ttaatggtac tagatag      1197


<210> SEQ ID NO 45
<211> LENGTH: 323
<212> TYPE: PRT
<213> ORGANISM: Mirabilis jalapa
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(323)
<223> OTHER INFORMATION: Mirabilis jalapa ADH-alpha

<400> SEQUENCE: 45

Ile Ala Ile Val Gly Phe Gly Asn Phe Gly Gln Phe Leu Gly Lys Glu
1               5                   10                  15

Ile Val Lys Gln Gly His Thr Val Leu Ala Tyr Ser Arg Ser Asp Tyr
            20                  25                  30

Leu Arg Ala Ala His Asn Ile Gly Val Lys Phe Phe Ser Asp Ala Asp
        35                  40                  45

Asp Leu Cys Glu Glu His Pro Gln Val Ile Leu Leu Cys Thr Ser Ile
    50                  55                  60

Leu Ser Thr Glu Arg Val Leu Arg Ser Leu Pro Leu His Arg Leu Arg
65                  70                  75                  80

Arg Ser Thr Leu Met Val Asp Val Leu Ser Val Lys Glu Phe Pro Arg
                85                  90                  95

Ser Leu Phe Leu Gln Leu Leu Pro Pro Asp Phe Asp Ile Leu Cys Thr
            100                 105                 110

His Pro Met Phe Gly Pro Asp Ser Gly Lys Ala Gly Trp Gly Gly Leu
       115                 120                 125

Pro Phe Val Phe Glu Lys Val Arg Val Gly Ser Asn Pro Thr Arg Ser
    130                 135                 140

Cys Arg Val Glu Ser Phe Leu Gly Ile Phe Gln Glu Ala Gly Cys Arg
145                 150                 155                 160

Met Val Glu Met Ser Cys Ala Glu His Asp Arg His Ala Ala Gly Ser
                165                 170                 175

Gln Phe Ile Thr His Met Met Gly Arg Val Leu Glu Lys Leu Ala Leu
            180                 185                 190

Glu Asp Thr Pro Ile Asn Thr Lys Gly Tyr Glu Ser Leu Leu Asn Leu
        195                 200                 205

Val Asp Asn Thr Ala Arg Asp Ser Phe Glu Leu Phe Tyr Gly Leu Phe
    210                 215                 220

Leu Tyr Asn Lys Asn Ala Met Glu Gln Leu Asp Arg Met His Trp Ala
225                 230                 235                 240

Phe Glu Thr Val Lys Gln Gln Leu Ser Gly Tyr Leu His Asp Leu Val
                245                 250                 255

Arg Lys Gln Leu Met Leu Glu Ser Ser Ser Asn Asp Asn Asn Asp Phe
            260                 265                 270

Val Gly Asn Tyr Tyr Asp Asn Asn Glu Asn Asp Lys Ser Ser Asp Glu
        275                 280                 285

Lys Lys Leu Met Leu Pro Ala Pro Gly Val Ala Ala Ala Ala Gln Ile
    290                 295                 300

Leu Pro Ser Ser Glu Arg Gln Gln Asn His Asp Leu Leu Tyr Ile Asn
305                 310                 315                 320
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Gly Arg Arg

<210> SEQ ID NO 46
<211> LENGTH: 972
<212> TYPE: DNA
<213> ORGANISM: Mirabilis jalapa
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(972)
<223> OTHER INFORMATION: Mirabilis jalapa ADH-alpha

<400> SEQUENCE: 46

atagcgatag ttgggtttgg taactttggt cagtttttgg gtaaagaaat agtaaagcaa 60

ggtcatactg ttttggctta ttcacgctct gattacttac gtgctgctca caacatcggc 120

gtcaaattct tttctgacgc cgatgacctt tgtgaggaac atcctcaggt gatactgcta 180

tgcacatcca tcctatcaac agagcgagtc cttcgctcac tccctctcca ccgcctgcgc 240

cgctcaacac tcatggtaga cgtactgtcg gtcaaggagt ttccccgttc attattcctt 300

caacttctac caccggactt tgacatcctg tgcacacacc ccatgtttgg acctgactca 360

ggcaaggccg ggtggggagg gctcccattc gtgtttgaaa aagtgcgagt tggatccaac 420

ccaacccgct cttgccgggt tgagtccttt cttggaatat tccaagaagc ggggtgtcgg 480

atggtggaaa tgagttgtgc agaacatgac aggcatgctg cagggtcaca gttcataact 540

cacatgatgg gtcgtgtttt ggagaaatta gcattagaag acactccaat taacacaaaa 600

ggatatgaaa gtttactgaa tttggttgat aacacggcaa gagatagctt tgagttgttt 660

tatggactgt ttttgtacaa caagaatgca atggaacaac ttgataggat gcattgggca 720

ttcgaaactg ttaagcaaca gttatctggt tacttacacg atctggttcg caaacaattg 780

atgttagaat cttcaagtaa tgataacaat gactttgtcg gtaattatta tgataataat 840

gaaaatgata agagtagtga tgaaaagaaa ttgatgcttc ctgctcctgg agttgcagct 900

gctgctcaga ttctaccttc ttctgaaagg caacaaaatc atgacttgct ctatatcaat 960

ggtcgtcgat ag 972

<210> SEQ ID NO 47
<211> LENGTH: 386
<212> TYPE: PRT
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(386)
<223> OTHER INFORMATION: BvADH-beta Boltardy red beet variety

<400> SEQUENCE: 47

Met Leu Ser Leu Ser Ser Thr Thr Thr Ala Lys Pro Ser Pro Ser Pro
1 5 10 15

Ser Pro Ala Asn Phe Pro Ala Lys Leu Ser Ser Leu Ser Thr Ile Thr
20 25 30

Thr Thr Leu Ser Phe Ser Pro Arg Arg Arg Tyr Phe His Gly Val Lys
35 40 45

Thr Leu Thr Ile Arg Ser Ile Asp Ala Ala Gln Phe Phe Asp Tyr Glu
50 55 60

Ser Lys Leu Ala Ala Ile Asn Thr Thr Ser Ser Ser Thr Ser Ser Ser
65 70 75 80

Tyr Ser Lys Leu Lys Ile Ala Ile Val Gly Phe Gly Asn Tyr Gly Gln

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               85                  90                  95

Phe Leu Ala Lys Thr Leu Val Ser Gln Gly His Thr Val Leu Ala Tyr
            100                 105                 110

Ser Arg Ser Asp Tyr Ser Lys Ile Ala Ala Asn Leu Gly Val Ser Tyr
       115                 120                 125

Phe Ser Asp Pro Asp Asp Leu Cys Glu Glu His Pro Glu Val Ile Met
    130                 135                 140

Leu Cys Thr Ser Ile Leu Ser Thr Glu Val Met Leu Asn Ser Leu Pro
145                 150                 155                 160

Leu Gln Arg Leu Lys Arg Ser Thr Leu Phe Val Asp Val Leu Ser Val
                165                 170                 175

Lys Glu Phe Pro Arg Asn Leu Phe Leu Gln Thr Leu Pro Ser Asp Phe
            180                 185                 190

Asp Ile Leu Cys Thr His Pro Met Phe Gly Pro Glu Ser Gly Lys Asn
        195                 200                 205

Gly Trp Gly Ser Leu Pro Phe Val Tyr Asp Lys Val Arg Ile Gly Lys
    210                 215                 220

Asp Glu Gly Arg Ile Lys Arg Cys Glu Ser Phe Leu Asp Val Phe Arg
225                 230                 235                 240

Arg Glu Gly Cys Arg Val Glu Glu Met Thr Cys Ala Glu His Asp Lys
                245                 250                 255

Phe Ala Ala Gly Ser Gln Phe Ile Thr His Phe Leu Gly Arg Val Leu
            260                 265                 270

Glu Lys Leu Asp Leu Glu Asp Thr Pro Ile Asn Thr Lys Gly Tyr Glu
        275                 280                 285

Ser Leu Leu Asn Leu Val Asp Asn Thr Ser Lys Asp Ser Phe Glu Leu
    290                 295                 300

Phe Tyr Gly Leu Phe Leu Tyr Asn Gln Asn Ala Met Glu Gln Leu Glu
305                 310                 315                 320

Arg Leu Asp Trp Ala Phe Glu Leu Val Lys Lys Gln Leu Phe Gly His
                325                 330                 335

Leu His Gly Leu Leu Arg Lys Gln Leu Phe Gly Phe Ser Glu Ile Asp
            340                 345                 350

Glu Arg Ile Gly Lys Ala Lys Glu Ile Lys Phe Leu Ser Asp Ala Ala
        355                 360                 365

Glu Gln Asn Gly Ser Ala Leu Ser Ala Arg Glu Asn Ala Asn Ser Glu
    370                 375                 380

Thr Asn
385


<210> SEQ ID NO 48
<211> LENGTH: 1161
<212> TYPE: DNA
<213> ORGANISM: Beta vulgaris
<220> FEATURE:
<221> NAME/KEY: misc_feature
<222> LOCATION: (1)..(1161)
<223> OTHER INFORMATION: BvADH-beta Boltardy red beet variety

<400> SEQUENCE: 48

atgctttctc tctcctccac aaccaccgca aaaccctcgc cgtcgccatc tccggcgaat     60

tttccggcaa aactttcttc tctctccacc atcaccacca ctctctcctt ctctcctcgc    120

cggagatatt ttcatggcgt caaaacccta acaattcgca gcatcgacgc tgcacaattc    180
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ttcgattacg aatcaaaact cgccgccatt aacacaacat cttcatctac atcttcatct 240

tattcgaaac tcaaaatcgc aatcgtaggt ttcggaaatt acggacaatt tctggcgaaa 300

accctagttt ctcaaggtca tactgttctc gcttattctc gctctgatta ctctaaaatc 360

gctgcgaatc tcggtgtttc ttacttttct gatcctgatg atctttgcga agaacatccc 420

gaggtaatta tgttgtgtac ttcgatttta tcaactgaag ttatgttgaa ttcgttacca 480

ttgcagcgac ttaaacgatc gacgcttttt gttgatgttt tatcggtgaa agaatttccg 540

cgtaatttgt ttcttcagac tttaccgtct gattttgata tattatgtac tcatcctatg 600

tttgggcctg aatctgggaa aaatggttgg ggaagtttgc cgtttgttta tgataaagtt 660

aggattggga aagatgaggg tagaattaag agatgtgaga gttttttgga tgtttttagg 720

agagaaggtt gtagggttga ggaaatgact tgtgctgagc atgataagtt tgcagcagga 780

tctcagttta taacacattt cttagggagg gttttggaga agcttgattt ggaggatacg 840

ccgattaata cgaaagggta tgagagtttg ttgaatttgg tggataatac gtcgaaggat 900

agtttcgagt tgttttatgg gttgtttttg tataatcaga atgctatgga gcagttagag 960

aggttagatt gggcgtttga gttggttaag aagcaattgt ttggacactt gcatgggttg 1020

ctaaggaaac agttgtttgg gttttctgag atagatgaac gtattgggaa ggcgaaggag 1080

atcaaatttc tctctgatgc tgcagaacag aatggctctg ccttgtctgc tagggagaat 1140

gcaaattcgg agacaaattg a 1161

<210> SEQ ID NO 49
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 49

ggttccgcgt ggatccctaa caattcgcag cat 33

<210> SEQ ID NO 50
<211> LENGTH: 34
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 50

aattcggaga caaattgaga attcatcgtg actg 34

<210> SEQ ID NO 51
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 51

ctggttccgc gtggatcctg cggtggaggt ggttcg 36

<210> SEQ ID NO 52
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 52

gttaatggta ctagatagga attcatcgtg actga 35

<210> SEQ ID NO 53
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 53

ctggttccgc gtggatccgc aatcgacgcc gcccaa 36

<210> SEQ ID NO 54
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 54

tcatcatcat catcttaaga attcatcgtg actga 35

<210> SEQ ID NO 55
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 55

ctggttccgc gtggatccgc cgctaccaat acctcc 36

<210> SEQ ID NO 56
<211> LENGTH: 35
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 56

aattcagaga tcaattgaga attcatcgtg actga 35

<210> SEQ ID NO 57
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 57

ctggttccgc gtggatcctg cgccgcctct gactcc 36

<210> SEQ ID NO 58
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 58

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tggtaataat tctagatagg aattcatcgt gactga 36

<210> SEQ ID NO 59
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 59

ctggttccgc gtggatccgc cgcgctgcca aacgact 37

<210> SEQ ID NO 60
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 60

aaatgttgag agaaattgag aattcatcgt gactga 36

<210> SEQ ID NO 61
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 61

ctggttccgc gtggatcctg ctcatcatca tcatcat 37

<210> SEQ ID NO 62
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 62

cgtcaacgat agatcatagg aattcatcgt gactga 36

<210> SEQ ID NO 63
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 63

ctggttccgc gtggatccat agcgatagtt gggtttg 37

<210> SEQ ID NO 64
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 64

tatcaatggt cgtcgatagg aattcatcgt gactga 36

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<210> SEQ ID NO 65
<211> LENGTH: 38
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 65

ctggttccgc gtggatcctg cacggccttc actaaaac 38

<210> SEQ ID NO 66
<211> LENGTH: 37
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 66

tcaatggatc aaagcggtag gaattcatcg tgactga 37

<210> SEQ ID NO 67
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 67

tcaagctgag gttacttttg aca 23

<210> SEQ ID NO 68
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 68

aagaagcatg atttagtggt ggt 23

<210> SEQ ID NO 69
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 69

tgcagcgact taaacgatcg 20

<210> SEQ ID NO 70
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 70

ttggggaagt ttgccgtttg 20

<210> SEQ ID NO 71
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 71

agttccctct gctgatatg 19

<210> SEQ ID NO 72
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 72

gtggttaatg gtactagata g 21

<210> SEQ ID NO 73
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 73

gcgaaggaga tcaaatttct 20

<210> SEQ ID NO 74
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 74

tcaatttgtc tccgaatttg c 21

<210> SEQ ID NO 75
<211> LENGTH: 26
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 75

atgatttcac tctcttcttt tcatcc 26

<210> SEQ ID NO 76
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 76

gatttagtgg tggttaatgg tactagatag 30

<210> SEQ ID NO 77
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 77

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atgctttctc tctcctccac 20

<210> SEQ ID NO 78
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 78

caaattcgga gacaaattga 20

<210> SEQ ID NO 79
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 79

tctatccttg catctctcag 20

<210> SEQ ID NO 80
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 80

tctccaaggg cgagtatgat 20

<210> SEQ ID NO 81
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 81

cattggttca ggaagtgcaa 20

<210> SEQ ID NO 82
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 82

cctttgattc atggcttcgt 20

<210> SEQ ID NO 83
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 83

tatcaaacga gggcacttc 19

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<210> SEQ ID NO 84
<211> LENGTH: 19
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 84

gatggtcttt gatagcagc 19

<210> SEQ ID NO 85
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 85

cttttcagtg gaattagccc acc 23

<210> SEQ ID NO 86
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 86

tggaacatta tggaagatat tggg 24

<210> SEQ ID NO 87
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 87

ggctggaaga gtgatcggag 20

<210> SEQ ID NO 88
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 88

acgctactgt tgagcatctt ca 22

<210> SEQ ID NO 89
<211> LENGTH: 56
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 89

gctgtcaacg atacgctacg taacggcatg acagtgtttt ttttttttt ttttt 56

<210> SEQ ID NO 90
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence

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<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 90

agctttacct cccaagtcat c 21

<210> SEQ ID NO 91
<211> LENGTH: 20
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic primer

<400> SEQUENCE: 91

ccaagattga caggcgttct 20

<210> SEQ ID NO 92
<211> LENGTH: 358
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: AtADH2

<400> SEQUENCE: 92

Met Leu Leu His Phe Ser Pro Ala Lys Pro Leu Ile Ser Pro Pro Asn
1 5 10 15

Leu Arg Arg Asn Ser Pro Thr Phe Leu Ile Ser Pro Pro Arg Ser Leu
20 25 30

Arg Ile Arg Ala Ile Asp Ala Ala Gln Ile Phe Asp Tyr Glu Thr Gln
35 40 45

Leu Lys Ser Glu Tyr Arg Lys Ser Ser Ala Leu Lys Ile Ala Val Leu
50 55 60

Gly Phe Gly Asn Phe Gly Gln Phe Leu Ser Lys Thr Leu Ile Arg His
65 70 75 80

Gly His Asp Leu Ile Thr His Ser Arg Ser Asp Tyr Ser Asp Ala Ala
85 90 95

Asn Ser Ile Gly Ala Arg Phe Phe Asp Asn Pro His Asp Leu Cys Glu
100 105 110

Gln His Pro Asp Val Val Leu Leu Cys Thr Ser Ile Leu Ser Thr Glu
115 120 125

Ser Val Leu Arg Ser Phe Pro Phe Gln Arg Leu Arg Arg Ser Thr Leu
130 135 140

Phe Val Asp Val Leu Ser Val Lys Glu Phe Pro Lys Ala Leu Phe Ile
145 150 155 160

Lys Tyr Leu Pro Lys Glu Phe Asp Ile Leu Cys Thr His Pro Met Phe
165 170 175

Gly Pro Glu Ser Gly Lys His Ser Trp Ser Gly Leu Pro Phe Val Tyr
180 185 190

Asp Lys Val Arg Ile Gly Asp Ala Ala Ser Arg Gln Glu Arg Cys Glu
195 200 205

Lys Phe Leu Arg Ile Phe Glu Asn Glu Gly Cys Lys Met Val Glu Met
210 215 220

Ser Cys Glu Lys His Asp Tyr Tyr Ala Ala Gly Ser Gln Phe Val Thr
225 230 235 240

His Thr Met Gly Arg Val Leu Glu Lys Tyr Gly Val Glu Ser Ser Pro
245 250 255

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```
Ile Asn Thr Lys Gly Tyr Glu Thr Leu Leu Asp Leu Val Glu Asn Thr
            260                 265                 270

Ser Ser Asp Ser Phe Glu Leu Phe Tyr Gly Leu Phe Met Tyr Asn Pro
        275                 280                 285

Asn Ala Leu Glu Gln Leu Glu Arg Leu Asp Met Ala Phe Glu Ser Val
    290                 295                 300

Lys Lys Glu Leu Phe Gly Arg Leu His Gln Gln Tyr Arg Lys Gln Met
305                 310                 315                 320

Phe Gly Gly Glu Val Gln Ser Pro Lys Lys Thr Glu Gln Lys Leu Leu
                325                 330                 335

Asn Asp Gly Gly Val Val Pro Met Asn Asp Ile Ser Ser Ser Ser Ser
            340                 345                 350

Ser Ser Ser Ser Ser Ser
        355


<210> SEQ ID NO 93
<211> LENGTH: 311
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: AaPDH

<400> SEQUENCE: 93

Met Ala Ile Leu Ser Ser Met Phe Asn Pro Ser Pro Pro Gln Gly Phe
1               5                   10                  15

Cys Lys Lys Asn Ile Ile Lys Ile Leu Lys Ser Leu Ser Met Gln Asn
            20                  25                  30

Val Leu Ile Val Gly Val Gly Phe Met Gly Gly Ser Phe Ala Lys Ser
        35                  40                  45

Leu Arg Arg Ser Gly Phe Lys Gly Lys Ile Tyr Gly Tyr Asp Ile Asn
    50                  55                  60

Pro Glu Ser Ile Ser Lys Ala Val Asp Leu Gly Ile Ile Asp Glu Gly
65                  70                  75                  80

Thr Thr Ser Ile Ala Lys Val Glu Asp Phe Ser Pro Asp Phe Val Met
                85                  90                  95

Leu Ser Ser Pro Val Arg Thr Phe Arg Glu Ile Ala Lys Lys Leu Ser
            100                 105                 110

Tyr Ile Leu Ser Glu Asp Ala Thr Val Thr Asp Gln Gly Ser Val Lys
       115                 120                 125

Gly Lys Leu Val Tyr Asp Leu Glu Asn Ile Leu Gly Lys Arg Phe Val
    130                 135                 140

Gly Gly His Pro Ile Ala Gly Thr Glu Lys Ser Gly Val Glu Tyr Ser
145                 150                 155                 160

Leu Asp Asn Leu Tyr Glu Gly Lys Lys Val Ile Leu Thr Pro Thr Lys
                165                 170                 175

Lys Thr Asp Lys Lys Arg Leu Lys Leu Val Lys Arg Val Trp Glu Asp
            180                 185                 190

Val Gly Gly Val Val Glu Tyr Met Ser Pro Glu Leu His Asp Tyr Val
        195                 200                 205

Phe Gly Val Val Ser His Leu Pro His Ala Val Ala Phe Ala Leu Val
    210                 215                 220

Asp Thr Leu Ile His Met Ser Thr Pro Glu Val Asp Leu Phe Lys Tyr
225                 230                 235                 240
```

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Pro Gly Gly Gly Phe Lys Asp Phe Thr Arg Ile Ala Lys Ser Asp Pro
                245                 250                 255

Ile Met Trp Arg Asp Ile Phe Leu Glu Asn Lys Glu Asn Val Met Lys
            260                 265                 270

Ala Ile Glu Gly Phe Glu Lys Ser Leu Asn His Leu Lys Glu Leu Ile
        275                 280                 285

Val Arg Glu Ala Glu Glu Glu Leu Val Glu Tyr Leu Lys Glu Val Lys
    290                 295                 300

Ile Lys Arg Met Glu Ile Asp
305                 310


<210> SEQ ID NO 94
<211> LENGTH: 279
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Synthetic: SyADH

<400> SEQUENCE: 94

Met Lys Ile Gly Val Val Gly Leu Gly Leu Ile Gly Ala Ser Leu Ala
1               5                   10                  15

Gly Asp Leu Arg Arg Arg Gly His Tyr Leu Ile Gly Val Ser Arg Gln
            20                  25                  30

Gln Ser Thr Cys Glu Lys Ala Val Glu Arg Gln Leu Val Asp Glu Ala
        35                  40                  45

Gly Gln Asp Leu Ser Leu Leu Gln Thr Ala Lys Ile Ile Phe Leu Cys
    50                  55                  60

Thr Pro Ile Gln Leu Ile Leu Pro Thr Leu Glu Lys Leu Ile Pro His
65                  70                  75                  80

Leu Ser Pro Thr Ala Ile Val Thr Asp Val Ala Ser Val Lys Thr Ala
                85                  90                  95

Ile Ala Glu Pro Ala Ser Gln Leu Trp Ser Gly Phe Ile Gly Gly His
            100                 105                 110

Pro Met Ala Gly Thr Ala Ala Gln Gly Ile Asp Gly Ala Glu Glu Asn
       115                 120                 125

Leu Phe Val Asn Ala Pro Tyr Val Leu Thr Pro Thr Glu Tyr Thr Asp
    130                 135                 140

Pro Glu Gln Leu Ala Cys Leu Arg Ser Val Leu Glu Pro Leu Gly Val
145                 150                 155                 160

Lys Ile Tyr Leu Cys Thr Pro Ala Asp His Asp Gln Ala Val Ala Trp
                165                 170                 175

Ile Ser His Leu Pro Val Met Val Ser Ala Ala Leu Ile Gln Ala Cys
            180                 185                 190

Ala Gly Glu Lys Asp Gly Asp Ile Leu Lys Leu Ala Gln Asn Leu Ala
        195                 200                 205

Ser Ser Gly Phe Arg Asp Thr Ser Arg Val Gly Gly Gly Asn Pro Glu
    210                 215                 220

Leu Gly Thr Met Met Ala Thr Tyr Asn Gln Arg Ala Leu Leu Lys Ser
225                 230                 235                 240
```

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```
Leu Gln Asp Tyr Arg Gln His Leu Asp Gln Leu Ile Thr Leu Ile Ser
                245                 250                 255

Asn Gln Gln Trp Pro Glu Leu His Arg Leu Leu Gln Gln Thr Asn Gly
            260                 265                 270

Asp Arg Asp Lys Tyr Val Glu
        275
```

1. A cDNA polynucleotide encoding a polypeptide having at least 90% sequence identity to a polypeptide selected from the group consisting of any one of SEQ ID NOS: 1-20, 43, 45, or 47 and functional fragments of any one of SEQ ID NOS: 1-20, 43, 45, or 47.

2. (canceled)

3. The cDNA polynucleotide of claim **1**, wherein the cDNA polynucleotide is codon-optimized for expression in a cell.

4. The cDNA polynucleotide of claim **3**, wherein the cell is a plant cell, bacterial cell, or fungal cell.

5. (canceled)

6. The cDNA of claim **1**, wherein the polypeptide maintains at least 50% of its ADH activity in the presence of 10 μM tyrosine.

7. A construct comprising a heterologous promoter operably linked to a polynucleotide encoding a polypeptide having at least 90% sequence identity to a polypeptide selected from the group consisting of any one of SEQ ID NOS: 1-20, 43, 45, or 47 and functional fragments of any one of SEQ ID NOS: 1-20, 43, 45, or 47.

8. The construct of claim **7**, wherein the heterologous promoter is a plant promoter.

9. The construct of claim **7**, wherein the heterologous promoter is an inducible promoter or a tissue-specific promoter.

10. A vector comprising the construct of claim **7**.

11. The vector of claim **10**, wherein the vector comprises a plasmid.

12. A cell comprising the construct of claim **7**.

13. The cell of claim **12**, wherein the cell is a plant cell.

14. The cell of claim **13**, wherein the plant cell is selected from a soybean plant cell, a mung bean plant cell, an opium poppy plant cell, a *quinoa* plant cell, an alfalfa plant cell, a rice plant cell, a wheat plant cell, a corn plant cell, a sorghum plant cell, a barley plant cell, a millet plant cell, an oat plant cell, a rye plant cell, a rapeseed plant cell, a beet plant cell, and a miscanthus plant cell.

15. (canceled)

16. A seed comprising the construct of claim **7**.

17. (canceled)

18. A plant comprising the construct of claim **7**.

19. The plant of claim **18**, wherein the plant is selected from a beet plant, a soybean plant, a mung bean plant, an opium poppy plant, a *quinoa* plant, an alfalfa plant, a rice plant, a wheat plant, a corn plant, a sorghum plant, a barley plant, a millet plant, an oat plant, a rye plant, a rapeseed plant, and a *miscanthus* plant.

20. (canceled)

21. A method for increasing production of at least one product of the tyrosine or HPP pathways in a cell comprising introducing the construct of claim **7** into the cell.

22. The method of claim **21**, wherein the cell is a plant cell.

23. The method of claim **22**, wherein the plant cell is selected from a soybean plant cell, a mung bean plant cell, an opium poppy plant cell, a *quinoa* plant cell, an alfalfa plant cell, a rice plant cell, a wheat plant cell, a corn plant cell, a sorghum plant cell, a barley plant cell, a millet plant cell, an oat plant cell, a rye plant cell, a rapeseed plant cell, a beet plant cell, and a *miscanthus* plant cell.

24. (canceled)

25. The method of claim **21**, wherein the product is selected from vitamin E, plastoquinone, a cyanogenic glycoside, a benzylisoquinoline alkaloid, rosmarinic acid, betalains, suberin, mescaline, morphine, salidroside, a phenylpropanoid compound, dhurrin, a tocochromanol, ubiquinone, lignin, a catecholamine, melanin, an isoquinoline alkaloid, hydroxycinnamic acid amide (HCAA), an amaryllidaceae alkaloid, hordenine, hydroxycinnamate, hydroxylstyrene, or tyrosine.

26. The method of claim **21**, further comprising purifying the product from the cell.

* * * * *