

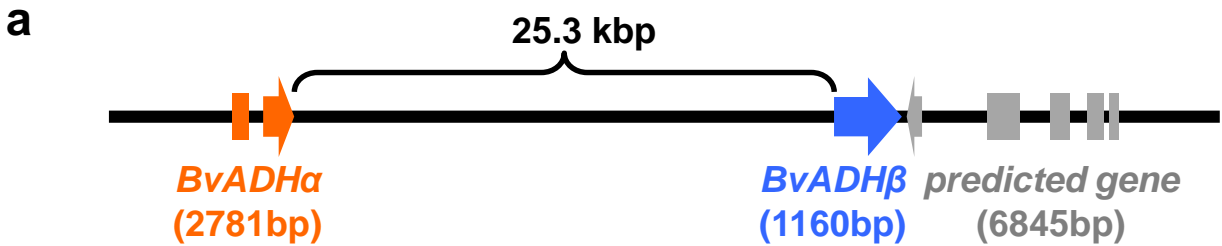
New Phytologist Supporting Information for the Article

# **Relaxation of Tyrosine Pathway Regulation Underlies the Evolution of Betalain Pigmentation in Caryophyllales**

Figure S1-S10 and Table S1-S4

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**b**

|                                 | <i>BvADH<math>\alpha</math></i> | <i>BvADH<math>\beta</math></i> | <i>AtADH1</i> | <i>AtADH2</i> | <i>GmPDH1</i> | <i>AaPDH</i> | <i>EcPDH</i> | <i>SyADH</i> |
|---------------------------------|---------------------------------|--------------------------------|---------------|---------------|---------------|--------------|--------------|--------------|
| <i>BvADH<math>\alpha</math></i> | 100                             | 66                             | 66            | 61            | 52            | 18           | 28           | 24           |
| <i>BvADH<math>\beta</math></i>  | 66                              | 100                            | 72            | 59            | 54            | 24           | 26           | 25           |
| <i>AtADH1</i>                   | 66                              | 72                             | 100           | 61            | 56            | 22           | 25           | 34           |
| <i>AtADH2</i>                   | 61                              | 59                             | 61            | 100           | 52            | 23           | 23           | 32           |
| <i>GmPDH1</i>                   | 52                              | 54                             | 56            | 52            | 100           | 23           | 23           | 29           |
| <i>AaPDH</i>                    | 18                              | 24                             | 22            | 23            | 23            | 100          | 21           | 28           |
| <i>EcPDH</i>                    | 28                              | 26                             | 25            | 23            | 23            | 21           | 100          | 23           |
| <i>SyADH</i>                    | 24                              | 25                             | 34            | 32            | 29            | 28           | 23           | 100          |

**Figure S1a and b. Physical location, homology, and phylogeny of *BvADH $\alpha$*  and *BvADH $\beta$* .** (a) The location and physical distance of *BvADH $\alpha$*  and *BvADH $\beta$*  on the chromosome 8 of the *B. vulgaris* genome. A nearby gene is indicated in gray. (b) Amino acid identity of ADH and PDH proteins from different plants and bacteria. *AaPDH*, *Aquifex aeolicus*; *AtADH1* and *AtADH2*, *Arabidopsis thaliana*; *GmPDH1*, *Glycine max*; *EcPDH*, *Escherichia coli*; and *SyADH*, *Synechocystis* sp. PCC6803. (c) See the next page.

C

Phylogenetic tree showing relationships between various plant species and their ADH/PDH enzymes. The tree is rooted at the top left. Bootstrap support values are indicated at the nodes. The tree is divided into several major clades:

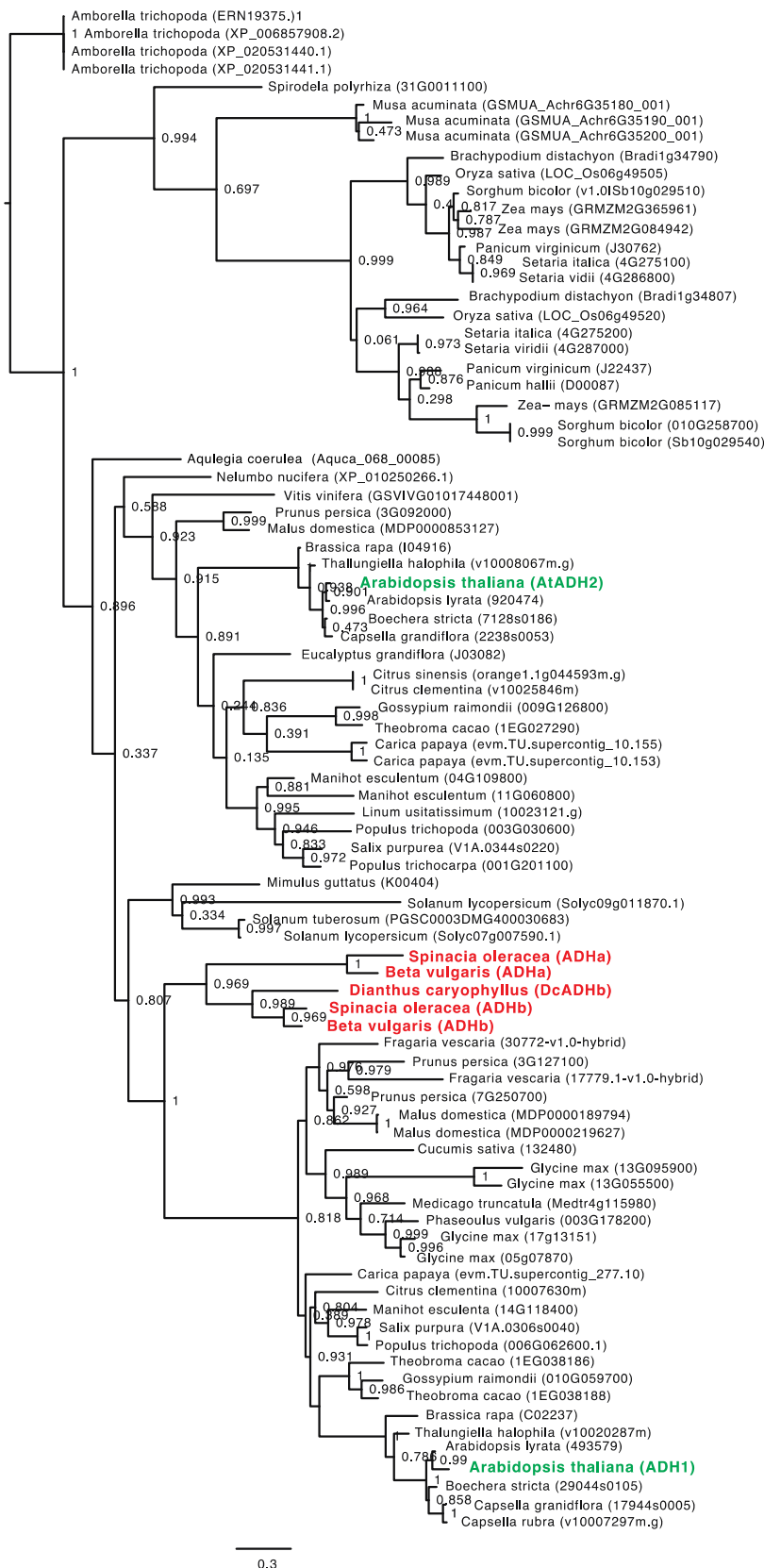
- PDH-like (Blue):** Includes *Brachypodium distachyon*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*, *Setaria viridii*, *Setaria italica*, *Panicum virginicum*, *Panicum hallii*, *Eucalyptus grandiflora*, *Theobroma cacao*, *Gossypium raimondii*, *Citrus clementina*, *Populus trichopoda*, *Salix purpurea*, *Mimulus guttatus*, *Solanum tuberosum*, *Solanum lycopersicum*, *Medicago truncatula*, *Glycine max*, *Medicago truncatula MtPDH1*, *Phaseolus vulgaris*, *Glycine max GmPDH2*, and *Glycine max GmPDH1*.
- ADH-like (Green):** Includes *Spirodela polyrhiza*, *Musa acuminata*, *Oryza sativa*, *Brachypodium distachyon*, *Sorghum bicolor*, *Zea mays*, *Panicum virginicum*, *Setaria italica*, *Setaria viridii*, *Brachypodium distachyon*, *Oryza sativa*, *Zea mays*, *Sorghum bicolor*, *Setaria italica*, *Setaria viridii*, *Panicum virginicum*, *Panicum hallii*, *Spinacia oleracea*, *Beta vulgaris*, *Dianthus caryophyllus*, *Mimulus guttatus*, *Solanum lycopersicum*, *Solanum tuberosum*, *Solanum lycopersicum*, *Prunus persica*, *Malus domestica*, *Eucalyptus grandiflora*, *Linum usitatissimum*, *Manihot esculentum*, *Manihot esculentum*, *Populus trichopoda*, *Populus trichocarpa*, *Salix purpurea*, *Populus trichocarpa*, *Gossypium raimondii*, *Theobroma cacao*, *Citrus sinensis*, *Citrus clementina*, *Carica papaya*, *Carica papaya*, *Brassica rapa*, *Thalungiella halophila*, *Arabidopsis thaliana*, *Arabidopsis lyrata*, *Boechera stricta*, *Boechera stricta*, *Capsella grandiflora*, *Fragaria vescaria*, *Prunus persica*, *Cucumis sativa*, *Glycine max*, *Glycine max*, *Medicago truncatula*, *Phaseolus vulgaris*, *Glycine max*, *Glycine max*, *Fragaria vescaria*, *Prunus persica*, *Malus domestica*, *Malus domestica*, *Carica papaya*, *Theobroma cacao*, *Gossypium raimondii*, *Theobroma cacao*, *Manihot esculenta*, *Salix purpurea*, *Populus trichopoda*, *Citrus clementina*, *Brassica rapa*, *Thalungiella halophila*, *Arabidopsis lyrata*, *Arabidopsis thaliana*, *Boechera stricta*, *Capsella grandiflora*, and *Capsella rubra*.
- Caryophyllales ADH candidates (Red):** Includes *Spinacia oleracea*, *Beta vulgaris*, and *Dianthus caryophyllus*.

Scale bar indicates inferred number of amino acid substitution per site.

PDH-like

ADH-like

**Figure S1c. (c)** Maximum likelihood<sup>10.2</sup> phylogeny of BvADHs together with other plant ADHs and PDHs. Previously characterized *Arabidopsis* ADH and legume PDH enzymes are indicated in green and blue, respectively. Red indicates ADH candidates from Caryophyllales, which are derived from a Caryophyllales-specific duplication event (98%). These Caryophyllales genes fall within in the clade containing *Arabidopsis ADH* genes. Numbers at branches indicate bootstrap support values. Scale bar indicates inferred number of amino acid substitution per site.

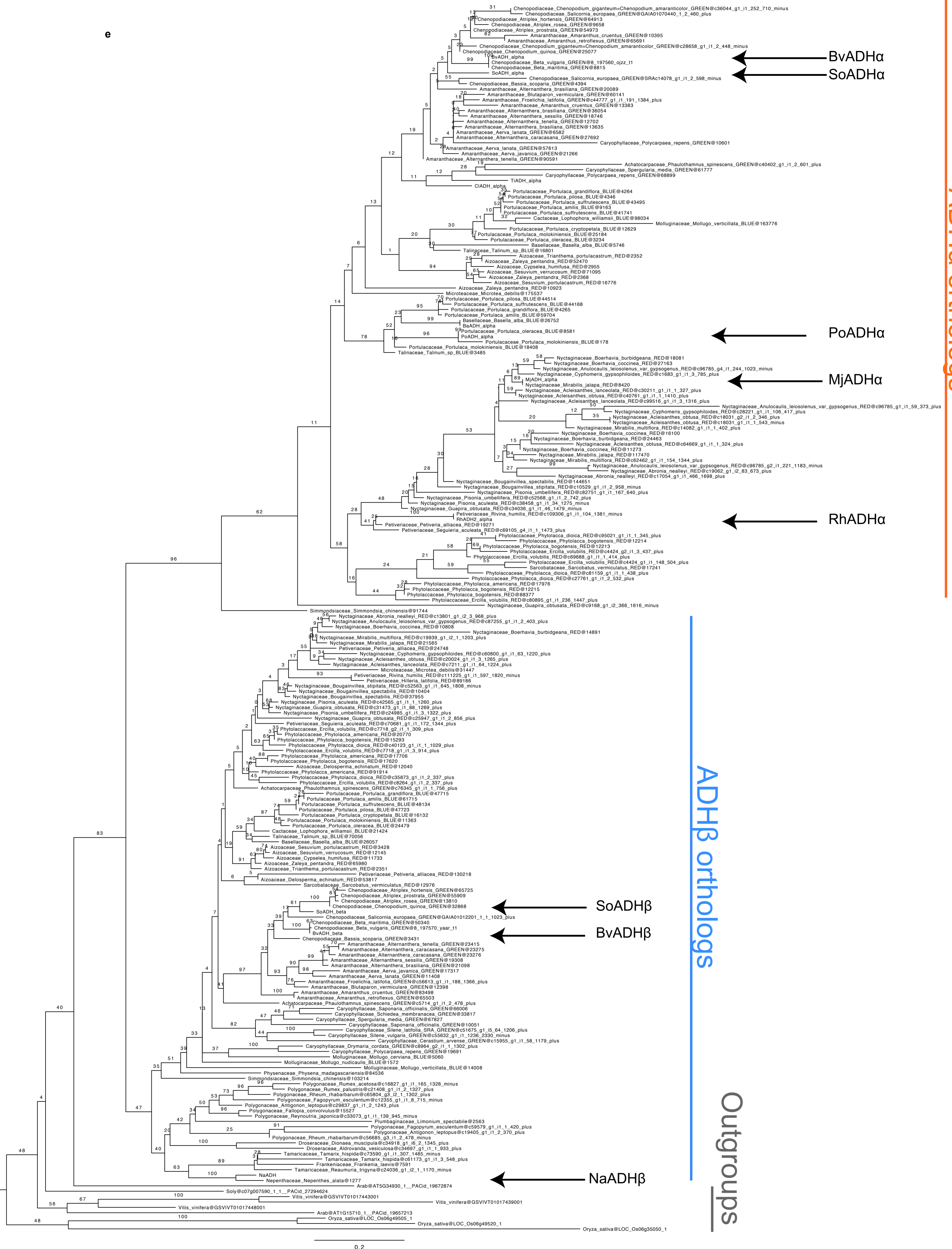
**d**

ADH-like

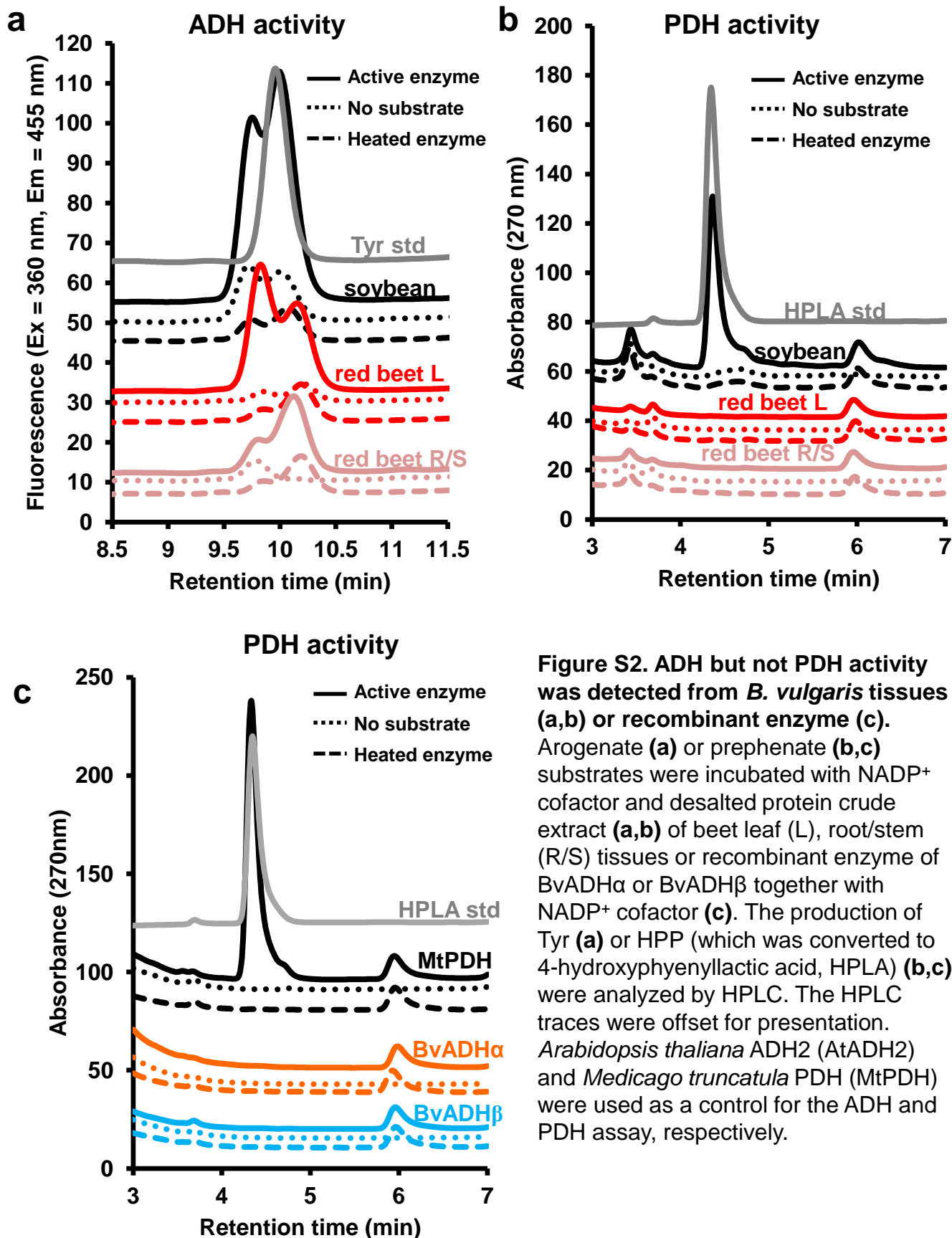
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**Figure S1d. (d)** Maximum likelihood phylogeny of BvADHs together with other plant ADHs only. Previously characterized *Arabidopsis* ADH enzymes are indicated in green. Red indicates ADH candidates from Caryophyllales, which are derived from a recent duplication event shown in a red circle. These analyses still allow us to claim that ADH $\alpha$  and ADH $\beta$  lineages fall within the ADH clade as they are nested with the eudicot clade. Numbers at branches indicate bootstrap support values. Scale bar indicates inferred number of amino acid substitution per site.

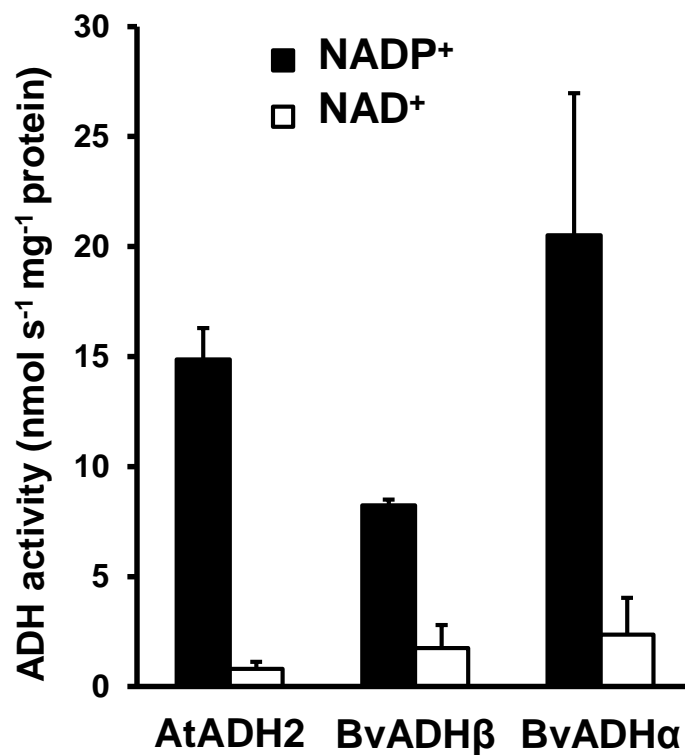
e



**Figure S1e. (e)** Maximum-likelihood phylogeny of *ADH* genes in Caryophyllales. The cartoon version of this tree is shown in **Figure 4a**. Numbers above branches indicate bootstrap support values. Scale bar indicates inferred number of amino acids substitution per site. *ADH* enzymes characterized in this study are indicated by arrows. Clades containing *BvADHα* and *BvADHβ* orthologs are indicated by orange and blue, respectively.



**Figure S2. ADH but not PDH activity was detected from *B. vulgaris* tissues (a,b) or recombinant enzyme (c).** Arogenate (a) or prephenate (b,c) substrates were incubated with NADP<sup>+</sup> cofactor and desalted protein crude extract (a,b) of beet leaf (L), root/stem (R/S) tissues or recombinant enzyme of BvADH $\alpha$  or BvADH $\beta$  together with NADP<sup>+</sup> cofactor (c). The production of Tyr (a) or HPP (which was converted to 4-hydroxyphenyllactic acid, HPLA) (b,c) were analyzed by HPLC. The HPLC traces were offset for presentation. *Arabidopsis thaliana* ADH2 (AtADH2) and *Medicago truncatula* PDH (MtPDH) were used as a control for the ADH and PDH assay, respectively.



**Figure S3. BvADHs prefer NADP<sup>+</sup> over NAD<sup>+</sup> as cofactor.** ADH activity was analyzed using NADP<sup>+</sup> or NAD<sup>+</sup> cofactor, which is expressed as the mean of three independent experiments  $\pm$  s.e.m. in nmol s<sup>-1</sup> mg<sup>-1</sup> of protein.

**a) Nucleotide sequence alignment of *BvADHα***

|                   |     |  |
|-------------------|-----|--|
| Redbeet1_BvADHα   | 1   | ATGATTTCACTCTCTTCTTTTCATCCTTCCTCCACCACCGCCACCGCCAC   |
| Yellowbeet_BvADHα | 1   | ATGATTTCACTCTCTTCTTTTCATCCTTCCTCCACCACCGCCACCGCCAC   |
| Whitebeet_BvADHα  | 1   | ATGATTTCACTCTCTTCTTTTCATCCTTCCTCCACCACCGCCACCGCCAC   |
| Sugarbeet_BvADHα  | 1   | ATGATTTCACTCTCTTCTTTTCATCCTTCCTCCACCACCGCCACCGCCAC   |
| Seabeet_BvADHα    | 1   | ATGATTTCACTCTCTTCTTTTCATCCTTCCTCCACCACCGCCACCGCCAC   |
| Redbeet2_BvADHα   | 1   | ATGATTTCACTCTCTTCTTTTCATCCTTCCTCCACCACCGCCACCGCCAC   |
|                   |     |  |
| Redbeet11_BvADHα  | 51  | CGCCGCGCGCCACC-----ACCCACC                           |
| Yellowbeet_BvADHα | 51  | CGCCGCGCGCGCCACC-----ACCCACC                         |
| Whitebeet_BvADHα  | 51  | CGCCGCGCGCGCCACC-----ACCCACC                         |
| Sugarbeet_BvADHα  | 51  | CGCCGCCACCGCCACC-----ACCCACC                         |
| Seabeet_BvADHα    | 51  | CGCCGCCACCGCCACCGCCACCGCCACCGCCACCACCCACC            |
| Redbeet2_BvADHα   | 51  | CGCCGCGCGCGCCACC-----ACCCACC                         |
|                   |     |  |
| Redbeet1_BvADHα   | 74  | CACCTCAACAATGTCGCTTTTTCCTCTCCTCCGTCGCATCTCTCGCTT     |
| Yellowbeet_BvADHα | 74  | CACCTCAACAATGTCGCTTTTTCCTCTCCTCCGTCGCATCTCTCGCTT     |
| Whitebeet_BvADHα  | 74  | CACCACAACAATGTCGCTTTTTCCTCTCCTCCGTCGCATCTCTCGCTT     |
| Sugarbeet_BvADHα  | 74  | CACCACAACAATGTCGCTTTTTCCTCTCCTCCATCGCATCTCTCGCTT     |
| Seabeet_BvADHα    | 101 | CACCACAACAATGTCGCTTTTTCCTCTCCTCCATCGCATCTCTCGCTT     |
| Redbeet2_BvADHα   | 74  | CACCTCAACAATGTCGCTTTTTCCTCTCCTCCGTCGCATCTCTCGCTT     |
|                   |     |  |
| Redbeet1_BvADHα   | 124 | CCTTTACGCCACCCTCGCCAACACCTTGTAGTTCGGTGC GG TGGAGGTGG |
| Yellowbeet_BvADHα | 124 | CCTTTACGCCACCCTCGCCAACACCTTGTAGTTCGGTGC GG TGGAGGTGG |
| Whitebeet_BvADHα  | 124 | CCTTTACGCCACCCTCGCCAACACCTTGTAGTTCGGTGC GG TGGAGGTGG |
| Sugarbeet_BvADHα  | 124 | CCTTTACGCCACCCTCGCCAACACCTTGTAGTTCGGTGC GG TGGAGGTGG |
| Seabeet_BvADHα    | 151 | CCTTTACGCCACCCTCGCCAACACCTTGTAGTTCGGTGC GG TGGAGGTGG |
| Redbeet2_BvADHα   | 124 | CCTTTACGCCACCCTCGCCAACACCTTGTAGTTCGGTGC GG TGGAGGTGG |
|                   |     |  |
| Redbeet1_BvADHα   | 174 | TTCGGCCTCCGAATCGGTATTTAACCGTGATAGTGCTGCTACTCGTGTTT   |
| Yellowbeet_BvADHα | 174 | TTCGGCCTCCGAATCGGTATTTAACCGTGATAGTGCTGCTACTCGTGTTT   |
| Whitebeet_BvADHα  | 174 | TTCGGCCTCCGAATCGGTATTTAACCGTGATAGTGCTGCTACTCGTGTTT   |
| Sugarbeet_BvADHα  | 174 | TTCGGCCTCCGAATCGGTATTTAACCGTGATAGTGCTGCTACTCGTGTTT   |
| Seabeet_BvADHα    | 201 | TTCGGCCTCCGAATCGGTATTTAACCGTGATAGTGCTGCTACTCGTGTTT   |
| Redbeet2_BvADHα   | 174 | TTCGGCCTCCGAATCGGTATTTAACCGTGATAGTGCTGCTACTCGTGTTT   |
|                   |     |  |
| Redbeet1_BvADHα   | 224 | CTAATGATCATCTTGACGTTAGTAAAAGAGATGTTAAGCTTAAGATTGCT   |
| Yellowbeet_BvADHα | 224 | CTAATGATCATCTTGACGTTAGTAAAAGAGATGTTAAGCTTAAGATTGCT   |
| Whitebeet_BvADHα  | 224 | CTAATGATCATCTTGACGTTAGTAAAAGAGATGTTAAGCTTAAGATTGCT   |
| Sugarbeet_BvADHα  | 224 | CTAATGATCATCTTGACGTTAGTAAAAGAGATGTTAAGCTTAAGATTGCT   |
| Seabeet_BvADHα    | 251 | CTAATGATCATCTTGACGTTAGTAAAAGAGATGTTAAGCTTAAGATTGCT   |
| Redbeet2_BvADHα   | 224 | CTAATGATCATCTTGACGTTAGTAAAAGAGATGTTAAGCTTAAGATTGCT   |
|                   |     |  |
| Redbeet1_BvADHα   | 274 | ATTATTGGGTTTGGTAACTTTGGCCAGTTTTTGGCTAAGACAATGGCTAA   |
| Yellowbeet_BvADHα | 274 | ATTATTGGGTTTGGTAACTTTGGCCAGTTTTTGGCTAAGACAATGGCTAA   |
| Whitebeet_BvADHα  | 274 | ATTATTGGGTTTGGTAACTTTGGCCAGTTTTTGGCTAAGACAATGGCTAA   |
| Sugarbeet_BvADHα  | 274 | ATTATTGGGTTTGGTAACTTTGGCCAGTTTTTGGCTAAGACAATGGCTAA   |
| Seabeet_BvADHα    | 301 | ATTATTGGGTTTGGTAACTTTGGCCAGTTTTTGGCTAAGACAATGGCTAA   |
| Redbeet2_BvADHα   | 274 | ATTATTGGGTTTGGTAACTTTGGCCAGTTTTTGGCTAAGACAATGGCTAA   |
|                   |     |  |
| Redbeet1_BvADHα   | 324 | GCAAGGTCATAGAGTGTTGGCTTACTCACGCTCGGACTACTCCCGCGCTG   |
| Yellowbeet_BvADHα | 324 | GCAAGGTCATAGAGTGTTGGCTTACTCACGCTCGGACTACTCCCGCGCTG   |
| Whitebeet_BvADHα  | 324 | GCAAGGTCATAGAGTGTTGGCTTACTCACGCTCGGACTACTCCCGCGCTG   |
| Sugarbeet_BvADHα  | 324 | GCAAGGTCATAGAGTGTTGGCTTACTCACGCTCGGACTACTCCCGCGCTG   |
| Seabeet_BvADHα    | 351 | GCAAGGTCATAGAGTGTTGGCTTACTCACGCTCGGACTACTCCCGCGCTG   |
| Redbeet2_BvADHα   | 324 | GCAAGGTCATAGAGTGTTGGCTTACTCACGCTCGGACTACTCCCGCGCTG   |
|                   |     |  |
| Redbeet1_BvADHα   | 374 | CTAAGGAGATCGGCGTCGAGTATTTTACTGACGCCGATGACCTCTGCGAG   |
| Yellowbeet_BvADHα | 374 | CTAAGGAGATCGGCGTCGAGTATTTTACTGACGCCGATGACCTCTGCGAG   |
| Whitebeet_BvADHα  | 374 | CTAAGGAGATCGGCGTCGAGTATTTTACTGACGCCGATGACCTCTGCGAG   |



|                           |     |   |
|---------------------------|-----|---|
| Sugarbeet_BvADH $\alpha$  | 374 | CTAAGGAGATCGGCGTCGAGTATTTTACTGACGCCGATGACCTCTGCGAG  |
| Seabeet_BvADH $\alpha$    | 401 | CTAAGGAGATCGGCGTCGAGTATTTTACTGACGCCGATGACCTCTGCGAG  |
| Redbeet2_BvADH $\alpha$   | 374 | CTAAGGAGATCGGCGTCGAGTATTTTACTGACGCCGATGACCTCTGCGAG  |
| Redbeet1_BvADH $\alpha$   | 424 | GAGCACCTGAGGTTATTCTGTTGTGCACAATCCATCCTCTCAACGGAGAA  |
| Yellowbeet_BvADH $\alpha$ | 424 | GAGCACCTGAGGTTATTCTGTTGTGCACAATCCATCCTCTCAACGGAGAA  |
| Whitebeet_BvADH $\alpha$  | 424 | GAGCACCTGAGGTTATTCTGTTGTGCACGTCCATCCTCTCAACGGAGAA   |
| Sugarbeet_BvADH $\alpha$  | 424 | GAGCACCTGAGGTTATTCTTTTGTGCACGTCCATCCTCTCAACGGAGAA   |
| Seabeet_BvADH $\alpha$    | 451 | GAGCACCTGAGGTTATTCTTTTGTGCACGTCCATCCTCTCAACGGAGAA   |
| Redbeet2_BvADH $\alpha$   | 424 | GAGCACCTGAGGTTATTCTGTTGTGCACAATCCATCCTCTCAACGGAGAA  |
| Redbeet1_BvADH $\alpha$   | 474 | GGTCCTCCGATCACTCCCCCTCCACCGGCTCCGTCGTTCAACCCTCTTTG  |
| Yellowbeet_BvADH $\alpha$ | 474 | GGTCCTCCGATCACTCCCCCTCCACCGGCTCCGTCGTTCAACCCTCTTTG  |
| Whitebeet_BvADH $\alpha$  | 474 | GGTCCTCCGATCACTCCCCCTCCACCGGCTCCGTCGTTCAACCCTCTTTG  |
| Sugarbeet_BvADH $\alpha$  | 474 | GGTCCTCCGATCACTCCCCCTCCACCGGCTCCGTCGTTCAACCCTCTTTG  |
| Seabeet_BvADH $\alpha$    | 501 | GGTCCTCCGATCACTCCCCCTCCACCGGCTCCGTCGTTCAACCCTCTTTG  |
| Redbeet2_BvADH $\alpha$   | 474 | GGTCCTCCGATCACTCCCCCTCCACCGGCTCCGTCGTTCAACCCTCTTTG  |
| Redbeet1_BvADH $\alpha$   | 524 | CGGATGTTCTCTCGGTCAAGGAATTTCCCTCGATCGCTCTTCCTTCAACTA |
| Yellowbeet_BvADH $\alpha$ | 524 | CGGATGTTCTCTCGGTCAAGGAATTTCCCTCGATCGCTCTTCCTTCAACTA |
| Whitebeet_BvADH $\alpha$  | 524 | CGGATGTTCTCTCGGTCAAGGAATTTCCCTCGATCGCTCTTCCTTCAACTA |
| Sugarbeet_BvADH $\alpha$  | 524 | CGGATGTTCTCTCGGTCAAGGAATTTCCCTCGATCGCTCTTCCTTCAACTA |
| Seabeet_BvADH $\alpha$    | 551 | CGGATGTTCTCTCGGTCAAGGAATTTCCCTCGATCGCTCTTCCTTCAACTA |
| Redbeet2_BvADH $\alpha$   | 524 | CGGATGTTCTCTCGGTCAAGGAATTTCCCTCGATCGCTCTTCCTTCAACTA |
| Redbeet1_BvADH $\alpha$   | 574 | CTTCCTAAGGACTTTGATATCCTATGCACCCACCCTATGTTTGGCCCAGA  |
| Yellowbeet_BvADH $\alpha$ | 574 | CTTCCTAAGGACTTTGATATCCTATGCACCCACCCTATGTTTGGCCCAGA  |
| Whitebeet_BvADH $\alpha$  | 574 | CTTCCTAAGGACTTTGATATCCTATGCACCCACCCTATGTTTGGCCCAGA  |
| Sugarbeet_BvADH $\alpha$  | 574 | CTTCCTAAGGACTTTGATATCCTATGCACCCACCCTATGTTTGGCCCAGA  |
| Seabeet_BvADH $\alpha$    | 601 | CTTCCTAAGGACTTTGATATCCTATGCACCCACCCTATGTTTGGCCCAGA  |
| Redbeet2_BvADH $\alpha$   | 574 | CTTCCTAAGGACTTTGATATCCTATGCACCCACCCTATGTTTGGCCCAGA  |
| Redbeet1_BvADH $\alpha$   | 624 | CTCGGGCAAAGACGGGTGGGGTGGACTACCTTTTGTGTTTCGATAAAGTTA |
| Yellowbeet_BvADH $\alpha$ | 624 | CTCGGGCAAAGACGGGTGGGGTGGACTACCTTTTGTGTTTCGATAAAGTTA |
| Whitebeet_BvADH $\alpha$  | 624 | CTCGGGCAAAGACGGGTGGGGTGGACTACCTTTTGTGTTTCGATAAAGTTA |
| Sugarbeet_BvADH $\alpha$  | 624 | CTCGGGCAAAGACGGGTGGGGTGGACTACCTTTTGTGTTTCGATAAAGTTA |
| Seabeet_BvADH $\alpha$    | 651 | CTCGGGCAAAGACGGGTGGGGTGGACTACCTTTTGTGTTTCGATAAAGTTA |
| Redbeet2_BvADH $\alpha$   | 624 | CTCGGGCAAAGACGGGTGGGGTGGACTACCTTTTGTGTTTCGATAAAGTTA |
| Redbeet1_BvADH $\alpha$   | 674 | GAGTCGGATCAGATCAGAGTCGGACATCTCGTGCTGAGGCATTCCTAGAC  |
| Yellowbeet_BvADH $\alpha$ | 674 | GAGTCGGATCAGATCAGAGTCGGACATCTCGTGCTGAGGCATTCCTAGAC  |
| Whitebeet_BvADH $\alpha$  | 674 | GAGTCGGATCAGATCAGAGTCGGACATCTCGTGCTGAGGCATTCCTAGAC  |
| Sugarbeet_BvADH $\alpha$  | 674 | GAGTCGGATCAGATCAGAGTCGGACATCTCGTGCTGAGGCATTCCTAGAC  |
| Seabeet_BvADH $\alpha$    | 701 | GAGTCGGATCAGATCAGAGTCGGACATCTCGTGCTGAGGCATTCCTAGAC  |
| Redbeet2_BvADH $\alpha$   | 674 | GAGTCGGATCAGATCAGAGTCGGACATCTCGTGCTGAGGCATTCCTAGAC  |
| Redbeet1_BvADH $\alpha$   | 724 | GTGTTTAGGAATGCCGGGTGTAGGATGGTGGAAATGAGTTGTGTTGATCA  |
| Yellowbeet_BvADH $\alpha$ | 724 | GTGTTTAGGAATGCCGGGTGTAGGATGGTGGAAATGAGTTGTGTTGATCA  |
| Whitebeet_BvADH $\alpha$  | 724 | GTGTTTAGGAATGCCGGGTGTAGGATGGTGGAAATGAGTTGTGTTGATCA  |
| Sugarbeet_BvADH $\alpha$  | 724 | GTGTTTAGGAATGCCGGGTGTAGGATGGTGGAAATGAGTTGTGTTGATCA  |
| Seabeet_BvADH $\alpha$    | 751 | GTGTTTAGGAATGCCGGGTGTAGGATGGTGGAAATGAGTTGTGTTGATCA  |
| Redbeet2_BvADH $\alpha$   | 724 | GTGTTTAGGAATGCCGGGTGTAGGATGGTGGAAATGAGTTGTGTTGATCA  |
| Redbeet1_BvADH $\alpha$   | 774 | TGACAAGCATGCAGCCGGGTCTCAATTTATTACACATATGATGGGACGAG  |
| Yellowbeet_BvADH $\alpha$ | 774 | TGACAAGCATGCAGCCGGGTCTCAATTTATTACACATATGATGGGACGAG  |
| Whitebeet_BvADH $\alpha$  | 774 | TGACAAGCATGCAGCCGGGTCTCAATTTATTACACATATGATGGGACGAG  |
| Sugarbeet_BvADH $\alpha$  | 774 | TGACAAGCATGCAGCCGGGTCTCAATTTATTACACATATGATGGGACGAG  |
| Seabeet_BvADH $\alpha$    | 801 | TGACAAGCATGCAGCCGGGTCTCAATTTATTACACATATGATGGGACGAG  |
| Redbeet2_BvADH $\alpha$   | 774 | TGACAAGCATGCAGCCGGATCTCAATTTATTACACATATGATGGGACGAG  |

|                           |      |  |
|---------------------------|------|--|
| Redbeet1_BvADH $\alpha$   | 824  | TTTTGGAGAAATTGGCCTTGAAAATACACCAATTAATACAAAAGGGTAC  |
| Yellowbeet_BvADH $\alpha$ | 824  | TTTTGGAGAAATTGGCCTTGAAAATACACCAATTAATACAAAAGGGTAC  |
| Whitebeet_BvADH $\alpha$  | 824  | TTTTGGAGAAATTGGCCTTGAAAATACACCAATTAATACAAAAGGGTAC  |
| Sugarbeet_BvADH $\alpha$  | 824  | TTTTGGAGAAATTGGCCTTGAAAATACACCAATTAATACAAAAGGGTAC  |
| Seabeet_BvADH $\alpha$    | 851  | TTTTGGAGAAATTGGCCTTGAAAATACACCAATTAATACAAAAGGGTAC  |
| Redbeet2_BvADH $\alpha$   | 824  | TTTTGGAGAAATTGGCCTTGAAAATACACCAATTAATACAAAAGGGTAC  |
|                           |      |  |
| Redbeet1_BvADH $\alpha$   | 874  | GAAAGTTTGTAAATTTGGTGGATAATACTGCAAGGGATAGTTTTGAGTT  |
| Yellowbeet_BvADH $\alpha$ | 874  | GAAAGTTTGTAAATTTGGTGGATAATACTGCAAGGGATAGTTTTGAGTT  |
| Whitebeet_BvADH $\alpha$  | 874  | GAAAGTTTGTAAATTTGGTGGATAATACTGCAAGGGATAGTTTTGAGTT  |
| Sugarbeet_BvADH $\alpha$  | 874  | GAAAGTTTGTAAATTTGGTGGATAATACTGCAAGGGATAGTTTTGAGTT  |
| Seabeet_BvADH $\alpha$    | 901  | GAAAGTTTGTAAATTTGGTGGATAATACTGCAAGGGATAGTTTTGAGTT  |
| Redbeet2_BvADH $\alpha$   | 874  | GAAAGTTTGTAAATTTGGTGGATAATACTGCAAGGGATAGTTTTGAGTT  |
|                           |      |  |
| Redbeet1_BvADH $\alpha$   | 924  | GTTTTACGGGTTGTTTTTGTACAATAAAAATGCAATGGAGCAATTGGATA |
| Yellowbeet_BvADH $\alpha$ | 924  | GTTTTACGGGTTGTTTTTGTACAATAAAAATGCAATGGAGCAATTGGATA |
| Whitebeet_BvADH $\alpha$  | 924  | GTTTTACGGGTTGTTTTTGTACAATAAAAATGCAATGGAGCAATTGGATA |
| Sugarbeet_BvADH $\alpha$  | 924  | GTTTTATGGGTTGTTTTTGTACAATAAAAATGCAATGGAGCAATTGGATA |
| Seabeet_BvADH $\alpha$    | 951  | GTTTTATGGGTTGTTTTTGTACAATAAAAATGCAATGGAGCAATTGGATA |
| Redbeet2_BvADH $\alpha$   | 924  | GTTTTACGGGTTGTTTTTGTACAATAAAAATGCAATGGAGCAATTGGATA |
|                           |      |  |
| Redbeet1_BvADH $\alpha$   | 974  | GAATGGATTGGGCTTTCGAGATGGTAAAAAGCAACTTTCGGGATATTTG  |
| Yellowbeet_BvADH $\alpha$ | 974  | GAATGGATTGGGCTTTCGAGATGGTAAAAAGCAACTTTCGGGATATTTG  |
| Whitebeet_BvADH $\alpha$  | 974  | GAATGGATTGGGCTTTCGAGATGGTAAAAAGCAACTTTCGGGATATTTG  |
| Sugarbeet_BvADH $\alpha$  | 974  | GAATGGATTGGGCTTTCGAGATGGTAAAAAGCAACTTTCGGGATATTTG  |
| Seabeet_BvADH $\alpha$    | 1001 | GAATGGATTGGGCTTTCGAGATGGTAAAAAGCAACTTTCGGGATATTTG  |
| Redbeet2_BvADH $\alpha$   | 974  | GAATGGATTGGGCTTTCGAGATGGTAAAAAGCAACTTTCGGGATATTTG  |
|                           |      |  |
| Redbeet1_BvADH $\alpha$   | 1024 | CATGATCTTGTTAGAAAACAATTGATGTTGGAGGGTAATAATGATCAAGC |
| Yellowbeet_BvADH $\alpha$ | 1024 | CATGATCTTGTTAGAAAACAATTGATGTTGGAGGGTAATAATGATCAAGC |
| Whitebeet_BvADH $\alpha$  | 1024 | CATGATCTTGTTAGAAAACAATTGATGTTGGAGGGTAATAATGATCAAGC |
| Sugarbeet_BvADH $\alpha$  | 1024 | CATGATCTTGTTAGAAAACAATTGATGTTGGAGGGTAATAATGATCAAGC |
| Seabeet_BvADH $\alpha$    | 1051 | CATGATCTTGTTAGAAAACAATTGATGTTGGAGGGTAATAATGATCAAGC |
| Redbeet2_BvADH $\alpha$   | 1024 | CATGATCTTGTTAGAAAACAATTGATGTTGGAGGGTAATAATGATCAAGC |
|                           |      |  |
| Redbeet1_BvADH $\alpha$   | 1074 | TGAGGTTACTTTTGACAAACCATTGATGCTTCCTTCTCCTACTATTAATC |
| Yellowbeet_BvADH $\alpha$ | 1074 | TGAGGTTACTTTTGACAAACCATTGATGCTTCCTTCTCCTACTATTAATC |
| Whitebeet_BvADH $\alpha$  | 1074 | TGAGGTTACTTTTGACAAACCATTGATGCTTCCTTCTCCTACTATTAATC |
| Sugarbeet_BvADH $\alpha$  | 1074 | TGAGGTTACTTTTGACAAACCATTGATGCTTCCTTCTCCTACTATTAATC |
| Seabeet_BvADH $\alpha$    | 1101 | TGAGGTTACTTTTGACAAACCATTGATGCTTCCTTCTCCTACTATTAATC |
| Redbeet2_BvADH $\alpha$   | 1074 | TGAGGTTACTTTTGACAAACCATTGATGCTTCCTTCTCCTACTATTAATC |
|                           |      |  |
| Redbeet1_BvADH $\alpha$   | 1124 | CTCCACAAATAGTTCCTCTGCTGATATGGCTGAGAAGAAGCATGATTTA  |
| Yellowbeet_BvADH $\alpha$ | 1124 | CTCCACAAATAGTTCCTCTGCTGATATGGCTGAGAAGAAGCATGATTTA  |
| Whitebeet_BvADH $\alpha$  | 1124 | CTCCACAAATAGTTCCTCTGCTGATATGGCTGAGAAGAAGCATGATTTA  |
| Sugarbeet_BvADH $\alpha$  | 1124 | CTCCACAAATAGTTCCTTCTGCTGATATGGCTGAGAAGAAGCATGATTTA |
| Seabeet_BvADH $\alpha$    | 1151 | CTCCACAAATAGTTCCTTCTGCTGATATGGCTGAGAAGAAGCATGATTTA |
| Redbeet2_BvADH $\alpha$   | 1124 | CTCCACAAATAGTTCCTCTGCTGATATGGCTGAGAAGAAGCATGATTTA  |
|                           |      |  |
| Redbeet1_BvADH $\alpha$   | 1174 | GTGGTGGTTAATGGTACTAGATAG                           |
| Yellowbeet_BvADH $\alpha$ | 1174 | GTGGTGGTTAATGGTACTAGATAG                           |
| Whitebeet_BvADH $\alpha$  | 1174 | GTGGTGGTTAATGGTACTAGATAG                           |
| Sugarbeet_BvADH $\alpha$  | 1174 | GTGGTGGTTAATGGTACTAGATAG                           |
| Seabeet_BvADH $\alpha$    | 1201 | GTGGTGGTTAATGGTACTAGATAG                           |
| Redbeet2_BvADH $\alpha$   | 1174 | GTGGTGGTTAATGGTACTAGATAG                           |

### b) Nucleotide sequence alignment of *BvADH $\beta$*

|                          |   |   |
|--------------------------|---|---|
| Sugarbeet_BvADH $\beta$  | 1 | ATGCTTTCTCTCTCCTCCACAACCACCGCAAACCCTCGCCGTCGCCATC |
| Yellowbeet_BvADH $\beta$ | 1 | ATGCTTTCTCTCTCCTCCACAACCACCGCAAACCCTCGCCGTCGCCATC |
| Redbeet1_BvADH $\beta$   | 1 | ATGCTTTCTCTCTCCTCCACAACCACCGCAAACCCTCGCCGTCGCCATC |

|                   |     |   |
|-------------------|-----|---|
| Whitebeet_BvADHβ  | 1   | ATGCTTTCTCTCTCCTCCACAACCACCGCAAACCCCTCGCCGTCGCCATC  |
| Seabeet_BvADHβ    | 1   | ATGCTTTCTCTCTCCTCCACAACCACCGCAAACCCCTCGCCGTCGCCATC  |
| Redbeet2_BvADHβ   | 1   | ATGCTTTCTCTCTCCTCCACAACCACCGCAAACCCCTCGCCGTCGCCATC  |
| Sugarbeet_BvADHβ  | 51  | TCCGGCGAATTTTCCGGCGAAACTTTCTTCTCTCTCCACCATCACCACCA  |
| Yellowbeet_BvADHβ | 51  | TCCGGCGAATTTTCCGGCGAAACTTTCTTCTCTCTCCACCATCACCACCA  |
| Redbeet1_BvADHβ   | 51  | TCCGGCGAATTTTCCGGCGAAACTTTCTTCTCTCTCCACCATCACCACCA  |
| Whitebeet_BvADHβ  | 51  | TCCGGCGAATTTTCCGGCGAAACTTTCTTCTCTCTCCACCATCACCACCA  |
| Seabeet_BvADHβ    | 51  | TCCGGCGAATTTTCCGGCGAAACTTTCTTCTCTCTCCACCATCACCACCA  |
| Redbeet2_BvADHβ   | 51  | TCCGGCGAATTTTCCGGCGAAACTTTCTTCTCTCTCCACCATCACCACCA  |
| Sugarbeet_BvADHβ  | 101 | CTCTCTCTTTCTCTCCTCGCCGGAGATATTTTCATGGCGTCAAACCCCTA  |
| Yellowbeet_BvADHβ | 101 | CTCTCTCTTTCTCTCCTCGCCGGAGATATTTTCATGGCGTCAAACCCCTA  |
| Redbeet1_BvADHβ   | 101 | CTCTCTCTTTCTCTCCTCGCCGGAGATATTTTCATGGCGTCAAACCCCTA  |
| Whitebeet_BvADHβ  | 101 | CTCTCTCTTTCTCTCCTCGCCGGAGATATTTTCATGGCGTCAAACCCCTA  |
| Seabeet_BvADHβ    | 101 | CTCTCTCTTTCTCTCCTCGCCGGAGATATTTTCATGGCGTCAAACCCCTA  |
| Redbeet2_BvADHβ   | 101 | CTCTCTCTTTCTCTCCTCGCCGGAGATATTTTCATGGCGTCAAACCCCTA  |
| Sugarbeet_BvADHβ  | 151 | ACAATTCGCAGCATCGACGCCGCACAATTCTTCGATTACGAATCAAACCT  |
| Yellowbeet_BvADHβ | 151 | ACAATTCGCAGCATCGACGCCGCACAATTCTTCGATTACGAATCAAACCT  |
| Redbeet1_BvADHβ   | 151 | ACAATTCGCAGCATCGACGCCGCACAATTCTTCGATTACGAATCAAACCT  |
| Whitebeet_BvADHβ  | 151 | ACAATTCGCAGCATCGACGCCGCACAATTCTTCGATTACGAATCAAACCT  |
| Seabeet_BvADHβ    | 151 | ACAATTCGCAGCATCGACGCCGCACAATTCTTCGATTACGAATCAAACCT  |
| Redbeet2_BvADHβ   | 151 | ACAATTCGCAGCATCGACGCCGCACAATTCTTCGATTACGAATCAAACCT  |
| Sugarbeet_BvADHβ  | 201 | TGCCGCCATTAACACAACCTCTTCGTCTTCATCTTCATCTTATTTCGAAGC |
| Yellowbeet_BvADHβ | 201 | TGCCGCCATTAACACAACCTCTTCGTCTTCATCTTCATCTTATTTCGAAGC |
| Redbeet1_BvADHβ   | 201 | TGCCGCCATTAACACAACCTCTTCGTCTTCATCTTCATCTTATTTCGAAGC |
| Whitebeet_BvADHβ  | 201 | TGCCGCCATTAACACAACCTCTTCGTCTTCATCTTCATCTTATTTCGAAGC |
| Seabeet_BvADHβ    | 201 | TGCCGCCATTAACACAACCTCTTCGTCTTCATCTTCATCTTATTTCGAAGC |
| Redbeet2_BvADHβ   | 201 | TGCCGCCATTAACACAACCTCTTCGTCTTCATCTTCATCTTATTTCGAAGC |
| Sugarbeet_BvADHβ  | 251 | TCAAAATCGCAATCGTAGGGTTCGGAAATTACGGACAATTTCTCGCGAAA  |
| Yellowbeet_BvADHβ | 251 | TCAAAATCGCAATCGTAGGGTTCGGAAATTACGGACAATTTCTCGCGAAA  |
| Redbeet1_BvADHβ   | 251 | TCAAAATCGCAATCGTAGGGTTCGGAAATTACGGACAATTTCTCGCGAAA  |
| Whitebeet_BvADHβ  | 251 | TCAAAATCGCAATCGTAGGGTTCGGAAATTACGGACAATTTCTCGCGAAA  |
| Seabeet_BvADHβ    | 251 | TCAAAATCGCAATCGTAGGGTTCGGAAATTACGGACAATTTCTCGCGAAA  |
| Redbeet2_BvADHβ   | 251 | TCAAAATCGCAATCGTAGGGTTCGGAAATTACGGACAATTTCTCGCGAAA  |
| Sugarbeet_BvADHβ  | 301 | ACCCTAGTTTCTCAAGGTCATACTGTTCTCGCTTATTCTCGCTCTGATTA  |
| Yellowbeet_BvADHβ | 301 | ACCCTAGTTTCTCAAGGTCATACTGTTCTCGCTTATTCTCGCTCTGATTA  |
| Redbeet1_BvADHβ   | 301 | ACCCTAGTTTCTCAAGGTCATACTGTTCTCGCTTATTCTCGCTCTGATTA  |
| Whitebeet_BvADHβ  | 301 | ACCCTAGTTTCTCAAGGTCATACTGTTCTCGCTTATTCTCGCTCTGATTA  |
| Seabeet_BvADHβ    | 301 | ACCCTAGTTTCTCAAGGTCATACTGTTCTCGCTTATTCTCGCTCTGATTA  |
| Redbeet2_BvADHβ   | 301 | ACCCTAGTTTCTCAAGGTCATACTGTTCTCGCTTATTCTCGCTCTGATTA  |
| Sugarbeet_BvADHβ  | 351 | CTCTAAAATCGCTGCGAATCTCGGCGTTTCTTACTTTTCTGATCCTGATG  |
| Yellowbeet_BvADHβ | 351 | CTCTAAAATCGCTGCGAATCTCGGCGTTTCTTACTTTTCTGATCCTGATG  |
| Redbeet1_BvADHβ   | 351 | CTCTAAAATCGCTGCGAATCTCGGCGTTTCTTACTTTTCTGATCCTGATG  |
| Whitebeet_BvADHβ  | 351 | CTCTAAAATCGCTGCGAATCTCGGCGTTTCTTACTTTTCTGATCCTGATG  |
| Seabeet_BvADHβ    | 351 | CTCTAAAATCGCTGCGAATCTCGGCGTTTCTTACTTTTCTGATCCTGATG  |
| Redbeet2_BvADHβ   | 351 | CTCTAAAATCGCTGCGAATCTCGGCGTTTCTTACTTTTCTGATCCTGATG  |
| Sugarbeet_BvADHβ  | 401 | ATCTTTGCGAAGAACATCCTGAGGTAATTATGTTGTGTACTTTCGATTTTA |
| Yellowbeet_BvADHβ | 401 | ATCTTTGCGAAGAACATCCTGAGGTAATTATGTTGTGTACTTTCGATTTTA |
| Redbeet1_BvADHβ   | 401 | ATCTTTGCGAAGAACATCCTGAGGTAATTATGTTGTGTACTTTCGATTTTA |
| Whitebeet_BvADHβ  | 401 | ATCTTTGCGAAGAACATCCTGAGGTAATTATGTTGTGTACTTTCGATTTTA |
| Seabeet_BvADHβ    | 401 | ATCTTTGCGAAGAACATCCTGAGGTAATTATGTTGTGTACTTTCGATTTTA |
| Redbeet2_BvADHβ   | 401 | ATCTTTGCGAAGAACATCCTGAGGTAATTATGTTGTGTACTTTCGATTTTA |

|                          |     |   |
|--------------------------|-----|---|
| Sugarbeet_BvADH $\beta$  | 451 | TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC  |
| Yellowbeet_BvADH $\beta$ | 451 | TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC  |
| Redbeet1_BvADH $\beta$   | 451 | TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC  |
| Whitebeet_BvADH $\beta$  | 451 | TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC  |
| Seabeet_BvADH $\beta$    | 451 | TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC  |
| Redbeet2_BvADH $\beta$   | 451 | TCAACTGAAGTTATGTTGAATTCGTTACCATTGCAGCGACTTAAACGATC  |
|                          |     |   |
| Sugarbeet_BvADH $\beta$  | 501 | GACGCTTTTTGTTGATGTTTTATCGGTGAAAAGAATTTCCGCGTAATTTGT |
| Yellowbeet_BvADH $\beta$ | 501 | GACGCTTTTTGTTGATGTTTTATCGGTGAAAAGAATTTCCGCGTAATTTGT |
| Redbeet1_BvADH $\beta$   | 501 | GACGCTTTTTGTTGATGTTTTATCGGTGAAAAGAATTTCCGCGTAATTTGT |
| Whitebeet_BvADH $\beta$  | 501 | GACGCTTTTTGTTGATGTTTTATCGGTGAAAAGAATTTCCGCGTAATTTGT |
| Seabeet_BvADH $\beta$    | 501 | GACGCTTTTTGTTGATGTTTTATCGGTGAAAAGAATTTCCGCGTAATTTGT |
| Redbeet2_BvADH $\beta$   | 501 | GACGCTTTTTGTTGATGTTTTATCGGTGAAAAGAATTTCCGCGTAATTTGT |
|                          |     |   |
| Sugarbeet_BvADH $\beta$  | 551 | TTCTTCAAACCTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG |
| Yellowbeet_BvADH $\beta$ | 551 | TTCTTCAAACCTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG |
| Redbeet1_BvADH $\beta$   | 551 | TTCTTCAAACCTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG |
| Whitebeet_BvADH $\beta$  | 551 | TTCTTCAAACCTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG |
| Seabeet_BvADH $\beta$    | 551 | TTCTTCAAACCTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG |
| Redbeet2_BvADH $\beta$   | 551 | TTCTTCAAACCTTTACCGTCTGATTTTGATATATTATGTACTCATCCTATG |
|                          |     |   |
| Sugarbeet_BvADH $\beta$  | 601 | TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA  |
| Yellowbeet_BvADH $\beta$ | 601 | TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA  |
| Redbeet1_BvADH $\beta$   | 601 | TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA  |
| Whitebeet_BvADH $\beta$  | 601 | TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA  |
| Seabeet_BvADH $\beta$    | 601 | TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA  |
| Redbeet2_BvADH $\beta$   | 601 | TTTGGGCCTGAATCTGGGAAAAATGGTTGGGGAAGTTTGCCTTTTGTTTA  |
|                          |     |   |
| Sugarbeet_BvADH $\beta$  | 651 | TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA  |
| Yellowbeet_BvADH $\beta$ | 651 | TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA  |
| Redbeet1_BvADH $\beta$   | 651 | TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA  |
| Whitebeet_BvADH $\beta$  | 651 | TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA  |
| Seabeet_BvADH $\beta$    | 651 | TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA  |
| Redbeet2_BvADH $\beta$   | 651 | TGATAAGGTTAGGATTGGGAAAGATGAGGGTAGAATTAAGAGATGTGAGA  |
|                          |     |   |
| Sugarbeet_BvADH $\beta$  | 701 | GTTTTTTGGATGTTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT |
| Yellowbeet_BvADH $\beta$ | 701 | GTTTTTTGGATGTTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT |
| Redbeet1_BvADH $\beta$   | 701 | GTTTTTTGGATGTTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT |
| Whitebeet_BvADH $\beta$  | 701 | GTTTTTTGGATGTTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT |
| Seabeet_BvADH $\beta$    | 701 | GTTTTTTGGATGTTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT |
| Redbeet2_BvADH $\beta$   | 701 | GTTTTTTGGATGTTTTTTAGGAGAGAAGGTTGTAGGGTTGAGGAAATGACT |
|                          |     |   |
| Sugarbeet_BvADH $\beta$  | 751 | TGTGCTGAGCATGATAAGTTTGCAGCAGGGTCTCAGTTTATAACACATTT  |
| Yellowbeet_BvADH $\beta$ | 751 | TGTGCTGAGCATGATAAGTTTGCAGCAGGGTCTCAGTTTATAACACATTT  |
| Redbeet1_BvADH $\beta$   | 751 | TGTGCTGAGCATGATAAGTTTGCAGCAGGGTCTCAGTTTATAACACATTT  |
| Whitebeet_BvADH $\beta$  | 751 | TGTGCTGAGCATGATAAGTTTGCAGCAGGGTCTCAGTTTATAACACATTT  |
| Seabeet_BvADH $\beta$    | 751 | TGTGCTGAGCATGATAAGTTTGCAGCAGGGTCTCAGTTTATAACACATTT  |
| Redbeet2_BvADH $\beta$   | 751 | TGTGCTGAGCATGATAAGTTTGCAGCAGGGTCTCAGTTTATAACACATTT  |
|                          |     |   |
| Sugarbeet_BvADH $\beta$  | 801 | CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA  |
| Yellowbeet_BvADH $\beta$ | 801 | CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA  |
| Redbeet1_BvADH $\beta$   | 801 | CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA  |
| Whitebeet_BvADH $\beta$  | 801 | CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA  |
| Seabeet_BvADH $\beta$    | 801 | CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA  |
| Redbeet2_BvADH $\beta$   | 801 | CTTAGGGAGGGTTTTGGAGAAGCTTGATTTGGAGGATACGCCGATTAATA  |
|                          |     |   |
| Sugarbeet_BvADH $\beta$  | 851 | CGAAAGGGTATGAGAGTTTGTGAAATTTGGTGGATAATACGTCGAAGGAT  |
| Yellowbeet_BvADH $\beta$ | 851 | CGAAAGGGTATGAGAGTTTGTGAAATTTGGTGGATAATACGTCGAAGGAT  |
| Redbeet1_BvADH $\beta$   | 851 | CGAAAGGGTATGAGAGTTTGTGAAATTTGGTGGATAATACGTCGAAGGAT  |
| Whitebeet_BvADH $\beta$  | 851 | CGAAAGGGTATGAGAGTTTGTGAAATTTGGTGGATAATACGTCGAAGGAT  |

|                          |      |  |
|--------------------------|------|--|
| Seabeet_BvADH $\beta$    | 851  | CGAAAGGGTATGAGAGTTTGTGGAATTTGGTGGATAATACGTCGAAGGAT |
| Redbeet2_BvADH $\beta$   | 851  | CGAAAGGGTATGAGAGTTTGTGGAATTTGGTGGATAATACGTCGAAGGAT |
| Sugarbeet_BvADH $\beta$  | 901  | AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA |
| Yellowbeet_BvADH $\beta$ | 901  | AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA |
| Redbeet1_BvADH $\beta$   | 901  | AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA |
| Whitebeet_BvADH $\beta$  | 901  | AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA |
| Seabeet_BvADH $\beta$    | 901  | AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA |
| Redbeet2_BvADH $\beta$   | 901  | AGTTTCGAGTTGTTTTATGGGTTGTTTTTGTATAATCAGAATGCTATGGA |
| Sugarbeet_BvADH $\beta$  | 951  | GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT |
| Yellowbeet_BvADH $\beta$ | 951  | GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT |
| Redbeet1_BvADH $\beta$   | 951  | GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT |
| Whitebeet_BvADH $\beta$  | 951  | GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT |
| Seabeet_BvADH $\beta$    | 951  | GCAGTTAGAGAGGTTAGATTGGGCAATTGAGTTGGTTAAGAAGCAATTGT |
| Redbeet2_BvADH $\beta$   | 951  | GCAGTTAGAGAGGTTAGATTGGGCGTTTGAGTTGGTTAAGAAGCAATTGT |
| Sugarbeet_BvADH $\beta$  | 1001 | TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG |
| Yellowbeet_BvADH $\beta$ | 1001 | TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG |
| Redbeet1_BvADH $\beta$   | 1001 | TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG |
| Whitebeet_BvADH $\beta$  | 1001 | TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG |
| Seabeet_BvADH $\beta$    | 1001 | TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG |
| Redbeet2_BvADH $\beta$   | 1001 | TTGGACACTTGCATGGGTTGCTAAGGAAACAGTTGTTTGGGTTTTCTGAG |
| Sugarbeet_BvADH $\beta$  | 1051 | ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC |
| Yellowbeet_BvADH $\beta$ | 1051 | ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC |
| Redbeet1_BvADH $\beta$   | 1051 | ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC |
| Whitebeet_BvADH $\beta$  | 1051 | ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC |
| Seabeet_BvADH $\beta$    | 1051 | ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC |
| Redbeet2_BvADH $\beta$   | 1051 | ATAGATGAACGTATTGGGAAGGCGAAGGAGATCAAATTTCTCTCTGATGC |
| Sugarbeet_BvADH $\beta$  | 1101 | TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG |
| Yellowbeet_BvADH $\beta$ | 1101 | TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG |
| Redbeet1_BvADH $\beta$   | 1101 | TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG |
| Whitebeet_BvADH $\beta$  | 1101 | TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG |
| Seabeet_BvADH $\beta$    | 1101 | TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG |
| Redbeet2_BvADH $\beta$   | 1101 | TGCAGAACAGAATGGCTCTGCCTTGTCTGCTAGGGAGAATGCAAATTCGG |
| Sugarbeet_BvADH $\beta$  | 1151 | AGACAAATTGA  |
| Yellowbeet_BvADH $\beta$ | 1151 | AGACAAATTGA  |
| Redbeet1_BvADH $\beta$   | 1151 | AGACAAATTGA  |
| Whitebeet_BvADH $\beta$  | 1151 | AGACAAATTGA  |
| Seabeet_BvADH $\beta$    | 1151 | AGACAAATTGA  |
| Redbeet2_BvADH $\beta$   | 1151 | AGACAAATTGA  |


### c) Amino acid sequence alignment of BvADH $\alpha$

|                           |    |   |
|---------------------------|----|---|
| Redbeet1_BvADH $\alpha$   | 1  | MISLSSFHPSSTTATATAAAAT-----THPPQQCPAFSSPPSHLSL      |
| Whitebeet_BvADH $\alpha$  | 1  | MISLSSFHPSSTTATATAAAAT-----THPPQQCPAFSSPPSHLSL      |
| Yellowbeet_BvADH $\alpha$ | 1  | MISLSSFDPSSSTTATATAAAAT-----THPPQQCPAFSSPPSHLSL     |
| Sugarbeet_BvADH $\alpha$  | 1  | MISLSSFHPSSTTATATAAATAT-----THPPQQCPAFSSPPSHLSL     |
| Seabeet_BvADH $\alpha$    | 1  | MISLSSFHPSSTTATATAAATATATAATATATTHPPQQCPAFSSPPSHLSL |
| Redbeet2_BvADH $\alpha$   | 1  | MISLSSFHPSSTTATATAAAAT-----THPPQQCPAFSSPPSHLSL      |
| Redbeet1_BvADH $\alpha$   | 42 | PLRHPRQHLVVRCGGGGSASESVFNRDSAATRVSNDHLDVSKRDVCLKIA  |
| Whitebeet_BvADH $\alpha$  | 42 | PLRHPRQHLVVRCGGGGSASESVFNRDSAATRVSNDHLDVSKRDVCLKIA  |
| Yellowbeet_BvADH $\alpha$ | 42 | PLRHPRQHLVVRCGGGGSASESVFNRDSAATRVSNDHLDVSKRDVCLKIA  |
| Sugarbeet_BvADH $\alpha$  | 42 | PLRHPRQHLVVRCGGGGSASESVFNRDSAATRVSNDHLDVSKRDVCLKIA  |
| Seabeet_BvADH $\alpha$    | 51 | PLRHPRQHLVVRCGGGGSASESVFNRDSAATRVSNDHLDVSKRDVCLKIA  |
| Redbeet2_BvADH $\alpha$   | 42 | PLRHPRQHLVVRCGGGGSASESVFNRDSAATRVSNDHLDVSKRDVCLKIA  |

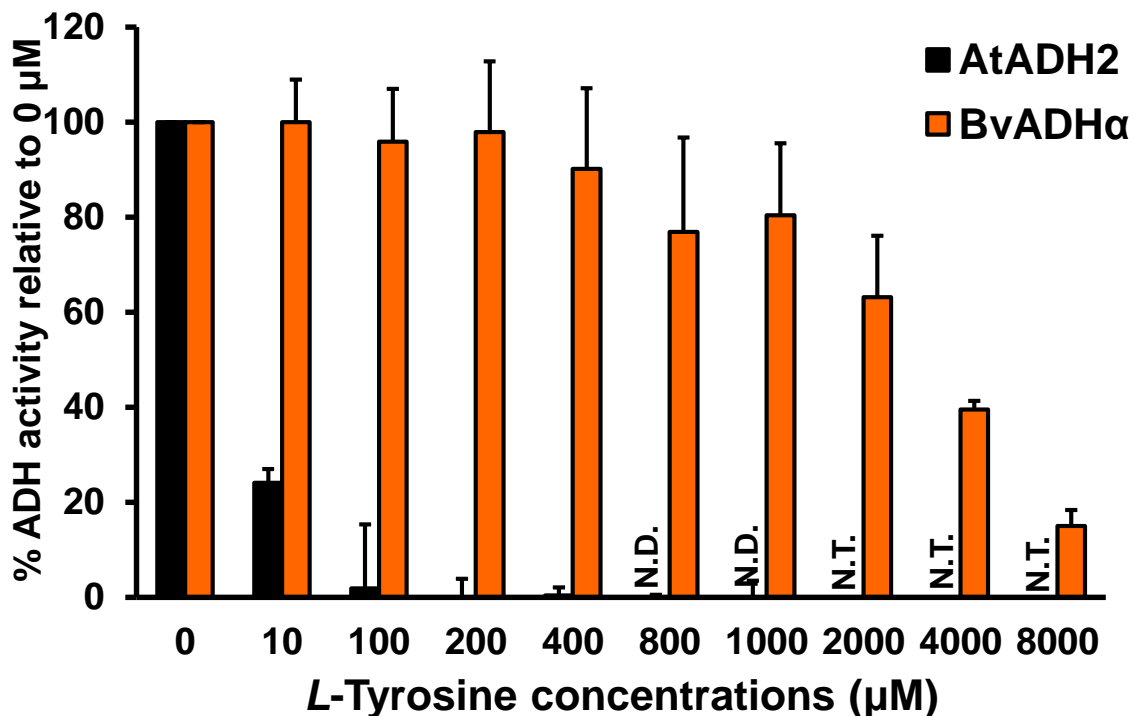
|                           |     |   |
|---------------------------|-----|---|
| Redbeet1_BvADH $\alpha$   | 92  | IIGFGNFGQFLAKTMAKQGHRVLAYSRSDYSRAAKEIGVEYFTDADDLCE  |
| Whitebeet_BvADH $\alpha$  | 92  | IIGFGNFGQFLAKTMAKQGHRVLAYSRSDYSRAAKEIGVEYFTDADDLCE  |
| Yellowbeet_BvADH $\alpha$ | 92  | IIGFGNFGQFLAKTMAKQGHRVLAYSRSDYSRAAKEIGVEYFTDADDLCE  |
| Sugarbeet_BvADH $\alpha$  | 92  | IIGFGNFGQFLAKTMAKQGHRVLAYSRSDYSRAAKEIGVEYFTDADDLCE  |
| Seabeet_BvADH $\alpha$    | 101 | IIGFGNFGQFLAKTMAKQGHRVLAYSRSDYSRAAKEIGVEYFTDADDLCE  |
| Redbeet2_BvADH $\alpha$   | 92  | IIGFGNFGQFLAKTMAKQGHRVLAYSRSDYSRAAKEIGVEYFTDADDLCE  |
|                           |     |   |
| Redbeet1_BvADH $\alpha$   | 142 | EHPEVILLCTSILSTEKVLRSPLHLRRLRRSTLFADVLSVKEFPRSLFLQL |
| Whitebeet_BvADH $\alpha$  | 142 | EHPEVILLCTSILSTEKVLRSPLHLRRLRRSTLFADVLSVKEFPRSLFLQL |
| Yellowbeet_BvADH $\alpha$ | 142 | EHPEVILLCTSILSTEKVLRSPLHLRRLRRSTLFADVLSVKEFPRSLFLQL |
| Sugarbeet_BvADH $\alpha$  | 142 | EHPEVILLCTSILSTEKVLRSPLHLRRLRRSTLFADVLSVKEFPRSLFLQL |
| Seabeet_BvADH $\alpha$    | 151 | EHPEVILLCTSILSTEKVLRSPLHLRRLRRSTLFADVLSVKEFPRSLFLQL |
| Redbeet2_BvADH $\alpha$   | 142 | EHPEVILLCTSILSTEKVLRSPLHLRRLRRSTLFADVLSVKEFPRSLFLQL |
|                           |     |   |
| Redbeet1_BvADH $\alpha$   | 192 | LPKDFDILCTHPMFGPDSGKDGWGGLPFVFDKVRVGSQDQRTSRAEAFLD  |
| Whitebeet_BvADH $\alpha$  | 192 | LPKDFDILCTHPMFGPDSGKDGWGGLPFVFDKVRVGSQDQRTSRAEAFLD  |
| Yellowbeet_BvADH $\alpha$ | 192 | LPKDFDILCTHPMFGPDSGKDGWGGLPFVFDKVRVGSQDQRTSRAEAFLD  |
| Sugarbeet_BvADH $\alpha$  | 192 | LPKDFDILCTHPMFGPDSGKDGWGGLPFVFDKVRVGSQDQRTSRAEAFLD  |
| Seabeet_BvADH $\alpha$    | 201 | LPKDFDILCTHPMFGPDSGKDGWGGLPFVFDKVRVGSQDQRTSRAEAFLD  |
| Redbeet2_BvADH $\alpha$   | 192 | LPKDFDILCTHPMFGPDSGKDGWGGLPFVFDKVRVGSQDQRTSRAEAFLD  |
|                           |     |   |
| Redbeet1_BvADH $\alpha$   | 242 | VFRNAGCRMVEMSCVDHDKHAAGSQFITHMMGRVLEKLALENTPINTKGY  |
| Whitebeet_BvADH $\alpha$  | 242 | VFRNAGCRMVEMSCVDHDKHAAGSQFITHMMGRVLEKLALENTPINTKGY  |
| Yellowbeet_BvADH $\alpha$ | 242 | VFRNAGCRMVEMSCVDHDKHAAGSQFITHMMGRVLEKLALENTPINTKGY  |
| Sugarbeet_BvADH $\alpha$  | 242 | VFRNAGCRMVEMSCVDHDKHAAGSQFITHMMGRVLEKLALENTPINTKGY  |
| Seabeet_BvADH $\alpha$    | 251 | VFRNAGCRMVEMSCVDHDKHAAGSQFITHMMGRVLEKLALENTPINTKGY  |
| Redbeet2_BvADH $\alpha$   | 242 | VFRNAGCRMVEMSCVDHDKHAAGSQFITHMMGRVLEKLALENTPINTKGY  |
|                           |     |   |
| Redbeet1_BvADH $\alpha$   | 292 | ESLLNLVDNTARDSFELFYGLFLYNKNAMEQLDRMDWAFEMVKKQLSGYL  |
| Whitebeet_BvADH $\alpha$  | 292 | ESLLNLVDNTARDSFELFYGLFLYNKNAMEQLDRMDWAFEMVKKQLSGYL  |
| Yellowbeet_BvADH $\alpha$ | 292 | ESLLNLVDNTARDSFELFYGLFLYNKNAMEQLDRMDWAFEMVKKQLSGYL  |
| Sugarbeet_BvADH $\alpha$  | 292 | ESLLNLVDNTARDSFELFYGLFLYNKNAMEQLDRMDWAFEMVKKQLSGYL  |
| Seabeet_BvADH $\alpha$    | 301 | ESLLNLVDNTARDSFELFYGLFLYNKNAMEQLDRMDWAFEMVKKQLSGYL  |
| Redbeet2_BvADH $\alpha$   | 292 | ESLLNLVDNTARDSFELFYGLFLYNKNAMEQLDRMDWAFEMVKKQLSGYL  |
|                           |     |   |
| Redbeet1_BvADH $\alpha$   | 342 | HDLVRKQLMLEGNNDQAEVTFDKPLMLPSPTINPPQIVPSADMAEKKHDL  |
| Whitebeet_BvADH $\alpha$  | 342 | HDLVRKQLMLEGNNDQAEVTFDKPLMLPSPTINPPQIVPSADMAEKKHDL  |
| Yellowbeet_BvADH $\alpha$ | 342 | HDLVRKQLMLEGNNDQAEVTFDKPLMLPSPTINPPQIVPSADMAEKKHDL  |
| Sugarbeet_BvADH $\alpha$  | 342 | HDLVRKQLMLEGNNDQAEVTFDKPLMLPSPTINPPQIVPSADMAEKKHDL  |
| Seabeet_BvADH $\alpha$    | 351 | HDLVRKQLMLEGNNDQAEVTFDKPLMLPSPTINPPQIVPSADMAEKKHDL  |
| Redbeet2_BvADH $\alpha$   | 342 | HDLVRKQLMLEGNNDQAEVTFDKPLMLPSPTINPPQIVPSADMAEKKHDL  |
|                           |     |   |
| Redbeet1_BvADH $\alpha$   | 392 | VVVNGTR   |
| Whitebeet_BvADH $\alpha$  | 392 | VVVNGTR   |
| Yellowbeet_BvADH $\alpha$ | 392 | VVVNGTR   |
| Sugarbeet_BvADH $\alpha$  | 392 | VVVNGTR   |
| Seabeet_BvADH $\alpha$    | 401 | VVVNGTR   |
| Redbeet2_BvADH $\alpha$   | 392 | VVVNGTR   |

#### d) Amino acid sequence alignment of BvADH $\beta$

|                          |    |  |
|--------------------------|----|--|
| Sugarbeet_BvADH $\beta$  | 1  | MLSLSSTTTAKPSPSPANFPAKLSSLSTITTTLSFSPPRRYFHGVKTL   |
| Yellowbeet_BvADH $\beta$ | 1  | MLSLSSTTTAKPSPSPANFPAKLSSLSTITTTLSFSPPRRYFHGVKTL   |
| Redbeet1_BvADH $\beta$   | 1  | MLSLSSTTTAKPSPSPANFPAKLSSLSTITTTLSFSPPRRYFHGVKTL   |
| Whitebeet_BvADH $\beta$  | 1  | MLSLSSTTTAKPSPSPANFPAKLSSLSTITTTLSFSPPRRYFHGVKTL   |
| Seabeet_BvADH $\beta$    | 1  | MLSLSSTTTAKPSPSPANFPAKLSSLSTITTTLSFSPPRRYFHGVKTL   |
| Redbeet2_BvADH $\beta$   | 1  | MLSLSSTTTAKPSPSPANFPAKLSSLSTITTTLSFSPPRRYFHGVKTL   |
|                          |    |  |
| Sugarbeet_BvADH $\beta$  | 51 | TIRSIDAAQFFDYESKLAainttsssssssySKLKIAIVGFGNYGQFLAK |
| Yellowbeet_BvADH $\beta$ | 51 | TIRSIDAAQFFDYESKLAainttsssssssySKLKIAIVGFGNYGQFLAK |
| Redbeet1_BvADH $\beta$   | 51 | TIRSIDAAQFFDYESKLAainttsssssssySKLKIAIVGFGNYGQFLAK |

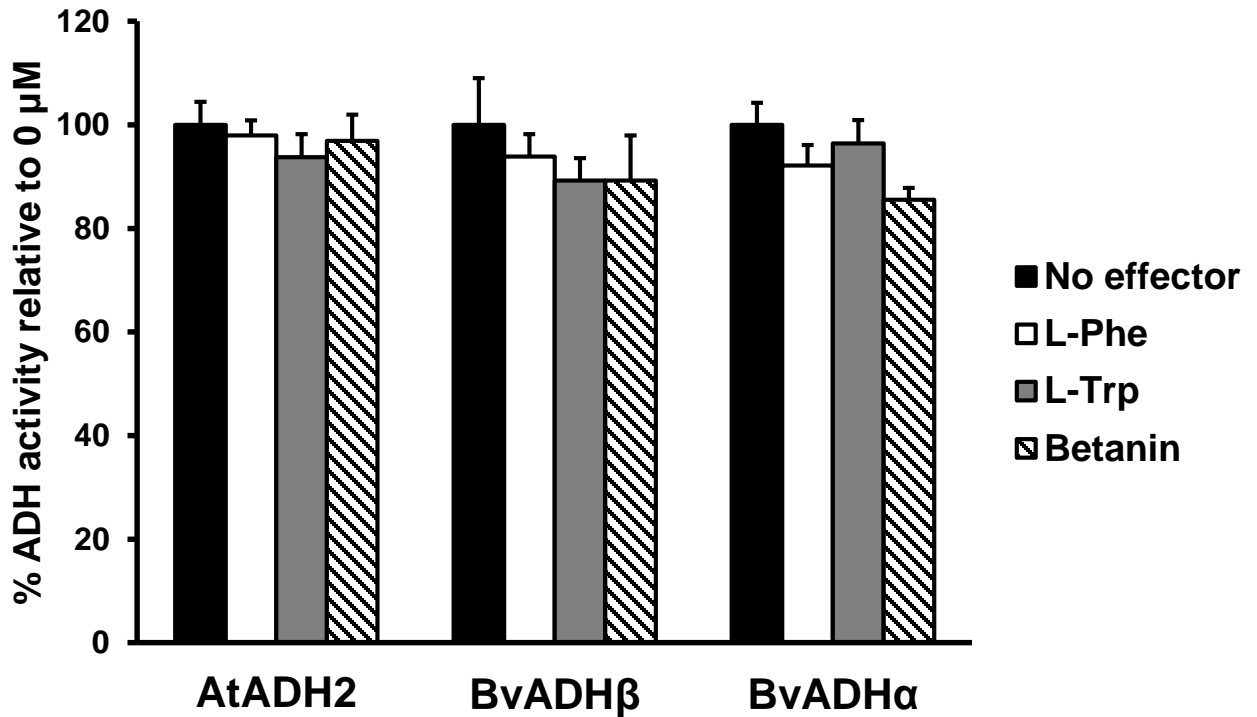
|                          |     |   |
|--------------------------|-----|---|
| Whitebeet_BvADH $\beta$  | 51  | TIRSIDAAQFFDYESKLA AINTTSSSSSSSSYSK LKIAIVGFGNYGQFLAK                             |
| Seabeet_BvADH $\beta$    | 51  | TIRSIDAAQFFDYESKLA AINTTSSSSSSSSYSK LKIAIVGFGNYGQFLAK                             |
| Redbeet2_BvADH $\beta$   | 51  | TIRSIDAAQFFDYESKLA AINTTSSSSSSSSYSK LKIAIVGFGNYGQFLAK                             |
|                          |     |  |
| Sugarbeet_BvADH $\beta$  | 101 | TLVSQGHTVLAYSRS DYSKIAANLGVSYFSDPDDLCEEHPEVIMLCTSIL                               |
| Yellowbeet_BvADH $\beta$ | 101 | TLVSQGHTVLAYSRS DYSKIAANLGVSYFSDPDDLCEEHPEVIMLCTSIL                               |
| Redbeet1_BvADH $\beta$   | 101 | TLVSQGHTVLAYSRS DYSKIAANLGVSYFSDPDDLCEEHPEVIMLCTSIL                               |
| Whitebeet_BvADH $\beta$  | 101 | TLVSQGHTVLAYSRS DYSKIAANLGVSYFSDPDDLCEEHPEVIMLCTSIL                               |
| Seabeet_BvADH $\beta$    | 101 | TLVSQGHTVLAYSRS DYSKIAANLGVSYFSDPDDLCEEHPEVIMLCTSIL                               |
| Redbeet2_BvADH $\beta$   | 101 | TLVSQGHTVLAYSRS DYSKIAANLGVSYFSDPDDLCEEHPEVIMLCTSIL                               |
| Sugarbeet_BvADH $\beta$  | 151 | STEVMLNSLPLQRLKRSTLFVDVLSVKEFPRNLF LQTLPSDFDILCTHPM                               |
| Yellowbeet_BvADH $\beta$ | 151 | STEVMLNSLPLQRLKRSTLFVDVLSVKEFPRNLF LQTLPSDFDILCTHPM                               |
| Redbeet1_BvADH $\beta$   | 151 | STEVMLNSLPLQRLKRSTLFVDVLSVKEFPRNLF LQTLPSDFDILCTHPM                               |
| Whitebeet_BvADH $\beta$  | 151 | STEVMLNSLPLQRLKRSTLFVDVLSVKEFPRNLF LQTLPSDFDILCTHPM                               |
| Seabeet_BvADH $\beta$    | 151 | STEVMLNSLPLQRLKRSTLFVDVLSVKEFPRNLF LQTLPSDFDILCTHPM                               |
| Redbeet2_BvADH $\beta$   | 151 | STEVMLNSLPLQRLKRSTLFVDVLSVKEFPRNLF LQTLPSDFDILCTHPM                               |
| Sugarbeet_BvADH $\beta$  | 201 | FGPESGKNGWGS LPFVYDKVRIKDEGRIKRCE SFLDVFRREGCRVEEMT                               |
| Yellowbeet_BvADH $\beta$ | 201 | FGPESGKNGWGS LPFVYDKVRIKDEGRIKRCE SFLDVFRREGCRVEEMT                               |
| Redbeet1_BvADH $\beta$   | 201 | FGPESGKNGWGS LPFVYDKVRIKDEGRIKRCE SFLDVFRREGCRVEEMT                               |
| Whitebeet_BvADH $\beta$  | 201 | FGPESGKNGWGS LPFVYDKVRIKDEGRIKRCE SFLDVFRREGCRVEEMT                               |
| Seabeet_BvADH $\beta$    | 201 | FGPESGKNGWGS LPFVYDKVRIKDEGRIKRCE SFLDVFRREGCRVEEMT                               |
| Redbeet2_BvADH $\beta$   | 201 | FGPESGKNGWGS LPFVYDKVRIKDEGRIKRCE SFLDVFRREGCRVEEMT                               |
| Sugarbeet_BvADH $\beta$  | 251 | CAEHDKFAAGSQ FITHFLGRVLEKLDLEDTP INTKGYESLLNLVDNTSKD                              |
| Yellowbeet_BvADH $\beta$ | 251 | CAEHDKFAAGSQ FITHFLGRVLEKLDLEDTP INTKGYESLLNLVDNTSKD                              |
| Redbeet1_BvADH $\beta$   | 251 | CAEHDKFAAGSQ FITHFLGRVLEKLDLEDTP INTKGYESLLNLVDNTSKD                              |
| Whitebeet_BvADH $\beta$  | 251 | CAEHDKFAAGSQ FITHFLGRVLEKLDLEDTP INTKGYESLLNLVDNTSKD                              |
| Seabeet_BvADH $\beta$    | 251 | CAEHDKFAAGSQ FITHFLGRVLEKLDLEDTP INTKGYESLLNLVDNTSKD                              |
| Redbeet2_BvADH $\beta$   | 251 | CAEHDKFAAGSQ FITHFLGRVLEKLDLEDTP INTKGYESLLNLVDNTSKD                              |
| Sugarbeet_BvADH $\beta$  | 301 | SFELFYGLFLYNQ NAMEQLERLDWAFELVKKQ LFGHLHGLLRKQLFGFSE                              |
| Yellowbeet_BvADH $\beta$ | 301 | SFELFYGLFLYNQ NAMEQLERLDWAFELVKKQ LFGHLHGLLRKQLFGFSE                              |
| Redbeet1_BvADH $\beta$   | 301 | SFELFYGLFLYNQ NAMEQLERLDWAFELVKKQ LFGHLHGLLRKQLFGFSE                              |
| Whitebeet_BvADH $\beta$  | 301 | SFELFYGLFLYNQ NAMEQLERLDWAFELVKKQ LFGHLHGLLRKQLFGFSE                              |
| Seabeet_BvADH $\beta$    | 301 | SFELFYGLFLYNQ NAMEQLERLDWAFELVKKQ LFGHLHGLLRKQLFGFSE                              |
| Redbeet2_BvADH $\beta$   | 301 | SFELFYGLFLYNQ NAMEQLERLDWAFELVKKQ LFGHLHGLLRKQLFGFSE                              |
| Sugarbeet_BvADH $\beta$  | 351 | IDERIGKAKEIKFLSDAAEQNGSALSARENANSETN  |
| Yellowbeet_BvADH $\beta$ | 351 | IDERIGKAKEIKFLSDAAEQNGSALSARENANSETN  |
| Redbeet1_BvADH $\beta$   | 351 | IDERIGKAKEIKFLSDAAEQNGSALSARENANSETN  |
| Whitebeet_BvADH $\beta$  | 351 | IDERIGKAKEIKFLSDAAEQNGSALSARENANSETN  |
| Seabeet_BvADH $\beta$    | 351 | IDERIGKAKEIKFLSDAAEQNGSALSARENANSETN  |
| Redbeet2_BvADH $\beta$   | 351 | IDERIGKAKEIKFLSDAAEQNGSALSARENANSETN  |

**Figure S4 . No amino acid changes were found in the mature protein coding region of BvADH $\alpha$  among different *B. vulgaris* varieties.** The *BvADH $\alpha$*  and *BvADH $\beta$*  genes were sequenced from five different varieties of domesticated (red 1 [*W357B*], red 2 [*Boltardy*], sugar, yellow, and white) and a wild beet (sea beet ascension number PI562585). In nucleotide sequence comparisons of *BvADH $\alpha$*  (a) and *BvADH $\beta$*  (b), several single nucleotide polymorphisms (SNPs) were found among varieties. Amino acid sequence alignments of *BvADH $\alpha$*  (c) and *BvADH $\beta$*  (d), 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.

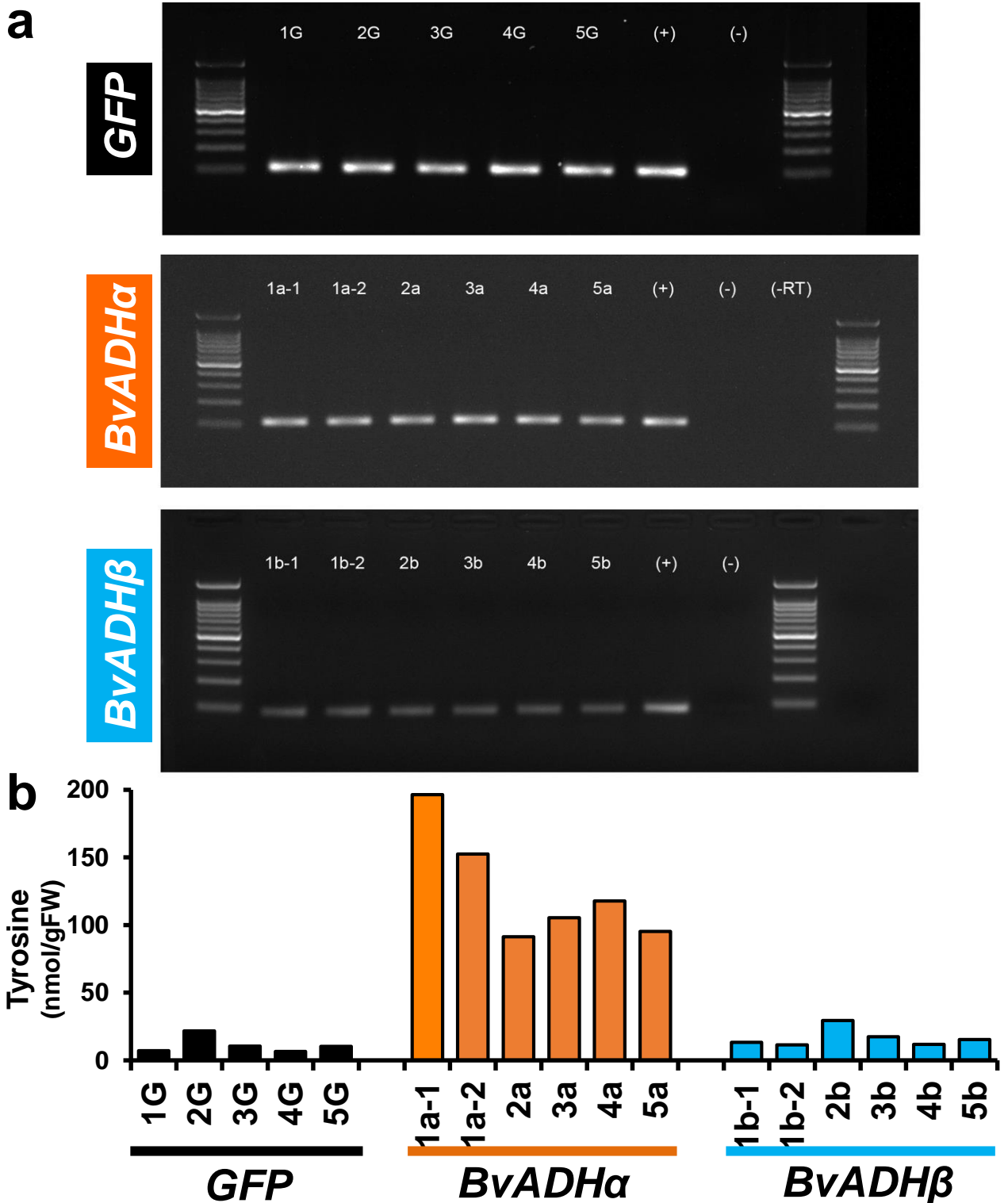


**Figure S5. Recombinant His-tagged BvADH $\alpha$  also exhibits reduced sensitivity to Tyr relative to AtADH2.** BvADH $\alpha$  and AtADH2 recombinant enzymes were also generated as 6xHis-tag proteins to determine if GST-tag affects Tyr sensitivity of BvADH $\alpha$ . The His-BvADH $\alpha$  recombinant enzyme still exhibited relaxed sensitive to Tyr inhibition. Data are expressed as the percentage of respective control activity without Tyr (0  $\mu\text{M}$ ) and the means of three independent experiments  $\pm$  s.e.m. N.D., not detectable; N.T., not tested.

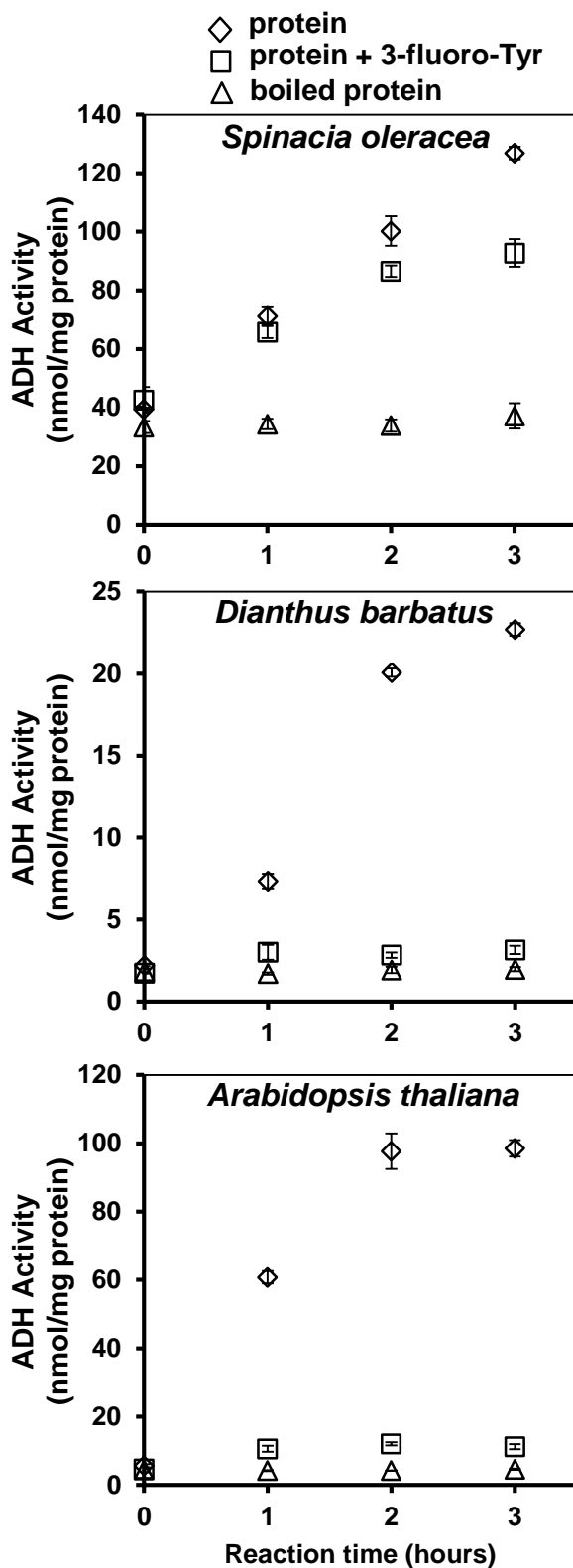




**Figure S6. BvADHs are not inhibited by phenylalanine, tryptophan, and betanin.** ADH activity of BvADH $\alpha$ , BvADH $\beta$  and AtADH2 was measured in the presence and absence of 1 mM final concentration of *L*-phenylalanine (*L*-Phe), *L*-tryptophan (*L*-Trp), and betanin as an effector. Data are expressed as the percentage of respective control activity without effector and the mean of three independent experiments  $\pm$  s.m.e. No significant reduction was observed by any effector treatment relative to respective no effector control ( $P < 0.05$ , student-*t* test).

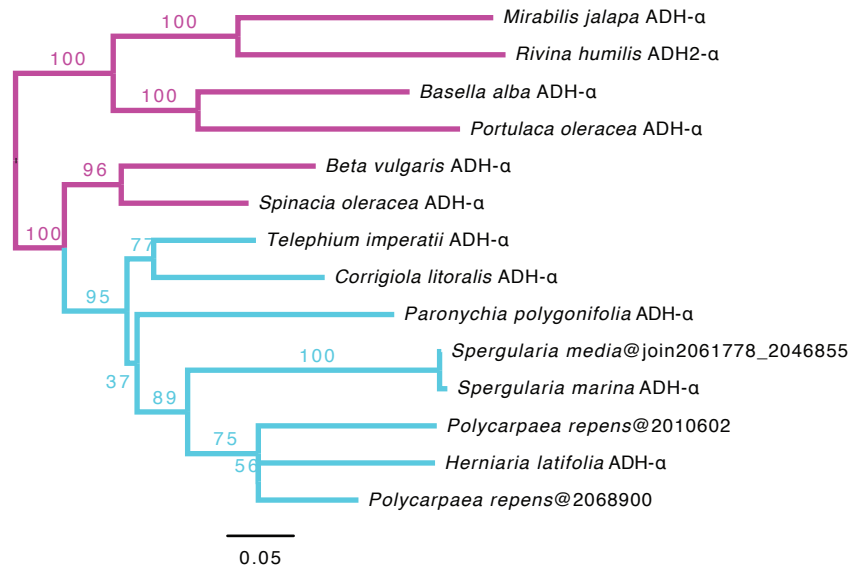


**Figure S7. Transgene expression and tyrosine levels of individual leaf samples of infiltrated *Nicotiana benthamiana*.** *Agrobacterium tumefaciens* carrying the construct of 35S::GFP, 35S::BvADH $\alpha$ , or 35S::BvADH $\beta$  was infiltrated to *Nicotiana benthamiana* leaves (sample names ending with G, a, and b, respectively). 1a-1 and 1a-2 are technical replicates of the same leaf infiltrated with 35S::BvADH $\alpha$ , so do 1b-1 and 1b-2 for 35S::BvADH $\beta$ . (a) Expression of respective transgenes shown by RT-PCR. (+) 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. (b) Tyrosine contents of individual samples. Two technical replicates showed very similar results. Means  $\pm$  s.e.m. of Tyr and other amino acids analysis are shown in **Figure 3** and **Table S2**.

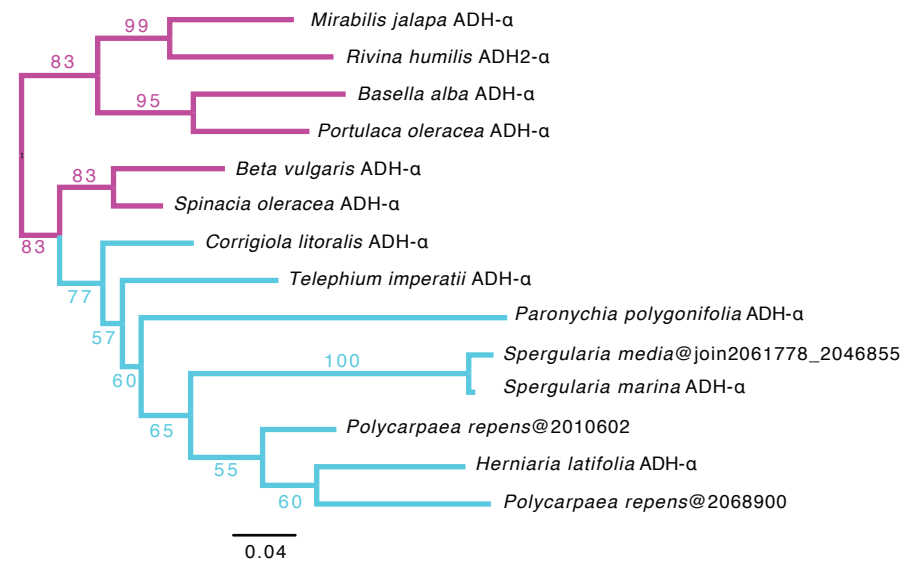


**Figure S8. Tyr sensitivity of ADH activity from plant tissues.** The plastid extracts of spinach (*Spinacia oleracea*), and the crude extracts of *Dianthus barbatus* and *Arabidopsis thaliana* were incubated with 1 mM arogenate substrate and 1 mM NADP<sup>+</sup> cofactor for indicated times. Plastids were isolated for spinach ADH assays to eliminate strong polyphenol oxidase activity present in the crude extracts. Data are means  $\pm$  s.e.m. ( $n = 4$ ). Activity increased linearly during the first two hours, which were used to calculate ADH activity presented in **Table 1**.

a) *ADH $\alpha$*  CDS



b) *ADH $\alpha$*  Peptide



**Figure S9. *ADH $\alpha$*  sequences used for testing relax selection. (a and b)** *ADH $\alpha$*  orthologs of Caryophyllaceae (blue, designated as test branches in RELAX analysis, **Table S4**), as compared to those betalain-producing Caryophyllales species (pink, designated as reference branches in RELAX analysis, **Table S4**). Blue branches showed no obvious acceleration of substitution in their coding sequences (CDS, **a**), whereas there was apparent acceleration in their peptide sequences (**b**). Tips marked with '@' are from assembled transcriptomes (see **Supporting Information Methods S1**). The rest of the sequences are from PCR and Sanger sequencing from DNA (*H. latifolia*, *S. marina*, and *P. polygonifolia*) or RNA.

|                |     |  |
|----------------|-----|--|
| AtADH2         | 1   | -----MLLHFS <sup>S</sup> PAKPLISPP-----N <sup>I</sup> RRNS <sup>P</sup> PTFLI <sup>S</sup> SPR <sup>R</sup> -----SLR   |
| BvADH $\beta$  | 1   | ---MLSLSS <sup>T</sup> TTAKPSPSPSPANFPAKLSS <sup>T</sup> STI <sup>T</sup> TTLSF <sup>S</sup> SPR <sup>R</sup> RYFHGVKTLT   |
| BvADH $\alpha$ | 1   | MISLSS <sup>F</sup> FHPSS <sup>T</sup> TATATAAAAA <sup>T</sup> THPPQQCPAF <sup>S</sup> SSPP <sup>S</sup> HL <sup>S</sup> SLPLR <sup>R</sup> PRQHLVVR <sup>C</sup> G  |
| AaPDH          | 1   | -----M <sup>A</sup> IL <sup>S</sup> SMFNP <sup>S</sup> SP <sup>P</sup> Q-----  |
| SyADH          | 1   | -----  |
| AtADH2         | 34  | IR <sup>A</sup> IDAA <sup>Q</sup> IFDYE <sup>E</sup> IQ <sup>L</sup> KSEYRKSS-----AL <sup>K</sup> IAV <sup>G</sup> FGNFG <sup>Q</sup> FLSK <sup>T</sup> LIR <sup>R</sup> IG  |
| BvADH $\beta$  | 52  | IRSIDAA <sup>Q</sup> FFDYE <sup>S</sup> SK <sup>L</sup> AAINTTSSSSSSSSYSK <sup>L</sup> KIAIVGFGNY <sup>Q</sup> QFLAK <sup>T</sup> LIVSQ <sup>G</sup>   |
| BvADH $\alpha$ | 56  | GGGSASE <sup>S</sup> VFN <sup>R</sup> SAATR <sup>V</sup> SVNDHLDVSKRDV <sup>K</sup> LKIAI <sup>G</sup> FGNFG <sup>Q</sup> FLAK <sup>T</sup> MAK <sup>Q</sup> G   |
| AaPDH          | 15  | --G <sup>F</sup> CKK <sup>N</sup> I <sup>I</sup> KILK <sup>S</sup> SMQ <sup>N</sup> -----M <sup>L</sup> IVG <sup>V</sup> GFMG <sup>G</sup> SE <sup>F</sup> AKSLRR <sup>S</sup> G   |
| SyADH          | 1   | -----M <sup>K</sup> IGV <sup>V</sup> GLGLIGAS <sup>L</sup> LAGD <sup>L</sup> LR <sup>R</sup> RG  |
| AtADH2         | 82  | H <sup>D</sup> L <sup>T</sup> THSR <sup>S</sup> D----YSDAANS <sup>I</sup> GAR <sup>F</sup> FDNPH <sup>D</sup> LCE <sup>Q</sup> HPD <sup>V</sup> L <sup>L</sup> CTSIL <sup>S</sup> TE <sup>S</sup> VLR  |
| BvADH $\beta$  | 107 | H <sup>T</sup> VLAYS <sup>R</sup> SD----Y <sup>S</sup> KIAANL <sup>G</sup> VSY <sup>F</sup> SDPDL <sup>C</sup> EEHPE <sup>V</sup> IML <sup>C</sup> TSIL <sup>S</sup> TE <sup>V</sup> MLN   |
| BvADH $\alpha$ | 111 | H <sup>R</sup> VLAYS <sup>R</sup> SD----Y <sup>S</sup> RAAKE <sup>I</sup> GV <sup>F</sup> YFTDAD <sup>D</sup> L <sup>C</sup> EEHPE <sup>V</sup> ILL <sup>C</sup> TSIL <sup>S</sup> TE <sup>K</sup> VLR   |
| AaPDH          | 54  | FKGKI <sup>Y</sup> GYDIN <sup>P</sup> ESI <sup>S</sup> KA <sup>V</sup> DLGI <sup>I</sup> DEG <sup>T</sup> TSIAK <sup>V</sup> ED <sup>F</sup> SPDF <sup>V</sup> ML <sup>S</sup> SP <sup>V</sup> RT <sup>F</sup> RE <sup>I</sup> A   |
| SyADH          | 24  | H <sup>Y</sup> L <sup>I</sup> GV <sup>S</sup> SR <sup>Q</sup> Q----S <sup>I</sup> CEKAVER <sup>Q</sup> LVD <sup>E</sup> AGD <sup>L</sup> LSLL <sup>Q</sup> TAK <sup>L</sup> I <sup>F</sup> L <sup>C</sup> PI <sup>Q</sup> LIL <sup>P</sup> TL  |
| AtADH2         | 133 | S <sup>F</sup> PF <sup>Q</sup> RLRR <sup>S</sup> TLE <sup>V</sup> DVLSV <sup>K</sup> EFPK <sup>A</sup> L <sup>F</sup> I <sup>K</sup> YLPK <sup>F</sup> FDIL <sup>C</sup> THPM <sup>F</sup> GPES <sup>G</sup> K <sup>H</sup> SW <sup>S</sup> G  |
| BvADH $\beta$  | 158 | SL <sup>P</sup> IQ <sup>R</sup> LKR <sup>S</sup> TLE <sup>V</sup> DVLSV <sup>K</sup> EFP <sup>R</sup> N <sup>L</sup> FL <sup>Q</sup> TLP <sup>S</sup> DFDIL <sup>C</sup> THPM <sup>F</sup> GPES <sup>G</sup> K <sup>N</sup> GW <sup>S</sup>  |
| BvADH $\alpha$ | 162 | SL <sup>P</sup> L <sup>H</sup> RLRR <sup>S</sup> TLE <sup>A</sup> DVLSV <sup>K</sup> EFP <sup>R</sup> SL <sup>F</sup> L <sup>Q</sup> LLPK <sup>D</sup> FDIL <sup>C</sup> THPM <sup>F</sup> GPDS <sup>G</sup> K <sup>D</sup> GW <sup>G</sup>  |
| AaPDH          | 109 | KKLSY <sup>I</sup> L <sup>S</sup> EDAT <sup>V</sup> T <sup>D</sup> QGS <sup>V</sup> K <sup>G</sup> KL <sup>V</sup> YDLE <sup>N</sup> L <sup>L</sup> GK <sup>R</sup> FV <sup>G</sup> G--H <sup>P</sup> IAG <sup>T</sup> E <sup>K</sup> SG <sup>V</sup> E <sup>Y</sup> SL  |
| SyADH          | 75  | EKL <sup>I</sup> PH <sup>L</sup> SP <sup>T</sup> AT <sup>V</sup> T <sup>D</sup> VAS <sup>V</sup> K <sup>T</sup> AIAEPAS <sup>Q</sup> LWS <sup>G</sup> -E <sup>I</sup> GG--H <sup>P</sup> MA <sup>G</sup> TAA <sup>Q</sup> GID <sup>G</sup> A <sup>E</sup>  |
| AtADH2         | 188 | L <sup>P</sup> FVYDK <sup>V</sup> RIG <sup>D</sup> AAS <sup>R</sup> Q--E <sup>R</sup> CEK <sup>F</sup> L <sup>R</sup> I <sup>F</sup> ENEG <sup>C</sup> KM <sup>V</sup> EMS <sup>C</sup> E <sup>K</sup> H <sup>D</sup> Y <sup>Y</sup> AAG <sup>S</sup> Q <sup>F</sup> IT  |
| BvADH $\beta$  | 213 | L <sup>P</sup> FVYDK <sup>V</sup> RIG <sup>K</sup> DEG <sup>R</sup> I--K <sup>R</sup> CE <sup>S</sup> F <sup>L</sup> D <sup>V</sup> ER <sup>R</sup> E <sup>G</sup> C <sup>R</sup> E <sup>E</sup> M <sup>C</sup> A <sup>H</sup> D <sup>K</sup> F <sup>A</sup> AG <sup>S</sup> Q <sup>F</sup> IT                             |
| BvADH $\alpha$ | 217 | L <sup>P</sup> FVFD <sup>K</sup> V <sup>R</sup> VGS <sup>D</sup> QS <sup>R</sup> T--S <sup>R</sup> AE <sup>A</sup> F <sup>L</sup> D <sup>V</sup> ER <sup>N</sup> AG <sup>C</sup> RM <sup>V</sup> EM <sup>S</sup> C <sup>V</sup> D <sup>H</sup> D <sup>K</sup> H <sup>A</sup> AG <sup>S</sup> Q <sup>F</sup> IT             |
| AaPDH          | 162 | DNLYEG <sup>K</sup> K <sup>V</sup> IL <sup>I</sup> PT <sup>K</sup> K <sup>T</sup> DK <sup>K</sup> RL <sup>K</sup> L <sup>V</sup> K <sup>R</sup> V <sup>I</sup> ED <sup>V</sup> G <sup>V</sup> V <sup>E</sup> Y <sup>M</sup> SP <sup>E</sup> L <sup>H</sup> D <sup>Y</sup> V <sup>F</sup> CV <sup>V</sup> SH <sup>I</sup> P |
| SyADH          | 127 | ENL <sup>F</sup> VNAP <sup>Y</sup> V <sup>I</sup> PT <sup>E</sup> Y <sup>T</sup> D <sup>P</sup> E <sup>Q</sup> LACL <sup>R</sup> SV <sup>E</sup> PL <sup>G</sup> V <sup>K</sup> I <sup>Y</sup> L <sup>C</sup> PE <sup>A</sup> D <sup>H</sup> D <sup>Q</sup> AV <sup>A</sup> W <sup>I</sup> SH <sup>I</sup> P               |
| AtADH2         | 241 | H <sup>T</sup> M <sup>G</sup> RVLE <sup>K</sup> Y <sup>G</sup> VE <sup>S</sup> SPINT <sup>K</sup> GYET <sup>L</sup> L <sup>L</sup> IVENT <sup>S</sup> SS <sup>D</sup> SFEL <sup>F</sup> YGL <sup>F</sup> MYN <sup>P</sup> NALE <sup>Q</sup> LE   |
| BvADH $\beta$  | 266 | H <sup>F</sup> L <sup>G</sup> RVLE <sup>K</sup> MDLE <sup>D</sup> TPINT <sup>K</sup> GYES <sup>L</sup> LLN <sup>L</sup> VDNT <sup>S</sup> SKDSFEL <sup>F</sup> YGL <sup>F</sup> LYN <sup>Q</sup> NAME <sup>Q</sup> LE  |
| BvADH $\alpha$ | 270 | H <sup>M</sup> M <sup>G</sup> RVLE <sup>K</sup> LAL <sup>E</sup> NT <sup>P</sup> INT <sup>K</sup> GYES <sup>L</sup> LLN <sup>L</sup> VDNT <sup>A</sup> DSFEL <sup>F</sup> YGL <sup>F</sup> LYN <sup>K</sup> NAME <sup>Q</sup> L <sup>D</sup>   |
| AaPDH          | 217 | H <sup>A</sup> VAFAL <sup>V</sup> DTL <sup>I</sup> H <sup>M</sup> S-----T <sup>P</sup> EV <sup>D</sup> L <sup>E</sup> K <sup>Y</sup> PG <sup>G</sup> CF <sup>K</sup> DF <sup>T</sup> RIAK <sup>S</sup> DP  |
| SyADH          | 182 | VM <sup>V</sup> SAAL <sup>I</sup> QACAGE <sup>K</sup> DG-----D <sup>I</sup> L <sup>K</sup> LAQN <sup>L</sup> ASS <sup>G</sup> FR <sup>D</sup> T <sup>S</sup> RVGG <sup>N</sup> P   |
| AtADH2         | 296 | R <sup>L</sup> DMAFE <sup>S</sup> VK <sup>K</sup> EL <sup>F</sup> GR <sup>L</sup> H <sup>Q</sup> QYR <sup>K</sup> Q <sup>F</sup> GG---E <sup>V</sup> QSP <sup>K</sup> KTE <sup>Q</sup> K <sup>L</sup> LN <sup>D</sup> GGV <sup>V</sup> PMND <sup>I</sup>   |
| BvADH $\beta$  | 321 | R <sup>L</sup> DWAFEL <sup>V</sup> K <sup>Q</sup> L <sup>F</sup> GR <sup>L</sup> H <sup>L</sup> GL <sup>L</sup> R <sup>K</sup> Q <sup>L</sup> FG <sup>F</sup> SE <sup>D</sup> ERIG <sup>K</sup> AKE <sup>I</sup> K <sup>F</sup> LS <sup>D</sup> AAE <sup>Q</sup> NGSAL   |
| BvADH $\alpha$ | 325 | R <sup>M</sup> DWAFEM <sup>V</sup> K <sup>Q</sup> LS <sup>G</sup> Y <sup>L</sup> H <sup>D</sup> L <sup>V</sup> R <sup>K</sup> Q <sup>L</sup> M <sup>L</sup> EG <sup>N</sup> D <sup>Q</sup> AEV <sup>T</sup> FD <sup>K</sup> PL <sup>M</sup> LP <sup>S</sup> PT <sup>I</sup> N <sup>P</sup> P <sup>Q</sup> IV               |
| AaPDH          | 257 | IM <sup>W</sup> RDIF <sup>L</sup> EN <sup>K</sup> EN <sup>V</sup> M <sup>K</sup> AIE <sup>G</sup> E <sup>K</sup> SL <sup>N</sup> HL <sup>K</sup> ELI <sup>V</sup> REA <sup>E</sup> E <sup>E</sup> L <sup>V</sup> EY <sup>L</sup> L <sup>K</sup> E <sup>V</sup> K <sup>I</sup> K <sup>R</sup> ME <sup>I</sup> D             |
| SyADH          | 224 | E <sup>L</sup> GTMMAT <sup>Y</sup> N <sup>Q</sup> RALL <sup>K</sup> SL <sup>Q</sup> DY <sup>R</sup> OH <sup>L</sup> D <sup>L</sup> IT <sup>L</sup> IS <sup>N</sup> Q <sup>Q</sup> W <sup>P</sup> EL <sup>H</sup> ELL <sup>Q</sup> QT <sup>N</sup> GDR <sup>D</sup> K <sup>Y</sup> V  |
| AtADH2         | 348 | SSSSSSSSSSSS-----  |
| BvADH $\beta$  | 376 | SARENANSETN-----   |
| BvADH $\alpha$ | 380 | PSADMAEKKHDLVVVNGTR  |
| AaPDH          | 312 | -----  |
| SyADH          | 279 | E-----   |

### Figure S10. Histidine 217 residue responsible for Tyr sensitivity of

***Aquifex aeolicus* PDH (AaPDH) is still present in BvADH $\alpha$ .** 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 BvADH $\alpha$ , BvADH $\beta$ , and Arabidopsis ADH (AtADH2) 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 BvADH $\alpha$

**Table S1.** Primers used as indicated in the text and methods

| Species (gene)  | Purpose | Primer name                | Primer sequence 5' to 3'                 |
|---|---------|----------------------------|--|
| <i>Beta vulgaris</i> (BvADH $\beta$ )                 | RT-PCR  | pHM0290SLNBvADH $\beta$ F  | GGTTCGCGTGGATCCCTAACAAATTCGCAGCAT        |
| <i>Beta vulgaris</i> (BvADH $\beta$ )                 | RT-PCR  | pHM0291SLNRBvADH $\beta$ R | AATTCGGAGACAAATTGAGAATTCATCGTGACTG       |
| <i>Beta vulgaris</i> (BvADH $\alpha$ )                | RT-PCR  | pHM0372SLNBvADH $\alpha$ F | CTGGTTCCGCGTGGATCCTGCGGTGGAGGTGGTTCCG    |
| <i>Beta vulgaris</i> (BvADH $\alpha$ )                | RT-PCR  | pHM0373SLNBvADH $\alpha$ R | GTTAATGGTACTAGATTAGGAATTCATCGTGACTGA     |
| <i>Arabidopsis thaliana</i> (AtADH2)                  | Cloning | pHM0384SLNAtADH $\alpha$ F | CTGGTTCCGCGTGGATCCGCAATCGACGCCGCCAA      |
| <i>Arabidopsis thaliana</i> (AtADH2)                  | Cloning | pHM0385SLNAtADH $\alpha$ R | TCATCATCATCATCTTAAGAATTCATCGTGACTGA      |
| <i>Spinacea oleracea</i> (SoADH $\beta$ )             | Cloning | pHM0582SoADH $\beta$ F     | CTGGTTCCGCGTGGATCCGCCGCTACCAATACCTCC     |
| <i>Spinacea oleracea</i> (SoADH $\beta$ )             | Cloning | pHM0583SoADH $\beta$ R     | AATTCAGAGATCAATTGAGAATTCATCGTGACTGA      |
| <i>Spinacea oleracea</i> (SoADH $\alpha$ )            | Cloning | pHM0584SoADH $\alpha$ F    | CTGGTTCCGCGTGGATCCTGCGCCGCTCTGACTCC      |
| <i>Spinacea oleracea</i> (SoADH $\alpha$ )            | Cloning | pHM0585SoADH $\alpha$ R    | TGGTAATAATTCTAGATAGGAATTCATCGTGACTGA     |
| <i>Nepenthes alata</i> (NaADH $\beta$ )               | Cloning | pHM0603SLNNaADH $\beta$ F  | CTGGTTCCGCGTGGATCCGCCGCTGCCAACGACT       |
| <i>Nepenthes alata</i> (NaADH $\beta$ )               | Cloning | pHM0604SLNNaADH $\beta$ R  | AAATGTTGAGAGAAATTGAGAATTCATCGTGACTGA     |
| <i>Portulaca oleracea</i> (PoADH $\alpha$ )           | RT-PCR  | pHM0609SLNPoADH $\alpha$ F | CTGGTTCCGCGTGGATCCTGCTCATCATCATCAT       |
| <i>Portulaca oleracea</i> (PoADH $\alpha$ )           | RT-PCR  | pHM0610SLNPoADH $\alpha$ R | CGTCAACGATAGATCATAGGAATTCATCGTGACTGA     |
| <i>Mirabilis jalapa</i> (MjADH $\alpha$ )             | Cloning | pHM0624SLNMjADH $\alpha$ F | CTGGTTCCGCGTGGATCCATAGCGATAGTTGGGTTTG    |
| <i>Mirabilis jalapa</i> (MjADH $\alpha$ )             | Cloning | pHM0625SLNMjADH $\alpha$ R | TATCAATGGTCTCGATAGGAATTCATCGTGACTGA      |
| <i>Rivina humilis</i> (RhADH $\alpha$ )               | Cloning | pHM0647SLNRhADH $\alpha$ F | CTGGTTCCGCGTGGATCCTGCACGGCCTTACTAAAAC    |
| <i>Rivina humilis</i> (RhADH $\alpha$ )               | Cloning | pHM0648SLNRhADH $\alpha$ R | TCAATGGATCAAAGCGGTAGGAATTCATCGTGACTGA    |
| <i>Beta vulgaris</i> (BvADH $\alpha$ )                | RT-PCR  | BvADH $\alpha$ q F         | TCAAGCTGAGGTTACTTTTGACA                  |
| <i>Beta vulgaris</i> (BvADH $\alpha$ )                | RT-PCR  | BvADH $\alpha$ q R         | AAGAAGCATGATTTAGTGGTGGT                  |
| <i>Beta vulgaris</i> (BvADH $\beta$ )                 | RT-PCR  | BvADH $\beta$ q F          | TGCAGCGACTTAAACGATCG                     |
| <i>Beta vulgaris</i> (BvADH $\beta$ )                 | RT-PCR  | BvADH $\beta$ q R          | TTGGGGAAGTTTGCCGTTTG                     |
| <i>Beta vulgaris</i> (BvADH $\alpha$ )                | RT-PCR  | pHM0793SLNBvADH $\alpha$ F | AGTTCCTCTGCTGATATG                       |
| <i>Beta vulgaris</i> (BvADH $\alpha$ )                | RT-PCR  | pHM0794SLNBvADH $\alpha$ R | GTGGTTAATGGTACTAGATAG                    |
| <i>Beta vulgaris</i> (BvADH $\beta$ )                 | qPCR    | pHM0791SLNBvADH $\beta$ F  | GCGAAGGAGATCAAATTTCT                     |
| <i>Beta vulgaris</i> (BvADH $\beta$ )                 | qPCR    | pHM0792SLNBvADH $\beta$ R  | TCAATTTGTCTCCGAATTTGC                    |
| <i>Beta vulgaris</i> (BvADH $\alpha$ )                | qPCR    | BvADH $\alpha$ F           | ATGATTTCACTCTCTTTTTCATCC                 |
| <i>Beta vulgaris</i> (BvADH $\alpha$ )                | qPCR    | BvADH $\alpha$ R           | GATTTAGTGGTGGTAAATGGTACTAGATAG           |
| <i>Beta vulgaris</i> (BvADH $\beta$ )                 | qPCR    | BvADH $\beta$ F            | ATGCTTTCTCTCTCCTCCAC                     |
| <i>Beta vulgaris</i> (BvADH $\beta$ )                 | qPCR    | BvADH $\beta$ R            | CAAATTCGGAGACAAATTGA                     |
| <i>Beta vulgaris</i> (BvActin)                        | qPCR    | pHM0001HMBvACT             | TCTATCCTTGATCTCTCAG                      |
| <i>Beta vulgaris</i> (BvActin)                        | qPCR    | pHM0002HMBvACT             | TCTCAAGGGCGAGTATGAT                      |
| <i>Beta vulgaris</i> (BvDODA)                         | qPCR    | pHM0003HMBvDODA            | CATTGGTTCAGGAAGTGCAA                     |
| <i>Beta vulgaris</i> (BvDODA)                         | qPCR    | pHM0004HMBvDODA            | CCTTTGATTCATGGCTTCGT                     |
| <i>Beta vulgaris</i> (BvMYB1)                         | qPCR    | pHM0576BvMYB1F             | TATCAAACGAGGGCACTTC                      |
| <i>Beta vulgaris</i> (BvMYB1)                         | qPCR    | pHM0577BvMYB1R             | GATGGTCTTTGATAGCAGC                      |
| <i>Beta vulgaris</i> (BvCYP76AD1)                     | qPCR    | pHM0005HMBvCYP76AD1        | CTTTTCAGTGAATTAGCCCCACC                  |
| <i>Beta vulgaris</i> (BvCYP76AD1)                     | qPCR    | pHM0006HMBvCYP76AD1        | TGGAACATTATGGAAGATATTGGG                 |
| GFP   | qPCR    | tGFP_q_F                   | GGCTGGAAGAGTGATCGGAG                     |
| GFP   | qPCR    | tGFP_q_R                   | ACGCTACTGTTGAGCATCTTCA                   |
| <i>Gene Racer oligoT</i>                              | RT-PCR  | GeneRacer OligoT           | GCTGTCAACGATACGCTACGTAACGGCATGACAGT(G)20 |
| Eukaryotic translational elongation factor 1 $\alpha$ | qPCR    | EF1 $\alpha$ q_F           | AGCTTTACCTCCCAAGTCATC                    |
| Eukaryotic translational elongation factor 1 $\alpha$ | qPCR    | EF1 $\alpha$ q_R           | CCAAGATTGACAGGCGTTCT                     |

**Table S2.** Sequences of Caryophyllales (ingroups) and non-Caryophyllales (outgroups) used in this study.

| Taxon                                    | Source    | Accession code          | Citation                           |
|--|-----------|-------------------------|------------------------------------|
| <b>Ingroups</b>                          |           |                         |                                    |
| Achatocarpaceae_Phaulothamnus_spinescens | Smith Lab | MJM1677                 | (Brockington <i>et al.</i> , 2015) |
| Aizoaceae_Cypselea_humifusum             | 1KP       | GJNX                    | (Matasci <i>et al.</i> , 2014)     |
| Aizoaceae_Delosperma_echinatum           | 1KP       | BJKT                    | (Matasci <i>et al.</i> , 2014)     |
| Aizoaceae_Sesuvium_portulacastrum        | 1KP       | HZTS                    | (Matasci <i>et al.</i> , 2014)     |
| Aizoaceae_Sesuvium_verrucosum            | 1KP       | EDIT                    | (Matasci <i>et al.</i> , 2014)     |
| Aizoaceae_Trianthemum_portulacastrum     | 1KP       | OMYK                    | (Matasci <i>et al.</i> , 2014)     |
| Aizoaceae_Zaleya_pentandra               | 1KP       | BERS                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Aerva_javanica             | 1KP       | HDSY                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Aerva_lanata               | 1KP       | PDQH                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Alternanthera_brasiliana   | 1KP       | ZBPY                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Alternanthera_caracasana   | 1KP       | OHKC                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Alternanthera_sessilis     | 1KP       | BWRK                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Alternanthera_tenella      | 1KP       | EYRD                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Amaranthus_cruentus        | 1KP       | XSSD                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Amaranthus_retroflexus     | 1KP       | WMLW                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Atriplex_hortensis         | 1KP       | ONLQ                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Atriplex_prostrata         | 1KP       | AAXJ                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Atriplex_rosea             | 1KP       | CBJR                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Bassia_scoparia            | 1KP       | WGET                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Beta_maritima              | 1KP       | FVXD                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Beta_vulgaris              | Genome    | v1.1                    | (Dohm <i>et al.</i> , 2014)        |
| Amaranthaceae_Blutaparon_vermiculare     | 1KP       | CUTE                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Chenopodium_amaranticolor  | SRA       | SRX151423               | (Zhang <i>et al.</i> , 2012)       |
| Amaranthaceae_Chenopodium_quinoa         | 1KP       | SMMC                    | (Matasci <i>et al.</i> , 2014)     |
| Amaranthaceae_Froelichia_floridana       | Smith Lab | MJM1665                 | (Brockington <i>et al.</i> , 2015) |
| Amaranthaceae_Salicornia_europaea        | SRA       | SRX302090               | (Fan <i>et al.</i> , 2013)         |
| Basellaceae_Basella_alba                 | 1KP       | CTYH                    | (Matasci <i>et al.</i> , 2014)     |
| Cactaceae_Lophophora_williamsii          | 1KP       | CPKP                    | (Matasci <i>et al.</i> , 2014)     |
| Cactaceae_Pereskia_aculeata              | 1KP       | JLOV                    | (Matasci <i>et al.</i> , 2014)     |
| Caryophyllaceae_Cerastium_arvense        | Smith Lab | MJM1767                 | (Brockington <i>et al.</i> , 2015) |
| Caryophyllaceae_Dianthus_caryophyllus    | Genome    | v1.0                    | (Yagi <i>et al.</i> , 2014)        |
| Caryophyllaceae_Drymaria_cordata         | Smith Lab | LCMsn                   | (Brockington <i>et al.</i> , 2015) |
| Caryophyllaceae_Polycarpaea_repens       | 1KP       | RXEN                    | (Matasci <i>et al.</i> , 2014)     |
| Caryophyllaceae_Saponaria_officinalis    | 1KP       | SKNL                    | (Matasci <i>et al.</i> , 2014)     |
| Caryophyllaceae_Schiedea_membranacea     | 1KP       | OLES                    | (Matasci <i>et al.</i> , 2014)     |
| Caryophyllaceae_Silene_latifolia         | 1KP       | FZQN                    | (Matasci <i>et al.</i> , 2014)     |
| Caryophyllaceae_Silene_latifoliaSRA      | SRA       | SRX118777–<br>SRX118782 | (Muyle <i>et al.</i> , 2012)       |

|   |           |           |                                    |
|---|-----------|-----------|------------------------------------|
| Caryophyllaceae_Silene_vulgaris         | SRA       | SRX096120 | N/A                                |
| Caryophyllaceae_Spergularia_media       | 1KP       | TJES      | (Matasci <i>et al.</i> , 2014)     |
| Droseraceae_Aldrovanda-vesiculosa       | Smith Lab | MJM1652   | (Brockington <i>et al.</i> , 2015) |
| Droseraceae_Dionaea_muscipula           | SRA       | SRX312294 | (Jensen <i>et al.</i> , 2015)      |
| Frankeniaceae_Frankenia_laevis          | 1KP       | WPYJ      | (Matasci <i>et al.</i> , 2014)     |
| Microteaceae_Microtea_debilis           | 1KP       | YNFJ      | (Matasci <i>et al.</i> , 2014)     |
| Molluginaceae_Mollugo_cerviana          | 1KP       | RNBN      | (Matasci <i>et al.</i> , 2014)     |
| Molluginaceae_Mollugo_nudicaulis        | 1KP       | SCAO      | (Matasci <i>et al.</i> , 2014)     |
| Molluginaceae_Mollugo_verticillata      | 1KP       | NXTS      | (Matasci <i>et al.</i> , 2014)     |
| Nepenthaceae_Nepenthes_alata            | 1KP       | WQUF      | (Matasci <i>et al.</i> , 2014)     |
| Nyctaginaceae_Abronia_carletonii        | Smith Lab | MJM1751   | (Brockington <i>et al.</i> , 2015) |
| Nyctaginaceae_Acleisanthes_lanceolata   | Smith Lab | MJM1741   | (Brockington <i>et al.</i> , 2015) |
| Nyctaginaceae_Acleisanthes_obtusa       | Smith Lab | MJM1697   | (Brockington <i>et al.</i> , 2015) |
| Nyctaginaceae_Anulocaulis_leiosolenus   | Smith Lab | SRX717838 | (Yang <i>et al.</i> , 2015)        |
| Nyctaginaceae_Boerhavia_burbridgeana    | 1KP       | VJPU      | (Matasci <i>et al.</i> , 2014)     |
| Nyctaginaceae_Boerhavia_coccinea        | 1KP       | ZBTA      | (Matasci <i>et al.</i> , 2014)     |
| Nyctaginaceae_Bougainvillea_spectabilis | 1KP       | JAFJ      | (Matasci <i>et al.</i> , 2014)     |
| Nyctaginaceae_Bougainvillea_stipitata   | Smith Lab | SRX718672 | (Yang <i>et al.</i> , 2015)        |
| Nyctaginaceae_Cyphomeris_gypsophiloides | Smith Lab | MJM1714   | (Brockington <i>et al.</i> , 2015) |
| Nyctaginaceae_Guapira_obtusata          | Smith Lab | SRX718384 | (Yang <i>et al.</i> , 2015)        |
| Nyctaginaceae_Mirabilis_jalapa          | 1KP       | JGAB      | (Matasci <i>et al.</i> , 2014)     |
| Nyctaginaceae_Mirabilis_multiflora      | Smith Lab | MJM1771   | (Brockington <i>et al.</i> , 2015) |
| Nyctaginaceae_Pisonia_aculeata          | Smith Lab | SRX718389 | (Yang <i>et al.</i> , 2015)        |
| Nyctaginaceae_Pisonia_umbellifera       | Smith Lab | SFB29     | (Brockington <i>et al.</i> , 2015) |
| Physenaceae_Physena_madagascariensis    | 1KP       | RUUB      | (Matasci <i>et al.</i> , 2014)     |
| Phytolaccaceae_Ercilla_volubilis        | Smith Lab | MJM1649   | (Brockington <i>et al.</i> , 2015) |
| Phytolaccaceae_Hillieria_latifolia      | 1KP       | SFKQ      | (Matasci <i>et al.</i> , 2014)     |
| Phytolaccaceae_Petiveria_alliacea       | 1KP       | AZBL      | (Matasci <i>et al.</i> , 2014)     |
| Phytolaccaceae_Phytolacca_americana     | 1KP       | BKQU      | (Matasci <i>et al.</i> , 2014)     |
| Phytolaccaceae_Phytolacca_bogotensis    | 1KP       | MRKX      | (Matasci <i>et al.</i> , 2014)     |
| Phytolaccaceae_Phytolacca_dioica        | Smith Lab | SFB31     | (Brockington <i>et al.</i> , 2015) |
| Phytolaccaceae_Rivina_humilis           | Smith Lab | SRX718277 | (Yang <i>et al.</i> , 2015)        |
| Phytolaccaceae_Segueria_aculeata        | Smith Lab | SRX718486 | (Yang <i>et al.</i> , 2015)        |
| Plumbaginaceae_Limonium_spectabile      | 1KP       | WOBD      | (Matasci <i>et al.</i> , 2014)     |
| Polygonaceae_Antigonon_leptopus         | Smith Lab | MJM1811   | (Brockington <i>et al.</i> , 2015) |
| Polygonaceae_Fagopyrum_esculentum       | SRA       | SRX112838 | N/A                                |
| Polygonaceae_Polygonum_convulvulus      | 1KP       | FYSJ      | (Matasci <i>et al.</i> , 2014)     |
| Polygonaceae_Polygonum_cuspidatum       | SRA       | SRX079484 | (Hao <i>et al.</i> , 2012)         |
| Polygonaceae_Rheum_nobile               | SRA       | SRX621187 | N/A                                |
| Polygonaceae_Rheum_rhabarbarum          | SRA       | SRX286365 | N/A                                |
| Polygonaceae_Rumex_acetosa              | SRA       | ERX190940 | N/A                                |



|                                       |        |                              |                                  |
|---------------------------------------|--------|------------------------------|----------------------------------|
| Polygonaceae_Rumex_palustris          | SRA    | ERX190941,<br>ERX190942      | N/A                              |
| Portulacaceae_Portulaca_amilis        | 1KP    | LDEL                         | (Matasci <i>et al.</i> , 2014)   |
| Portulacaceae_Portulaca_cryptopetala  | 1KP    | LLQV                         | (Matasci <i>et al.</i> , 2014)   |
| Portulacaceae_Portulaca_grandiflora   | 1KP    | CPLT                         | (Matasci <i>et al.</i> , 2014)   |
| Portulacaceae_Portulaca_molokiniensis | 1KP    | UQCB                         | (Matasci <i>et al.</i> , 2014)   |
| Portulacaceae_Portulaca_oleracea      | 1KP    | EZGR                         | (Matasci <i>et al.</i> , 2014)   |
| Portulacaceae_Portulaca_pilosa        | 1KP    | IWIS                         | (Matasci <i>et al.</i> , 2014)   |
| Portulacaceae_Portulaca_suffruticosa  | 1KP    | GCYL                         | (Matasci <i>et al.</i> , 2014)   |
| Sarcobataceae_Sarcobatus_vermiculatus | 1KP    | GIWN                         | (Matasci <i>et al.</i> , 2014)   |
| Simmondsiaceae_Simmondsia_chinensis   | 1KP    | CVDF                         | (Matasci <i>et al.</i> , 2014)   |
| Talinaceae_Talinum_sp                 | 1KP    | LKKX                         | (Matasci <i>et al.</i> , 2014)   |
| Tamaricaceae_Reaumuria_trigyna        | SRA    | SRX099851,<br>SRX105466      | N/A                              |
| Tamaricaceae_Tamarix_hispida          | SRA    | All 8 runs in<br>PRJNA170420 | (Wang <i>et al.</i> , 2014)      |
| <b>Outgroups</b>                      |        |                              |                                  |
| Arabidopsis_thaliana                  | Genome | Accessed May 28,<br>2014     | (Goodstein <i>et al.</i> , 2012) |
| Oryza_sativa                          | Genome | Accessed Apr 21,<br>2015     | (Goodstein <i>et al.</i> , 2012) |
| Solanum_lycopersicum                  | Genome | Accessed May 28,<br>2014     | (Goodstein <i>et al.</i> , 2012) |
| Vitis_vinifera                        | Genome | Accessed Apr 21,<br>2015     | (Goodstein <i>et al.</i> , 2012) |

**Table S3. Amino acid levels of *Nicotiana benthamiana* leaves expressing GFP, BvADH $\alpha$ , or BvADH $\beta$ .**

Agrobacteria carrying the 35S::GFP, 35S::BvADH $\alpha$ , or 35S::BvADH $\beta$  construct were infiltrated to *Nicotiana benthamiana* leaves and the levels of amino acids were analyzed after three days post-infiltration. Data are mean  $\pm$  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.

| <b>Amino Acids</b>     | <b>35S::GFP</b>   | <b>35S::BvADH<math>\alpha</math></b> | <b>35S::BvADH<math>\beta</math></b> |
|------------------------|-------------------|--------------------------------------|-------------------------------------|
| alanine                | 99.8 $\pm$ 15.5   | 93.0 $\pm$ 14.8                      | 88.1 $\pm$ 20.0                     |
| glycine                | 15.5 $\pm$ 1      | 17.5 $\pm$ 2.1                       | 13.6 $\pm$ 0.2                      |
| valine                 | 23.9 $\pm$ 9.7    | 23.8 $\pm$ 8.3                       | 22.1 $\pm$ 8.4                      |
| leucine                | 21.3 $\pm$ 10.4   | 21.8 $\pm$ 9.2                       | 18.8 $\pm$ 8.3                      |
| isoleucine             | 13.8 $\pm$ 7      | 13.3 $\pm$ 5.7                       | 13.3 $\pm$ 6.7                      |
| proline                | 154.8 $\pm$ 67.4  | 126.7 $\pm$ 56.3                     | 137.3 $\pm$ 75.4                    |
| methionine             | 2.8 $\pm$ 0.4     | 3.1 $\pm$ 0.4                        | 2.6 $\pm$ 0.2                       |
| serine                 | 57.4 $\pm$ 8      | 58.6 $\pm$ 11.7                      | 43.9 $\pm$ 3.9                      |
| threonine              | 69.4 $\pm$ 7.5    | 67.8 $\pm$ 8.6                       | 58.1 $\pm$ 6.5                      |
| phenylalanine          | 10.8 $\pm$ 0.7    | 5.9 $\pm$ 1.2*                       | 9.7 $\pm$ 0.7                       |
| aspartic acid          | 173.5 $\pm$ 45.5  | 176.8 $\pm$ 40.6                     | 132.7 $\pm$ 41.5                    |
| glutamic acid          | 941.6 $\pm$ 45.8  | 968.1 $\pm$ 91.6                     | 746.4 $\pm$ 111.4                   |
| ornithine <sup>a</sup> | 54.9 $\pm$ 1.6    | 56.2 $\pm$ 2.4                       | 48.4 $\pm$ 2.9                      |
| asparagine             | 6.8 $\pm$ 1.2     | 6.9 $\pm$ 1.5                        | 4.9 $\pm$ 1.0                       |
| glutamine              | 345.2 $\pm$ 116.1 | 348.7 $\pm$ 138.4                    | 291.3 $\pm$ 107.7                   |
| tyrosine               | 11.2 $\pm$ 2.8    | 116.8 $\pm$ 15.1*                    | 17.2 $\pm$ 3.2                      |

<sup>a</sup>Arginine was quantified as its non-enzymatic degradation product ornithine.

**Table S4. 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 | $\omega$ 1 (purifying selection) | $\omega$ 2 (nearly neutral) | $\omega$ 3 (positive selection) |
|-----------------------------|---------|--------|---------|-------|------------|----------------------------------|-----------------------------|---------------------------------|
| <b>Partitioned MG94xREV</b> | -5484.8 | 38     | 11046.5 | 2.23  | Reference  | 0.0743 (100%)                    |                             |                                 |
|                             |         |        |         |       | Test       | 0.166 (100%)                     |                             |                                 |
| <b>Null</b>                 | -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%)                     |
| <b>Alternative</b>          | -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)

## Supplemental References for Table S2

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