

**Table S1. Primer and gene accession numbers**

Maize Gene	Primer No	Sequence	ACCESSION NO.				
			Maize			Rice	Sorghum
			MAP	GENOMIC	cDNA		
HYD3	1409	TGGCCTGGACGAGCTTGTTAG	10.05	AZM5_1855 <sup>a</sup> AY844957 <sup>e</sup>	BM382572 <sup>e</sup> / AY844958 <sup>e</sup>	NA	NA
	995	CACACTCATTGGCCCACTCGC					
HYD4	1408	TGGCCTGGACGAGCTGGAAAA	2.03	AZM5_1857 <sup>a</sup>	BG320875 <sup>e</sup> / AY844956 <sup>e</sup>	LOC_Os04g48880 <sup>c</sup> (HYD1)	Sbi_0.9426 <sup>d</sup> (HYD1)
	1011	GGCTCGCGCATCCAAACC					
HYD5	1513	GCAGCCCATCAGATACATCA	9.07	AZM5_23363 <sup>a</sup>	DR817188 <sup>e</sup>	LOC_Os03g03370 <sup>c</sup> (HYD2)	NA
	1514	GCAAGCAGGTCTTATTGGGT					
HYD6	1419	GAGATCAAGAAGAGGATCAGGAG	1.01	AZM5_7254 <sup>a</sup>	BQ619575 <sup>e</sup>	LOC_Os10g38940 <sup>c</sup> (HYD3)	Sbi_0.29550 <sup>d</sup> (HYD2)
	1420	GATCTAATGTGAGGAGAAGGTCAA					
CYP97A	1344	CTGGAGCCATCTGAAAGTCA	5	ZmGSStuc11- 12-04.50592.1 <sup>b</sup>	DV169913 <sup>e</sup>	LOC_Os02g57290 <sup>c</sup>	Sbi_0.9326 <sup>d</sup>
	1345	GGACCAAATCCAAACGAGAT					
CYP97C	1348	GTTGACATTGGATGTGATTGG	3.05	NA	BE552887 <sup>e</sup>	LOC_Os10g39930 <sup>c</sup>	Sbi_0.11094 <sup>d</sup>
	1349	AACCAACCTTCCAGTATGGC					

<sup>a</sup> tigr.org; <sup>b</sup> plantgdb.org; <sup>c</sup> gramene.org; <sup>d</sup> phytozome.org; <sup>e</sup> NCBI

**Table S2. Predicted structural characteristics of HYD proteins.**

Gene	Accession #		Map Position	Transit peptide		Mature peptide		Isoelectric point (pI)		# Transmembrane domains (residues)
	Genomic	cDNA		Residues	Mass (kD)	Residues	Mass (kD)	Mature protein	Un-processed	
HYD3	AZM5_1855 <sup>a</sup> AY844957 <sup>b</sup>	BM382572 <sup>b</sup> / AY844958 <sup>b</sup>	10.05	69	7.1	250	27.5	6.32	8.81	4 (22-41; 53-73; 107-124; 129-151)
HYD4	AZM5_1857 <sup>a</sup>	BG320875 <sup>b</sup> / AY844956 <sup>b</sup>	2.03	68	7.0	241	26.5	9.51	9.99	4 (42-61; 73-94; 127-144; 149-171)
HYD5	AZM5_23363 <sup>a</sup>	DR817188 <sup>b</sup>	9.07	73	7.1	220	24.5	8.11	10.28	4 (12-31; 44-64; 99-116; 121-143)
HYD6	AZM5_7254 <sup>a</sup>	BQ619575 <sup>b</sup>	1.01	56	5.8	243	26.6	9.62	9.88	4 (36-55; 67-83; 122-139; 144-166)

<sup>a</sup> tigr.org; <sup>b</sup> NCBI

**Table S3. Transcript profiling of *HYD* genes in maize diversity lines and Pearson correlation values. A. Transcript profiling of *HYD3* in maize diversity lines. B-F. Transcript profiling of other hydroxylase genes in maize diversity lines that did not exhibit correlation between transcripts and betacarotene level. Transcripts were measured quantitatively from endosperm collected at the indicated DAP. Parentheses show SD, standard deviation. The fold reduction in transcripts (15/25 DAP) was calculated by dividing the 15 DAP value by the 25 DAP value. Quantitative RT-PCR was performed using gene specific primers (Table S1) and normalized to actin, as previously described (Li et al., 2008a). Values are expressed as the mean of three RT-PCR replicates +/- standard deviation.  $\beta$ -carotene levels in mature kernels are from published values (Harjes et al., 2008). G. Pearson correlation values for all *HYD* transcripts compared to betacarotene levels.**

**Table S3A. Transcript profiling of *HYD3* in maize diversity lines.**

Inbred	$\beta$ -carotene $\mu\text{g/g}$	Relative transcript levels of <i>HYD3</i> (SD)			Fold reduction 15/25 DAP
		15 DAP	20 DAP	25 DAP	
<b>A619</b>	6.11	408.4 (14.01)	0.23 (0.13)	0.08 (0.02)	5105
<b>B73</b>	0.57	163.96 (19.04)	135.97 (49)	90.23 (5.07)	1.8
<b>B37</b>	1.15	482.32 (111.6)	246.51(67.39)	100.46 (8.07)	4.8
<b>CI.7</b>	13.63	515.15 (98.79)	26.40 (4.87)	0.12 (0.01)	4293
<b>CI31A</b>	0.82	791.72 (73.78)	164.71 (30.5)	88.47 (14.8)	9.0
<b>DE3</b>	13.34	147.93 (14.36)	2.70 (0.48)	0.05 (0)	2959
<b>KUI2007</b>	3.15	240.32 (13.9)	317.84 (47.2)	352.26 (64.5)	0.7
<b>NC300</b>	1.90	363 (21.3)	164.52 (62.5)	99.53 (21.31)	3.7
<b>SD44</b>	1.95	350.64 (65.4)	5.79 (1.76)	4.45 (0.19)	64.9
<b>TZI18</b>	2.36	375.8 (7.36 )	159.10 (8.2)	130.43 (15.4)	2.9

**Table S3B-F. Transcript profiling of other hydroxylase genes in maize diversity lines that did not exhibit correlation between transcripts and betacarotene level.**

**B**

Inbred	$\beta$ -carotene	Relative transcript levels of <i>HYD4</i> (SD)			Fold reduction
	$\mu\text{g/g}$	15 DAP	20 DAP	25 DAP	15/25 DAP
A619	6.11	1.43 (0.08)	3.32 (0.17)	2.5 (0.10)	0.57
B73	0.57	1.83 (0.34)	2.3 (0.51)	2.8 (0.46)	0.65
B37	1.15	4.12 (0.63)	9.67 (0.49)	8.11 (0.69)	0.51
CI.7	13.63	3.66 (0.83)	3.74 (0.40)	2.73 (0.19)	1.34
CI31A	0.82	2.37 (1.15)	1.23 (0.37)	1.71 (0.13)	1.39
DE3	13.34	2.4 (0.47)	3.54 (0.76)	1.81 (0.12)	1.32
KUI2007	3.15	1.04 (0.31)	6.69 (1.12)	8.98 (2.15)	0.12
NC300	1.90	1.3 (0.52)	1.5 (0.22)	2.02 (0.10)	0.64
SD44	1.95	1.73 (0.62)	5.61 (0.19)	4.69 (1.90)	0.37
TZI18	2.36	1.18 (0.82)	3.5 (0.29)	3.46 (0.34)	0.34

**C**

Inbred	$\beta$ -carotene	Relative transcript levels of <i>HYD5</i> (SD)			Fold reduction
	$\mu\text{g/g}$	15 DAP	20 DAP	25 DAP	15/25 DAP
A619	6.11	0.03 (0.01)	0.02 (0.00)	0.01 (0.00)	2.93
B73	0.57	0.01 (0.00)	0.05 (0.02)	0.05 (0.02)	0.19
B37	1.15	0.06 (0.04)	0.15 (0.02)	0.01 (0.01)	6.47
CI.7	13.63	0.05 (0.00)	0.06 (0.00)	0.02 (0.02)	2.09
CI31A	0.82	0.03 (0.01)	0.01 (0.00)	0.01 (0.01)	2.64
DE3	13.34	0.01 (0.01)	0.01 (0.00)	0.01 (0.01)	1.08
KUI2007	3.15	0.02 (0.01)	0.03 (0.00)	0.05 (0.01)	0.35
NC300	1.90	0.01 (0.00)	0.01 (0.00)	0.02 (0.01)	0.6
SD44	1.95	0.21 (0.08)	0.05 (0.04)	0.09 (0.01)	2.23
TZI18	2.36	0.11 (0.04)	0.03 (0.00)	0.03 (0.01)	3.18

**D**

Inbred	$\beta$ -carotene	Relative transcript levels of <i>HYD6</i> (SD)			Fold reduction
	$\mu\text{g/g}$	15 DAP	20 DAP	25 DAP	15/25 DAP
A619	6.11	0.01 (0.00)	0.02 (0.01)	0.01 (0.00)	0.71
B73	0.57	0.08 (0.00)	0.33 (0.01)	0.19 (0.02)	0.43
B37	1.15	0.07 (0.01)	0.26 (0.02)	0.11 (0.03)	0.66
CI.7	13.63	0.02 (0.01)	0.09 (0.03)	0.08 (0.06)	0.23
CI31A	0.82	0.05 (0.02)	0.03 (0.01)	0.14 (0.02)	0.35
DE3	13.34	0.01 (0.00)	0.21 (0.02)	0.01 (0.00)	0.7
KUI2007	3.15	0.01 (0.00)	0.11 (0.00)	0.71 (0.17)	0.01
NC300	1.90	0.01 (0.00)	0.01 (0.00)	0.02 (0.01)	0.61
SD44	1.95	0.01 (0.00)	0.23 (0.05)	0.23 (0.04)	0.03
TZI18	2.36	0.08 (0.05)	0.17 (0.02)	0.02 (0.00)	4.78

E

Inbred	$\beta$ -carotene	Relative transcript levels of CYP97A (SD)			Fold reduction
	$\mu\text{g/g}$	15 DAP	20 DAP	25 DAP	15/25 DAP
A619	6.11	0.12 (0.03)	1.97 (0.43)	0.8 (0.21)	0.15
B73	0.57	0.21(0.03)	0.11 (0.00)	0.14 (0.01)	1.5
B37	1.15	0.04 (0.01)	0.9 (0.12)	0.39 (0.12)	0.1
CI.7	13.63	0.04 (0.01)	1.05 (0.02)	0.09 (0.01)	0.44
CI31A	0.82	0.13 (0.07)	0.71(0.11)	0.22 (0.07)	0.59
DE3	13.34	0.08 (0.00)	0.57 (0.11)	0.1 (0.03)	0.8
KUI2007	3.15	0.34 (0.02)	2.42 (0.31)	0.38 (0.02)	0.89
NC300	1.90	0.07 (0.01)	0.7(0.14)	0.27 (0.07)	0.26
SD44	1.95	0.04 (0.02)	0.53 (0.21)	0.22 (0.02)	0.18
TZI18	2.36	0.43 (0.01)	2.37 (0.21)	0.65 (0.12)	0.65

F

Inbred	$\beta$ -carotene	Relative transcript levels of CYP97C (SD)			Fold reduction
	$\mu\text{g/g}$	15 DAP	20 DAP	25 DAP	15/25 DAP
A619	6.11	0.32 (0.00)	4.06 (0.92)	1.2 (0.17)	0.27
B73	0.57	0.51 (0.02)	0.68 (0.14)	0.33 (0.13)	1.55
B37	1.15	0.13 (0.09)	3.3 (0.03)	1.76 (0.51)	0.07
CI.7	13.63	0.42 (0.02)	5.33 (0.80)	0.73 (0.46)	0.58
CI31A	0.82	0.27 (0.16)	4.17 (0.13)	1.57 (0.21)	0.17
DE3	13.34	0.44 (0.00)	2.3 (0.35)	1.25 (0.26)	0.35
KUI2007	3.15	0.15 (0.02)	1.22 (0.23)	3.1 (0.28)	0.05
NC300	1.90	0.1 (0.00)	1.86 (0.10)	0.62 (0.13)	0.16
SD44	1.95	0.41 (0.11)	4.2 (0.35)	1.61 (0.11)	0.14
TZI18	2.36	0.4 (0.03)	2.41 (0.48)	1.73 (0.17)	0.23

**Table S3G. Pearson correlation values for all HYD transcripts compared to betacarotene levels.**

<b>HYD gene</b>	<b>DAP</b>	<b><math>\beta</math>-carotene</b>
<b>HYD3</b>	15	-0.17 (0.63 )
	20	-0.58 (0.07)
	25	-0.42 (0.21 )
	15/25	<b>0.79 (0.005)</b>
<b>HYD4</b>	15	0.35 (0.30)
	20	-0.08 (0.81)
	25	-0.30 (0.38)
	15/25	0.60 (0.06)
<b>HYD5</b>	15	-0.20 (0.56)
	20	-0.171 (0.63)
	25	-0.33 (0.34)
	15/25	-0.15 (0.66)
<b>HYD6</b>	15	-0.48 (0.15 )
	20	-0.13 (0.70 )
	25	-0.25 (0.47)
	15/25	-0.12(0.73)
<b>CYP97A</b>	15	-0.29 (0.40 )
	20	0.006 (0.98)
	25	-0.27 (0.44)
	15/25	-0.02 (0.93)
<b>CYP97C</b>	15	0.37 (0.28 )
	20	0.36 (0.30)
	25	-0.19 (0.58 )
	15/25	0.02 (0.94)