



## Supplementary Materials:

**Table S1.** The primers used in this study.

Primer Name	Orientation	Sequence (5'-3')	Annotations
BnaC02.TMT.a_Fc	Forward	AATCCCATACACAGCCACA	specific primers for
BnaC02.TMT.a_Rc	Reverse	CTAAAATAGACAACGTAGCC	
BnaC02.TMT.a-XhoI-F	Forward	CCGCTCGAGATGAAAGCGACTCTCGCAC	nested primers for
BnaC02.TMT.a-ClaI-R	Reverse	CCATCGATGAGAGGCTTCTGGCAAGTG	
35S_P	Forward	GACCCTTCCTCTATATAAGGAAGTTC	DNA genotyping
BnTMT_R	Reverse	GAGAGGCTTCTGGCAAGTG	
BnGAPDH_F-qPCR	Forward	CCGCTTCCTTCAACATCATT	RT-qPCR
BnGAPDH_R-qPCR	Reverse	GTCGCAGCTTTCTCGAGTCT	
BnTMT_F-qPCR	Forward	GCCTCCCAAGGCACAAAGT	
BnTMT_R-qPCR	Reverse	TCCGCTATTCCTTCTCGCAG	
BnFAD2_F-qPCR	Forward	GTTTCCTCGTGTGATCACTTAC	
BnFAD2_R-qPCR	Reverse	TGATAATGCGGCATCGTGGA	
BnFAD3_F-qPCR	Forward	TATAAGGGCGGCCATTCTTAAGCA	
BnFAD3_R-qPCR	Reverse	AGATAGCCCAGAACAGGGTTCCTT	

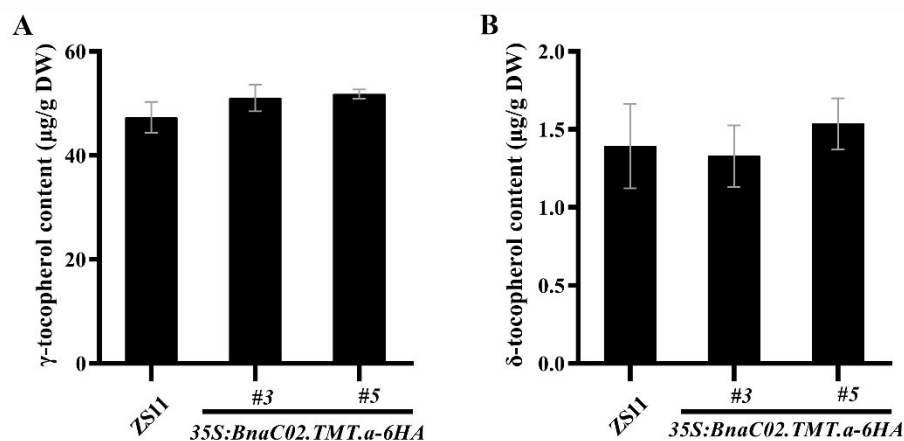
**Table S2.** The standard curve of  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\delta$ -tocopherol.

Tocopherol Forms	Standard Curve	Linearity Range ( $\mu\text{g/mL}$ )	Retention Time (min)
$\alpha$ -tocopherol	$y = 4.3411x - 3.7525$ ; $R^2 = 0.9996$	0.4–200	12.445
$\beta$ -tocopherol	$y = 6.2545x - 0.6437$ ; $R^2 = 0.9999$	0.4–200	11.209
$\gamma$ -tocopherol	$y = 6.7402x - 3.6822$ ; $R^2 = 0.9996$	0.4–200	10.771
$\delta$ -tocopherol	$y = 4.9310x - 1.0052$ ; $R^2 = 0.9998$	0.4–200	9.443

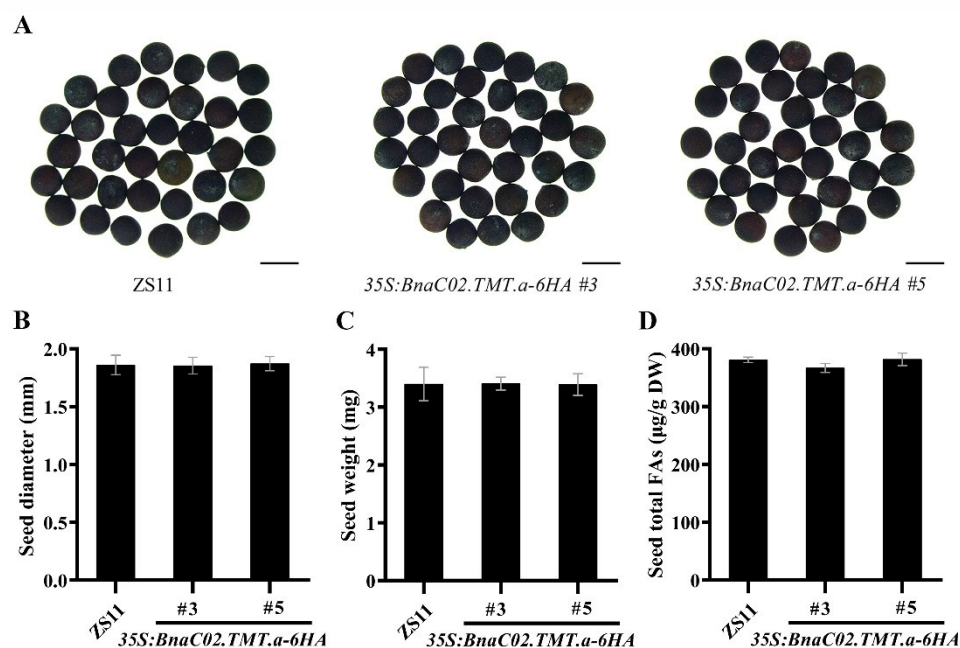
Percent Identity						
	1	2	3	4	5	
Divergence	1	89.3	89.2	88.4	89.5	1
	2	11.6	98.3	98.6	98.0	2
	3	11.6	1.8	97.4	98.8	3
	4	12.6	1.5	2.6	96.5	4
	5	11.3	2.1	1.2	3.6	5
	1	2	3	4	5	

At $\gamma$ -TMT (NP\_176677.1)  
 BnaC02.TMT.a (BnaC02T0331100ZS)  
 BnaC02.TMT.b (BnaC02T0197500ZS)  
 BnaA02.TMT.a (BnaA02T0247300ZS)  
 BnaA02.TMT.b (BnaA02T0154300ZS)

**Figure S1.** Percent identity (up) and divergence (down) of the amino acid sequences of the  $\gamma$ -TMT protein from *A. thaliana* and *B. napus*. 1: At $\gamma$ -TMT (NP\_176677.1); 2: BnaC02.TMT.a (BnaC02T0331100ZS); 3: BnaC02.TMT.b (BnaC02T0197500ZS); 4: BnaA02.TMT.a (BnaA02T0247300ZS); 5: BnaA02.TMT.b (BnaA02T0154300ZS). Divergence and percent identity were calculated by comparing sequence pairs to the phylogeny reconstructed by MegAlign.



**Figure S2.** γ-tocopherol (A) and δ-tocopherol (B) content of wild-type ZS11 and 35S:*BnaC02.TMT.a-6HA* plants in mature seed of rapeseed. Error bars denote standard deviation.



**Figure S3.** Analysis of seed coat color, seed size, seed weight and seed total FAs among wild-type ZS11 and 35S:*BnaC02.TMT.a-6HA* plants. (A) Microscopic observations of randomly selected mature seeds. Bars = 2000 μm. (B) Quantitative comparisons of seed size (length and width) between ZS11 and 35S:*BnaC02.TMT.a-6HA* plants. (C) Quantitative comparisons of seed weight between ZS11 and 35S:*BnaC02.TMT.a-6HA* plants. In (B) and (C), Values represent means ± SD (n = 5), and each of the three assays for each biological replicate contained 200 seeds from 8 individual plants grown in different pots arranged randomly within one of three blocks. (D) Seed total FA content in seeds among ZS11 and 35S:*BnaC02.TMT.a-6HA* plants.

BnaC02. TMT. a (BnaC02T0331100ZS)	MKATLAPPSSLISLPRHKVSSLRSPSLLLQSQRPSSALM-TTASRGSAVATAATSSAE	(59)
BnaA. VTE4. a1 (EU637012. 1)	MKATLA-PSSLISLPRHKVSSLRSPSLLLQSQRPSSALMTTTASRGSAVATAATSSFE	(59)
	* * *	
BnaC02. TMT. a (BnaC02T0331100ZS)	ALREGIAEFYNETSGLWEEIWGDHMHGFDYDPSSVQLSDSGHREAQIRMIEESLRFAGV	(119)
BnaA. VTE4. a1 (EU637012. 1)	ALREGIAEFYNETSGLWEEIWGDHMHGFDYDPSSVQLSDSGHREAQIRMIEESLRFAGV	(119)
BnaC02. TMT. a (BnaC02T0331100ZS)	TEEEKKIKRVVDVCGIGGSSRYIASKFGAECIGITLSPVQAKRANDLAAAQSLSHKVSF	(179)
BnaA. VTE4. a1 (EU637012. 1)	TEEEKKIKRVVDVCGIGGSSRYIASKFGAECIGITLSPVQAKRANDLAAAQSLSHKVSF	(179)
BnaC02. TMT. a (BnaC02T0331100ZS)	QVADALDQPFEDGIFDLVWSMESGEHMPDKAKFVKELVRVTAPGGRIIIVTWCHRNLSQG	(239)
BnaA. VTE4. a1 (EU637012. 1)	QVADALEQPFEDGIFDLVWSMESGEHMPDKAKFVKELVRVAAPGGRIIIVTWCHRNLSPG	(239)
	* * *	
BnaC02. TMT. a (BnaC02T0331100ZS)	EESLQPWEQNLLDRICKTFYLPWCSTSDYVELLQSLSLQDIKADWSENVAPFWPAVIR	(299)
BnaA. VTE4. a1 (EU637012. 1)	EEALQPWEQNLLDRICKTFYLPWCSTSDYVDLLQSLSLQDIKADWSENVAPFWPAVIR	(299)
	* *	
BnaC02. TMT. a (BnaC02T0331100ZS)	TALTWKGLVSLLRSGMKSIGALTMPLMIEGYKKGVIKFGIITCQKPL	(347)
BnaA. VTE4. a1 (EU637012. 1)	TALTWKGLVSLLRSGMKSIGALTMPLMIEGYKKGVIKFGIITCQKPL	(347)

**Figure S4.** Alignment of protein sequences of BnaC02.TMT.a from *B. napus* 'ZS11' and BnaA.VTE4.a1 from *B. napus* 'Express'. The alignment was conducted using the MUSCLE program (<http://www.ebi.ac.uk/Tools/msa/muscle/>) and the different amino acids are indicated by asterisks. S-adenosylmethionine binding sites are underlined.