Data S1 Materials and methods

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1 Sample preparation and Toc analysis

Samples used to extract seed Toc were prepared according to Hussain *et al*. (2013). Oilseed rape seeds (1 g) were ground using a mortar and pestle with 1 ml of n-hexane to produce meal-sized forms. About 0.5 g of the meal was transferred to a 15 ml PE tube. Subsequently, 5 ml of n-hexane was added to the tubes and vortexed for 45 s. The samples were then placed in a mechanical shaker cabin for 1 h in the dark. Afterwards, the mixture was centrifuged at 7000×*g* for 7 min at 4 °C. An aliquot of the extract (supernatant) was collected and dried in a vacuum freeze dryer. For trimethylsilylation, 50 μl of N,O-bis(trimethylsilyl) trifluoroacetamide (BSTFA) was added to the samples and incubated at 70 °C for 40 min (Grob and Barry, 2004). 1 ml of *n*-hexane was added to each tube, vortexed for 30 s, and centrifuged at 9000×*g* for 3 min. Subsequently, 0.8 ml of the supernatant was collected and stored at –20 °C in 1.5 ml GC vials until the Toc were subjected to GC analysis.

2 GC parameters and temperature programming

A "GC-2014" system comprising an AOC-20i auto injector (Shamidzu Corporation, Kyoto, Japan), an Rxi®-5Sil mass spectrometry (MS) column (fused silica; 30 m long with an inner diameter of 0.25 mm and a thickness of 0.25 μm), and a flame ionization detector (FID), was used for Toc analysis. A splitless inlet was used to inject 1 μl of samples into the capillary column. To set the optimum temperature program, pre-experimental trials were conducted by running Toc standards on the GC. After several trials, the following optimum temperature program was obtained. Oven temperature was increased from 180 °C to 260 °C at 8 °C/min, further increased to 280 °C at 2 °C/min, and maintained constant for 13 min. The inlet temperature was set to 290 °C and the detector temperature to 300 °C. N was used as the carrier gas at a constant flow rate of 1.2 ml/min. The detector (FID) air, H2, and make-up gas (N) flow rates were set at 136, 35, and 45 ml/min, respectively.

3 GC data computation and statistical analysis

Shimadzu GC Solution computer software was programmed to calculate the peak areas, height, and concentrations of the standards and the samples for Toc detection. Reference standards, such as α-, β-, γ-, δ-, and T-Toc, and BSTFA for silylation were obtained from Sigma-Aldrich (Shanghai). Liquid chromatography-grade *n*-hexane, supplied by Sigma-Aldrich (Shanghai), was used as a solvent. Standards of T-Toc and its isoforms (α-, β, γ-, and δ-Toc) were run on the GC using the temperature program described in Section 2.3. The peaks corresponding to individual isoform standards were distinguished from those of total (mixed) Toc by their retention times. The standard curve used to quantify the experimental samples was then calibrated. The

peaks in the experimental samples were identified by comparison of their retention times with those of the Toc standards. Statistical analyses were performed using the MSTAT-C software version 2.10 for DOS (MSTATC version 2.10, 1989). Data were subjected to ANOVA appropriate for a randomized complete block design with three factors without splitting. The level of statistical significance was accepted at $P \leq 0.01$ or $P \leq 0.05$. Fisher's (LSD) test was used for multiple comparisons of the means (Steel and Torrie, 1980).

References

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