

Polymorphisms in *FAD3A* and *FAD3B* Genes that Determine the Fatty Acid Composition of Flax Oil

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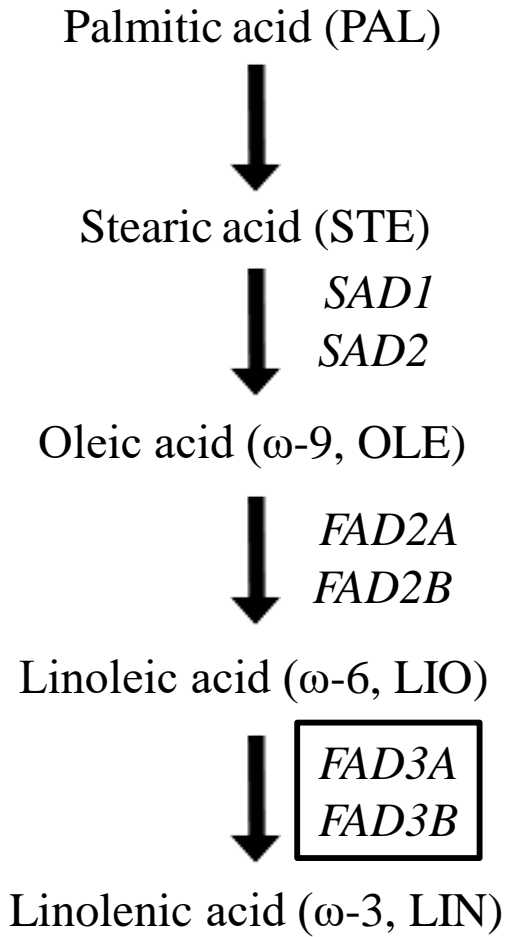
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- Flax oil is used in pharmaceuticals, food, feed, paints, varnishes, etc
- Directions of flax oil use are determined by the ratio of fatty acids, mainly linolenic (omega-3) and linoleic (omega-6) ones
- The purpose of our study was to reveal polymorphisms in *FAD3A* and *FAD3B* genes that determine the fatty acid composition of flax oil and to develop a test system for their identification

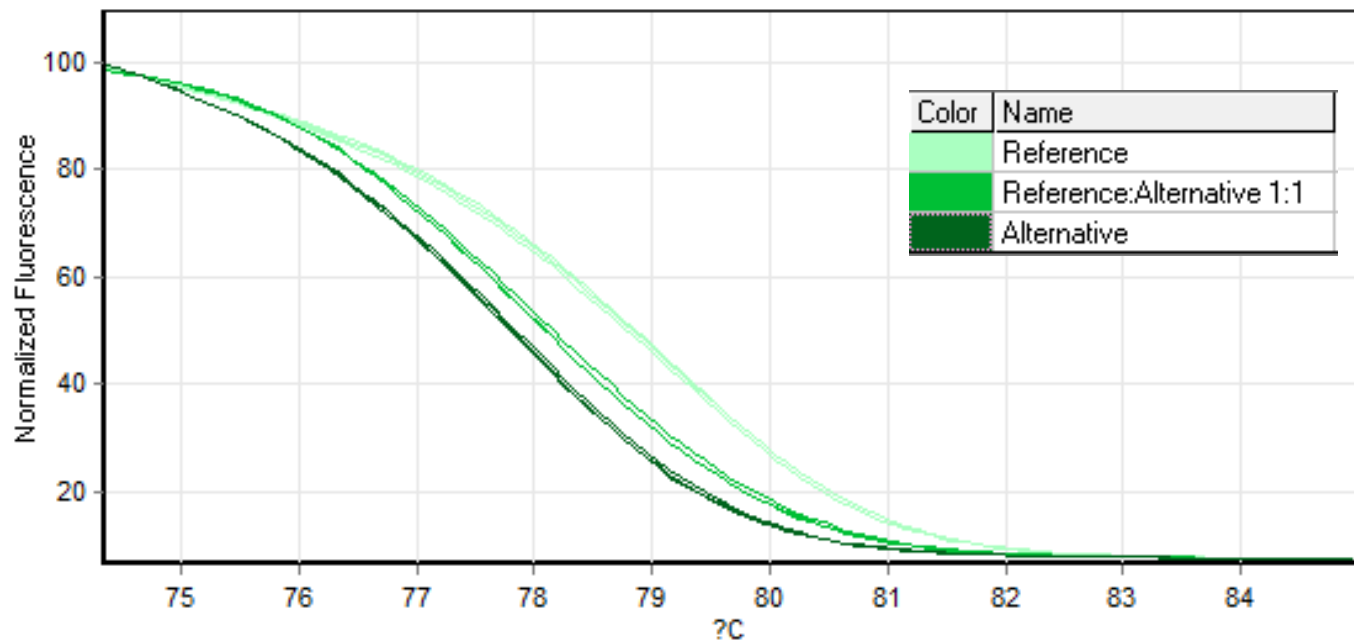


Synthesis of fatty acids



Methods and Algorithms

- 84 flax cultivars/lines with diverse content of LIO (from 11.9% to 72.4%) and LIN (from 2.7% to 65.3%) in the oil (provided by the Institute for Flax, Torzhok, Russia)
- Deep sequencing of *FAD3A* and *FAD3B* on MiSeq (Illumina, USA) in a 600-cycle format
- Identification of polymorphisms in *FAD3* genes using VarScan
- Correlation analysis between the fatty acid composition and polymorphisms in *FAD3A* and *FAD3B*

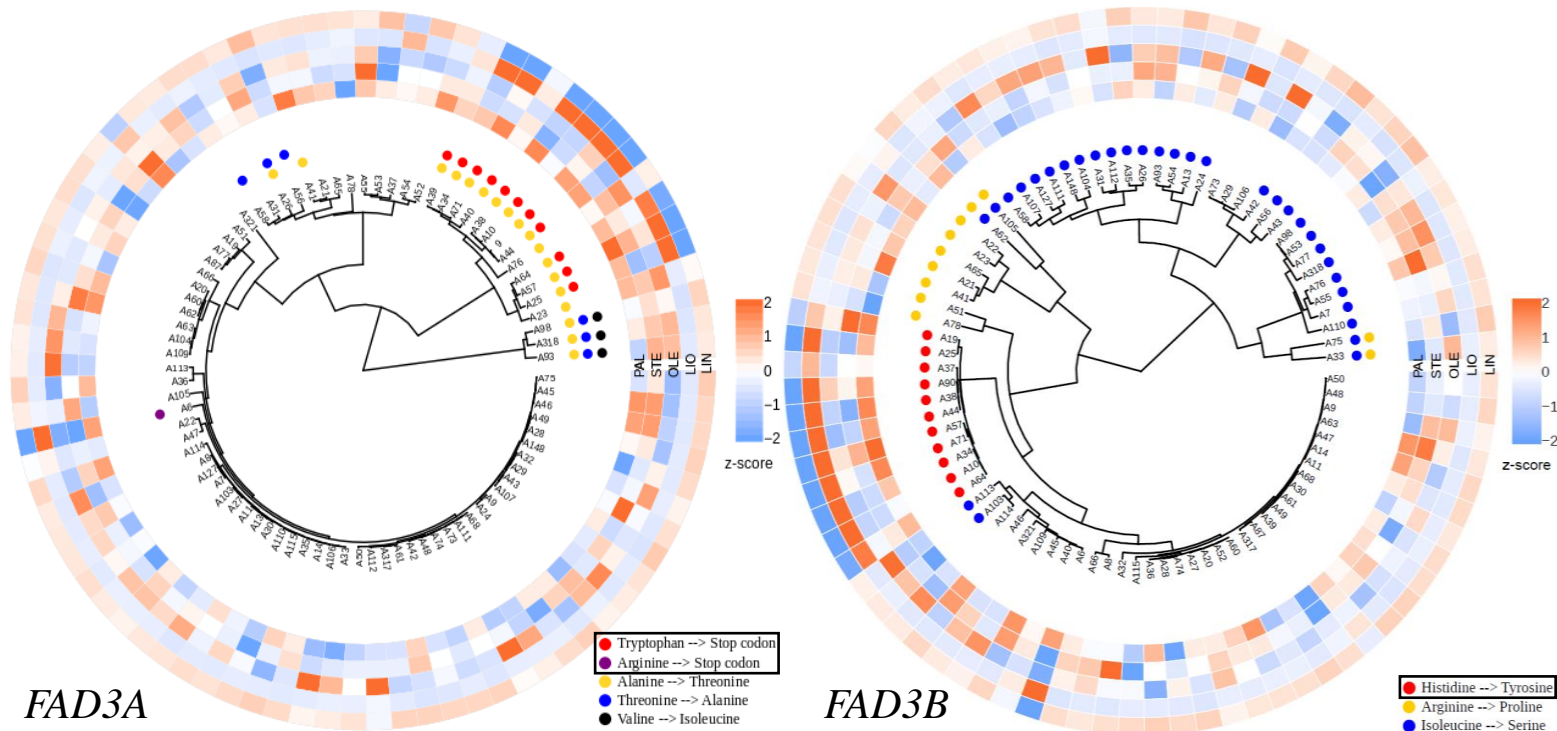


- The HRM analysis on Rotor-Gene Q (Qiagen, Netherlands) for identification of the SNPs in *FAD3A* and *FAD3B* genes that are responsible for the ratio of LIN and LIO

Homozygous and heterozygous states are distinguished clearly by temperature shift in the melt curves

Results

- Deep sequencing led to the gene coverage of about 300x and enabled the identification of 91 and 62 polymorphisms in *FAD3A* and *FAD3B* genes respectively
- Three polymorphisms were revealed to be responsible for the LIN/LIO ratio: CP027631.1:16092348 (tryptophan to stop codon substitution) and CP027631.1:16090340 (arginine to stop codon) in the *FAD3A* gene and CP027622.1:1035655 (histidine to tyrosine) in the *FAD3B* gene. Coordinates are given according to the *Linum usitatissimum* reference genome (assembly GCA_000224295.2/ASM22429v2)
- All except one of the samples with low LIN and high LIO had both the tryptophan to stop codon substitution in *FAD3A* and the histidine to tyrosine substitution in *FAD3B*, while all cultivars and lines with high LIN and low LIO had not these mutations. Samples with only one of the two mutations had medium content of LIN and LIO. The rest low-LIN sample contained arginine to stop codon substitution in *FAD3A*
- HRM-based test system for the identification of the three SNPs responsible for the LIN content was developed. Homozygous and heterozygous states for analyzed SNPs were distinguished clearly by temperature shift in melt curves



The developed test system can increase the efficiency of breeding of flax cultivars with the targeted content of linolenic and linoleic acids

Clusterization of studied flax cultivars and lines based on *FAD3A* and *FAD3B* polymorphisms. The color scale represents the content of fatty acids in the oil

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