

# Molecular markers based on SNPs in *FAD3* genes for determination of linolenic acid content in flax seed

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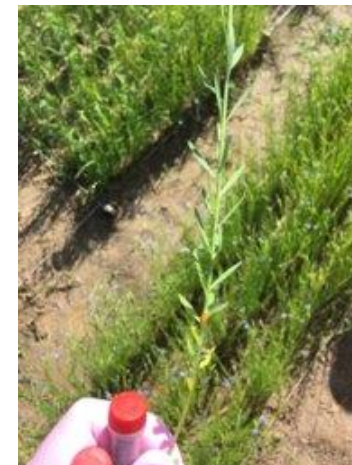
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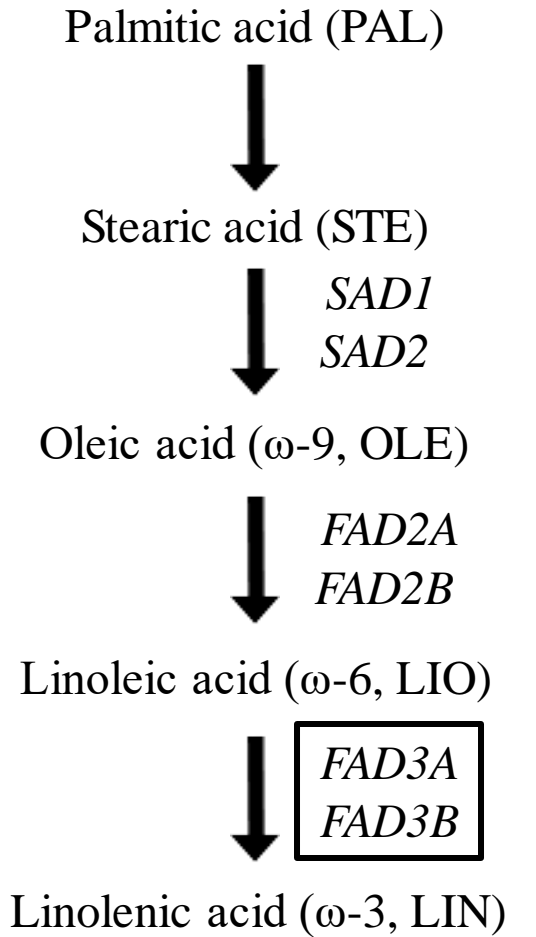
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- Flax seeds are used in pharmaceutical, food, feed, and polymer productions
- The way of flax seed use mainly depends on the ratio of linolenic and linoleic acids in the oil
- The purpose of our study was to identify polymorphisms in *FAD3* genes affecting the content of LIN and LIO in flax seeds and to develop a test system for their identification

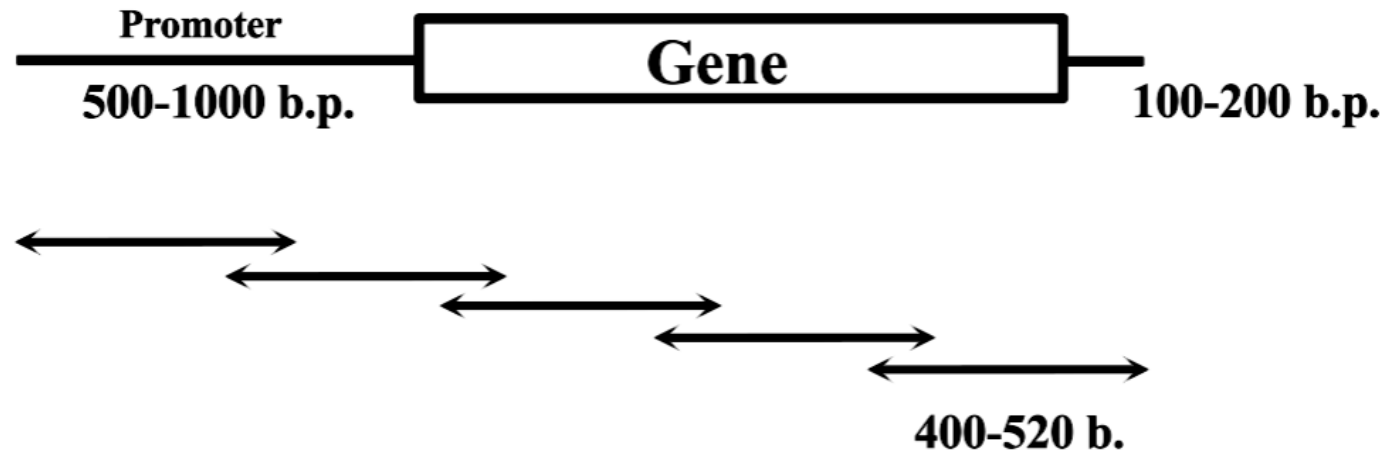


## Synthesis of fatty acids



# Materials and Methods

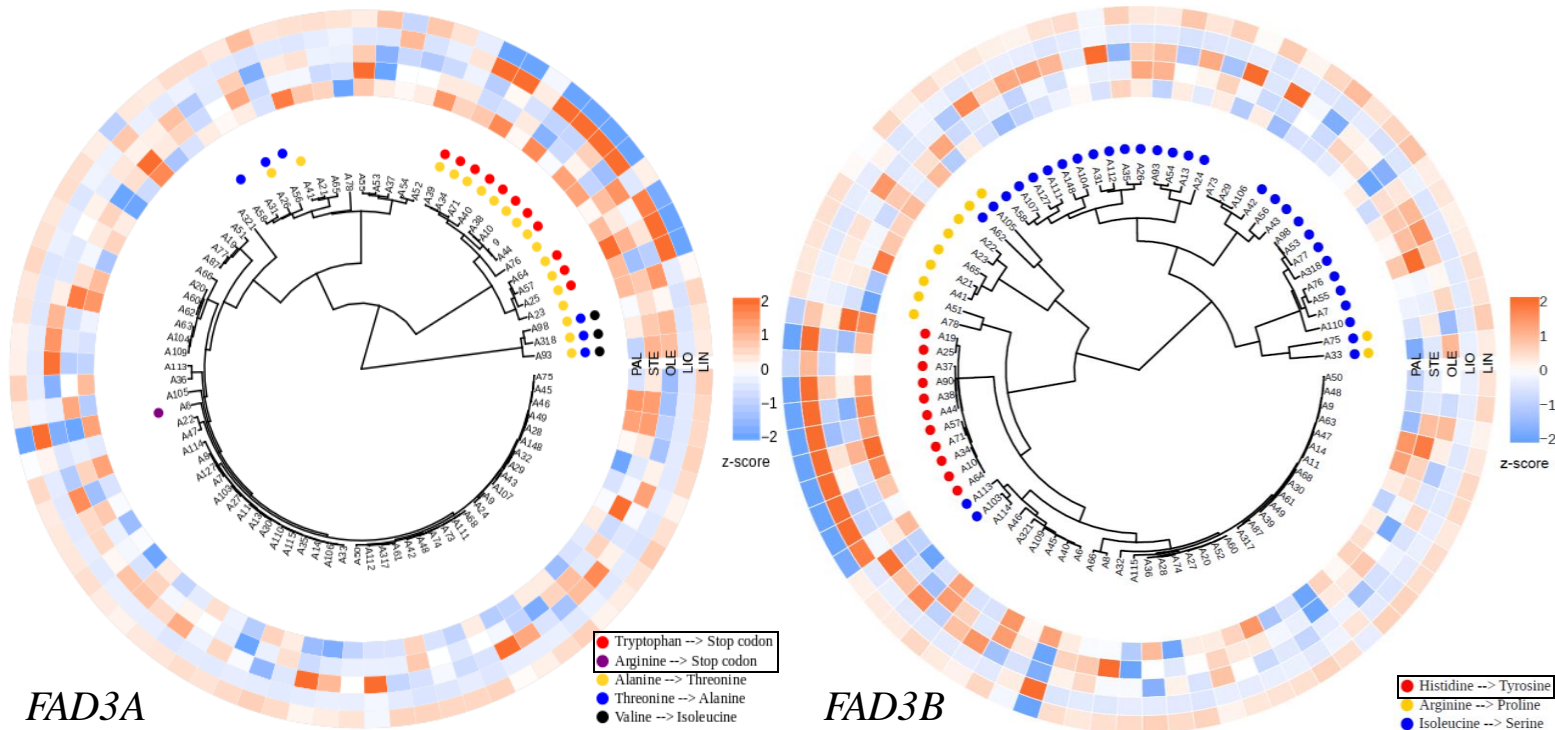
- 84 flax cultivars/lines with diverse content of LIO (from 11.9% to 72.4%) and LIN (from 2.7% to 65.3%) in the oil
- Deep sequencing of *FAD3A* and *FAD3B* on MiSeq (Illumina, USA) in a 600-cycle format
- Identification of polymorphisms in *FAD3* genes using VarScan and freeBayes
- Correlation analysis 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 (single nucleotide polymorphisms) in *FAD3A* and *FAD3B* genes that are responsible for the ratio of linolenic and linoleic acids in flax oil



*Primer design for FAD3 genes amplification*

# 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*
- Based on data on these polymorphisms, an HRM-based test system for the identification of the three SNPs responsible for the LIN content was developed



Molecular markers can increase the efficiency of breeding by obtaining pure improved cultivars and are necessary for the development of the marker-assisted selection 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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