



**National Research
Council Canada**

**Plant Biotechnology Institute
Lipid Biotechnology Group
Saskatoon**

Genomics and the Production of Industrial Oil Feedstocks in Brassica Crops

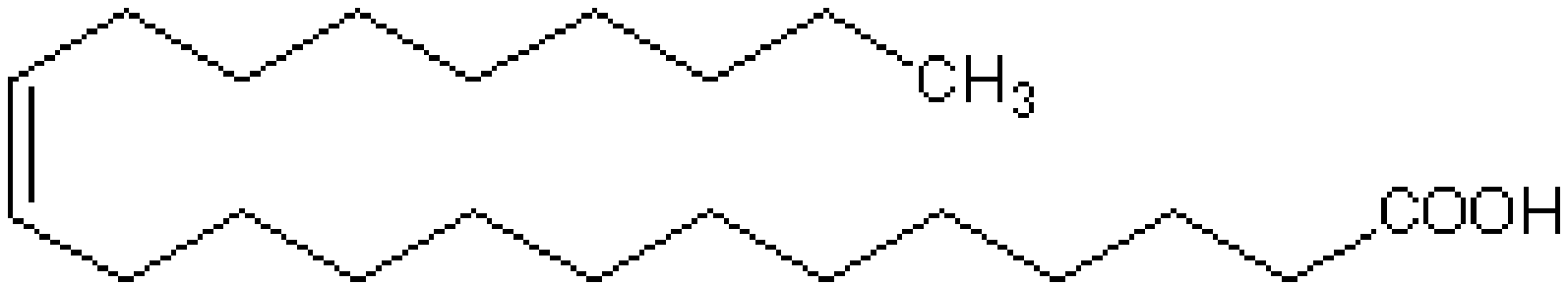
by

Ela Mietkiewska and David Taylor

Goal:

**Isolation and characterization of strategic genes for
high trierucin production**

Erucic acid – C22:1

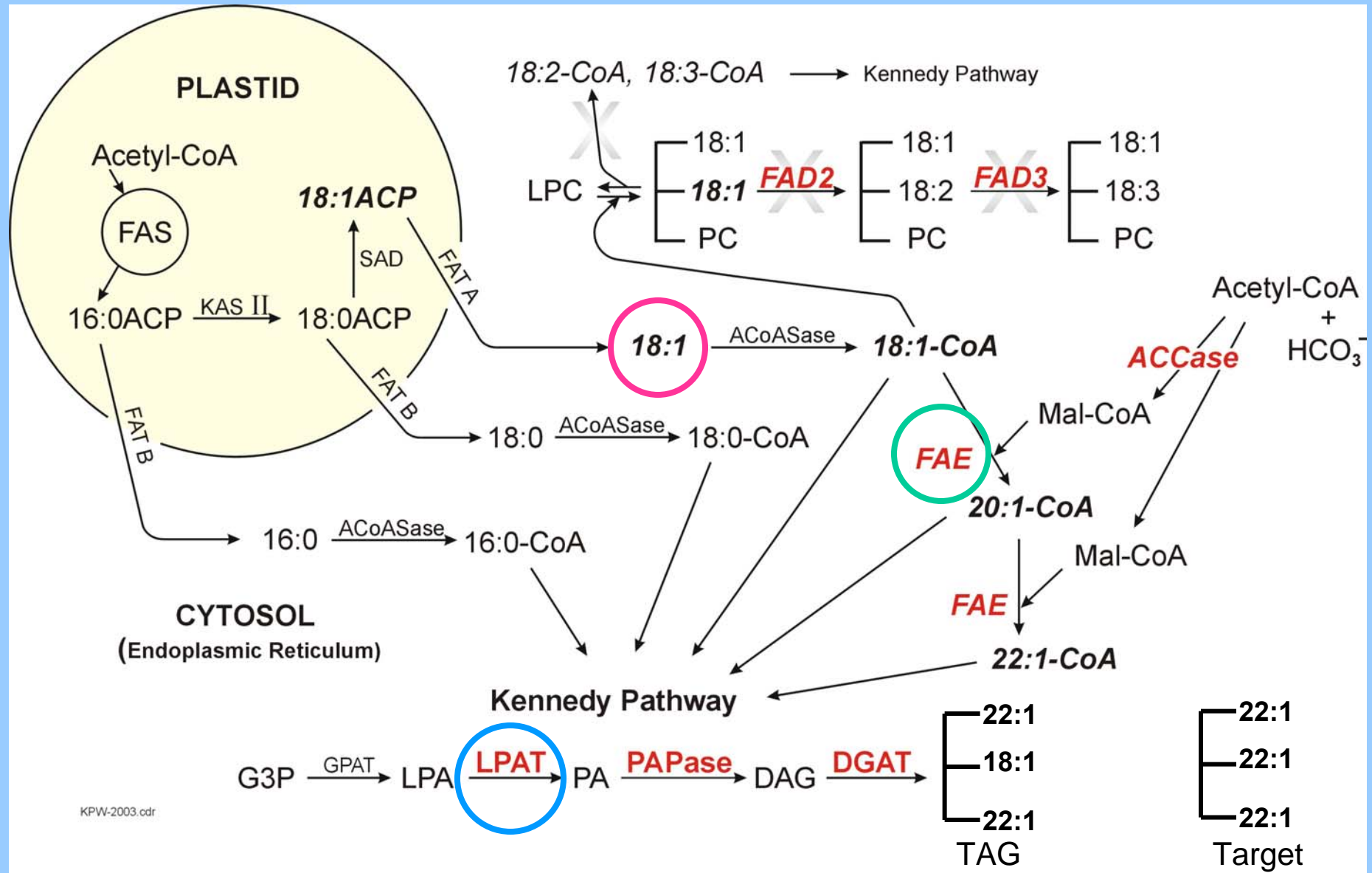


Applications:

- plastic film manufacture
- nylon 13,13 synthesis
- lubricant industries

Source:

- *Brassica napus* - seeds



Source of strategic genes

Tropaeolum majus
(garden nasturtium)



Fatty acid distribution (wt%)

	16:0	18:0	18:1	18:2	18:3	20:0	20:1	22:0	22:1	24:0	24:1
TAG	0.7	0	2.1	trace	0.4	0	16.6	0.5	78.3	0	1.4
sn-1	4	4.4	2.8	0.8	0	0	13.2	0	64.7	0	1
sn-2	1.5	0.9	3	0	0.4	0	12.1	0.3	75.7	0	0.5
sn-3	2.1	4.1	2.2	0	0	0	14.3	1.5	73.6	0	2.2



Isolation of strategic genes for high trierucin production

**Approach: Identification of target ESTs
(Subtracted cDNA libraries)**

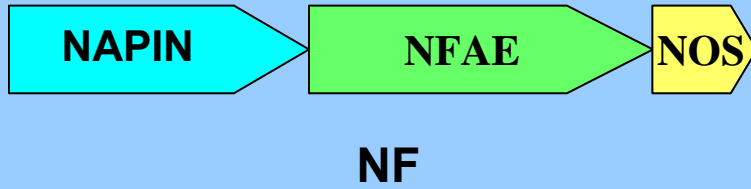
FA metabolism related cDNA

#clusters	sequence homology	hits
1	E-14-3-3 protein	3
2	lipase/acylhydrolase	16
3	oleosin isoform B	2
4	phospholipase D 1 precursor	2
5	lipid transfer protein	3
6	fatty acid elongase	10
7	long-chain-fatty-acid--CoA ligase	1
8	Acyl-CoA binding protein	1
9	fatty acid multifunctional protein	1
10	acyl-CoA oxidase ACX3	1
11	putative lysosomal acid lipase	1
12	palmitoyl-protein thioesterase	1
13	acyl-CoA dehydrogenase	1
14	acetyl-CoA synthetase	1
15	sterol delta-7 reductase	1
16	stearoyl-ACP desaturase	1

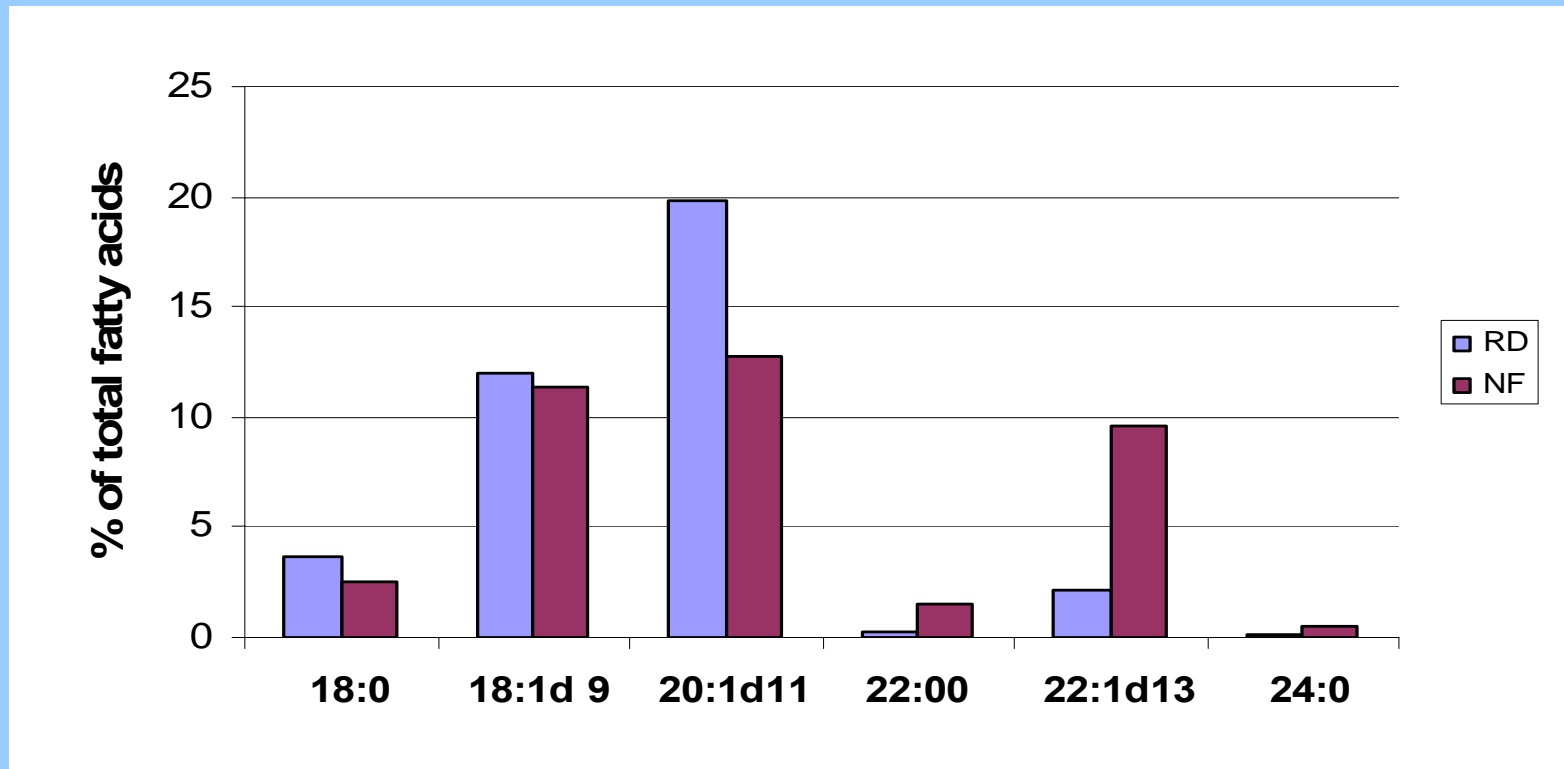
Nasturtium fatty acid elongase - *FAE*

- 1. cDNA clone of 1765 bp– GenBank # AY082610
ORF → 504 aa**

Expression of nasturtium *FAE* in *Arabidopsis* seeds-T₂ (25 lines)

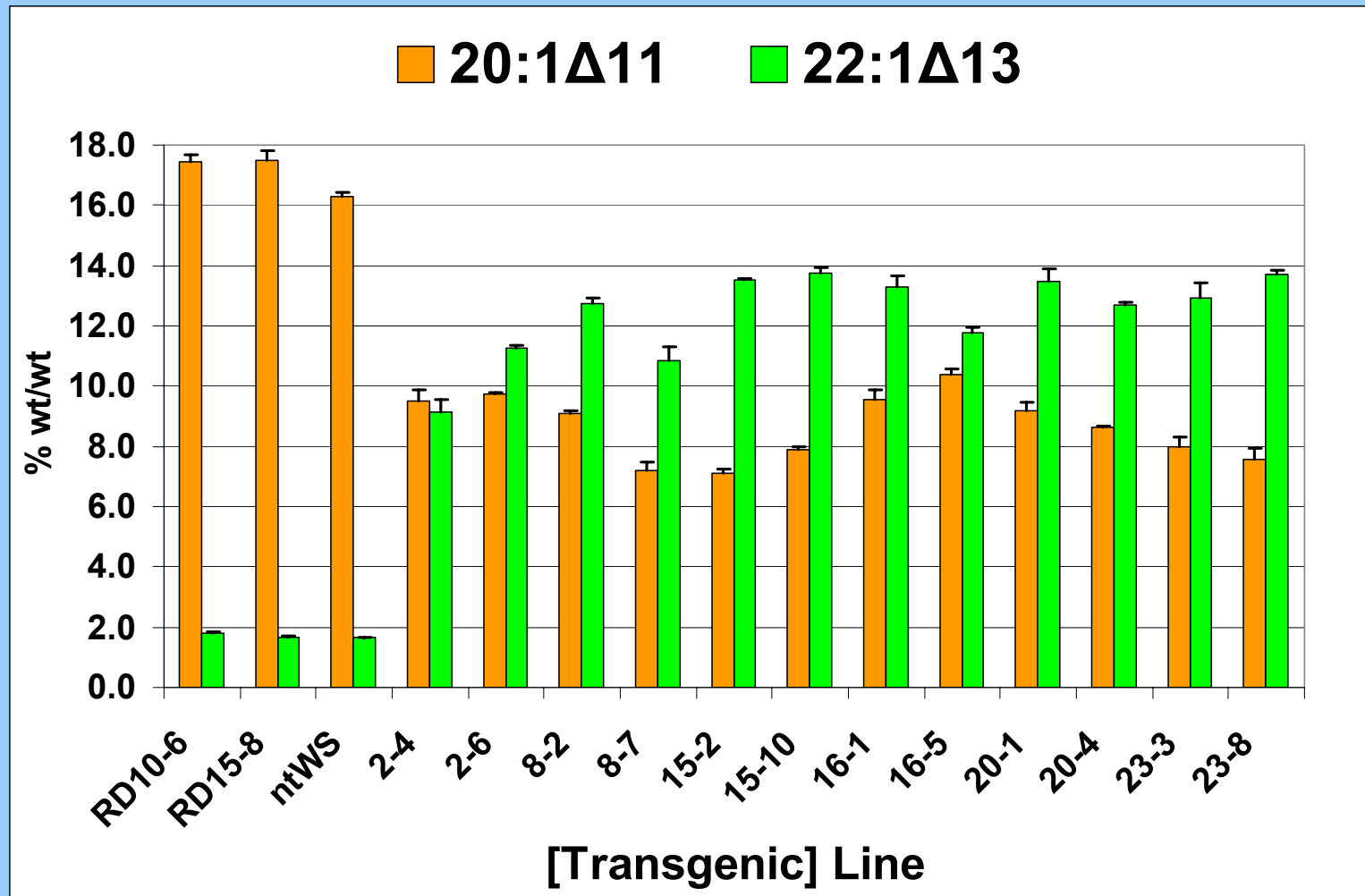


RD-empty vector



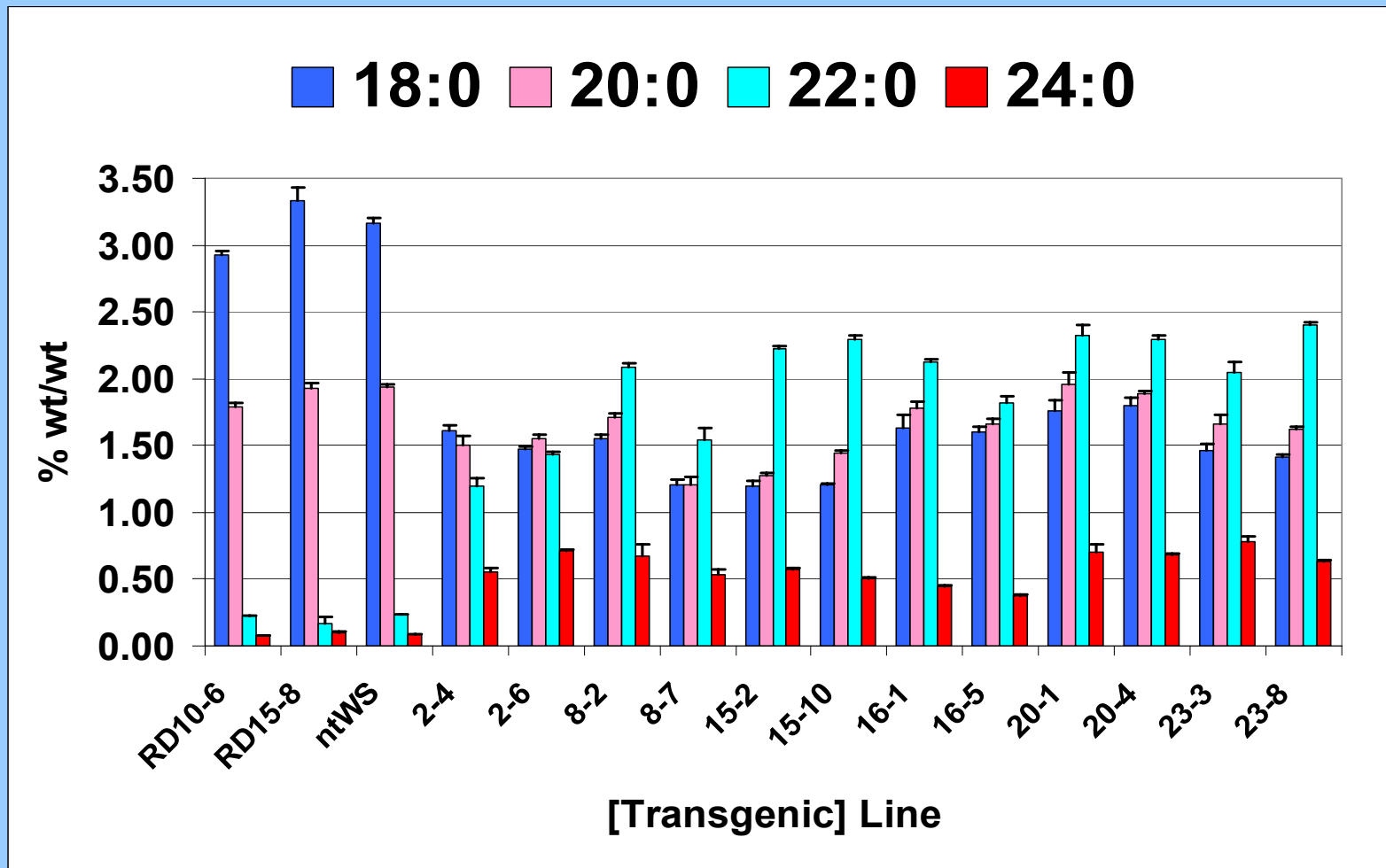
Mietkiewska et al., Plant Physiol. (2004)

Fatty acid composition of *Arabidopsis* seeds (T₃, Ho lines)



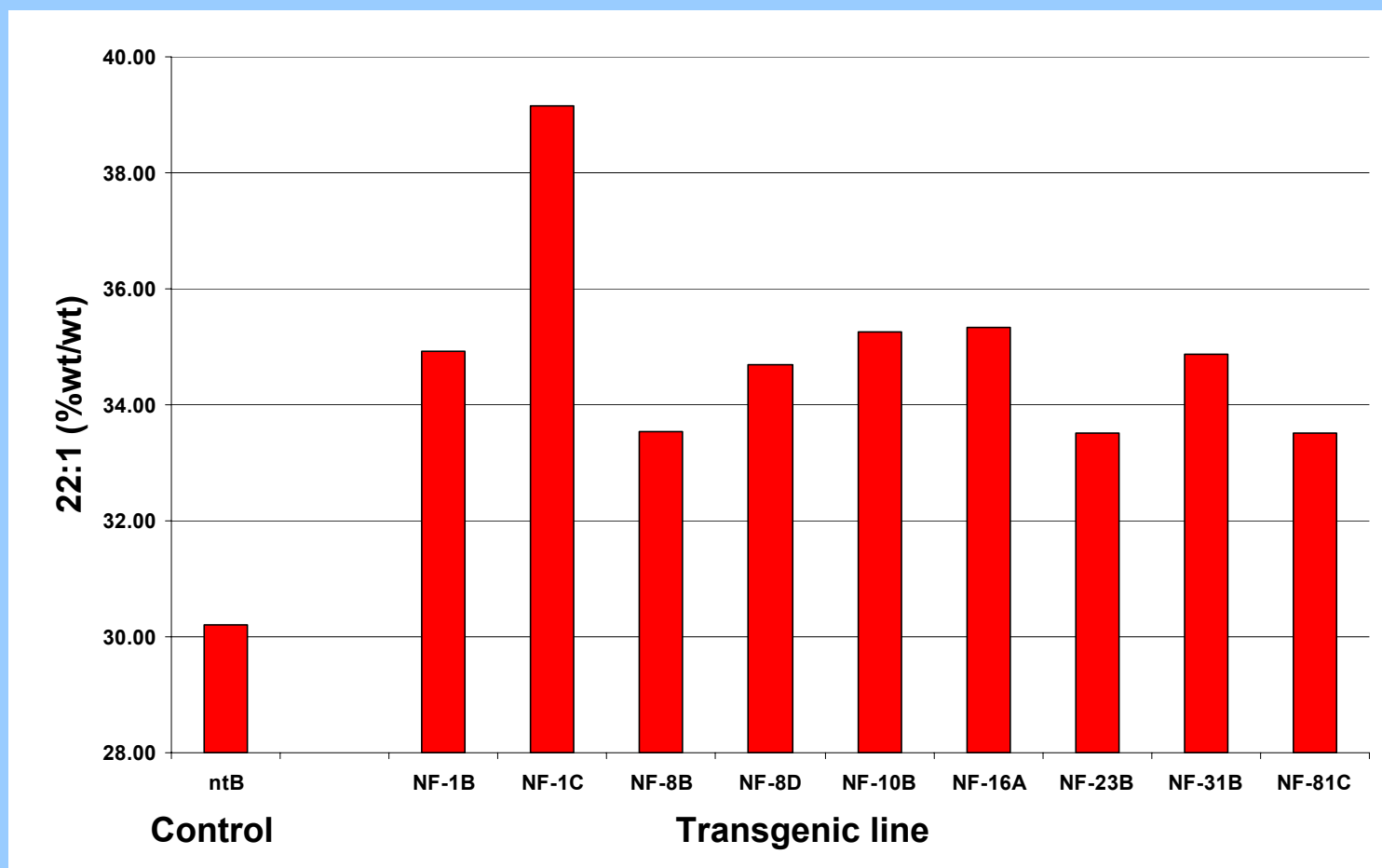
Mietkiewska et al., Plant Physiol. (2004)

Fatty acid composition of *Arabidopsis* seeds (T₃, Ho lines)



Mietkiewska et al., Plant Physiol. (2004)

Accumulation of erucic acid in *B.carinata* (T₁ seeds) transformed with nasturtium *FAE*



Conclusions

- **Genomic tools allowed to isolate/identify genes involved in fatty acid metabolism in nasturtium**
- **The nasturtium *FAE* gene encodes a condensing enzyme involved in the biosynthesis of very long chain fatty acids, preferentially utilizing monounsaturated (20:1) and saturated acyl substrates**
- **Nasturtium *FAE* may have a strong engineering impact when strongly expressed in a seed-specific manner in high erucic acid Brassicaceae**