

# Establishment & Application of Biotechnologies in *Camelina sativa*



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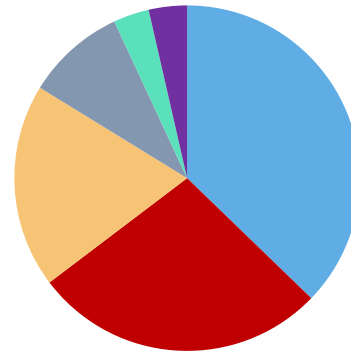
**15<sup>th</sup> International Rapeseed Congress**  
**19.06.2019**

# Glucosinolates accumulating in seeds affect the nutritional value



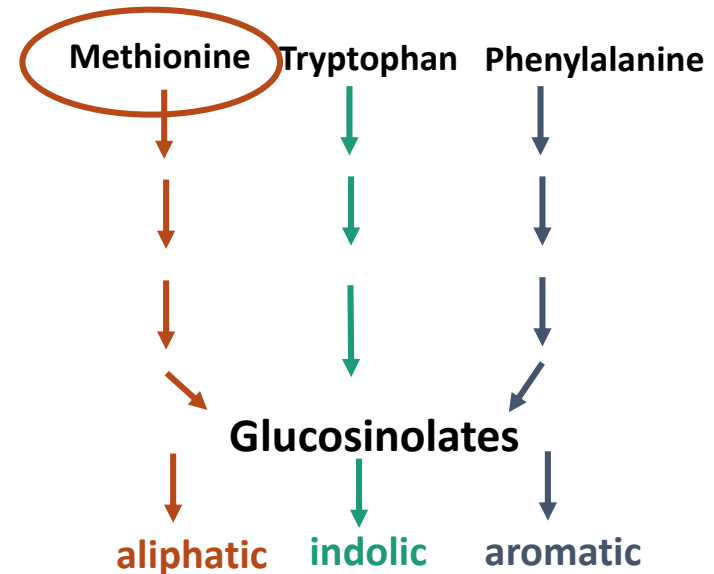
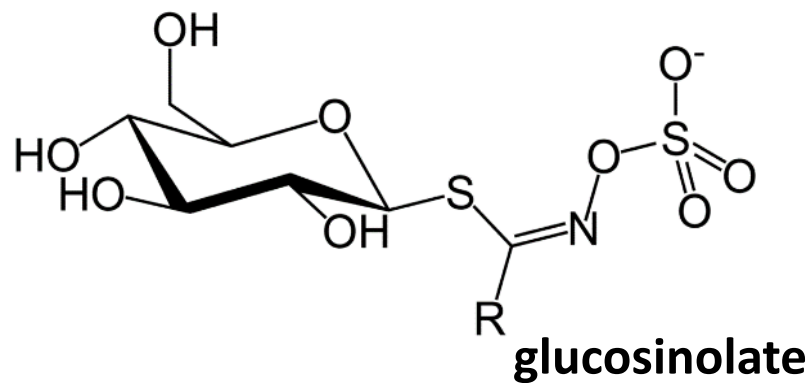
*Camelina sativa*

## Fatty acid composition of camelina oil



- $\alpha$ -Linolenic acid (18:3)
- Linoleic acid (18:2)
- Oleic acid (18:1)
- Palmitic acid (16:0)
- Erucic acid (22:1)
- Stearic acid (18:0)

## HIGH ALIPHATIC GLUCOSINOLATE (HAG1 = *MYB28*)

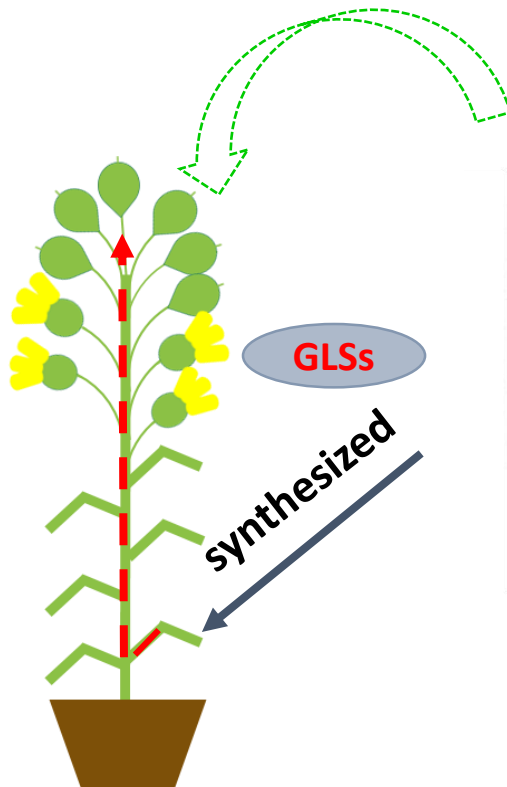


Camelina, Ehrensing & Guy, 2008

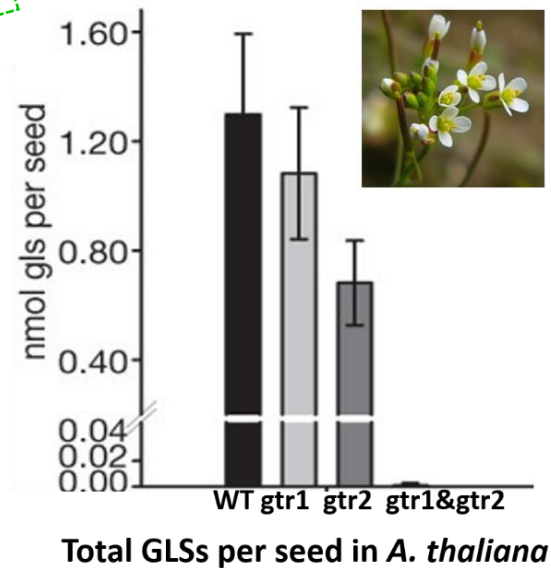
# Glucosinolates are imported to seeds

## GLUCOSINOLATE TRANSPORTER genes (*GTR1*, *GTR2*)

- essential for transport and accumulation of glucosinolates (GLSs) in seeds



*Camelina sativa*



Nour-Eldin et al., Nature 2012



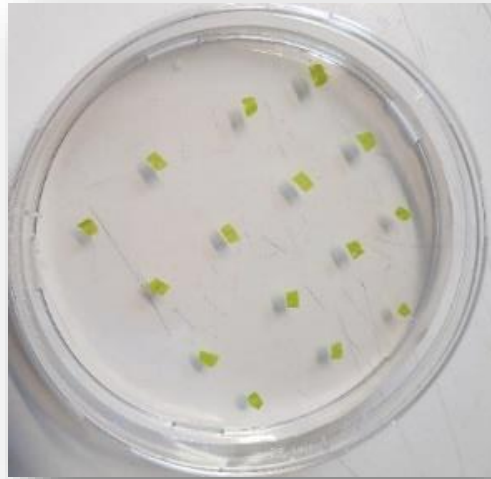
60-70% reduced GLSs in seeds of *B.rapa* & *B.juncea*

Nour-Eldin et al., Nat. Biotechnology 2017

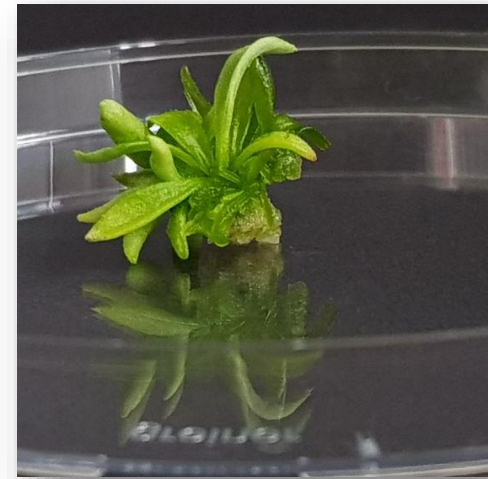
# Plant Regeneration via Adventitious Shoot Formation

## Explants:

- Hypocotyl
- Cotyledon
- True leaf ✓



Leaf segments from seedlings



Shoot formation



Regenerated plant



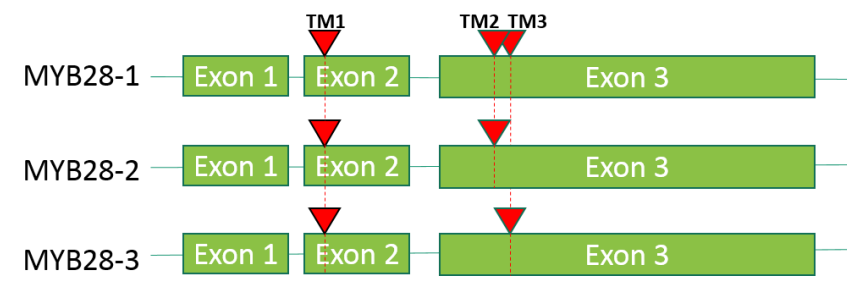
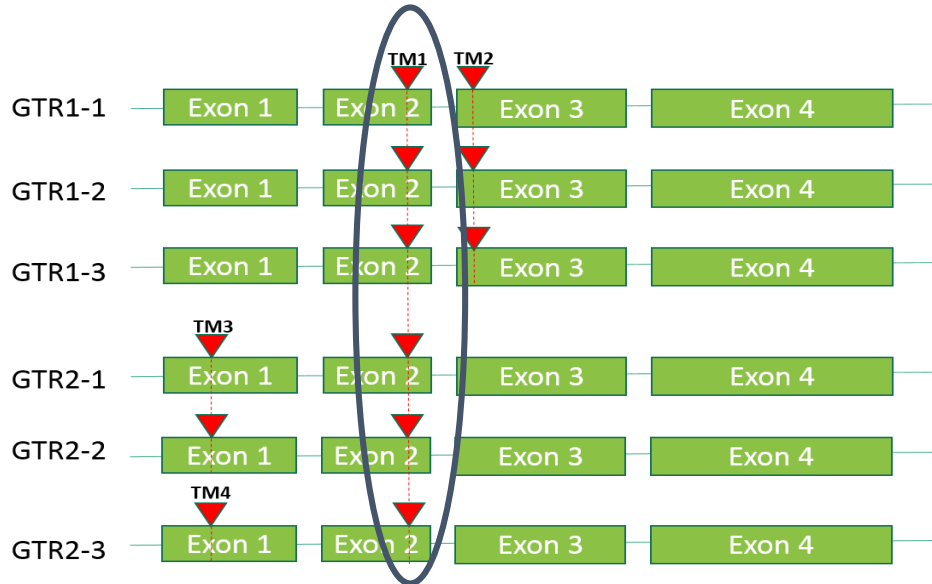
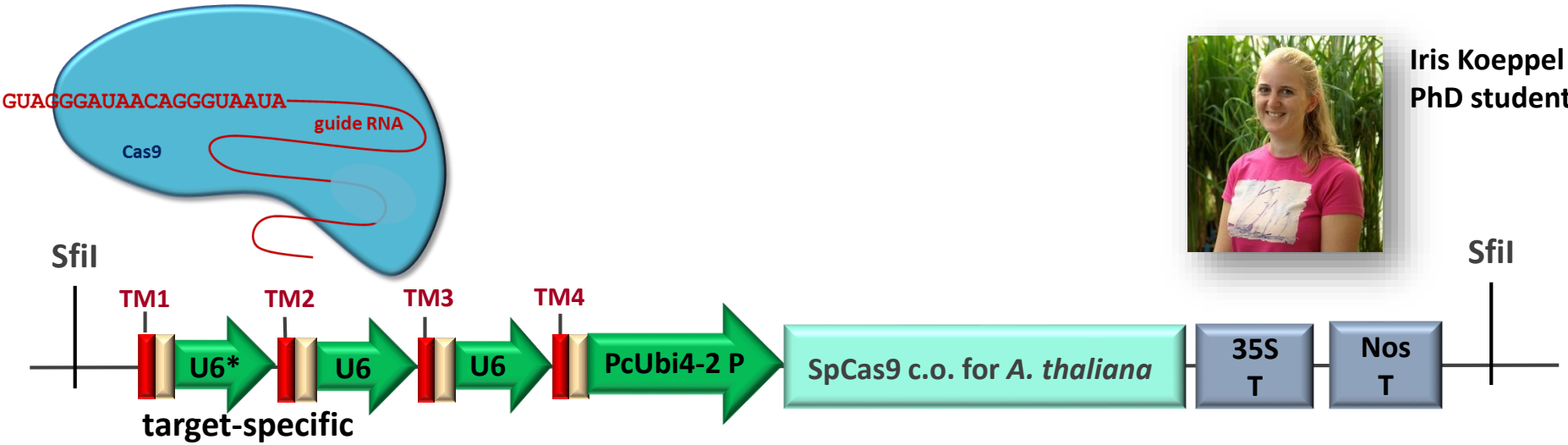
Plants on rooting medium



# Selection of gRNA/Cas9 Target Motifs (TM) in *GTRs* & *MYB28*



Iris Koepfel  
PhD student



\*AtU6-26 P


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**RNAfold WebServer**



# Sequence results of mutations in *MYB28* & *GTR1*

Wild type  
Mutant plant 7

```

*           300           *           320           *
ACGGTGTGGAAAGAGCTGTAGACTG-CGATGGACCAATTACCTTAA
ACGGTGTGGAAAGAGCTGTAGACTGT-CGATGGACCAATTACCTTAA

           420           *           440           *           4
ACGTTTCTATGTTTATATATATAACAAACAAACAAAAAATGTGTG
ACGTTTCTATGTTTATATATATAACAAACAAACAAAAAATGTGTG

           540           *           560           *           580
ATTATCATCTTAATTCTGTCTTCGGTCTATCTATTCATCTTCTTT
ATTATCATCTTAATTCTGTCTTCGGTCTATCTATTCATCTTCTTT

           680           *           700
AAAAAACGTTTGGTTGAGCAGGGTATTGATCCCGTGACACACAAG
AAAAAACGTTTGGTTGAGCAGGGTATTGATCCCGTGACACACAAG

*           800           *           820           *
ACGGTCGAGCTCAATGCCTTCTCTGTCTCCACCTCTATCCGGTTG
ACGGTCGAGCTCAATGCCTTCT--GTCTCCACCTCTATCCGGTTG

PAM
    
```

TM1 – one insertion

MYB28



TM2 – two deletions

GTR1

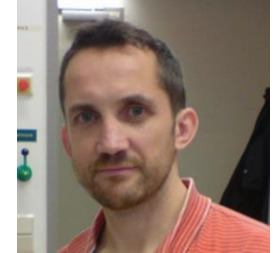


```

*           200           *           220           *
Line 15 TTGCTGCTTTCCCTCTGTGTTGACACTTACTTTGGTCGCTACAAGACTCTC
wild type TTGCTGCTTTCCCTCTG-TGACACTTACTTTGGTCGCTACAAGACTCTC
Line 16 TTGCTGCTTTCCCTCTGTGTTGACACTTACTTTGGTCGCTACAAGACTCTC
line 13 TTGCTGCTTTCCCTCTGATGACACTTACTTTGGTCGCTACAAGACTCTC

PAM
    
```

TM1  
single base  
insertions



Dr. Georg Hölzl

# Doubled haploids (DH):

Individuals with the chromosome number of haploids being doubled

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## *In vitro*

- Ovule/ovary/culture
- Anther/microspore culture

## *In vivo*

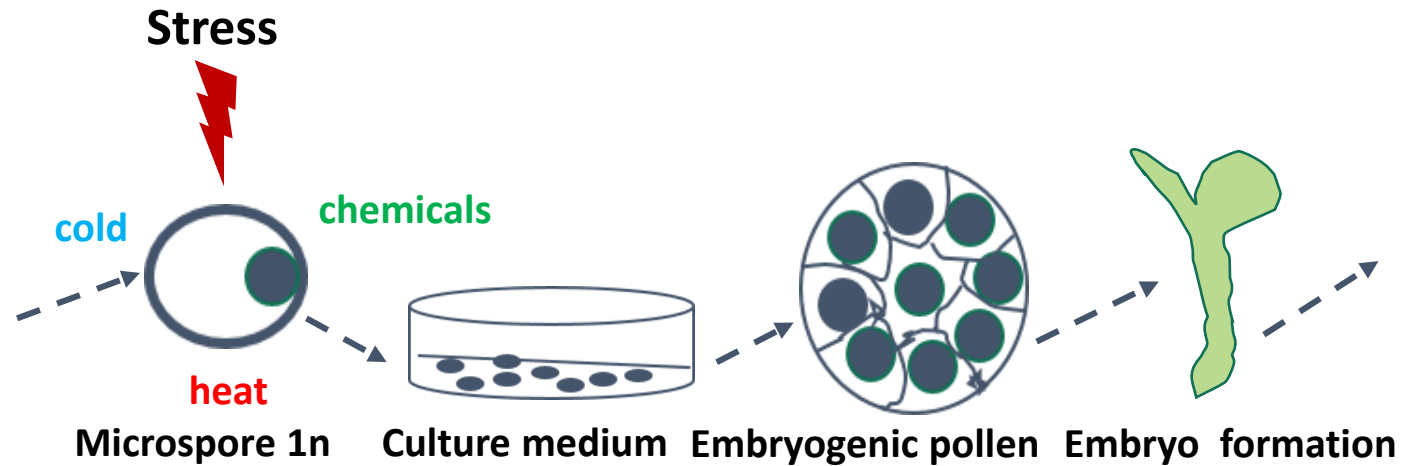
- Spontaneous
- Hybridization

# Doubled haploids:

Individuals with the chromosome number of haploids being doubled

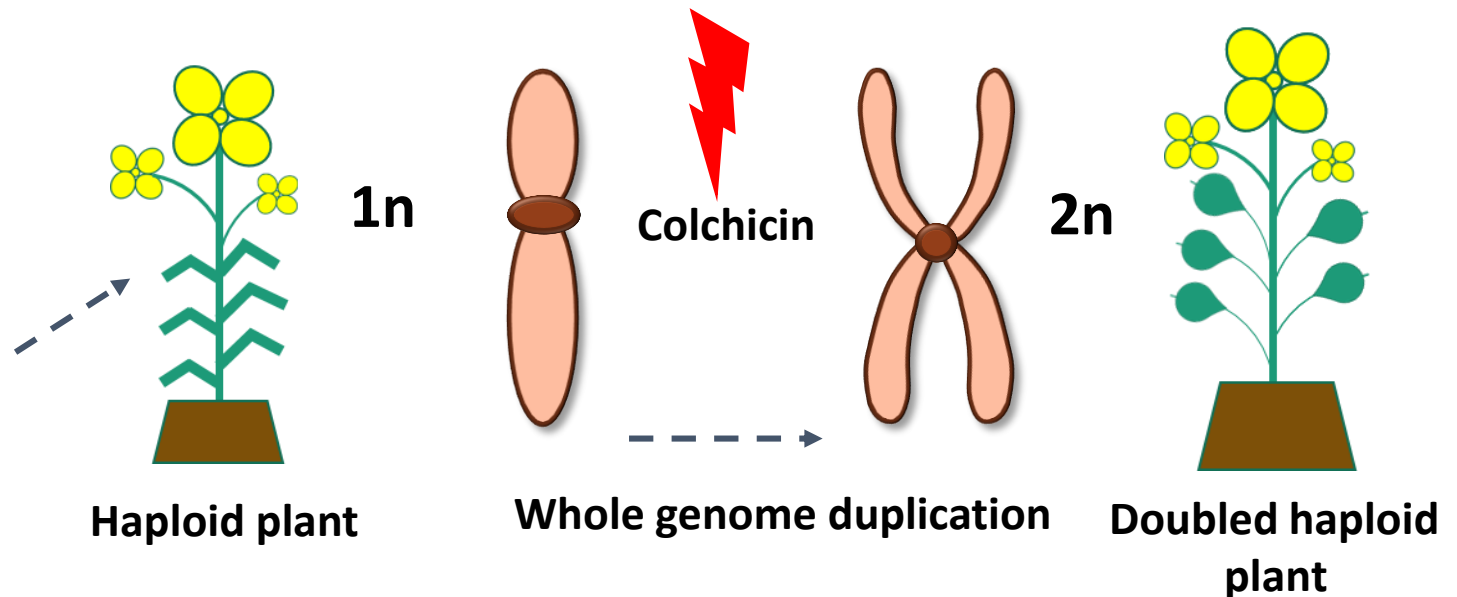
## *In vitro*

- Ovule/ovary/culture
- Microspore culture



## *In vivo*

- Spontaneous
- Hybridization





# Regeneration from Microspores



Cotyledonary stage



Plants on rooting medium



DH plant in soil

# Summary & Outlook

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- ✓ **Adventitious shoot formation from seedling explants is established**
- ✓ **First doubled haploids from isolated microspores are obtained**
  
- **Experiments on genetic transformation are in progress**
- **Knockout of candidate genes by gRNA/Cas9 is in progress**

# Acknowledgments

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**PRB group members**



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*Leibniz*  
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Association



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# Regeneration from Microspores

