Canola Pathology Workshop

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The use of Acyl-CoA-binding proteins (ACBPs) in disease resistance and frost tolerance

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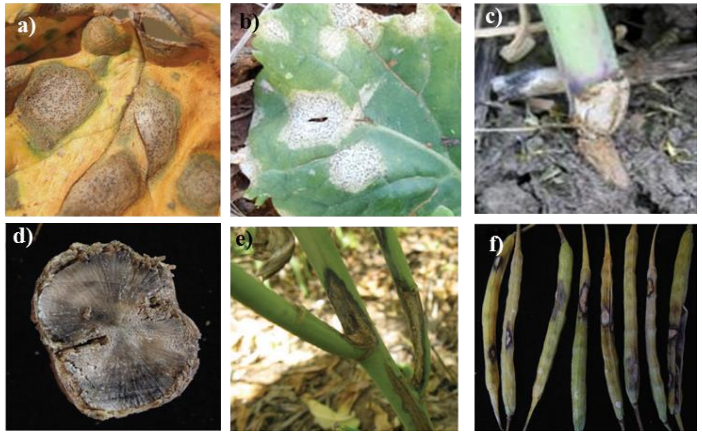
Rice ACBP5

Fungal diseases
blackleg, *Sclerotinia*, *Alternaria*

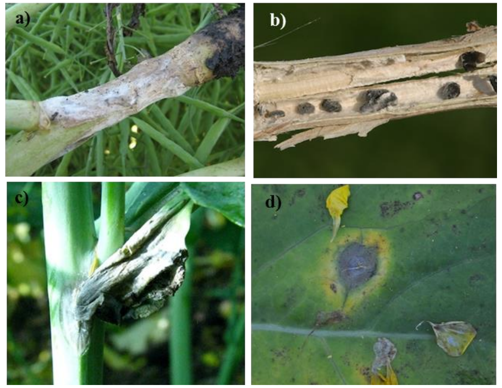
~40% yield losses

blackleg



http://www.cesaraustralia.com/sustainable-agriculture/pestfacts-south-eastern/past-issues/2016-2/pestfacts-issue-no-5-14th-july-2016/blackleg-disease-of-canola-in-2016/

Sclerotinia



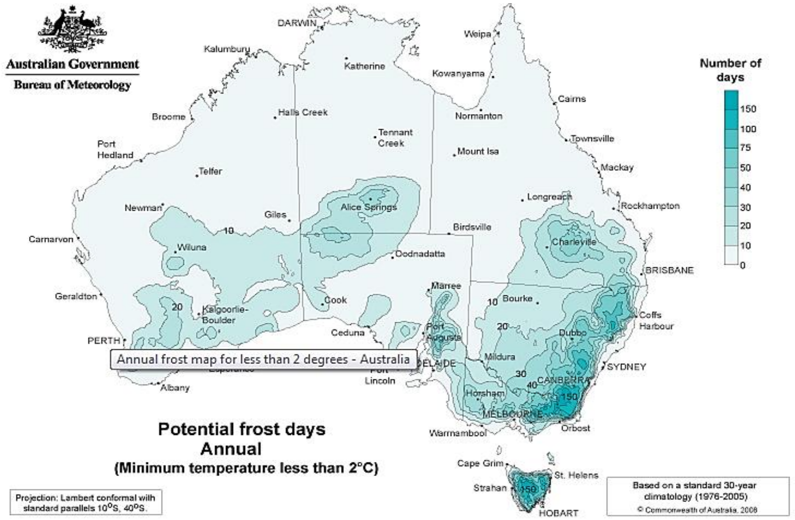
https://www.canolawatch.org/2013/01/09/sclerotinia-stem-rot-management/

Arabidopsis ACBP6

Low temperature stress
Frost conditions

~ 360 million dollars to the agricultural industry annually

Department of Primary Industries and Regional Development https://www.agric.wa.gov.au/mycrop/diagnosing-frost-damage-canola and Grain Research and Development Corporation https://grdc.com.au/__data/assets/pdf_file/0020/203735/grdcbpgfrostpulses.pdf.pdf



source: http://www.bom.gov.au/jsp/ncc/climate_averages/frost/index.jsp Bureau of Meteorology, accessed 04/10/2018

Rapid-Cycling *B. napus* as a trait testing model

Tissue Culture Optimisation

- ***Explants***: Cotyledons
- ***Callus Induction***: NAA 0.1-0.2 mg/L; BAP 0.5-1.0 mg/L **≥ 88%**
- ***Shoot Induction***: Silver nitrate essential

  NAA 0.1 mg/L; BAP 1.0 mg/L; $AgNO_3$ 5 mg/L **70%**
- ***Root Induction:*** IBA 1-2 mg/L **60-65%**

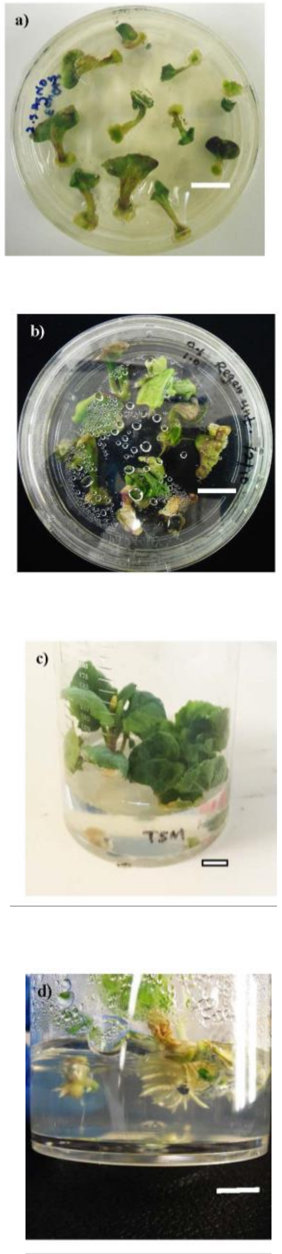
Improve Transformation Efficiency

Reduce *Agrobacterium* colony incubation time

Delay initial antibiotic selection

Remove MS liquid medium from explant preparation

- **Efficiency improved from 2-5% to >10%**



Rice *ACBP5* cDNA for enhanced disease resistance in *Brassica napus*

- Blackleg
- Sclerotinia stem rot disease

Rice Acyl-CoA binding protein family- 6 ACBPs from ACBP1-ACBP6 (size 91-655 aa)

OsACBP5------------ class III (Large ACBPs: 569aa)

Highly expressed

Reproductive phase

High salinity

Rice blast fungal (*Magnaporthe grisea)* infection

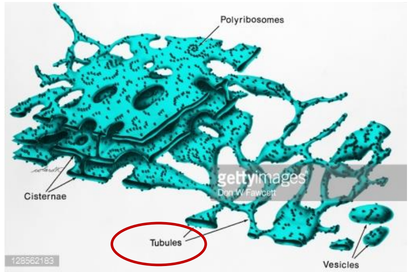
Localisation

In the tubular region of ER, where vesicles bud-off

Suggested roles

Synthesis/export of phospholipids and lipid droplets from ER

Pathogenesis related (PR) proteins release from ER



In *Arabidopsis*

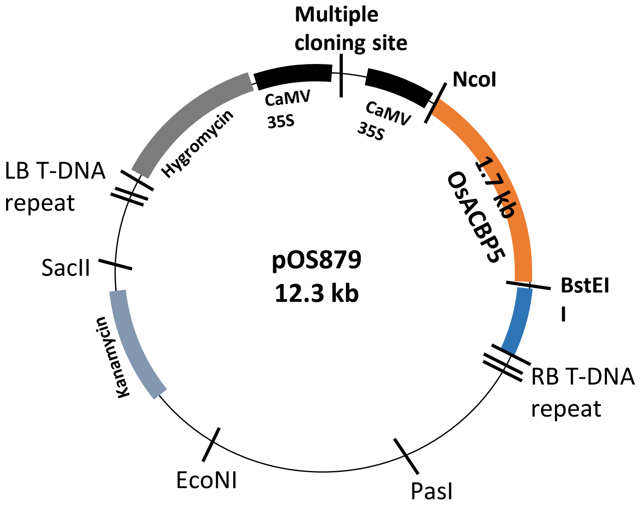
Resistance to *Pseudomonas syringae, Rizoctonia solani, Botrytis cinerea, Alternaria brassicicola*

Expression of cell-wall related proteins – help inhibit cell wall degrading enzymes, cell wall remodelling

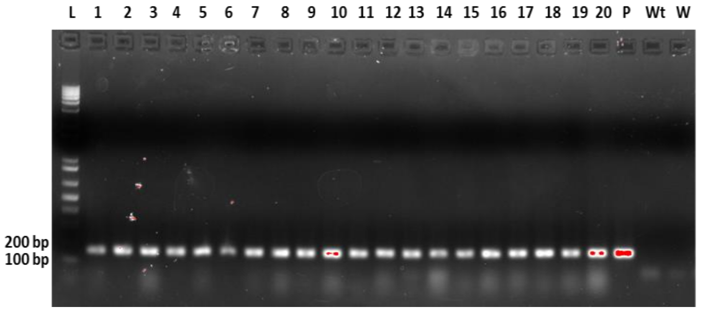
✓ Transformation

Plasmid construct pOS879

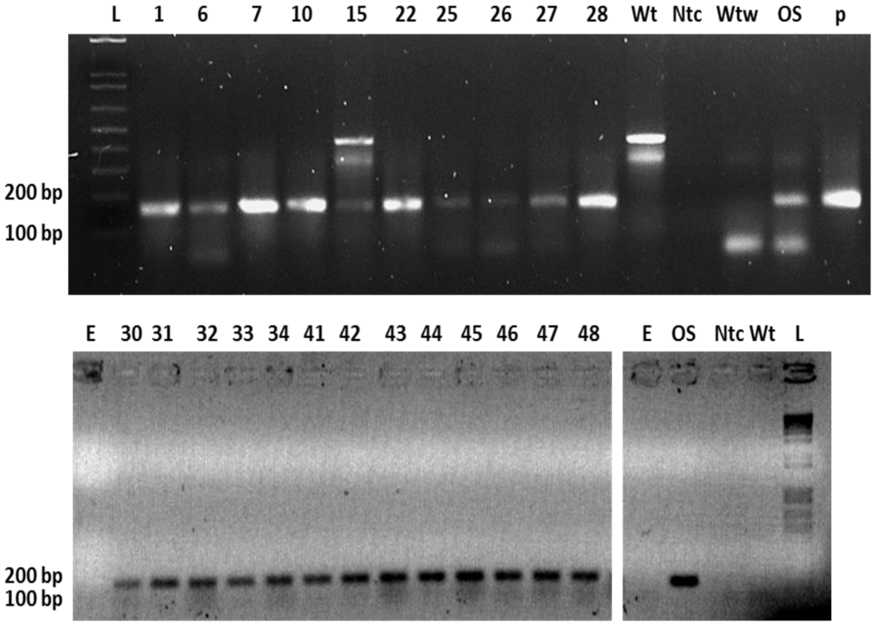
Rice ACBP5 cDNA driven by Cauliflower mosaic virus 35S promoter



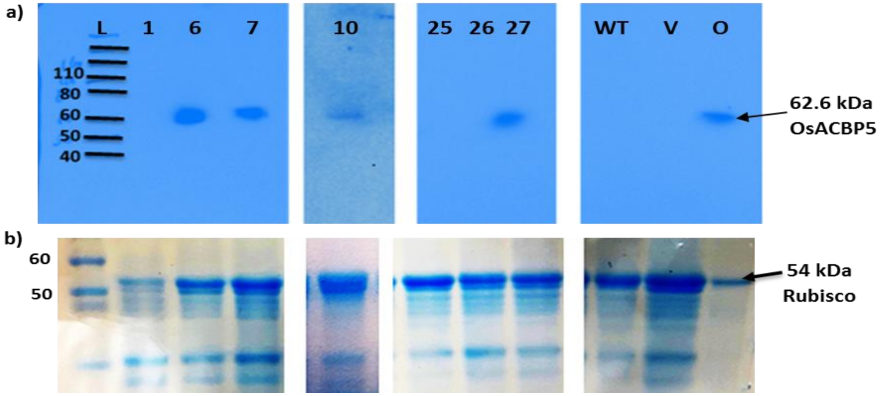
✓ Genotyping



✓ Semi quantitative-RT-PCR



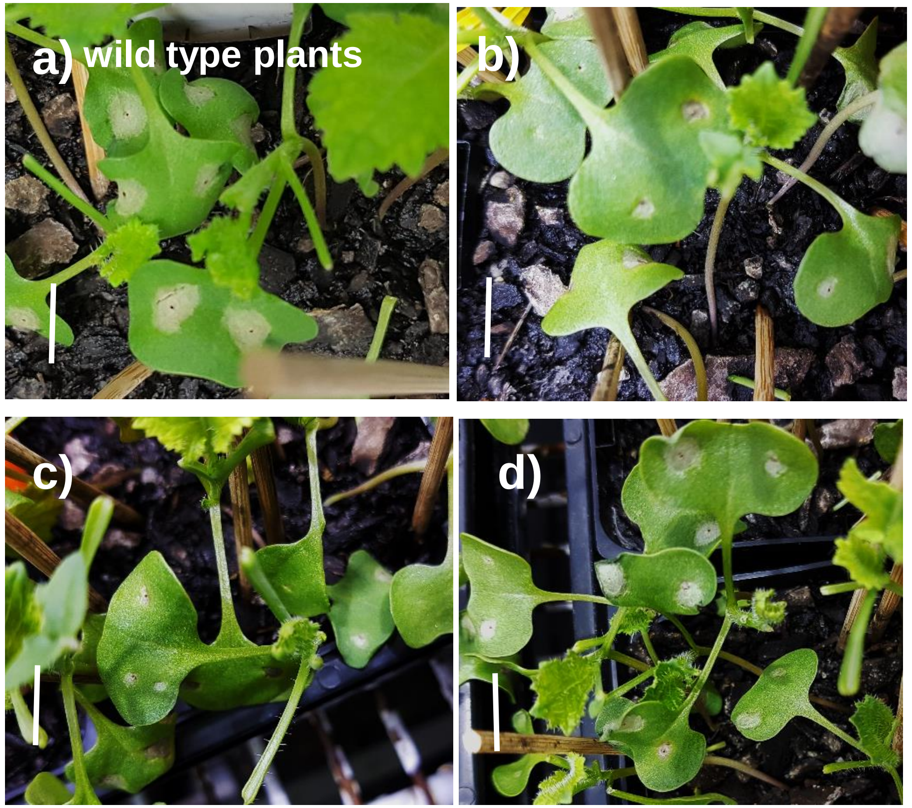
✓ Western blot analysis



Molecular verification of independent OsACBP5-OE rapid-cycling *B. napus* lines established in the glasshouse using PCR, reverse transcription PCR and western blot analysis

Blackleg assay on 12-day-old cotyledons

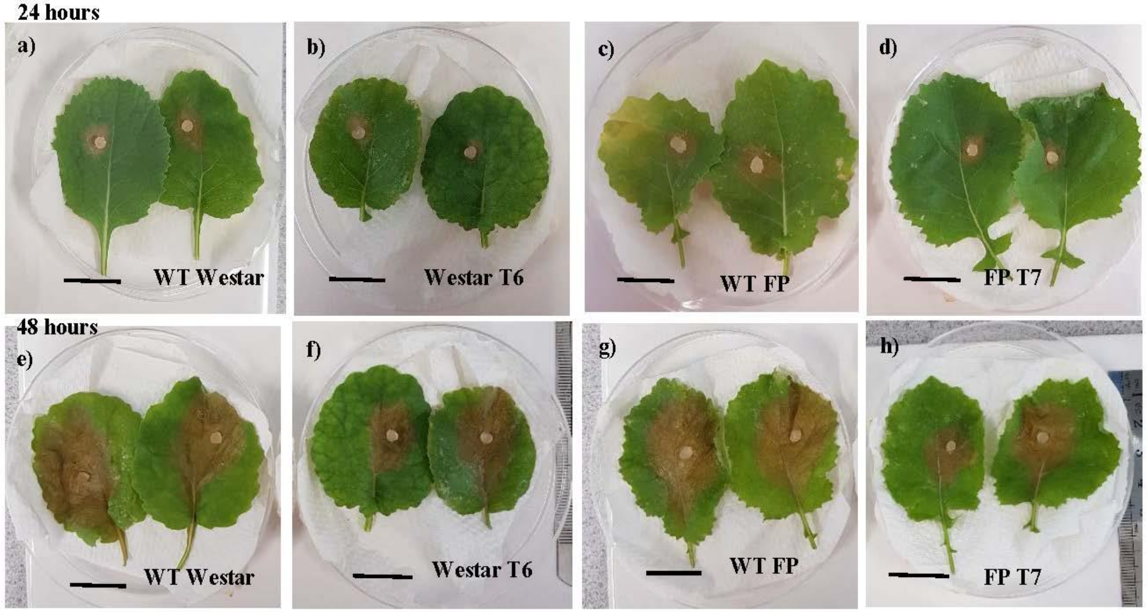
Genotype	Average lesion diameter (mm)	Average mean disease score	Average median disease score
Westar			
Line 6	5.0^{c}	5.5^{cd}	5.4^{b}
Line 27	4.5^{d}	5.0^{d}	5.1^{bc}
Wild type Westar	6.0^{b}	6.2^{ab}	6.4^{a}
Vector control W	6.8^{a}	6.7^{a}	6.9^{a}
Rapid-cycling			
Line 7	2.9^{e}	3.5^{f}	3.6^{d}
Line 10	3.5^{e}	4.3^{e}	4.4^{cd}
Wild type RC	5.4^{c}	6.0^{b}	6.0^{ab}
Vector control RC	5.1^{cd}	5.8^{bc}	5.7^{ab}



✓ Lesion diameter and mean and median disease scores- lower in OsACBP5 plants

Sclerotinia agar plug assay

Genotype	Average lesion diameter (mm)	
	24 hours after inoculation	48 hours after inoculation
Westar		
Line 6	8.5^{d}	21.5^{a}
Line 27	9.2^{cd}	23.7^{a}
Wild type Westar	10.8^{ab}	24.0^{a}
Vector control W	12.1^{a}	27.2^{a}
Rapid-cycling		
Line 7	8.8^{cd}	24.8^{a}
Line 10	10.3^{bc}	25.4^{a}
Wild type RC	11.5^{ab}	26.1^{a}
Vector control RC	11.2^{ab}	26.5^{a}



Detached leaves after inoculation with *Sclerotinia sclerotiorum*: After 24 h and 48 h

- ✓ OsACBP5 transgenic plants- more resistant to *Sclerotinia* after 24 h
- ✓ OsACBP5 may help acquire inducible defence

Evaluation of low temperature/frost tolerance in transgenic rapid-cycling *B. napus* plants expressing the *Arabidopsis* ACBP6

-at the vegetative, flowering and seed setting stages

Original transgenic plant development by Eden Tongson, Faculty of Vet. and Ag Sci Univ of Melb.

Arabidopsis Acyl-CoA binding protein family- 6 ACBPs from ACBP1-ACBP6 (size 10-73 kDa)

AtACBP6

Smallest ACBP protein (10 kDa) in *Arabidopsis - ha*s acyl-CoA-binding domain only

Highly expressed

cotyledons, developing embryos and flowers

low temperature

Localisation

cytoplasmic region

Suggested roles - mediate cold tolerance

phospholipid metabolism – by binding and trafficking phosphatidylcholine

In *Arabidopsis*

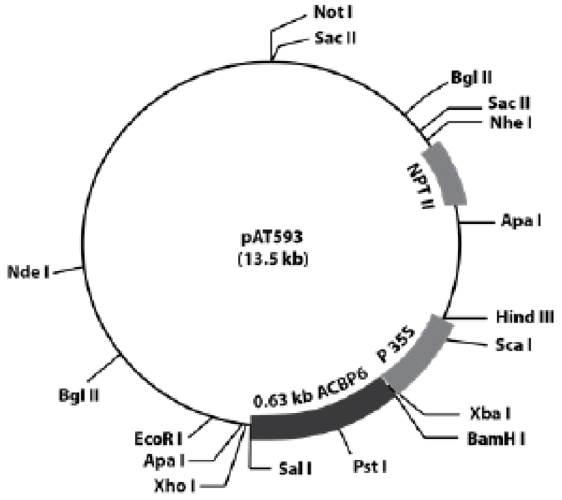
Increased phospholipase Dδ –*stabilise cell membrane*

Reduction of phosphatidyl choline (PC) and increase in phosphatidic acid (PA)- *nonlamellar phase lipid and inhibit phospholipase A activity*

✓ Transformation

Plasmid construct pAT593

Arabidopsis ACBP6 cDNA driven by Cauliflower mosaic virus 35S promoter



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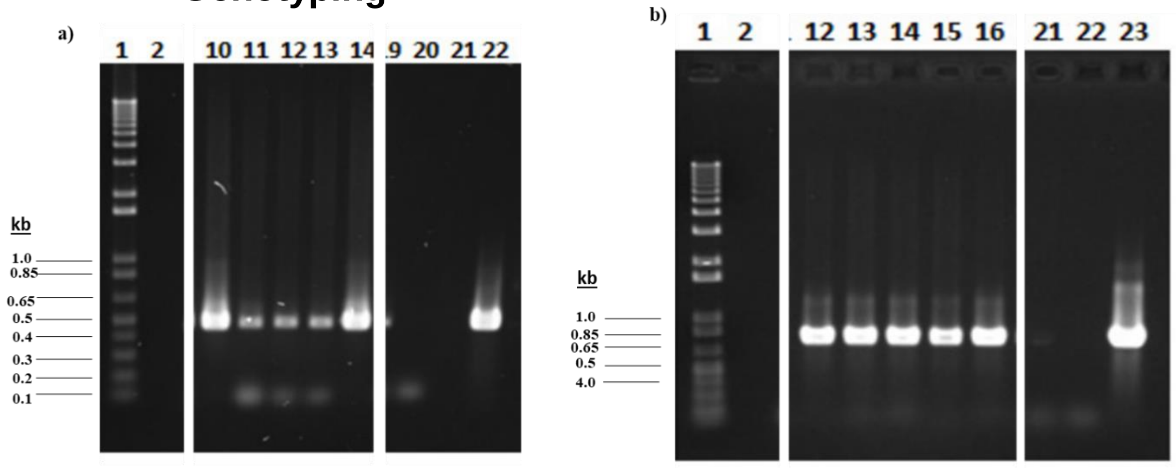
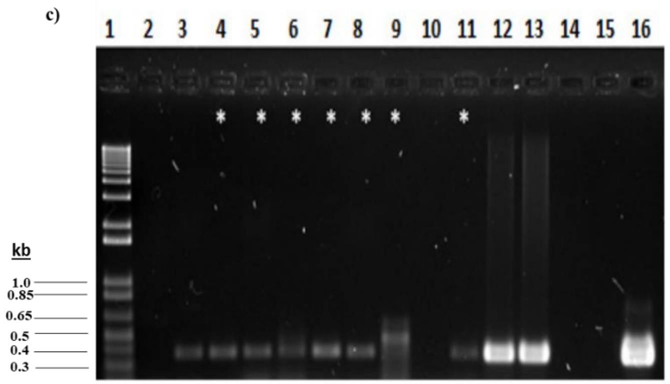
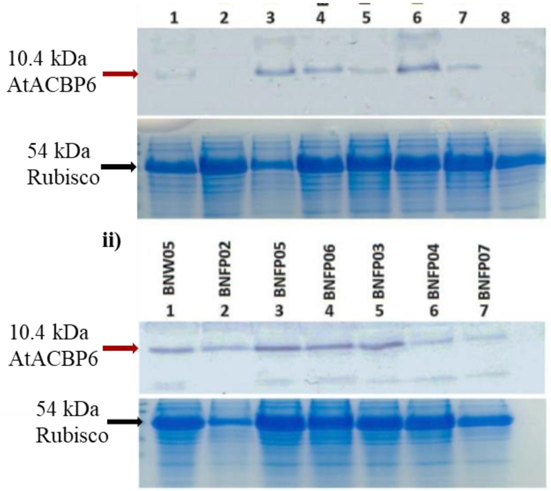


✓ Semi quantitative-RT-PCR



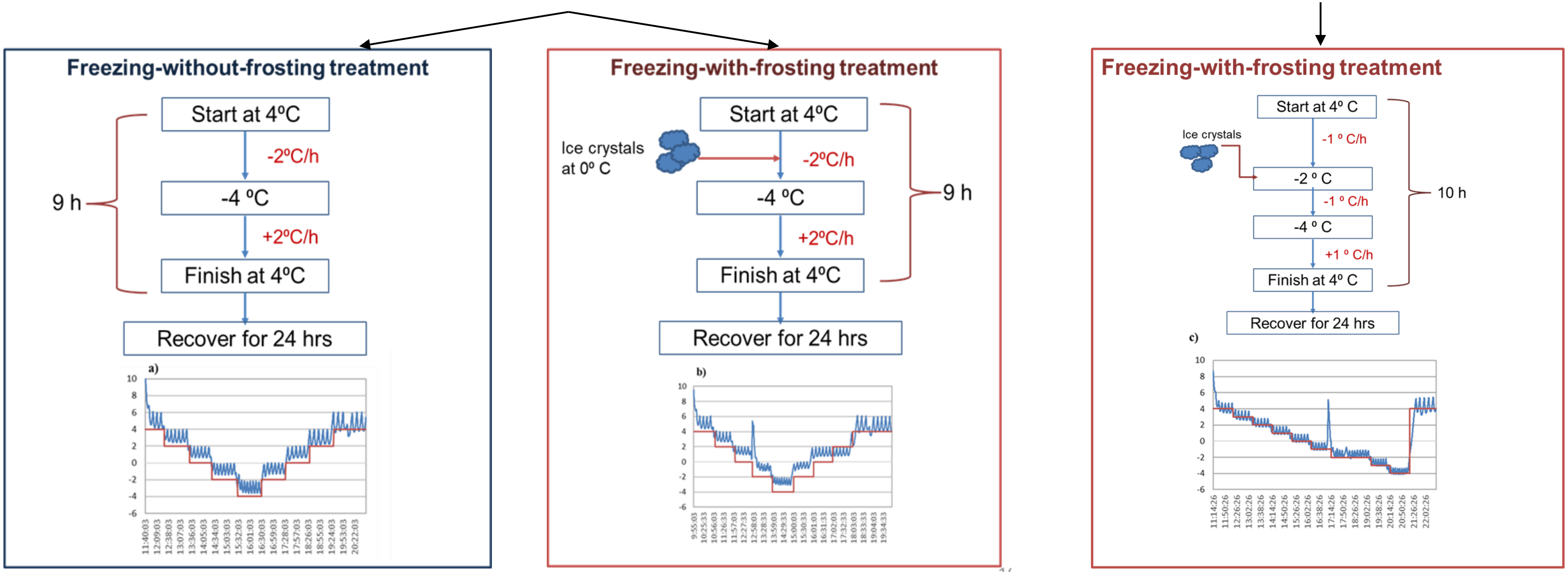
✓ Western blot analysis



Molecular verification of independent AtACBP6-OE rapid-cycling *B. napus* lines established in the glasshouse using PCR, reverse transcription PCR and western blot analysis.

Cold treatments at the vegetative stage (5-6 leaves)

Cold treatment at the flowering and seed-setting stage



Cold-acclimation- 4ºC for 7 days before the freezing treatment

★ Non-acclimation- at ambient temperature until the treatment

Measurements

- Electrolyte leakage
- MINI-PAM II fluorescence yield parameters
- Recovery and the yield
  - Bleaching of leaves
  - Emergence of new shoots
  - No. of flowers, Inflorescences, pods

    -At 4, 6, 8, 11 weeks
- Seed Viability

Statistical Analysis

Minitab 17,

One-way ANOVA of genotype

two-way ANOVA of genotype*treatment (CA/NA)

5% confidence interval

Fisher's least significant difference (protected) test

Varying degrees of recovery after freezing treatment

Emergence of new shoot buds

% Electrolyte leakage – cell membrane damage

Black letters- one-way ANOVA of genotype

Vegetative plants

Plant Line	Freezing- without-frosting treatment
109	7.6[b]
111	11.1[ab]
1	13.8[ab]
81	8.6[b]
Transgenic (avg)	10.3
WT	16.8[a]

Vegetative plants

Plant Line	Freezing-with-frosting treatment
109	20.0[c]
111	34.8[b]
1	30.2[bc]
81	20.0[c]
Transgenic (avg)	26.2
WT	66.8[a]

Flowering plants

Plant Line	Freezing-with-frosting treatment
109	69.5[ab]
111	69.6[ab]
1	68.6[b]
81	60.6[b]
Transgenic (avg)	67.1
WT	81.1[a]

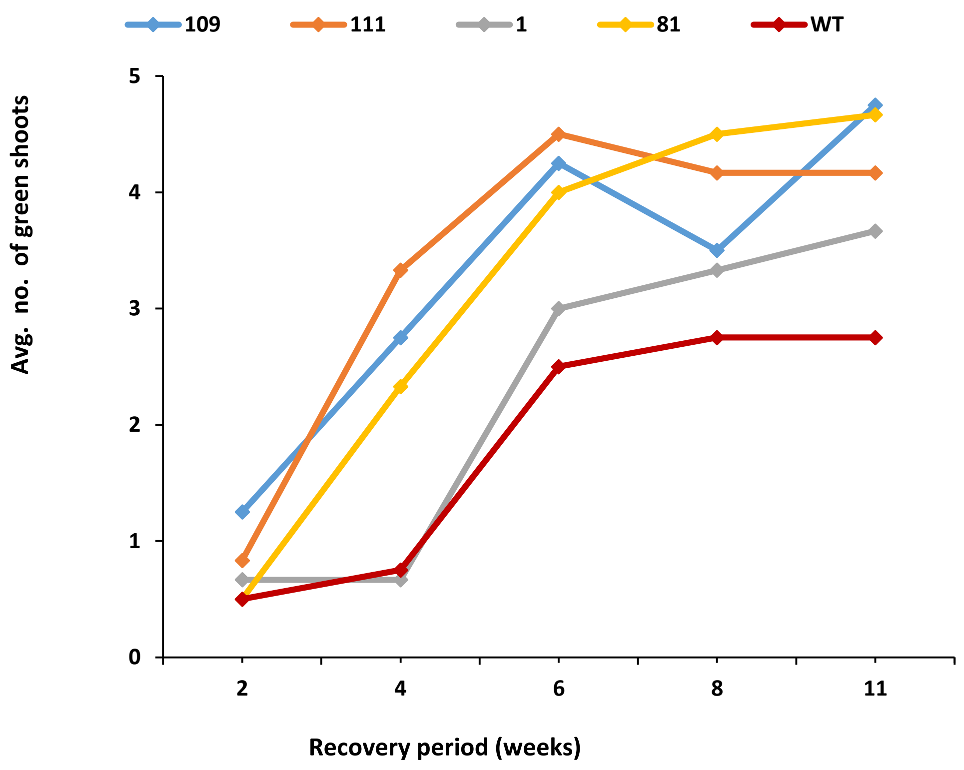
Seed setting plants

Plant Line	Freezing-with-frosting treatment
109	45.1[b]
1	32.9[c]
81	41.3[bc]
Transgenic (avg)	39.8
WT	69.1[a]

- ✓ Less electrolyte leakage from the transgenic plants

Recovery of the vegetative plants after the freezing-without-frosting treatment

Shoots



- ✓ More shoots in transgenic plants

Yield measurements of the vegetative plants after freezing-without-frosting treatment

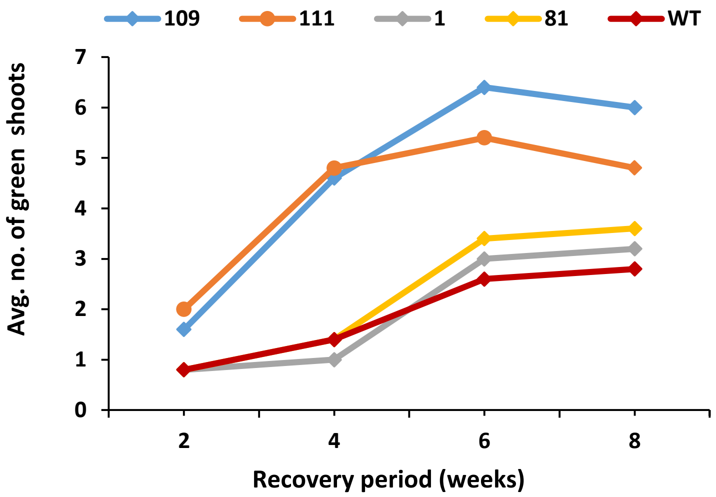
Parameter	*Fresh Biomass in g*	*Total pod weight in g (Dry)*
Genotype (g)		
109	9.36^{a}	0.62^{a}
111	6.34^{b}	0.49^{ab}
1	5.66^{b}	0.43^{ab}
81	5.70^{b}	0.26^{b}
Transgenic (avg)	6.8	0.45
WT	6.13^{b}	0.29^{b}

parameter	*Harvest Index (HI)*	*Dry seed weight in grams*
Genotype (g)		
109	0.20^{ab}	0.41^{a}
111	0.20^{ab}	0.28^{ab}
1	0.25^{a}	0.30^{ab}
81	0.11^{b}	0.13^{b}
Transgenic (avg)	0.19	0.28
WT	0.09^{b}	0.15^{b}

- ✓ Seed yield and harvest index of *AtACBP6* transgenic plants were higher than WT

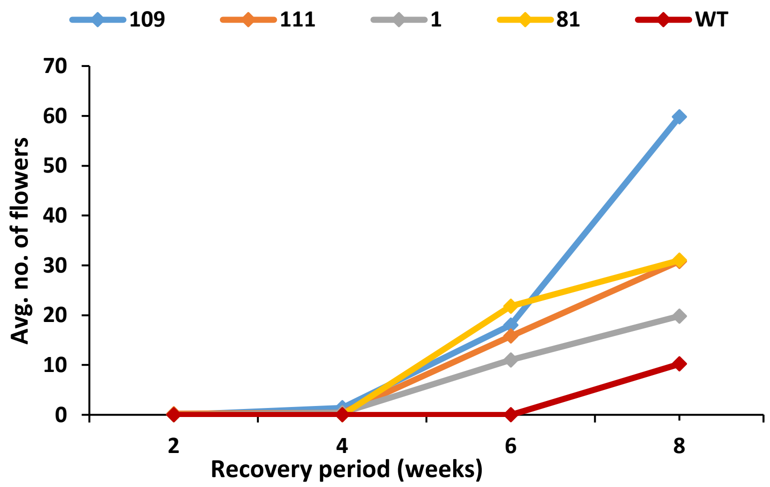
Recovery of the vegetative plants subjected to freezing-with-frosting treatment

Shoots



- ✓ More shoots in transgenic plants

Flowers



- ✓ More flowers in transgenic plants

Recovery of the vegetative plants after freezing-with-frosting treatment

Shoots

- ✓ More shoots in transgenic plants

Genotype (g)	*Avg. number of shoots at 4 weeks*	*Avg. number of shoots at 6 weeks*	*Avg. number of shoots at 8 weeks*
109	4.6^{a}	6.4^{a}	6.0^{a}
111	4.8^{a}	5.4^{a}	4.8^{a}
1	1.0^{b}	3.0^{a}	3.2^{a}
81	1.4^{b}	3.4^{a}	3.6^{a}
Transgenic (avg)	3.0	4.6	4.4
WT	1.4^{b}	2.6^{a}	2.8^{a}

Flowers

- ✓ More flowers and inflorescences in transgenic plants

Genotype (g)	*Avg. number of flowers at 6 weeks*	*Avg. number of flowers at 8 weeks*	*Avg. number of inflorescences at 8 weeks*
109	18^{ab}	59.8^{a}	8.0^{a}
111	15.8^{ab}	30.8^{b}	3.0^{b}
1	11.0^{b}	19.8^{bc}	2.2^{b}
81	21.8^{a}	31.0^{b}	2.2^{b}
Transgenic (avg)	16.7	35.4	3.9
WT	0.0^{c}	10.2^{c}	2.2^{b}

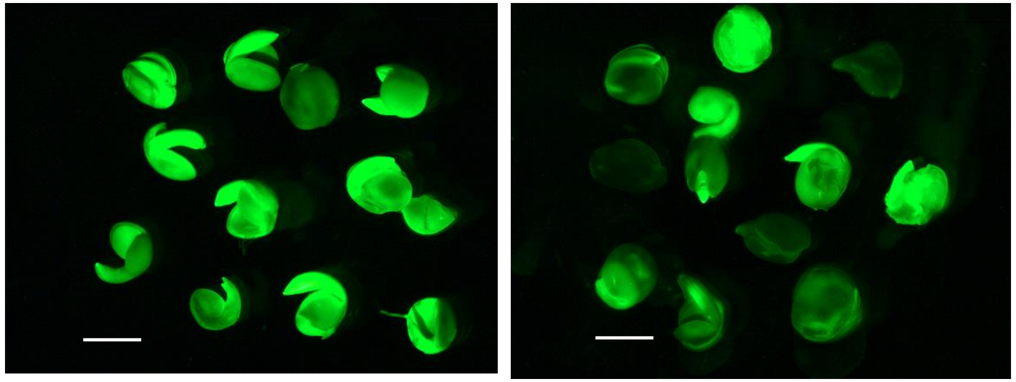
Analysis of seeds from plants subjected to freezing-with-frosting treatment

Plant line	*% Fully viable seeds as seen by FDA staining*
109	73.4^{a}
1	52.9^{ab}
81	40.3^{bc}
Transgenic (avg)	55.5
WT	19.5^{c}

Plant line	*Seed germination percentage*
109	80^{a}
1	76.5^{a}
81	18.85^{b}
Transgenic (avg)	58.4
WT	31.3^{b}

*Correlation coefficient between FDA method and seed germination (R)= 0.58**

% Fully viable seeds



90.9% 33.3%

✓ *AtACBP6* transgenic plants had more viable seeds and better germination % than WT

Conclusions

In freezing (+/- frost) treated plants the presence of ACBP6 showed:

1. Reduced electrolyte leakage
2. Improved recovery
3. Higher flower production
4. Higher harvest index
5. Higher seed viability

- Line 109 showed the strongest tolerance , 81 the weakest

- cold/freezing tolerance ability was not enhanced by cold-acclimation of the transgenic plants

Future directions

- Testing the *OsACBP5* and *AtACBP6* genes in commercial canola cultivars in more extensive trials
- Testing *OsACBP5* plants for high salinity stress

Molecular experiments:

- Assessing the expression of AtACBP6 during freezing treatment
- Assessing the expression of *OsACBP5* during pathogen infection
- Transcriptome analysis of AtACBP6 and *OsACBP5* transgenic plants

Thank you!

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