January 2011 CCOVI lecture series

"Empowering growers: using molecular tools to select for freeze-tolerant grapes"

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Outline of presentation

- Example trait: Berry color
  - genes and alleles
  - select visually or with molecular marker
- Freezing tolerance
  - complex trait
  - genes involved in freezing tolerance
  - selection with molecular markers
- Conclusions and future work
- Acknowledgements

Berry color has a genetic basis

Cabernet franc

X

Sauvignon blanc

Cabernet sauvignon

Based on Bowers and Meredith 1997

Berry color

- Berry color is determined by several genes

A → B → C → D → berry color: red

- Different variants of genes = alleles
- Grape plant has 2 alleles for each gene (diploid)

A → B → C → X → D → X → berry color: white

Berry color
VvMybA1 gene: red or white allele

Cabernet franc

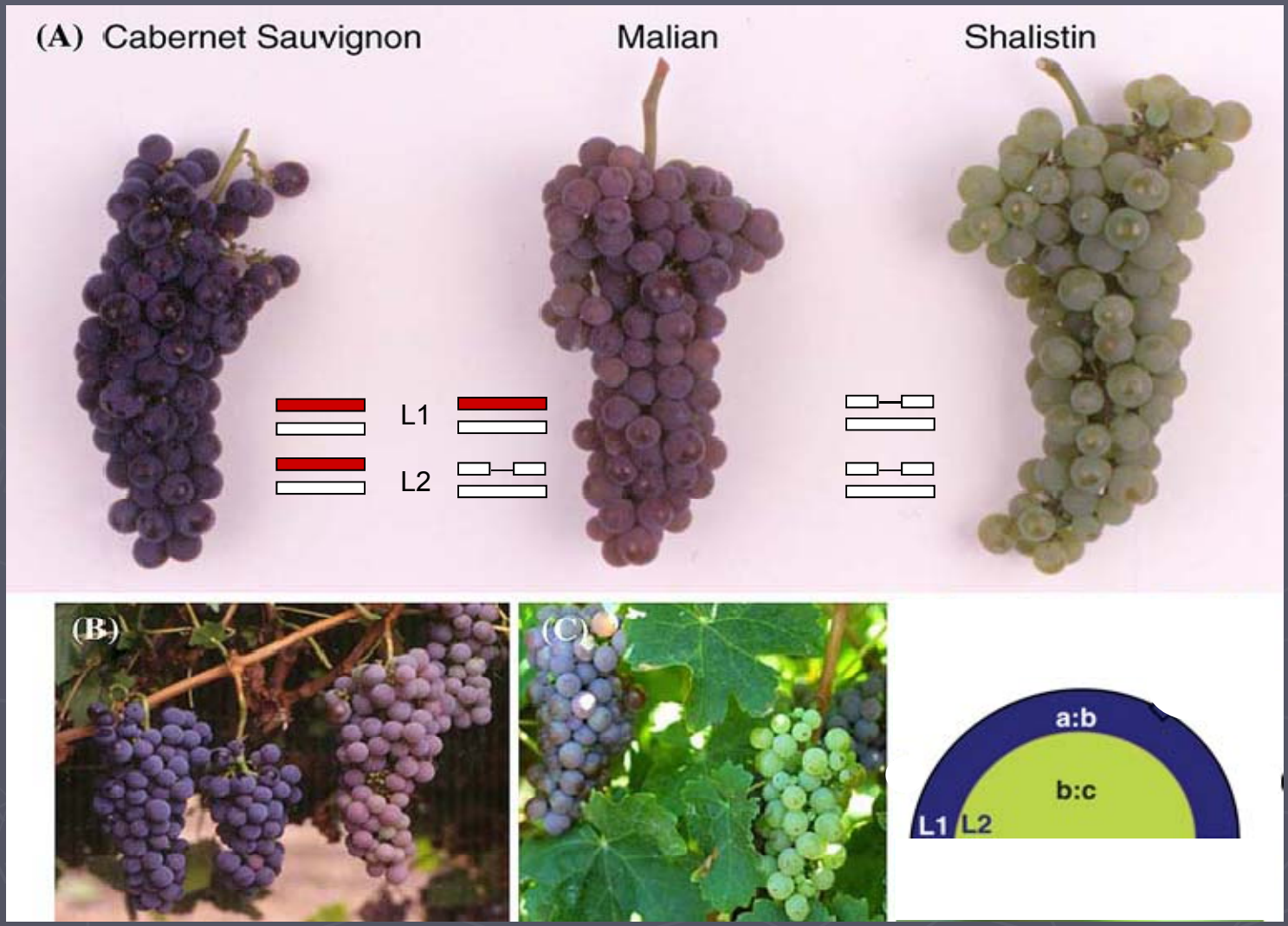
Sauvignon blanc

X

Cabernet sauvignon

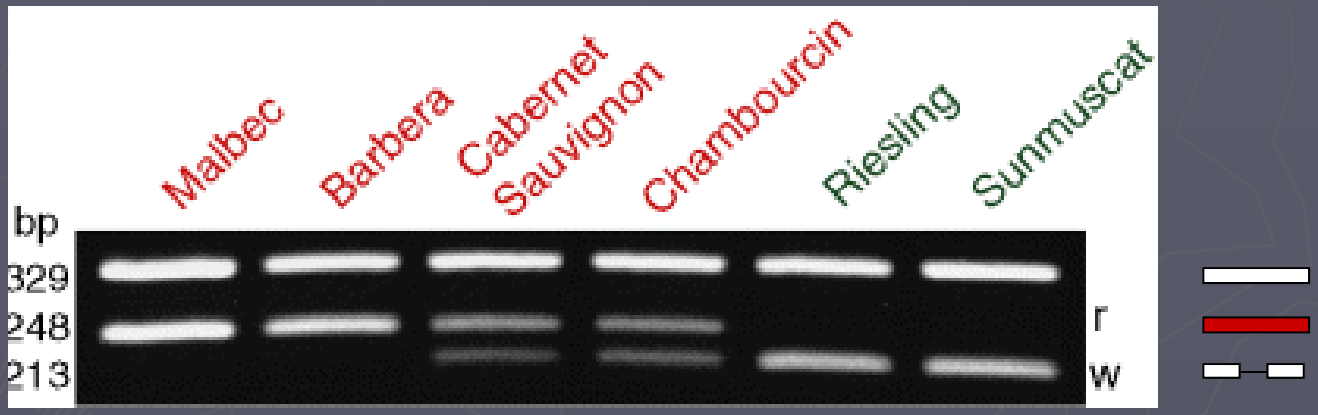
Walker et al. as quoted in Pelsy 2010

Berry color – sports show gene mutations



VvMybA1- Kobayashi et al. 2004; Walker et al. 2006

Molecular technique (PCR) can determine which allele(s) are present

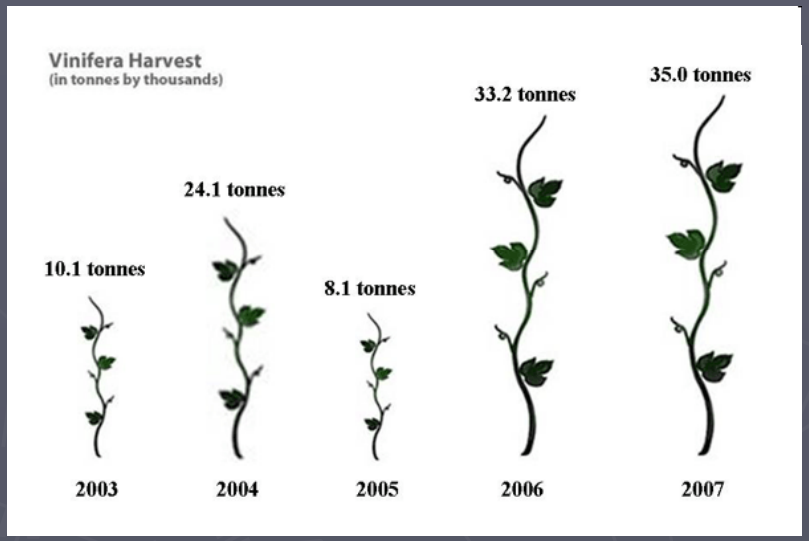


- All cultivars with red berries have the red allele
- All cultivars with white berries lack the red allele

CAN SELECT FOR RED BERRY COLOR BY LOOKING OR BY SELECTING RED ALLELE

Fig. 4 Walker et al. 2007

Freezing temperatures in Ontario cause yield loss of wine grapes



- Incorporate genetic basis for superior freezing tolerance = allele from wild grape *Vitis riparia*
- Cross *V. riparia* with cultivated grape *V. vinifera*
- Backcross with wine cultivars to restore wine quality

Grape growers of Ontario website

Selecting for superior freezing tolerance

- Analysis of freezing tolerance is not easy

  Need cold period to trigger freezing tolerance

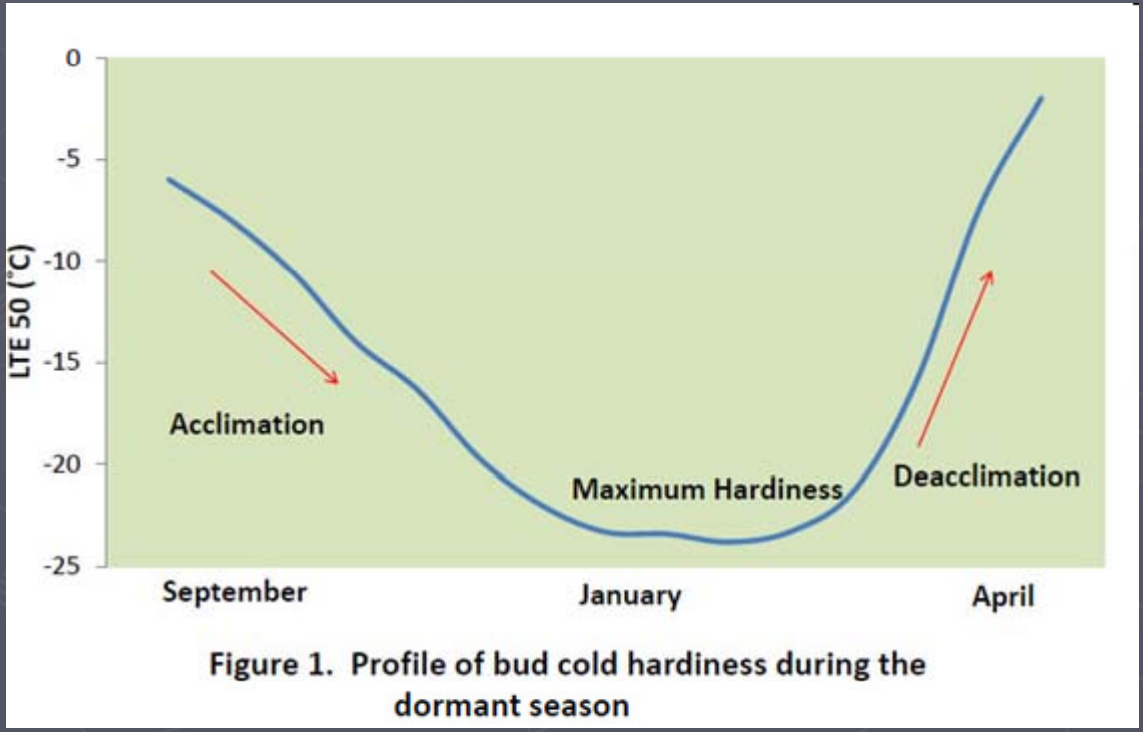
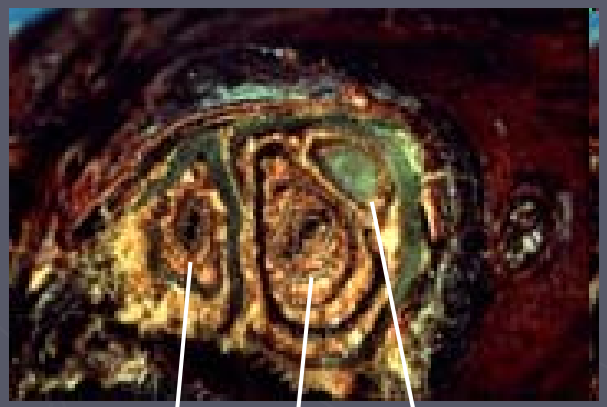


Figure 1. Profile of bud cold hardiness during the dormant season

CCOVI Vine Alert website

Analyzing freezing tolerance (FT)



Compound bud

Dr. Thomas Zabadal, Michigan State University

BUD LT50 ^{0}C	NOV	DEC	JAN
Riesling	-21.2	-23.5	-25.6
Chardonnay	-21.5	-22.7	-23.2
Gewurtztraminer	-20.8	-21	-23.1
Pinot Blanc	-19.3	-20.5	-24.8

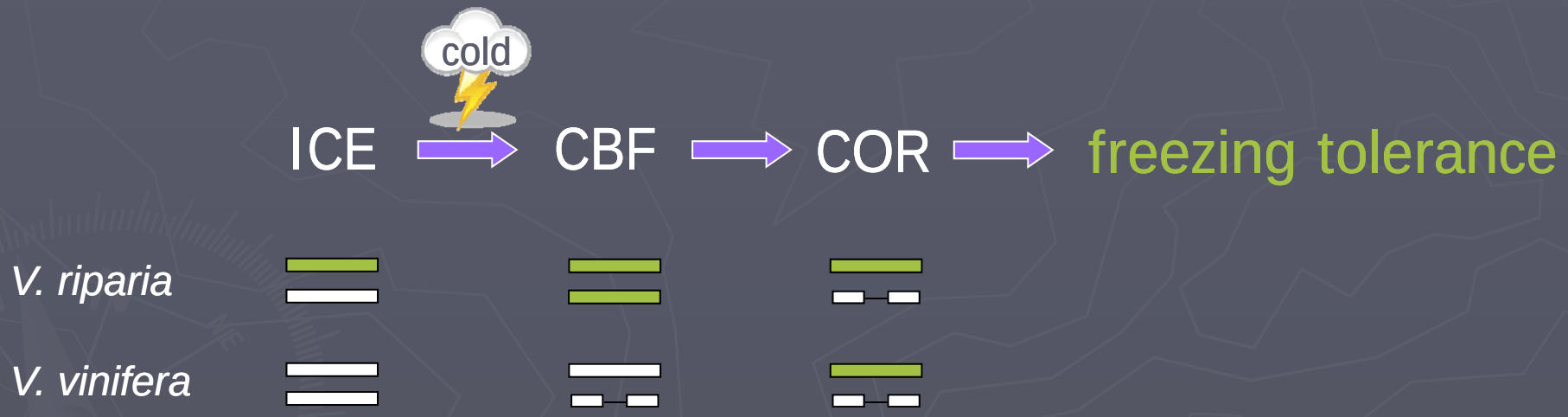
Quamme et al. 1973; Gusta et al. 2009

Collaborative project by AAFC, the Grape Growers of Ontario and CCOVI

- Maximum freezing tolerance varies depending on genetic background and growing conditions
- One-time-a-year field test under nature's control

Selecting for superior freezing tolerance

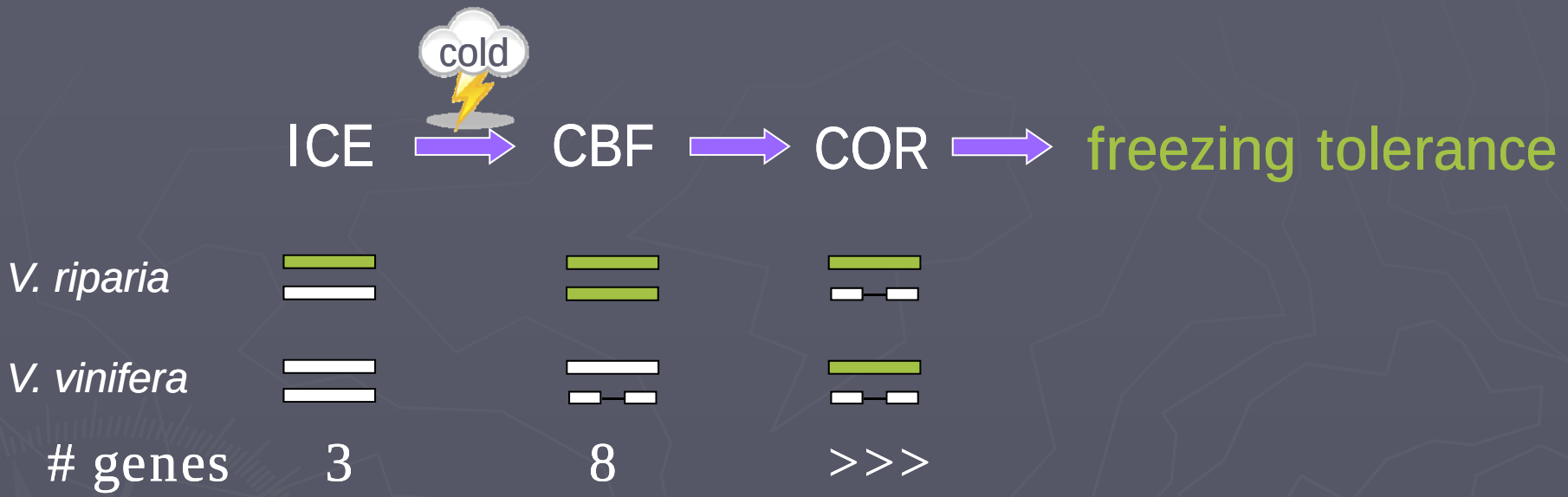
- Field selection for freezing tolerance is not easy,
  Try molecular selection based on alleles?



- Sequence genes from *V. riparia* and *V. vinifera*
- Design molecular markers for alleles

Model for *Arabidopsis* Chinnusamy et al. 2007

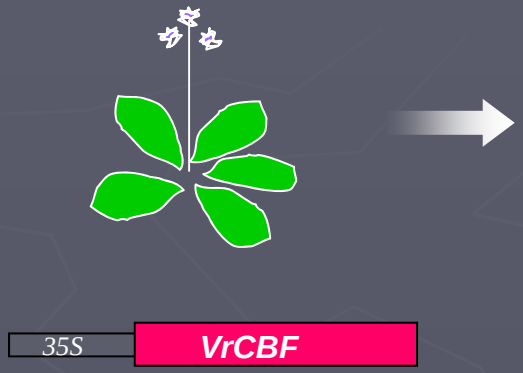
Selecting for superior freezing tolerance

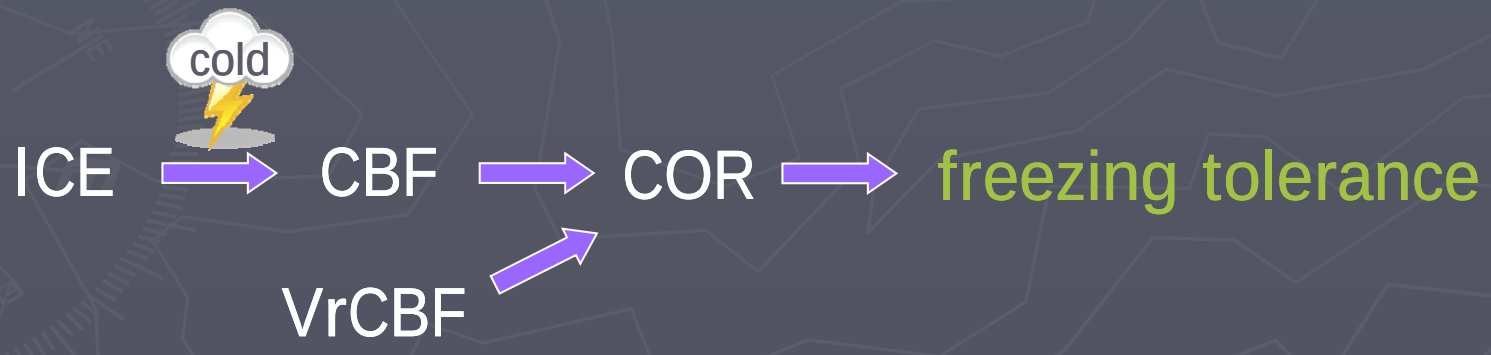


- Probably need a combination of superior genes (alleles) to obtain superior freezing tolerance
- WHICH ALLELES FROM WHICH GENES?

  Test *V. riparia CBF1* and *CBF4 (VrCBF1/VrCBF4)*

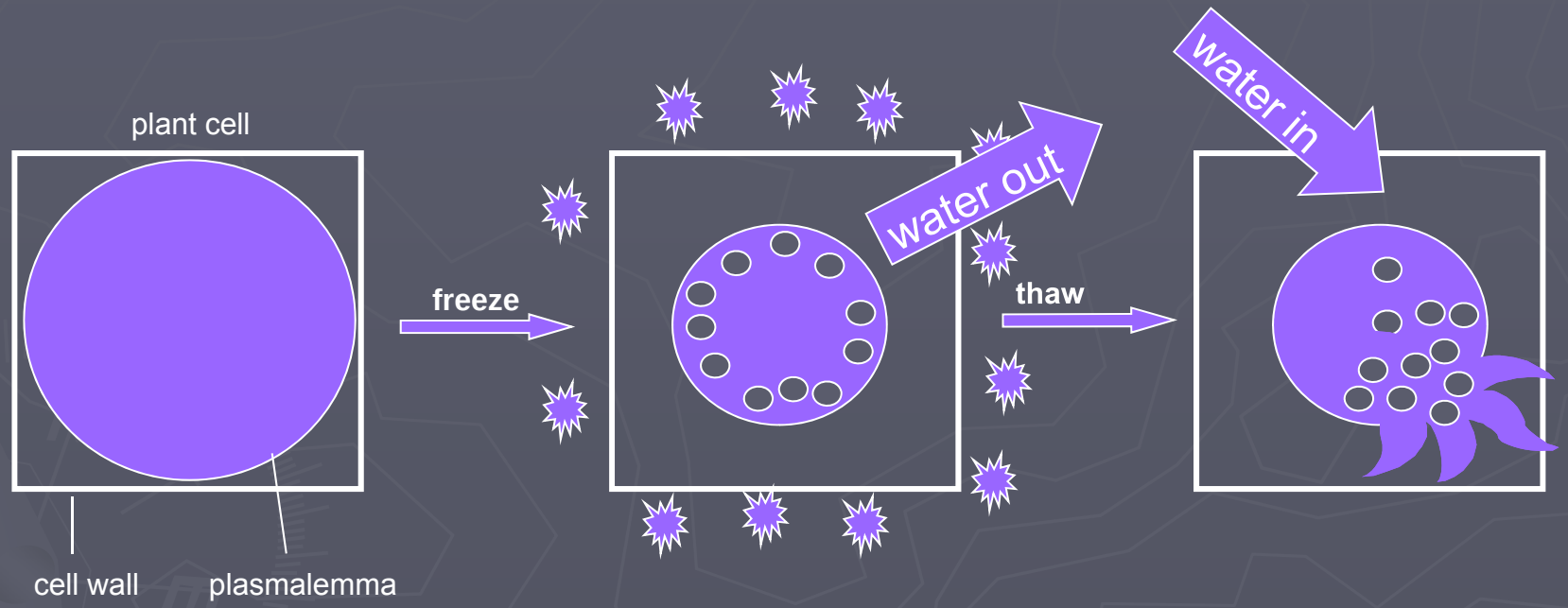
Test *VrCBF1* and *VrCBF4* in Arabidopsis





Analyzing freezing tolerance

- Expose plants to freezing temperatures, then thaw

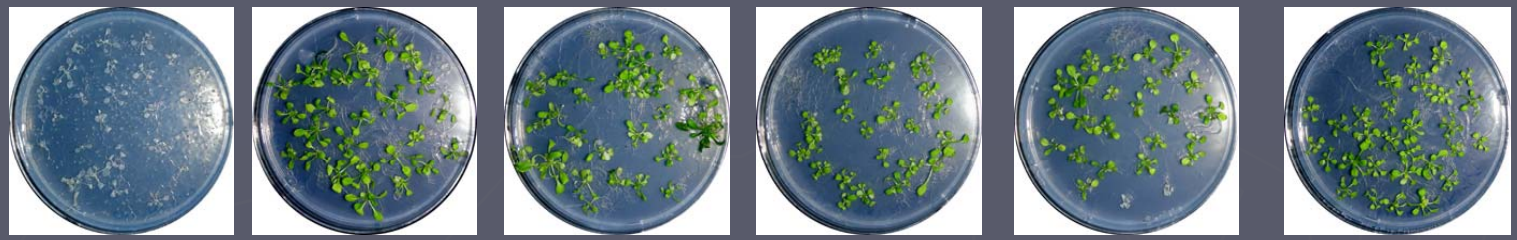


- Quantify ions = electrolytes with conductivity meter

  FT plants leak only at much lower temperatures

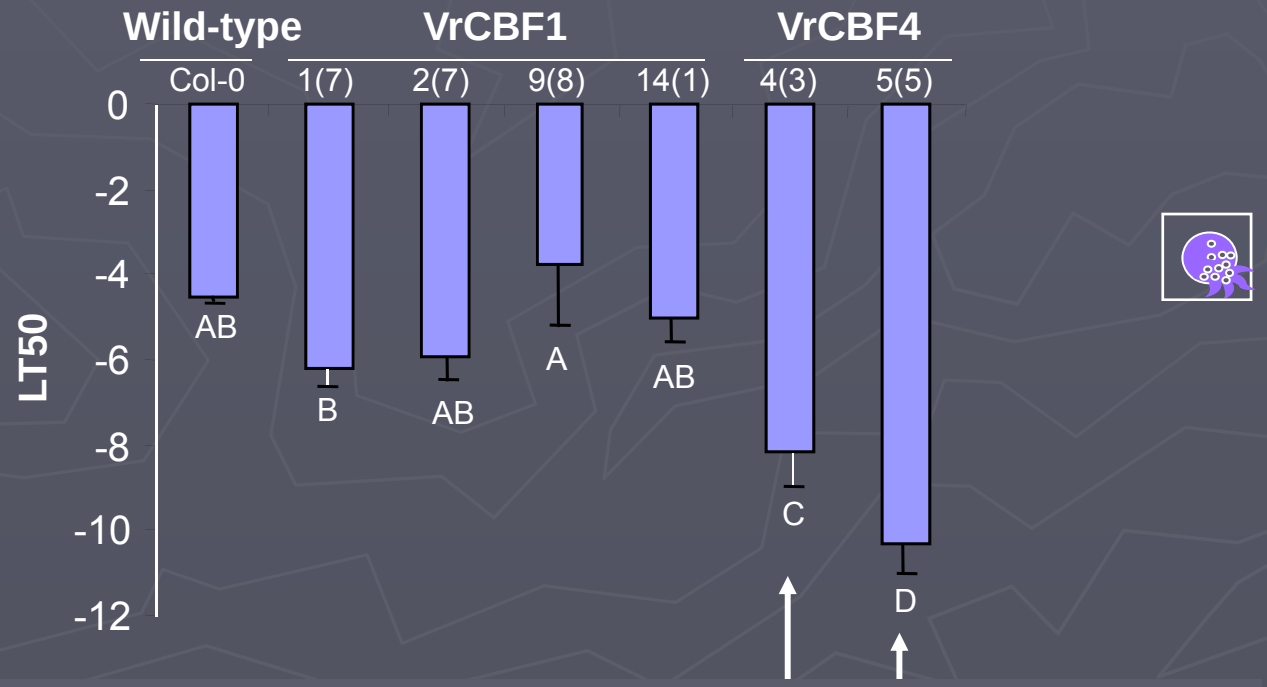
Based on Gilmour et al. 1988

Vitis CBF1 and 4 increase freezing tolerance



Col-0 | 1(7) | 2(7) | 9(8) | 14(1) | 4(3)

Wild type | VrCBF1 | VrCBF4



Highest freezing tolerance with VrCBF4

Vitis CBFs increase drought tolerance

Col-0 | Wild type

1(7)

2(7)

VrCBF1

9(8)

14(1)

4(3)

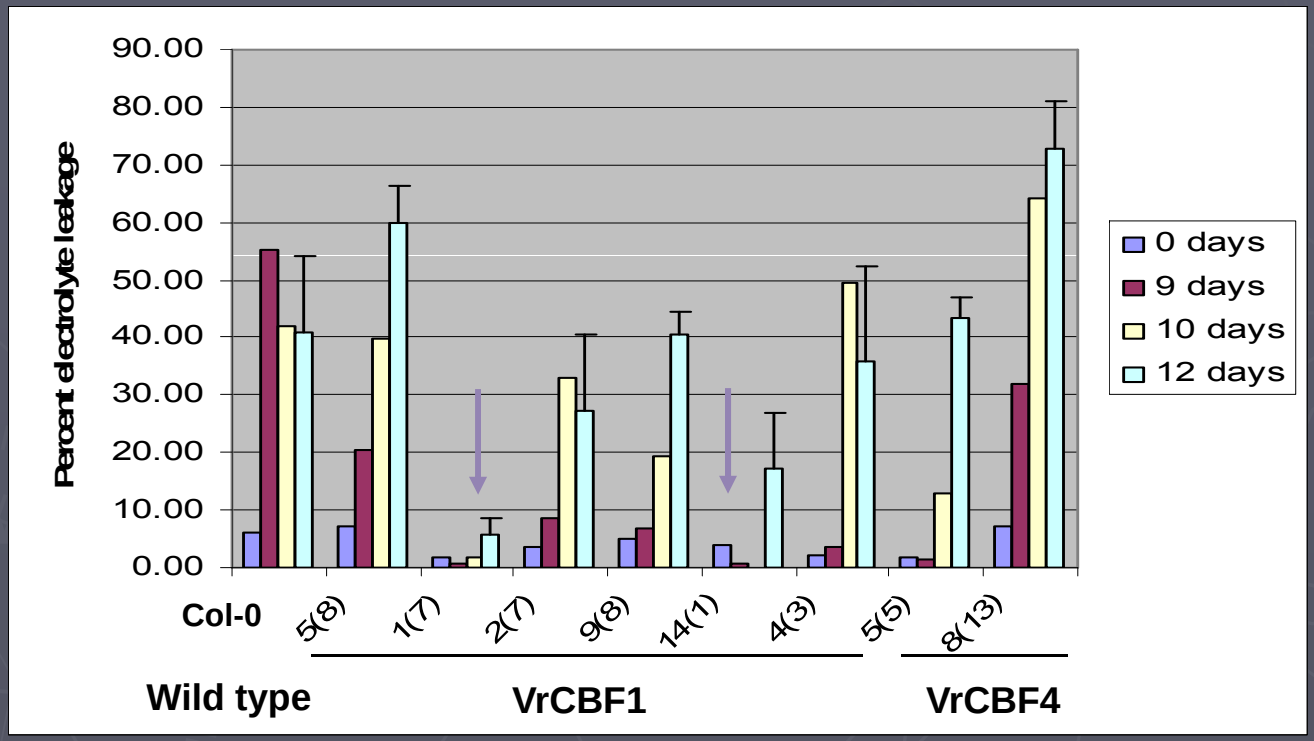
VrCBF4

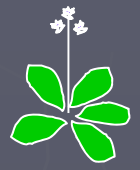
5(5)

Both VrCBF1 and VrCBF4 increase drought tolerance

Siddiqua and Nassuth submitted

Vitis CBFs increase drought tolerance

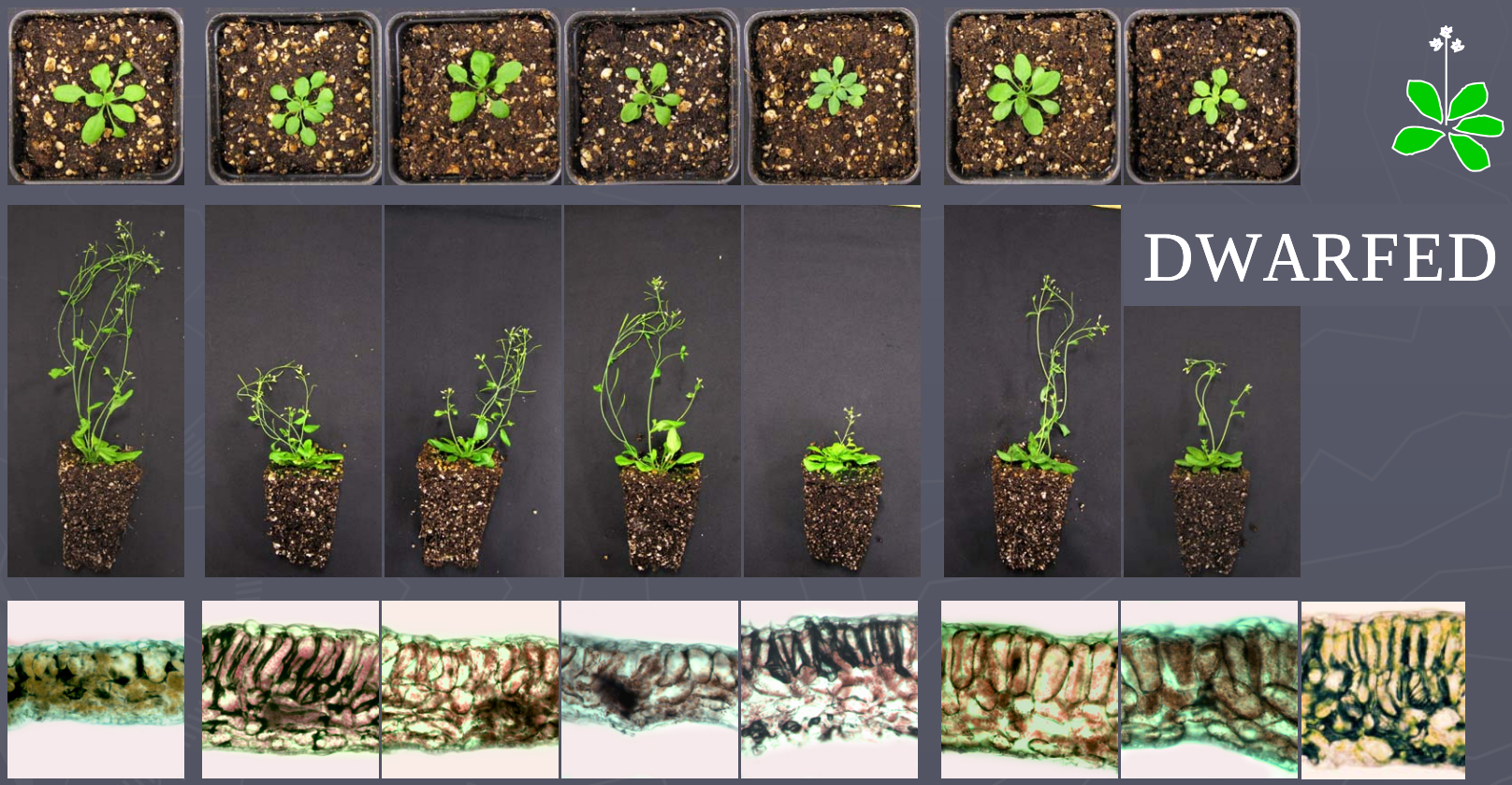




Highest drought tolerance with VrCBF1

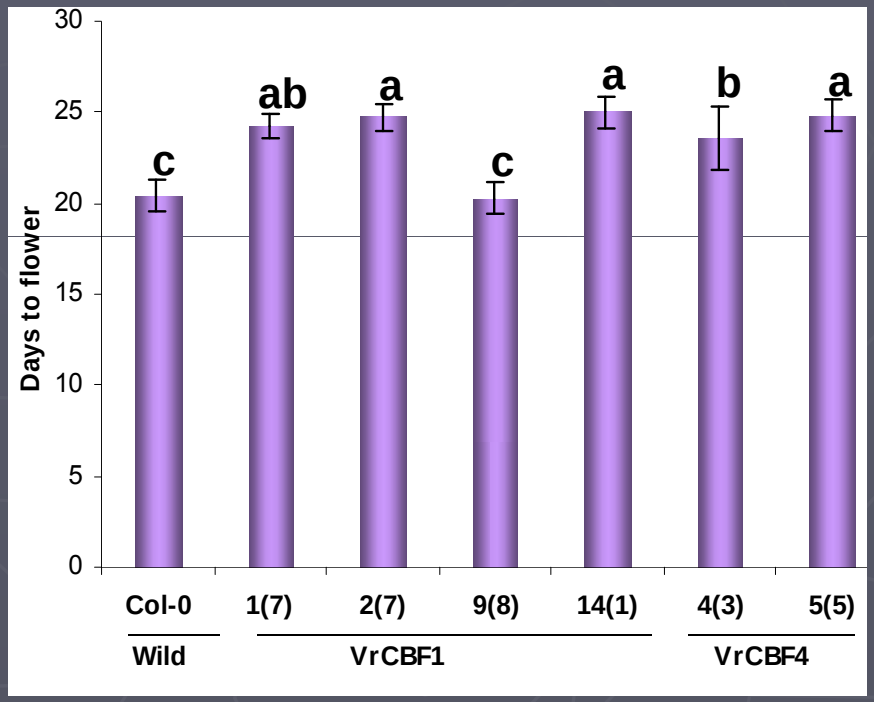
Siddiqua and Nassuth submitted

However: *Vitis* CBFs also affect plant growth and development



Siddiqua and Nassuth submitted

Vitis CBFs affect flowering

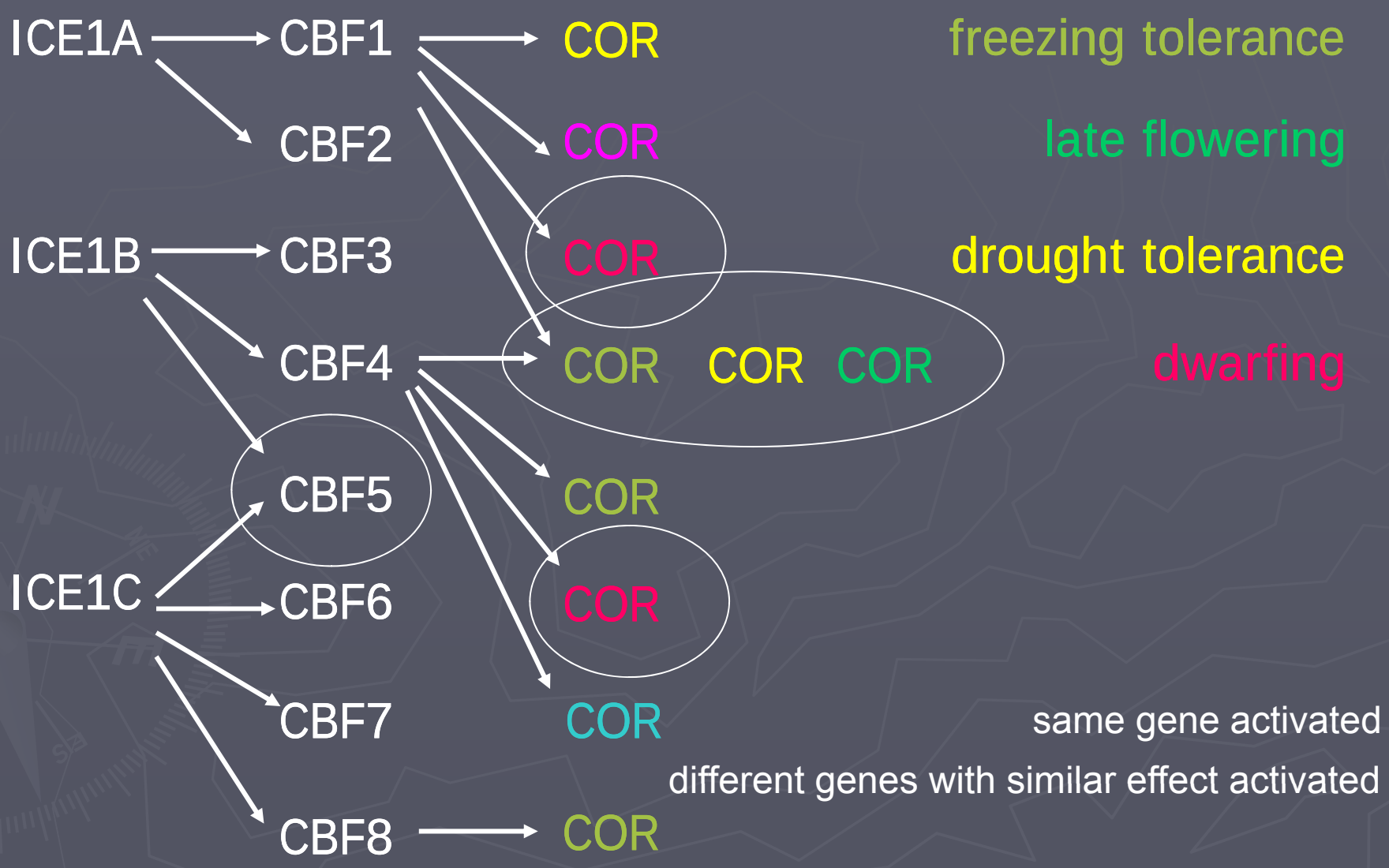


days to flower

VrCBF1 and VrCBF4 DELAY FLOWERING

Siddiqua and Nassuth submitted

Hypothetical model

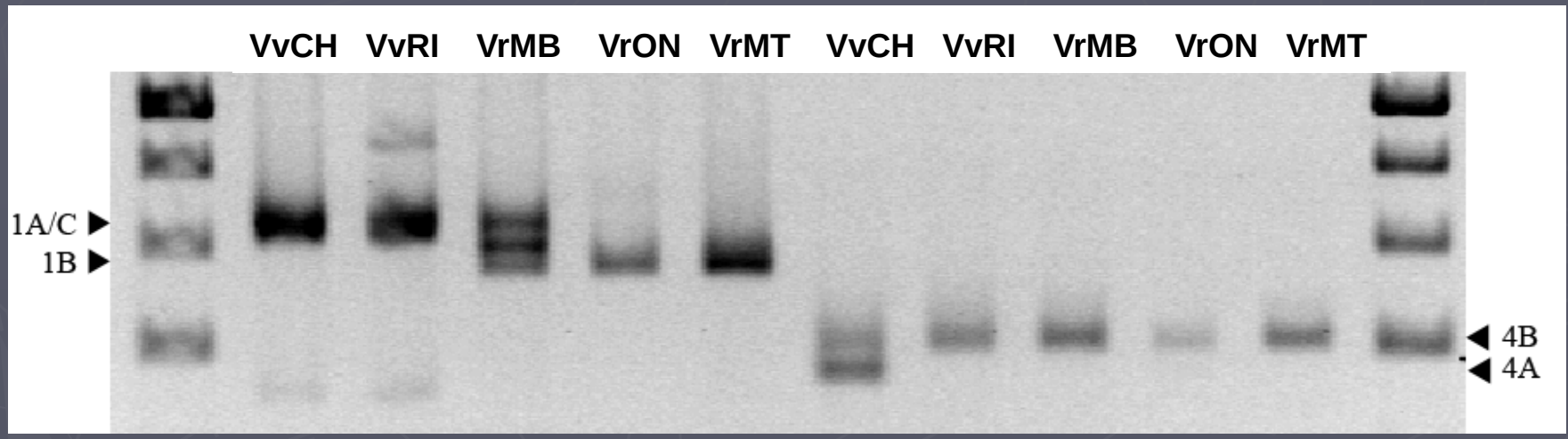


Selecting for superior freezing tolerance

- Field selection for freezing tolerance is not easy
- Probably need a combination of superior genes (alleles) to obtain superior freezing tolerance
- “Freezing tolerance” genes (alleles) are also involved in other traits (timing of expression important)

DETERMINE CORRELATION BETWEEN ALLELE(S) AND FREEZING TOLERANCE

Analysis with molecular markers



Nassuth et al. 2011

Analysis with molecular markers

- Alleles of

CBF1B and CBF4B are candidate freezing tolerance markers

***Vitis* Accessions**	*CBF1* allele		*CBF4* allele	
	A/C	B	A	B
	333/345 bp	303 bp	192 bp	216 bp
Chardonnay	+	–	+	+
Riesling	+	–	–	+
MB	+	+	–	+
QC	–	+	–	+
ON	–	+	–	+
MT	–	+	–	+
ND	–	+	–	+
MN	–	+	–	+
IA	–	+	–	+
NH	–	+	–	+
VT	–	+	–	+
NY	–	+	–	+
WI	–	+	–	+
CT	–	+	–	+
NJ	–	+	–	+
CO	–	+	–	+
MO	–	+	–	+
KS	–	+	–	+
FR	+	+	–	+
Tocai	+	–	–	–
Freisia	+	–	+	+
Felicia	+	+	+	+

Nassuth et al. 2011

CONCLUSIONS & FUTURE WORK
(Basic research)

- *Vitis* species have several CBF pathway genes
  - 3 *ICE* - 8 *CBF* - many *COR* genes
- *VrCBF1* and *VrCBF4* impart stress tolerance
  - VrCBF4 best for freezing tolerance
  - VrCBF1 best for drought tolerance
  - Both affect growth and flowering time
  - Different (Vv) alleles might be less optimal
- Analyze further *Vitis ICE* and *CBF* genes
  - Determine which genes are desirable
  - Transient expression/transformation system

CONCLUSIONS & FUTURE WORK
(Applied research, partly in ORF application)

- Develop molecular markers
  - Currently for alleles of 7 putative FT genes
- Test markers on additional *V. vinifera* cultivars
  - Merlot, Sauvignon blanc, Cabernet franc, Pinot noir
- Test progeny of *V. vinifera* Riesling x *V. riparia*
  - Correlate markers with bud hardiness in field*
  - Develop laboratory freezing test
- Backcross superior progeny with vinifera cultivar*
  - Select backcross progeny with FT markers

*In collaboration with Fisher and Somers

Thank you

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