“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

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 ➔ D ➔ berry color: white
Berry color
VvMybA1 gene: red or white allele

Walker et al. as quoted in Pelsy 2010
Berry color – sports show gene mutations

(A) Cabernet Sauvignon  Malian  Shalistein

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
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)

Maximum freezing tolerance varies depending on genetic background and growing conditions

One-time-a-year field test under nature’s control

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Quamme et al. 1973; Gusta et al. 2009

Collaborative project by AAFC, the Grape Growers of Ontario and CCOVI
Selecting for superior freezing tolerance

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

Sequence genes from \textit{V. riparia} and \textit{V. vinifera}

Design molecular markers for alleles

Model for \textit{Arabidopsis}  Chinnusamy et al. 2007
Selecting for superior freezing tolerance

Ice → CBF → COR → freezing tolerance

V. riparia

V. vinifera

# genes 3 8 >>>

► 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**

Highest freezing tolerance with VrCBF4
*Vitis* CBFs increase drought tolerance

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: \textit{Vitis} CBFs also affect plant growth and development

\begin{center}
\begin{tabular}{c c c c c c c c}
\textbf{Col-0} & 1(7) & 2(7) & 9(8) & 14(1) & 4(3) & 5(5) & \textbf{Col-0 (in cold)} \\
Wild type & VrCBF1 & VrCBF4 & \\
Siddiqua and Nassuth submitted
\end{tabular}
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Vitis CBFs affect flowering

VrCBF1 and VrCBF4 DELAY FLOWERING

Siddiqua and Nassuth submitted
Hypothetical model

ICE1A → CBF1 → COR
CBF2 → COR
CBF3 → COR
CBF4 → COR
CBF5 → COR
ICE1B → CBF3
CBF4 → COR
CBF5 → COR
CBF6 → COR
CBF7 → COR
CBF8 → COR

Freezing tolerance
Late flowering
Drought tolerance
Dwarfing

Same gene activated
Different genes with similar effect activated
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

VvCH  VvRI  VrMB  VrON  VrMT  VvCH  VvRI  VrMB  VrON  VrMT

1A/C  1B

VrCBF1 alleles

VrCBF4 alleles

Nassuth et al. 2011
Analysis with molecular markers

- Alleles of CBF1B and CBF4B are candidate freezing tolerance markers

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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