An efficient molecular design breeding strategy for grape coloring trait based on MYB haplotypes

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Grapevine is one of the most important fruit trees in China.

- Wide distribution
- High yields
- High economic benefits
- Mainly consumed as fresh fruits
Anthocyanin composition is responsible for the color diversity of grape berries.
The MYB haplotype is the genetic determinant of grape color

MybA1 and MybA2, inherited together, can be regarded as the color locus.

Haplotype composition at the color locus is a major genetic determinant of skin color

A model of the evolutionary differentiation of MYB haplotypes at the color locus in *Vitis* species

(Azuma et al. 2017)
Our goals and questions raised

Assisting in breeding of high quality cultivars with favorable coloration

Can we achieve early prediction of color diversification according to the *MYB* haplotype composition?
The 213 grape varieties were classified into 7 categories according to the depth of the coloration.
Identification of *MYB* haplotype composition of the 213 grape varieties by PCR and sequencing

**VvMybA1**

- **VvMybA1a**
  - 1559 bp
  - DNA quality control by the amplification of *actin*

- **VvMybA1b**
  - 1675 bp
  - YES
  - 846 bp
  - 1035 bp + sequencing
  - 999 bp + sequencing

- **VvMybA1c**
  - YES
  - 2105 bp

- **VvMybA1**
  - YES
  - 312 bp

- **VvlMybA1**
  - 1675 bp
  - YES
  - 846 bp
  - 1035 bp + sequencing
  - 999 bp + sequencing

**MybA1 allele identification**
Identification of **MYB** haplotype composition of the 213 grape varieties by PCR and sequencing

DNA quality control by the amplification of *actin*

**MybA2** allele identification
MYB haplotype composition of 211 grape varieties can be identified by PCR and sequencing except ‘Yuanruhei’ (*V. vinifera*) and ‘Olarra Queen’ (*V. labrusca*).
MYB haplotype composition of ‘Yuanruihei’ and ‘Olarra Queen’

HapA
HapC-N

‘phased phase’

HapC-Rs
HapG

‘repulsive phase’

MYB haplotype composition of ‘Yuanruihei’ and ‘Olarra Queen’ could be

✓ either A/C-N or G/C-Rs
✓ identified by self-crossing
Haplotype composition of ‘Yuanruihei’ was A/C-N.
Identification of *MYB* haplotype composition of ‘Olarra Queen’

<table>
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<th>Code</th>
<th>VvMybA1a</th>
<th>VvMybA1b</th>
<th>VvMybA1c</th>
<th>VvMybA1SUB</th>
<th>VvMybA1BEN</th>
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Haplotype composition of ‘Yuanruhei’ was A/C-N.
Is coloration and haplotype composition closely correlated?

- A total of 8 haplotypes and 19 haplotype compositions were identified;
- HapC-N and HapE2 showed stronger effect than HapC-Rs, HapB and HapE1;
- And thus VlmybA2 might have stronger effect than Vlmyb1-2 regarding coloration;
- The more functional alleles, the darker the skin tended to be.
Can we achieve early color prediction during cross breeding?

a) Haplotype identification and berry coloration

‘Muscat Hamberg’ ♀ AC-Rs × ‘Crimson seedless’ ♂ AC-Rs

F1 A/A : A/C-Rs : C-Rs/C-Rs

Expected 1 2 1
Observed a 18 32 19

Berry color

\(^a\)Chi square value=0.391, \(P\) value=0.82

b) The berry color observation and quantification

Anthocyanin concentration (mg/kg FW)

\(A/A\) < \(A/C-Rs\) < \(C-Rs/C-Rs\)

Haplotype composition
Can we achieve early color prediction during cross breeding?

a) Haplotype identification and berry coloration

‘Cuibao seedless’ ♀
A/A

‘Qiuhongbao’ ♂
A/C-Rs

F1:
A/A

Expected
1

Observed
25

Chi square value=2.29,
\(P\) value=0.13

b) The berry color observation and quantification

Berry color

\(^a\)Chi square value=2.29,
\(P\) value=0.13
Summary

Early prediction of color diversification can be achieved by the identification of the *MYB* haplotype composition.

- The berry coloration matched with the haplotype composition in 99.1% of the investigated varieties;
- AC-N and AE2 tended to show deeper colors;
- The more functional alleles it contained, the darker the skin color tended to be.

An efficient molecular design breeding strategy for grape coloring trait based on *MYB* haplotypes.

213 grape varieties

*MYB* haplotype composition

Closely correlated

Color index

Verifications:

- ‘Muscat Hamberg’ × ‘Crimson seedless’
- Cuibao seedless’ × ‘Qiuhongbao’

Confirmed
Multiple target breeding traits

Selection of multiple candidate parents with favorable target traits from the germplasm

MYB haplotype database cross-check

Preference parents with MYB haplotype generating target color trait

More hybrids with desirable target traits including berry color
Acknowledgements

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Thank you for your attention!