南京農業大學
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COLLEGE OF HORTICULTURE

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

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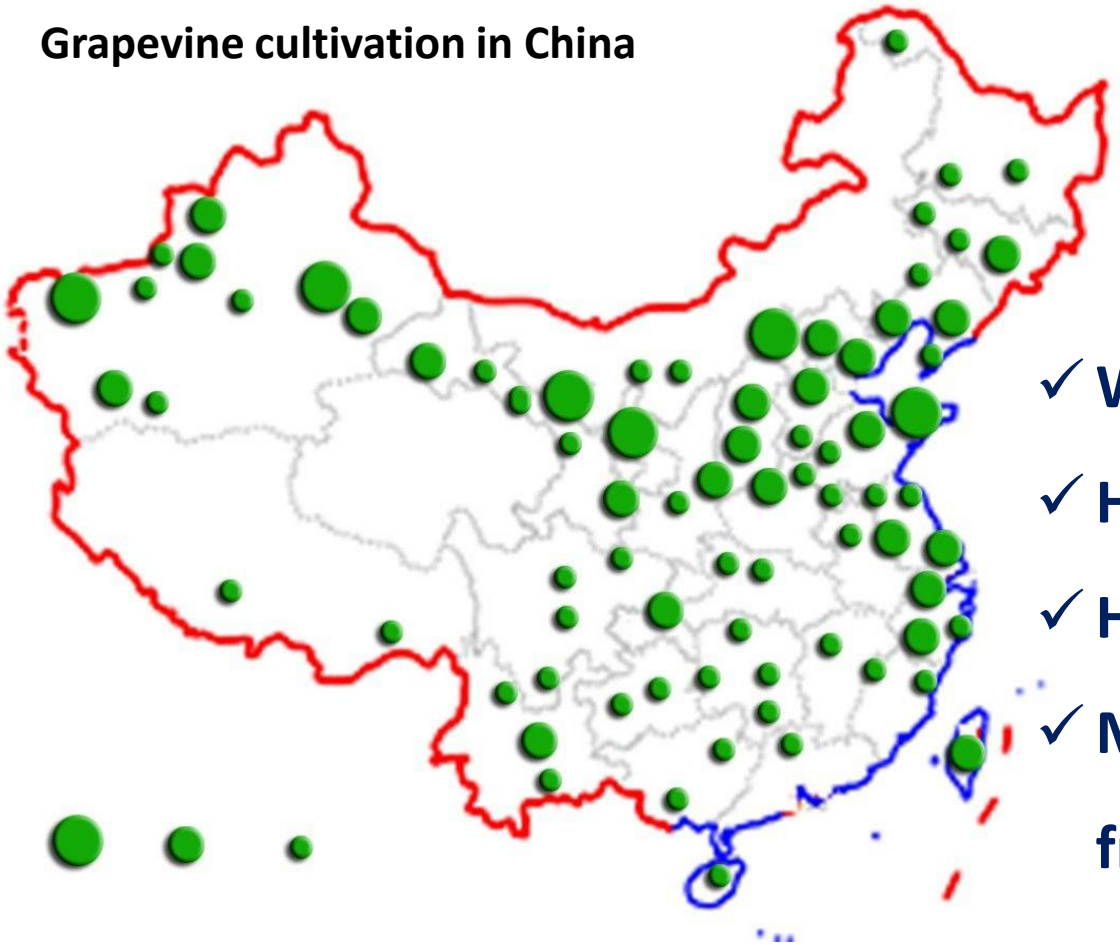
Grapevine is one of the most important fruit trees in China

Grapevine cultivation in China



- ✓ Wide distribution
- ✓ High yields
- ✓ High economic benefits
- ✓ Mainly consumed as fresh fruits

Anthocyanin composition and color diversity

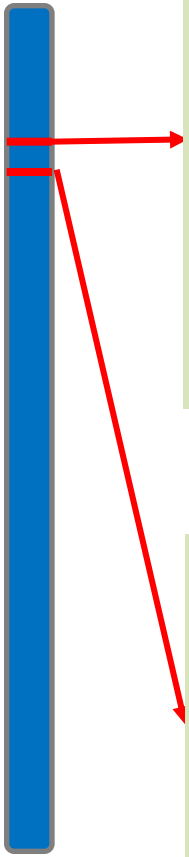
Anthocyanin composition is responsible for the color diversity of grape berries.

The *MYB* haplotype is the genetic determinant of grape color

Color locus

MybA1
MybA2

Chr2



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

Alleles

VvmybA1a

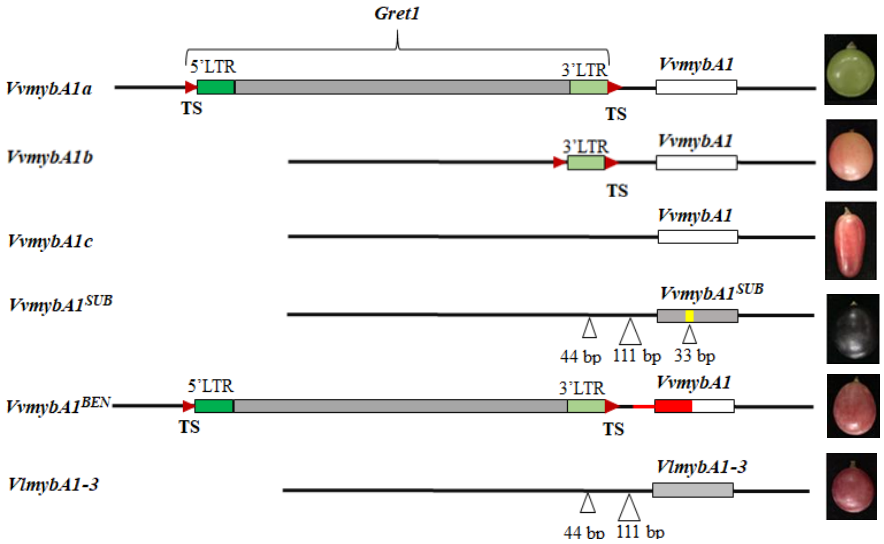
VvmybA1b

VvmybA1c

VvmybA1^{SUB}

VvmybA1^{BEN}

*VlmybA1-3**



Gret1: grapevine retrotransposon1
LTR: long terminal repeat
TS: target site for the *Gret1* insertion

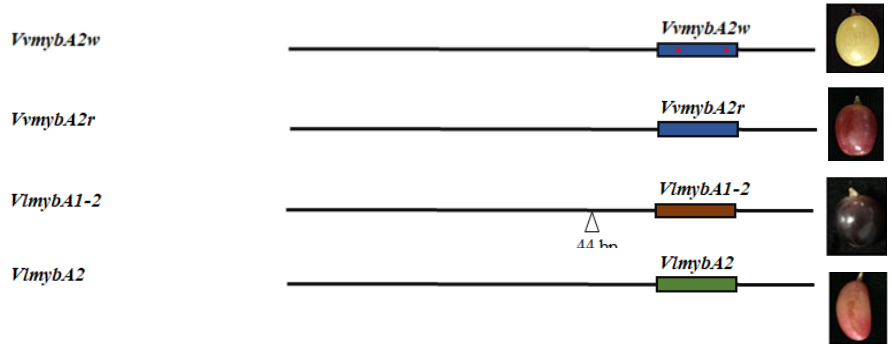
Alleles

VvmybA2w

VvmybA2r

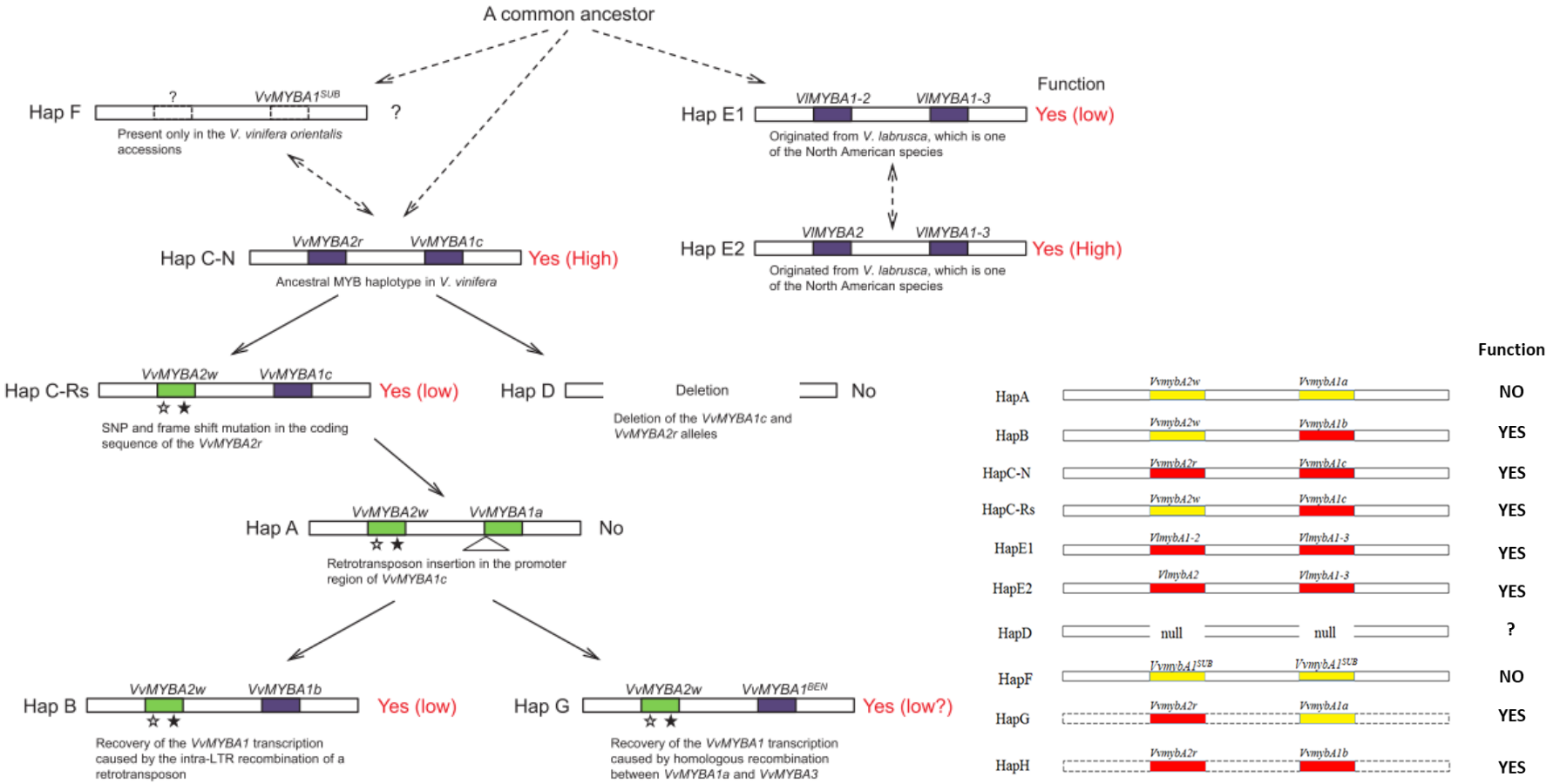
*VlmybA1-2**

*VlmybA2**



(Kobayashi et al. 2004, Walker et al. 2006, Azuma et al. 2009, 2011)

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

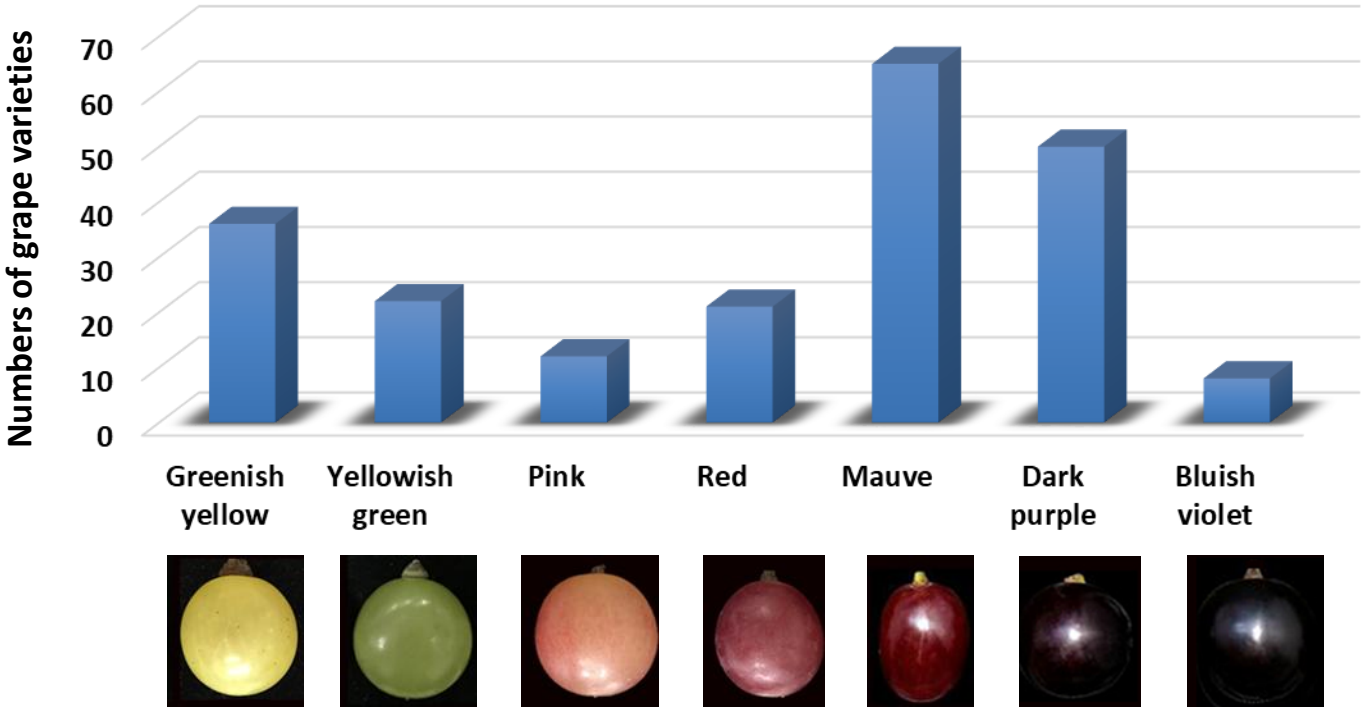
(Azuma et al. 2017)

Our goals and questions raised

Assisting in breeding of high quality cultivars with favorable coloration

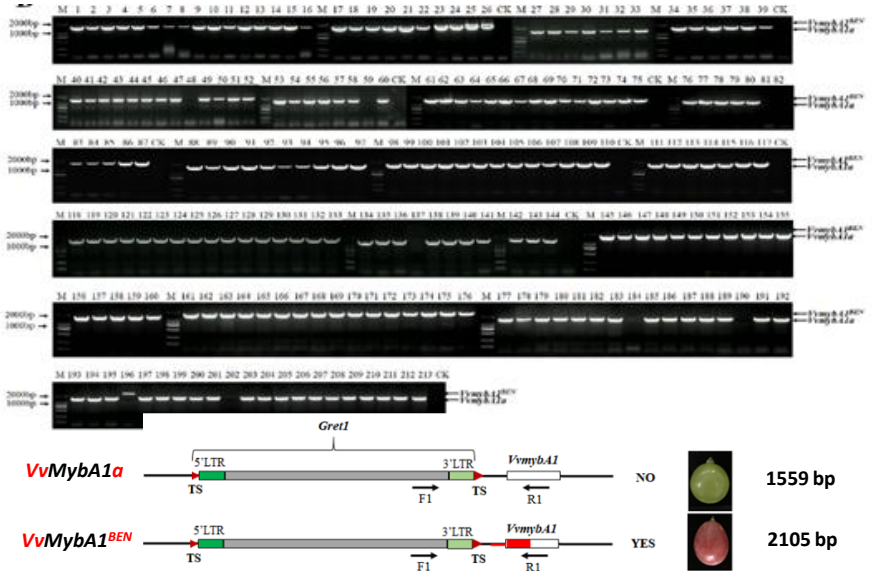
Can we achieve early prediction of color diversification according to the *MYB* haplotype composition?

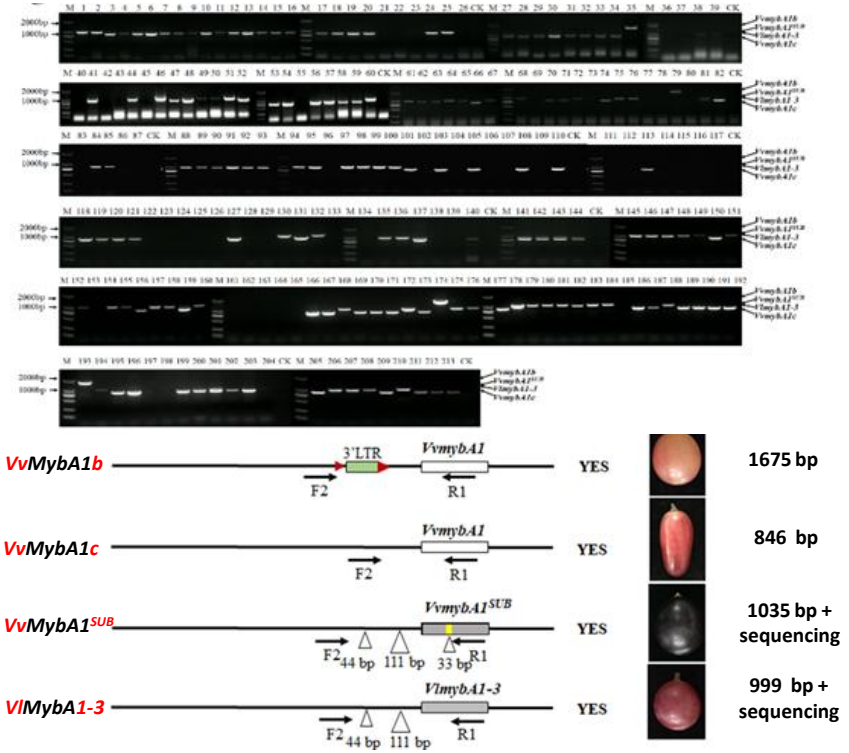
Coloration classification of the 213 investigated grape varieties

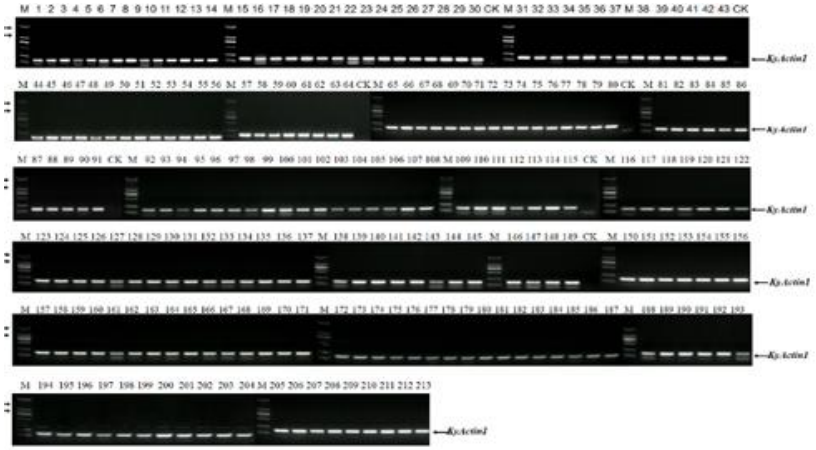


The 213 grape varieties were classified into 7 categories according to the depth of the coloration.

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



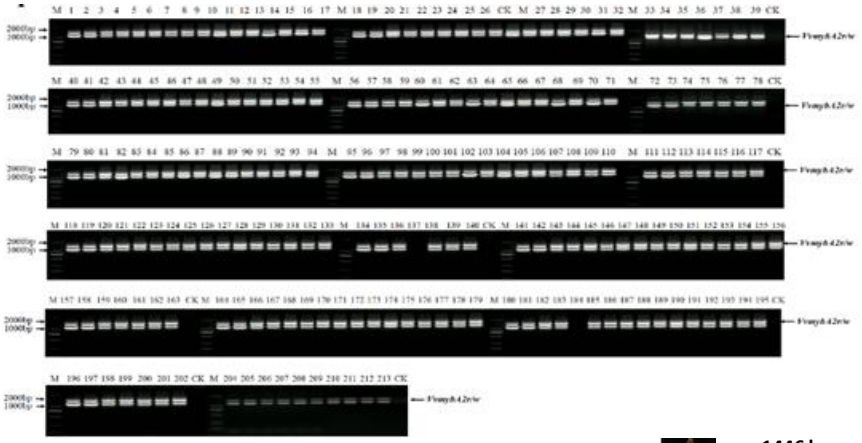


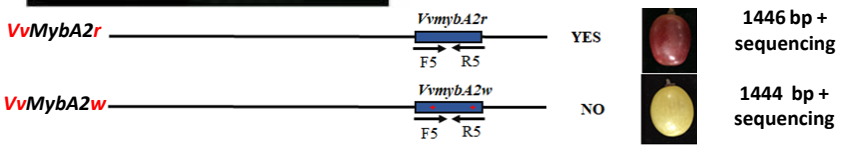


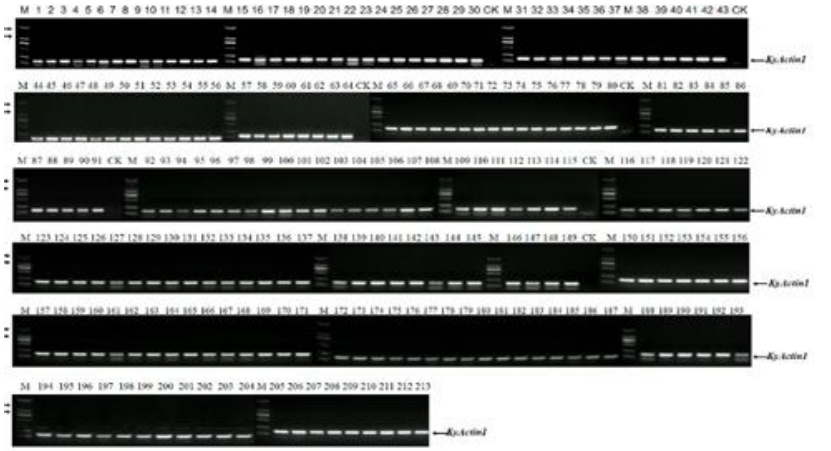
DNA quality control by the amplification of *actin*

***MybA1* allele identification**

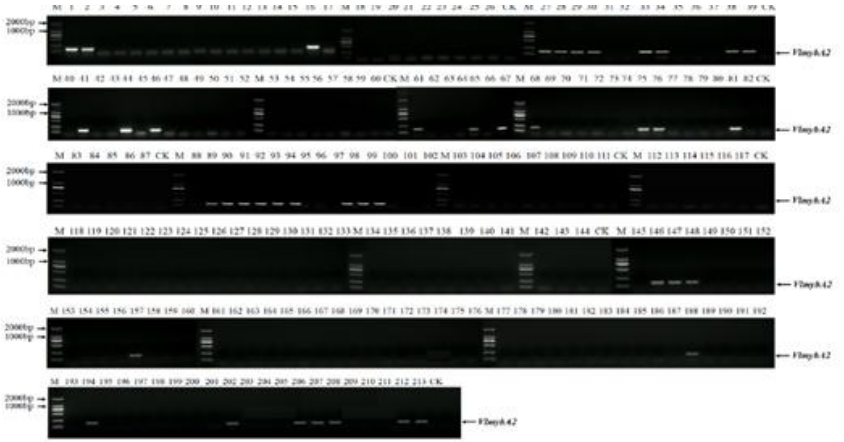
Identification of *MYB* haplotype composition of the 213 grape varieties by PCR and sequencing

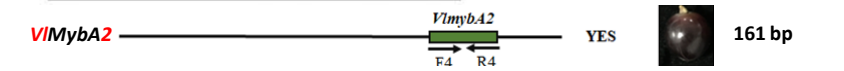


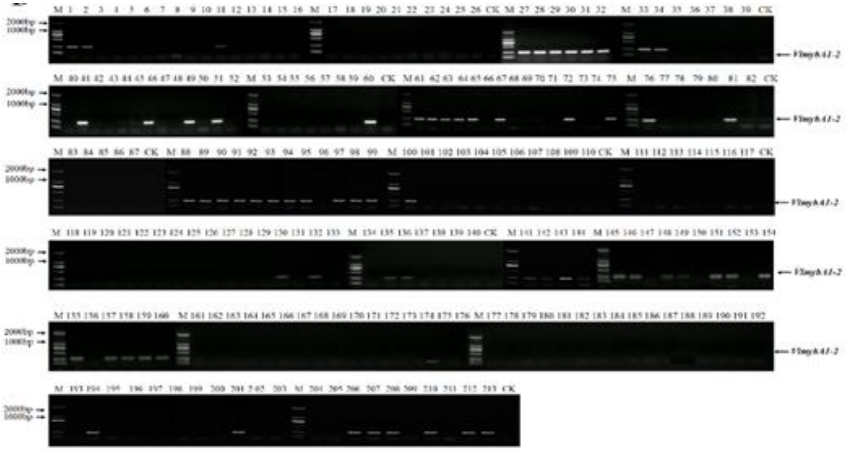


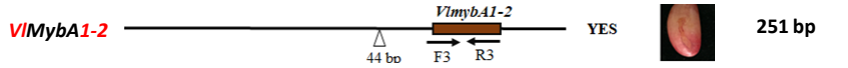


DNA quality control by the amplification of *actin*



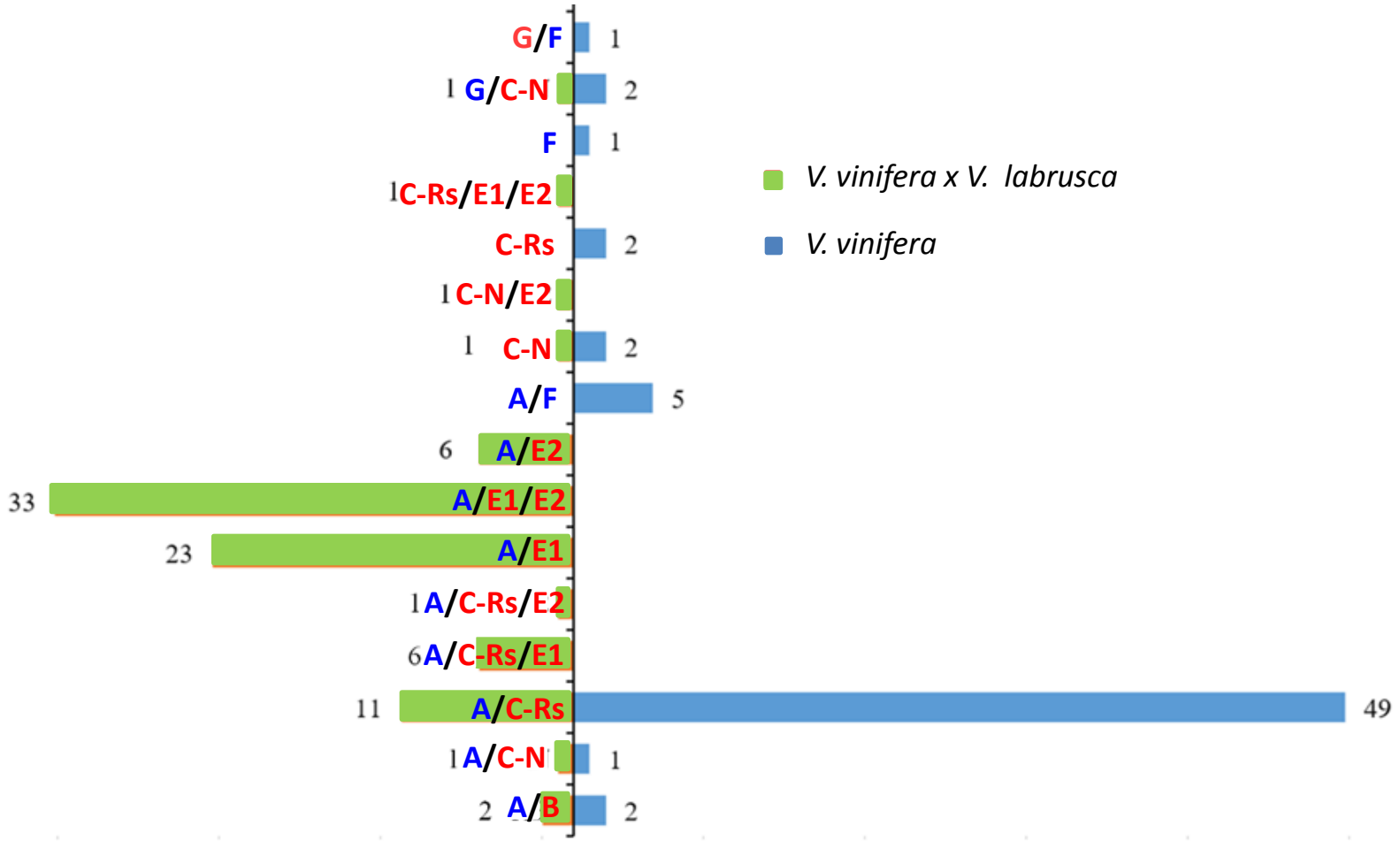






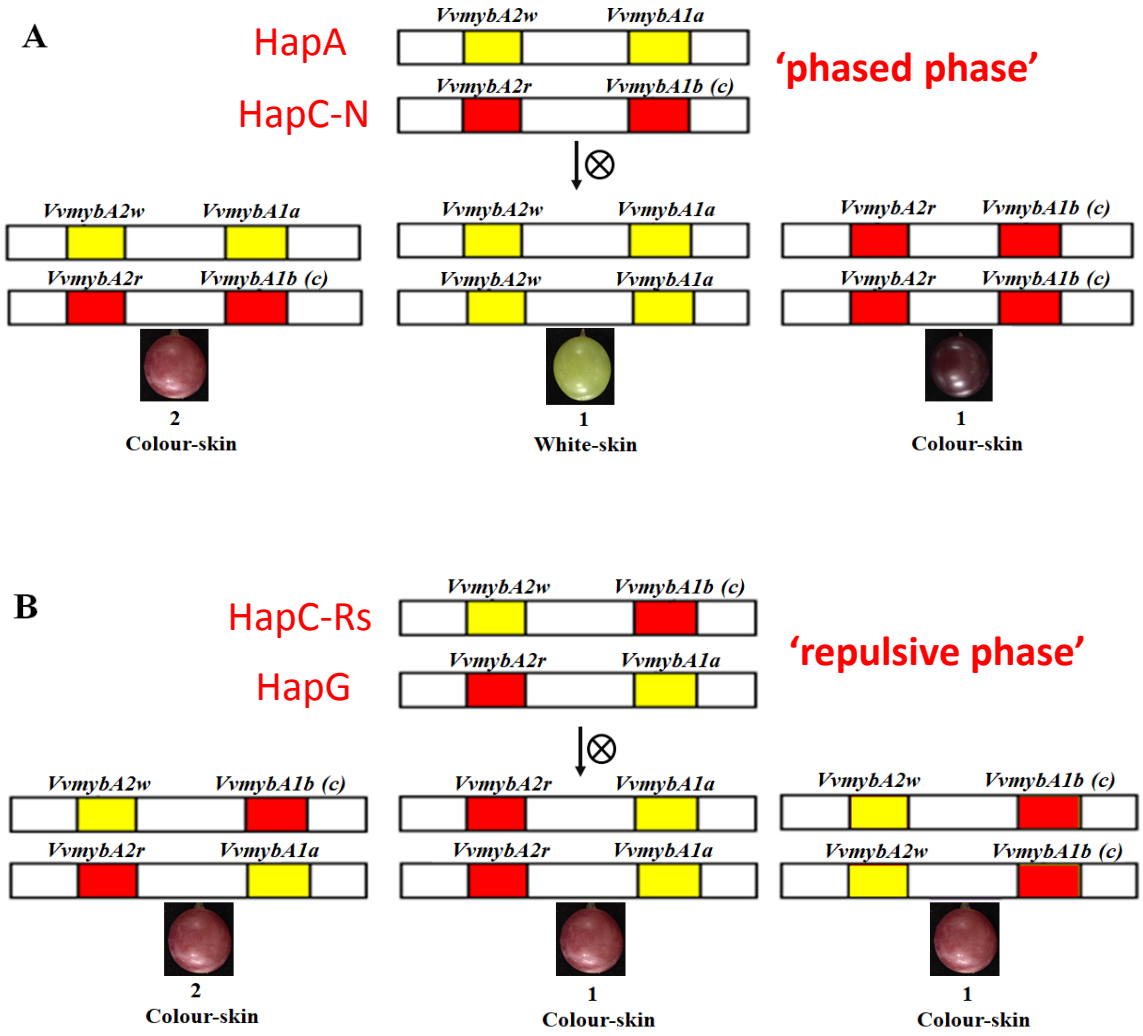
MybA2 allele identification

MYB haplotype composition of the investigated grape varieties



MYB haplotype composition of 211 grape varieties can be identified by PCR and sequencing except 'Yuanruihei' (*V. vinifera*) and 'Olarra Queen' (*V. labrusca*).

MYB haplotype composition of 'Yuanruihei' and 'Olarra Queen'



MYB haplotype composition of 'Yuanruihei' and 'Olarra Queen' could be

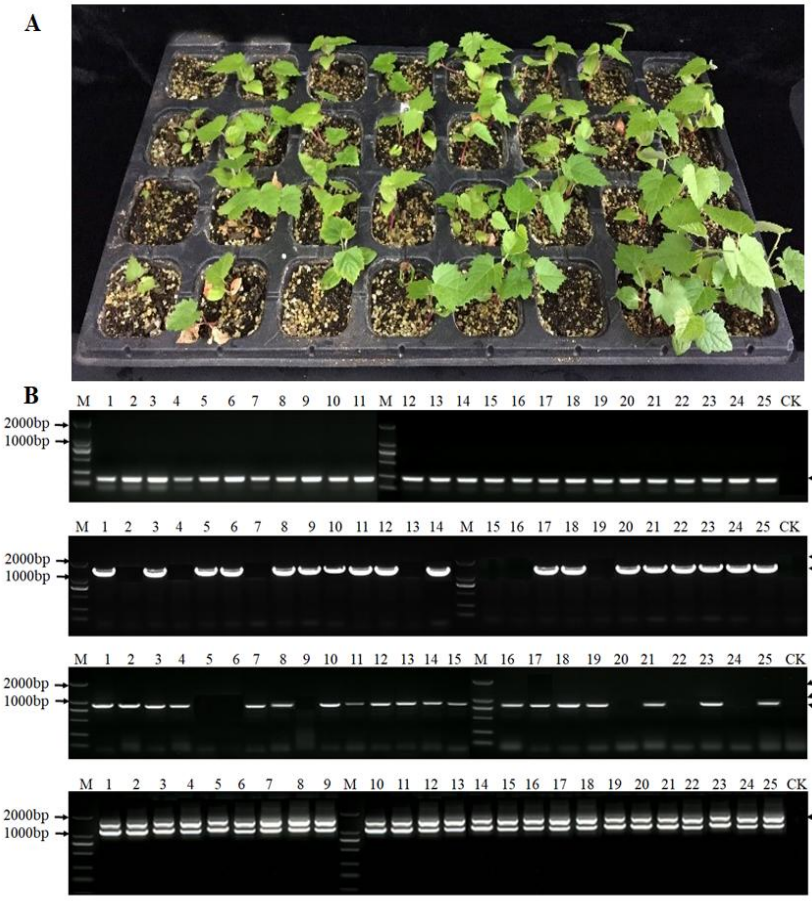
- ✓ **either A/C-N or G/C-Rs**
- ✓ **identified by self-crossing**

Identification of *MYB* haplotype composition of ‘Yuanruihei’

Haplotype composition of ‘Yuanruihei’ was A/C-N.

Code	*VvMybA1a*	*VvMybA1b*	*VvMybA1c*	*VvMybA1^{SUB}*	*VvMybA1^{BEN}*	*VvMybA2r/w*	Haplotype composition
1	+					2w	A
2	+					2w	A
3	+					2w	A
4	+					2w	A
5	+					2w	A
6	+					2w	A
7	+					2w	A
8	+					2w	A
9	+					2w	A
10	+		+			2w/2r	AC-N
11	+		+			2w/2r	AC-N
12	+		+			2w/2r	AC-N
13	+		+			2w/2r	AC-N
14	+		+			2w/2r	AC-N
15	+		+			2w/2r	AC-N
16	+		+			2w/2r	AC-N
17	+		+			2w/2r	AC-N
18	+		+			2w/2r	AC-N
19	+		+			2w/2r	AC-N
20	+		+			2w/2r	AC-N
21	+		+			2w/2r	AC-N
22	+		+			2w/2r	AC-N
23	+		+			2w/2r	AC-N
24	+		+			2w/2r	AC-N
25	+		+			2w/2r	AC-N
26			+			2r	C-N
27			+			2r	C-N
28			+			2r	C-N
29			+			2r	C-N
30			+			2r	C-N
31			+			2r	C-N
32			+			2r	C-N
33			+			2r	C-N

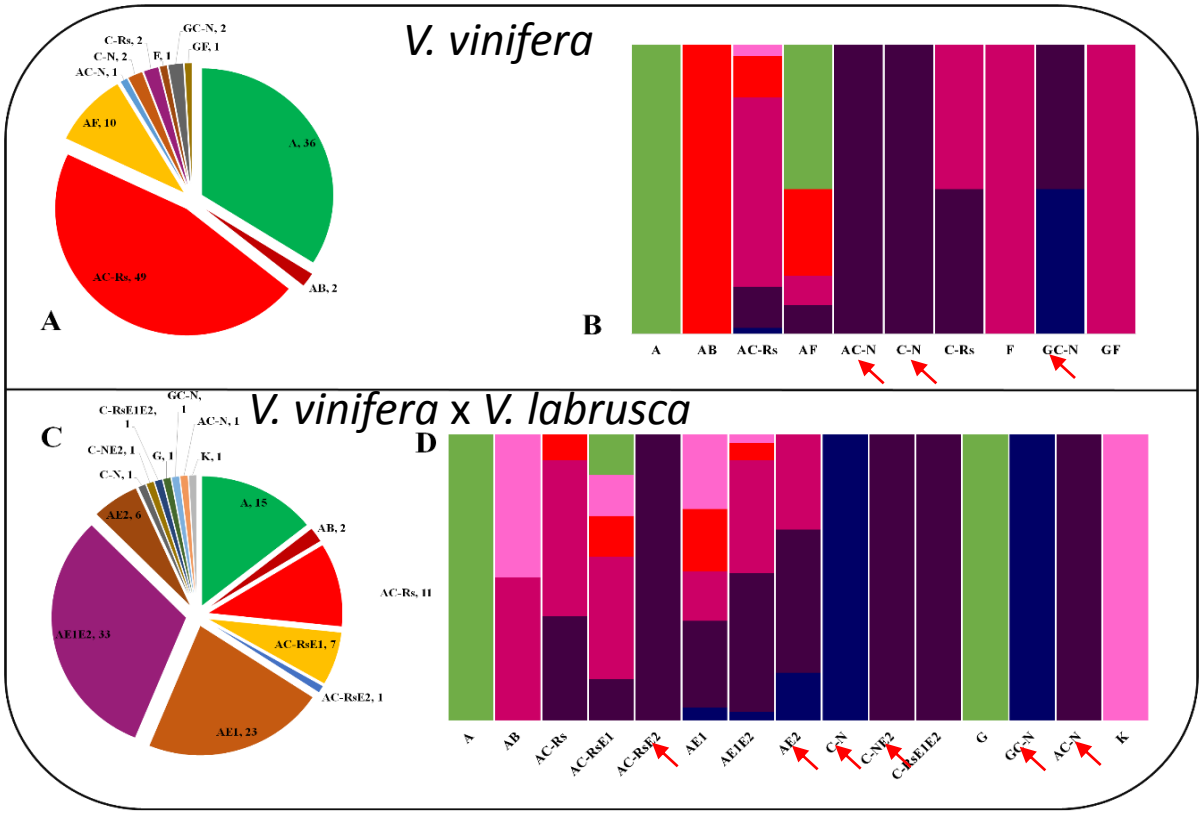
Identification of *MYB* haplotype composition of ‘Olarra Queen’

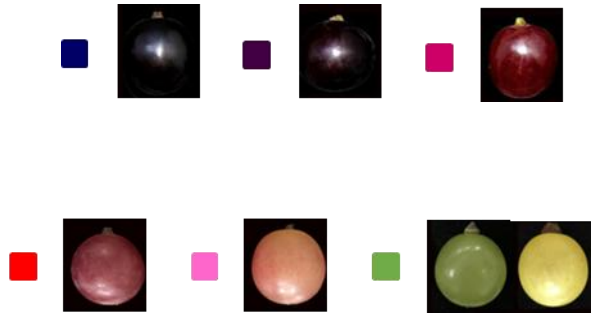


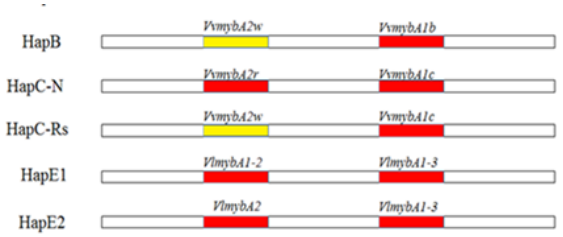
Code	*VvMybA1a*	*VvMybA1b*	*VvMybA1c*	*VvMybA1^{SUB}*	*VvMybA1^{BEN}*	*VvMybA2r/w*	Haplotype composition
1	+					2w	A
2	+					2w	A
3	+					2w	A
4	+					2w	A
5	+					2w	A
6	+					2w	A
7	+		+			2w/2r	AC-N
8	+		+			2w/2r	AC-N
9	+		+			2w/2r	AC-N
10	+		+			2w/2r	AC-N
11	+		+			2w/2r	AC-N
12	+		+			2w/2r	AC-N
13	+		+			2w/2r	AC-N
14	+		+			2w/2r	AC-N
15	+		+			2w/2r	AC-N
16	+		+			2w/2r	AC-N
17	+		+			2w/2r	AC-N
18	+		+			2w/2r	AC-N
19			+			2r	C-N
20			+			2r	C-N
21			+			2r	C-N
22			+			2r	C-N
23			+			2r	C-N
24			+			2r	C-N
25			+			2r	C-N

Haplotype composition of ‘Yuanruihei’ 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.

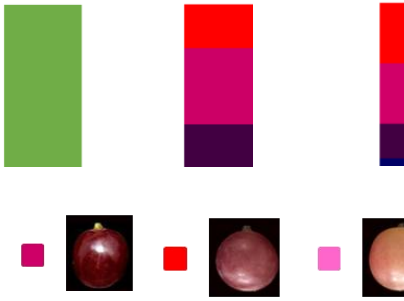
Can we achieve early color prediction during cross breeding?

a) Haplotype identification and berry coloration

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

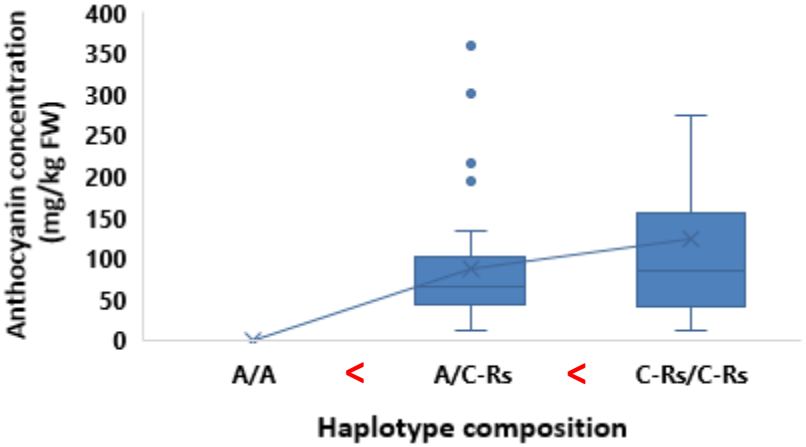
F1	A/A	:	A/C-Rs	:	C-Rs/C-Rs
Expected	1		2		1
Observed [a]	18		32		19

Berry color



[a]Chi square value=0.391, *P* value=0.82

b) The berry color observation and quantification



Can we achieve early color prediction during cross breeding?

a) Haplotype identification and berry coloration

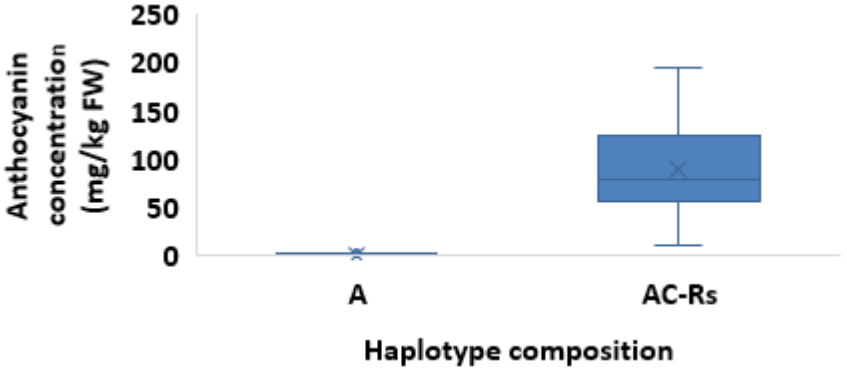
‘Cuibao seedless’ ♀ A/A × ‘Qiuhongbao’ ♂ A/C-Rs

F1	A/A	:	A/C-Rs
Expected	1		1
Observed [a]	25		38

Berry color

[a]Chi square value=2.29, *P* value=0.13

b) The berry color observation and quantification



Summary

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

Confirmed

Verification

- ✓ 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

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

Closely correlated

MYB haplotype composition

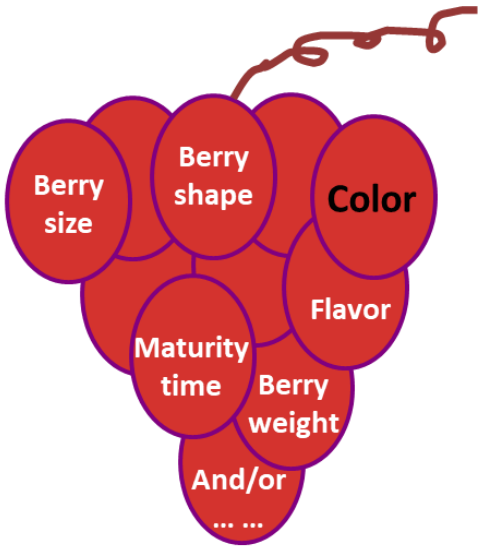
Color index

213 grape varieties

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

Potential strategy for the way forward

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

↓ x

More hybrids with desirable target traits including berry color

Acknowledgements

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Thank you for your attention!

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