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Analysis of Allele-Specific Expression (ASE) in three Grapevine Cultivars reveals Pervasive and Tissue-specific Allelic Imbalance

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DEGLI STUDI
DI UDINE**

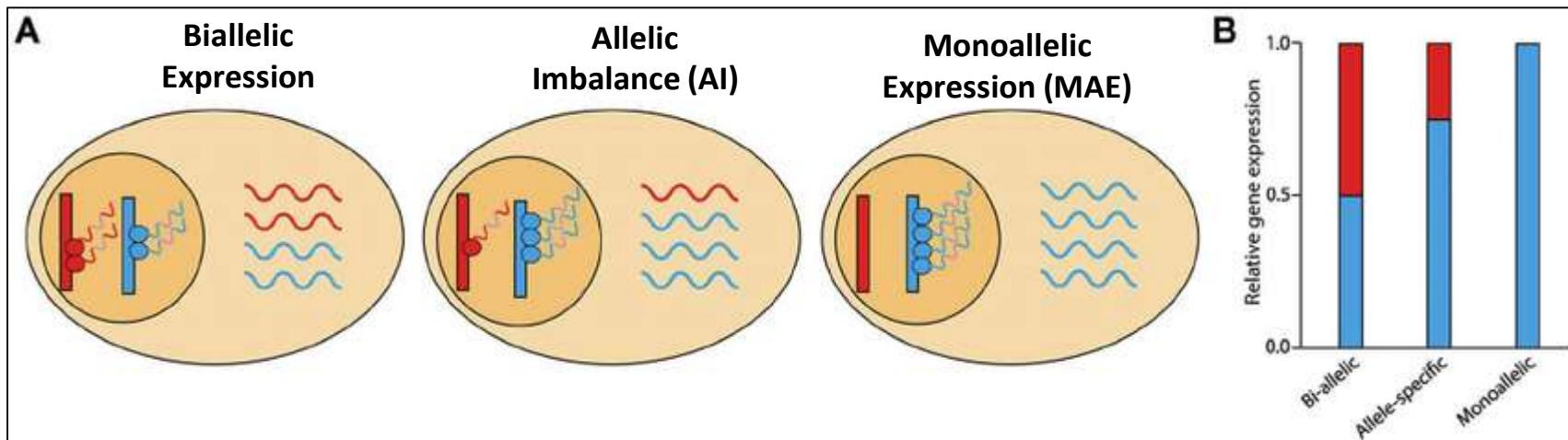


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Allele-Specific Expression (ASE)



Adapted from Kukurba et al. (2014)

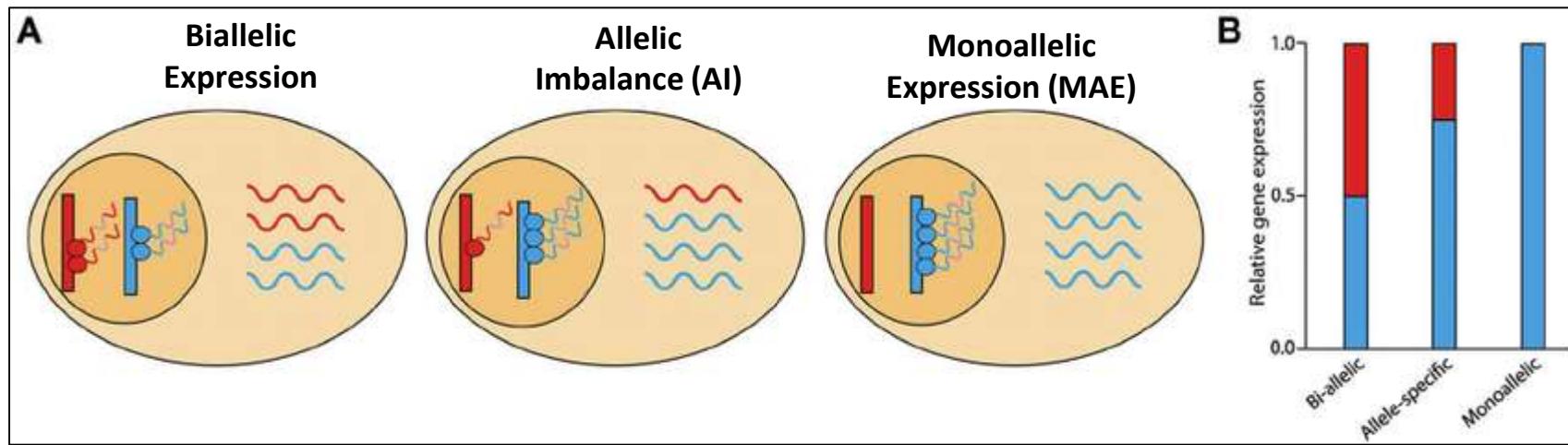
Magnitude and direction of ASE

Allelic Imbalance → Fisher's test $q.\text{value} \leq 0.05$

→ Fold-change ($\text{FC} \geq 1.25, \text{FC} \geq 1.5, \text{FC} \geq 2.0$)

↓

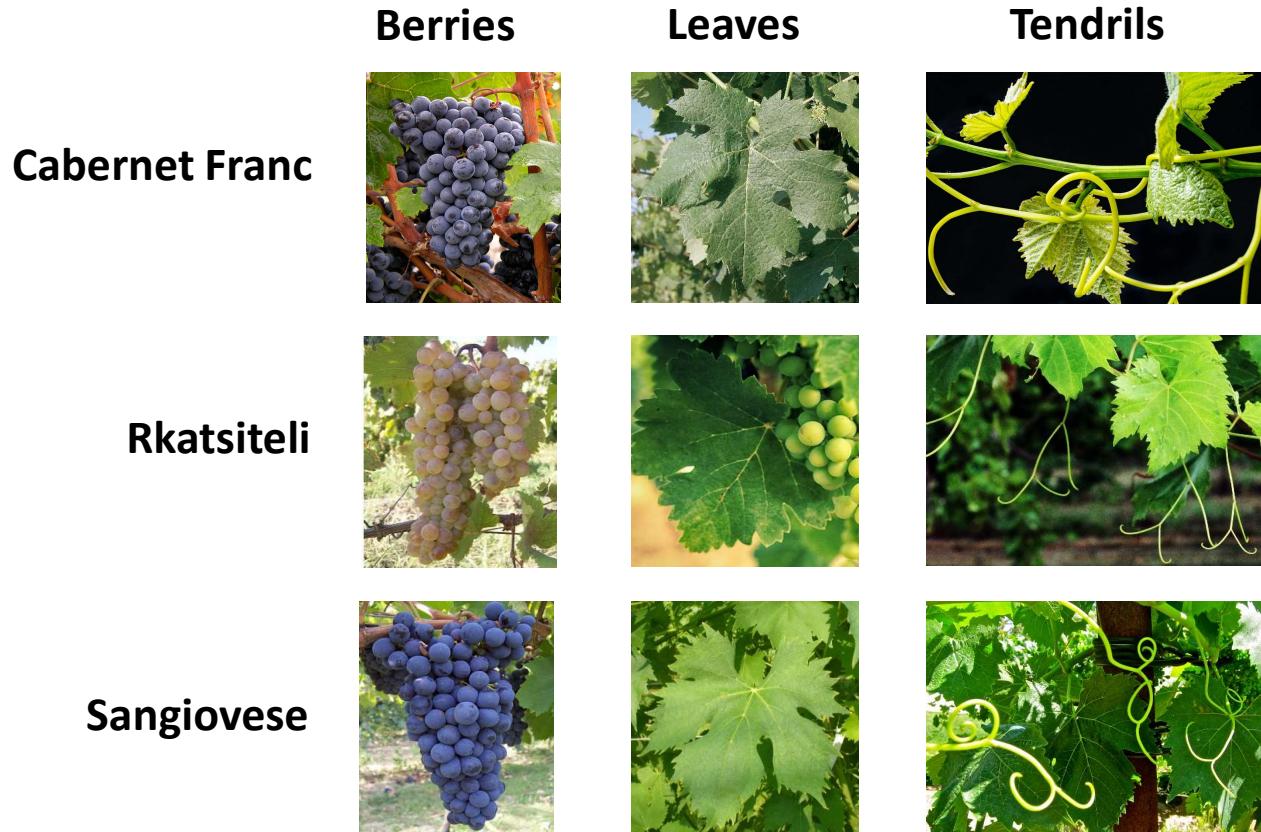
ratio between the read count of the two haplotypes
(HapA/HapB)



Adapted from Kukurba et al. (2014)

- Genes showing allelic imbalance (AI) affect several phenotypic traits
- Innovative breeding projects to obtain improved crops with a better potential of adaptation to changing environments.

ASE analysis of RNA-Seq data in Grapevine

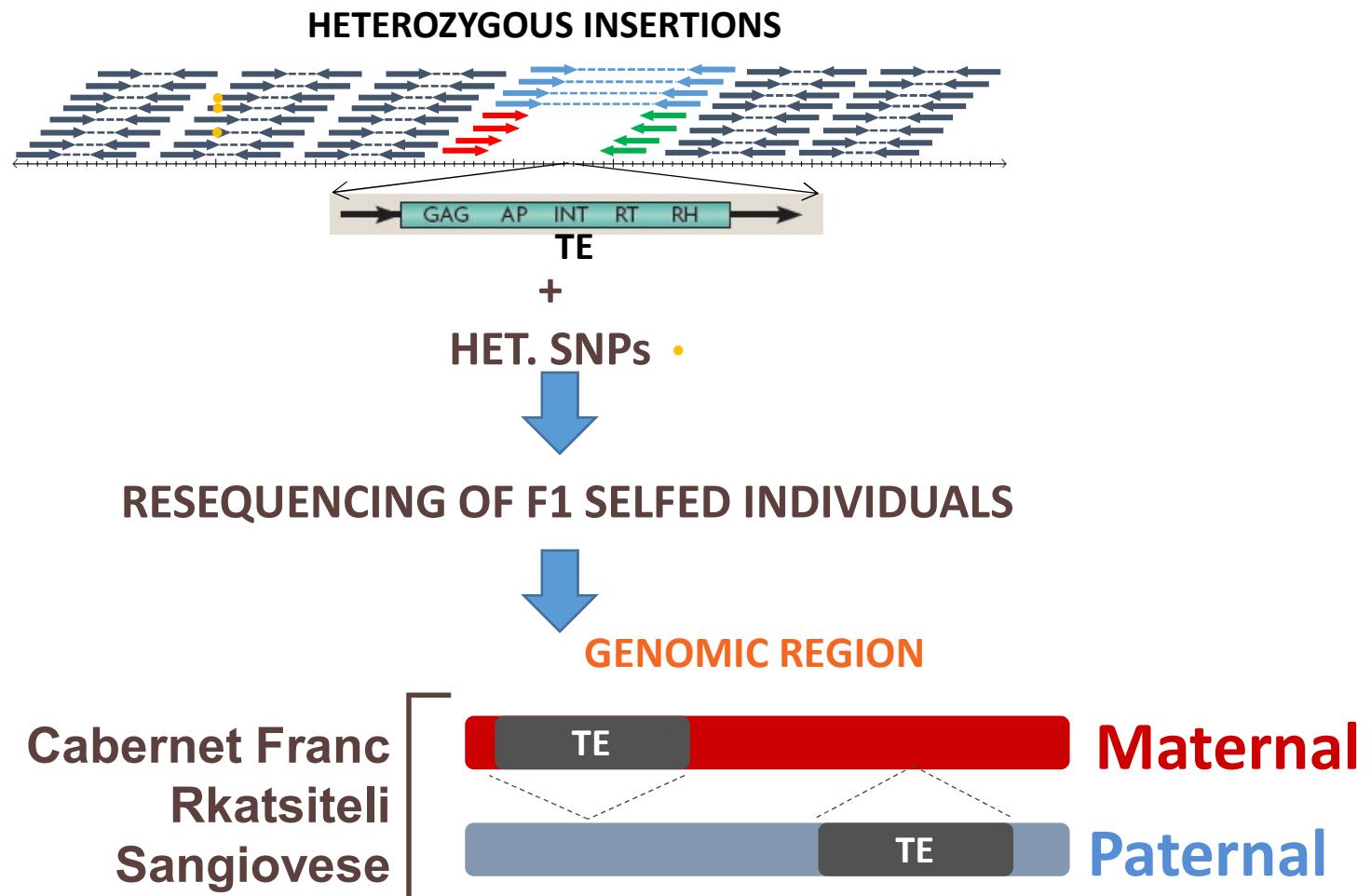


ALLIM (Allelic Imbalance metre) is an user-friendly software tool

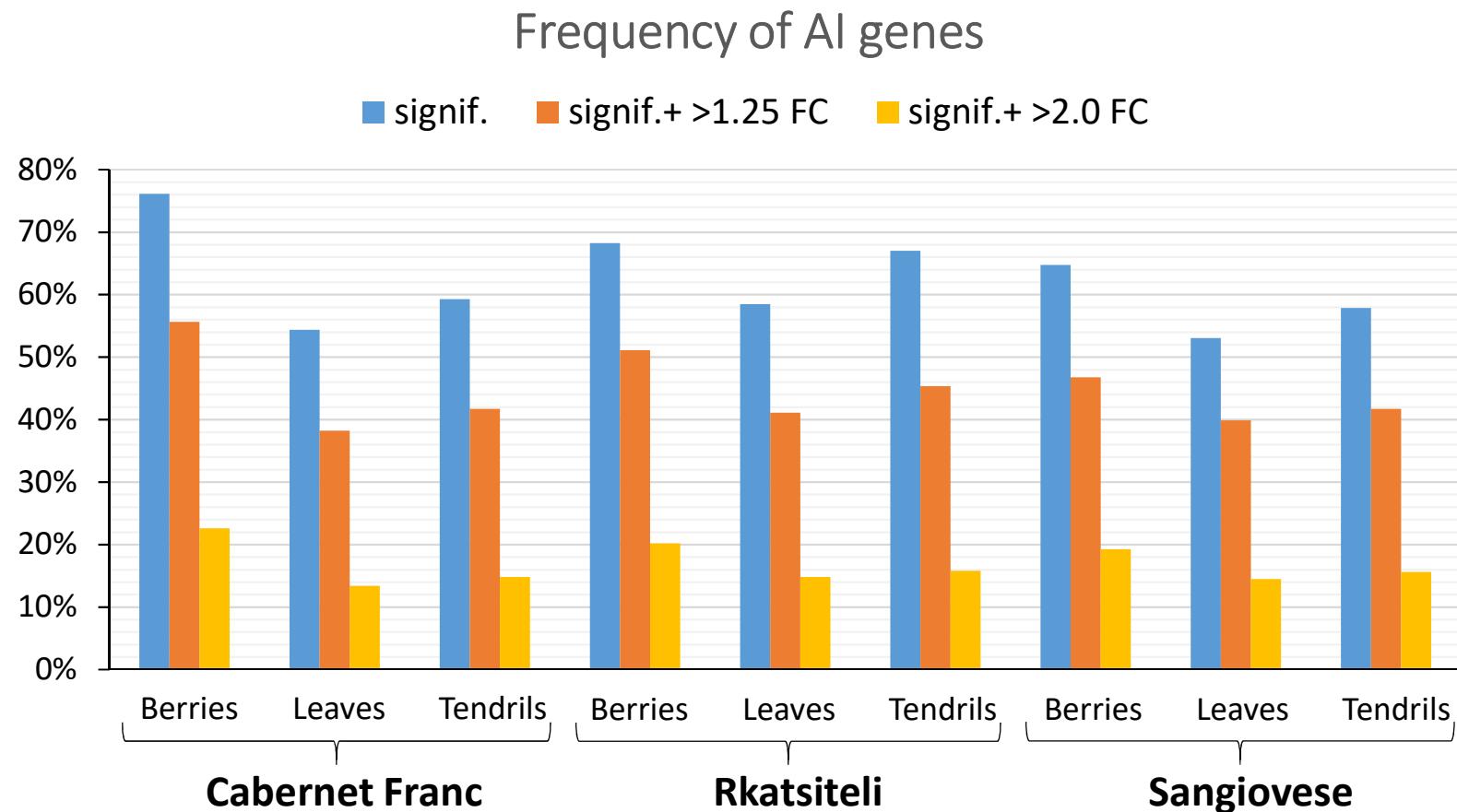
Pandey et al. (2013)

Haplotype phasing

Phase inference of SNPs and SVs allows to perform allele-specific analysis of gene expression in heterozygous individuals



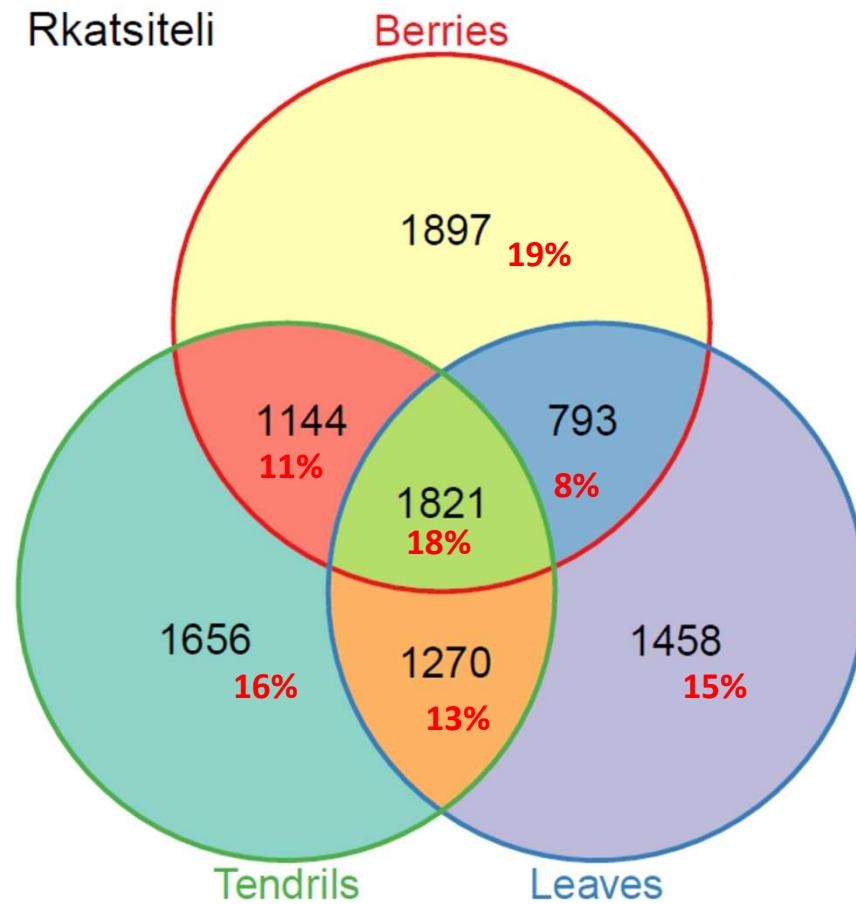
ASE analysis of RNA-Seq data in 3 cultivars



***Cis*-regulatory variation as measured by ASE is pervasive in grapevine:
more frequent in berries than in leaves or tendrils**

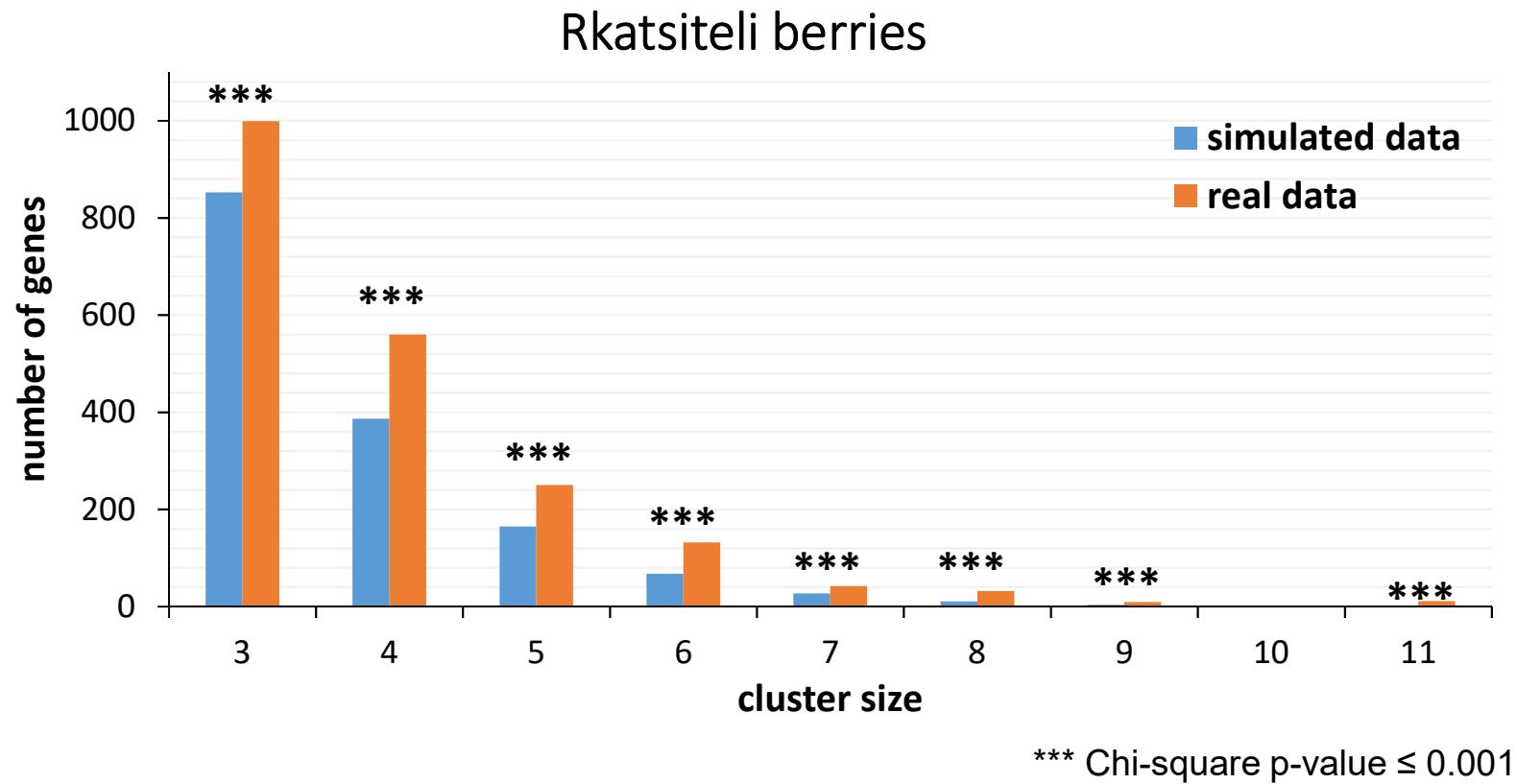
ASE analysis of RNA-Seq data in 3 cultivars

Frequency of AI genes tissue-specific ($FC \geq 1.25$) in Rkatsiteli



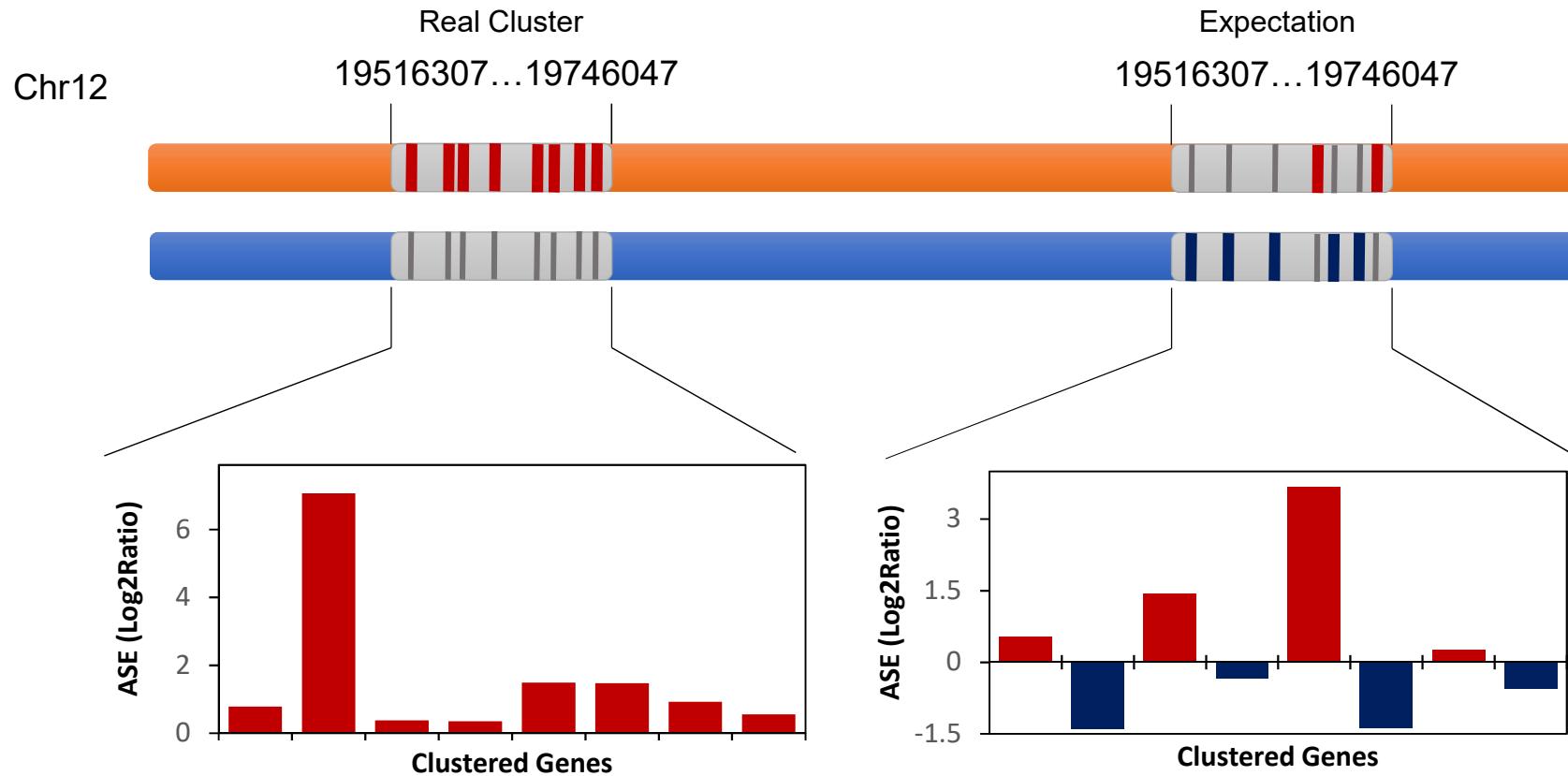
A large proportion (50%) of genes showing ASE is tissue-regulated

Coordinated expression of neighbouring genes



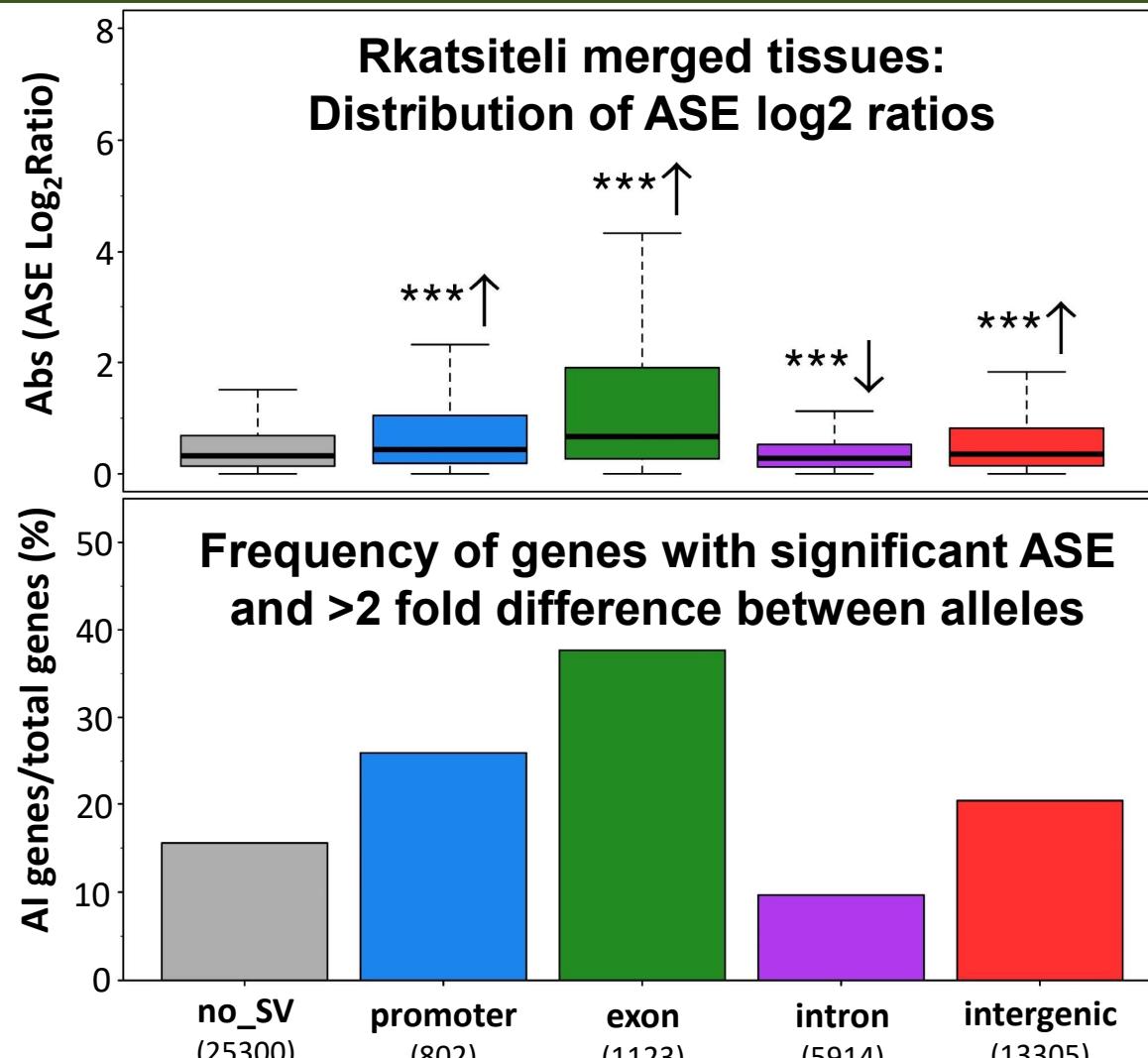
Genes with AI displays significant clustering of expression, greater than expected by chance.

Coordinated expression of neighbouring genes



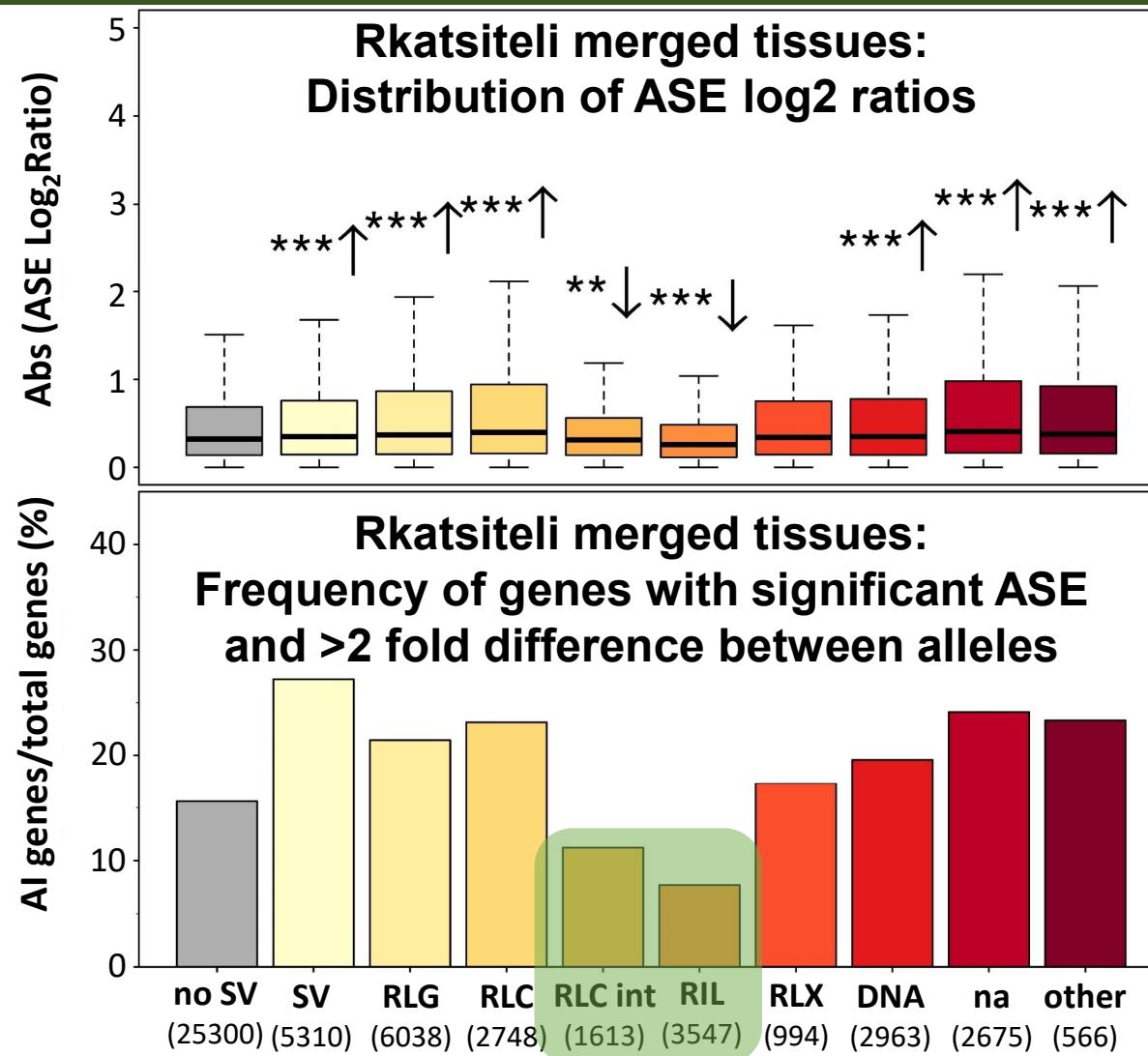
Neighbouring genes with AI are more likely to express the same allele and to be controlled by the same *cis*-regulatory elements or chromatin domains.

SVs and Allelic Expression



Genes with SVs show higher frequency of significant ASE except for SVs involving introns

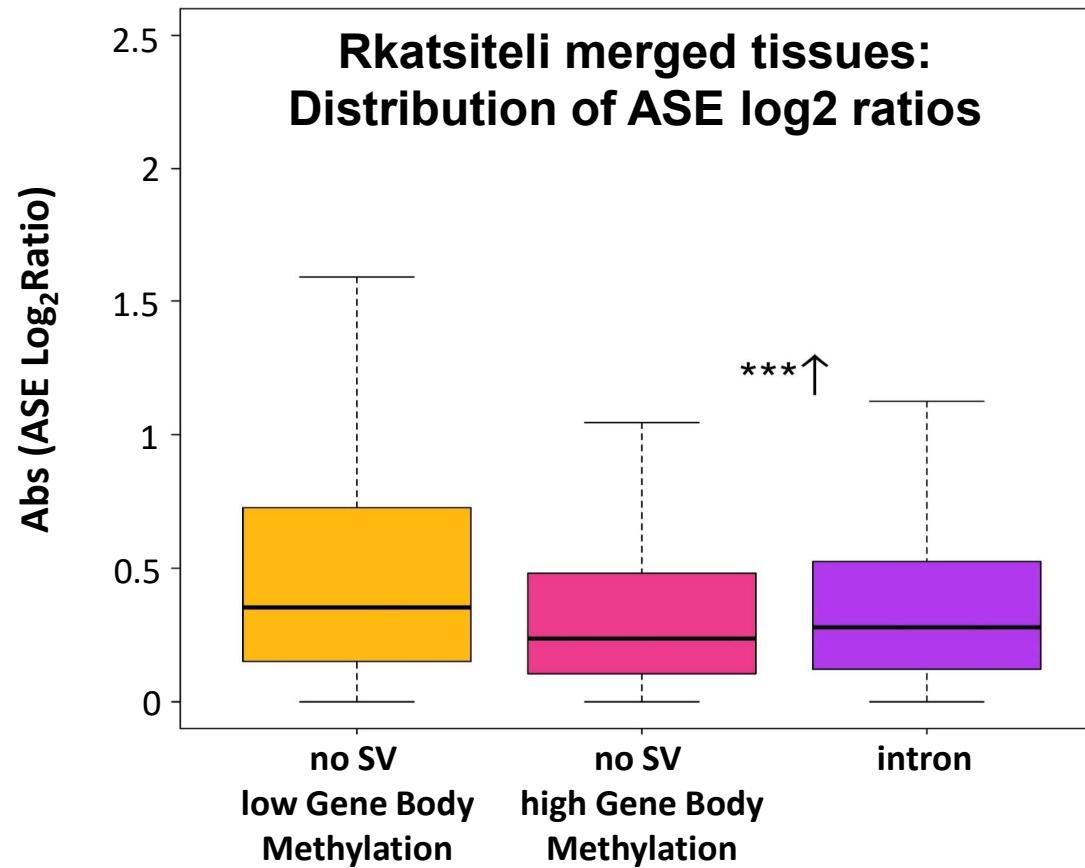
TEs and Allelic Expression



Most TE superfamilies increase frequency of significant ASE

Introns and Gene Body Methylation (GbM)

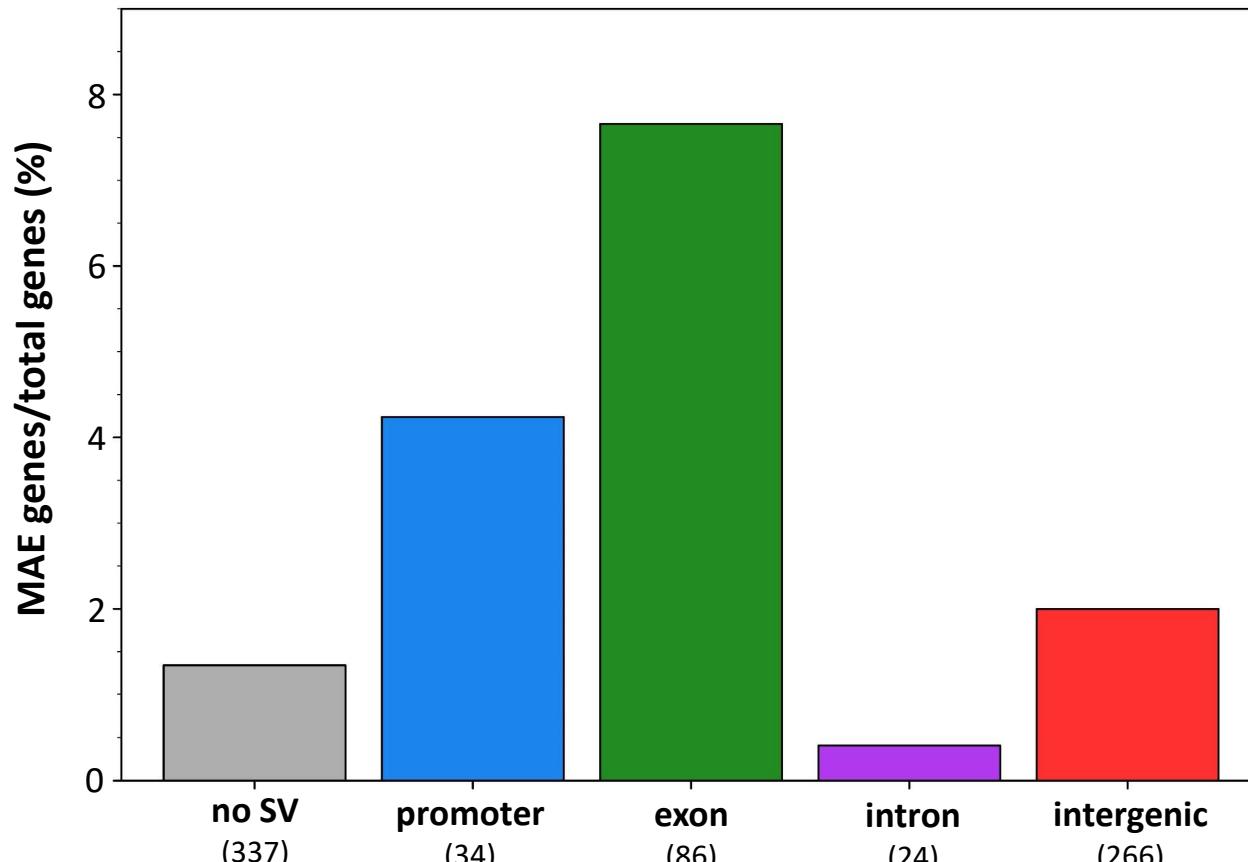
GbM is most frequent in constitutively and highly expressed genes
TEs in introns are associated with high GbM



At high GBM, genes with SVs show higher frequency of significant ASE
also for those that insert into introns

SVs and Monoallelic Expression (MAE)

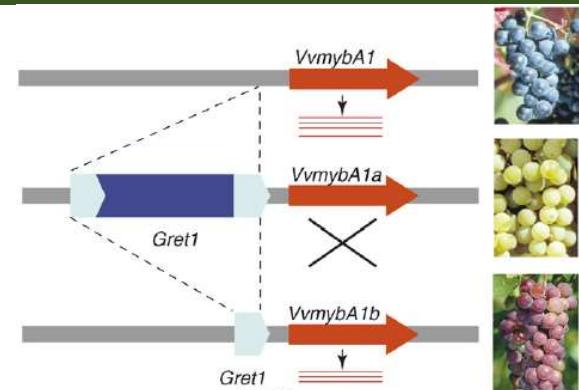
Rkatsiteli merged tissues:
Frequency of genes with MAE



Genes with SVs in promoter and exons show higher frequency of monoallelic expression

SVs and Monoallelic Expression (MAE): berries color

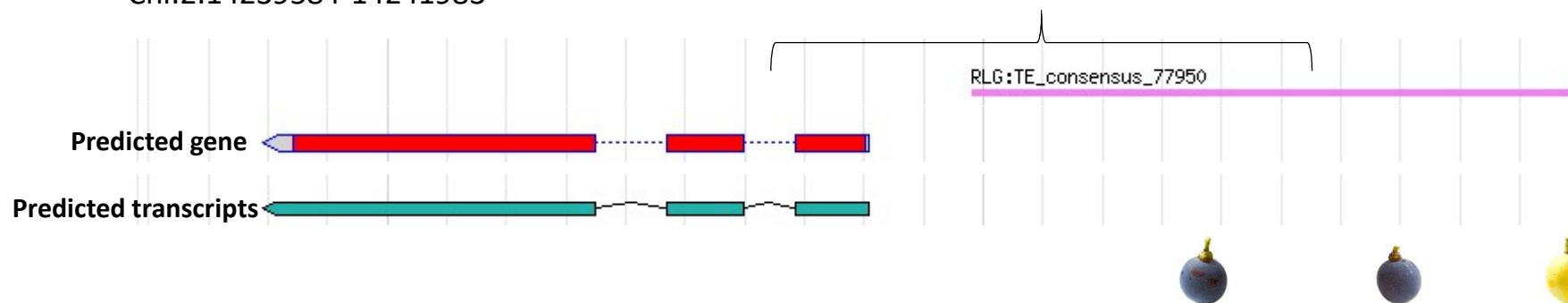
The insertion of the retroelement *Gret1* in the promoter region of *VvmybA1* was in strong association with the white-fruited phenotype



Current Opinion in Plant Biology

Chr.2:14239584-14241983

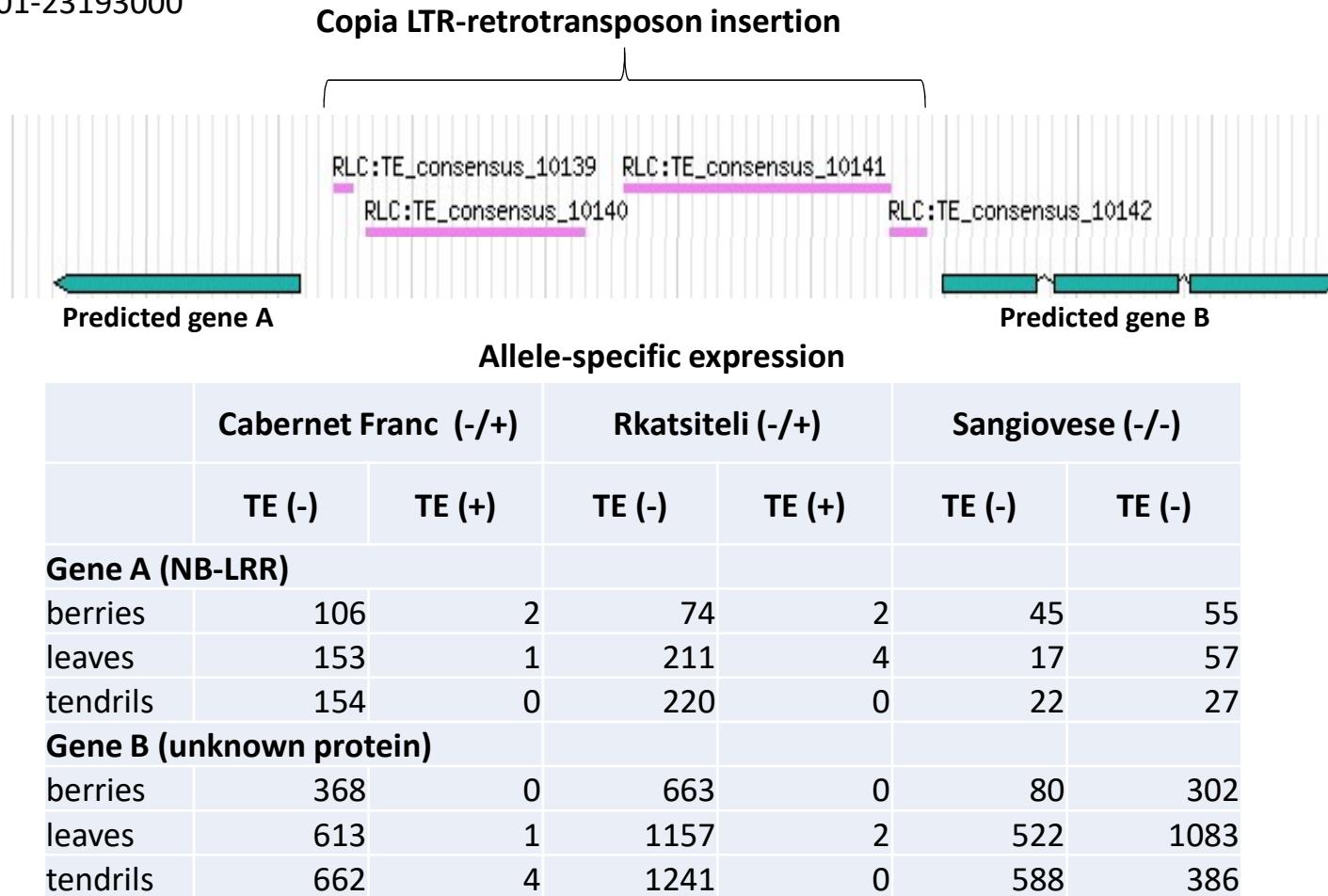
Gret1 insertion



	cultivars	Cabernet Franc		Sangiovese			cultivars	Cabernet Franc	Sangiovese	Rkatsiteli
		TE (-)	TE (+)	TE (-)	TE (+)		TE genotype	-/+	-/+	+/-
ASE	berries	1454	11	2428	24	FPKM	berries	35.5	27.0	4.8

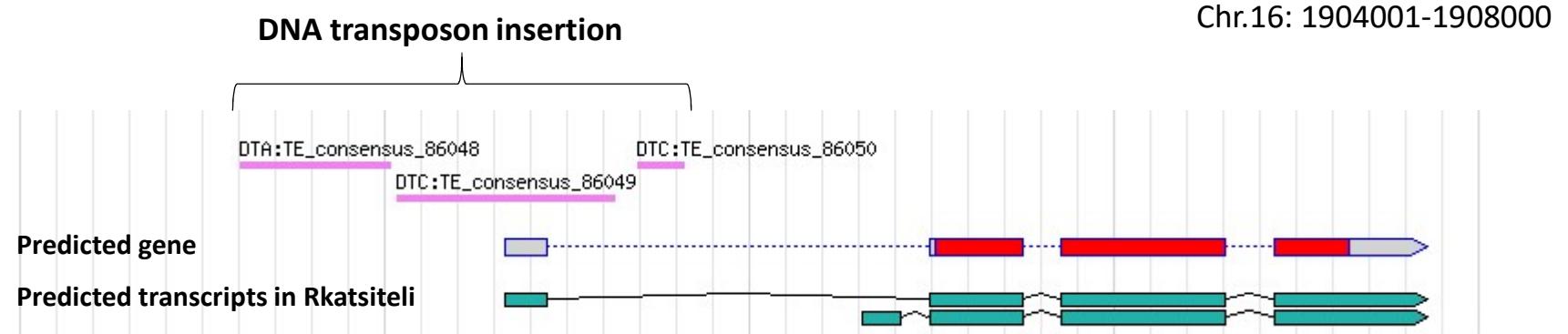
SVs and Monoallelic Expression (MAE)

Chr.5: 23183001-23193000



TE insertion shuts down expression of 2 genes (under bidirectional promoter)

SVs and Monoallelic Expression (MAE)



Rkatsiteli allele-specific expression

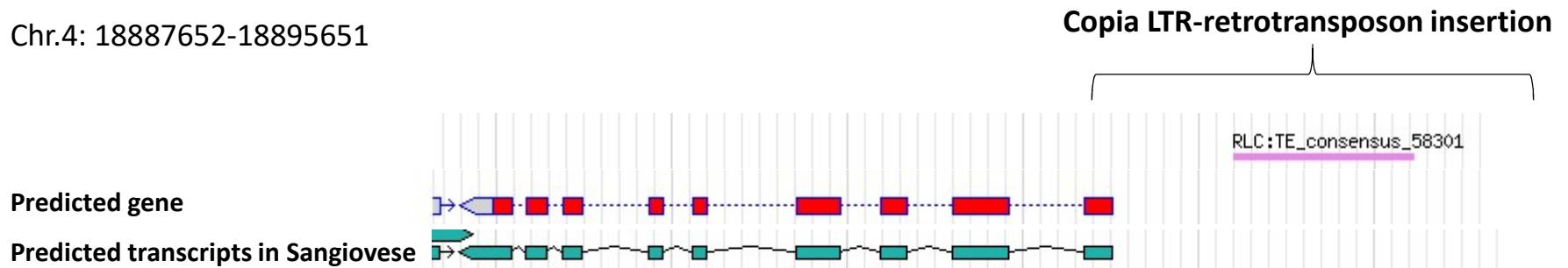
	TE (-)	TE (+)
berries	0	410
leaves	2	352
tendrils	2	714

	cultivars	Sangiovese	Rkastiteli
	TE genotype	-/-	-/+
	leaves	0,0	25,4
FPKM	tendrils	0,0	43,3
	berries	0,0	34,0

TE insertion increases expression of a myB transcription factor and changes gene structure

SVs and Monoallelic Expression (MAE)

Chr.4: 18887652-18895651



Sangiovese allele-specific expression

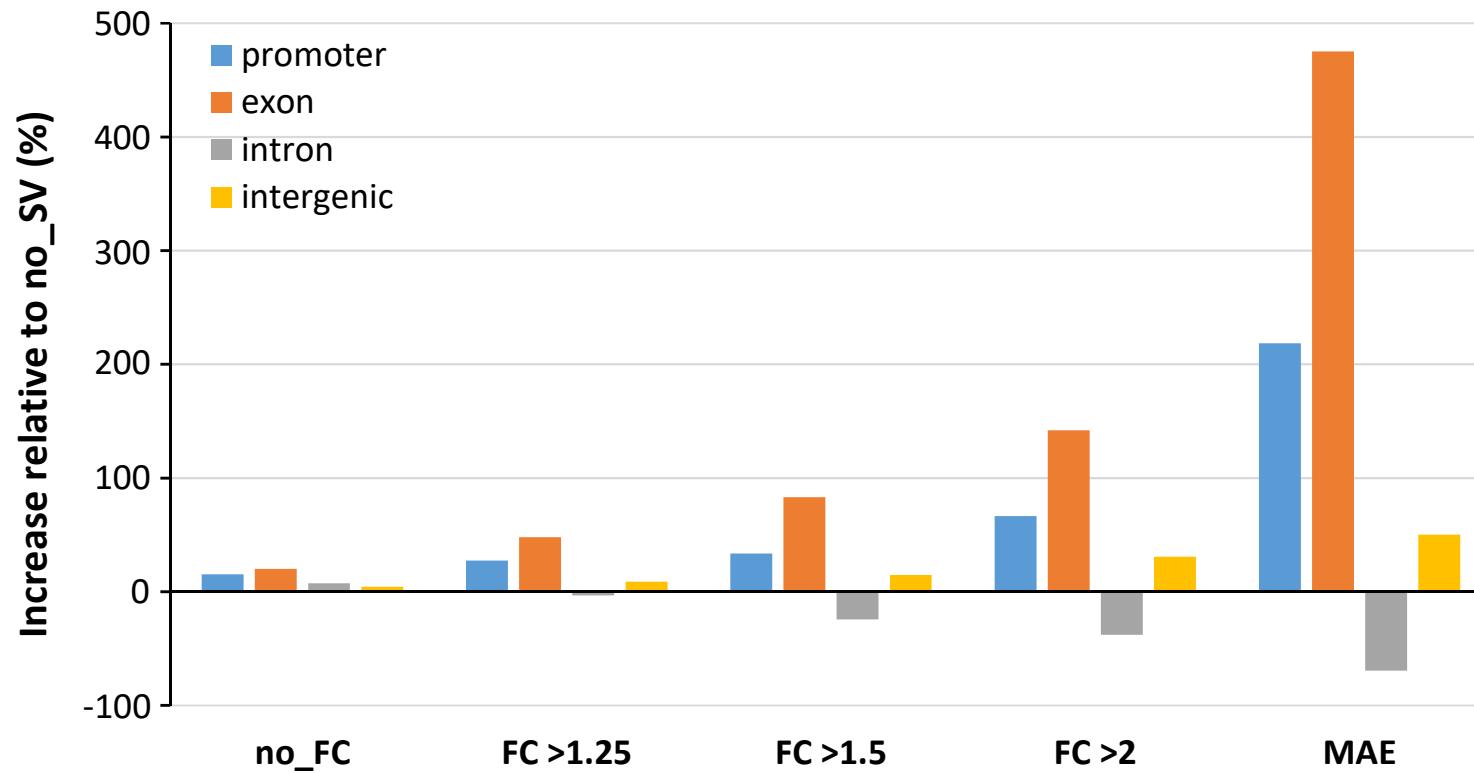
	TE (-)	TE (+)
berries	8	352
leaves	7	1043
tendrils	20	2015

	cultivars	Cabernet Franc	Rkatsiteli	Sangiovese
	TE genotype	-/-	-/-	-/+
	berries	0,0	0,0	4,7
FPKM	leaves	0,3	0,1	13,8
	tendrils	0,0	0,1	16,2

TE insertion activates expression of an abscisic acid 8-hydroxylase

TE-SVs affect Allele-Specific Expression

Enrichment of ASE in structural variants loci



Increasing the magnitude of AI, genes showing ASE are more strongly associated with SVs

Conclusion

- In grapevine, ASE is pervasive among cultivars and tissues
- ASE is tissue-specific and often is monoallelic
- Structural variation affects allelic expression of flanking genes
 - SVs affect the expression in all genomic compartments
 - SVs affect the expression also in introns, depending on gene-body methylation status
 - TE insertions can knock-out genes, contributing to genetic load
 - TE insertions can produce new patterns of gene expression

Acknowledgments



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