An Investigation of the Role of the Regulatory Gene *VvMYBA1* in Colour, Flavour and Aroma Metabolism Using Transgenic Grapevines

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TABLE OF CONTENTS

TABLE OF CONTENTS ..... **III**

ABSTRACT ..... **X**

DECLARATION ..... **XII**

ACKNOWLEDGEMENTS ..... **XIII**

LIST OF TABLES AND FIGURES ..... **XV**

ABBREVIATIONS ..... **XVII**

Chapter 1: Introduction **1**

1.1 Introduction **2**

1.2 The Grapevine **2**

1.3 Grape and wine flavour **3**
1.3.1 Grape contribution to wine flavour 3
1.3.2 Red and white wine flavour: what is the difference? 4
1.3.3 Factors contributing to red and white wine flavour differences 5
1.3.4 Is there a link between berry colour and wine flavour/aroma? 6

1.4 Anthocyanins: their synthesis and regulation **7**
1.4.1 Anthocyanins 7
1.4.2 The anthocyanin biosynthesis pathway 9
1.4.3 Transcriptional regulation of the anthocyanin biosynthesis pathway 12

1.5 Evidence linking MYB transcription factors to the regulation of anthocyanin and flavour/aroma biosynthesis **15**

1.6 Natural and transgenic grape varieties with altered *VvMYBA* gene expression used in this study **16**

1.7 Summary and project aims **21**
1.7.1 Aims/objectives 21

Chapter 2: Transcriptomic analysis of berries with altered *VvMYBA* gene expression in transgenic grapevines **24**

2.1 Introduction 26
2.1.1 Specific aims of this research 26

2.2 Materials and methods 27
2.2.1 Plant Material 27
2.2.2 RNA extractions 27
2.2.3 Experimental plan 28
2.2.4 cDNA synthesis, labelling and microarray experiments 28
2.2.5 Analysis of microarray data 29
2.2.5.1 Identification of genes with significantly altered expression levels in transgenic berries 29
2.2.5.2 Identification of genes that were modulated in relation to VvMYBA1 gene expression consistently in Chardonnay and Shiraz 29
2.2.5.3 Identification of genes with differential expression changes when comparing skin and whole berry Chardonnay datasets 30
2.2.5.4 Analysis of gene expression trends in relation to berry colour in Shiraz 30
2.2.6 Bioinformatics 30

2.3 Results 31
2.3.1 Altering *VvMYBA* gene expression in grapevine changes transcription in many cellular pathways 31
2.3.1.1 The expression of some flavonoid pathway genes was altered in transgenic berries 31
2.3.1.2 Transcription in other cellular pathways was also altered in transgenic berries expressing VvMYBA1 32
2.3.2 Comparison of Chardonnay and Shiraz microarray datasets 34
2.3.2.1 The transcription of some flavonoid pathway genes was consistently altered by VvMYBA gene expression 38
2.3.2.2 VvMYBA gene expression consistently altered the transcription levels of potential flavour/aroma biosynthetic genes 38
2.3.2.3 Two potential acyl-transferase genes were upregulated in red berries 38
2.3.2.4 Other genes were affected by VvMYBA gene expression consistently in both cultivars 39
2.3.3 Over-expression of VvMYBA1 in Chardonnay results in differential transcriptomic changes in skins compared to whole berries 39
2.3.3.1 Genes with increased up-regulation in whole berries compared to skins 39
2.3.3.2 Genes with increased up-regulation in skins compared to whole berries 40
2.3.3.3 Genes with increased down-regulation in whole berries compared to skins 40
2.3.3.4 Genes with increased down-regulation in skins compared to whole berries 40
2.3.4 Analysis of gene expression changes in red, rose and white Shiraz berries 52
2.3.4.1 Expression trends 1 and 2 67
2.3.4.2 Expression trends 3 and 4 67
2.3.4.3 Expression trends 5 and 6 68
2.3.4.4 Expression trends 7 and 8 68
2.3.4.5 Expression trends 9-12 69

2.4 Discussion 69
2.4.1 Over-expression of *VvMYBA* in white berries has greater effects on berry transcription than its silencing in red berries 69
2.4.2 *VvMYBA* gene expression has a large impact on global transcription 70

2.4.2.1 VvMYBA affects the expression of flavonoid biosynthetic genes and may do this in a cultivar-specific manner *72*
2.4.2.2 VvMYBA may have a role in defence and stress responses *73*
2.4.3 VvMYBA affects global transcription in a tissue-specific manner 74
2.4.4 Discussion on expression trends of genes in Shiraz microarray data in relation to berry colour 75
2.4.4.1 Genes whose expressions were altered in a manner consistent with VvMYBA gene expression and anthocyanin concentrations *75*
2.4.4.2 Some genes were modulated but did not follow VvMYBA gene expression and anthocyanin concentrations *77*
2.4.5 VvMYBA regulates a narrow set of genes consistently across cultivars and is a positive regulator of anthocyanin biosynthesis and transport 79
2.4.6 The identification of two putative anthocyanin acyltransferases up-regulated by VvMYBA 80
2.4.7 The role of VvMYBA in flavour/aroma metabolism 81

2.5 Conclusion 82

Chapter 3: An investigation into the role of VvMYBA in the synthesis of flavour and aroma compounds in the cultivated grapevine *Vitis vinifera L.* 83

3.1 Introduction 85
3.1.1 The phenylpropanoid pathway produces substrates used in the synthesis of volatile phenylpropanoids/benzenoids and flavonoids 85
3.1.2 Links between flavonoid related MYB transcription factors and terpene synthesis 88
3.1.3 Terpene biosynthesis 88
3.1.3.1 The MEP pathway *89*
3.1.4 How can anthocyanin and terpene biosynthesis be co-ordinately regulated? 91
3.1.5 The research question: Does VvMYBA have a role in flavour/aroma biosynthesis in grapevine? 91

3.2 Materials and methods 91
3.2.1 Chemicals 91
3.2.2 Plant material 92
3.2.2.1 Transgenic berries used in microfermentations *92*
3.2.2.2 Cabernet Sauvignon, Malian and Shalistin used in microfermentations *92*
3.2.2.3 Shade and light experiment for microfermentations *92*
3.2.2.4 Cabernet Sauvignon, Malian and Shalistin developmental series for RNA extractions *93*
3.2.3 Microfermentations 93
3.2.3.1 Transgenic grapes *93*
3.2.3.2 Pressed berry fermentations *93*
3.2.3.3 Fermentations on free-run juice *94*
3.2.3.4 Yeast preparation *94*
3.2.3.5 Fermentation conditions *95*
3.2.4 Determination of wine anthocyanin concentrations 95

3.2.4.1 Spectrophotometric assay *95*
3.2.4.2 HPLC *95*
3.2.5 Initial analysis of volatile wine compounds 96
3.2.6 Analysis of monoterpenes in wine 96
3.2.7 RNA extractions and cDNA synthesis 97
3.2.8 qPCR analysis 97

3.3 Results 98
3.3.1 Analysis of flavour and aroma compounds in wines made from berries with altered *VvMYBA* gene expression and colour 98
3.3.1.1 Flavour compounds altered by VvMYBA gene expression with similar trends in different cultivars *101*
3.3.1.2 Flavour compounds altered by VvMYBA gene expression with different trends in different cultivars *102*
3.3.2 Further analysis of monoterpenes in wines using a linalool standard 106
3.3.3 Gene expression analysis of monoterpene biosynthesis genes identified from analysis of microarrays experiments 108
3.3.4 Linalool synthase is down-regulated in red grapes 110
3.3.5 The effect of light exclusion from Cabernet Sauvignon, Malian and Shalistin berries on wine linalool abundance 112
3.3.6 The expression of monoterpene precursor biosynthesis genes in Cabernet Sauvignon, Malian and Shalistin berries 114
3.3.6.1 MEP pathway gene expression in whole berries *114*
3.3.6.2 MEP pathway gene expression in skins *114*

3.4 Discussion 120
3.4.1 Altering *VvMYBA* gene expression in berries changes flavour compounds present in their wines 120
3.4.1.1 VvMYBA has an effect on the presence of volatile phenylpropanoids and benzenoids *121*
3.4.1.2 VvMYBA has a negative effect on the abundance of linalool *122*
3.4.1.3 VvMYBA expression changed volatile production in a variety specific manner *122*
3.4.1.4 Rose wines were different from red and white wines *123*
3.4.1.5 Altering VvMYBA gene expression resulted in more flavour differences in wines from ferments on pressed berries *124*
3.4.2 Considerations for further investigation into flavour differences of wine affected by VvMYBA 125
3.4.3 Differential expression of genes involved in monoterpene biosynthesis due to VvMYBA may account for altered levels of monoterpenes in wines 126
3.4.4 Anthocyanins may cause a shading effect, down-regulating monoterpene accumulation 127

3.5 Conclusion 130

Chapter 4: Characterisation of a Serine Carboxypeptidase-like gene up-regulated in *Vitis vinifera* berries expressing *VvMYBA* 131

4.1 Introduction 132
4.1.1 Anthocyanin acylation in grapevine 132
4.1.2 The SCPL acyltransferase family 132
4.1.2.1 How do the SCPL acyltransferases differ from other acyltransferases?..... 133
4.1.3 SCPLs as anthocyanin acyltransferases 134
4.1.4 Specific aims of this research..... 134

4.2 Materials and Methods 135
4.2.1 Plant material 135
4.2.2 Nucleic acid extraction and cDNA synthesis..... 135
4.2.3 Analysis of gene expression..... 136
4.2.4 Obtaining the DNA sequences of *VvSCPL1* 136
4.2.5 Sequencing the 5' UTR of the *VvSCPL1* gene in CS and PN 136
4.2.6 Transient promoter-binding luciferase activity assays..... 137
4.2.7 Production of genetically modified tobacco containing the *VvSCPL1* gene 138
4.2.8 Analysis of anthocyanins in transgenic tobacco flowers 139
4.2.9 Bioinformatics..... 140

4.3 Results..... 140
4.3.1 *VvSCPL1* belongs to the serine carboxypeptidase-like gene family..... 140
4.3.2 Sequencing *VvSCPL1* coding region and whole gene in Cabernet Sauvignon and Pinot Noir 143
4.3.3 Determining the correct gene structure (exon/intron structure) and start codon position of the *VvSCPL1* gene 143
4.3.4 Pattern of gene expression of *VvSCPL1* over berry development 150
4.3.4.1 Gene expression of VvSCPL1 in Cabernet Sauvignon, Malian, and Shalistin 150
4.3.4.2 Gene expression of VvSCPL1 in Cabernet Sauvignon and Pinot Noir berry development 152
4.3.5 VvMYBA does not activate the promoter of *VvSCPL1* 154
4.3.6 Analysis of putative acyltransferase activity of VvSCPL1 *in planta* 156

4.4 Discussion 158
4.4.1 Bioinformatic analysis of *VvSCPL1* suggests an anthocyanin acyltransferase function 158
4.4.2 VvSCPL1 may be located in the vacuole 158
4.4.3 *VvSCPL1* is transcribed pre- and post-veraison and is not directly regulated by VvMYBA..... 159
4.4.4 *VvSCPL1* sequence and expression in Cabernet Sauvignon and Pinot Noir does not match acylated anthocyanin phenotypes of these cultivars..... 161
4.4.5 VvSCPL1 did not function as an anthocyanin acyltransferase in tobacco 162

4.5 Conclusion 164

Chapter 5: Characterisation of a grapevine anthocyanin acyltransferase gene (*VvAnAT*) 167

Chapter 6: Conclusions and future perspectives 207

6.1 VvMYBA regulates the later stages of anthocyanin biosynthesis, modification and transport 208

6.2 Linalool synthesis is reduced in red berries expressing *VvMYBA* 210

6.3 Anthocyanins cause a shading effect in red berries that reduces linalool accumulation..... 211

6.4 Identification of a putative acyltransferase belonging to the SCPL family and a grapevine anthocyanin acyltransferase..... 212

6.5 Implications of the research and future perspectives..... 212

Appendices..... 215

Appendix A Total soluble sugar in berries using in microarray experiments..... 216

Appendix B Plant ID and transformant lines of berries used in microarray experiments 217

Appendix C Genes with altered expression in transgenic 'white' or 'rose' Shiraz separated into gene expression trends 9-12..... 218

Appendix D Sugar concentrations in berries used to make wines..... 221

Appendix E Anthocyanin concentration in wines 222

Appendix F Primers used in this study..... 223

Appendix G Volatile compounds with significantly altered abundances in Cabernet Sauvignon, Malian and Shalistin wines made from pressed berry ferments..... 226

Appendix H Volatile compounds with significantly altered abundances in Cabernet Sauvignon, Malian and Shalistin wines made from ferments on free-run juice..... 228

Appendix I Volatile compounds with significantly altered abundances in wines of transgenic 'rose' and 'white' Shiraz with controls 229

Appendix J Volatile compounds with significantly altered abundances in wines of transgenic Chardonnay and controls 230

Appendix K Alignment of VvSCPL1 with homologous proteins that have been functionally characterised 233

Appendix L Description of the SignalP 4.1 prediction output 236

Appendix M Alignment of two annotation of the VvSCPL1 gene 237

Appendix N Supplementary data for manuscript in Chapter 5 239

Reference List 246

ABSTRACT

Anthocyanins are flavonoid compounds responsible for most of the red, purple and blue colours of leaves, fruit and flowers of many plant species. They are produced through the anthocyanin biosynthesis pathway and in grapevine the VvMYBA1 and VvMYBA2 transcription factors are responsible for the transcriptional activation of genes encoding enzymes required for their synthesis. White grapevine cultivars contain inactive versions of the *VvMYBA1* and *VvMYBA2* genes and hence cannot produce anthocyanins in berries. While much is now known about anthocyanin biosynthesis in grapevine, there are still some genes involved in anthocyanin modification and transport which have not yet been identified. In several other plant species recent research has established a link between anthocyanin biosynthesis and the synthesis of volatile aroma compounds.

In this research project, the aim was to further characterise VvMYBA and its role in anthocyanin and flavour metabolism. To do this, transgenic and natural mutant grapevines in which berry colour has been altered due to differential expression of *VvMYBA* genes were used. Two different approaches were taken to investigate the effect of *VvMYBA* gene expression on the transcriptome and flavour metabolism in berries, with the aim of linking transcriptomic changes to metabolomic changes. Microarray analysis was performed to identify differences in global transcription levels in berries differing in their *VvMYBA* gene expression. Microscale wines were also made from both whole berries and free run juice and volatile wine flavour/aroma compounds were analysed using HS-SPME-GC/MS.

This research has shown that the presence of VvMYBA in berries does have an effect on the abundance of volatile flavour/aroma compounds in wines; however this was often in a cultivar specific manner. One conserved difference was that red wines, made from berries expressing *VvMYBA*, contained less linalool compared to white wines, made from berries not expressing *VvMYBA*. Light exclusion studies and transcript analysis of genes associated with linalool metabolism have suggested that the accumulation of anthocyanins in red grapes may cause a shading effect which down-regulates linalool synthesis.

From microarray studies, two putative acyltransferase genes were identified, one belonging to the BAHD protein family and the other to the serine carboxypeptidase-like (SCPL) family. At the commencement of this study, no anthocyanin acyltransferases had been identified in grapevine and it was hypothesised that one or both of these genes could have this function.

Acylation of anthocyanins has been shown to change the hue of the pigment in the fruit and flowers of various plant species, and to increase their stability in products such as wine. Gene expression studies, bioinformatics analyses and *in vitro* and *in planta* functional assays were used to characterised these two genes. Through these studies the first *Vitis vinifera* anthocyanin acyltransferase gene (*VvAnAT*) was identified. VvAnAT belongs to the BAHD acyltransferase protein family and recombinant enzyme kinetic studies show that it can utilise a range of CoA thioester acyl donors and shows a preference towards monoglucoside anthocyanins as the acyl acceptor substrate. Using promoter activation assays the ability of the VvMYBA1 transcription factor to activate the transcription of the *VvAnAT* gene was shown. The putative SCPL gene did not function as an anthocyanin acyltransferase in *in planta* experiments; further studies are required to understand the function of this gene.

The outcomes of this PhD project have added to the current understanding of anthocyanin synthesis and its regulation in grapevine. Knowledge and identification of a grapevine anthocyanin acyltransferase gene can be used in breeding programs aiming to improve grapevine cultivars that cannot currently produce acylated anthocyanins, and hence increase their potential wine colour stability properties.

DECLARATION

This work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Amy Rinaldo and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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SIGNED DATE 12/6/14

Amy Rinaldo

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LIST OF TABLES AND FIGURES

Figure 1.1 - General anthocyanin structure found in grapevine. 8
Figure 1.2 - A schematic of the general flavonoid biosynthetic pathway. 10
Figure 1.3 - Proposed model of how the MYB/bHLH/WD40 complex activates flavonoid structural genes by binding to responsive elements in their promoters. 14
Figure 1.4 - Cabernet Sauvignon bud sports, Malian and Shalistin 18
Figure 1.5 - Transgenic grapevines with altered pigmentation and Vv*MYBA1* gene expression. 20
Figure 2.1: Pie chart representing number of genes with altered expression in transgenic 'red' Chardonnay categorised by function.....33
Figure 2.2: Comparison of numbers of genes with altered transcription in response to *VvMYBA* gene expression in transgenic Chardonnay and Shiraz berries 35
Table 2.1: Genes with altered transcription levels in response to *VvMYBA* gene expression in a consistent manner in both transgenic Chardonnay and Shiraz berries.....36
Table 2.2: Genes with altered expression in transgenic 'red' Chardonnay berries have differential expression when comparing skin transcriptomes to those of whole berries.....41
Figure 2.3: Representation of gene expression trends of genes with altered transcription in transgenic 'rose' and 'white' Shiraz compared to control berries 53
Table 2.3: Genes with altered expression in transgenic 'white' or 'rose' Shiraz separated into gene expression trends (1-8, see Figure 2.3) 54
Figure 3.1: How the phenylpropanoid biosynthesis pathway links anthocyanin and volatile aroma compound biosynthesis.....87
Figure 3.2: The methylerythritol phosphate (MEP) pathway.....90
Figure 3.3: Anthocyanin differences in wines made from berries with altered *VvMYBA* gene expression.....100
Table 3.1: Normalised peak areas of flavour compounds with significant differences in the different coloured wines of Cabernet Sauvignon, Chardonnay and Shiraz, and their colour mutants with altered *VvMYBA* gene expression.....104
Table 3.2: Monoterpenes in wines made from Cabernet Sauvignon, Chardonnay and Shiraz and their colour mutants with altered *VvMYBA* gene expression 107
Figure 3.4: Transcript levels of genes with links to monoterpene biosynthesis in berry skins of Cabernet Sauvignon, Malian and Shalistin.....109

Figure 3.5: Transcript levels of a linalool synthase gene in berries of Cabernet Sauvignon, Malian and Shalistin 111
Figure 3.6: Shade effect on linalool abundance in wines made from Cabernet Sauvignon, Malian and Shalistin berries. 113
Figure 3.7: Transcript levels of MEP pathway genes in whole berries of Cabernet Sauvignon and its colour sports 117
Figure 3.8: Transcript levels of MEP pathway genes in berry skins of Cabernet Sauvignon and its colour sports 119
Figure 4.1: Signal P 4.1 prediction result obtained using the VvSCPL protein sequence 142
Figure 4.2: Schematic comparing exon/intron structure of two annotations of the *VvSCPL1* gene sequence 144
Figure 4.3: Agarose gel of 5' RACE fragments obtained from Cabernet Sauvignon and Pinot Noir *VvSCPL1* transcripts in berries 147
Figure 4.4: Sequence alignment of *VvSCPL1* transcript 5' ends from 5' RACE of Cabernet Sauvignon and Pinot Noir mRNA 149
Figure 4.5: Transcript levels of *VvSCPL1* over the development of Cabernet Sauvignon, Malian and Shalistin berries 151
Figure 4.6: Transcript levels of *VvSCPL1* over the development of Cabernet Sauvignon and Pinot Noir berries 153
Figure 4.7: VvMYBA1 trascriptional activation assays of *VvUFGT* and *VvSCPL1* gene promoters 155
Figure 4.8: Chromatograms of anthocyanins in wildtype and transgenic tobacco expressing *VvSCPL1* 157

ABBREVIATIONS

Units

°C	degrees Celcius
aa	amino acid
bp/kb/Mb	base pairs/kilobase pairs/megabase pairs
g	relative centrifugal force
g; mg; µg	gram; milligram; microgram
h	hour
kPa	kilopascal
L; ml; µl	litre; millilitre; microlitre
M; mM; µM; nM	molar (moles per L); millimolar; micromolar; nanomolar
min	minute
s	second
TTS	total soluble solids
Vol	volume
w/v	weight per volume
wpf	weeks post flowering
wpv	weeks post veraison

Flavonoid pathway

anthoMATE	anthocyanin multidrug and toxic efflux transporter
4CL	4-coumaroyl CoA ligase
ANR	anthocyanidin reductase
bHLH	basic helix-loop-helix
CHI	chalcone isomerase
CHS	chalcone synthase
DFR	dihydroflavonol 4-reductase
F3'5'H	flavonoid 3',5'-hydroxylase
F3H	flavanone-3-hydroxylase
F3'H	flavonoid 3'-hydroxylase
FAOMT	flavanol and anthocyanidin-glucoside 3',5'-*O*-methyltransferase
FGT	flavonol glucosyltransferase
FLS	flavonol synthase
GST	glutathione-S-transferase
LAR	leucoanthocyanidin reductase
LDOX	leucoanthocyanidin dioxygenase
MYB	transcription factor family named after the first gene identified in the family *Myeloblast*
MYC	transcription factor family named after the first gene identified in the family *myelocytomatosis viral oncogene*
PA	Proanthocyanidin (condensed tannins)

Flavonoid pathway continued....

PAL	phenylalanine ammonia lyase
R2R3-MYB	class of MYB TFs containing a two-repeat R2R3 DNA binding domain
UFGT	UDP-glucose flavonoid 3-*O*-glucosyltransferase
WD40 TF	A class of transcription factors containing tandem repeats of a structural motif terminating in a tryptophan-aspartic acid (W-D) dipeptide
WDR	tryptophan-aspartic acid repeat protein
WRKY TF	A class of DNA binding transcription factors that contain a conserved WRKYGOK amino acid sequence

Methylerythritol (MEP) pathway and linalool synthesis

bOci	E-β-ocimene synthase
CDP-ME	4-(cytidine 5'-diphospho)-2-C-methyl-D-erythritol
CDP-MEP	2-phospho-4-(cytidine 5'-di-phospho)-2-C-methyl-D-erythritol
CMK	CDP-ME kinase
CMS	CDP-ME synthase
DMAPP	dimethylallyl diphosphate
DXP	1-deoxy-D-xylulose 5-phosphate
DXR	DXP reductoisomerase
DXS	DXP synthase
G3P	glyceraldehyde 3-phosphate
GPP	geranyl diphosphate
GPS	GPP synthase
HDS	HMBPP synthase
HMBPP	4-hydroxy-3-methylbut-2-enyl diphosphate
IDI	IPP isomerase
IDS	IPP/DMAPP synthase
IPP	isopentenyl prenyldiphosphate
MCS	ME-cPP synthase
ME-cPP	2-C-methyl-D-erythritol 2,4-cyclodiphosphate
MEP	methylerythritol

General

35S	35S constitutive promoter from the *Cauliflower Mosaic Virus*
A, C, G, T	adenine, cytosine, guanine, thymine
ABC transporter	ATP-binding cassette transporter
AMP	adenosine monophosphate
ANOVA	analysis of varience
ATP	adenosine triphosphate
BAHD	A gene and protein family named after the first letter of the first 4 characterised proteins BEAT, AHCT HCBT and DAT

General continued...

BLAST	basic local alignment search tool
cDNA	complementary DNA
cp	cycle threshold
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DNA	deoxyribonucleic acid
ELIP	early light-inducible protein
EST	expressed tag sequence
ER	endoplasmic reticulum
FC	fold change
GC	gas chromatography
gDNA	genomic DNA
HPLC	high performance liquid chromatography
HS	headspace
LC	liquid chromatography
MS	mass spectrometry
NADH	nicotinamide adenine dinucleotide
NCBI	National Centre for Biotechnology Information
N-terminal	amino-terminal
PLACE	plant cis-acting regulatory DNA elements
qPCR	quantitative polymerase chain reaction
QTL	quantitative trait locus
RACE	rapid amplification of cDNA ends
RNA	ribonucleic acid
RT-PCR	reverse transcription-polymerase chain reaction
SAM	significance analysis of microarray
SCP	serine carboxypeptidase
SCPL	serine carboxypeptidase-like
SMT	1-*O*-β-sinapoylglucose:L-malate sinapoyltransferase
SPME	solid phase microextraction
TF	transcription factor
UTR	untranslated region
UV	ultra violetlight