An examination of malate metabolism in *Vitis vinifera* during fruit ripening and in response to elevated vineyard temperature

by

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Abbreviations

ADH	Alcohol dehydrogenase
ADP	Adenosine diphosphate
ANGIS	Australian National Genomic Information Service
AOX	Alternative oxidase
ATP	Adenosine triphosphate
BLAST	Basic Local Alignment Search Tool
BSA	Bovine serum albumin
cDNA	Complementary DNA
CH/BL	Chamber / blower heat treatment
CoA	Coenzyme A
COX	Cytochrome oxidase
Ct	Cycle threshold
DEDTC	Diethyldithiocarbamate
DFCI	Dana Farber Cancer Insitute
DNA	Deoxyribonucleic acid
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
EST	Expressed sequence tag
H_2O_2	Hydrogen peroxide
KCl	Potassium chloride
KCN	Potassium cyanide
MDH	Malate dehydrogenase
ME	Malic enzyme
MES	2-(*N*-morpholino)ethanesulfonic acid
MPA	Metaphosphoric acid
NAD	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide, reduced
NADP	Nicotinamide adenine dinucleotide phosphate
NADPH	Nicotinamide adenine dinucleotide phosphate, reduced
NAD(P)	NAD and NADP
NAD(P)H	NADH and NADPH
NCBI	National Centre for Biotechnology Information

OAA	Oxaloacetate
OG	Octyl gallate
OTC	Open top chamber heat treatment
PCR	Polymerase chain reaction
PDC	Pyruvate decarboxylase
PEG	Polyethylene glycol
PEP	Phospho*enol*pyruvate
PEPC	Phospho*enol*pyruvate carboxylase
PEPCK	Phospho*enol*pyruvate carboxykinase
PEPCkin	Phospho*enol*pyruvate carboxylase kinase
PK	Pyruvate kinase
PMSF	Phenylmethylsulfonyl fluoride
PVP	Polyvinylpyrrolidone
PVPP	Polyvinylpolypyrrolidone
RNA	Ribonucleic acid
mRNA	Messenger RNA
RT-PCR	Reverse transcriptional PCR
qRT-PCR	Quantitative real-time PCR
SA	Surface area
T_a	PCR cycle annealing temperature
T_m	PCR cycle melting temperature
TCA	Tricarboxylic acid
TES	3-(*N*-morpholino)propanesulfonic acid
TMPD	*N*,*N*,*N*',*N*'-tetramethyl-p-phenylenediamine
WGS	Whole genome shotgun
bp / kb	Base pairs / kilobases
˚C	Degrees, Celsius
g/mg/µg/ng	Gram / milligram / microgram / nanogram
L/ml/µl	Litre / millilitre / microlitre
M	Molar (moles.litre^{-1})
mM/µM/nM	Millimolar / micromolar / nanomolar
v/v	volume / volume
w/v	weight / volume

Summary

Acidity is an important characteristic for many fruits, particularly in aspects of fruit biochemistry and sensory quality. In grapes (*Vitis vinifera*), one of the most important agricultural fruiting crops, the use of fruit with low acidity and high pH in winemaking can increase risk of microbial spoilage and undesirable fermentative outcomes. Levels of the organic acid malate can determine acidity and pH in many fruits, through species- and cultivar-specific regulation of developmental and environmental responses. Malate is also extensively involved in primary and secondary metabolic pathways that can be critical to fruit development and ripening. The grape berry demonstrates particularly striking changes in malate accumulation and degradation during development and in response to vine temperature, likely driven by changes in metabolic pathways involving the acid. If harvested late in the season, or if ripening berries are exposed to unusually warm temperatures, the fruit are likely to contain less-than-optimal acid due to reduced levels of malate. Despite the considerable influence that berry acidity imparts on berry and wine quality, the mechanisms of regulation for malate metabolism in response to changes in grape berry development and vine temperature are still largely unknown. This thesis contains an evaluation of the activities and transcripts of enzymes in *V. vinifera* fruit that are involved in processes such as glycolysis, gluconeogenesis, CO_2 assimilation, respiration and fermentation, each involving malate either directly or indirectly. Enzymes from purified grape berry mitochondrial preparations were also explored, providing evidence for two activities that were, until now, undetermined in grapes: NAD-dependent malic enzyme and alternative oxidase. A developmental series of grapes from fruit set until harvest maturity displayed the expected pattern of pre-véraison malate accumulation, and post-veraison malate loss. Activities or transcripts that were differentially regulated with development included NADP-dependent malic enzyme, PEP carboxylase, PEP carboxykinase, pyruvate, orthophosphate dikinase, alcohol dehydrogenase and the terminal oxidases of the mitochondrial transport chain. To test the effect of elevated vine temperature on grape berry malate, field-based heat trials were established at numerous developmental stages. Temperature experiments were set up as mild, long-term heating trials to mimic a climatic shift, as well as shorter, more intense trials to represent heat-wave events. Effects of raising bunch temperature during the day and night were also investigated. Heating of grapevines at the post-set developmental stage (i.e. malate accumulation phase) led to higher berry malate,

particularly when bunches were heated at night. Heating of grapevines around the véraison and pre-harvest stages (i.e. malate degradation phase) generally led to lower berry malate, unless bunches were also heated at night, or if the majority of malate had already been lost from the fruit before the time of treatment. Data indicated that the temperature-sensitive changes in grape berry malate during the day and/or night at different developmental stages could be linked to alterations in PEP carboxylase, PEP carboxykinase, pyruvate, orthophosphate dikinase and NAD-dependent malic enzyme. Implications of such shifts in metabolism are discussed in detail within the text.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

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Statement of the contributions of jointly authored papers

1. **Sweetman, C., Deluc, L.G., Cramer, G.R., Ford, C.M., Soole, K.L.** Regulation of malate metabolism in grape berry and other developing fruits. Review published in *Phytochemistry* on 15th September, 2009.

Author Contributions: CS surveyed the literature, analysed the data and drafted/constructed the manuscript. KLS and CMF contributed to the structure and editing of the manuscript. LGD and GRC assisted with provision and analysis of the microarray data, and with editing of the manuscript.

2. **Sweetman, C., Ford, C.M., Soole, K.L.** Developmental regulation of malate and its involvement in primary and secondary metabolism of *Vitis vinifera* fruit. Manuscript in preparation for submission to *Physiologia Plantarum*.

Author Contributions: CS designed and conducted all research experiments, analysed the data, and drafted/constructed the manuscript. KLS and CMF contributed to the research ideas and design, and the editing of the manuscript.

3. **Sweetman, C., Ford, C.M., Soole, K.L.** Importance of mitochondrial respiration and acid metabolism in *Vitis vinifera* berry development and ripening. Manuscript in preparation for submission to *Physiologia Plantarum*.

Author Contributions: CS designed and conducted all research experiments, analysed the data, and drafted/constructed the manuscript. KLS and CMF contributed to the research ideas and design, and the editing of the manuscript.

4. **Sweetman, C., Soar, C.J., Sadras, V.O., Ford, C.M., Soole, K.L.** Effects of elevated temperature on malate and other aspects of fruit primary metabolism in *Vitis vinifera*. Manuscript in preparation for submission to *The Journal of Experimental Botany*.

Author Contributions: CS assisted with experimental design of the CH/BL field experiment, collected materials, conducted all laboratory research experiments, analysed the data, and drafted/constructed the manuscript. KLS and CMF contributed to the research ideas and the editing of the manuscript. VOS designed field experiments and assisted with data analysis and editing of the manuscript.

The following authors agree that the Statement of the contributions of jointly authored papers accurately describes their contribution to research manuscripts 1., 2., 3., and 4. and give consent to their inclusion in this thesis.

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