The Role of Calcium in the Cell Wall of Grape Berries

By Bradleigh James Hocking

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy

School of Agriculture, Food & Wine

Faculty of Science

The University of Adelaide

September 2015

Table of contents

Abstract

Calcium has defined roles in plant signalling, water relations and cell wall interactions. Calcium nutrition impacts fruit quality by facilitating developmental and stress response signalling, stabilising membranes, and modifying cell wall properties through cross-linking of de-esterified pectins. The importance of calcium in fruit development and ripening is reviewed, experimental work probing the relationship between calcium nutrition and fruit development in grape berries is undertaken.

Relationships between calcium uptake and pectin modification were investigated in a survey of red, white, and table grape varieties collected from two sites varying in calcium levels. Grapes harvested at the Barossa site showed higher calcium concentrations within apoplastic fluid, skin and mesocarp tissues than those from Waite. Chenin Blanc had higher apoplastic calcium content than other varieties. Fluorescent immuno-labelling revealed de-esterified pectin localisation in the middle lamella of all varieties with punctillate staining patterns observed in Grenache and Thompson Seedless. A negative correlation between apoplastic pH and apoplastic calcium concentration was observed. Shiraz was the only variety to demonstrate any significant difference between sites in apoplastic pH and apoplastic calcium activity.

Effects of low and high calcium supply in grapevines were investigated. Low calcium grown Shiraz showed early berry softening and onset of berry weight loss. High calcium grown Shiraz showed delayed and asynchronous fruit development. Berry hydration assays indicated that early onset of berry weight loss in low calcium grown berries was a result of higher post-veraison berry transpiration. High calcium grown berries demonstrated lower berry water uptake rate pre-veraison, and lower berry transpiration rates throughout development. Whole vine physiology was assessed in Chenin Blanc; high calcium grown vines demonstrated reduced transpiration and net assimilation rates compared to basal and low calcium grown vines.

An image analysis macro was developed for quantification of cell vitality (with fluorescein diacetate; FDA) and pectin de-esterification (with propidium iodide; PI) staining patterns. Chenin Blanc maintained higher PI staining in skin tissue than Shiraz throughout development; higher magnification imaging revealed this staining to be localised to the epidermis and peripheral vasculature of Chenin Blanc berries.

Transmission electron microscopy demonstrated cuticle localisation of de-esterified pectin in Chenin Blanc and Shiraz berries, particularly of low calcium grown berries; low levels of calcium-pectin cross-linkages and high rates of berry transpiration result in increased movement of de-esterified pectin from the epidermis into the cuticle. Shiraz cuticle de-esterified pectin levels increased throughout development, indicating pectin solubilisation. Chenin Blanc showed strong de-esterified pectin labelling in epidermal and hypodermal cell walls, consistent with patterns visualised using PI staining. Low calcium grown Chenin Blanc berries showed a higher Botrytis infection rate than basal or high calcium grown berries.

Differences in calcium accumulation and pectin modification contribute to varietal diversity in ripening physiology. Berries supplied with low calcium are early softening and susceptible to shrivel and Botrytis infection, whereas high calcium supply results in changes in vine physiology, including delayed and asynchronous berry development.

Declaration

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name in any university or other tertiary institution 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. In addition, I certify that no part of this work will, in the future, be used in a submission in my name for any other degree or diploma in any university or tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

The author acknowledges that copyright of published works within this thesis resides with the copyright holder(s) of those works.

I also give permission for the digital version to be made available on the web, via the University's digital research repository, the Library Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

…….. …….

Bradleigh James Hocking

Acknowledgements

I thank my supervisors Associate Professor Matthew Gilliham, Associate Professor Rachel Burton, and Professor Steve Tyerman, for their ongoing guidance and support throughout this project. Particular thanks go to Matt for his persistence and encouragement. I greatly appreciate the time Matt has spent reading and discussing this work.

I thank the University of Adelaide, Wine 2030, and the Farrer Memorial Trust, for their support of this project in the form of resources and funding.

I thank my lab colleagues for sharing their enthusiasm and expertise. Particular thanks go to Wendy Sullivan for experimental assistance and Johannes Scharwies for sharing his designs.

I thank the various others who have made significant contributions; Dr Cassandra Collins for her viticultural advice and guidance in data analysis, Cameron Nowell for his assistance in image analysis, Chris Fiebiger for kindly providing experimental material from his vineyard.

I thank my family for their unshakeable faith in me, and all of the things they have taught me. I thank Asmini Athman for her wonderful support and encouragement, without which I would not have completed this journey. I thank my friends for expressing their love of life in conversation and celebration.

List of abbreviations

ABA	Abscisic acid
AGJ	Artificial grape juice
ANOVA	Analysis of variance
apo	apoplast
Ara	Arabinan
BNS	Basal nutrient solution
Cel	Cellulose
Ca^{2+}	Calcium ion
CEC	Cation exchange capacity
CI	Coulure Index
C_m	Membrane capacitance
cyt	cytosol
DAA	Days after anthesis
DW	Dry weight
FDA	Fluorescein di-acetate
GA	Gibberellic acid
Gal	Galactan
HCS	High calcium solution
HG	Homogalacturonan
IAA	Indole acetic acid
ICP-AES	Inductively coupled plasma atomic emission spectroscopy
IRGA	Infra-red gas analyser
ISE	Ion selective electrode
LCS	Low calcium solution
LGO	Live green ovary
MI	Millerandage Index
OGA	Oligogalacturonide
PCA	Principal component analysis
PG	Polygalacturonase
PI	Propidium iodide
PID	PINOID
PIN	PIN-FORMED
PM	Plasma membrane
PME	Pectin methyl-esterase
PMEI	Pectin methyl-esterase inhibitor
QTL	Quantitative trait loci
R_e	Extracellular resistance
R_h	Hydraulic resistance
R_i	Intracellular resistance
R_T	Xylem hydraulic resistance
RG-I	Rhamnogalacturonan-I
RG-II	Rhamnogalacturonan-II
SD	Standard deviation
TEM	Transmission electron microscopy
WAK	Wall associated kinase
XG	Xyloglucan