

The Role of Calcium in the Cell Wall of Grape Berries

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Table of contents

Abstract.....	i
Declaration.....	iii
Acknowledgements.....	iv
List of abbreviations.....	v
Chapter 1 Apoplastic calcium influences hormonal signalling, fruit water status and cell wall composition during fruit ripening	1
1.1 Introduction	1
1.2 Plant calcium uptake, delivery and storage.....	2
1.3 Calcium and water relations in fruit.....	7
1.4 Calcium-cell wall interactions during fruit development	11
1.5 Calcium-hormone interactions during fruit development.....	18
1.6 Conclusion.....	21
Chapter 2 Varietal differences in berry physical properties, nutrient accumulation and pectin distribution.....	23
2.1 Introduction	23
2.2 Materials and Methods.....	23
2.2.1 Bunch and berry sampling.....	23
2.2.2 Microscopy	24
2.2.3 Histological staining.....	25
2.2.4 Immunofluorescent staining.....	25
2.2.5 Berry physical testing.....	26
2.2.6 Apoplast fluid centrifuge method.....	27
2.2.7 Nutrient analysis; low volume apoplast method.....	28
2.2.8 Total soluble sugars, pH and titratable acidity methods.....	29
2.2.9 Apoplastic ion selective electrode (ISE), pH and calcium activity measurements.....	29
2.2.10 Principal component analysis	30
2.3 Results	30
2.3.1 Berry histological staining.....	30
2.3.2 Berry immunofluorescent staining.....	31
2.3.3 Berry physical properties	31
2.3.4 Berry tissue nutrient composition.....	35
2.3.5 Principal component analysis	39
2.4 Discussion	41
2.4.1 Berry phenolics.....	41
2.4.2 Berry cell morphology.....	41
2.4.3 Berry pectin distribution	42
2.4.4 Berry physical changes.....	44
2.4.5 Berry apoplast interactions	44
2.5 Conclusion.....	46
Chapter 3 Grapevine calcium nutrition	48

3.1	Introduction	48
3.2	Materials and Methods	48
3.2.1	<i>Fruiting cuttings; Mullins method modifications</i>	48
3.2.2	<i>Hydroponics system design</i>	49
3.2.3	<i>Biomechanical testing</i>	50
3.2.4	<i>Leaf water potential</i>	50
3.2.5	<i>Gas exchange measurements</i>	50
3.2.6	<i>Root hydraulic conductance</i>	51
3.2.7	<i>ICP-AES analysis</i>	51
3.2.8	<i>Bunch reproductive measures</i>	51
3.3	Results	52
3.3.1	<i>Effect of calcium treatments upon calcium uptake</i>	52
3.3.2	<i>Shiraz berry development responses to modified calcium nutrition</i>	55
3.3.3	<i>Berry class diversity</i>	62
3.3.4	<i>Vine physiology</i>	63
3.3.5	<i>Chenin Blanc nutrient uptake from veraison to late harvest</i>	65
3.4	Discussion	70
3.4.1	<i>Calcium deficiency accelerates berry ripening</i>	70
3.4.2	<i>Elevated calcium impairs berry development</i>	72
3.4.3	<i>Calcium impact on vine fruitset and reproductive indices</i>	73
3.4.4	<i>Chenin Blanc nutrient accumulation consistent with other varieties</i>	74
3.5	Conclusion	74
Chapter 4	Berry ripening physiology; calcium uptake and cell wall modification	76
4.1	Introduction	76
4.2	Materials and Methods	76
4.2.1	<i>Grapevine material</i>	76
4.2.2	<i>Immunogold labelling and transmission electron microscopy</i>	76
4.2.3	<i>Berry staining methods; FDA and PI</i>	77
4.2.4	<i>MATLAB/ImageJ image analysis</i>	78
4.2.5	<i>Berry electrical impedance spectroscopy</i>	79
4.2.6	<i>Berry rehydration assay</i>	79
4.2.7	<i>Biomechanical testing</i>	80
4.2.8	<i>Botrytis wounding assay</i>	80
4.3	Results	81
4.3.1	<i>Cell wall modifications from veraison to late harvest</i>	81
4.3.2	<i>Fresh berry staining</i>	86
4.3.3	<i>Berry electrical impedance spectroscopy</i>	89
4.3.4	<i>Berry physical changes</i>	91
4.3.5	<i>Berry water relations</i>	93
4.3.6	<i>Botrytis susceptibility</i>	94
4.4	Discussion	95
4.4.1	<i>Effect of calcium on pectin distribution</i>	95
4.4.2	<i>Effect of calcium on berry softening</i>	98

4.4.3	<i>Effect of calcium on berry water relations</i>	99
4.4.4	<i>Effect of calcium on cell vitality</i>	100
4.4.5	<i>Effect of calcium on Botrytis susceptibility</i>	103
4.5	Conclusion	104
Chapter 5 General Discussion and Conclusion		105
5.1	Grapevine calcium nutrition	105
5.2	Berry calcium physiology	107
5.3	Implications of calcium nutrition for fruit quality	107
5.4	Future perspectives and Conclusion	110
Appendices		112
Appendix 1		112
Appendix 2		113
Appendix 3		114
Appendix 4		123
Appendix 5		125
Appendix 6		129
Appendix 7		133
Appendix 8		134
Reference list		135

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

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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