Understanding the Physiological Mechanisms of Ripening in Capsicum

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Abstract

Capsicum (*Capsicum annuum* L.) is considered a non-climacteric fruit, exhibiting limited respiration and ethylene levels. The physiological mechanisms of ripening in capsicum have not been fully understood to date, especially the probable reason behind the non-climacteric behaviour. In this thesis, the protein (Chapter 3) and metabolite (Chapter 4) profiles of capsicum at different ripening stages have been reported. Several proteomic and metabolic candidates, for example ACC oxidase (ACO) enzyme, sugars (glucose, fructose, sucrose) and malate were chosen and analysed in different tissue types (peel, pulp and seeds/placenta) and cultivars with different ripening times (Chapter 5). The results suggested that some of these candidates were differentially present in different tissues and cultivars which implied that ripening could be regulated spatially and temporally.

Furthermore, proteomic analysis also identified an ACO isoform 4 (CaACO4) which was found during capsicum ripening onset and corresponded to the increase in the overall ACO activity (Chapter 3). The expression of several *ACO* isoforms including *CaACO4*, and other identified *ACC synthase* (*ACS*) and *Ethylene receptor* (*ETR*) isoforms were therefore characterised to shed some light on their roles in this non-climacteric fruit (Chapter 6). *CaACO4* was the only *ACO* isoform expressed significantly higher during capsicum ripening onset, confirming the earlier proteomic results. The expression of several *ACS* and *ETR* isoforms, normally associated with the climacteric increase of ethylene in tomato (a close relative of capsicum), was also limited as was ACS activity and ACC content. The production of ACC, as an ethylene precursor, may therefore be the rate-limiting step for ethylene production in non-climacteric capsicum. The postharvest application of ethylene did not promote capsicum ripening or induce the expression of most *ACO*, *ACS* and *ETR* isoforms, suggesting they are not regulated by ethylene as usually observed in climacteric fruit such as tomato. However, 1-methylcyclopropene

treatment significantly delayed capsicum ripening postharvest, particularly when applied at Breaker stage (the onset of ripening), suggesting that blocking ethylene perception could affect ripening and that the basal level of ethylene normally produced in non-climacteric fruit may be (partially) required for ripening (Chapter 6).

Other proteomic candidates such as *Copper chaperone*, *TCP chaperone*, *Cysteine synthase* and *Spermidine synthase* were also isolated and investigated due to their possible roles in capsicum ripening. However, unlike *CaACO4*, the RNA expression of these candidates did not follow their respective proteomic trends, suggesting a regulation at the post-translational level (Chapter 7). The identified candidates including *CaACO4* are now a resource for further investigation to identify factors that may be involved in capsicum ripening.

Thesis 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 other 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.

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Abbreviations

1-MCP	1-methylcyclopropene
2DGE	2D-gel electrophoresis
ABA	abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
ACO	ACC oxidase enzyme
ACS	ACC synthase enzyme
ADK	adenosine kinase
ANOVA	analysis of variance
ATP	adenosine triphosphate
βME	β-mercaptoethanol
B	Breaker capsicum fruit
BR1	Breaker Red 1 capsicum fruit
BR2	Breaker Red 2 capsicum fruit
CaGAPdH	*Capsicum annuum glyceraldehyde 3-phosphate dehydrogenase*
CCH	copper chaperone
cv.	cultivar
CYS	cysteine synthase
DAA	days after anthesis
DR	Deep Red capsicum fruit
ETR	ethylene receptor
G	Green capsicum fruit
GC-MS	gas chromatography-mass spectrometry
IPG	immobilised pH gradient
LC-MS	liquid chromatography-mass spectrometry
LR	Light Red capsicum fruit
l.s.d.	least significant difference
Met	methionine
MW	molecular weight
PCR	polymerase chain reaction
pI	isoelectric point
qPCR	quantitative real-time polymerase chain reaction
rin	*ripening inhibitor*
RT	room temperature
SAM	S-adenosyl-methionine
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SPS	spermidine synthase
sqRT-PCR	semi-quantitative Reverse Transcriptase-polymerase chain reaction
TCA/acetone	trichloroacetic acid/acetone
TCA cycle	tricarboxylic acid cycle
TF	transcription factor
Tris	tris(hydroxymethyl)amino methane