بِسْمِ اللهِ الرَّحْمٰنِ الرَّحِيْمِ

The biochemical consequences of ascorbate deficiency in *Arabidopsis thaliana*

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I certify that all material in this thesis which is not my own work has been identified and that no material is included for which a degree has been previously approved from this or any other University

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Dedication

I would like to dedicate this thesis to my mother whose wonderful support and encouragement enabled me to complete my task successfully Alhamdulillah.

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AsA	Ascorbic acid
NADPH	Nicotinamide adenine dinucleotide phosphate (reduced form)
JA	Jasmonic acid
SA	Salicylic acid
ABA	Abscic acid
Prx	Peroxidase
AOX	Ascorbate oxidase
CW	Cell wall
vtc	Ascorbate deficient mutants
LC-MS	Liquid chromatography mass spectrometry
ESI	Electrospray ionisation
CE	Collision Energy
FW	Fresh weight
TIC	Total ion chromatogram
EIC	Extracted ion chromatogram
m/z	Mass to charge ratio
Rt	Retention time
KMPA	Kernal mass peak algorithm
HL	High light
LL	Low light
NCED	9-cis-epoxicarotenoid dioxygenase
F3H	Flavanone 3-hydroxylase
FLS	Flavonol synthase
LDOX	Leucoanthocyanidin dioxygenase

Abstract

Biochemical consequences of ascorbate deficiency were studied in the leaf tissue of *Arabidopsis thaliana* ascorbate-deficient *vtc* mutants with a view of understanding the relationship between ascorbate, stress response and metabolism. Ascorbate is an important antioxidant and is also a cofactor for 2-oxoglutarate-dependent dioxygenases, which are involved in the biosynthesis of a number of metabolites. The response of wild type (Col-0) and *vtc1*, *vtc2-1*, *vtc2-2* and *vtc3-1* mutants to high light intensity, wounding and salinity was investigated using a metabolomics and proteomics approach. Metabolite profiling and comparative proteomics were performed by liquid chromatography-quadrupole time of flight mass spectrometry (LC-QToF MS) and targeted analysis of plant hormones and flavonoids by liquid chromatography triple-quadrupole mass spectrometry (LC-QQQ-MS). These combined analyses revealed the effect of ascorbate deficiency and stress on metabolites and cell wall proteins.

LC-QToF-MS based untargeted metabolite profiling methodologies were developed for analysis of metabolites on a large scale. Using this method about 3000-5000 metabolites (mass-retention time pairs) could be reproducibly detected in *A. thaliana* leaf extract and aligned between samples. Approximately 1000 metabolites were differentially expressed between WT and *vtc* mutants in different experiments. Of these, twenty eight compounds were confirmed to be differentially expressed by LC-QQQ-MS between WT and *vtc* mutants, and eight of these compounds were positively identified and validated with standards.

The plant hormones abscisic acid (ABA), salicylic acid (SA) and jasmonic acid (JA) have all been implicated in plant stress responses and differences in their accumulation in some of the *vtc* mutants have been reported. A systematic study of the response to stress of these hormones in several *vtc* mutants was carried out using LC- QQQ- MS. While some of the mutants showed increased SA and SA-glycoside accumulation, stress-induced ABA and JA accumulation was generally unaffected. Methods for identifying the metabolites in a targeted manner by LC- QQQ-MS was developed and were shown that all *vtc* mutants were impaired in the accumulation of anthocyanin in response to HL treatment. In strong contrast to anthocyanin, flavonol glycosides were not affected by ascorbate deficiency. Therefore, ascorbate deficiency has a specific effect on the anthocyanin biosynthesis.

Ascorbate occurs in the plant cell wall and isolation of apoplastic fluid showed that all *vtc* mutants have decreased apoplastic ascorbate compared to WT. Ionically-bound proteins were from the cell wall of *A. thaliana* leaves. Peroxidase specific activity in this fraction tended to be higher in *vtc* mutants than WT. High light intensity also increased peroxidase activity in WT and *vtc* mutants. To determine which peroxidase isoenzyme caused increased peroxidase activity, ionically-bound cell wall N-glycosylated proteins were isolated by Concanavalin A chromatography and analysed by LC-QToF-MS. Comparison of WT and *vtc2-2* grown in low light and high light identified 937 peptides significantly different between WT and *vtc2-2* and some are also affected by light intensity. Specifically, peroxidases 33 and 34 had increased abundance in *vtc2-2*.

The results show that ascorbate deficiency causes a detectable change in the metabolome of *A. thaliana* leaves, with specific effects on anthocyanin accumulation being detected. Ascorbate deficiency also influences the expression of cell wall proteins. Peroxidase activity is increased, and this response could be related to the increased pathogen resistance reported in *vtc* mutants.