Manipulation of ascorbic acid levels in *Arabidopsis thaliana*.

by

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Abstract

Vitamin C (ascorbic acid) is one of the most essential organic compounds required by the human body for normal metabolic function. Unfortunately, this valuable nutrient is not produced in the human body but most plants and animal can produce this molecule. Although ascorbic acid was not isolated until the early part of the twentieth century, it was known that eating limes and other citrus fruits could ward off the affects of scurvy as early as the 1500's. Ascorbate serves many critical functions in plants as well as the human body. In both, it works as a cofactor in the production of hydroxyproline-rich compounds and helps protect molecules such as proteins, lipids and fatty acids from oxidation. Although the biochemical pathway in animals has been known since the 1950's (Jackel et al., 1950), the exact process by which ascorbic acid is made in plants has eluded scientists. It was shown in 1963 that the inversion of the hexose carbon chain, which occurs in the animal pathway, is not a possible mode of synthesis in plants (Loewus, 1963). As an alternative, a non-inversion pathway was proposed, which achieves ascorbic acid using D-mannose and L-galactose as intermediates, referred to as the Smirnoff-Wheeler pathway (Wheeler et al., 1998). It was shown that transforming lettuce (cv. Grand Rapids and Black Seeded Simpson) and tobacco (cv. Xanthi) with the terminal enzyme in the animal biosynthetic pathway (GLO; L-gulono-γ-lactone oxidase) increases the ascorbic acid content between 4 and 7 fold. It was also shown through feeding studies that wild type tobacco plants had elevated ascorbate levels when fed the animal precursor (Jain and Nessler, 2000). These data suggest that at least part of the animal pathway could be present in plants, along with the Smirnoff-Wheeler (1998) pathway.

To further investigate this discovery, wild type and ascorbic acid-deficient *Arabidopsis thaliana* were transformed with the *glo*. Homozygous lines of these transformants were generated and the ascorbic acid levels were compared to the untransformed wild type and mutant plants. Although the wild type plants containing *glo* did not show a significant increase in ascorbic acid production, all five of the *vtc* mutant lines had an increased ascorbic acid content relative to wild type level. These data suggest that an alternative pathway is present in plants that does not require many of the steps in the published Smirnoff-Wheeler (1998) pathway to produce ascorbic acid.

Dedicated to my parents.

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Nomenclature

AP-1	Activator protein-1
cDNA	Complementary deoxyribonucleic acid
Col-0	*A. thaliana* Columbia ecotype
DHA	Dehydroascorbate
DKG	Diketo-D-gluconate
DNA	Deoxyribonucleic acid
EMS	Ethane methyl sulfonate mutation
ER	Endoplasmic reticulum
GalDH	L-Galactose dehydrogenase
GalLD	L-Galactono-γ-lactone dehydrogenase
GalUR	D-Galacturonic acid reductase
GDP	Guanine diphosphate
GLO	L-Gulono-γ-lactone oxidase
L-AsA	L-Ascorbic acid
Leu	*A. thaliana* Landsberg erecta ecotype
L-Gal	L-Galactono-γ-lactone
L-Gul	L-Gulono-γ-lactone
mRNA	Messenger ribonucleic acid
NAD^+	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
NBT	Nitroblue tetrazolium
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
ROS	Reactive oxygen species
UDP	Uridine diphosphate
vtc	Vitamin C deficient mutated locus
WT	Wild type