



# Ascorbic acid regulation in leaves and fruits of tomato ecotypes infected by Eggplant Mottled Dwarf Virus



A. Zarrillo, M. Minutolo, D. Alioto, A. Errico\*

Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, NA, Italy

## ARTICLE INFO

### Keywords:

EMDV  
Antioxidant  
Vitamin C  
Secondary metabolite  
Ascorbic acid  
Ascorbate

## ABSTRACT

Changes in ascorbic acid levels and gene expression of its biosynthetic pathway were studied in *Solanum lycopersicum* plants inoculated with *Eggplant Mottled Dwarf Virus* (EMDV). Data showed that EMDV inoculation caused a significant decrease of tAsA in the leaves of two ecotypes at 7 dpi, before symptom appearance, and at 21 dpi when symptoms were most evident and was restored at 35 dpi when symptoms remained constant. Biosynthetic gene expression decreased at 7 and 21 dpi. In Tondo Giallo the tAsA levels were restored at 35 dpi; which coincided with an upregulation of almost all genes. In immature fruits of Maiese, the decrease of tAsA was accompanied by a substantial down-regulation of genes in L-galactose and/or L-galactonic acid pathways while in Tondo Giallo the constant level of tAsA was associated with an increase of ASA and with an upregulation of gene expression. This could be a direct consequence of a high ascorbate turnover and a low level of oxidative stress. In ripe fruits, the higher tAsA content observed in Maiese was associated with a general upregulation of gene expression, while in Tondo Giallo it could depend on a high rate of ascorbate recycling as suggested by the upregulation of the gene *MDHAR2*. Collectively these results provide evidence that the interaction between *Solanum lycopersicon* and EMDV affects ascorbic acid quality and accumulation in relation to tomato ecotype, plant developmental stage, and fruit ripening stage.

## 1. Introduction

Tomato (*Solanum lycopersicum*) is one of the major vegetable crops grown worldwide. Tomatoes and tomato products are particularly rich in nutritional compounds such as ascorbic acid (AsA), also called vitamin C or ascorbate. AsA represents a fundamental water-soluble antioxidant for animals and plants. In plants, AsA content is highly dependent on genetic diversity and genome plasticity of individual genotype. It is also influenced by environmental conditions such as water status, fertilization levels, and pathogen interaction. This metabolite is mainly synthesized via L-galactose pathway as described by Wheeler et al. (1998). Other biosynthetic routes, including through D-galacturonic acid, myo-inositol and L-gulose, augment the L-galactose pathway, working in different tissues or under different physiological

conditions (Gallie 2013; Zhang et al., 2016). After synthesis, AsA can be used as an enzymatic cofactor or as an antioxidant reacting with reactive oxygen species (ROS), a group of chemically unstable molecules and ions derived from oxygen metabolism. ROS over production can affect cells by damaging nucleic acids, lipids, and proteins. Thus, to avoid damage to cellular processes and to maintain plant growth, development and productivity, the equilibrium between ROS production and detoxification must be tightly regulated. AsA can detoxify ROS directly or by ascorbate peroxidase (APX) action, which catalyzes the electron transfer from AsA to hydrogen peroxide in order to form water and monodehydroascorbate (MDHA) (Gallie 2013). MDHA can spontaneously degrade into dehydroascorbate (DHA) and AsA or become again AsA via monodehydroascorbate reductase (MDHAR), and DHA can be reprocessed to AsA via dehydroascorbate reductase (DHAR)

**Abbreviations:** tAsA, total ascorbic acid; AsA, reduced ascorbic acid; ACCS, 1-aminocyclopropane-1-carboxylic acid synthase; GGP, GDP-L-galactosephosphorylase; GDH, L-galactose dehydrogenase; GMP, GDP-D-mannose pyrophosphorylase; GME, GDP-D-mannose-3',5' epimerase; PGal, polygalacturonase; GalUR, galacturonate reductase; GLDH, galacto-lactone dehydrogenase; GlyH, glycosyl hydrolase; β-Glu, beta-glucuronidase; PME, pectin methyltransferase; MDHAR, monodehydroascorbate reductase; DHAR, dehydroascorbate reductase; AO, ascorbate oxidase; APX, ascorbate peroxidase

\* Corresponding author.

E-mail address: [angela.errico@unina.it](mailto:angela.errico@unina.it) (A. Errico).

<http://dx.doi.org/10.1016/j.scienta.2017.07.043>

Received 21 June 2017; Received in revised form 24 July 2017; Accepted 25 July 2017

Available online 04 August 2017

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**Table 1**  
List of accession ID and primer sequences of genes analyzed in this study.

Gene	Accession ID	Primers
GDP-L-galactose phosphorylase 1 (GGP1)	Solyc06g073320.2.1	gatcgtcagcagaagaagcct ggtttcgcaagcagtgacatc
GDP-L-galactose phosphorylase 2 (GGP2)	Solyc02g091510.2.1	tgccctgaggaggagtgacaga agcccaacaacaatcgttca
L-galactose dehydrogenase (GDH)	Solyc01g106450.2.1	atgagacactcccgcctt aacggatctttccagctgtct
GDP-D-mannose pyrophosphorylase 1 (GMP1)	Solyc03g096730.2.1	ttcggctactcggctcaggc tcgacaagtggctttgggac
GDP-D-mannose pyrophosphorylase 2 (GMP2)	Solyc06g051270.2.1	gtaccgggttgaggccatta aatcaaccagtggctttggg
GDP-D-mannose pyrophosphorylase 3 (GMP3)	Solyc09g011220.2.1	agagaagcagctccatgccca ccaatgtccatcaaaaagcc
GDP-D-mannose-3',5' epimerase (GME)	Solyc09g082990.2.1	tcgctgtgataggagggt tcaccgagtggttcgactga
1-Aminocyclopropane-1-carboxylic acid synthase (ACCS)	Solyc02g091990.2.1	gccatgcgacgtataaaagga ctggtgattagtgcgttgg
polygalacturonase1 (PGal1)	Solyc08g082170.2	ccattcaagtcagcccttt ggtagagcatgcaccagtttt
polygalacturonase2 (PGal2)	Solyc06g009200.2.1	tgagagcagcatgtcaatc aaatgttctcgttgggaataca
galacturonate reductase (GalUR)	Solyc02g063420.2.1	tggtggttaagagcagaagc cgaactggtttagaggctga
galacto-lactone dehydrogenase (GLDH)	Solyc10g079470.2.1	gatgcatacaatcaagcgcg agataggatgcggttgggtc
glycosyl hydrolase (GlyH)	Solyc01g074030.2.1	aatcggttaactctggcaactga cagcagcatgagaagaagt
beta-glucuronidase (β-Glu)	Solyc10g085220.1.1	ttcagctgtgcatgggttg aaacaagattgcgaccactgt
pectin methylesterase (PME)	Solyc12g098340.1.1	taacatttgcctgaggaagatgca ttttgaagtgtttgatccattc
elongation factor 1 alpha (EF-1a)	Solyc06g005060.2.1	gaccaacctctctgaggct atggcctctgggctcatta
monodehydro ascorbate reductase1 (MDHAR1)	Solyc09g009390.2.1	cacaactctattcaaaaggcaag tgttttgaagaacgcatctgtc
monodehydro ascorbate reductase2 (MDHAR2)	Solyc08g081530.2.1	cactgttaaccggcttctcat gtttgcctctcaccaccagc
dehydro ascorbate reductase (DHAR)	Solyc05g054760.2.1	ccatctcgaggtggtctctg gctttcagcacaactcactt
ascorbate oxidase (AO)	Solyc04g054690.2.1	catgggctgatggaacagaa ccctggcacaataggacatt
ascorbate peroxidase1 (APX1)	Solyc06g005150.2.1	tacgggaggacctgatgttcc tctggtttgtctctctgcca
ascorbate peroxidase2 (APX2)	Solyc01g111510.2.1	agacctccgcgctctcatc cgaagcatgatcggagcac
ascorbate peroxidase3 (APX3)	Solyc06g005160.2.1	agcagtttcccacctctcc caacaccagccaactgatgg
ascorbate peroxidase4 (APX4)	Solyc06g060260.2.1	atggggaggtgatgtgtcc gcttctctctctggcact

(Mellidou et al., 2012). Furthermore, AsA can act as cofactor in ethylene biosynthesis (Van Loon et al., 2006; Gallie 2013). Ethylene synthesis is tightly regulated and involves two enzymes, 1-aminocyclopropane-1-carboxylic acid oxidase encoded by multi-gene families (Barry et al., 2000) and 1-aminocyclopropane-1-carboxylate synthase (ACCS) (Barry and Giovannoni 2006). It has been reported that biotic stresses often affect ascorbic acid biosynthesis and content. Pathogens (viruses, bacteria, fungi) may attack certain plants producing disease symptoms (compatible response); whereas in others they may be blocked by plant defense mechanisms causing little or no disease at all (incompatible response). In both compatible and incompatible interactions, pathogens, such as viruses, may alter plant primary and secondary metabolism that results in changes in metabolites, enzyme profiles, and gene expression (Jahangir et al., 2009). Previous studies have tended to focus on AsA alterations and gene regulation of incompatible interactions rather than compatible virus-plant interaction (Fujiwara et al., 2016). Studies have reported that peroxidation via ROS increases in many compatible plant-virus interactions (Clarke et al., 1998; Riedle-Bauer 2000; Hernández et al., 2004). Analysis of ascorbic acid in response to different plant-virus interactions has been variable. An increase of this metabolite has been described in tomato fruits infected with a mild strain of CMV (Tsuda et al., 2005) while a decrease has been recorded in tomato fruits affected by *Tomato yellow leaf curl virus* (TYLCV) (Tajul et al., 2011) and in sunflower leaves affected by *Tomato spotted wilt virus* (TSWV) (Kotakadi et al., 2012). Other studies have revealed that the antioxidant response and ROS accumulation often correlate with the severity of plant symptoms. This has been observed in *Nicotiana benthamiana* infected with two strains of *Pepper mild mottle virus* (PMMoV) and in *Cucumis sativus* and *Cucurbita pepo* infected with CMV and ZYMV (Hakmaoui et al., 2012; Riedle-Bauer 2000). AsA accumulation in plants may also depend on the biosynthesis of ethylene, which is highly increased by virus infection, as reported in leaves of tobacco systemically infected with CMV or TMV (Jameson and Clarke 2002; Van Loon et al., 2006). The changes in ascorbic acid and ethylene content have been correlated with the ability of a pathogen to suppress or activate host gene expression (Gambino et al., 2012). Relatively little information exists on AsA regulation in compatible virus-plant systems, further studies on these interactions could lead to the identification of key genes useful in tomato breeding programs to improve plant virus tolerance as well as to enhance plant nutraceutical properties (Khan et al., 2011; Locato et al., 2013).

Here, we report the ascorbic acid metabolism and related gene expression in a compatible pathosystem consisting of two tomato ecotypes, selected for their contrasting phenotypes and shelf life, and infected with the *Eggplant Mottled Dwarf Virus* (EMDV), a monopartite, negative, single strand RNA virus, belonging to *Rhabdoviruses* (Martelli et al., 2011).

## 2. Materials and methods

### 2.1. Plant material, virus inoculation and detection

Two tomato (*Solanum lycopersicum*) ecotypes from the Campania region of Southern Italy were used in this study: Maiese and Tondo Giallo. Maiese, is a perishable ecotype, characterized by round red fruits intended for the fresh market, while Tondo Giallo is characterized by round yellow-orange fruits with a long shelf life, usually stored as fresh crop during summer and suitable for winter and spring consumption. Seeds, provided by local farmers, were sown in 12 cm plastic

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