



Is monodehydroascorbate reductase activity in leaf tissue critical for the maintenance of yield in tomato?

Vincent Truffault^{a,b,1}, Gisèle Riqueau^a, Cécile Garchery^a, Hélène Gautier^b, Rebecca G. Stevens^{a,*}

^a Institut National de la Recherche Agronomique, INRA, UR1052, Génétique et Amélioration des Fruits et Légumes, CS60094, 84143 Montfavet, France

^b INRA, UR1115, Plantes et Systèmes de culture Horticoles, CS40509, 84914 Avignon Cedex 9, France

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ABSTRACT

Ascorbate redox metabolism and growth have been shown to be linked and related to the activity of enzymes that produce or remove the radical monodehydroascorbate, the semi-oxidized form of ascorbate (ascorbate oxidase or peroxidase and monodehydroascorbate reductase respectively). Previous work in cherry tomato has revealed correlations between monodehydroascorbate reductase and ascorbate oxidase activity and fruit yield: decreased whole plant MDHAR activity decreases yield while decreased whole plant ascorbate oxidase activity increases yield under unfavourable environmental conditions. We aimed to investigate if similar effects on yield are obtained in a large-fruited variety of tomato, Moneymaker. Furthermore we wished to establish whether previously observed effects on yield in cherry tomato following changes in whole plant enzyme activity could be reproduced by reducing MDHAR activity in fruit only by using a fruit-specific promoter in cherry tomato (West Virginia 106). In Moneymaker, RNAi lines for monodehydroascorbate reductase did not show significant yield decrease compared to control lines when plants were grown under optimal or non-optimal conditions of carbon stress generated by mature leaf removal. In addition, we show that a decrease in monodehydroascorbate reductase activity in fruit of cherry tomato had no effect on yield compared to a reduction in whole-plant monodehydroascorbate reductase activity: we therefore show that whole plant MDHAR activity is necessary to maintain yield in *cherry* tomato, suggesting that the carbon source in autotrophic tissue is more important than fruit sink activity. The present data also revealed differences between cherry and large fruited tomato that could be linked to a source of genetic variability in the response to monodehydroascorbate metabolism in tomato: maybe the domestication of tomato towards large-fruited lines could have affected the importance of MDHAR in yield maintenance.

1. Introduction

The redox status of plant cells influences plant growth and development through the network involving reactive oxygen species, antioxidants and hormones (Bartoli et al., 2013; Considine and Foyer, 2013; Kocsy et al., 2013; Schippers et al., 2016). As ascorbate is present in high concentrations in all cells, its redox status, which is maintained by the enzymes of the ascorbate-glutathione cycle, is important in controlling the redox state of the cell. Under optimal growth conditions, ascorbate and glutathione redox couples are maintained in a highly reduced state. The reduced form of ascorbate is oxidized, enzymatically or directly, into monodehydroascorbate (a radical; MDHA), with the loss of a single electron; dehydroascorbate (DHA) is then generated from the disproportionation of the radical form or the loss of a second

electron from MDHA. Recycling of ascorbate is carried out by monodehydroascorbate reductase (MDHAR) which is an NADH-dependent enzyme and dehydroascorbate reductase (DHAR) which uses glutathione as an electron donor. Two DHAR genes have been mapped in tomato (Stevens et al., 2007) and MDHAR also belongs to multigene family as shown by the presence of three isoforms in tomato (Stevens et al., 2007) and six in *Arabidopsis* (Lisenbee et al., 2005). The isoforms, localized in different organelles, can have different biological effects on global metabolism; the isoform targeted in this study, MDHAR3, is a cytosolic and peroxisomal enzyme (whereas MDHAR1 and MDHAR2 have been identified respectively in chloroplasts and in peroxisomes; (Gest et al., 2013; Haroldsen et al., 2011)). Dual targeting to both peroxisomes and cytosol has also been reported for glutathione reductase which is another ascorbate-glutathione cycle enzyme (Kataya

Abbreviations: MDHA(R), monodehydroascorbate (reductase); DHA(R), dehydroascorbate (reductase); WT, wild type; RNAi, RNA interference

* Corresponding author.

E-mail address: Rebecca.stevens@inra.fr (R.G. Stevens).

¹ Present address: Centre Technique Interprofessionnel des Fruits et Légumes, CTIFL, 35 allée des Sapins, 44483, Carquefou, France.

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and Reumann, 2010). It appears that chloroplastic MDHAR activity correlates positively with ascorbate content (Li et al., 2010), while MDHAR3 has shown negative correlations with ascorbate content (Gest et al., 2013; Haroldsen et al., 2011) in different genotypes.

Ascorbate and the activity of ascorbate recycling enzymes have been shown to be correlated to plant growth and yield. For example, in rice, over-expression of DHAR led to an improvement in terms of yield and biomass which was associated with an increase in photosynthetic activity, but also to better tolerance of environmental stress in the field (Kim et al., 2013). The authors suggested this was related to the co-activation of MDHAR, ascorbate peroxidase and glutathione reductase leading to higher ascorbate content and a less oxidized state of the ascorbate pool. High ascorbate content resulting from over-expression of the biosynthetic pathway genes also led to positive effects on growth and biomass in *Arabidopsis* (Lisko et al., 2013). It has also been shown that silencing of ascorbate oxidase activity improved yield in cherry tomato under unfavourable conditions (Garchery et al., 2013) and the opposite effect was seen on reducing MDHAR3 activity, again in cherry tomato: yield and vegetative growth were decreased with more pronounced effects being seen under conditions of carbon limitation (Truffault et al., 2016). At a cellular level, ascorbate metabolism and growth (cell division and expansion) are often closely related: the reduced form of ascorbate seems to stimulate mitosis (de Pinto et al., 1999; Liso et al., 1984) unlike DHA which is an inhibitor of cell cycle progression (Potters et al., 2000). It has also been shown that the radical MDHA stimulates cell expansion (Gonzalez-Reyes et al., 1994). Ascorbate oxidase activity also seems to stimulate cell expansion (Kato and Esaka, 2000) and the enzyme is suggested to regulate auxin levels at least in root meristems (Kerk et al., 2000).

These studies point to pleiotropic effects of the ascorbate molecule and its oxidized forms on both whole plant and cellular physiology. We have been particularly interested in how the ascorbate redox state controls growth processes, in particular yield, under different conditions. The genetic background is also a deciding factor in the establishment of yield as different genotypes have very different harvest indices and growth habits (determinate or indeterminate). Domestication has increased the harvest index of many crops, including tomato (Tanksley, 2004), and in tomato has led to breeding of larger-fruited genotypes (Cong et al., 2002; Frary et al., 2000) typically with harvest indices of up to 60–65% (Ho, 1984; van der Ploeg et al., 2007). Cherry tomatoes represent an intermediate type of tomato between cultivated tomato (*Solanum lycopersicum*) and the closest wild ancestor (*Solanum pimpinellifolium*) and are probably the first domesticated form (Nesbitt and Tanksley, 2002; Ranc et al., 2008), their harvest index tends to be much lower compared to modern large-fruited varieties, presumably because of increasing sink strength of fruit during domestication.

We have previously identified differences in yield in cherry tomato when enzymes controlling ascorbate recycling are manipulated (Garchery et al., 2013; Truffault et al., 2016). The yield phenotypes appeared to be correlated to photosynthesis and sugar metabolism and particularly the environmental conditions. Our hypothesis was that carbon metabolism and/or translocation of sugars from leaves to fruits was affected in these plants. To test this hypothesis we developed two parallel experiments firstly we observed effects on yield in a large-fruited tomato where the harvest index (the ratio of total fruit yield to total plant biomass) is higher than in cherry tomato and therefore may be subject to different controls determining yield. For this the cultivated genotype Moneymaker was chosen. We studied the impact of modified ascorbate recycling in Moneymaker, under optimal growth conditions and conditions of carbon limitation, applied by leaf removal. Secondly we tested the hypothesis that sugar allocation was affected between leaves and fruit as previous results had been shown using plants with reduced whole-plant MDHAR activity. Thus, we investigated the modification of ascorbate recycling in fruits only as compared to the whole plant by using a fruit-specific promoter to control MDHAR silencing.

We show that MDHAR activity does not significantly affect yield in Moneymaker. Also a reduction in fruit MDHAR activity does not affect yield in contrast to a reduction in whole plant MDHAR activity.

2. Materials and methods

2.1. Plant material

2.1.1. Moneymaker

Solanum lycopersicum L. cv Moneymaker cotyledons were transformed using the MDHAR RNAi construct as previously described (Gest et al., 2013) (Solyc09g009390) and the AO RNAi construct described in Garchery et al. (2013) (Solyc04g054690). The presence of the relevant transgene was verified by PCR. The six independent RNAi lines silenced for the MDHAR isoform used in this paper were MD-2MK, MD-3MK, MD-6MK, MD-25MK, MD-26MK and MD-29MK. Moneymaker RNAi lines for AO were labeled AO-2MK, AO-3MK, AO-4MK, AO-21MK, AO-22MK, AO-24MK. Lines studied were from the T1 generation, selected on kanamycin and were heterozygous or homozygous for the transgene and selected based on the reduction in enzymatic activity. Wild type plants were used as controls, WT_MK1 and WT_MK2, were from two separate seed bulks and treated independently during the experiments.

2.1.2. West Virginia 106

Solanum lycopersicum L. cv West Virginia 106 (cherry tomato) was transformed with the RNAi MDHAR construct (see above) described in Gest et al. (2013). In the present study, the independent line under-expressing MDHAR3 is mds5. This line, conjointly with 2 others independent lines under-expressing MDHAR3 (mds3 and mds42), have been previously characterized in terms of MDHAR activity and yield (Truffault et al., 2016). The RNAi fragment was also cloned into a Gateway™ compatible vector, pK8GWIWG-PPC2-B4, where the 35S promoter was replaced with a fruit specific promoter from the phosphoenolpyruvate carboxylase gene which is highly expressed during the phase of rapid fruit growth: this promoter has therefore been used to express or silence genes in tomato fruit as described previously (Fernandez et al., 2009; Guillet et al., 2012). Following transformation, measurements of MDHAR activity were carried out on fully-expanded leaves and red-ripe fruit of individual T0 plants. The lines were chosen based on the fact that they respected the following criteria (i) no change in leaf MDHAR activity compared to untransformed control lines (ii) a decrease in fruit MDHAR activity compared to untransformed or other control plants. On this basis we were able to select the following lines, the percentage fruit MDHAR activity compared to control (100%) is shown in brackets: Pmds1 (24%), Pmds4 (9%), Pmds5 (16%), Pmds6 (17%), Pmds14 (13%), Pmds15 (17%). T- is a non-transgenic segregating sibling fruit promoter plant. The non-transformed control line was labeled WT.

2.2. Growth conditions and experimental design

2.2.1. Moneymaker

Moneymaker plants for the yield experiments were grown in a Venlo-type greenhouse located in Avignon (44°N), France. The experiment took place in Autumn 2012 in accordance with commercial practice in terms of plant nutrition and pest control. Plants were potted into 5L-pots containing potting compost. Water was supplied to plants using a drip irrigation system to maintain 20–30% drainage. The day/night temperature in the greenhouse was maintained at 22 °C/16 °C and relative humidity at 70–80%. Flowers were mechanically pollinated three times a week and side shoots removed as they appeared. Total fruit yield was estimated by a precise counting of the number of fruit per truss and weight of the fruits from the first six trusses. For the leaf removal experiment (carbon limitation) only 1 leaf was kept per truss, other leaves were removed (see Fig. 1) once fruit were set. 6 plants per condition and genotype were used as replicates.

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