Evolution of the Metabolic Network Leading to Ascorbate Synthesis and Degradation Using *Marchantia polymorpha* as a Model System

Jarrod Creameans, Karina Medina-Jiménez, Tanya Gómez-Díaz, Viankail Cedillo-Castelán, Dulce Flores-Martínez, Adolfo Aguilar-Cruz, Omar Altehua-Lopez, Grecia Lopez-Ramirez, Ana E. Dorantes-Acosta, John L. Bowman, Argelia Lorence, and Mario A. Arteaga-Vazquez

Abstract In plants, L-ascorbic acid (AsA) is a functional enzyme cofactor, a major antioxidant, and a modulator of several biological processes including photosynthesis, photo-protection, cell wall growth and expansion, tolerance to environmental stresses, and synthesis of other molecules. One of the major roles of AsA in plants is detoxifying reactive oxygen species (ROS) such as singlet oxygen or peroxide radicals. ROS are produced when plants undergo biotic or abiotic stresses and if accumulated in high concentrations, can cause damage to macromolecules such as nucleic acids, membrane lipids, and proteins. Until now, little study has been done on ascorbate metabolism in liverworts. Bryophytes (liverworts, hornworts, and mosses) comprise the earliest diverging land plant lineages that came about approximately 360–450 million years ago between the Ordovician and Devonian periods. The ancient liverwort *Marchantia polymorpha* is an emergent model system specifically suited to use in the study of the evolution of different biosynthetic pathways.

J. Creameans
Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR, USA

K. Medina-Jiménez · T. Gómez-Díaz · D. Flores-Martínez · A. Aguilar-Cruz
O. Altehua-Lopez · G. Lopez-Ramirez · A. E. Dorantes-Acosta · M. A. Arteaga-Vazquez (✉)
Instituto de Biotecnologia y Ecologia Aplicada (INBIOTECA), Universidad Veracruzana, Xalapa, Veracruz, Mexico
e-mail: maarteaga@uv.mx

V. Cedillo-Castelán
Instituto de Biotecnologia (IBT), Universidad Nacional Autonoma de Mexico (UNAM), Cuernavaca, Morelos, Mexico

J. L. Bowman
School of Biological Sciences, Monash University, Melbourne, VIC, Australia

A. Lorence (✉)
Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR, USA

Department of Chemistry and Physics, Arkansas State University, Jonesboro, AR, USA
e-mail: alorence@astate.edu

M. A. Hossain et al. (eds.), *Ascorbic Acid in Plant Growth, Development and Stress Tolerance*, https://doi.org/10.1007/978-3-319-74057-7_16

In this chapter, basal levels of both reduced and oxidized AsA in *M. polymorpha* are reported. Comparative and functional genomics experiments in combination with precursor feeding experiment are also discussed in order to provide valuable insights on the evolution of the AsA biosynthetic pathways.

Keywords Marchantia · Liverworts · Vitamin C · Ascorbic acid · Ascorbate pathways · Pathway evolution

1 Introduction

There are many roles that L-ascorbic acid (AsA, a.k.a. vitamin C) plays in the biochemistry of plants and of them, two are especially important to study in modern plant biotechnology: (1) The antioxidant properties of AsA and (2) AsA's contributions as a modulator of biological processes including photosynthesis, photoprotection, cell wall growth and expansion, tolerance to environmental stresses, and synthesis of other molecules (Smirnoff and Wheeler 2000; Gest et al. 2013). The former of these important functions is in detoxifying reactive oxygen species (ROS), both in enzymatic and nonenzymatic detoxification.

Reactive oxygen species are produced in response to various stresses, biotic and abiotic alike, and serve as messengers for plants to indicate that a change in plant biochemistry is necessary. ROS play a key role in cell signaling processes such as growth, development, response to biotic and abiotic stresses, and apoptosis; however, increased levels cause excessive oxidative stress to macromolecules such as membrane lipids, proteins, and nucleic acids, eventually leading to cellular damage (Bailey-Serres and Mittler 2006). This role in cell signaling is especially apparent in periods with prevalent drought and salinity stress as ROS have a dual role in sensing cellular redox state and in retrograde signaling (Golldack et al. 2014). It has been proposed that different abiotic stresses result in different ROS signatures that determine the specificity of the acclimation response and help tailor the plant to the stress situation (Choudhury et al. 2017).

Liverworts, known collectively with hornworts and mosses as Bryophytes, are the earliest diverging land plant lineages arising approximately 360–450 million years ago between the Ordovician and Devonian periods (Bowman et al. 2016). Species of the genus *Marchantia* are liverworts that have recently emerged as excellent model systems specifically suited to study the evolution of different biosynthetic pathways, including the various routes to AsA. The genus *Marchantia* has been used as a model for almost two centuries (Bowman 2016) and was used in early genome sequencing of chloroplasts (Ohyama et al. 1986) and mitochondria (Oda et al. 1992). *Marchantia* has also been used for sex chromosome sequencing in plants with haploid systems, including gene organization of the Y chromosome (Yamato et al. 2007) and in the study of sex differentiation and determination (Oda et al. 1992).

The interest in *Marchantia* could be accredited in part to its relatively small genome size (230 Mb) with only 20,000 protein coding genes and the fact that gene

families present on it consist of mostly fundamental components (Berger et al. 2016). Liverworts are also being utilized as bioindicators in an increasing number of environmental monitoring programs due to their tolerance to abiotic stresses in different environments (Paciolla and Tommasi 2003). There is more than enough rationale warranting further study on the evolution of biosynthetic pathways in *Marchantia polymorpha*, especially in the context of abiotic stress on plants.

In this chapter, basal levels of both reduced and oxidized AsA were reported in *M. polymorpha*. Bioinformatics approaches to confirm the presence of genes and transcripts of ascorbate biosynthetic and recycling genes in the *Marchantia* genome and transcriptome are also reported. Precursor feeding studies in *Marchantia* in vitro cultures are discussed. We show evidences suggesting that L-galactose, *myo*-inositol, and L-gulono-1,4-lactone are precursors of ascorbate in *Marchantia*. Once combined, this data indicates that *Marchantia* possesses the metabolic machinery to synthesize ascorbate using more than one pathway.

2 Effect of Exogenous Ascorbate in the Phenotype of *Marchantia* Cultures

Levels of ascorbate in plants and animals vary greatly over several orders of magnitude (Pauling 1970; Herrero-Martínez et al. 1998; Gest et al. 2013; Akram et al. 2017). In plants, concentrations of AsA as low as 0.1–0.6 micromol per gram fresh weight (μmol/g FW) have been reported in the moss *Hypnum plumaeforme* (Sun et al. 2010) while levels as high as 170 μmol/g FW have been found in Camu Camu (*Myrciaria dubia)* fruits (Justi et al. 2000; Gest et al. 2013). Variation in AsA levels is also observed within members of the same genus or species. For example, *Solanum pennellii* contains five times more AsA than its domesticated relative *Solanum lycopersicum* (Stevens et al. 2007; Gest et al. 2013).

Information on the role of AsA in basal land plant lineages is very scarce and dominated mainly by reports on the determination of concentration levels for a reduced number of species. In the case of *M. polymorpha*, concentration levels of AsA fall within the low range 0.3 μmol/g FW (Paciolla and Tommasi 2003). Experimental evidence indicates that AsA in *M. polymorpha* is involved in the removal of hydrogen peroxide but the pool of AsA levels upon desiccation decline rapidly (Paciolla and Tommasi 2003). Interestingly, it has been reported that *M. polymorpha* methanol extracts exhibit antioxidant properties and can reduce the formation of free radicals, ROS, and oxidative stress in HEK293 human embryonic kidney cell lines exposed to lead (Saputra et al. 2016).

In order to characterize the effects of exogenous AsA on the development of *M. polymorpha* gametophytes, we established hydroponic cultures that allowed us to provide a constant supply of AsA over the course of the experiments. Gemmae from in vitro cultured *M. polymorpha* plants (accession Takaragaike-1) were grown in hydroponic cultures (half-strength Gamborg media supplemented with 1% sucrose,

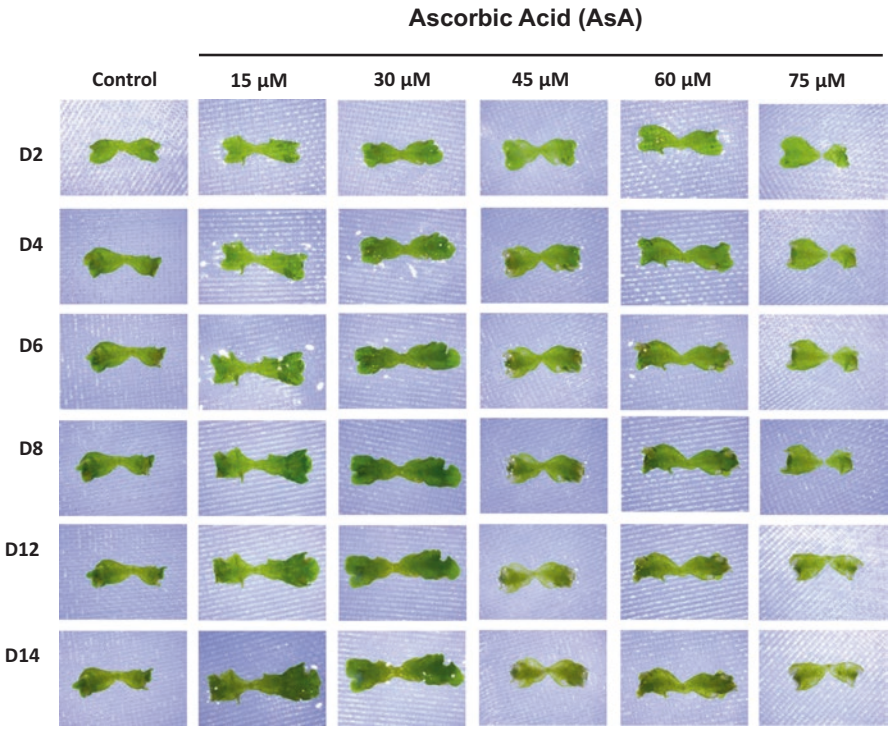

Fig. 1 Exogenous AsA induces dramatic effects on *Marchantia polymorpha* development. *Marchantia* gemmae germinated in hydroponic cultures were grown for 7 days under optimal conditions and then fed with increasing concentrations of AsA during (15, 30, 45, 60, and 75 µM) 14 days (14D). AsA was added to the hydroponic cultures every 24 h and development of thalli was recorded every 24 h. Relevant phenotypes at 2 (D2), 4 (D4), 6 (D6), 8 (D8), 12 (D12), and 14 (D14) days are shown. Phenotypically noticeable effects were observed in plants grown under 45 µM of AsA for 4 days (D4) as evidenced by the arrest of growth, the presence of brown patches around the apical notches and subsequent chlorosis. In sharp contrast, plants exposed to 15 and 30 µM showed increased growth relative to control plants grown under the same conditions but in the absence of AsA

1% agar, and different concentrations of AsA) in a growth chamber at 22 °C under an 18 h light/6 h dark photoperiod regime. First, gemmae were grown in hydroponic cultures without AsA for 7 days in order to allow for the establishment of the dorsoventral pattern, the germination of rhizoids, and the proper development of thalli. On the eighth day after culture, the media was supplemented with different concentrations of AsA that were applied at the same time every 24 h. We documented the development of each individual plant upon exposure to five different concentrations (15, 30, 45, 60, and 75 µM) of AsA. Concentrations as low as 45 µM induced cell death as evidenced by the presence of brown patches located around the apical meristems of the gemmae (also known as apical notches) (Fig. 1). In addition to cell death, exposure to higher concentrations of AsA (from 60 µM to above) induces photo-bleaching and a reduction in growth rate. In sharp contrast, 15 and

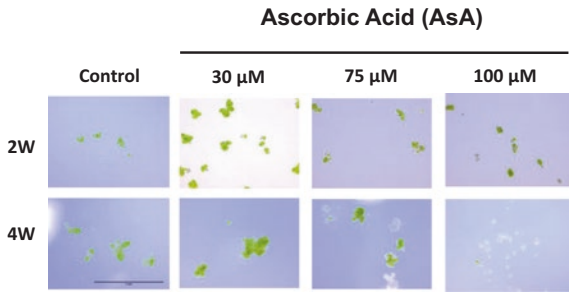

Fig. 2 *Marchantia* sporelings tolerate higher concentrations of exogenous AsA relative to developing thalli. *Marchantia* spores germinated directly in hydroponic cultures supplemented with increasing concentrations of AsA (30, 75, and 100 µM) during 4 weeks (4 W). Development of sporelings was recorded every 24 h and relevant phenotypes are shown after 2 (2 W) and 4 (4 W) weeks. Spores germinated in 75 µM show germination rates and growth patterns at 2 W and 4 W similar to that observed in control spores grown under the same conditions but in the absence of AsA. Similar to that observed in developing thalli, developing spores showed increased growth relative to control plants 2 W after exposure. Spores grown at 100 µM of AsA did germinate and develop for 2 W but ultimately turned chlorotic and died at 4 W

30 µM AsA treatments had positive effects on plant growth. We performed similar experiments on developing sporelings and observed that sporelings are more tolerant to AsA (Fig. 2).

3 Interrogating the *Marchantia polymorpha* Genome to Identify Ascorbate Biosynthetic and Recycling Genes

In order to gain insight into the evolutionary history of the genetic machinery involved in the biosynthesis and recycling of AsA in land plants, we used the well-characterized genetic framework from *Arabidopsis thaliana* as a template to interrogate publicly available Embryophyte genomes and transcriptomes from the Phytozome database (Goodstein et al. 2012), including the recently released *Marchantia polymorpha* genome (phytozome.org) (Bowman et al. 2017). First, we used the repertoire of AsA-related genes from *A. thaliana* to screen the *M. polymorpha* genome and transcriptomes available from Phytozome, the Joint Genome Institute genome-sequencing project (http://www.jgi.doe.gov/) and the Sequence Read Archive (SRA) from the National Center for Biological Information (NCBI) (https://www.ncbi.nlm.nih.gov/sra), using a combination of reciprocal BLAST (Altschul et al. 1990; Gish and States 1993) and conserved domains-based sequence similarity searches using Pfam (Finn et al. 2016) and HMMER (Finn et al. 2011). For phylogenetic inference we employed the Maximum Likelihood (ML) criterion and selected the substitution model that best fit our data from the Akaike Information Criterion (AIC) given its close proximity to the ML method (Anisimova and Gascuel 2001). The AIC estimates the expected distance between the model and the True

Value (deLeeuw 1992) and therefore considers how well the model does fit to the data and its associated variance. The AIC value was calculated using the Prottest software (Darriba et al. 2011) which gave us the best phylogenetic model. With the smallest AIC, the best model of amino acid replacement to infer protein evolution of our data is Whelan and Goldman, which uses an approximate maximum-likelihood method. Branch support was calculated with the approximate Likelihood Ratio Test (aLRT) and the SH (Shimodaira–Hasegawa) correction (Shimodaira 2002) with the software PhyML 3.0 (Guindon et al. 2010). While aLRT calculates verisimilitude logarithms almost as conventional LRT, the branch support made with aLRT-SH has the advantage of being faster and requiring less computational time relative to Bootstrap. The results could differ between both approaches as a consequence of small samples or different levels of divergence (Anisimova and Gascuel 2006). Manual inspection and editing of trees was performed with Geneious version 10.2.3. Based on our phylogenetic inferences, we found homologs for all Arabidopsis genes involved in the biogenesis and recycling of AsA except for two regulators of the pathway, namely *ASCORBATE MANNOSE/GALACTOSE PATHWAY REGULATOR 1* (*AMR1*-AT1G65770) and *ETHYLENE RESPONSE FACTOR 98* (ERF98-AT3G23230) (reviewed in Lisko et al. 2014) (Fig. 3).

Similar to what has been reported for other gene families, the great majority of homologous AsA-related gene families present in the *Marchantia* genome exhibit a reduced number of members per family relative to most of the sequenced genomes from land plants (Fig. 3). In order to explore the evolution of the AsA pathway in land plants, we used the incredible collection of genomic information deposited in Phytozome for the identification of homologous genes through BLAST and a subsequent analysis of the Family History and Gene Ancestry views that rely on the analysis of relationships through Inparanoid analysis and a combination of Smith–Watermann alignments (based on BLOSUM45, gap opening and extension penalty of −12 and −2, respectively, up to a gap length of 50 aa, with zero extension cost after), for each gene in the pathway (Goodstein et al. 2012). The presence and number of genes (including isoforms) are shown in Fig. 3.

3.1 The D-Mannose/L-Galactose (Man/Gal) Pathway

We identified single copy genes for all enzymes involved in the D-mannose/L-galactose (Man/Gal) pathway. Mapoly0082s0088: phosphomannose isomerase (*PMI1*-At3g02570) and *PMI2* (At1g67070); Mapoly0004s0121: phosphomannose mutase (*PMM*-At2g45790); Mapoly0034s0043: GDP-D-mannose pyrophosphorylase (*VITAMIN C1-VTC1*-At2g39770) and *VTC1*-like (At3g55590); Mapoly0101s0064: GDP-D-mannose-3,5-epimerase (*GME*-At5g28840); Mapolv31013666m: GDP-L-galactose phosphorylase (*VTC2*-At4g26850 and *VTC5*-At5g55120); Mapoly0002s0010: L-galactose-1-phosphate phosphatase (*VTC4*-At3g02870); Mapoly0002s0285: L-galactose dehydrogenase (*GalDH*- At4g33670); and Mapoly0077s0021: L-galactono-1,4-lactone dehydrogenase (GLDH- At3g47930) (Fig. 4).

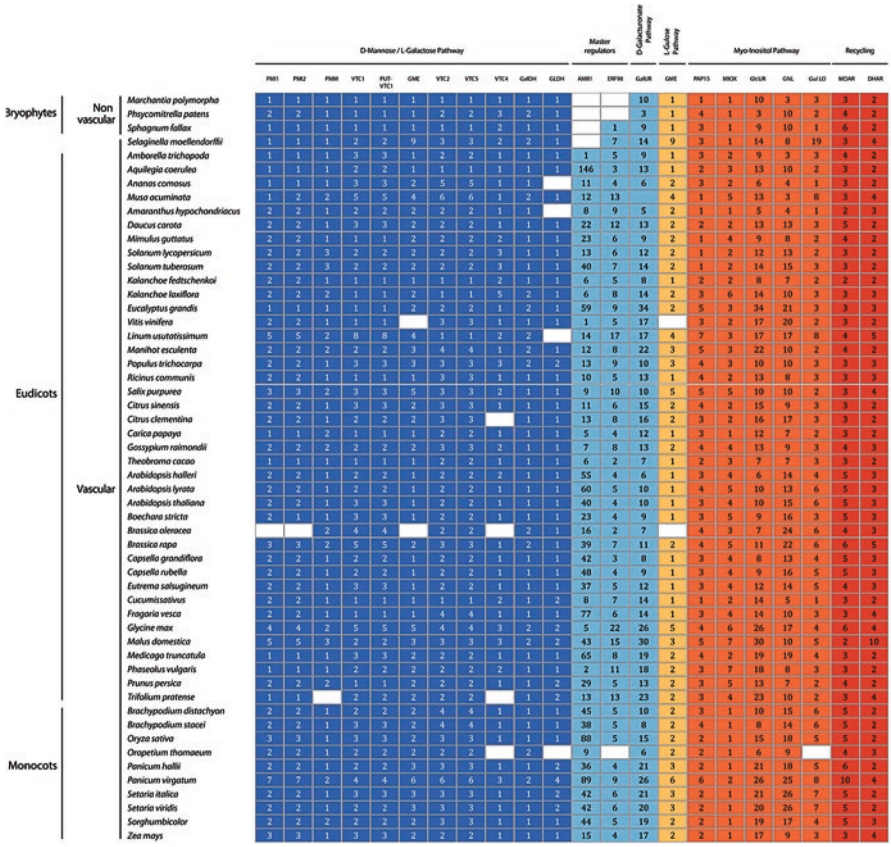

Fig. 3 Conservation of gene families involved in the biogenesis and recycling of AsA in land plants. Sequence similarity searches using BLAST, HMMER, and pfam combined with a phylogenetic analysis allowed us to identify homologous genes involved in the biogenesis and recycling of AsA in *Marchantia*. The number of homologous genes (including isoforms) for each gene family in land plants is indicated inside each colored box and was obtained from the gene ancestry and gene family analysis in phytozome.org. Absence of homologous sequences is represented by blank boxes without a number

3.2 The D-Galacturonate (GalUR) Pathway

For the GalU pathway, we identified 11 *M. polymorpha* genes (Mapolv31014624m, Mapolv31005742m, Mapolv31011830m, Mapolv31011827m, Mapolv31023497m, Mapolv31002560m, Mapolv31005838m, Mapolv31002670m, Mapolv31023489m, Mapolv31011434m, Mapolv31008564m, Mapolv31019915m, Mapolv31011412m, Mapolv31021855m, and Mapolv31004812m) homologous to both copies of the Arabidopsis D-galacturonate reductase (*GalUR*-At1g59950 and At1g59960). This is quite interesting as in this case the number of members in the family in *Marchantia* is higher than that present in Arabidopsis. Undergoing functional genomic approaches

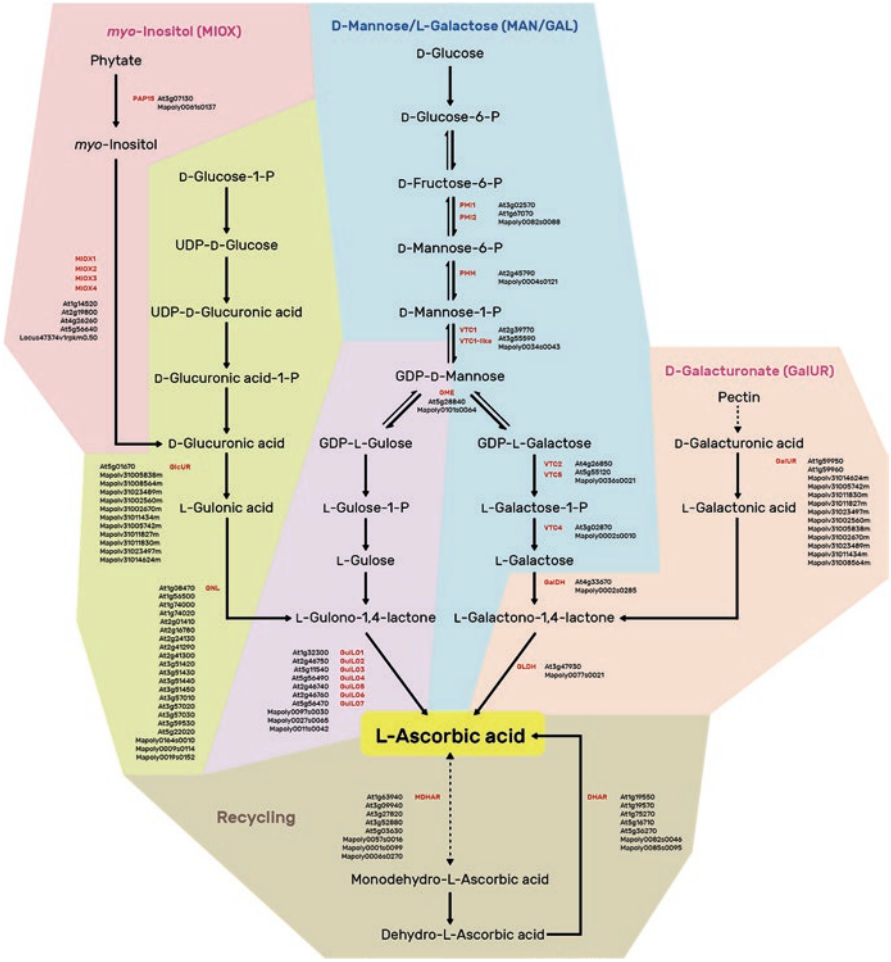

Fig. 4 Pathways involved in the biogenesis and recycling of AsA in *Arabidopsis thaliana* and *Marchantia polymorpha*. The four tested metabolic paths leading to the production of AsA in *Arabidopsis* are depicted. Abbreviated loci names of experimentally identified components for each path are highlighted in red. Each path is shown with a different color. *Arabidopsis* and *Marchantia* loci identifiers are shown next to the abbreviated loci names.

as part of a collaboration between the Lorence and the Arteaga laboratories through forward (genetic screens of mutant plants resistant and oversensitive to AsA) and reverse genetics (CRISPR-Cas based genome edition) will help us unravel the functions and contributions of each member of the family to the pathway (Fig. 4).

3.3 *The* myo-*Inositol (MIOX) Pathway*

In the case of the MIOX pathway, we identified a single homolog (Mapoly0061s0137) for the PAP15 (At3g07130) phytase. We were not able to find a homolog of the *myo*-inositol oxygenase gene in *Marchantia* in the publicly available version of the genome but we were able to identify a homolog in a publicly available transcriptome (Sharma et al. 2014) from immature antheridiophores and in an unpublished transcriptome from mixed tissues (Bowman et al., unpublished). The deduced open reading frame (ORF) from the composite transcriptional units (Locus47374v1rpkm0) is homolog to all four Arabidopsis *myo*-inositol oxygenase genes (MIOX1-At1g14520, MIOX2-At2g19800, MIOX4-At4g26260, and MIOX5-At5g56640).

The next enzyme in the inositol pathway to AsA is D-glucuronate reductase (GlcUR). The Lorence Laboratory has characterized the enzyme encoded by At5g01670 and confirmed that it is a functional reductase of uronic acids with no substrate preference between D-glucuronate and D-galacturonate. Arabidopsis plants overexpressing this gene possess enhanced ascorbate, while knockouts have diminished content of this antioxidant (Yactayo-Chang 2011). While the D-glucuronate reductase (*GlcUR*- At5g01670) family in *Marchantia* is also large with 11 members (Mapolv31005838m, Mapolv31008564m, Mapolv31023489m, Mapolv31002560m, Mapolv31002670m, Mapolv31011434m, Mapolv31005742m, Mapolv31011827m, Mapolv31011830m, Mapolv31023497m, Mapolv31014624m), the gluconolactonase (*GNL*-At1g08470, At1g56500, At1g74000, At1g74020, At2g01410, At2g16780, At2g24130, At2g41290, At2g41300, At3g51420, At3g51430, At3g51440, At3g51450, At3g57010, At3g57020, At3g57030, At3g59530, At5g22020) family in *Marchantia* shows a remarkable reduction with only three members (Mapoly0164s0010, Mapoly0009s0114, Mapoly0019s0152) (Fig. 4). The Lorence Laboratory has characterized the enzyme encoded by At1g56500 and confirmed that it is functional gluconolactonase (GNL). This GNL isoform is localized in chloroplasts. Knockouts on this gene have lower AsA content compared to wild-type controls. Arabidopsis overexpressers and complemented lines (knockouts overexpressing the functional gene) have higher AsA than wild type, enhanced tolerance to high light stress, improved photosynthetic efficiency, and higher seed yield (Yactayo-Chang 2016; Yactayo-Chang and Lorence 2016).

The last enzyme that participates in the intersect between the *myo*-inositol and the L-gulose pathways to ascorbate is L-gulono-1,4-lactone oxidase (GulLO). The *GulLO* gene family in *Marchantia* is composed by three members (Mapoly0097s0030, Mapoly0027s0065, Mapoly0011s0042) that correspond to seven members in Arabidopsis (GulLO1-At1g32300, GulLO2-At2g46750, GulLO3-At5g11540, GulLO4-At5g56490, GulLO5-At2g46740, GulLO6-At2g46760, GulLO7-At5g56470). The Lorence Laboratory has recently characterized GulLO5 and has confirmed that this enzyme possesses oxidase activity towards L-GulL (Aboobucker et al. 2017).

Wheeler et al. proposed that GulLO was lost in all photosynthetic eukaryotes and functionally replaced with GLDH (Wheeler et al. 2015). However, genetic and biochemical evidence from different groups indicates that the *Arabidopsis* genome

does contain genes encoding GulLO enzymes (Maruta et al. 2010; Aboobucker et al. 2017). Interestingly, overexpression of the rat GulLO enzyme in Arabidopsis can functionally rescue vitamin C (*vtc*) mutants with a concomitant increase in AsA levels (Radzio et al. 2003), and evidences from different groups have demonstrated the effective conversion of L-Gul into AsA in different plant species (Baig et al. 1970; Davey et al. 1999; Pallanca and Smirnoff 1999; Jain and Nessler 2000; Radzio et al. 2003; Davey et al. 2004; Imai et al. 2009; Li et al. 2010; Mellidou et al. 2012; Aboobucker et al. 2017). Based on these evidences and taking into account that irrespective of the considerations inherent to any overexpression experiment and to the potential substrate specificity, the data indicates that plants fed with a specific substrate for GulLO do produce AsA and that AsA levels are increased when increasing the expression of GulLO (reviewed in Lisko et al. 2014). Given there are seven GulLO genes in Arabidopsis, genetic redundancy is hard to overcome in order to functionally characterize the GulLO gene family in that model; however, taking into account there are only three *GulLO* genes in *Marchantia* and the feasibility of generating mutant edited alleles, we are focusing our efforts to functionally test the involvement of GulLO in the biosynthesis of AsA in *Marchantia*.

3.4 Ascorbate Recycling

The monodehydroascorbate reductase (MDHAR-At1g63940, At3g09940, At3g27820, At3g52880, At5g03630) and dehydroascorbate reductase (DHAR-At1g19550, At1g19570, At1wg75270, At5g16710, At5g36270) gene families that regulate the rapid regeneration of reduced AsA in plants are represented in *Marchantia* by three (Mapoly0057s0016, Mapoly0001s0099, Mapoly0006s0270) and two (Mapoly0082s0046 and Mapoly0085s0095) members, respectively (Fig. 4).

4 Precursor Feeding Studies as a Proxy to Test the Operation of Ascorbate Pathways in *Marchantia*

Based on the fact that we detected the presence of *Marchantia* genes and transcripts with significant homology to the genes involved in AsA metabolism, next we interrogated the function of the various pathways to AsA by doing feeding studies with nonradioactive precursors. For this purpose, six ascorbate precursors were added to *Marchantia* cultures growing in B5 growth media (Fig. 5). The tissue with media supplemented with AsA showed the lowest *in planta* AsA concentration of any of the samples, followed by the control, which included no precursors added into the media. This result indicates that feedback inhibition took place, causing decreased AsA synthesis. D-Galacturonate and L-gulose feeding led to modest ascorbate increases but were not found to be significant when statistical analysis was done in comparison to the control. Three precursors, however, did cause

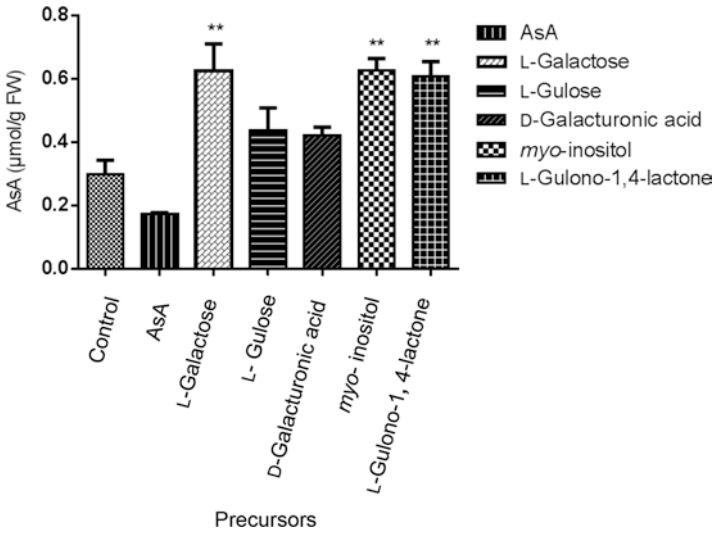

Fig. 5 L-Galactose, *myo*-inositol, and L-gulono-1,4 lactone feeding significantly increased the ascorbate content in *Marchantia*. In vitro *Marchantia* cultures were grown on B5 media; once enough biomass was obtained, cultures were transferred to B5 media supplemented with various ascorbate precursors. Tissue samples were collected and flash frozen 48 h after feeding. Reduced, oxidized, and total ascorbate were determined using an enzyme-based spectrophotometric method. One-way ANOVA and Tukey's post hoc test were performed at significance level of 0.05. p-value < 0.01 was indicated by **. Five biological replicates were used in these assays

significant changes in ascorbate content after analysis using the one-way ANOVA and Tukey's post hoc statistical tests. These precursors were L-galactose, *myo*-inositol, and L-gulono-1,4-lactone. The precursor that resulted in the highest levels of AsA in the thalli was L-galactose, followed by *myo*-inositol and L-gulono-1,4-lactone. These results indicate the operation of at least two of the proposed ascorbate pathways to AsA in *Marchantia*.

5 Conclusions

In summary, *M. polymorpha*, one of the earliest diverging land plants, contains homologous genes to both classical and alternative pathways for the biosynthesis and recycling of AsA and given its characteristics as a powerful model for functional genomics it will surely help aid current efforts to understand how evolution has shaped the biosynthetic pathways of AsA and its role during plant development and responses to the environment in land plants.

Feeding studies with nonradioactive (a.k.a. cold) precursors indicate the functionality in *Marchantia* of both the D-mannose/L-galactose and the *myo*-inositol pathways to ascorbate. Follow-up studies will shed light into the role of specific pools of AsA in the response and adaptation of basal plants to specific stresses.

Acknowledgements Work in the Lorence Laboratory was supported by a sub-award from the NIH Grant # P20 GM103429 from the IDeA Networks of Biomedical Research Excellence (INBRE) program of the National Institute of General Medical Sciences and by the Plant Imaging Consortium (PIC) funded by the National Science Foundation Award Numbers IIA-1430427 and IIA-1430428. AL also thanks startup funds provided by the Arkansas Biosciences Institute, the major research component of the Arkansas Tobacco Settlement Proceeds Act. The Arteaga Group thanks support from the Universidad Veracruzana Cuerpo Académico CA-UVER-234 and funding provided by UCMEXUS-CONACYT grant 1994144OAC7, Consejo Nacional de Ciencia y Tecnología grants CB158550 and CB158561, Jeunes équipes associées à l'IRD (JEAI) grant EPIMAIZE, Royal Society Newton Advance Fellowship #RG79985 and Agropolis Foundation grant EPIMAIZE. G.L.R. is the recipient of a scholarship from the National System of Researchers (SNI) through the Universidad Veracruzana. D.F.M., A.A.C. and O.O.L. are recipients of a graduate scholarship from CONACYT. M.A.A.V. is the recipient of a Royal Society Research Fellowship. All members of the Arteaga Group would like to collectively thank Jocelyn E. Arteaga-Vázquez for her consistent help and support provided during the preparation of figures.

J.L. Bowman thanks funds provided by the Australian Research Council DP160100892.

References

Aboobucker SI, Suza WP, Lorence A (2017) Characterization of two *Arabidopsis* L-gulono-1,4-lactone oxidases, AtGulLO3 and AtGulLO5, involved in ascorbate biosynthesis. React Oxyg Spec 4(12):1–29

Akram NA, Shafiq F, Ashraf M (2017) Ascorbic acid-a potential oxidant scavenger and its role in plant development and abiotic stress tolerance. Front Plant Sci 8:613

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215:403–410

Anisimova M, Gascuel O (2001) A general empirical model of protein evolution derived from multiple protein families using a maximum-likelihood approach. Mol Biol Evol 18(5):691–699

Anisimova M, Gascuel O (2006) Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. Syst Biol 55(4):539–552

Baig MM, Kelly S, Loewus F (1970) L-ascorbic acid biosynthesis in higher plants from L-gulono-1,4-lactone and L-galactono-7,4-lactone. Plant Physiol 46:277–280

Bailey-Serres J, Mittler R (2006) The roles of reactive oxygen species in plant cells. Plant Physiol 141:311

Berger F, Bowman JL, Kohchi T (2016) Marchantia. Curr Biol 26(5):R186–R187

Bowman JL (2016) A brief history of *Marchantia* from Greece to genomics. Plant Cell Physiol 57(2):210–229

Bowman JL, Araki T, Kohchi T (2016) *Marchantia*: past, present and future. Plant Cell Physiol 57(2):205–209

Bowman JL, Kohchi T, Yamato KT, Jenkins J, Shu S, Ishizaki K, Yamaoka S, Nishihama R, Nakamura Y, Berger F, Adam C, Aki SS, Althoff F, Araki T, Arteaga-Vazquez MA, Balasubrmanian S, Barry K, Bauer D, Boehm CR, Briginshaw L, Caballero-Perez J, Catarino B, Chen F, Chiyoda S, Chovatia M, Davies KM, Delmans M, Demura T, Dierschke T, Dolan L, Dorantes-Acosta AE, Eklund DM, Florent SN, Flores-Sandoval E, Fujiyama A, Fukuzawa H, Galik B, Grimanelli D, Grimwood J, Grossniklaus U, Hamada T, Haseloff J, Hetherington AJ, Higo A, Hirakawa Y, Hundley HN, Ikeda Y, Inoue K, Inoue SI, Ishida S, Jia Q, Kakita M, Kanazawa T, Kawai Y, Kawashima T, Kennedy M, Kinose K, Kinoshita T, Kohara Y, Koide E, Komatsu K, Kopischke S, Kubo M, Kyozuka J, Lagercrantz U, Lin SS, Lindquist E, Lipzen AM, Lu CW, De Luna E, Martienssen RA, Minamino N, Mizutani M, Mizutani M, Mochizuki N, Monte I, Mosher R, Nagasaki H, Nakagami H, Naramoto S, Nishitani K, Ohtani M,

Okamoto T, Okumura M, Phillips J, Pollak B, Reinders A, Rövekamp M, Sano R, Sawa S, Schmid MW, Shirakawa M, Solano R, Spunde A, Suetsugu N, Sugano S, Sugiyama A, Sun R, Suzuki Y, Takenaka M, Takezawa D, Tomogane H, Tsuzuki M, Ueda T, Umeda M, Ward JM, Watanabe Y, Yazaki K, Yokoyama R, Yoshitake Y, Yotsui I, Zachgo S, Schmutz J (2017) Insights into Land Plant Evolution Garnered from the Marchantia polymorpha Genome. Cell 171:287–304

Choudhury FK, Riveri RM, Blumwald E, Mitler R (2017) Reactive oxygen species, abiotic stress and stress combination. Plant J 90:856–867

Darriba D, Taboada GL, Doallo R, Posada D (2011) ProtTest 3: fast selection of best-fit models of protein evolution. Bioinformatics 27(8):1164–1165

Davey MW, Gilot C, Persiau G, Østergaard J, Han Y, Bauw GC, Van Montagu MC (1999) Ascorbate biosynthesis in Arabidopsis cell suspension culture. Plant Physiol 121:535–543

Davey MW, Franck C, Keulemans J (2004) Distribution, developmental and stress responses of antioxidant metabolism in *Malus*. Plant Cell Environ 27:1309–1320

deLeeuw J (1992) Introduction to Akaike (1973) information theory and an extension of the maximum likelihood principle. In: Kotz S, Johnson NL (eds) Breakthroughs in statistics, Springer series in statistics. Springer, New York, NY

Finn RD, Clements J, Eddy SR (2011) HMMER web server: interactive sequence similarity searching. Nucleic Acids Res 39:W29–W37

Finn RD, Coggill P, Eberhardt RY, Eddy SR, Mistry J, Mitchell AL, Potter SC, Punta M, Qureshi M, Sangrador-Vegas A, Salazar GA, Tate J, Bateman A (2016) The Pfam protein families database: towards a more sustainable future. Nucleic Acids Res 44(D1):D279–D285

Gest N, Gautier H, Stevens R (2013) Ascorbate as seen through plant evolution: the rise of a successful molecule? J Exp Bot 64(1):33–53

Gish W, States DJ (1993) Identification of protein coding regions by database similarity search. Nat Genet 3:266–272

Golldack D, Li C, Mohan H, Probst N (2014) Tolerance to drought and salt stress in plants: unraveling the signaling networks. Front Plant Sci 5(151):1–10

Goodstein D, Shu S, Howson R, Neupane R, Hayes RD, Fazo J, Mitros T, Dirks W, Hellsten U, Putnam N, Rokhsar DS (2012) Phytozome: a comparative platform for green plant genomics. Nucleic Acids Res 40:D1178–D1186

Guindon S, Dufayard JE, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol 59:307–321

Herrero-Martínez JM, Simó-Alfonso E, Deltoro VI, Calatayud A, Ramis-Ramos G (1998) Determination of L-ascorbic acid and total ascorbic acid in vascular and nonvascular plants by capillary zone electrophoresis. Anal Biochem 265:275–281

Imai T, Ban Y, Terakami S, Yamamoto T, Moriguchi T (2009) L-Ascorbate biosynthesis in peach: cloning of six L-galactose pathway-related genes and their expression during peach fruit development. Physiol Plant 136:139–149

Jain AK, Nessler CL (2000) Metabolic engineering of an alternative pathway for ascorbic acid biosynthesis in plants. Mol Breed 6:73–78

Justi KC, Visentainer JV, Evelazio de Souza N, Matsushita M (2000) Nutritional composition and vitamin C stability in stored camucamu (*Myrciaria dubia*) pulp. Arch Latinoam Nutr 50:405–408

Li M, Ma F, Liang D, Li J, Wang Y (2010) Ascorbate biosynthesis during early fruit development is the main reason for its accumulation in kiwi. PLoS One 5:e14281. https://doi.org/10.1371/journal.pone.0014281

Lisko K, Siddique IA, Torres R, Lorence A (2014) Engineering elevated vitamin C in plants to improve their nutritional content, growth, and tolerance to abiotic stress. In Phytochemicals – biosynthesis, function and application R Jetter (ed). Recent Adv Phytochem 44: 109–128

Maruta T, Ichikawa Y, Mieda T, Takeda T, Tamoi M, Yabuta Y, Ishikawa T, Shigeoka S (2010) The contribution of Arabidopsis homologs of L-gulono-1,4-lactone oxidase to the biosynthesis of ascorbic acid. Biosci Biotechnol Biochem. 74:1494–7

Mellidou I, Keulemans J, Kanellis AK, Davey MW (2012) Regulation of fruit ascorbic acid concentrations during ripening in high and low vitamin C tomato cultivars. BMC Plant Biol 34:239

Oda K, Yamato T, Ohta E, Nakamura Y, Takemura M, Nozato N, Akashi K, Kanegae T, Ogura Y, Kohchi K, Ohyama K (1992) Gene Organization Deduced from the Complete Sequence of Liverwort Marchantia-Polymorpha Mitochondrial-DNA. A Primitive Form of Plant Mitochondrial Genome. Journal of Molecular Biology 223:1–7

Ohyama K, Fukuzawa H, Kohchi T, Shirai H, Sano T, Sano S, Umesono K, Shiki Y, Takeuchi M, Chang Z, Aota S, Inokuchi H, Ozeki H (1986) Chloroplast gene organization deduced from complete sequence of liverwort Marchantia polymorpha chloroplast DNA. Nature 322:572–574

Paciolla C, Tommasi F (2003) The ascorbate system in two bryophytes: *Brachythecium velutinum* and *Marchantia polymorpha*. Biol Plant 47(3):387–393

Pallanca JE, Smirnoff N (1999) Ascorbic acid metabolism in pea seedlings. A comparison of D-glucosone, L-sorbosone, and L-galactono-1,4-lactone as ascorbate precursors. Plant Physiol 120:453–461

Pauling L (1970) Evolution and the need for ascorbic acid. Proc Natl Acad Sci U S A 67(4):1643–1648

Radzio JA, Lorence A, Chevone BI, Nessler CL (2003) L-Gulono-1,4-lactone oxidase expression rescues vitamin C-deficient Arabidopsis (vtc) mutants. Plant Mol Biol 53(6):837–844

Saputra HM, Mandia S, Retnoaji B, Wijayanti N (2016) Antioxidant properties of liverwort (*Marchantia polymorpha* L.) to lead-induced oxidative stress on HEK293 cells. J Biol Sci 16(3):77–85

Sharma N, Jung CH, Bhalla PL, Singh MB (2014) RNA sequencing analysis of the gametophyte transcriptome from the liverwort, Marchantia polymorpha. PLoS One 9(5):e97497

Shimodaira H (2002) An approximately unbiased test of phylogenetic tree selection. Syst Biol 51:492–508

Smirnoff N, Wheeler GL (2000) Ascorbic acid in plants: biosynthesis and function. Crit Rev Biochem Mol Biol 35(4):291–314

Stevens R, Buret M, Duffé P, Garchery C, Baldet P, Rothan C, Causse M (2007) Candidate genes and quantitative trait loci affecting fruit ascorbic acid content in three tomato populations. Plant Physiol 143:1943–1953

Sun SQ, He M, Cao T, Li JL (2010) Antioxidative responses related to H_2O_2 depletion in *Hypnum plumaeforme* under the combined stress induced by Pb and Ni. Environ Monit Assess 163:303–312

Wheeler G, Ishikawa T, Pornsaksit V, Smirnoff N (2015) Evolution of alternative biosynthetic pathways for vitamin C following plastid acquisition in photosynthetic eukaryotes. elife 4:e06369

Yactayo-Chang J. (2011) Stable co-expression of vitamin C enhancing genes for improved production of a recombinant therapeutic protein, hIL-12, in *Arabidopsis thaliana*. MS Thesis, Arkansas State University, Jonesboro, AR

Yactayo-Chang J. (2016) The role of the chloroplastic and endoplasmic reticulum ascorbate subcellular pools in plant physiology. PhD thesis, Arkansas State University, Jonesboro, Arkansas, USA

Yactayo-Chang JP, Lorence A. (2016) Method of improving chloroplast function. US Patent pending

Yamato KT, Ishizaki K, Fujisawa M, Okada S, Nakayama S, Fujishita M, Bando H, Yodoya K, Hayashi K, Bando T, Hasumi A, Nishio T, Sakata R, Yamamoto M, Yamaki A, Kajikawa M, Yamano T, Nishide T, Choi SH, Shimizu-Ueda Y, Hanajiri T, Sakaida M, Kono K, Takenaka M, Yamaoka S, Kuriyama C, Kohzu Y, Nishida H, Brennicke A, Shin-i T, Kohara Y, Kohchi T, Fukuzawa H, Ohyama K (2007) Gene organization of the liverwort Y chromosome reveals distinct sex chromosome evolution in a haploid system. Proc Natl Acad Sci U S A. 104:6472–7