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ORIGINAL ARTICLE

Deficiency of GDP-L-galactose phosphorylase, an enzyme required for ascorbic acid synthesis, reduces tomato fruit yield

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Abstract

Main conclusion **Reduced GDP-L-galactose phosphorylase expression and deficiency of ascorbic acid content lead to decreased fruit set and yield in tomato plants.**

Abstract Reduced GDP-L-galactose phosphorylase expression and deficiency of ascorbic acid content lead to decreased fruit set and yield in tomato plants. GDP-L-galactose phosphorylase (GGP) catalyzes the first step committed to ascorbic acid synthesis. The participation of GDP-L-galactose phosphorylase and ascorbate in tomato fruit production and quality was studied in this work using two *Sl*GGP1 deficient EMS Micro-Tom mutants. The *Sl*GGP1 mutants display decreased concentrations of ascorbate in roots, leaves, flowers, and fruit. The initiation of anthesis is delayed in *ggp1* plants but the number of flowers is similar to wild type. The number of fruits is reduced in *ggp1* mutants with an increased individual weight. However, the whole fruit biomass accumulation is reduced in both mutant lines. Fruits of the *ggp1* plants produce more ethylene and show higher firmness and soluble solids content than the wild type after the breaker stage. Leaf CO_2 uptake decreases about 50% in both *ggp1* mutants at saturating light conditions; however, O_2 production in an enriched CO_2 atmosphere is only 19% higher in wild type leaves. Leaf conductance that is largely reduced in both mutants may be the main limitation for photosynthesis. Sink-source assays and hormone concentration were measured to determine restrictions to fruit yield. Manipulation of leaf area/fruit number relationship demonstrates that the number of fruits and not the provision of photoassimilates from the source restricts biomass accumulation in the *ggp1* lines. The lower gibberellins concentration measured in the flowers would contribute to the lower fruit set, thus impacting in tomato yield. Taken as a whole these results demonstrate that ascorbate biosynthetic pathway critically participates in tomato development and fruit production.

Keywords Antioxidant · Ascorbate · Fruit · GGP · Ripening · Tomato · Yield

Introduction

Ascorbate is one of the most abundant compounds in plants and there is great interest in its multiple functions as an antioxidant and enzyme cofactor (Foyer and Noctor 2011; Smirnoff 2018). It is synthesized via GDP-mannose

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and GDP-L-galactose and the first enzyme in this pathway considered to be specific to ascorbate synthesis is GDP-L-galactose phosphorylase (Dowdle et al. 2007; Laing et al. 2007; Linster et al. 2007). It is encoded by paralogues in various species, including arabidopsis (*VTC2* and *VTC5*). Double *vtc2 vtc5* mutants which are unable to make ascorbate are not viable but can be rescued by ascorbate supplementation (Dowdle et al. 2007; Lim et al. 2016). A range of other *vtc* mutants and transgenic plants in different parts of the ascorbate biosynthesis pathway with 10–20% of wild type ascorbate concentrations grow relatively normally but exhibit various subtle developmental changes, increased sensitivity to environmental stresses and increased basal resistance to pathogens (Pavet et al. 2005; Barth et al. 2006; Senn et al. 2016; Caviglia et al. 2018; Plumb et al. 2018). Therefore, it is apparent that relatively severe decreases in ascorbate still enable its essential functions while higher concentrations must be assumed to be beneficial. Notably, high light intensity increases ascorbate concentration in leaves, associated with its role in removal of hydrogen peroxide and in photoprotection (Asada 1999; Bartoli et al. 2006). GDP-L-galactose phosphorylase expression is strongly controlled by light and repressed by high ascorbate in part via a conserved upstream open reading frame (uORF) in the 5′-UTR. This, along with over-expression experiments, strongly supports its role in controlling ascorbate biosynthesis (Dowdle et al. 2007; Gao et al. 2011; Yoshimura et al. 2014; Laing et al. 2015; Macknight et al. 2017; Li et al. 2018).

Tomatoes are of interest as a source of ascorbate in the diet and control of its synthesis and functions have been investigated by altering expression of various biosynthesis genes (Alhagdow et al. 2007; Gilbert et al. 2009, 2016). The availability of tomato plants with GDP-L-galactose phosphorylase deficiency enables the role of this enzyme and ascorbate in fruit production and quality (Baldet et al. 2013). Two *GGP* genes encode GDP-L-galactose phosphorylase in tomato with complementary function, and *SlGGP1* is about thousand time more expressed than *SlGGP2* (Massot et al. 2012). The *Slggp1* mutant, although expressing *GGP2*, had low ascorbate concentration in its leaves. When this *Slggp1* mutant was submitted to high irradiance conditions chlorophyll bleaching was observed (Baldet et al. 2013). In addition, transformed tomato with decreased GDP-L-galactose phosphorylase expression display increased damage when exposed to chilling (Wang et al. 2013; Yang et al. 2017). These results provide evidence for the increased susceptibility of GDP-L-galactose phosphorylase deficient tomato plants to stress. However, studies focused in modifications at the level of the fruit have not been done yet. The work focuses on the effects of GDP-L-galactose phosphorylase expression and associated ascorbate deficiency on tomato fruit yield and quality.

Material and methods

The experiments were carried out with *Solanum lycopersicum* L cv Micro-Tom plants with two lines deficient in expression of the *GGP1* gene encoding GDP-L-galactose phosphorylase. The two EMS mutant Micro-Tom lines used here, GGP-5261 and GGP-49C12 were, respectively, from the NBRP-Tomato population (Tsukuba-Japan) and TILLING-Tomato collection (Bordeaux-France). They are truncation and splice junction mutants, respectively (Baldet et al. 2013). Plants were grown hydroponically in an air-conditioned greenhouse during spring and summer seasons under an irradiance of 700 µmol photons m^{-2} s^{-1} at midday and temperatures average of 25 ± 2 and 20 ± 3 °C during the day and night, respectively. Each experiment included 10 plants of each genotype and the harvest was taken when half mutant plants were bearing the first fully red ripe fruit.

Exogenous treatment of ascorbic acid was initiated when seedlings were twenty days-old and finished after the development of the fourth inflorescence. Each plant received 1 mL of 20 mM ascorbic acid solution (including 0.01% tween 20 as a surfactant) wetting all above ground organs (mainly in the adaxial side of the leaves). The treatment was repeated four times a week.

Sink-source experiment

A sink-source experiment was performed leaving only 4 inflorescences on each plant. The treatments consisted of plants with one (1F) or two (2F) tomatoes per inflorescence, two levels of leaf pruning (−L and =L, for 50 and 75% of leaf area removal, respectively) and a control without organ removal (leaving only 4 inflorescences). Three independent experiments were carried out including at least five plants per treatment for each genotype (i.e., 75 plants for each experiment). Measurements were made when half mutant plants got at least one red fruit.

Ascorbate determination

Ascorbate was determined with a HPLC system (Shimadzu LC-10Atvp solvent delivery module and Shimadzu UV–Vis SPD-10Avp detector) as previously described (Bartoli et al. 2006). Root, leaf, flower, and fruit tissues were ground in 6% (v/v) trifluoracetic acid, centrifuged at 13000×g for 5 min and supernatants used for the measurements. Total AA was determined after the treatment of an aliquot with 5 mM dithiothreitol (DTT). Oxidized AA was calculated as the difference between samples with or without DTT.

Photosynthesis measurements

CO_2 assimilation was measured in fully developed leaves with an infrared gas analyzer (PLC 6, Cirus-2 PPSystems) at saturating irradiance (1200 µmol photon m^{-2} s^{-1}, A_{max}). In addition, photosynthesis was measured as O_2 evolution under saturating irradiance and CO_2 concentration (P_{pot}). Leaf discs were placed in a gas tight chamber equipped with a Clark type electrode (Hansatech, UK). Saturating CO_2 atmosphere was generated including a mate imbibed with 1 M $NaHCO_3$ (Walker 1987). Photosynthetic electron transport rate was determined with a modulated chlorophyll fluorescence system (FMS-2, Hansatech Instruments Ltd., Norfolk, UK) and calculated according to Genty et al. (1989). Chlorophyll fluorescence quenching analysis was carried out with a CF Imager (Technologica Ltd., Colchester, UK) as described by Lim et al. (2016) and the chlorophyll fluorescence parameters calculated according to Baker (2008). Leaf temperature was measured with a thermographic camera (FLUKE Ti 400) with an emissivity of 0.95. Measurements were taken to well watered plants exposed at 700 µmol photons m^{-2} s^{-1} during midday inside the greenhouse.

Ripening parameters

Fruit ethylene production was measured with a gas chromatograph system (Konik, KNK-3000-HGRC) including an alumina column and a flame ionization detector as previously reported (Bartoli et al. 1996). Firmness was measured in detached tomato fruit with a texture analyzer (TA. XT.PLUS, Micro Systems TM Goldalming, Surrey, UK) using a 2.5 mm diameter flat probe. The measurements were obtained by fruit deformation for a distance of 0.5 mm at 0.25 mm s^{-1} and 5.9 g trigger force. The maximum force was recorded and results expressed in force *g*. Total soluble solids were measured as previously described (Gergoff et al. 2016).

Hormone determination

Plant hormones were measured in whole flower tissues sampled at anthesis and collected from several plants of each independent experiment. About 100 mg of lyophilized tissues were added with 1% (v/v) AcH (40 mg ml^{-1}) and $^2H_5$-indoleacetic acid (IAA), $^2H_2$-gibberellin (GA), $^2H_2$-GA4, $^2H_2$-GA8, $^2H_5$-zeatin and $^2H_6$-ABA (OlChemIm Ltd., Olomouc, Czech Republic) as internal standards. The aqueous solution was partitioned 3 times with ethyl acetate at pH 3. Organic fractions were combined, evaporated and then resuspended in methanol for hormone determination by liquid chromatography-mass spectrometry with electrospray ionization (Waters Corp., New York, NY, USA). An Alliance 2695 (Separation Module, Waters, USA) quaternary pump equipped with a Restek C18 column (2.1 × 100 mm) (Restek, USA) was used to analyze the samples. A binary solvent system used for elution consisted of 0.2% (v/v) acetic acid in H_2O and methanol. MS/MS assays were done with a Micromass Quatro Ultima TM mass spectrometer (Micromass, Manchester City, UK) as described by Masciarelli et al. (2014).

Results

Growth, development, and photosynthesis in GDP-L-galactose phosphorylase mutants.

The two GDP-L-galactose phosphorylase mutants had 34–50% of wild type ascorbate in their leaves, roots, flowers, and fruit (Fig. 1a–d). Ascorbate concentration was lower in roots and the proportion of DHA was much higher than in leaves, flowers, and fruit. Exogenous ascorbate increased its concentration in leaves of the mutants by 66–79% but not in wild type plants (Table 1).

The vegetative biomass and leaf area at harvest were similar in all genotypes (Table 2). However, the mutants both allocated less biomass to roots (15% compared to 23% in wild type) and more to stems (20% compared to 13% in wild type). Therefore, the mutants were visibly different since they had larger internodes (Supplementary Fig. S1). The fruit fresh weight per plant was decreased in both mutants but the individual fruit weight was increased (Table 2). The mutants had a similar number of flowers but decreased fruit setting, and consequently, lower number of tomatoes than those of the wild type. Exogenous ascorbate supplementation increased fruit setting in both mutants but not in wild type plants (Table 1). In addition, anthesis was delayed in both mutants. Impairment of fruit setting may be the consequence of altered hormone concentrations. Consequently, the concentrations of various hormones in flowers at anthesis were measured (Table 3). All three gibberellins measured were decreased in the mutants but indole acetic acid (IAA), zeatin, and ABA showed no differences.

The CO_2 assimilation and electron transport rates (ETR) of the mutants were 50 and 90%, of wild type, respectively (Fig. 2a, b). In addition, photosynthetic O_2 production under a saturating CO_2 atmosphere, was 19% higher in wild type than in the mutants (Fig. 2c). Consistent with the small effect on ETR and O_2 assimilation under saturating CO_2, there was no significant effect on photochemical quenching (qP), PSII maximum operating quantum efficiency (Fv'/Fm') and PSII operating efficiency (Fq'/Fm'; φPSII) (Fig. 3). Non photochemical quenching (NPQ), which is impaired in ascorbate deficient arabidopsis mutants (Müller-Moule et al. 2002) was not affected in the mutants. Stomatal conductance was 60% higher in wild type than in the mutants

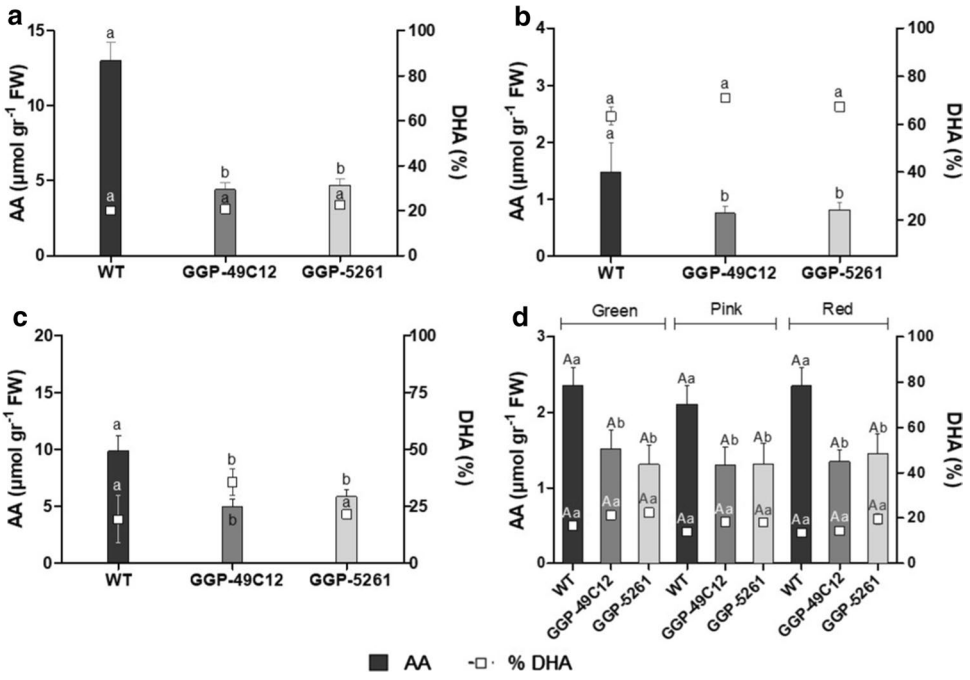

Fig. 1 Ascorbate concentrations in leaves (**a**), roots (**b**), flowers (**c**) and fruit (**d**) of *ggp1* mutants and wild type plants grown under greenhouse conditions. The values were obtained from at least three independent experiments and expressed as the means ± S.D. (ANOVA, $P < 0.05$). Lower and upper case letters indicate statistical differences between genotypes and stages, respectively

Table 1 Effect of 20 mM ascorbate supplement in ascorbate content and fruit setting in tomato wt and *GGP* deficient plants. One mL of ascorbate solution was sprayed on each plant four times a week

Treatment	Leaf ascorbate content µmol g^{-1}FW	Fruit setting %
Wt	10.0 ± 0.6	62.7 ± 10.3
Wt + AA	11.3 ± 0.33	57.3 ± 10.9
GGP 5261	5.0 ± 0.6	25.0 ± 8.6
GGP 5261 + AA	8.3 ± 0.9*	38.3 ± 6.4*
GGP P49 C12	4.3 ± 0.3	29.7 ± 1.2
GGP P49 C12 + AA	7.7 ± 1.2*	40.0 ± 1.5*

Values are means ± SD ($n = 3$)

*Indicates statistical differences with non-treated plants (ANOVA $P < 0.05$)

(Fig. 2d). Consequently, leaf temperature measured by infrared thermal imaging was 1.8–2.5 °C higher in the mutants (Supplementary Fig. S2).

The effect of GDP-L-galactose phosphorylase mutation on fruit quality

Fruit firmness decreased along ripening but both GDP-L-galactose phosphorylase mutants kept higher firmness than wild type at the red stage (Fig. 4a). Ethylene production was higher in the mutant lines than wild type fruit at the breaker and red stages (Fig. 4b). Soluble solids content (Brix) increased during fruit ripening and was also higher in mutants compared with wild type at breaker stage (Fig. 4c). Other ripening parameters such as pH and titratable acidity show no differences between genotypes (Data not shown).

The effect of source-sink manipulation on fruit yield characteristics

To investigate the relative importance of source or sink limitations on fruit yield various fruit or leaf pruning treatments were performed (Table 4). Fruit production was not modified in wild type or mutant plants after leaf removal. However, when plants were limited to two tomatoes per inflorescence (i.e., eight tomatoes per plant) the mutants kept their fruit weight per plant as in control treatment but it drastically decreased in all genotypes when only one fruit per inflorescence is maintained. For an easier interpretation of these results a principal component analysis is shown in Fig. 5. It shows a contrasting relationship between different traits such as fruit number and yield *versus* individual fruit fresh weight. Component 1 distinguishes treatments independently of the genotypes and component 2 the wild type from mutants (more markedly for fruit removal treatment).

Discussion

Arabidopsis *vtc2* mutants, which are knockouts of one of the two genes encoding GDP-L-galactose phosphorylase, have ~ 20% of wild type ascorbate (Dowdle et al. 2007; Barth et al. 2010) and have small decreases in rosette leaf area and biomass, the extent most likely being sensitive to growth conditions (Lim et al. 2016; Caviglia et al. 2018; Plumb

Table 2 Yield and vegetative parameters in wt and *ggp1* mutants tomato plants. Measurements were done when half mutant plants reached one mature fruit

	Fruit yield (g FW plant^{-1})	Individual fruit weight (g FW)	n° fruit per plant	n° flowers per plant	Fruit set (%)	Days to anthesis	Vegetative biomass (g FW)	Leaf area (cm^2)	Leaf biomass (g DW)	Stem biomass (g DW)	Root biomass (g DW)
Wild type	109.9 ± 15.9A	2.52 ± 1.82A	40.16 ± 9.15A	50.1 ± 10.7A	74.0 ± 8.1A	53.06 ± 4.7A	58.06 ± 15.3A	853.2 ± 165A	3.5 ± 0.90A	0.7 ± 0.12A	1.23 ± 0.32A
GGP 5261	88.31 ± 7.5B	3.29 ± 2.27B	24.0 ± 5.67B	54.4 ± 9.8A	42.5 ± 10.2B	59.3 ± 6.5 B	59.6 ± 8.8A	887.8 ± 236.7A	3.6 ± 0.85A	1.1 ± 0.24B	0.77 ± 0.16B
GGP P49C12	70.7 ± 14.4B	4.08 ± 2.36C	19.25 ± 5.04B	45.4 ± 37 A	42.6 ± 12.7B	58.3 ± 5.24B	56.7 ± 12.68A	906.0 ± 116.1A	3.7 ± 0.27A	1.2 ± 0.21B	0.91 ± 0.14B

The values were obtained from three independent experiments and expressed as means ± SD (ANOVA $P < 0.05$). Letters indicates statistical differences between genotypes

et al. 2018). Here, the tomato GDP-L-galactose phosphorylase mutants, which have 34–50% of wild type ascorbate in all organs, are similarly unaffected in total vegetative biomass but do have reduced allocation to roots. The reason for the effect on root growth is not evident but could be associated with re-allocation to shoots to compensate for decreased CO_2 assimilation. Effects of the GDP-L-galactose phosphorylase mutations are more evident in reproductive development, the mutants being slower to anthesis and having markedly decreased fruit set and number. However, early flowering has been reported in various ascorbate deficient Arabidopsis mutants (Kotchoni et al. 2009). Whatever the delaying or accelerating effect, these results together suggest that the plant ascorbic acid concentration is linked to the regulation of time to flowering. This effect may be a species- but also an environmental-dependent phenomenon. The decrease in fruit number is associated with increased fruit size in the mutants. Larger fruit size is most easily explained by decreased competition between fruits for assimilate, since the fruit removal experiment increased individual fruit weight in all genotypes. Critically, ascorbate supplementation increased fruit set in the mutants, suggesting that ascorbate is important for this process. This observation also suggests that assimilate limitation (Ruan et al. 2012) may be only partially responsible for reduced fruit set in this case. To further investigate the cause of reduced fruit set, the effect of GDP-L-galactose phosphorylase mutations on flower hormones was investigated. GA1, GA4, and GA8 were decreased in the mutants. The chemical inhibition of gibberellin synthesis in tomato reduces fruit setting and this effect is reversed by exogenous application of the hormone (Serrani et al. 2007). Therefore, the low GA concentration in the flowers of the mutants could contribute to low fruit setting. Ethylene treatment decreases fruit set in tomato, possibly by inhibiting GA synthesis (Shinozaki et al. 2015). Arabidopsis *vtc2* mutants have increased ethylene production (Caviglia et al. 2018). Therefore, if ascorbate deficiency also increases ethylene in tomato, it is possible that this could be the cause of decreased GA and fruit setting.

The large decrease in CO_2 assimilation in the mutants was not matched by a large decrease in biomass, although overall fruit yield was significantly less (~ 72% of wild type). As noted above this could be partly attributed to lower fruit set. Measuring photosynthesis under high CO_2 and by chlorophyll fluorescence showed that the mutations do not limit photosynthetic capacity, but the limitation is most likely caused by partial stomatal closure. Since the stomatal conductance was measured around midday, it is possible that over the course of the light period, stomata are more open in morning/evening, thus minimizing an overall effect on assimilation. Furthermore, decreasing GDP-L-galactose phosphorylase expression in tomato in another study also decreased ascorbate content by 50%

Table 3 Concentrations of several hormones in flowers of *ggp1* mutants and wild type tomato plants. Samples were taken at anthesis combining flowers from different plants

µg g^{-1}DW	GA1	GA4	GA8	AIA	Zeatin	ABA
Wt	2.85 ± 0.15A	19.5 ± 1.9A	1.59 ± 0.08A	1.13 ± 0.07A	1.26 ± 0.08A	16.2 ± 0.6A
GGP-5261	1.77 ± 0.06B	9.3 ± 0.3B	0.34 ± 0.03B	1.11 ± 0.03A	1.44 ± 0.2A	16.0 ± 1.1A
GGPP49C12	2.20 ± 0.11B	7.7 ± 0.4B	0.28 ± 0.02B	1.06 ± 0.02A	1.14 ± 0.03A	18.1 ± 1.1A

Data are shown as means ± S.D. from 3 independent experiments. Letters indicates statistical differences between genotypes (ANOVA, $P < 0.05$)

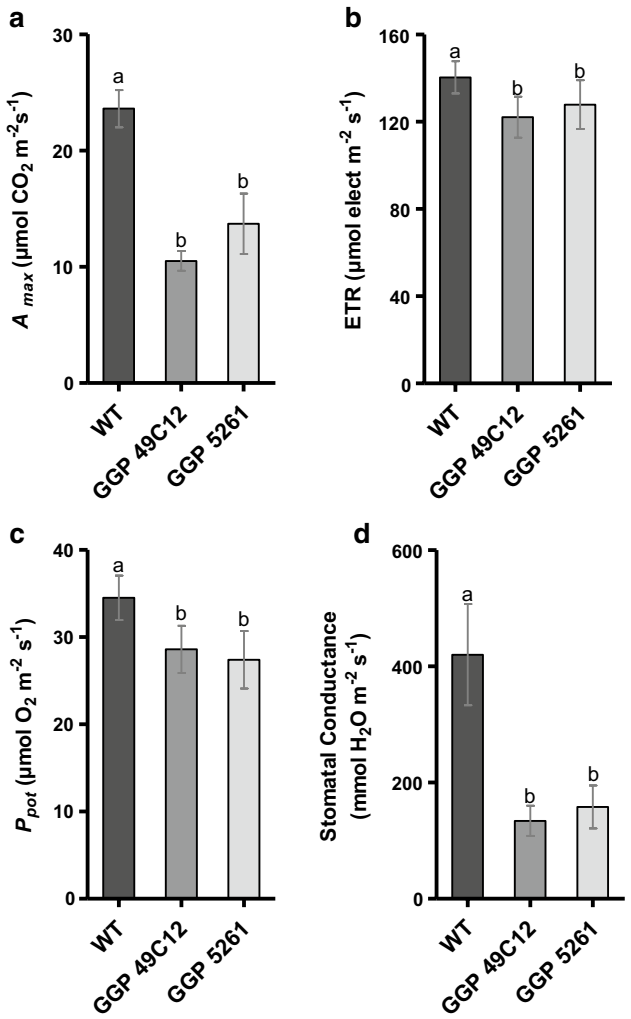

Fig. 2 Leaf gas exchange measurements in *ggp1* mutants and wild type plants as CO_2 uptake (**a**), ETR (**b**), O_2 production at saturating CO_2 (**c**) and stomatal conductance (**d**) grown under greenhouse conditions. The values were obtained from at least three independent experiments and expressed as the means ± SD. (ANOVA $P < 0.05$). Lower and upper case letters indicate statistical differences between genotypes

while having no effect on CO_2 assimilation under non-stressed conditions (Wang et al. 2013). Similarly, Arabidopsis *vtc2-1* has a similar CO_2 assimilation rate to wild type (Senn et al. 2016). Therefore, studies to date show that ascorbate deficiency (at least to 20% of wild type), has minimal effect on photosynthesis and vegetative biomass.

The source-sink experiment demonstrates that the number of fruit is crucial to determine fruit yield in tomato and that the higher abortion of fruit observed in *ggp1* mutants does not seem to be the consequence of an insufficient provision of photoassimilates by the leaves. Similarly, Tanaka and Fujita (1974) demonstrate in assays partially removing fruit and leaves that source is not limiting yield in tomato and also that fruit size has a limited flexibility. These authors conclude that unraveling the processes establishing fruit number is an important task. In this context, the present work, using GDP-L-galactose phosphorylase deficient mutants and especially exogenous ascorbic acid supplementation, demonstrates that ascorbic acid concentration is an important factor in establishing fruit number and consequently for the improvement of tomato yield.

Ascorbic acid constitutes an important nutritional attribute of fruit (Lee and Kader 2000). However, the impact of decreased ascorbate concentration on other fruit characteristics has scarcely been addressed. Previous reports on tomato mutants in enzymes earlier in the mannose/L-galactose biosynthetic pathway (GDP-mannose pyrophosphorylase and GDP-mannose-3′,5-epimerase) show effects on fruit development and quality (Gilbert et al. 2009, 2016; Zhang et al. 2013) but these may be caused by effects on cell wall composition as well as ascorbate deficiency itself. Decreased GDP-L-galactose phosphorylase activity in the current experiments is likely to affect ascorbate synthesis more specifically. Among effects observed here in the fruit of GDP-L-galactose phosphorylase mutants is an increase in ethylene production. *Vtc2* arabidopsis plants also produce more ethylene (Caviglia et al. 2018). Ethylene production is associated with a stimulation of cell wall degrading enzymes during fruit ripening (Osorio et al. 2011). However, the results observed in GDP-L-galactose phosphorylase mutants suggest that other processes prevail, leading to increased fruit firmness. For example, changes in cuticles may also delay fruit softening (Saladié et al. 2007). The modifications observed in GDP-L-galactose phosphorylase tomato lines (including increased soluble solids) suggest that ascorbate influences some aspects of fruit quality.

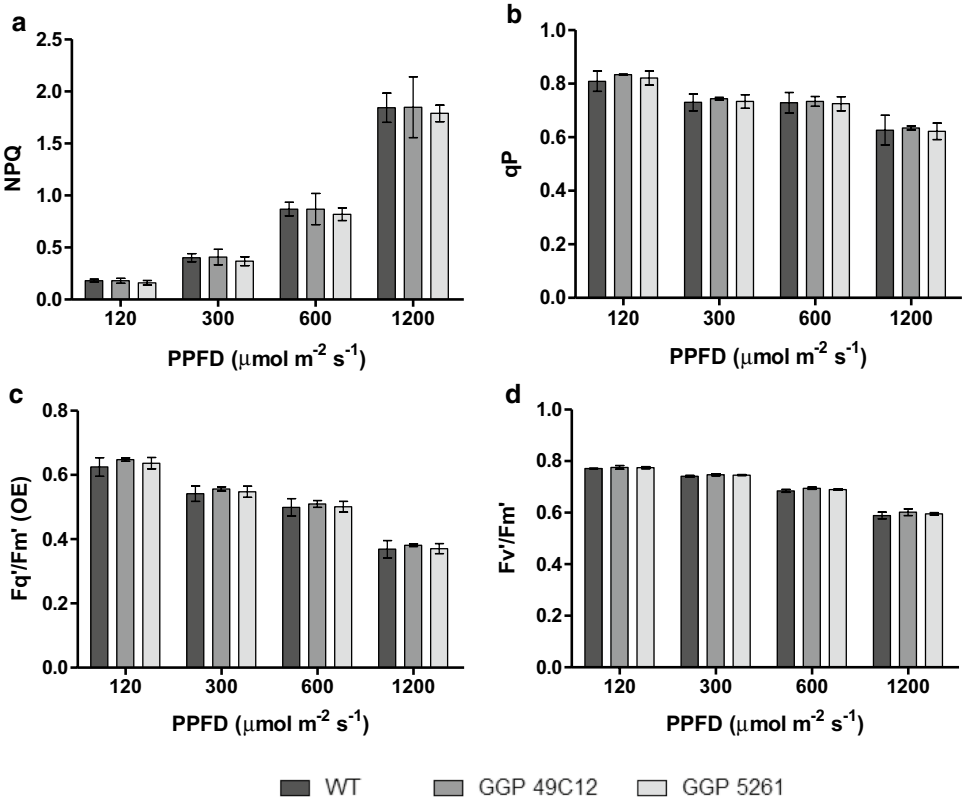

Fig. 3 Photosynthetic characteristics of *ggp1* mutants and wild type plants measured by chlorophyll fluorescence imaging. The plants were grown in 16 h day, 21C day (150 µmol m^{-2} s^{-1})/19C dark, 55 RH day/50 RH dark for 4 weeks before transferring to an irradiance of 500 µmol m^{-2} s^{-1} for 4 days. *NPQ* non-photochemical quenching (**a**), *qP* photosystem II efficiency factor (**b**), Fq'/Fm', PSII operating efficiency (**c**), Fv'/Fm', PSII maximum efficiency (**d**). The values were obtained from three independent experiments and expressed as the means ± SD

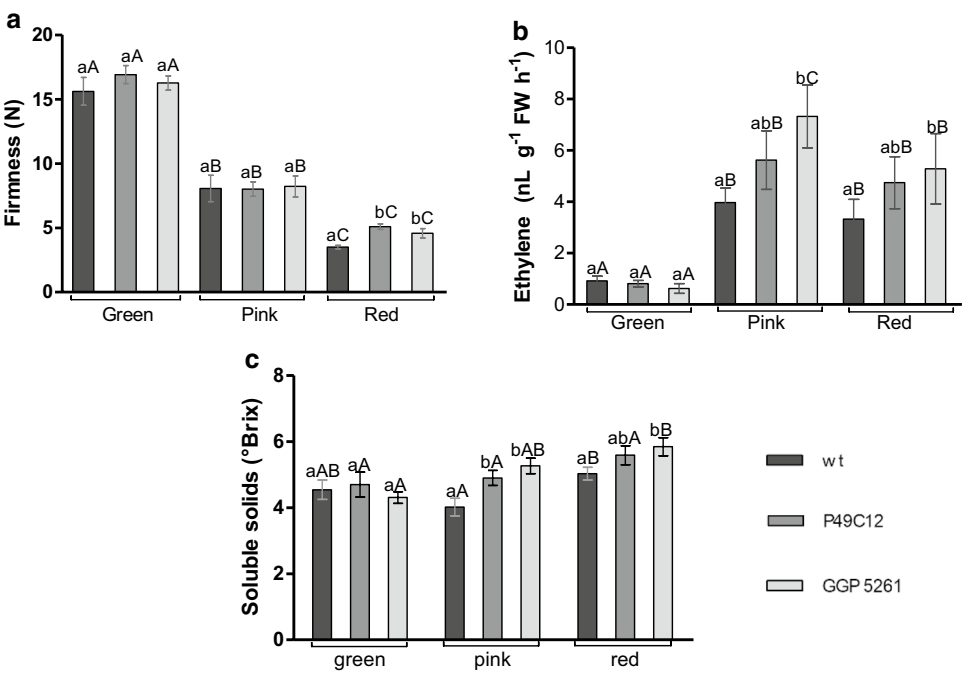

Fig. 4 Firmness (**a**), ethylene production (**b**) and soluble solids content (**c**) in fruit taken from *ggp1* mutants and wild type plants grown under greenhouse conditions at green mature, breaker and red stages. The values were obtained from at least three independent experiments and expressed as the means ± SD (ANOVA $P < 0.05$). Lower and upper case letters indicate statistical differences between genotypes and stages, respectively

Table 4 Biomass accumulation in different organs of wt and *GGP1* deficient tomato plants limiting the size of source and sink tissues

	Fruit yield g FWfr pl^{-1}	Individual FW g FW fr^{-1}	Fruit number fr pl^{-1}	Flower number fl pl^{-1}	Leaf g DW pl^{-1}	Stem g DW pl^{-1}	Root g DW pl^{-1}	Total vegetative g DW pl^{-1}
1F wt	18.7 ± 3.8B	3.60 ± 0.31B	4.0 ± 0	*	2.51 ± 0.2A	1.96 ± 0.3A	1.97 ± 0.5B	6.44 ± 1.0 A
2F wt	30.4 ± 2.3C	4.49 ± 0.16B	8.0 ± 0	*	2.38 ± 0.1A	1.89 ± 0.1A	1.75 ± 0.2B	6.01 ± 0.4 A
C wt	59.0 ± 2.6A	2.63 ± 0.36A	23.3 ± 1.6A	29.3 ± 2.3A	2.39 ± 0.2A	1.85 ± 0.1AC	1.65 ± 0.2AB	5.89 ± 0.6 A
–L wt	59.1 ± 2.7A	2.36 ± 0.23A	21.7 ± 0.75A	29.2 ± 0.9A	1.32 ± 0.09B	1.41 ± 0.03BC	1.25 ± 0.14AB	3.97 ± 0.2 B
=L wt	56.7 ± 0.5A	2.48 ± 0.29A	21.5 ± 1.3A	29.7 ± 1.6A	1.15 ± 0.10B	1.27 ± 0.04B	0.90 ± 0.09B	3.32 ± 0.1 B
1F GGP 5261	20.9 ± 2.3B	4.98 ± 1.02B	4.0 ± 0	*	1.75 ± 0.1A	1.82 ± 0.1A	0.99 ± 0.2A	4.57 ± 0.2 AB
2F GGP 5261	39.1 ± 2.30A	5.47 ± 0.41B	8.0 ± 0	*	2.03 ± 0.26A	1.73 ± 0.23A	1.09 ± 0.15A	4.85 ± 0.6 A
C GGP 5261	51.8 ± 1.7A	3.75 ± 0.22 A	14.7 ± 0.8A	29.3 ± 4.0A	2.05 ± 0.1A	1.41 ± 0.2A	1.0 ± 0.1A	4.6 ± 0.2 AB
–L GGP 5261	48.2 ± 2.7A	2.97 ± 0.82 A	17.0 ± 1.4A	27.0 ± 0.5A	1.42 ± 0.13AB	1.52 ± 0.14A	0.95 ± 0.12A	3.90 ± 0.4 AB
=L GGP 5261	46.2 ± 6.5A	3.27 ± 0.58 A	13.7 ± 1.8A	26.7 ± 2.3A	0.89 ± 0.14B	1.45 ± 0.22A	0.72 ± 0.13A	3.06 ± 0.5 B
1F GGP P49	26.3 ± 2.8**B**	5.76 ± 0.59**B**	4.0 ± 0	*	1.89 ± 0.2**A**	1.72 ± 0.2**A**	1.44 ± 0.2**A**	5.07 ± 0.6 **A**
2F GGP P49	43.1 ± 5.4A	6.73 ± 0.75B	8.0 ± 0	*	1.78 ± 0.15A	1.57 ± 0.16A	1.07 ± 0.08AB	4.43 ± 0.3 AB
C GGP P49	49.6 ± 5.3A	3.69 ± 0.60A	11.7 ± 2.9A	29.0 ± 3.0A	1.94 ± 0.1A	1.59 ± 0.2A	1.09 ± 0.1AB	4.65 ± 0.4 AB
–L GGP P49	55.4 ± 2.1**A**	3.62 ± 0.48**A**	16.2 ± 1.4**A**	30.7 ± 3.6**A**	1.30 ± 0.08**AB**	1.73 ± 0.08**A**	1.02 ± 0.04**AB**	4.04 ± 0.1 **AB**
=L GGP P49	49.2 ± 2.7A	3.70 ± 0.57A	15.4 ± 2.4A	33.4 ± 4.2A	0.96 ± 0.05B	1.44 ± 0.13A	0.76 ± 0.09B	3.16 ± 0.2 B

*Flowers were cut to establish a limited number of fruits

The treatments consisted in control plants that were cultivated leaving only 4 trusses (C); limited number of fruit where each plant kept 4 or 8 fruits (1F and 2F, respectively); and two levels of defoliation (−L and =L)

The values were obtained from three independent experiments and expressed as means ± SD (ANOVA $P < 0.05$). Letters indicate significant differences between treatments for the same genotype

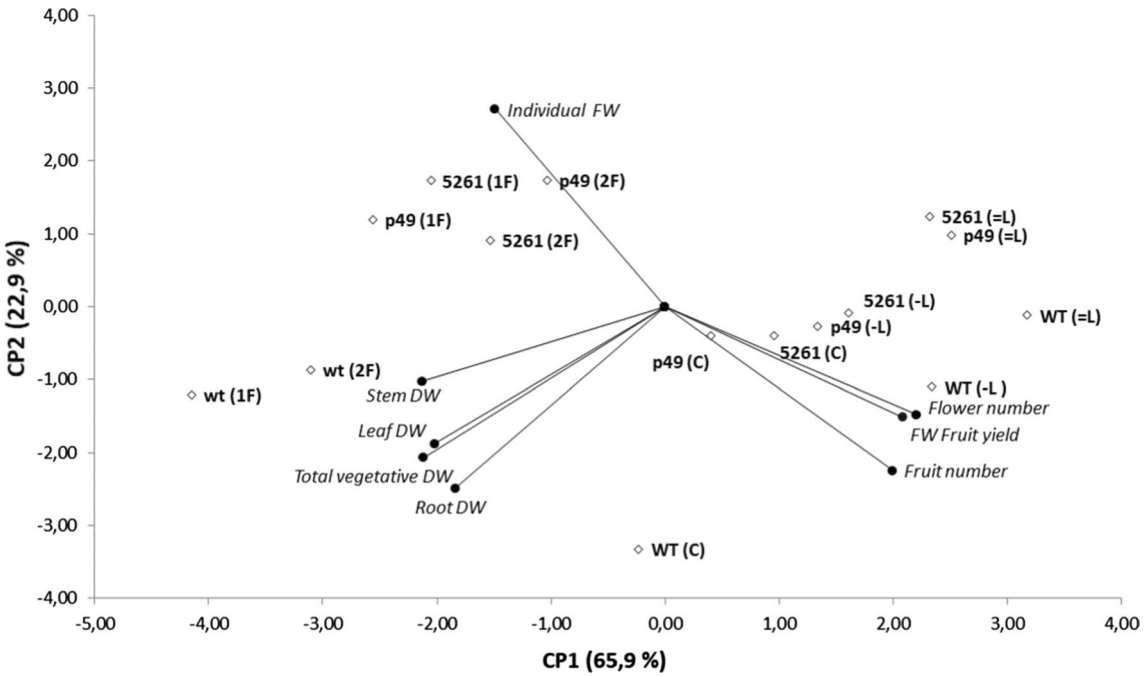

Fig. 5 Bi-plot of the first and second principal components for tomato development traits. Each parameter is represented by dark continuous lines and genotype-treatment combination by symbols (unfilled diamond). The treatments consisted in control plants that were cultivated leaving only 4 trusses (C); limited number of fruit where each plant kept 4 or 8 fruits (1F and 2F, respectively); and two levels of defoliation (−L and =L)

Conclusions

Tomato mutants in the ascorbate biosynthesis the *GGP1* isoform of GDP-L-galactose phosphorylase contain 34–50% of wild type ascorbate. The results suggest that this decrease in GDP-L-galactose phosphorylase expression and ascorbate concentration influence tomato fruit set, possibly via decreased GA concentration, final fruit size and some aspects of quality. A higher flower abortion is directly caused by ascorbate deficiency. Fruit size increases in compensation, but not sufficiently to prevent a decrease in total fruit biomass. Supply of assimilates for biomass and fruit production is unlikely to be a major limiting factor.

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