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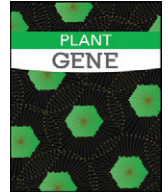

# Gene transcript accumulation and enzyme activity of β-amylases suggest involvement in the starch depletion during the ripening of cherry tomatoes

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A B S T R A C T

The flavor of tomato fruits is mostly influenced by the accumulation of sugars and organic acids. During fruit ripening a conversion of starch to sugars occurs, which modulates significantly the taste and consequently the quality of the ripe tomato fruits. β-Amylases, a group of major starch hydrolytic enzymes involved in starch degradation were examined in developing cherry tomatoes. Our results suggest that the enzyme activity and the gene transcript accumulation of plastidial β-amylase isoenzymes were elevated during the late stages of fruit development indicating a participation of the enzyme in starch depletion and in the increase of total soluble sugar levels in ripe tomatoes.

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## 1. Introduction

The flavor of tomato fruits, one of the most important quality traits, is mostly influenced by the accumulation of sugars and organic acids (Klee and Giovannoni, 2011). During the early stages of fruit development, tomato fruits accumulate high levels of starch coinciding with rapid fruit growth, cell division and enlargement (Wang et al., 1993; Moore et al., 2002). In contrast, during fruit ripening the conversion of starch to sugars occurs along with other metabolic changes and contributes to the texture, flavor and nutrient composition of the fruits (Moore et al., 2002). It is reported that fruits mostly accumulate starch up to 20 to 25 days after flowering, while starch degradation occurs 35 to 40 days after flowering (Luengwilai and Beckles, 2009).

Beta-amylases (EC 3.2.1.2-bAmy) are a class of hydrolases that remove β-maltose units from the non-reducing end of polyglucans and are involved in starch degradation in all plant tissues (Sparla et al., 2006; Peroni et al., 2008). bAmy is mostly associated with the later stages of fruit development and has been extensively studied in fruits containing high levels of starch (15–20% of fresh weight), such as mango and banana (do Nascimento et al., 2006; Peroni et al., 2008). In tomato fruits, starch phosphorylase was considered to be the predominant enzyme of starch breakdown during fruit maturation (Robinson et al., 1988). However, later research found that bAmy played an important role in the hydrolytic starch breakdown pathway (Purgatto et al., 2001; Fulton et al., 2008). Furthermore, Bassinello et al. (2002) emphasized the importance of bAmy in the hydrolytic breakdown pathway in mangoes, pears and also in corn.

In contrast to fruits such as mango and banana where the role of bAmy during ripening has been adequately investigated, in most fruits with lower starch levels, such as the tomato, only scarce information exists about the actual role of bAmy during fruit maturation (do Nascimento et al., 2006; Peroni et al., 2008). However, according to Schaffer and Petreikov (1997), the process of starch breakdown could also considerably affect the sugar levels, and consequently the quality, of the low-starch fruits.

The present study attempts to investigate the role of bAmy, probably one of the most important enzymes of the hydrolytic starch breakdown pathway during fruit development and maturation of cherry tomatoes. For this purpose, the starch content, the bAmy enzyme activity and the transcript accumulation of bAmy genes were examined in developing cherry tomatoes.

*Abbreviations:* bAmy, β-amylase; UBQ, ubiquitin; ImG, immature green; MG, mature green; Br, breaker; RR, red ripe.

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## 2. Materials and methods

### 2.1. Plant material and growth conditions

Plants of cherry tomato (*Solanum lycopersicum* L. var. *cerasiforme* cv. 'Conchita F1' de Ruiter seeds, Melbourne Australia), a productive hybrid with a long shelf life, were cultivated in a glasshouse of the Agricultural University of Athens, Greece between December and May. Mean minimum and maximum temperatures in the greenhouse were 15.7 ± 2.0 and 26.6 ± 4.3 °C, respectively, (Spring, {March–May}) and 12.9 ± 1.9 and 23.9 ± 4.4 °C (Winter, {Oct.–Feb.}). Average solar radiation was 318.75 μmol/$m^2$ s. Fruits were harvested systematically at the following stages: immature green (ImG — diameter 15 mm) (25 DAF — days after flowering), mature green (MG — 38 DAF), breaker (Br — 44 DAF) (<10% red color than red ripe-measured with a Konica — Minolta CR-400 Chroma Meter), and red ripe (RR — 52 DAF). Each harvest was carried out at 11 am and replicated three times. For the experiments at least 20 whole fruits of each lot were ground to a fine powder with liquid nitrogen except for the assessment of the starch content.

### 2.2. Assessment of starch content

Glucose, fructose, and sucrose contents were measured by HPLC in ethanolic extracts of tomato pulp, following the method of Piccaglia and Galleti (1988), with slight modifications. Sugars were extracted from 0.5 g of homogenized flesh in 2 ml 80% (*v*/*v*) ethanol at 65 °C for 25 min. The suspension was centrifuged at 5500 *g* for 15 min and the supernatant was retained for the analysis of sugars. The procedure was then repeated on the resulting pellet and the supernatants from both extractions were combined and evaporated to dryness at 65 °C with the aid of continuous ventilation (N2). The pellet was then dissolved in 3 ml water (HPLC-grade) and the aqueous solution was filtered (Macherey–Nagel Chromafil PET 20/15 MS; pore size 0.20 μm) and injected into an HPLC equipped with a refractive index detector (ERC-7511; Erma, Tokyo, Japan) and a Supelco Supelcosil LC-NH2 column (5 μm; 25 cm 4.6 mm; Sigma-Aldrich, St. Louis, MO, USA) maintained at 30 °C. The eluent was 80% (*v*/*v*) acetonitrile + 20% (*v*/*v*) $H_2O$ (HPLC-grade; Fisher Scientific, Hampton, NH, USA), at a flow rate of 1 ml $min^{-1}$. The ethanol-insoluble residue was used to measure starch content, according to the method of Dekker and Richards (1971) using amyloglycosidase (EC 3.2.1.3) from *Aspergillus niger* (Sigma-Aldrich) to release glucose. The glucose content was subsequently determined colorimetrically following the method of Barham and Trinder (1972), using a glucose oxidase/peroxidase (GOD-POD) kit (Biosis Ltd., Athens, Greece), and a spectrophotometer set at 510 nm (Lambda 1A; Perkin-Elmer, Waltham, MA, USA). A reference curve was created using standard starch concentrations (0, 25, 50, 100, 150, 250, 500, 750, 1000 mg/l).

### 2.3. Enzyme extraction and bAmy activity

For the enzyme activity assessment, 1 g of powdered fruits from all stages of fruit development was powdered using liquid nitrogen, and mixed at a 1:1 (*w*/*v*) with an extraction medium as proposed by Stenzel et al. (2003) with some modifications. The extraction buffer contained 50 mM Tris HCl pH 7.5, 15% glycerol, 0.02% bovine serum albumin, 2 mM reduced glutathione, 4% PVP-40 (MP Biochemicals, Eschwege Germany), 0.1% Triton X and 20 mM $Na_2SO_3$. The extracts were centrifuged at 15,000 ×*g* for 15 min at 4 °C. The protein content of supernatants was determined using the Bradford method, before the enzyme activity tests. The protocol Betamyl-3 (Megazyme Wicklow, Ireland) was used for the determination of beta amylase activity according to the manufacturer's instructions. The produced p-nitrophenol was assessed photometrically at 400 nm in a Shimadzu UV 160A spectrophotometer.

### 2.4. qPCR analysis

By performing BLAST searches (Altschul et al., 1997) among the databases of the National Center for Biotechnology Information (NCBI) using the appropriate keywords, the registered nucleotide sequences for tomato *bAmy* and for the reference genes were identified and recorded (Supplementary data). With the use of the online alignment tool of NCBI the highly similar sequences were grouped (Supplementary data) and gene specific primers were designed for the identified sequences coding for isoenzymes of tomato bAmy using Beacon designer v 7.01 software (Premier Biosoft, Palo Alto, USA) (Table 1). For the topology prediction of the bAmy isoenzymes the online tool TargetP 1.1 was used (Emanuelsson et al., 2000). For each identified tomato *bAmy* sequence the name of similar *bAmy* sequence from *Arabidopsis thaliana* is provided. No significantly similar *bAmy* sequence *Arabidopsis thaliana* was found for tomato *bAmy3* (Table 1, Supplementary data). Total RNA was isolated from each sample using RNeasy extraction Kit following the manufacturer's instructions (Qiagen, Hilden Germany). The quantity and quality of total RNA were assessed by spectrophotometric and electrophoretic analysis, measuring the absorbance at 260 nm and the absorbance ratio of 260/280 nm in a Nanodrop spectrophotometer (Thermo, Wilmington USA) and by 1.5% *w*/*v* agarose-gel electrophoresis. To eliminate total DNA, samples were treated with RNAse free DNase I (Takara, Otsu Shiga Japan) according to the manufacturer's instructions.

**Table 1**
Primer sequence of genes used for qPCR.

| Gene | Encoded enzyme/protein | Primer sequence | Accession no. |
|---|---|---|---|
| *bAmy1* | Putative plastidial beta amylase 1 | Sense: GTTCCACTTGCTGGGGAGAA<br>Antisense TGTTCGCGGCATTTGTTAGC | NM_001247627.1<br>Similar to AAY89374 from tobacco<br>(Ren et al., 2007) and NM_113297.2 from Arabidopsis. |
| *bAmy2* | Putative plastidial beta amylase 2 | Sense: GTG CAA ACT GCT CAC CAG AA<br>Antisense: CTT CCG ACA TGC TCT TCA CA | XM_004245796 similar to AJ250341 ct-bAmy from Arabidopsis (Lao et al., 1999) |
| *bAmy3* | Putative plastidial beta amylase 3 | Sense: CAA ACA GTA TGC CGA GAG CA<br>Antisense: AGG AAG TTT GCC ACA AAT GG | NM_001247123.1<br>(Aoki et al., 2010) similar to NM_121872.2 from Arabidopsis. |
| *bAmy4* | Putative cytosolic beta amylase1 | Sense: AGGGAGCTGAAGAACCAAGC<br>Antisense: ATAGCTCTTCGCTGTCGCTC | XM_004229839.2<br>Similar to XM007051752 bamy 7 from *Theobtoma cacao* and NM_130151.6 from Arabidopsis (Lin et al., 1999) |
| *bAmy5* | Putative plastidial beta amylase 4 | Sense: GAGCAATCCCCATCACCACA<br>Antisense: TGACGAAGTCCAGCAAGCAT | XM_004244394.1<br>Similar to XM_007040835 bamy 2 from Theobtoma cacao. Translation similar to NM_123898.2 from Arabidopsis (Motamayor et al., 2013) |
| *UBQ* | Ubiquitin | Sense: gcagactataacatccagaaagag<br>Antisence: aacaacaaagcacacacagccatc | X73156.1 |
| 18s | 18s ribosomal | Sense: ccgtcgcttggcattttcat<br>Antisence: ttggttcccattcccagacg | AF179442 |

The complete DNA removal was tested with primers designed against the ubiquitous expressed gene of UBIQUITIN (*UBQ*), while *S. lycopersicum* genomic DNA was used as a positive control.

First strand cDNA was reverse transcribed by the Affinity Script™ Multi Temperature reverse transcriptase using oligo primers according to the manufacturer's instructions (Stratagene, Santa Clara USA). The resulting first-strand cDNA was then normalized for the expression of the house-keeping gene of *UBQ*. qPCR experiments were conducted as previously described (Tsaniklidis et al., 2014). qPCR reactions were performed on a MX-3005P system (Stratagene, Santa Clara USA) using Kapa Probe Fast Universal 2 × qPCR Master Mix (Kapa, Woburn, USA) and gene-specific primers, following the manufacturer's instructions. For all samples, the qPCR reaction was performed in triplicate for each gene. The expression levels of a *S. lycopersicum UBQ* gene were used as an internal standard for normalization. Ribosomal *r18s* was used in order to validate the results. Data were analyzed as previously described (Tsaniklidis et al., 2014), PCR efficiency (E) for each amplicon was calculated employing linear regression of the log (fluorescence) per cycle number data, using LinRegPCR software. For all samples, qPCR reaction was performed in triplicate.

### 2.5. Total soluble solids assay

The total soluble solids were assayed using a Schmidt & Haensch HR32B refractometer.

### 2.6. Statistical analysis

Statistical analysis was performed using Statgraphics Centurion (Statpoint Technologies, Warrenton, USA). Significant differences between treatments were determined by one-way ANOVA and post-hoc comparisons by least significant difference ($p < 0.05$).

## 3. Results and discussion

### 3.1. Starch levels in developing cherry tomatoes

Starch is the major reserve carbohydrate and its metabolism is a complex process that is fundamental to the growth and quality of plants (Chang et al., 2013). The increase of simple sugar levels at the expense of starch levels is one of the main processes of fruit maturation (Klee and Giovannoni, 2011). Tomato, melon, grape, peach and strawberry accumulate soluble sugars mainly during fruit development that are mostly imported by the phloem, in contrast to the kiwifruit and banana that store a large amount of starch that is only released as soluble sugars after the fruit has reached maturity (Nardozza et al., 2013). The starch levels of cherry tomatoes rapidly declined during the development and maturation of the fruits reaching negligible levels at the RR stage (Fig. 1). This finding is in accordance to the reports of Luengwilai and Beckles (2009)) and Centeno et al. (2011), who observed lower starch levels in ripe tomatoes in comparison to tomatoes at the MG stage. In addition, our research group recently reported that sucrose and fructose levels in seeded and parhenocarpic cherry tomato fruits (cv. 'Conchita F1') significantly increased in the course of maturation (Rounis et al., 2015). This observed escalation in simple sugar levels mostly coincides with the climacteric (Breaker stage) and is mainly the result of two parallel processes: rapid starch degradation along with the faster simple sugar import by the phloem (Beckles, 2012). The pattern of fluctuation of starch levels during fruit development can be attributed to the progressive degradation of starch during fruit development. It should also be taken into consideration that the starch contents of tomato seeds are rather limited in comparison to seeds of other plants (Schaffer and Petreikov, 1997). In accordance to these findings, the fluctuation of the total soluble levels, an important marker of fruit quality, of developing cherry tomatoes was inversely proportional to the fluctuation of starch levels (Fig. 1).

### 3.2. bAmy activity

Starch metabolism is closely related to the activity of the main enzymes of starch biosynthesis and starch degradation (Chang et al., 2013). According to Manners (1985), bAmy is the key enzyme that initiates starch degradation in most plant tissues, especially in the endosperm. bAmy activity in cherry tomato fruit showed a peak at Br stage followed by a decline at the RR stage (Fig. 1). Interestingly, the sharp decrease in starch concentration observed between the Br and RR stages of fruit development coincided with increased levels of bAmy enzyme activity in developing cherry tomatoes (Fig. 1). In contrast, the considerably lower activity of the enzyme at ImG and MG stages suggests that bAmy probably holds a more important role in starch degradation during the late stages, while at the early stages of fruit development other enzymes such as isoamylases and starch

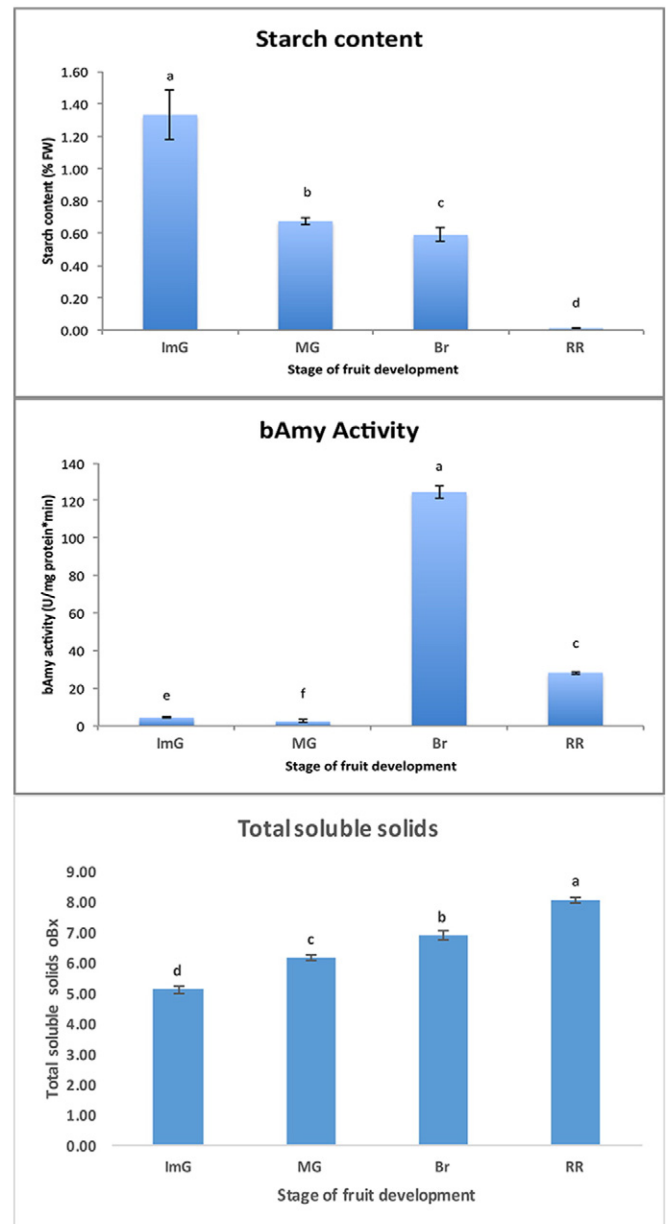


**Fig. 1.** Starch levels, bAmy enzyme activity and total soluble solids levels in developing cherry tomato fruits. ImG: immature green (Img)-15 mm diameter (25 DAF {days after flowering}); Mg: mature green (38 DAF); Br: breaker (44 DAF); RR: red ripe (52 DAF). Bars represent means (+SD) of three biological and three technical replicates. Indicator letters in common denote a lack of a significant difference.

phosphorylase may be more important for starch regulation (Peroni et al., 2008). Additionally, Sparla et al. (2006) indicated that the bAmy protein has redox dependency while Almeida and Huber (1999) reported a significant drop of pH in the apoplast between MG and Br stages in developing tomato fruits; by extension this could have affected the enzyme activity of the cytosolic bAmy. Moreover, the metabolic events during occurring during fruit maturation could also have an effect on the bAmy activity (Bian et al., 2011). The observations of Zhang and Wang (2002) and Peroni et al. (2008) in developing apple and mango fruits revealed that the patterns of bAmy activity in these fruits bear considerable pattern similarities with the pattern of bAmy enzyme activity in cherry tomatoes.

### 3.3. Transcript accumulation of bAmy genes during fruit development

*In silico* analysis revealed six possible genes coding for bAmy isoenzymes in the tomato genome, highly similar to characterized genes from Arabidopsis and cocoa, whose relevant coded proteins probably have diverse subcellular localizations (Lao et al., 1999; Lin et al., 1999; Ren et al., 2007; Aoki et al., 2010; Motamayor et al., 2013) (Table 1). However, the expression of only five of six *bAmy* genes was detected in cherry tomatoes of the cv Conchita (Supplementary data). *bAmy* genes exhibited distinct fluctuating patterns of transcript accumulation during fruit development in cherry tomatoes. *bAmy 1*, *bAmy 2* and *bAmy 3* peaked at late stages of fruit development, while *bAmy 4* and *bAmy 5* transcript levels did not change significantly (Fig. 2). Nardozza et al. (2013) reported that in kiwi fruits the cytosolic *bAmy* (BAM1) expression peaked later in fruit development, while the expression of a cytosolic *bAmy* peaked at earlier stages. Hence, it was suggested that while the plastidial β-amylases were critical for starch degradation inside the organelles, the cytosolic isoenzymes were probably involved in cytosolic starch or carbon turnover during cell divisions and fruit development. The contribution of the cytosolic bAmy could be direct, or it could be functioning as a maltose sensor regulating starch metabolism (Fulton et al., 2008; Takahashi et al., 2012). Our results indicate that cytosolic *bAmy* genes were expressed in cherry tomatoes but it should

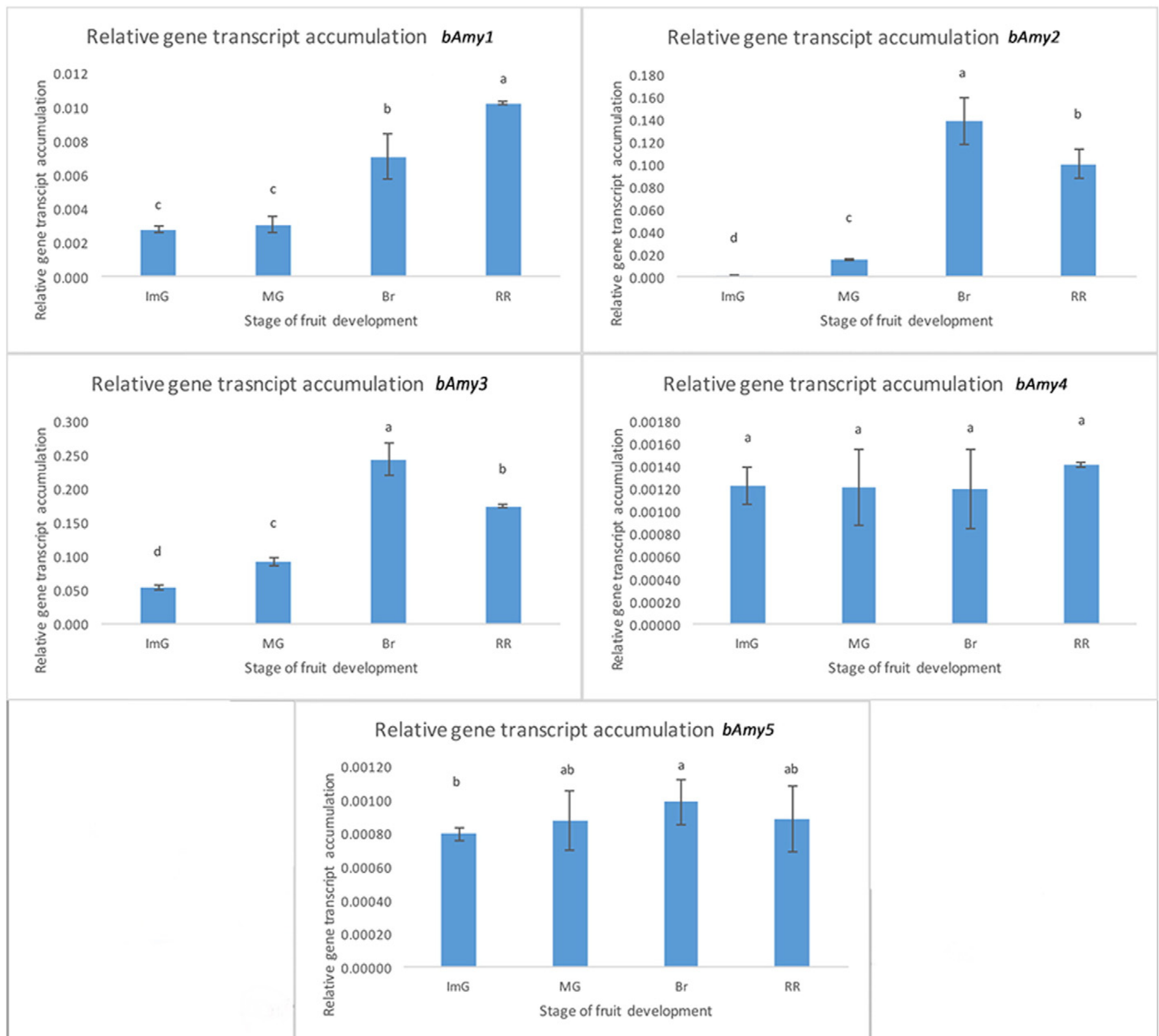


**Fig. 2.** Accumulation of *bAmy* gene transcripts in developing cherry tomato fruits. ImG: immature green (Img)-15 mm diameter (25 DAF); Mg: mature green (38 DAF); Br: breaker (44 DAF); RR: red ripe (52 DAF). Bars represent means (+SD) of three biological and three technical replicates. Indicator letters in common denote a lack of a significant difference.

be noted that the expression levels were about 100 fold lower than the platidial isoenzymes (Fig. 2). In general, the transcript accumulation of genes coding for plastidial *bAmy* isoenzymes correlated to the pattern of enzyme activity exhibiting higher levels of expression during Br and RR stages of fruit development (Fig. 2). bAmy is mostly associated with the processes of the late fruit development and ripening. Indeed, in several fruits such as mangoes (Peroni et al., 2008) and kiwis (Richardson et al., 2011), the transcript accumulation of most *bAmy* genes significantly increased in the late stages of fruit development, similarly to the genes of plastidial *bAmy* isoenzymes of 'Concita' cherry tomatoes (Fig. 2). In bananas, do Nascimento et al. (2006) correlated the high levels of the bAmy gene transcript accumulation to the ethylene emissions during the climacteric rise of respiration. Moreover, the elevated transcript accumulation of most plastidial *bAmy* genes in cherry tomatoes at Br stage could also suggest a possible effect of climacteric on the expression of bAmy genes. Furthermore, Maeo et al. (2001) reported that higher sugar levels could induce the transcription of *bAmy* genes. The higher glucose and fructose levels at the late stages of fruit development reported by our research group and other researchers (Rounis et al., 2015; Klee and Giovannoni, 2011), could have an effect on the accumulation of transcripts of the *bAmy* genes. Overall, plastidial *bAmy* genes exhibited comparably elevated expression levels at Br and RR compared to ImG and MG stages. In accordance with these findings at the early stages of fruit development, the activity of the bAmy enzyme was significantly lower.

### 3.4. Conclusion

Our results of bAmy enzyme activity and gene transcript accumulation of the plastidial isoenzymes indicate that the enzyme mostly participated in the events of the late development and maturation of tomato fruits, contributing to the depletion of starch and the increase of the total soluble solids.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.plgene.2015.10.004.

## References


Almeida, D.P.F., Huber, D.J., 1999. Apoplastic pH and inorganic ion levels in tomato fruit: a potential means for regulation of cell wall metabolism during ripening. Physiol. Plant. 105, 506–512.

Altschul, S.F., Madden, T.L., Schaffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J., 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25, 3389–3402.

Aoki, K., Yano, K., Suzuki, A., Kawamura, S., Sakurai, N., Suda, K., Kurabayashi, A., Suzuki, T., Tsugane, T., Watanabe, M., Ooga, K., Torii, M., Narita, T., Shin, I.T., Kohara, Y., Yamamoto, N., Takahashi, H., Watanabe, Y., Egusa, M., Kodama, M., Ichinose, Y., Kikuchi, M., Fukushima, S., Okabe, A., Arie, T., Sato, Y., Yazawa, K., Satoh, S., Omura, T., Ezura, H., Shibata, D., 2010. Large-scale analysis of full-length cDNAs from the tomato (*Solanum lycopersicum*) cultivar Micro-Tom, a reference system for the Solanaceae genomics. BMC Genomics 11, 210–215.

Barham, D., Trinder, P., 1972. An improved color reagent for the determination of blood glucose by oxidase system. Analyst 97, 142–145.

Bassinello, P.Z., Cordenunsi, B.R., Lajolo, F.M., 2002. Amylolytic activity in fruits: comparison of different substrates and methods using banana as model. J. Agric. Food Chem. 50, 5781–5786.

Beckles, D.M., 2012. Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) fruit. Postharv. Biol. Technol. 63, 129–140.

Bian, W., Barsan, C., Egea, I., Purgatto, E., Chervin, C., Zouine, M., Latché, A., Bouzayen, M., Pech, J.C., 2011. Metabolic and molecular events occurring during chromoplast biogenesis. J. Bot., 289859 http://dx.doi.org/10.1155/2011/289859.

Centeno, D.C., Osorio, S., Nunes-Nesi, A., Bertolo, A.L., Carneiro, R.T., Araujo, W.L., Steinhauser, M.C., Michalaska, J., Rohrma, J., Geigenberger, P., Oliver, S.N., Stitt, M., Carrari, F., Rose, J.K., Fernie, A.R., 2011. Malate plays a crucial role in starch metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest softening. Plant Cell 23, 162–184.

Chang, L., Xiao, Y.M., She, L.F., Xia, Y.P., 2013. Analysis of gene expression and enzyme activities related to starch metabolism in *Lycoris sprengeri* bulbs of different sizes. Sci. Hortic. 161, 118–124.

Dekker, R.H.F., Richards, G.N., 1971. Determination of starch in plant materials. J. Sci. Food Agric. 22, 441–444.

do Nascimento, J.R.O., Junior, A.V., Bassinello, P.Z., Cordenunsi, B.R., Mainardi, J.A., Purgatto, E., Lajolo, F.M., 2006. Beta-amylase expression and starch degradation during banana ripening. Postharv. Biol. Technol. 40, 41–47.

Emanuelsson, O., Nielsen, H., Brunak, S., von Heijne, G., 2000. Predicting subcellular localization of proteins based on their N-terminal amino acid sequence. J. Mol. Biol. 300, 1005–1016.

Fulton, D.C., Strttler, M., Mettler, T., Vaughan, C.K., Li, J., Francisco, P., Gil, M., Reinhold, H., Eicke, S., Messerli, G., et al., 2008. β-Amylase 4, a noncatalytic protein required for starch breakdown, acts upstream of three active beta-amylases in *Arabidopsis* chloroplasts. Plant Cell 20, 1040–1058.

Klee, H.J., Giovannoni, J.J., 2011. Genetics and control of tomato fruit ripening and quality attributes. Annu. Rev. Genet. 45, 41–59.

Lao, N.T., Schoneveld, O., Mould, R.M., Hibberd, J.M., Gray, J.C., Kavanagh, T.A., 1999. An *Arabidopsis* gene encoding a chloroplast-targeted beta-amylase. Plant J. 20, 519–527.

Lin, X., Kaul, S., Rounsley, S., Shea, T.P., Benito, M.I., Town, C.D., Fujii, C.Y., Mason, T., Bowman, C.L., Barnstead, M., Feldblyum, T.V., Buell, C.R., Ketchum, K.A., Lee, J., Ronning, C.M., Koo, H.L., et al., 1999. Sequence and analysis of chromosome 2 of the plant *Arabidopsis thaliana*. Nature 402, 761–768.

Luengwilai, K., Beckles, D.M., 2009. Starch granules in tomato fruit show a complex pattern of degradation. J. Agric. Food Chem. 57, 8480–8487.

Maeo, K., Tomiya, T., Hayashi, K., Akaike, M., Morikami, A., Ishiguro, S., Nakamura, K., 2001. Sugar-responsible elements in the promoter of a gene for beta-amylase of sweet potato. Plant Mol. Biol. 46, 627–637.

Manners, D.J., 1985. Starch. In: Dey, P.M., Dixon, R.A. (Eds.), Biochemistry of Storage Carbohydrates in Green Plants. Academic Press, New York (149–203 pp.).

Moore, S., Vrebalov, J., Payton, P., Giovannoni, J., 2002. Use of genomics tools to isolate key ripening genes and analyse fruit maturation in tomato. J. Exp. Bot. 53, 2023–2030.

Motamayor, J.C., Mockaitis, K., Schmutz, J., Haiminen, N., Iii, D.L., Cornejo, O., Findley, S.D., Zheng, P., et al., 2013. The genome sequence of the most widely cultivated cacao type and its use to identify candidate genes regulating pod color. Genome Biol. 14, R53.

Nardozza, S., Boldingh, H.L., Osorio, S., Hohne, M., Wohlers, M., Gleave, A.P., MacRae, E.A., Richardson, A.C., Atkinson, R.G., Sulpice, R., Fernie, A.R., Clearwater, M.J., 2013. Metabolic analysis of kiwifruit (*Actinidia deliciosa*) berries from extreme genotypes reveals hallmarks for fruit starch metabolism. J. Exp. Bot. 64, 5049–5063.

Peroni, F.A.G., Koike, C., Louro, R.P., Purgatto, E., Do Nascimento, J.R.O., Lajolo, F.M., et al., 2008. Mango starch degradation. II. The binding of α-amylase and β-amylase to the starch granule. J. Agric. Food Chem. 56, 7416–7421.

Piccaglia, R., Galletti, G.C., 1988. Sugar and sugar alcohol determination in feedstuffs by HRGC, HPLC and enzymic analysis. J. Sci. Food Agric. 45, 203–213.

Purgatto, E., Lajolo, F.M., Do Nascimento, J.R., Cordenunsi, B.R., 2001. Inhibition of beta-amylase activity, starch degradation and sucrose formation by indole-3-acetic acid during banana ripening. Planta 212, 823–828.

Ren, G., Healy, R.A., Horner, H.T., James, M.G., Thornburg, R.W., 2007. Expression of starch metabolic genes in the developing nectaries of ornamental tobacco plants. Plant Sci. 173, 621–637.

Richardson, A., Boldingh, H., McAtee, P., Gunaseelan, K., Luo, Z., Atkinson, R., David, K., Burdon, J., Schaffer, R., 2011. Fruit development of the diploid kiwifruit, *Actinidia chinensis* 'Hort16A'. BMC Plant Biol. 11, 182–196.

Robinson, N.L., Hewitt, J.D., Bennett, A.B., 1988. Sink metabolism in tomato fruit. 1. Developmental changes in carbohydrate metabolising enzymes. Plant Physiol. 87, 727–730.

Rounis, V., Skarmoutsos, K., Tsaniklidis, G., Nikoloudakis, N., Delis, C., Karapanos, I., Aivalakis, G., 2015. Seeded and parthenocarpic cherry tomato fruits exhibit similar sucrose, glucose and fructose levels, despite dissimilarities in UGPase and SPS gene expression and enzyme activity. J. Plant Growth Regul. 34, 47–56.

Schaffer, A.A., Petreikov, M., 1997. Sucrose to starch metabolism in tomato fruit undergoing transient starch accumulation. Plant Physiol. 113, 84–99.

Sparla, F., Costa, A., Lo Schiavo, F., Pupillo, P., Trost, P., 2006. Redox regulation of a novel plastid-targeted beta-amylase of *Arabidopsis*. Plant Physiol. 141, 840–850.

Stenzel, I., Hause, B., Maucher, H., Pitzschke, A., Miersch, O., Ziegler, J., Ryan, C.A., Wasternack, C., 2003. Allene oxide cyclase dependence of the wound response and vascular bundle-specific generation of jasmonates in tomato: amplification in wound signalling. Plant J. 33, 577–589.

Takahashi, I., Kuboi, T., Fujiwara, T., Hara, M., 2012. Overexpression of an extraplastidic β-amylase which accumulates in the radish taproot influences the starch content of *Arabidopsis thaliana*. Plant Biol. 29, 447–455.

Tsaniklidis, G., Delis, C., Nikoloudakis, N., Katinakis, P., Passam, H.C., Aivalakis, G., 2014. L-Ascorbic acid metabolism in parthenocarpic and seeded cherry tomatoes. Plant Growth Regul. 72, 141–153.

Wang, F., Sanz, A., Brenner, M.L., Smith, A., 1993. Sucrose synthase, starch accumulation, and tomato fruit sink strength. Plant Physiol. 101, 321–327.

Zhang, D., Wang, Y., 2002. Beta-amylase in developing apple fruits: activities, amounts and sub cellular localization. Sci. China Life Sci. 45, 429–440.