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Gene Expression, Activity and Localization of Beta-Galactosidases during Late Ripening and Postharvest Storage of Tomato Fruit

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Abstract: Beta-galactosidases (*β-GAL*s) hold a key role in both fruit softening and the increase of total soluble solids during maturation. Despite determining both quality and potential postharvest longevity, *β-GAL* activity during ripening, with a special focus on the postharvest period, has not been adequately addressed in a spatial and temporal manner. This study focused on the regulation of gene expression in relation to the total *β-GAL* enzyme activity during the ripening of tomato fruit attached on the plant, as well as harvested fruit ripened for 5 d at 4, 10, or 25 °C. The transcription of genes coding for *β-GAL* isoenzymes was significantly affected by both the fruit maturation stage (unripe vs. red ripe) and postharvest storage temperature. Cold stressed tomatoes (4 °C) exhibited a remarkably higher transcription of most *β-GAL* genes compared to on-plant red ripe fruit and to fruit exposed to either 10 or 25 °C, indicating a low temperature response. However, enzymatic activity and water-soluble pectin content increased with elevated temperature exposure, peaking in fruit stored at 25 °C. *β-GAL* activity was present in the pericarp, while it was less detected in locular parenchyma. These findings highlight the dual role of *β-GAL* not only in maturation, but also in the metabolism during postharvest homeostasis and cold acclimation of tomato fruit.

Keywords: beta-galactosidase; cold stress; fruit quality; gene expression; *Solanum lycopersicum* L.; soluble pectins

1. Introduction

Fruit softening during ripening determines the potential postharvest life and is one of the most important fruit quality indices [1]. It is a character of texture, generally associated with organized modifications in the two layers of cell wall (i.e., primary cell wall, middle lamella). These layers hold a highly complex and dynamic structure, with alterations being genetically programmed, but also being affected by environmental conditions and cultivation practices. Primary cell walls are composed of polysaccharides, which include cellulose fibrils, hemicellulose (neutral or weakly acidic glycans), and pectin [characterized by a high content of galacturonic acid (GalA) residue], while proteins, phenolic compounds, and ions

are also present. Pectins have a high content of GalA. They can be linear and unbranched (homogalacturonan) or with conserved side chains of neutral sugars (rhamnogalacturonan II or RG-II) in contrast to Rhamnogalacturonan I (RG-I), which consists of alternate GalA and rhamnose residues, linear or branched with arabinan and galactan side chains [2]. By contrast, the middle lamella consists only of homogalacturonans and some proteins [3].

Softening is largely the outcome of the degradation and solubilization of polysaccharides caused by several cell wall degrading enzymes, mainly pectinesterase, polygalacturonase, and b-galactosidase (*β-GAL*), resulting in both a rise in loosely cell wall-attached pectin and a reduction in the covalently linked pectin [4]. These processes have been, and continue to be, the focus of experimentation aimed towards a better understanding of the underlying mechanisms, and in this way gaining a better insight into extending fruit shelf life and an increase of market value [5].

*β-GAL*s consist of a group of hydrolases catalyzing the breakdown of the terminal non-reducing β-D-galactosyl residues from oligosaccharides and polysaccharides, as well as glycoproteins and glycolipids [6]. *β-GAL* is implicated in several physiological processes and plays a pivotal role in seed germination, cell growth, and fruit ripening, where extensive cell wall loosening takes place [2,7–9]. Indeed, fruit *β-GAL*'s contribution is crucial since it disrupts the cell wall by cleaving the galactose residues from RG-I early in ripening, thus causing texture changes related to fruit softening [10,11]. Particularly, this galactan degradation increases pectin solubilization through RG-I solubility and facilitates the access of pectinesterase and polygalacturonase to their substrates—particularly to homogalacturonan [12].

In the tomato genome, seventeen putative *β-GAL*-coding isoenzymes have been identified with diverse physiological roles and subcellular localizations. From these isoenzymes, at least seven are known to be expressed in fruit participating in the ripening process [6,13]. The combined activity of these seven isoenzymes has been shown to contribute to the total *β-GAL* enzymic activity. This contribution, together with the transcription of the corresponding genes, was found to be rather dissimilar in tomato fruit during early and late ripening [6,9,14,15]. Apart from on-plant maturation, *β-GAL* remains active during postharvest life and, in this way, it can influence fruit quality until consumption [1,16]. Tomato fruit are globally among the most important agricultural commodities [17,18], and thus, data about the regulation of the enzymatic systems directly associated to the consumer-perceived quality is of extreme importance [19]. Besides the involvement of *β-GAL*s at early maturation, they are among the major hydrolases that also regulate the events of late maturation contributing greatly to fruit softening and total soluble solids index, thereby shaping the taste [20]. Although the importance of *β-GAL* during the tomato fruit maturation is sufficiently documented [13], the regulation of the enzyme and the possible correlations to several important quality traits are largely unknown during the fruit postharvest life. Moreover, in real life scenarios, harvested tomatoes are stored at temperatures below the recommended (<10 °C) until consumption. The interplay among the cold stress, ripening, and *β-GAL* enzymatic activity of tomato fruit remains unclear as the gene expression provokes these events following the onset of cold storage.

Despite the undisputed role of hydrolases during critical physiological stages affecting quality, the activity of *β-GAL* in the course of ripening, with a special focus on the postharvest period, has received rather limited attention. Since fruit-localized *β-GAL* isoenzymes participate in the shaping of fruit quality, the present work was undertaken to analyze the regulation of gene expression in relation to the total *β-GAL* enzyme activity, total soluble solids, and texture of tomato fruit during late on-plant maturation and during short-term (5 d) postharvest storage at different temperatures, such as 4, 10, or 25 °C. We suggest that *β-GAL*s, apart from their role in fruit softening during late maturation to over-maturation, hold an important function in the physiological reactions during chilling.

2. Materials and Methods

2.1. Plant Material and Growth Conditions

Tomato *Solanum lycopersicum* L. cv. Chondrokatsari, a Greek traditional variety with excellent organoleptic properties and relatively high yield, was studied. Plants were grown under a random block design in a greenhouse compartment located in the southern region of Peloponnese (Kalamata, Greece) and were subject to standard agronomic management practices in the region. The growth period spanned from March to October. The air temperature, relative air humidity, and photosynthetically active radiation were recorded at canopy height throughout the cultivation period using cross-checked sensors linked to a datalogger. During cultivation, the mean air temperature was 23.2 ± 2.1 °C (range: 15.9–26.8), while the mean relative air humidity was 56 ± 8% (range: 30–80). No artificial illumination was employed during cultivation. The average daily light integral was 10.3 ± 0.4 mol m^{-2} day^{-1} (range: 8.6–12.8) (LI-250A, LI-COR, Lincoln, NE, USA).

Fifteen fruit for each treatment at the appropriate stage of maturation (at Commercial Maturity/turning-CM) were tagged and left to mature on the plants (i.e., they remained intact), while others were harvested. The intact fruit were harvested after an additional five day period compared to the harvested fruit. Following this period, the fruit had reached the Red Ripe (RR) stage. The harvested fruit were sampled at the CM stage. Each harvest was carried out at 11:00 am (22–24 °C during harvest). The harvested fruit were stored for five days at 4, 10 and 25 °C (further referred as 5 d 4 °C, 5 d 10 °C, and 5 d 25 °C, respectively) (Supplementary Material). These treatments correspond to common practices for retaining tomatoes in households (4 or 25 °C), or in the processing industry (10 °C), respectively [5] (Supplementary data S1).

Plants with a uniform appearance (intact fruit of similar size with no obvious abnormalities) were selected for measurements. At least 20 fruit per treatment (separated in three lots for biological replicates) were used for each assay. For all gene expression and enzyme activity assays, the samples were immediately frozen in liquid nitrogen after collection or treatment, homogenized using a pestle and mortar, and eventually stored at −80 °C. For each experiment, segments of at least 20 fruit were used as the sample.

2.2. Fruit Firmness and Total Soluble Solids Assay

The firmness of each fruit was evaluated using a Chatillon DFIS-10 penetrometer (John Chatillon, Greensboro, NC, USA) mounted on a Chatillon TCM 201-M using a 6.3 mm-needle with a penetration of 0.6 cm at a constant speed of 20 cm per min^{-1}. The firmness was expressed in Newtons (N). The total soluble solids were also assessed using a HR32B refractometer (Schmidt & Haensch, Berlin, Germany) (Table 1; Figures 1 and 2).

Table 1. Total soluble solids and firmness of tomato fruits (cv Chondrokatsari) during their late maturation and after postharvest storage (5 d) at different temperatures (4, 10 and 25 °C). Significant differences between treatments were determined by one-way ANOVA and false discovery rate correction (FDR) ($p < 0.05$).

	Stage		**Duration/Storage Temperature**		
	Commercial Maturity (CM)	**Red Ripe (RR)**	**5 d 4 °C**	**5 d 10 °C**	**5 d 25 °C**
Total soluble solids (° Brix)	8.20	9.70	8.90	9.30	10.40
SD	0.42	0.57	0.40	0.54	0.48
Homogenous groups	c	ab	bc	b	a
Firmness (N)	1.15	0.94	1.03	0.96	0.73
SD	0.03	0.06	0.08	0.05	0.06
Homogenous groups	a	b	a	b	c

Figure 1. Positive, neutral, and negative affinities across traits in *Solanum lycopersicum* L. cv. Chondrokatsari. Plants were cultivated across maturity stages (CM, RR) and postharvest treatments (5 d 4 °C, 5 d 10 °C, 5 d 25 °C). Positive correlations are portrayed by blue circles, while negative associations are indicated by red circles. The intensity of color corresponds to the correlation coefficient (r) ranging from -1 to 1 (scale). The larger size of circles indicates statistically significant values (non-significant, $p = 0.05$, $p = 0.01$, $p = 0.001$ respectively).

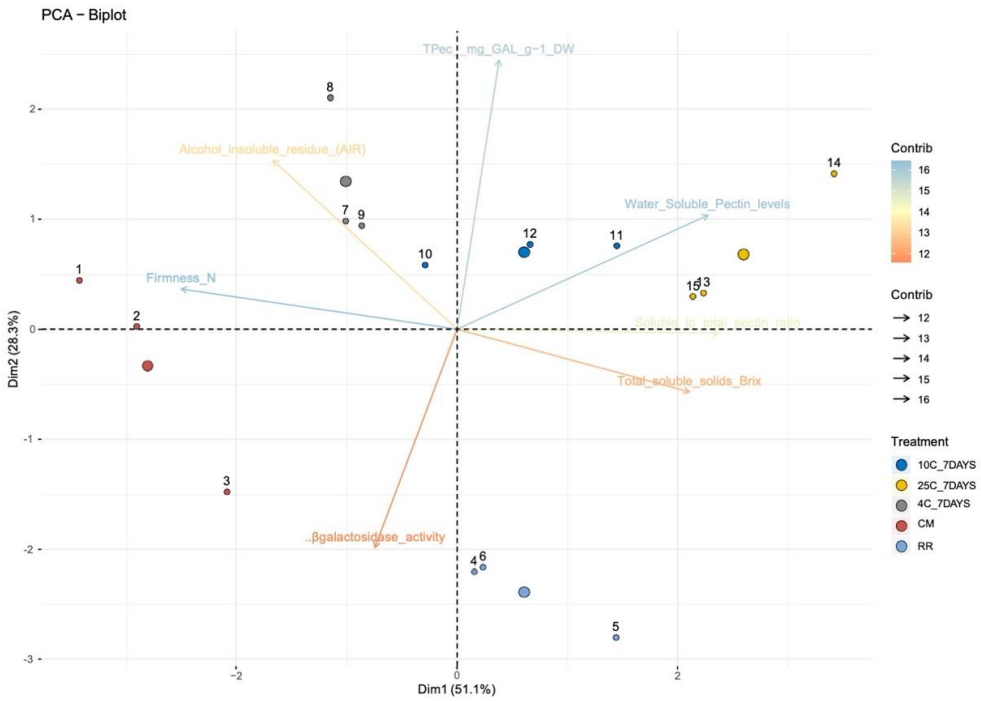

Figure 2. Biplot Principal coordinate analysis across stages. Larger dots indicate mean values calculated from discrete biological replications. The contribution of each trait in the two dimensions is indicated by a gradient scale and color intensity (scale). Vectors near the plot center have lower cos2 values. Narrow angles among variables indicate affinity and wide angles a negative correlation.

2.3. qPCR Expression Analysis

The total RNA was isolated from each sample using the RNeasy extraction Kit (Qiagen, Hilden Germany). The quantity and quality of total RNA were assessed using a spectrophotometric and electrophoretic analysis, measuring both the absorbance at 260 nm and the absorbance ratio of 260/280 nm in Nanodrop (Thermo, Wilmington, DE, USA), as well as by 1.5% *w*/*v* agarose-gel electrophoresis. The elimination of total DNA and the cDNA synthesis were performed with RNAse free DNaseI (Takara, Otsu Shiga, Japan) and Affinity ScriptTM Multi Temperature reverse transcriptase (Stratagene, Santa Clara, CA, USA), respectively, according to the manufacturer's instructions. The complete DNA removal was confirmed with primers designed against Ubiquitin (*UBQ*), while *Solanum lycopersicum* genomic DNA was used as a positive control. *UBQ* was also used for the normalization of the first-strand cDNA. Gene-specific primers for tomato *β-GAL* [4] and *UBQ* genes were designed using a Beacon designer v 7.01 (Supplementary data S2). Quantitative real time PCR reactions were performed on a MX-3005P system (Stratagene, Santa Clara, CA, USA) using Kapa Fast Universal 2X qPCR Master Mix (Kapa, Woburn, MA, USA). The expression levels of *Solanum lycopersicum UBQ* were used for normalization. The relative quantification of gene expression was performed as previously described [21–23]. For all samples, qPCR reactions were performed in triplicates. A pairwise fixed reallocation randomization test was performed via the REST-xl package, as reported by Pfaffl [24]. The *UBQ* gene was used as a reference gene and the Actin gene was used for the verification of the results. Transcription profiles were processed: relative values were standardized (median-centered across each treatment), and hierarchical clustering was performed using the R version 3.6.0 and the Package 'gplots'. Individual expression data for all studied *β-GAL* genes is provided in the Supplementary data section of this manuscript (Supplementary data S3).

2.4. Enzyme Extraction and Estimation of Total β-GAL Enzyme Activity

The extraction of the crude enzyme was performed with 4 mL of the extraction buffer (0.2 mM Na_2HPO_4, pH adjusted at 4.6 with 0.1 M citric acid) with 0.05 g PVP per 1 g of tissue. The *β-GAL* assay was conducted using the method described by Gao and Schaffer [23] for α-Galactosidase assay, replacing the enzyme substrate. The reaction mixture contained 200 mL Mc Invaine solution (0.2 mM Na_2HPO_4 with pH adjusted at 4.6 with 0.1 M citric acid) and 200 mL 3% *w*/*v* 4-Nitrophenyl β-D-galactopyranoside (Sigma-Aldirch, St. Louis, MO, USA). Thirty μg of total protein was used in the reaction extract (estimated with Bradford protein quantification). The reaction mixture was incubated at 30 °C for 30 min and was terminated with the addition of 1 mL 0.5 M Na_2CO_3 (pH 10.5). The production of nitrophenol was spectrophotometrically assessed at 410 nm using a UV-160A photometer (Shimadzu, Tokyo, Japan) (Figure 3).

2.5. Extraction Procedure of Alcohol Insoluble Residue (AIR) for Total and Soluble Pectins

The alcohol insoluble residue (AIR) extraction procedure was carried out as previously described [25–27], after few modifications, as described below. A sample (0.3 g) from freeze-dried tissue was dissolved in 10 mL methanol using an Ultra-Turrax (T25, Ika Labortechnik, Staufen im Breisgau, Germany) for 1 min. The homogenate was filtered (Machery-Nagel MN 615 Ø 90 mm) and then rinsed in sequence and twice with each solvent, which were 20 mL acidified methanol (0.01 N HCl), 20 mL chrorophorm:methanol (1:1), 20 mL 80% acetone diluted with double distilled water, and 25 mL 100% acetone. The filter and the residue (AIR) were dried at 50 °C for 24 h and the AIR was powdered with a mortar and pestle and stored at −20 °C. For each sample, the respective AIR was considered for the final calculations.

The total pectin was extracted from AIR (5 mg) according to Wrolstad et al. [28]. The extraction of soluble pectin was conducted according to Kafkaletou et al. [27] after some modifications, as described below. The AIR (10 mg) was mixed with 1 mL double distilled water, vortexed, placed in ultrasonic bath (20 min), and centrifuged (14,000× *g* for 15 min).

All steps were repeated four times and the combined supernatants were used for the soluble pectin's determination.

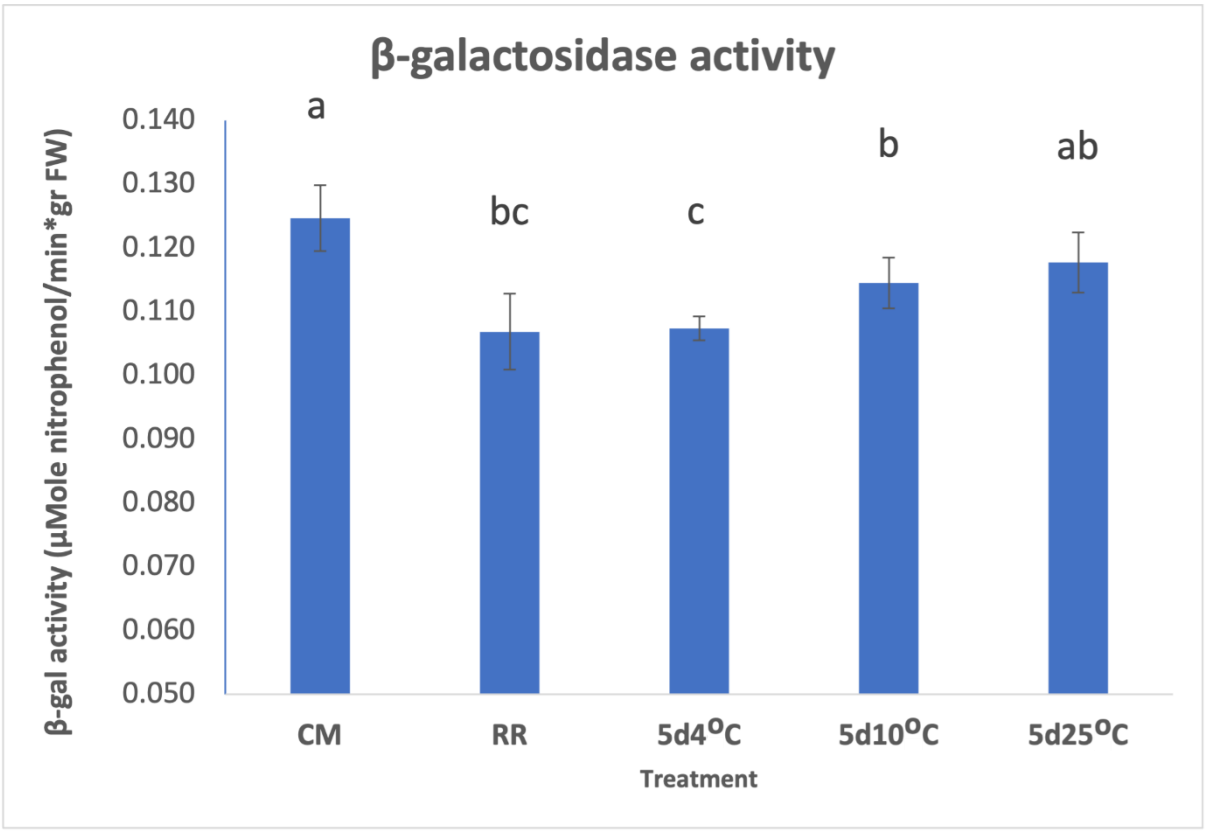

Figure 3. *β-GAL* enzyme activity in tomato fruit cv. Chondrokatsari during late maturation and after postharvest storage (5 d) at different temperatures (4, 10 and 25 °C). Significant differences between treatments were determined by one-way ANOVA and false discovery rate (FDR) correction. Bars represent means (±SE) of three biological replications.

The content of pectin in both extractions of the total and soluble pectin was estimated colorimetrically for the galacturonic acid (GalA) concentration [29]. GalA anhydrate was used for a standard curve and results were expressed as GA equivalents on a dry weight basis (mg g^{-1}) (Figure 4).

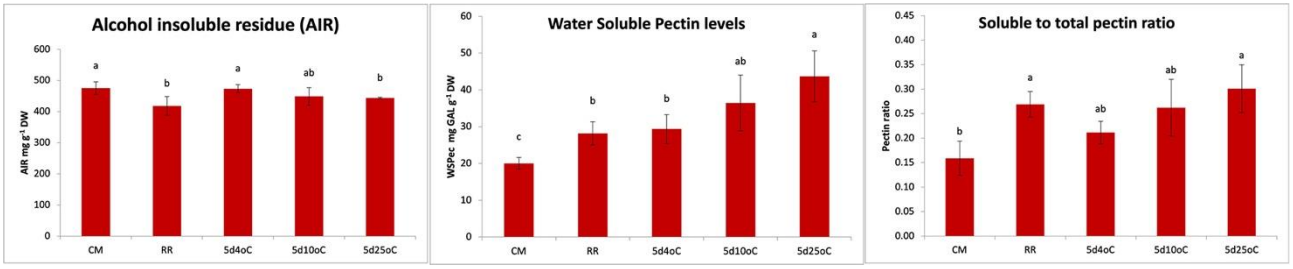

Figure 4. Alcohol insoluble residue (AIR), water soluble pectin, and soluble total pectin of tomato fruit cv. Chondrokatsari during their late maturation and after postharvest storage (5 d) at different temperatures (4, 10 and 25 °C). Significant differences between treatments were determined by one-way ANOVA and false discovery rate (FDR) correction. Bars represent means (±SE) of three biological replications.

2.6. In situ Localization of β-GAL Activity

Sections of tomato fruit (20 mm) were fixed in a mixture containing 2% Polyvinylpyrrolidone, 2% paraformaldehyde (40%), 1 mM Dithiothreitol (Sigma-Aldrich, St. Louis USA), and 0.025% BSA for 20 min at room temperature (25 °C) and then stored overnight at 5 °C. The activity of *β-GAL* was visualized according to the method described by Tsaniklidis et al. [5], with minor modifications. 5-Bromo-4-chloro-3-indolyl β-D-galactopyranoside (XGal) (Sigma-Aldrich, St. Louis, MO, USA) was used in the reaction mixture. The reaction mixture contained 50 mM CH_3COONa (pH 5.0), 1 mM beta X Gal and 0.03% NBT (Figure 5).

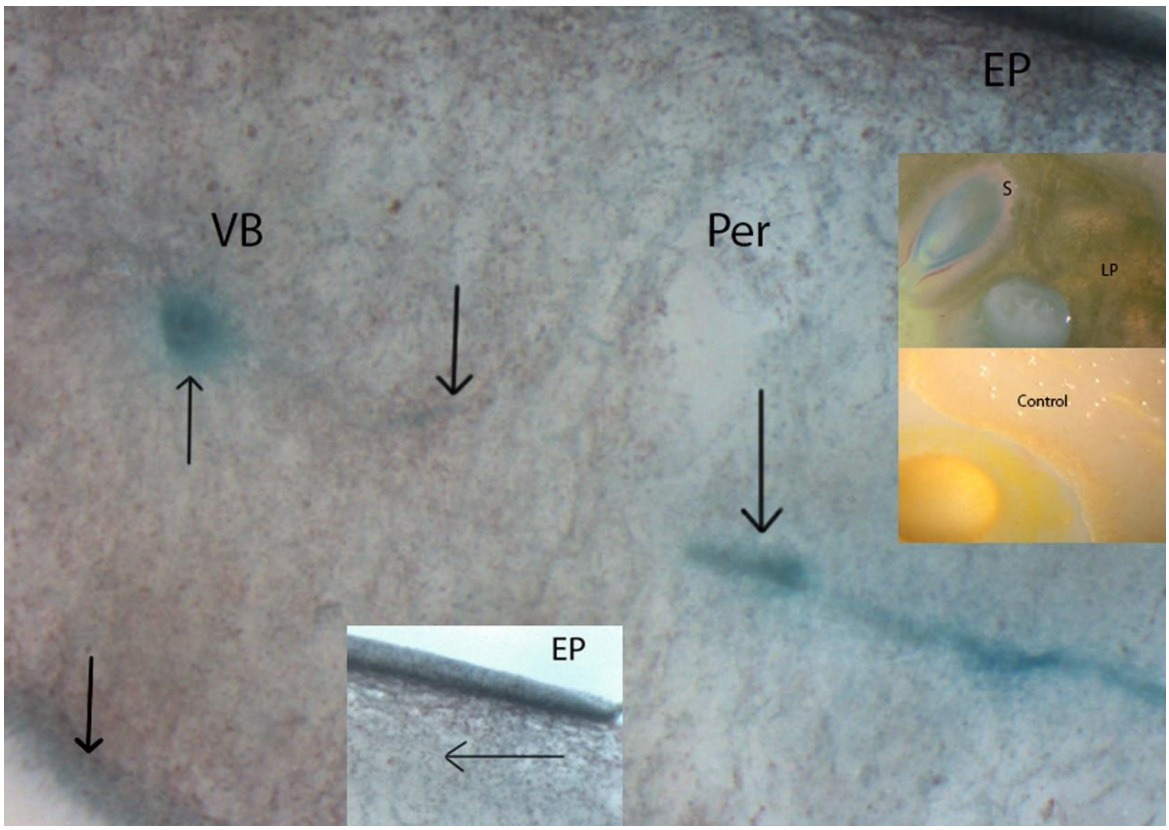

Figure 5. Localization of the *β-GAL* activity on different tomato fruit cv. Chondrokatsari segments at CM stage of fruit maturation. The enzyme activity was visualized as a darker stain. In1; control. VB; vascular bundle, LP; locular parenchyma, Per; pericarp, EP; epidermis, S; seed.

2.7. Statistical Analysis

For the experimental units, eigenvalues were extracted and the most contributing variables for each dimension were computed and identified. The first two eigenvalues cumulated about 80% of the total variance and were retained to produce the principal components. A biplot principal component analysis (PCA) was produced to depict associations across the treatments. Individuals were grouped (by discrete color) and variables by their contribution to the principal components (gradient colors). A correlation plot was also computed in order to depict positive and negative associations across the variables inquired. False discovery rate (FDR) tests were performed using the "multcomp" package. The "corrplot", "FactoMineR", "factoextra", and "readxl" libraries were used under the R-studio integrated development environment (RStudio suite V 1.2.5033).

3. Results and Discussion

3.1. β-GAL during Late On-Plant Tomato Maturation

The study of seven genes coding for *β-GAL* isoenzymes transcription levels revealed that all of them were expressed in fruit during their maturation and postharvest storage

at different temperature regimes i.e., 4, 10, and 25 °C (Figure 6). Pattern similarities were evident for most genes coding *β-GAL* isoenzymes. During late on-plant maturation, the transcription of *β-GAL 2*, *5* genes was higher in CM fruit than in RR fruit, while for *β-GAL 4* and *7* no significant differences were found between the two late maturation stages. *β-GAL 3* expression was only detected at the RR stage. These findings suggest that during late maturation, the regulation of the transcription some isoenzymes of *β-GAL* is rather independent for each gene coding for *β-GAL* isoenzyme despite any sequence similarities (Figure 6). Although all gene expressions coding for softening enzymes usually perform under a basis of orchestrated ordered cell wall restructuring and turnover, the observed regulation of *β-GAL* suggests a more complex regulation of the isoenzymes [21]. The higher transcription of some of *β-GAL* genes, at CM than in RR, was accompanied by a significantly higher enzymatic activity on CM stage (Figures 3 and 6). Similar results are presented by Smith et al. [22], who reported a higher transcription of *β-GAL 1* in the turning stage of fruit maturation in comparison to the RR one. However, another tomato study showed that the down-regulation of *β-GAL 1* in transgenic tomato fruit did not affect firmness [14]. Moreover, Posé and colleagues [23] revealed that a reduction of cell wall polysaccharides takes place during late maturation of tomato fruit, which is in accordance with our findings regarding the total soluble solid levels (Table 1).

Simpler sugars were accumulated because of the polysaccharides breakdown, and firmness was lower at the RR stage when compared to the CM stage (Table 1), suggesting a reduced availability of *β-GAL* substrate at the RR stage. Moreover, the content of soluble pectin was higher in the RR stage compared to the CM stage (Figure 4).

A correlation analysis across the five stages of experimental units indicated that several traits are indeed interconnected (Figure 1). Positive correlations were detected among the soluble-to-total pectin ratio and the water soluble pectin levels, as well as the AIR and firmness traits. On the other hand, firmness seems to be negatively correlated with beta-galactosidase activity, as well as to water soluble pectin levels and the soluble-to-total pectin ratio.

The present results are in general agreement with other observations stated that the earliest events in fruit softening are a loss of pectic galactan side chains and the depolymerization of glycans; these processes were later followed by pectin solubilization during mid-maturation [2,12]. This increase in pectin solubilization is an apparent maturation characteristic [24], accompanied by firmness reduction, as shown previously [17].

In order to identify and quantify the components that regulate the connections across developmental stages and treatments, a biplot PCA was conducted (Figure 2). Eigenvalues were examined to determine the number of considered principal components. The first two dimensions explained approximately 80% of the total variance percentage (Supplementary data S4). The level of significant contribution of morphological traits to the PCA was estimated by using the cos2 index (Supplementary data S5). Among these descriptors, firmness, pectin levels, and Tpec had a profound imprint for the categorization of the experimental units. A PCA based on the first two components revealed the complex relationships among treatments (Supplementary data S5). The first axis revealed that the most significant discrimination was based on the maturity index since Red Ripe (RR) tomatoes were clearly demarcated from Commercial Maturity (CM) fruits. Nonetheless, the exposure of tomato to low temperatures had a significant affect in the fruit homeostasis that was reflected on the second PCA axis. It appears that, as cold stress is prolonged (4 °C 7 days), the physiological responses become more intense. The biplot analysis also depicts negative connections of beta galactosidase activity with TPec values and that firmness is also negatively correlated to the total soluble content as well as water soluble pectin levels.

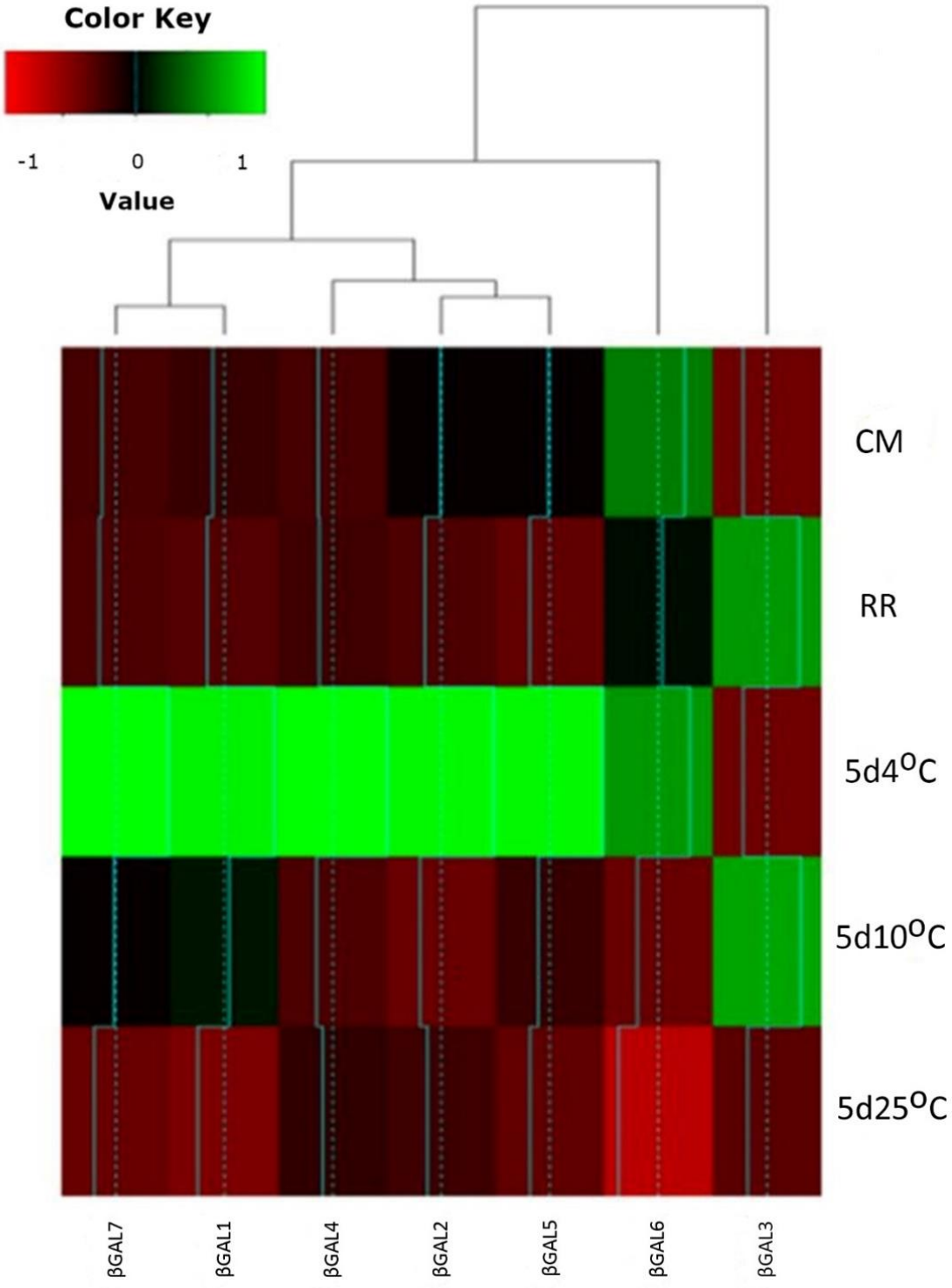

Figure 6. Heatmap of the relative transcription for tomato *β-GAL* genes (cv. Chondrokatsari) during their late maturation on-plant and after postharvest storage (5 d) at different temperatures (4, 10 and 25 °C). Relative mRNA abundance was assessed by real-time RT-qPCR, employing three independent biological replicates.

While considerable similarities were found in the fluctuation of the transcription of *β-GAL 4*, the results of cv. Chondrokatsari differed from those of the cv. Rutgers, as studied by Smith and Gross [6] concerning the accumulation of the transcripts of *β-GAL 6* and *7* genes, probably reflecting maturation and firmness discrepancies among different cultivars. Moreover, *β-GAL 6* has been shown to participate in specific physiological processes in

tomato fruit, such as the resistance to fruit cracking in ripe fruit, a trait which is also described to be cultivar-dependent. Its higher expression may be related to biosynthetic activity by transglycosylation and reverse hydrolysis resulting in cell wall strengthening, as in earlier maturity stages [6]. In contrast, the down-regulation of *β-GAL 6* increased fruit cracking and a thickening of the cuticle [30,31] The dataset presented in this study clearly indicates that the expression of certain genes is cultivar-specific by comparison; although, the general pattern of gene expression coding *β-GAL* isoenzymes bears some similarities. A high dependency, but with low sensitivity, of a *β-GAL* on the expression of a gene coding for the ethylene biosynthetic enzyme 1-aminocyclopropane-1-carboxylate oxidase has been demonstrated in apple fruit. However, the low sensitivity to ethylene concentration was overcome by a longer exposure time, exhibiting the role of *β-GAL* early in maturing fruit with low ethylene production [32]. In the Japanese pear (*Pyrus pyrifolia* L.), as in the case of tomato cultivar Chondrokatsari, the transcription of most *β-GAL* genes (e.g., *β-GAL 3*) peaked during late maturation (RR stage). Instead, the *β-GAL 4* was only expressed in the later stages of maturation, suggesting that this gene is late maturation specific [33]. Therefore, as noted earlier in other species and tomatoes, the expression of specific genes depends on the maturity stage [6]. Finally, the localization of the enzymatic activity of *β-GAL* (Figure 5), apart from the seeds, mostly in the tomato fruit pericarp and specifically at the cell walls, correlates with the contribution of the enzyme in the cell wall modifications during late maturation, resulting in fruit softening [4,34].

Interestingly, the distribution of the enzymatic activity of *β-GAL* was comparable to glutamate dehydrogenase (GDH) activity in tomato fruit [35]. Additionally, Van de Poel et al. [36], who studied the critical events of fruit maturation in several tissues of tomato fruit—especially the ethylene biosynthesis that orchestrates the climacteric maturation—indicated that the pericarp is the main site of these processes, being in agreement with our findings regarding the localization *β-GAL* activity (Figure 5).

3.2. β-GAL during Postharvest Tomato Storage

The transcription of all genes, except *β-GAL 3*, peaked in fruit stored at 4 °C for 5 d, suggesting that they respond to cold stress (Figure 6). The 4 °C temperature regime is considerably below the recommended storage temperature (10–12 °C) and can cause chilling injury, even though it is popular for household storage [35]. It is not uncommon for both gene transcription and activities of either hydrolytic (e.g., α-galactosidase) or redox-regulating enzymes (e.g., glutamate dehydrogenase) in tomato fruit to be stimulated after short-term cold storage [35]. Interestingly, here the level of soluble pectin was comparable to that of the RR fruit, indicating that a hydrolysis of polysaccharides towards simpler ones may take place to protect fruit from chilling stress (Figure 4) [37], although AIR, the ratio of soluble-to=-total pectin (Figure 4), and firmness, as shown previously [17], were all higher than in the RR. The similar levels of soluble pectin in RR, and in fruit stored at 4 °C, are in line with the similar total activity of *β-GAL* (Figure 3). It is worth noting that polysaccharides liberate signaling molecules, contribute to mechanical support, but also to tolerance in response to biotic [38] and abiotic [39] stress. Moreover, [40] showed that *β-GAL* 5 activity can hydrolyze β-(1→3) and β-(1→6)-linked galacto-oligosaccharides. These processes probably act as a means to maintain a physiological balance in enzymatic activities, even though the optimum temperature can be up to 50 °C for some isoenzymes [3,41]. By contrast, Bai et al. [42] discovered the down regulation of *β-GAL* at low temperatures, which might be ascribed to different genotypes.

Postharvest treatments significantly affected fruit across maturity stages (Supplementary data S1). Saltveit [43] indicated that the maturation of tomatoes is accelerated in harvested fruit at 20 °C. The physiological parameters and the enhanced accumulation of total soluble pectin (Figure 4) of the fruit stored at 25 °C shows that the maturation process has been accomplished and that over-maturation was likely reached, which can explain the rather low transcription of most *β-GAL* genes (Figure 6). In contrast, the fruit retain a comparably high *β-GAL* activity level (Figure 3), probably because the storage

temperature favors it, and due to the high half-life of the enzymes [44]. Similarly to the results of this experiment, in the storage of peach fruit the expression of *β-GAL* genes did not follow similar patterns where most genes exhibited reduced expression, especially in harvested fruit stored at 25 °C. Differences in the expression patterns of *β-GAL* genes were also found between two peach varieties [11]. The storage of CM tomatoes inside the recommended range of storage (circa 10 °C) resulted in a low *β-GAL* activity, and a marginal effect on the gene transcription indicated that their maturation continues, albeit at a slower pace compared to the other treatments (Figure 4). These results are comparable to those of Mwaniki et al. [1] in pears stored at 1 °C (which falls in the recommended temperature range), who observed the retention of the transcription levels of most genes coding for *β-GAL* isoenzymes at comparable levels to those of fruit at late stages of maturation. In apples, storage temperature also affected the *β-GAL* activity. Storage at room temperature resulted in a significant increase of the *β-GAL* activity, whereas no significant differences were observed in the enzyme activity during storage for the same period inside the recommended temperature range (0 °C; [45]).

4. Conclusions

The transcription of genes coding for *β-GAL* isoenzymes was significantly affected by the fruit maturation stage and storage temperature during tomato postharvest life. In the fruit on-plant maturation, i.e., during preharvest life, some genes' transcription levels and enzyme activities were higher at the Commercial Maturity (CM) stage than at the Red Ripe (RR) stage, suggesting a different regulation of the enzyme that can probably be attributed to the reduction of the substrate availability. Thereafter, during the postharvest life, it was evident that the chilling storage temperatures (4 °C) led to a dramatic increase of transcription levels which, in turn, suggests a role for several isoenzymes in cold stress response. In contrast, over-maturated fruit exhibited high *β-GAL* activity and, generally, lower gene transcription. The fruit stored at temperatures inside the recommended temperature range (10 °C) retained gene transcription levels comparable to the RR fruit, while they exhibited low *β-GAL* activity. Furthermore, while the progress of tomato fruit maturation is ongoing during the post-harvest period, maturation proceeds at a significantly slower pace as compared to the maturation rate of the fruit that remained on-plant. The results suggest that the quality of the harvested tomato fruit might be better maintained, and shelf life more prolonged, using proper postharvest handling practices and treatments.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agriculture12060778/s1, Supplementary data S1: Tomato fruits from treatments; Supplementaty data S2: Primers used for qPCR (with gene name and NCBI accession number); Supplementary data S3: gene expression of *β-GAL* genes; Supplementary data S4: The first seven principal components and percentages of attributed variation; Supplementary data S5: Quality of representation (cos2) of the variables on factor map.

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Abbreviations

AIR, alcohol insoluble residue; CM, commercial maturity; GalA, galacturonic acid; RG-I, rhamnogalacturonan-I; RR, red ripe; UBQ, Ubiquitin; *β-GAL*, Beta-galactosidases.

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