TOMATO PLANTS INCREASE THEIR TOLERANCE TO LOW TEMPERATURE IN A CHILLING ACCLIMATION PROCESS ENTAILING COMPREHENSIVE TRANSCRIPTIONAL AND METABOLIC ADJUSTMENTS

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ABSTRACT

Low temperature is a major environmental stress that seriously compromises plant development, distribution and productivity. Most crops are from tropical origin and, consequently, chilling sensitive. Interestingly, however, some tropical plants, are able to augment their chilling tolerance when previously exposed to suboptimal growth temperatures. Yet, the molecular and physiological mechanisms underlying this adaptive process, termed chilling acclimation, still remain practically unknown. Here, we demonstrate that tomato plants can develop a chilling acclimation response, which includes comprehensive transcriptomic and metabolic adjustments leading to increased chilling tolerance. More important, our results reveal strong resemblances between this response and cold acclimation, the process whereby plants from temperate regions raise their freezing tolerance after exposure to low, non-freezing temperatures. Both chilling and cold acclimation are regulated by a similar set of transcription factors and hormones, and share common defense mechanisms, including the accumulation of compatible solutes, the mobilization of antioxidant systems and the rearrangement of the photosynthetic machinery. Nonetheless, we have found some important divergences that may account for the freezing sensitivity of tomato plants. The data reported in this manuscript should foster new research into the chilling acclimation response with the aim of improving tomato tolerance to low temperature.

Keywords: Chilling acclimation, Low temperature, Tomato, Transcriptome analysis, Metabolome, Hormone signaling, Sugar accumulation, Antioxidant mechanisms, Photosynthetic machinery.

INTRODUCTION

Low temperature (LT) is one of the main environmental factors limiting plant geographical distribution and crop production. LT reduces water availability for the plant, decreases membrane fluidity, and causes an imbalance between the light energy absorbed by photosystems and the energy consumed by metabolic reactions, compromising plant growth and survival (Ruelland *et al.* 2009). These negative effects of LT are exacerbated if temperatures fall below zero. However, not all plants show the same degree of sensitivity to LT. Indeed, plants can be classified in three groups depending on their sensitivity to subzero and chilling (0-10°C) temperatures: freezing-tolerant, chilling-tolerant and chilling-sensitive (Guy 1990). Interestingly, many freezing-tolerant plants from temperate regions are able to cold acclimate, increasing their constitutive freezing tolerance after exposure to low, non-freezing temperatures (Levitt 1980).

Relevant progress in understanding cold acclimation has been made through the study of the C-repeat Binding factors (CBFs), a family of transcriptional regulators that play a central role in this adaptive response (Medina *et al.* 2011). The expression of *CBFs* is swiftly induced by LT and is tightly controlled. The CBFs, in turn, regulate the expression of a myriad of cold-regulated genes that are required for the correct development of cold acclimation (Medina *et al.* 2011). Plant hormones also have a significant function in this process. LT induces changes in the levels of abscisic acid (ABA), ethylene (ET), jasmonic acid (JA) and gibberellins (GA), and genetic evidence have shown that ABA, ET, JA and GA signaling contribute to the regulation of cold acclimation (Shi *et al.* 2015). CBFs, plant hormones and other signaling components are integrated into this adaptive response to finally promote the different physiological and metabolic adjustments needed to increase plant freezing tolerance. These adjustments include the accumulation of sugars and several amino acids to prevent cellular dehydration, the activation of anthocyanin biosynthesis and detoxifying

enzymes to protect membranes and other cellular components against oxidative stress, and the modification of the photosynthetic machinery to limit photoinhibition (Ruelland *et al.* 2009).

Cold acclimation takes place in most freezing-tolerant plants, including some economically important crops such as wheat, rye and barley. Nevertheless, a limited, but relevant, capacity to acclimate to LT has also been observed in chilling-sensitive species. Previous growth at suboptimal temperatures (10-15°C) reduces chilling-induced photoinhibition and injury in vegetative tissues of maize (Nie *et al.* 1992), soybean (Cabané *et al.* 1993), sweet pepper (Liu *et al.* 2001) and rice (Kuk *et al.* 2003). This alleviation of chilling injury symptoms may, ultimately, lead to increased chilling survival, as evidenced in maize where a short incubation at 14°C elicits an adaptive response, termed chilling acclimation, that significantly raises seedling survival to prolonged exposure to 4°C (Anderson *et al.* 1994). The mechanisms regulating this increased chilling tolerance are poorly understood but may be related, to some extent, to the cold acclimation process occurring in freezing-tolerant plants. In this context, exogenous treatments with ABA augment chilling tolerance in maize (Anderson *et al.* 1994) resembling ABA effect on cold acclimation in temperate species (Lång *et al.* 1989). Moreover, maize chilling acclimation has been associated with the accumulation of xanthophyll-cycle pigments (Haldimann 1997) and anthocyanin (Pietrini *et al.* 2002), which are responses associated with cold acclimation in freezing-tolerant plants (Ruelland *et al.* 2009).

Because of their tropical/subtropical origin, many crops including cotton, maize, rice, and tomato are chilling-sensitive (Lyons 1973). Nonetheless, they are cultivated largely outside their native geographical range, oftentimes close to their climatic limit where chilling tolerance becomes crucial. Tomato, the most important horticultural crop, is very sensitive to temperatures below 10°C (Lyons 1973) and fails to cold acclimate

106 (Zhang *et al.* 2004). Yet, some results were reported describing an enhancement in
107 chilling survival after a brief exposure of tomato seedlings to 10°C (King *et al.* 1988).
108 More recently, it was also shown that previous incubation of tomato plants at
109 suboptimal growth temperatures (10-12ºC) ameliorates chilling injury symptoms
110 including photoinhibition, membrane peroxidation and reduction of CO_2 assimilation
111 (Zhou *et al.* 2012). Despite these studies, however, the ability of tomato to acclimate to
112 chilling temperatures remains to be determined. In this work, a multidisciplinary
113 approach, including physiological, genetic, biochemical and molecular analysis, was
114 used to characterize the capacity of tomato plants to chilling acclimate and to identify
115 molecular and physiological mechanisms underlying this adaptive response.
116

MATERIALS AND METHODS

Plant material and growth conditions

Solanum lycopersicum Moneymaker and Ailsa Craig cultivars, and *notabilis* (Burbidge *et al*. 1999), *procera* (Bassel *et al*. 2008) and *never-ripe* (Lanahan *et al*. 1994) mutants were used in this work. Seeds were germinated on moistened filter paper in petri dishes at 25°C for 5 days in the dark, before being transferred to pots containing COMPO SANA Universal substrate (COMPO GmbH, Münster, Germany). Seedlings were grown in a chamber set at 25°C with a 12h photoperiod (day/night) and a photosynthetically active radiation of 100 µmol m^{-2} s^{-1}. Leaf tissue samples from three-week-old plants were utilized in all experiments. To acclimate to chilling temperatures, tomato plants were incubated at 10°C with a 12h photoperiod (day/night) and a photosynthetically active radiation of 40 µmol m^{-2} s^{-1} for different periods of time. Chilling tolerance was analyzed by exposing the plants at 4°C under continuous light with a photosynthetically active radiation of 40 µmol m^{-2} s^{-1} for different periods of time. Subsequently, the plants were transferred to standard growth conditions (25ºC) for recovery and survival score after 10 days.

Gene expression analysis

For RNA sequencing (RNAseq) experiments, total RNAs were extracted from Moneymaker plants exposed 0, 3, 24 and 48 hours at 10°C using Purezol reagent (Bio-Rad), treated with DNase I (Roche), and further purified with the RNeasy MinElute Cleanup Kit (Qiagen). RNA samples were utilized to make the cDNA libraries, each library being generated from an equivalent mixture of three independent RNA preparations. Constructing the libraries and subsequent sequencing was performed by the staff of the Beijing Genome Institute by means of Illumina HiSeq™ 2000 technology. Approximately, 44 million single-end 50-base reads per sample were generated and more than 85% of reads mapped to the tomato ITAG2.3 reference genome using SOAP2.21 (Li *et al.* 2009) with default parameters. Gene expression

levels were calculated using the RPKM (reads per kilobase per million reads) method (Mortazavi *et al.* 2008). Differentially expressed genes (DEGs) were identified by using the algorithm developed by Audic & Claverie (1997) to obtain a *P*-value for each gene between any pair of samples. Then, a False Discovery Rate (FDR) analysis was performed to determine the threshold of *P*-values in multiple tests. We set a FDR≤0.001 and a fold change ≥2 as cutoffs for any given DEG. Because of the limitations of this algorithm (Auer & Doerge, 2010), all important DEG candidates were validated by quantitative PCR (qPCR) (see below). BLAST (2.2.3) and BLAST2GO (2.2.5) were employed for the functional annotation of differentially expressed genes (DEGs), and gene ontology (GO) and Kyoto encyclopedia of genes and genomes (KEGG) for pathway enrichment analysis. Significantly enriched GO terms were established through a hypergeometric test followed by a Bonferroni correction to calculate a *P*-value for each term. We established a $P<0.05$ as cutoff for a significantly enriched GO term. For KEGG pathway enrichment analysis, a hypergeometric test followed by a false discovery rate analysis was realized, and a cutoff of $q<0.05$ was established to determine enriched KEGG pathways.

Quantitative PCR (qPCR) assays were performed as described (Catalá *et al.* 2014). The *SlEF1A* gene was used as a reference in all experiments. Primers utilized for qPCR analysis are listed in Supporting Information Table S1. All reactions were performed in triplicate employing three different RNA samples than those used to making the cDNA libraries for RNAseq experiments. Fold change was calculated by means of the $\Delta\Delta$CT method (Livak & Schmittgen 2001).

Hormone measurements

Determination of free 1-aminocyclopropane-1-carboxylic acid (ACC), a precursor of ET, was performed as described by Bulens *et al.* (2011) with minor modifications: 0.5 g of fresh weight were extracted in 4 mL of 5% sulfosalicylic acid and ACC content was

estimated indirectly by measuring the ethylene liberated from extracts after treatment with NaOCl in the presence of Hg^{2+}. Spikes of defined amounts of ACC were added for reaction efficiency normalization, and a calibration curve of diluted pure ethylene was employed for ACC quantification. GAs, ABA and JA were extracted and purified following the method of Seo *et al.* (2011). Then, hormones were separated using an autosampler and reverse phase UHPLC chromatography (2.6 µm Accucore RP-MS column, 50 mm length x 2.1 mm i.d.; ThermoFisher Scientific). Separated hormones were analyzed in a Q-Exactive mass spectrometer (Orbitrap detector; ThermoFisher Scientific) by targeted Selected Ion Monitoring (SIM), and their concentrations in the extracts determined using embedded calibration curves and the Xcalibur 2.2 SP1 build 48 and TraceFinder programs. Deuterium-labeled hormones were utilized as internal standards for quantification, except in the case of JA, for which we used dhJA. In all cases, hormone measurements were performed in quintuplicate employing fully independent tissue samples.

Metabolite profiling

The relative levels of primary metabolites were determined following the GC-MS protocols described in Rambla *et al.* (2015). Metabolite accumulation was measured in quintuplicate utilizing fully independent tissue samples.

Anthocyanin and chlorophyll determination

Anthocyanin content was determined as outlined in Solfanelli *et al.* (2006) with minor modifications. Anthocyanins were extracted from approximately 100 mg of grounded tissue with 1% HCl in methanol. Extracts were cleared by centrifugation and chlorophylls were removed by chloroform extraction. The aqueous partition was recovered and anthocyanin content was established by measuring absorbance at 530 nm. Chlorophylls were extracted in dimethylformamide. Appropriate dilutions of extracts were used to quantify absorbance at 647 and 664 nm. Chlorophyll content was

calculated from these measurements as reported in Porra *et al.* (1989). Anthocyanin and chlorophyll determinations were performed in triplicate employing fully independent tissue samples.

Statistical Analysis

Data sets were analyzed with the Prism 6 software (GraphPad Software Inc., USA). Comparisons between two groups were made utilizing the Student's t-test. Comparisons between different treatments and the control were made using the one-way ANOVA test. Correlation plots were computed from log_2-transformed values showing the relationship between qPCR results (x-axis) and the corresponding data from RNAseq (y-axis).

Accession numbers

The complete RNAseq data from this publication have been submitted to the GEO database (http://www.ncbi.nlm.nih.gov/geo/) and assigned the identifier accession GSE78154.

RESULTS

Tomato plants increase their chilling tolerance after exposure to suboptimal growth temperature

Tomato plants subjected to 4°C developed chlorosis and wilting symptoms after 5 days, and many of them died when LT treatment was prolonged for more than 10 days (Fig. 1a). Interestingly, however, a dramatic increase in chilling tolerance was observed when plants were incubated one week at suboptimal growth temperature (10°C) before being exposed to 4ºC for 10 days (Fig. 1b), demonstrating that tomato plants can acclimate to chilling temperature.

To determine the time tomato plants require to chilling acclimate, we tested their survival to a prolonged chilling exposure (18 days at 4°C) after being acclimated at 10°C for 0, 2, 4, 6 or 8 days. Results showed that maximal survival was achieved after four days of acclimation at 10ºC (Fig. 1c). Then, we estimated the differences between acclimated (10ºC, 4 days) and nonacclimated tomato plants in terms of days of survival when exposed to 4ºC. To this, we determined the number of days plants must be subjected to 4ºC in order that 50% of them die (Lethal Exposure 50, LE_{50}). Our data revealed that, under our experimental conditions, chilling acclimation increased the LE_{50} approximately in 6 days (Fig. 1d). Chilling acclimated tomato plants still developed normally and produced tomato fruits when returned to standard conditions (25°C) (not shown). Together, these results indicated that tomato plants significantly increase their chilling tolerance after being exposed several days to suboptimal growth temperatures.

Chilling acclimation response in tomato plants entails a large transcriptome reprogramming

The increase in freezing tolerance after cold acclimation is a very complex process that involves many physiological and biochemical adjustments largely regulated by changes in gene expression (Kaplan *et al.* 2007). We reasoned that the observed increase in

tomato chilling tolerance after exposure to suboptimal growth temperature might involve a similar transcriptomic response. To check this hypothesis, we determined changes in gene expression in 3-week-old tomato plants subjected to 10ºC for 3, 24 or 48 hours by means of RNAseq analysis. Results revealed a large transcriptome reprogramming taking place in response to suboptimal growth temperature with 1537, 4404 and 4600 DEGs (FDR<0.001, $|\log_2|>1$) at 3, 24 and 48 hours, respectively (Supporting Information Table S2-S4). Interestingly, we found more upregulated than downregulated genes at all time points (1008, 2871 and 3313 *vs.* 529, 1533 and 1287, respectively) (Supporting Information Table S2-S4). RNAseq data were validated by confirming the expression patterns of 8 induced and 8 repressed genes through qPCR experiments (Fig. 2a-b). Indeed, we found a high correlation between both RNAseq and qPCR datasets (Fig. 2c), thus corroborating the transcriptomic data.

Identified DEGs could be pooled into two main groups, depending on whether their expression patterns were transient or stable. Among the 4559 upregulated genes, 1246 (27.2%) showed transient induction, with 526 (11.5%) being transiently induced only at 3 hours, 75 (1.6%) at 3-24 hours, and 645 (14.1%) at 24 hours. In contrast, 2151 (47.2%) DEGs exhibited stable induction, with 304 (6.7%) displaying increased expression at all time points and 1847 (40.5%) at 24-48 hours. We also identified 1059 (23.2%) DEGs which were upregulated exclusively at 48 hours, and 103 (2.3%) that, intriguingly, were upregulated at both 3 and 48 hours (Fig. 3a). Among the 2156 repressed genes, we found that 869 (40.3%) presented transient repression, with 246 (11.4%) being transiently repressed particularly at 3 hours, 76 (3.5%) at 3-24 hours, and 547 (25.4%) at 24 hours. Furthermore, we recognized 910 (42.2%) DEGs showing stable downregulation, 176 (8.2%) of which displayed decreased expression at all time points, and 734 (34%) at 24-48 hours. Finally, we identified 346 (16%) DEGs that were downregulated specifically at 48 hours, and 31 that were downregulated at 3 and 48 hours (Fig. 3a).

After functional annotation of DEGs, we performed GO classification and GO term enrichment analysis as a first approach to understand the molecular changes that are produced in tomato plants during chilling acclimation. Enrichment analysis indicated that one of the functions that dominated the early steps of chilling acclimation (3 hours) was transcription factor activity (Fig. 3b). Other GO terms that were enriched at an early stage were related to stress responses as well as hormone biosynthesis and signaling. At later stages (24 and 48 hours), there were a significant enrichment of DEGs associated with biosynthesis of metabolite precursors and aromatic compounds, photosynthesis, transcription and translation (Fig. 3b). Further insight into the transcriptomic response of tomato plants to suboptimal growth temperature was gained through pathway-based analysis using the KEGG database (Fig. 3c). During the early phases of chilling acclimation, we found enriched pathways related to plant-pathogen interaction, hormone and phosphatidylinositol signaling, and circadian rhythm. At later steps, the enriched terms found were associated with an adjustment of metabolism, and photosynthetic and translation machineries (Fig. 3c).

All in all, our results indicated that chilling acclimation in tomato plants involves a large transcriptome reprogramming that would take place in, at least, two main phases. First, an early response, after a few hours of suboptimal growth temperature exposure, characterized by transient changes in the expression of genes encoding stress-related proteins, including transcription factors and proteins implicated in hormone biosynthesis and signaling. Second, a later response, after 24 hours of exposure, that is distinguished by stable changes in gene expression leading to a comprehensive adjustment of plant metabolism, photosystems, and transcription and translation machineries.

Chilling acclimation response in tomato plants involves transcription factors associated with cold acclimation in freezing tolerant plants

As mentioned above, GO term enrichment analysis underscored the relevance of transcription factor activity in early stages of the adaptive response that leads to an increase in tomato chilling survival. Notably, among the 156 DEGs encoding putative transcription factors which expression was induced after 3 hours of exposure to 10ºC (Supporting Information Table S5) we found 18 that had been associated with cold acclimation in freezing-tolerant species (Fig. 4a) (Medina *et al.* 2011; Meissner *et al.* 2012; Franklin *et al.* 2014; Park *et al.* 2015; Shi *et al.* 2015). qPCR analysis confirmed the induction of several of these relevant cold-signaling mediators, including *CBF1*, *CBF2*, *REVEILLE1 (REV1)* and *RESPONSIVE TO HIGH LIGHT 41 (RHL41)* (Fig. 4b). No transcription factor associated with cold acclimation was among the 47 transcription factors that were downregulated in tomato plants subjected to 10°C for 3 hours (Supporting Information Table S5). These findings strongly suggested that the chilling acclimation process in tomato shares some molecular mechanisms with the cold acclimation response of freezing-tolerant plants.

Phytohormones regulate chilling acclimation response in tomato plants

Another category that stood out among the GO terms enriched during the early stages of chilling acclimation in tomato was “response to hormone stimulus” (Fig. 3b). Furthermore, when the pathway enrichment analysis was taken into account, one of the enriched pathways that appeared at 3 hours of exposure to 10ºC was also “plant hormone signal transduction” (Fig. 3c). Thus, we investigated which genes were responsible for these results. We found numerous DEGs encoding proteins related with hormone biosynthesis and signaling, especially ABA, ET, GA and JA (Fig. 5a). Interestingly, in numerous instances their differential expression extended well beyond the 3 hour range. Moreover, we also identified a number of genes associated with hormone biosynthesis and signaling that were induced or repressed after 24 hours

(Fig. 5a), indicating that the action of this kind of genes was not restricted to the early stages of the chilling acclimation process as the term enrichment analysis suggested. qPCR analysis confirmed the altered expression patterns of various tomato genes involved in ABA, ET, GA and JA biosynthesis and signaling in response to suboptimal growth temperature (Fig. 5b), validating again the RNAseq data.

Phytohormones play a key role in regulating the correct development of cold acclimation in freezing-tolerant plants (Shi *et al.* 2015). Considering the results described above, we decided to explore whether they could also have a similar function in tomato chilling acclimation. With this aim, we first measured the levels of ABA, ACC, bioactive GAs and JA during the first 48 hours of exposure to 10ºC. A gradual increase in ABA and ACC levels was revealed as tomato plants responded to suboptimal growth temperature (Fig. 5c). Conversely, bioactive GAs and JA decreased after 24 hours of treatment (Fig. 5c). These findings strongly suggested that phytohormones might, in fact, regulate chilling acclimation in tomato. Then to definitively assess their role in chilling acclimation, we took advantage of selected tomato mutants affected in hormone biosynthesis or signaling and analyzed their capacities to chilling acclimate. Specifically, we examined the *never-ripe* mutant, an ET insensitive mutant (Lanahan *et al.* 1994), the *procera* mutant, which displays a constitutive GA response (Bassel *et al.* 2008), and the *notabilis* mutant, that has reduced levels of ABA (Burbidge *et al.* 1999). Both *never-ripe* and *procera* mutants showed an increased tolerance to 4ºC after chilling acclimation compared to wild-type Ailsa Craig (WT) plants (Fig. 5d-e). On the contrary, the capacity to chilling acclimate of the *notabilis* mutant was dramatically reduced compared to that of the WT (Fig. 5d-e). Together, all these data provide genetic and molecular evidence that ABA, ET and GA regulate the capacity of tomato plants to acclimate to chilling temperatures in a similar way as they regulate the cold acclimation process in freezing-tolerant species (Shi *et al.* 2015).

Chilling acclimation in tomato plants results in accumulation of amino acids and mobilization of antioxidant mechanisms

Term enrichment analysis of our transcriptomic profiling experiment in tomato plants (Fig. 3b-c) suggested that, after 24 hours of exposure to 10ºC, transcription factors and hormone signaling triggered a comprehensive adjustment of plant metabolism. Since the regulation of chilling acclimation by transcription factors and phytohormones was reminiscent of that of cold acclimation, we investigated the possibility that metabolic changes previously linked to this adaptive response could also be involved in tomato chilling acclimation. One of such metabolic adjustments is the accumulation of sugars and compatible solutes (Ruelland *et al.* 2009). Remarkably, the expression of several genes encoding key enzymes in sugar and amino acids biosynthesis, including *SUCROSE PHOSPHATE SYNTHASE (SPS)*, *RAFFINOSE SYNTHASE (RFS)*, *GALACTINOL SYNTHASE (GOLS)*, *PYRROLINE-5-CARBOXYLATE REDUCTASE (P5CR)*, *GLUTAMATE DECARBOXYLASE (GAD)* and *S-ADENOSYLMETHIONINE DECARBOXYLASE (SAMDC)*, was induced when tomato plants were subjected to 10ºC (Supporting Information Table S2-S4; Fig. 6a). Moreover, a few genes encoding enzymes associated with the degradation of sugars and amino acids like *B-FRUCTOFURANOSIDASE (INV)* and *PROLINE DEHYDROGENASE (PDH)* were downregulated at 10ºC (Supporting Information Table S2-S4; Fig. 6a). These results were validated by analyzing the expression of some representative genes through qPCR analysis (Fig. 6b). Consistent with the results obtained for *P5CR*, *PDH, SAMDC and GAD*, we found an accumulation of several amino acids and polyamines, namely proline, gamma-aminobutyric acid (GABA) and putrescine, in tomato plants exposed to suboptimal growth temperature (Fig. 6c-d). Intriguingly, however, in spite of the induction observed for *GOLS*, *SPS* and *RFS* expression, we did not observe a significant accumulation of compatible sugars like fructose, glucose or sucrose (Fig. 6c-d). Moreover, other complex sugars such as raffinose or stachyose were below the limit of detection of our GC-MS analysis. These data indicated that the accumulation of

amino acids and polyamines, but not of compatible sugars, plays a relevant role in tomato chilling acclimation.

Another relevant metabolic adjustment linked to cold acclimation is the accumulation of flavonoids and anthocyanins as part of a reactive oxygen species (ROS) scavenging system (Ruelland *et al.* 2009). In relation with this, most genes encoding enzymes implicated in the biosynthesis of anthocyanins and flavonoids including, *ANTHOCYANIDIN 3-O-GLUCOSYLTRANSFERASE (UGT78D2)*, *CYANIDIN 3-O-GLUCOSIDE 2-O-GLUCURONOSYL TRANSFERASE (UGT94B1)* and *CHALCONE SYNTHASE (CHS)*, as well as numerous genes coding for enzymes with antioxidant activity, like *L-ASCORBATE PEROXIDASE (APX)* and *SUPEROXIDE DISMUTASE (SODB)*, were also found to be differentially expressed in tomato plants after exposure to 10ºC (Supporting Information Table S2-S4; Fig. 7a). qPCR analysis confirmed the upregulation of several of these genes (Fig. 7b), corroborating once more our RNAseq data. Consistent with the expression results, the levels of anthocyanins increased in chilling-acclimated plants (Fig. 7c). The chilling acclimation process in tomato plants, therefore, also involves the mobilization of various antioxidant mechanisms.

Chilling acclimation in tomato plants includes adjustments in the light harvesting complex

Both, GO term and KEGG pathway enrichment analysis indicated that the tomato late response to suboptimal growth temperature might involve a rearrangement of the photosynthetic apparatus (Fig. 3b-c). Low temperature reduces the rate of biochemical reactions but does not affect light harvesting reactions, disrupting, therefore, the balance between the energy absorbed by photosystems and that consumed by metabolism (Allen & Ort 2001; Ruelland *et al.* 2009). Interestingly, during chilling acclimation we observed a general repression of tomato genes encoding proteins related with the light harvesting complex and a transient induction of genes encoding

412 subunits D1 and D2 of the photosystem II, which are particularly susceptible to chilling
413 injury (Allen & Ort 2001). In addition, the induction of genes encoding enzymes related
414 with chlorophyll breakdown was also detected (Supporting Information Table S2-S4;
415 Fig. 8a). qPCR expression analysis of selected genes, including *PS-I LIGHT*
416 *HARVESTING COMPLEX SUBUNIT 1 (LHCa1)*, *PS-II LIGHT HARVESTING*
417 *COMPLEX SUBUNIT 1 (LHCb1)*, *CHL-b-REDUCTASE* (*NYC1*) and
418 *CHLOROPHYLLASE* (*CLH*), confirmed their regulation by 10°C (Fig. 8b). These data
419 suggested that growth at suboptimal temperature could elicit a reduction of the light
420 harvesting capacity in tomato plants. This possibility was investigated by comparing
421 chlorophyll content in control and chilling acclimated plants. Results confirmed that, in
422 fact, exposure to 10°C reduced chlorophyll content and increased the chlorophyll a/b
423 ratio (Fig. 8c). Other protection mechanisms of photosystems against photoinhibition,
424 such as lipid membrane desaturation, were also considered. The expression levels of
425 genes encoding relevant enzymes in the biosynthesis of unsaturated fatty acids (i.e.,
426 *FATTY ACID DESATURASE 3 (FAD3)*, *FAD5*, *FAD7* and *FAD8*) in our tomato global
427 transcriptomic survey revealed that either were not differentially expressed or were
428 repressed by suboptimal growth temperature (Supporting Information Table S2-S4;
429 Supporting Information Fig. S1). Together, these results indicated that chilling
430 acclimation involves a rearrangement of the photosynthetic machinery, downregulating
431 the expression of light harvesting complex genes and reducing plant chlorophyll
432 content which, in turn, probably reduces the amount of light absorbed. In addition, the
433 induction of genes encoding subunits D1 and D2 may facilitate the turnover of these
434 photosystem II important components. Lipid membrane desaturation, however, does
435 not appear to contribute to chilling acclimation under our experimental conditions.
436

DISCUSSION

Plants from tropical regions are highly sensitive to low non-freezing temperatures and have not the capacity to cold acclimate (Lyons 1973). Yet, some of them have evolved a limited, but significant, adaptive response to increase their tolerance to low non-freezing temperatures in response to suboptimal growth temperatures that has been termed chilling acclimation (Cabané *et al.* 1993; Anderson *et al.* 1994). In this work, we present conclusive data demonstrating that tomato plants have the ability to chilling acclimate. We show that this adaptive response involves extensive transcriptional and metabolic adjustments, and has strong resemblances with cold acclimation. Both chilling and cold acclimation are regulated by a similar set of transcription factors and hormones, and share common protection mechanisms, including the accumulation of compatible solutes, the mobilization of antioxidant systems and the readjustment of the photosynthetic machinery.

Chilling sensitivity in tropical plants has been primarily studied in the context of chilling injury during fruit development, and conclusions from these studies cannot be directly extrapolated to plant survival. Research focused on whole plants has revealed that some species have the ability to increase their survival to chilling temperatures after exposure to suboptimal growth temperatures. In this sense, a three-day incubation at 14°C raises maize seedling survival to 4-6°C (Anderson *et al.* 1994). Likewise, overnight incubation at 10°C increments the survival of tomato seedlings to 2°C (King *et al.* 1988). In agreement with these findings, our results show that exposure to 10°C for 4 days induces tomato chilling acclimation, substantially increasing (≥ 50%) the time that tomato plants may survive to prolonged chilling (4ºC). Nonetheless, despite this enlarged tolerance to 4ºC, chilling-acclimated tomato plants are still sensitive to freezing temperatures (data not shown). Chilling acclimation in tomato entails a major transcriptome reprogramming, with 4559 and 2156 genes being induced and repressed, respectively. Thus, the expression of around 19% of all predicted genes

encoded by the tomato genome (ITAG2.4) seems to be regulated during chilling acclimation. These genes can be organized in two main groups, according to their expression patterns. The first group would be composed of DEGs showing transient regulation during the adaptive response (i.e., 3, 24 and 3-24 hours), and would include 27% of all upregulated genes and 40% of all downregulated genes. The second group would consist of DEGs displaying a permanent (i.e., at 3-24-48 and 24-48 hours) regulation, and would contain 47% of all induced genes and 42% of all downregulated genes. In addition, the expression of 23.2% of all upregulated genes and 16% of all repressed genes is altered exclusively after 48 hours, indicating that the transcriptome reprogramming is extended well throughout the development of the chilling acclimation response.

After functional annotation of DEGs, we used GO term and KEGG pathway enrichment analysis to identify molecular mechanisms underlying chilling acclimation in tomato plants. Results pointed out that transcription factor activity and hormone biosynthesis are relevant at early stages of the adaptive response. Among the 156 genes that are early induced at 10°C and encode transcription factors (Supporting Information Table S5), we found 18 that have been described to regulate the cold acclimation process in freezing tolerant plants (Medina *et al.* 2011; Meissner *et al.* 2012; Franklin *et al.* 2014; Park *et al.* 2015; Shi *et al.* 2015). This strongly suggests that both cold and chilling acclimation processes may share some particular regulatory elements. In fact, it has been reported that ectopic expression of *SlCBF1* increases freezing tolerance in transgenic Arabidopsis (Zhang *et al.* 2004) and a recent study has demonstrated that *CBF1*, and possibly *CBF2* and *CBF3*, regulate constitutive chilling tolerance in tomato (Wang *et al.* 2016). Our results demonstrate that two of these tomato *CBF* genes, *CBF1* and *CBF2* are upregulated under conditions that allow chilling acclimation. Furthermore, although *CBF3* was not included in the DEG list obtained from 3 hours at 10ºC due to the high stringency of the selection conditions, qPCR analysis showed that

it was also rapidly induced at suboptimal growth temperature (Supporting Information Fig. S2). Elucidating the role of these transcription factors in chilling acclimation, however, requires further research. Interestingly, some of the tomato genes identified that are induced at 10ºC and encode transcription factors, including *PSEUDO-RESPONSE REGULATOR 5 (PPR5)*, *DWARF AND DELAYED FLOWERING 1 (DDF1)*, *TIME OF CAB EXPRESSION 1 (TOC1)*, *ELONGATED HYPOCOTYL 5 (HY5)*, or *PHYTOCLOCK 1 (PCL1)* (Supporting Information Table S2-S4), have been shown to be regulated by the circadian clock in other species (Bieniawska *et al*. 2008), strongly suggesting a substantial influence of the clock on the observed changes in gene expression during chilling acclimation. The global expression analysis unveiled, moreover, that several genes encoding key enzymes in ABA and ET biosynthesis are induced at early stages of chilling acclimation in tomato. In addition, different genes encoding GA deactivating enzymes and negative regulators of JA biosynthesis are also concomitantly upregulated. Consistent with these results, our hormone measurements uncovered a rapid increase in ABA and ACC levels and a simultaneous decrease in the levels of bioactive GAs and JA when tomato plants are exposed to 10ºC, indicating important early hormone adjustments in response to suboptimal growth temperature. The physiological characterization of tomato mutants affected in hormone biosynthesis or signaling provided conclusive genetic evidence that ABA, ET and GA play a critical role in the correct development of chilling acclimation. Thus, while the ET- and GA-signaling mutants *never-ripe* and *procera*, respectively, display increased capacity to chilling acclimate, the capacity of the ABA-deficient mutant *notabilis* is impaired. Our findings demonstrate that, paralleling their function in the cold acclimation process, ABA, ET and GA regulate the capacity of tomato plants to acclimate to chilling temperatures.

At later stages of tomato chilling acclimation, diverse processes related with the secondary metabolism and the adjustment of photosynthetic apparatus become

relevant. Thus, the expression of numerous genes encoding important enzymes for amino acid biosynthesis is induced during this process and, accordingly, the levels of several amino acids, including GABA, putrescine and proline, which have been positively correlated with constitutive tolerance to chilling temperatures in tomato plants (Hsieh *et al.* 2002; Kim *et al.* 2002; Malekzadeh *et al.* 2014), augment upon exposure to 10ºC. We also observed a rise in the expression of most genes encoding key enzymes for anthocyanin biosynthesis late during tomato chilling acclimation and, coherently, a subsequent increase in anthocyanins. In Arabidopsis, where genes involved in anthocyanin biosynthesis are also upregulated during cold acclimation (Schulz *et al*. 2015), these pigments have been reported to have a protective role in preventing chilling-induced photoinhibition (Harvaux & Kloppstech 2001). It is, therefore, tempting to speculate the same function for the accumulation of anthocyanins in tomato plants in response to chilling conditions. Genes encoding enzymes related with antioxidant activities were also noticed to be induced after exposing tomato plants to 10ºC for 48 hours. These results are consistent with the modest but significant increment in catalase, ascorbate peroxidase and superoxide dismutase activities detected when tomato plants are subjected to suboptimal growth temperature for three days (Zhou *et al.* 2012). The rise in antioxidant activity should protect tomato cell membranes against chilling injury, as illustrated by the decrease in lipid peroxidation that takes place when tomato plants are incubated a week at 4°C (Zhou *et al*. 2012).

The transcriptome analysis indicates that the expression of several light harvesting complex genes also changes at later stages of tomato chilling acclimation, which, in all likelihood, should contribute to reduce the levels of the antenna complex and, consequently, to shorten the amount of light absorbed. The observed changes in chlorophyll content and chlorophyll a/b ratios are compatible with this idea. In addition, the upregulation of genes encoding subunits D1 and D2 would facilitate the turnover of

549 these important components of photosystem II which are particularly affected by
550 chilling. Photosynthesis is one of the most sensitive cellular processes to a decrease in
551 temperature. Low temperatures cause an imbalance between the light energy
552 absorbed by photosystems I and II and the energy consumed by CO_2 fixation and
553 photorespiration causing photodamage (Allen & Ort 2001). Therefore, it is not
554 surprising that chilling acclimation includes mechanisms to prevent photoinhibition. In
555 fact, this kind of mechanisms have been evidenced in different studies in which plants
556 incubated at suboptimal growth temperatures were found to experience just a small
557 reduction in photosystem efficiency compared to control plants (Haldimann 1997; Kuk
558 *et al.* 2003; Zhou *et al.* 2012).
559
560 Our study reveals strong similarities between chilling and cold acclimation. We have
561 already mentioned that a similar set of transcription factors is upregulated in both
562 processes. In addition, we demonstrate that several phytohormones that play a key
563 role in increasing freezing tolerance are also essential for correct development of
564 chilling acclimation. Likewise, several metabolic adjustments are common, including
565 amino acid and anthocyanin biosynthesis, and the activation of antioxidant systems
566 (Kaplan *et al.* 2004, 2007; Catalá *et al.* 2011). Intriguingly, however, we did not find a
567 significant accumulation of compatible sugars during chilling acclimation in tomato
568 plants despite the induction of several genes encoding enzymes implicated in sugar
569 biosynthesis. Since sugar accumulation has long been known to be crucial in cold
570 acclimation (Kaplan *et al.* 2007), this may constitute a fundamental difference with
571 chilling acclimation. Another interesting divergence between these adaptive responses
572 concerns the mobilization of components of the tricarboxylic acid (TCA) cycle. While
573 cold acclimation in Arabidopsis correlates with an augmentation in citrate, succinate
574 and malate (Kaplan *et al.* 2004), we did not observe a similar accumulation in chilling-
575 acclimated tomato plants (Supporting Information Fig. S3). Moreover, Arabidopsis cold
576 acclimation results in the induction of crucial genes in the biosynthesis of unsaturated

fatty acids such as *FAD3*, *FAD7* and *FAD8* (Shi *et al.* 2011), and an enhancement of membrane lipid desaturation (Uemura *et al.* 1995). We failed, however, to find an upregulation of these genes in tomato plants exposed to 10ºC (Supporting Information Fig. S1). It is possible, therefore, that the failure of tomato plants to cold acclimate may be related with an inability to adjust some of these metabolic processes in response to suboptimal growth temperatures.

Based on the results described in this work, a model summarizing the putative regulatory mechanisms involved in the correct development of chilling acclimation in tomato plants is presented in Figure 9. According to this model, suboptimal growth temperature is rapidly signaled through a number of transcription factors and different hormones that subsequently trigger an extensive transcriptional reprogramming. Then, as a consequence of this reprogramming, a comprehensive battery of metabolic adjustments, including the accumulation of compatible solutes, the mobilization of antioxidant systems and the rearrangement the photosynthetic machinery, takes place leading to the increased tolerance to chilling temperatures that follows chilling acclimation. We expect that the results unraveled by this work will foster new research into the elucidation of the molecular mechanisms underlying the chilling acclimation process, which could substantially contribute to improve LT tolerance in tomato.

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744

745 **FIGURE LEGENDS**

746 **Figure 1. Chilling acclimation in tomato plants.** (a) Representative plants grown
747 under control conditions (25ºC) and after being exposed to 4ºC for 5 or 10 days. (b)
748 Effect of chilling acclimation on LT tolerance. Tomato plants grown at 25ºC were either
749 directly subjected at 4°C (control) or incubated at 10°C for 7 days prior to 4°C
750 treatment (acclimated). Pictures were taken after 10 days at 4°C. (c) Determination of
751 the time required for chilling acclimation. Tomato plants were exposed to 10°C for the
752 indicated times and then incubated at 4°C for 18 days. After 10 days of recovery at
753 25°C, survivals were scored. Mean and SEM values from three independent
754 experiments are presented. Statistically significant differences with control plants were
755 determined by one-way ANOVA followed by Fisher's LSD test and are designated by
756 asterisks: **, (P<0.01); ***, (P<0.001). (d) Time-course survival assay for non-
757 acclimated (NA) and chilling-acclimated (CA) (4d, 10ºC) tomato plants. Three-week-old
758 plants were subjected to 4°C for the specified times, and then allowed to recover at
759 25°C and scored for survival after 10 days. Dashed lines illustrate LE_{50}. Data shown
760 are the mean and SEM of three independent experiments.
761
762 **Figure 2. Validation of transcriptomic data.** (a,b) qPCR analysis of 8 upregulated (a)
763 and 8 downregulated (b) genes in tomato plants exposed at 10ºC for the indicated
764 periods of time. Mean and SEM values from three independent experiments using
765 three different RNA samples are shown. Statistically significant differences with control
766 plants were calculated by one-way ANOVA and are designated by asterisks: *,
767 (P<0.05); **, (P<0.01); *** (P<0.001). (c) Correlation analysis between RNAseq fold
768 change data (y-axis) and qPCR fold change data (x-axis) for genes analyzed in (a) and
769 (b).
770
771 **Figure 3. Gene expression reprogramming during chilling acclimation in tomato**
772 **plants**. (a) Venn diagrams displaying the overlap among DEGs identified in tomato

plants exposed at 10ºC for 3, 24 and 48 hours. Numbers in parenthesis show the percentage with respect to the total of upregulated or downregulated genes. (b) Selected GO terms enriched among DEGs identified in tomato plants subjected to 10ºC for the indicated times. Color panels display the *P*-value of GO term enrichment. (c) KEGG pathway enrichment analysis among DEGs identified in tomato plants exposed for the designated periods of time to 10ºC. Color panels illustrate the significance level of enrichment.

Figure 4. Transcription factors related to chilling acclimation in tomato have been involved in cold acclimation in freezing tolerant species. (a) Heatmap of DEGs encoding transcription factors in tomato plants subjected to 10°C for the indicated times that have been involved in cold acclimation in freezing tolerant species. Color panels display the $\log_2$ value of fold change. (b) qPCR analysis of genes encoding selected transcription factors from (a) in tomato plants exposed for 3 hours to 10ºC. Mean and SEM values from three independent experiments using three different RNA samples are shown. Statistically significant differences with control plants were determined by t-student test and are designated by asterisks: **, (*P*<0.01); ***, (*P*<0.001).

Figure 5. Phytohormones regulate tomato chilling acclimation. (a) Heatmap showing DEGs identified encoding proteins related with hormone biosynthesis and signaling in tomato plants subjected to 10ºC for the specified times. Color panels illustrate the $\log_2$ value of fold change. (b) qPCR analysis of genes selected from (a) in tomato plants exposed for 0, 3, 24 and 48 hours to 10ºC. Mean and SEM values from three independent experiments using three different RNA samples are presented. Statistically significant differences with control plants were determined by one-way ANOVA followed by Fisher's LSD test and are designated by asterisks: *, (*P*<0.05); **, (*P*<0.01); ***, (*P*<0.001). (c) Levels of ABA, ACC, GA and JA in tomato plants grown at

10ºC for the indicated times. Mean and SEM values are displayed from 5 independent experiments. Statistically significant differences with control plants were determined and are denoted as in (b). (d) Time-course survival assay of chilling acclimated (4d, 10ºC) tomato mutants affected in hormone biosynthesis or signaling after being incubated at 4ºC for the indicated times. Mean and SEM values are shown from 3 independent experiments. (e) Representative plants from the analyzed mutant genotypes grown 20 days at 4°C and subsequently recovered 10 days at 25°C.

Figure 6. Amino acids, but not compatible sugars, accumulate during chilling acclimation in tomato plants. (a) Heatmap displaying DEGs encoding proteins related with sugar and amino acid biosynthesis in tomato plants exposed to 10ºC for the indicated times. Color panels illustrate the $\log_2$ value of fold change. (b) qPCR analysis of selected genes from (a) in tomato plants subjected for 0, 3, 24 and 48 hours to 10ºC. Mean and SEM values from three independent experiments using three different RNA samples are presented. Statistically significant differences with control plants were determined by one-way ANOVA followed by Fisher's LSD test and are designated by asterisks: *, (P<0.05); **, (P<0.01); ***, (P<0.001). (c) Heatmap showing a $\log_2$ transformation of metabolite fold change in tomato plants grown at 10ºC for the specified times. (d) Accumulation of abiotic stress-related metabolites in tomato plants incubated to 10ºC for 0, 3, 24 and 48 hours. Mean and SEM values from 5 independent experiments are shown. Statistically significant differences with control plants were determined and are denoted as in (b).

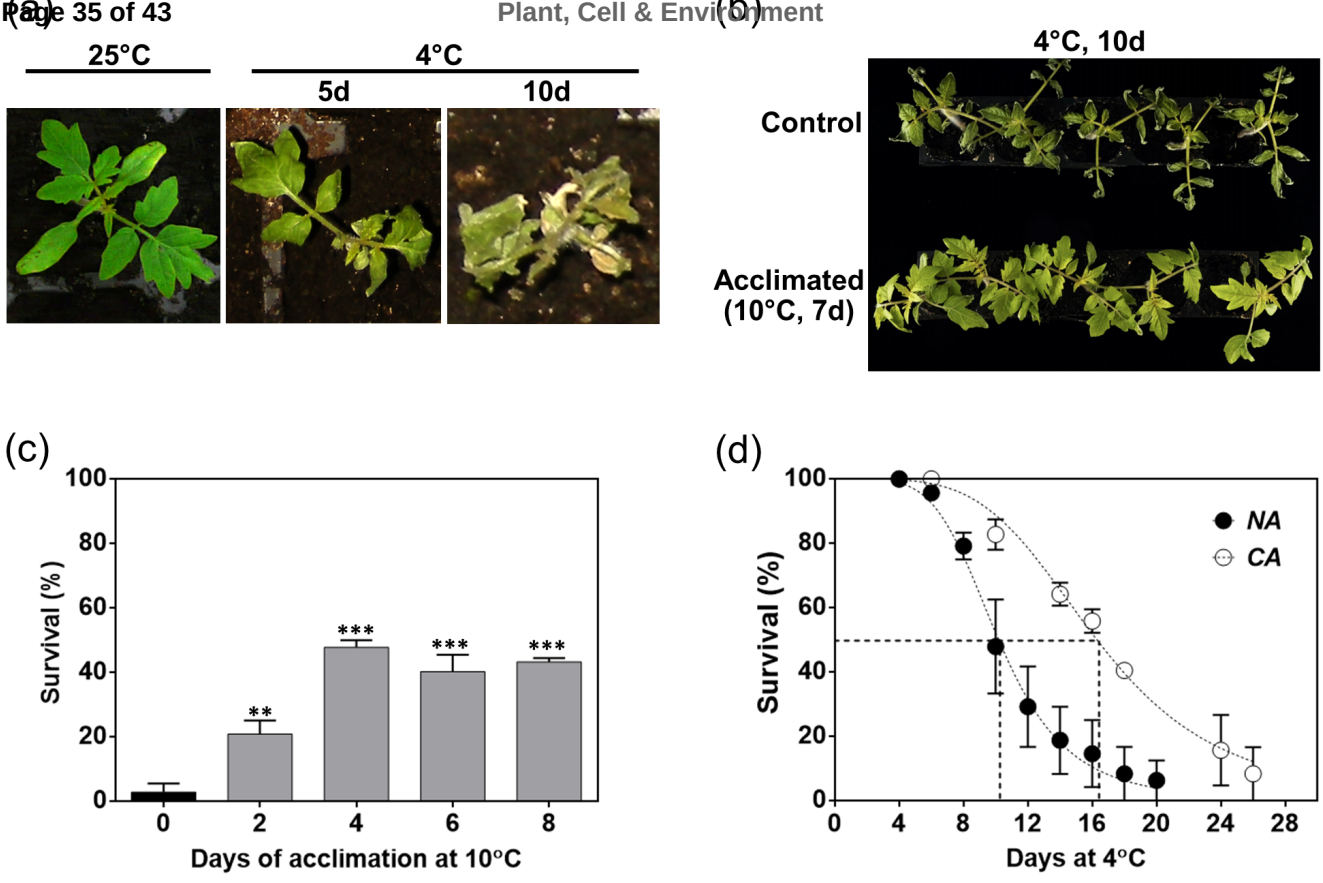
Figure 7. Antioxidant mechanisms are mobilized in response to suboptimal growth temperature in tomato plants. (a) Heatmap displaying representative DEGs encoding proteins involved in anthocyanin and flavonoid biosynthesis, and other antioxidant systems, in tomato plants subjected to 10ºC for the indicated times. Color panels illustrate the $\log_2$ value of fold change. (b) qPCR analysis of selected genes

from (a) in tomato plants grown at 10ºC for 0, 3, 24 and 48 hours. Mean and SEM values from three independent experiments using three different RNA samples are shown. Statistically significant differences with control plants were determined by one-way ANOVA followed by Fisher's LSD test and are designated by asterisks: *, (P<0.05); **, (P<0.01); ***, (P<0.001). (c) Anthocyanin accumulation in non-acclimated (NA) and chilling acclimated (4d, 10ºC) tomato plants (CA). Mean and SEM values from three independent experiments are presented. Asterisks denote statistically significant differences calculated by t-student test at P<0.01.

Figure 8. Readjustments of the photosynthetic machinery during chilling acclimation in tomato plants. (a) Heatmap showing DEGs encoding proteins related with photosynthesis in tomato plants exposed for the indicated times to 10ºC. Color panels illustrate the $\log_2$ value of fold change. (b) qPCR analysis of selected genes implicated in light capture and electron transport in tomato plants subjected to 10ºC for 0, 3, 24 and 48 hours. Mean and SEM values from three independent experiments using three different RNA samples are presented. Statistically significant differences with control plants were calculated by one-way ANOVA and are designated by asterisks: *, (P<0.05); **, (P<0.01); *** (P<0.001). (c) Chlorophyll content and chlorophyll a/b ratio in control (NA) and chilling acclimated (4d, 10ºC) tomato plants (CA). Mean and SEM values from three independent experiments are shown. Asterisks display statistically significant differences calculated by t-student test: *, (P<0.05); **, (P<0.01).

Figure 9. Proposed model for the correct development of chilling acclimation in tomato plants. In response to suboptimal growth temperature, rapid changes in the levels of several transcription factors and different hormones take place triggering an extensive transcriptional reprogramming. This transcriptional reprogramming would then contribute to the comprehensive metabolic adjustments, including the

accumulation of compatible solutes, the mobilization of antioxidant systems and the rearrangement the photosynthetic machinery, that are required for the correct development of chilling acclimation. Arrowheads and end lines indicate positive and negative regulation, respectively.


(a)
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(b)
4°C, 10d
Control
Acclimated
(10°C, 7d)
(c)
Survival (%)
Days of acclimation at 10°C
**
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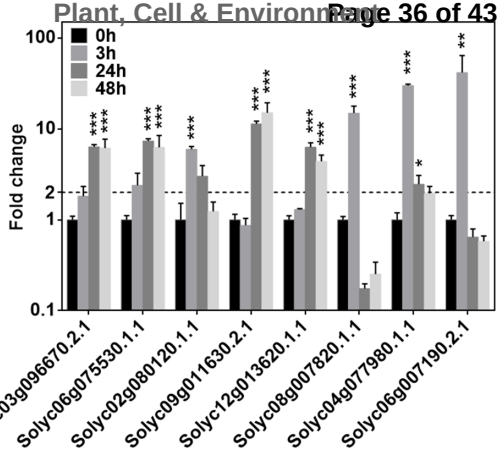
(d)
Survival (%)
Days at 4°C
NA
CA

(a)


0h
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Solyc02g080120.1.1
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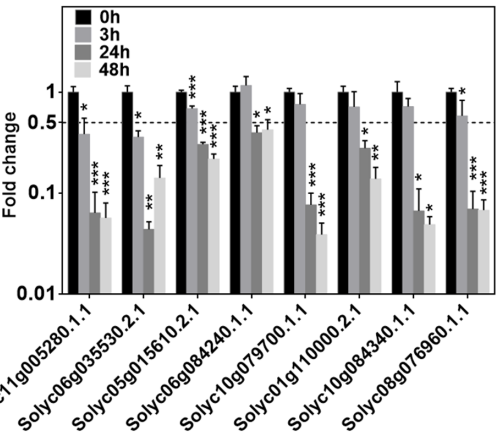
Solyc06g007190.2.1

(b)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Solyc11g005280.1.1
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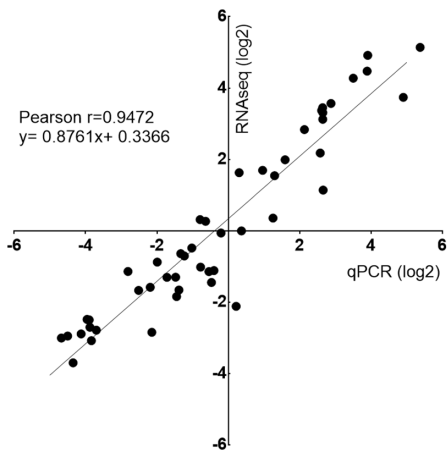
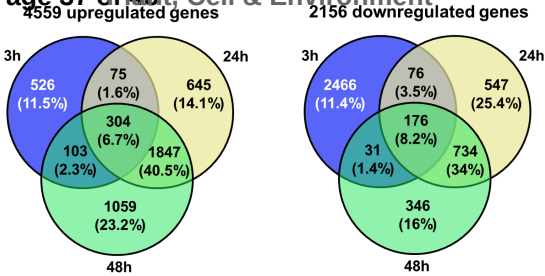
Solyc05g015610.2.1
Solyc06g084240.1.1
Solyc10g079700.1.1
Solyc01g110000.2.1
Solyc10g084340.1.1
Solyc08g076960.1.1

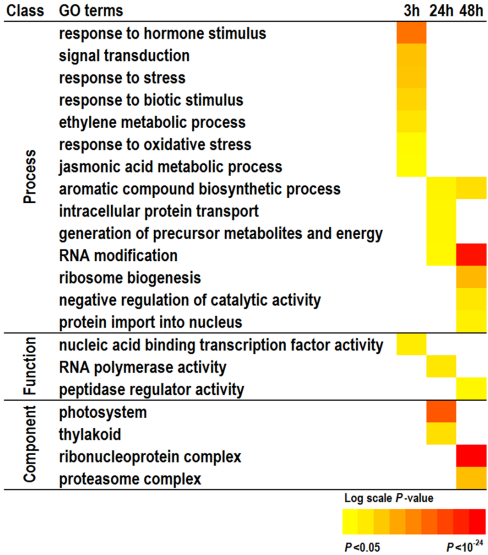
(c)


Pearson r=0.9472
y= 0.8761x+ 0.3366
RNAseq (log2)
qPCR (log2)
6
4
2
-2
-4
-6

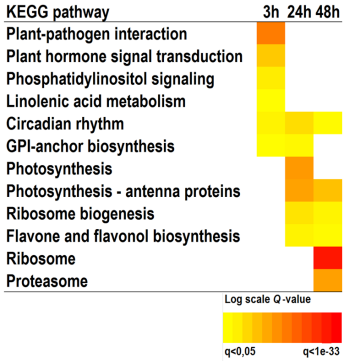
(a)


4559 upregulated genes
3h
24h
48h
526 (11.5%)
75 (1.6%)
645 (14.1%)
304 (6.7%)
103 (2.3%)
1847 (40.5%)
1059 (23.2%)
2156 downregulated genes
3h
24h
48h
2466 (11.4%)
76 (3.5%)
547 (25.4%)
176 (8.2%)
31 (1.4%)
734 (34%)
346 (16%)

(b)


Class
GO terms
3h
24h
48h
Process
response to hormone stimulus
signal transduction
response to stress
response to biotic stimulus
ethylene metabolic process
response to oxidative stress
jasmonic acid metabolic process
aromatic compound biosynthetic process
intracellular protein transport
generation of precursor metabolites and energy
RNA modification
ribosome biogenesis
negative regulation of catalytic activity
protein import into nucleus
Function
nucleic acid binding transcription factor activity
RNA polymerase activity
peptidase regulator activity
Component
photosystem
thylakoid
ribonucleoprotein complex
proteasome complex
Log scale P-value
P<0.05
P<10-24

(c)

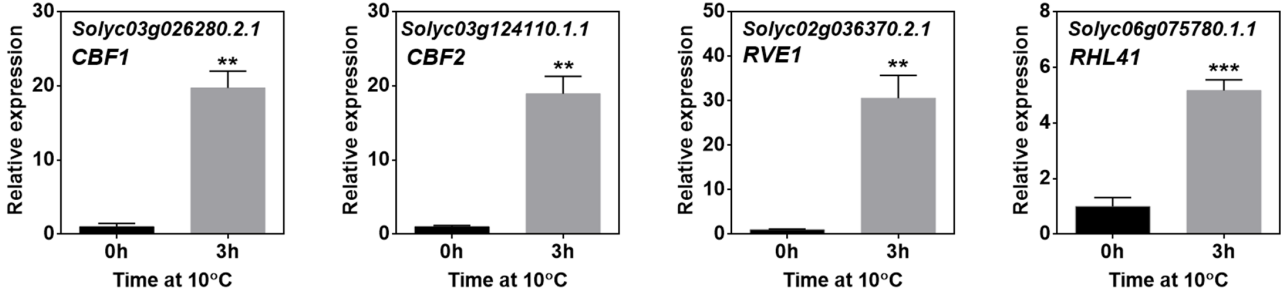

KEGG pathway
3h
24h
48h
Plant-pathogen interaction
Plant hormone signal transduction
Phosphatidylinositol signaling
Linolenic acid metabolism
Circadian rhythm
GPI-anchor biosynthesis
Photosynthesis
Photosynthesis - antenna proteins
Ribosome biogenesis
Flavone and flavonol biosynthesis
Ribosome
Proteasome
Log scale Q-value
q<0,05
q<1e-33

(a)

Transcript	Symbol	Annotation	3h	24	48h
Solyc03g026280.2.1	*CBF1*	*C-REPEAT/DRE BINDING FACTOR 1*			
Solyc03g124110.1.1	*CBF2*	*C-REPEAT/DRE BINDING FACTOR 2*			
Solyc08g007820.1.1	*CBF4*	*C-REPEAT/DRE BINDING FACTOR 4*			
Solyc08g007830.1.1	*DDF1*	*DWARF AND DELAYED FLOWERING 1*			
Solyc12g088390.1.1	*STZ*	*SALT TOLERANCE ZINC FINGER*			
Solyc10g086370.1.1	*RGA1*	*REPRESSOR OF GA1*			
Solyc03g113720.2.1	*ARR6*	*ARABIDOPSIS RESPONSE REGULATOR 6*			
Solyc10g005030.2.1	*PRR5*	*PSEUDO-RESPONSE REGULATOR 5*			
Solyc07g053230.2.1	*MYB15*	*MYB DOMAIN PROTEIN 15*			
Solyc12g007070.1.1	*HSF-C1*	*HEAT SHOCK TRANSCRIPTION FACTOR C1*			
Solyc02g036370.2.1	*RVE1*	*REVEILLE 1*			
Solyc06g005680.2.1	*PCL1*	*PHYTOCLOCK 1*			
Solyc03g081240.2.1	*PRR5*	*PSEUDO-RESPONSE REGULATOR 5*			
Solyc06g075780.1.1	*RHL41*	*RESPONSIVE TO HIGH LIGHT 41*			
Solyc04g077980.1.1	*STZ*	*SALT TOLERANCE ZINC FINGER*			
Solyc01g107170.2.1	*STZ*	*SALT TOLERANCE ZINC FINGER*			
Solyc03g115770.2.1	*TOC1*	*TIMING OF CAB EXPRESSION 1*			
Solyc08g061130.2.1	*HY5*	*ELONGATED HYPOCOTYL 5*			

< -8 > 8

(b)



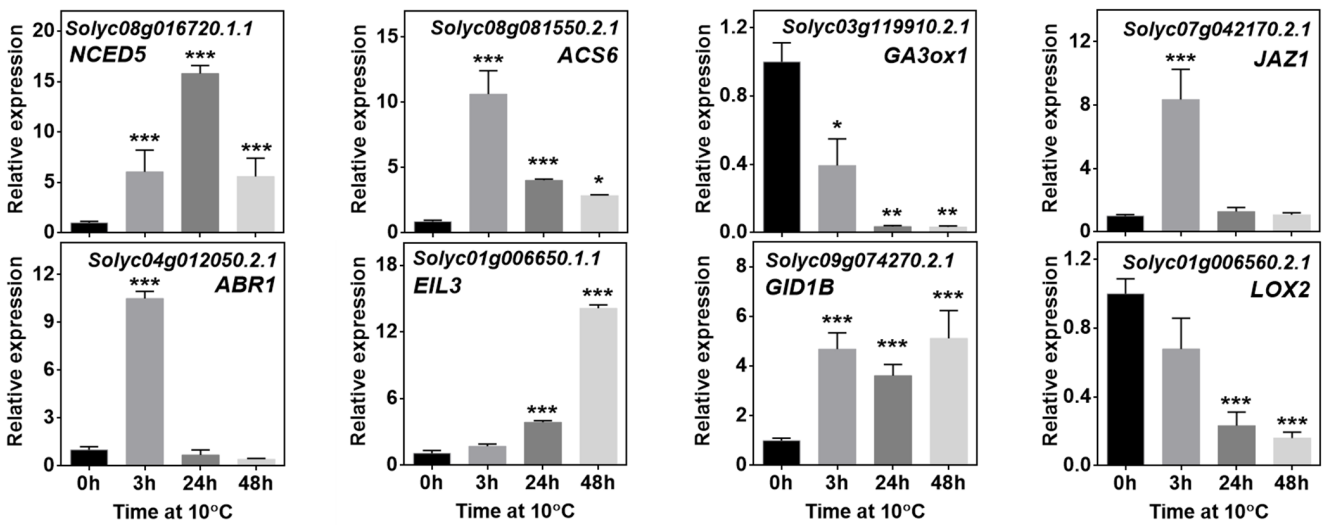
(a)

Hormone	Transcript	Symbol	Annotation
ABA	Solyc08g016720.1.1	NCED5	NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 5
	Solyc07g056570.1.1	NCED3	NINE-CIS-EPOXYCAROTENOID DIOXYGENASE 3
	Solyc02g090890.2.1	ABA1	ABA DEFICIENT 1
	Solyc04g012050.2.1	ABR1	ABA REPRESSOR 1
	Solyc04g078840.2.1	ABF2	ABA RESPONSIVE ELEMENTS-BINDING FACTOR 2
	Solyc01g108080.2.1	ABF3	ABA RESPONSIVE ELEMENTS-BINDING FACTOR 3
Ethylene	Solyc02g063540.1.1	ACS8	1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID SYNTHASE 8
	Solyc08g081550.2.1	ACS6	1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID SYNTHASE 6
	Solyc02g081190.2.1	ACO4	1-AMINOCYCLOPROPANE-1-CARBOXYLIC ACID OXIDASE 4
	Solyc03g093560.1.1	ERF5	ETHYLENE RESPONSIVE ELEMENT BINDING FACTOR 5
	Solyc01g006650.1.1	EIL3	ETHYLENE-INSENSITIVE3-LIKE 3
Gibberellin	Solyc06g084240.1.1	CPS1	ENT-COPALYL DIPHOSPHATE SYNTHETASE 1
	Solyc06g035530.2.1	GA20ox2	GIBBERELLIN 20 OXIDASE 2
	Solyc03g119910.2.1	GA3ox1	GIBBERELLIN 3 OXIDASE 1
	Solyc10g007570.2.1	GA2ox1	GIBBERELLIN 2 OXIDASE 1
	Solyc07g061730.2.1	GA2ox2	GIBBERELLIN 2 OXIDASE 2
	Solyc02g080120.1.1	GA2ox8	GIBBERELLIN 2 OXIDASE 8
	Solyc09g074270.2.1	GID1B	GA INSENSITIVE DWARF1B
	Solyc01g098390.2.1	GID1C	GA INSENSITIVE DWARF1C
Jasmonate	Solyc07g042170.2.1	JAZ1	JASMONATE-ZIM-DOMAIN PROTEIN 1
	Solyc01g109150.2.1	AOS	ALLENE OXIDE SYNTHASE
	Solyc08g029000.2.1	LOX1	LIPOXYGENASE 1
	Solyc01g006560.2.1	LOX2	LIPOXYGENASE 2
	Solyc03g122340.2.1	LOX3	LIPOXYGENASE 3

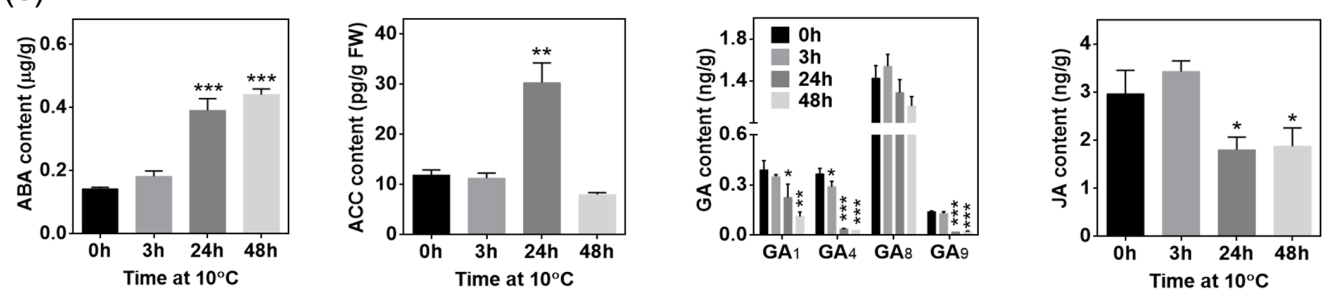
3h 24 48h

< -4 > 4

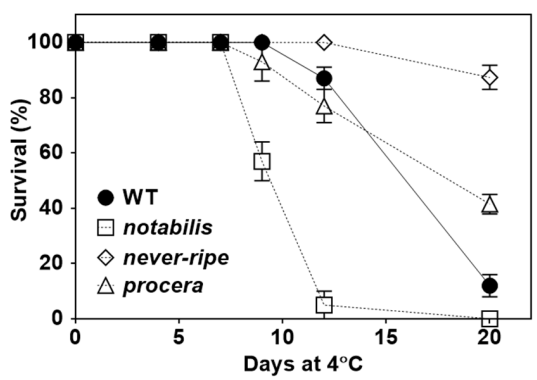
(b)



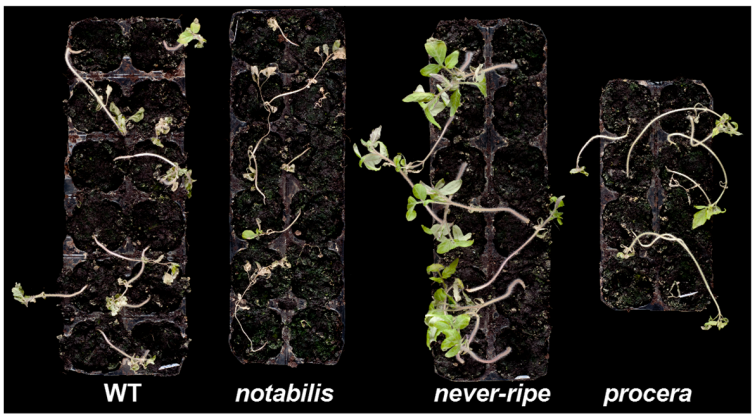
(c)



(d)

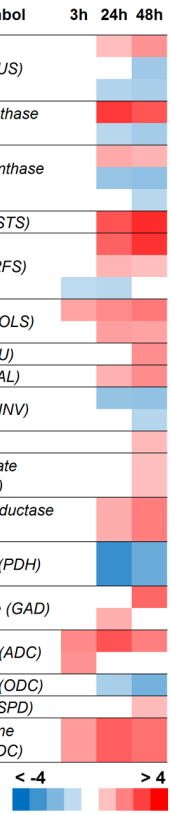


(e)

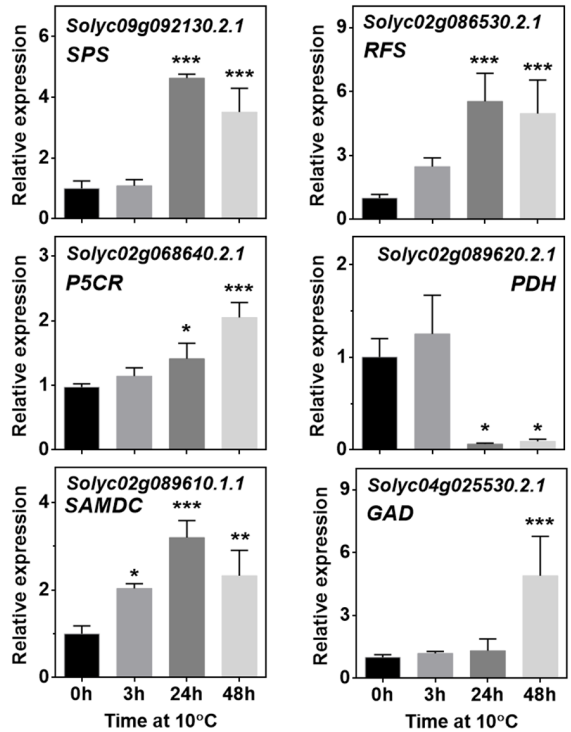


(a)

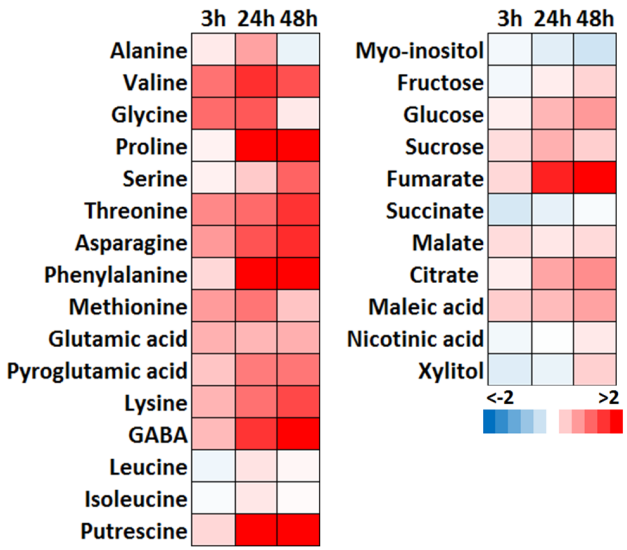
Metabolic pathway	Transcript	Annotation and symbol
Sugar accumulation	Solyc07g042550.2.1	Sucrose synthase (SUS)
	Solyc07g042520.2.1	
	Solyc12g009300.1.1	
	Solyc09g092130.2.1	Sucrose phosphate synthase (SPS)
	Solyc08g042000.2.1	
	Solyc02g071590.1.1	Trehalose phosphate synthase (TPS)
	Solyc02g072150.2.1	
	Solyc07g062140.2.1	
	Solyc01g079300.2.1	Stachyose synthase (STS)
	Solyc02g086530.2.1	Raffinose synthase (RFS)
	Solyc07g065980.2.1	
	Solyc07g007920.1.1	
	Solyc02g084980.2.1	Galactinol synthase (GOLS)
	Solyc01g079170.2.1	
Sugar breakdown	Solyc03g094020.2.1	a-glucosidase (AGLU)
	Solyc06g050130.2.1	a-galactosidase (AGAL)
	Solyc10g083300.1.1	b-fructofuranosidase (INV)
	Solyc03g121680.1.1	
	Solyc08g082860.2.1	Trehalase (TREA)
Proline accumulation	Solyc06g019170.2.1	Pyrroline-5-carboxylate synthetase (P5CS)
	Solyc02g068640.2.1	Pyrroline-5-carboxylate reductase (P5CR)
Proline degradation	Solyc02g089620.2.1	Proline dehydrogenase (PDH)
GABA accumulation	Solyc04g025530.2.1	Glutamate decarboxylase (GAD)
	Solyc03g098240.2.1	
Polyamine accumulation	Solyc10g054440.1.1	Arginine decarboxylase (ADC)
	Solyc01g110440.2.1	
	Solyc03g098300.1.1	Ornithine decarboxilase (ODC)
	Solyc05g005710.2.1	Spermidine synthase (SPD)
	Solyc02g089610.1.1	S-adenosylmethionine decarboxylase (SAMDC)



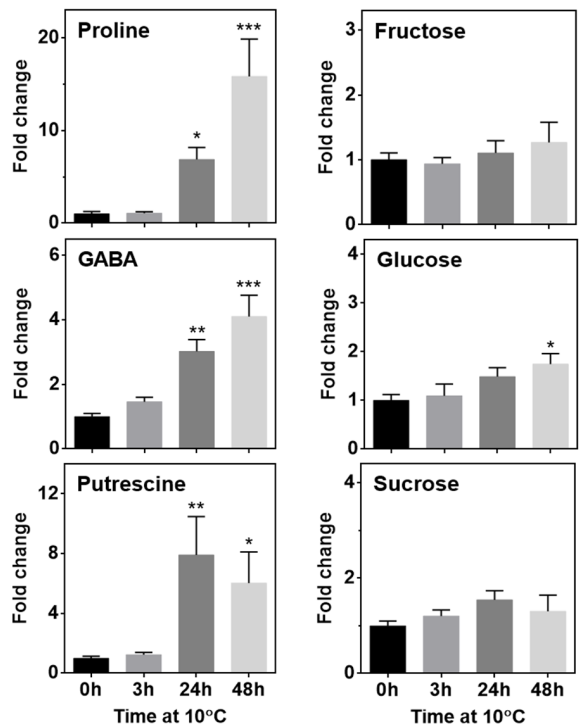
(b)



(c)



(d)

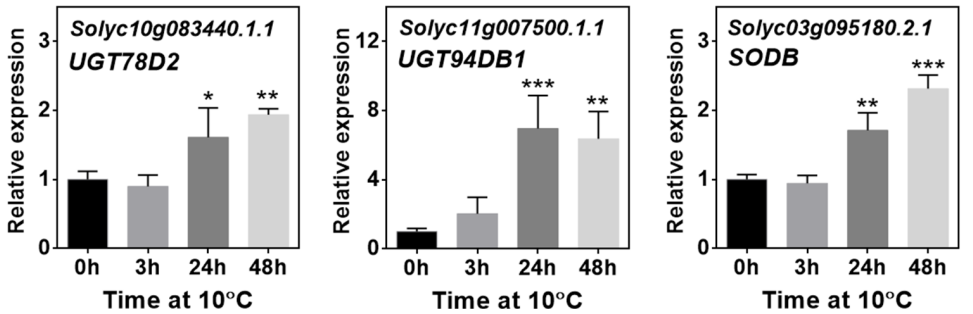


(a)

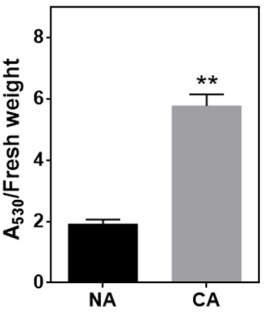
Metabolic pathway	Transcript	Annotation and symbol	3h	24h	48h
Anthocyanin biosynthesis	Solyc06g073080.2.1	Flavanone 3-hydroxylase (F3H)			
	Solyc04g009860.2.1				
	Solyc02g083860.2.1				
	Solyc03g080190.2.1				
	Solyc10g083440.1.1	Anthocyanidin 3-O-glucosyltransferase (UGT78D2)			
	Solyc11g007500.1.1	Cyanidin-3-O-glucoside 2-O-glucuronosyl transferase (UGT94B1)			
	Solyc09g092490.2.1	UDP-glucose: anthocyanin 5-O-glucosyltransferase (5-GT)			
Flavonoid synthesis	Solyc01g111080.2.1	Chalcone synthase (CHS)			
	Solyc09g091510.2.1				
	Solyc05g053550.2.1				
	Solyc05g052240.2.1	Chalcone isomerase (CHI)			
	Solyc05g010320.2.1				
Antioxidant system	Solyc06g005150.2.1	L-ascorbate peroxidase (APX)			
	Solyc06g005160.2.1				
	Solyc12g094620.1.1	Catalase (CAT)			
	Solyc03g095180.2.1	Superoxide dismutase (SODB)			
	Solyc02g021140.2.1				
	Solyc01g067740.2.1				

< -8 > 8

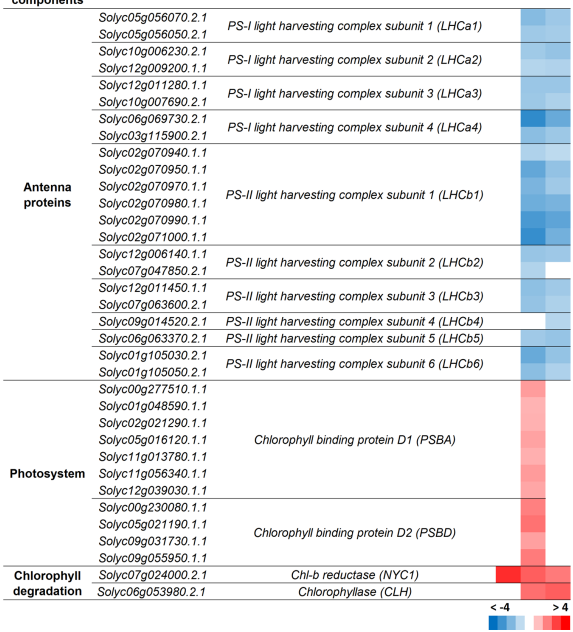
(b)



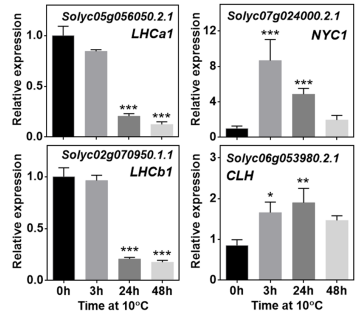
(c)



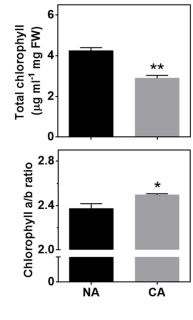
(a)

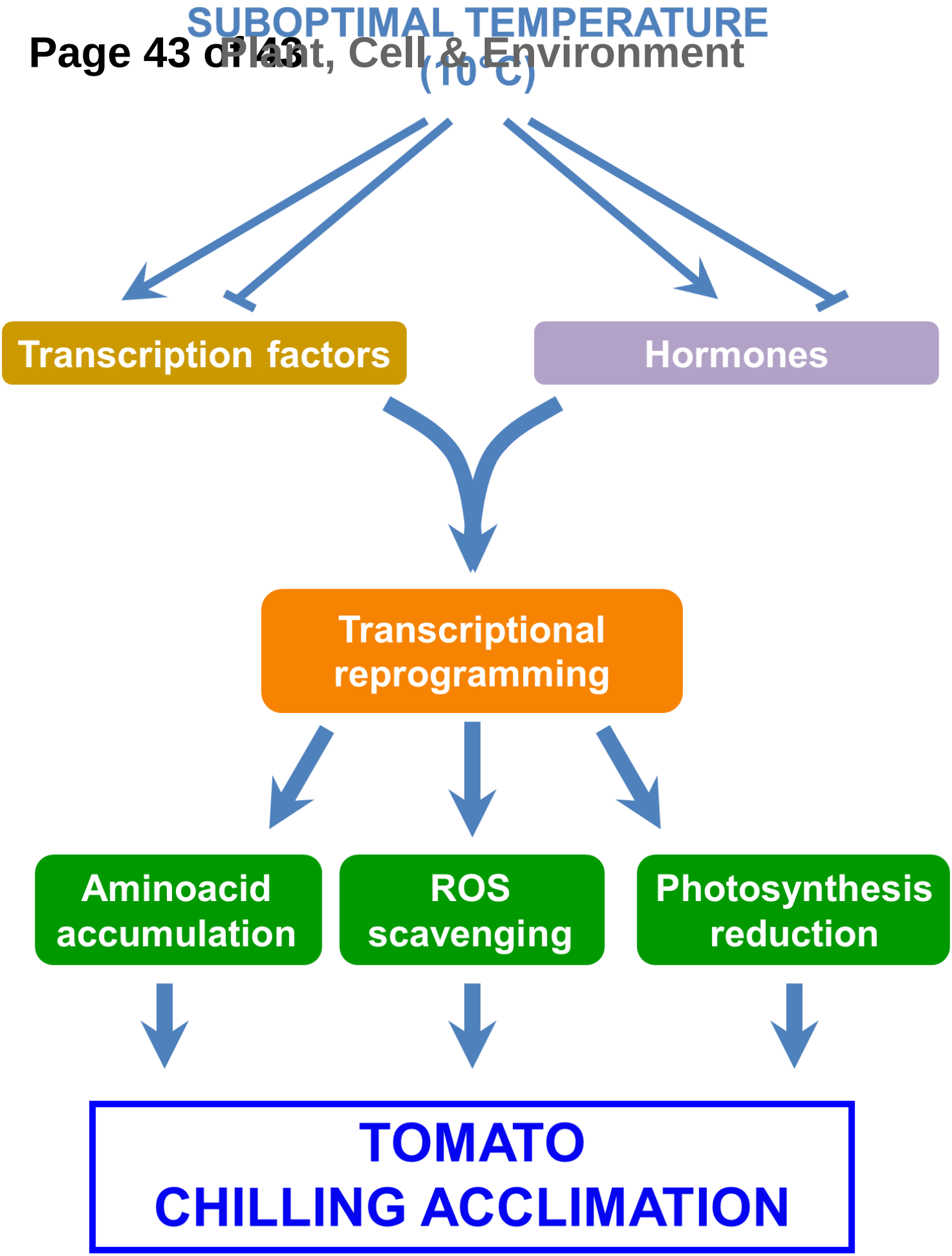

Photosyntetic components
Transcript
Annotation and symbol
3h 24h 48h
Antenna proteins
Photosystem
Chlorophyll degradation
Solyc05g056070.2.1
Solyc05g056050.2.1
PS-I light harvesting complex subunit 1 (LHCa1)
Solyc10g006230.2.1
Solyc12g009200.1.1
PS-I light harvesting complex subunit 2 (LHCa2)
Solyc12g011280.1.1
Solyc10g007690.2.1
PS-I light harvesting complex subunit 3 (LHCa3)
Solyc06g069730.2.1
Solyc03g115900.2.1
PS-I light harvesting complex subunit 4 (LHCa4)
Solyc02g070940.1.1
Solyc02g070950.1.1
Solyc02g070970.1.1
Solyc02g070980.1.1
Solyc02g070990.1.1
Solyc02g071000.1.1
PS-II light harvesting complex subunit 1 (LHCb1)
Solyc12g006140.1.1
Solyc07g047850.2.1
PS-II light harvesting complex subunit 2 (LHCb2)
Solyc12g011450.1.1
Solyc07g063600.2.1
PS-II light harvesting complex subunit 3 (LHCb3)
Solyc09g014520.2.1
PS-II light harvesting complex subunit 4 (LHCb4)
Solyc06g063370.2.1
PS-II light harvesting complex subunit 5 (LHCb5)
Solyc01g105030.2.1
Solyc01g105050.2.1
PS-II light harvesting complex subunit 6 (LHCb6)
Solyc00g277510.1.1
Solyc01g048590.1.1
Solyc02g021290.1.1
Solyc05g016120.1.1
Solyc11g013780.1.1
Solyc11g056340.1.1
Solyc12g039030.1.1
Chlorophyll binding protein D1 (PSBA)
Solyc00g230080.1.1
Solyc05g021190.1.1
Solyc09g031730.1.1
Solyc09g055950.1.1
Chlorophyll binding protein D2 (PSBD)
Solyc07g024000.2.1
Chl-b reductase (NYC1)
Solyc06g053980.2.1
Chlorophyllase (CLH)
< -4
> 4

(b)


Solyc05g056050.2.1
LHCa1
Solyc07g024000.2.1
NYC1
Solyc02g070950.1.1
LHCb1
Solyc06g053980.2.1
CLH
Relative expression
0h 3h 24h 48h
Time at 10°C

(c)


Total chlorophyll (μg ml-1 mg FW)
Chlorophyll a/b ratio
NA CA


SUBOPTIMAL TEMPERATURE
(10°C)
Transcription factors
Hormones
Transcriptional reprogramming
Aminoacid accumulation
ROS scavenging
Photosynthesis reduction
TOMATO
CHILLING ACCLIMATION