Generation of fruit postharvest gene datasets and a novel motif analysis tool for functional studies: uncovering links between peach fruit heat treatment and cold storage responses

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Abstract

Main Conclusion A survey of developed fruit gene-specific datasets and the implementation of a novel cis-element analysis tool indicate specific transcription factors as novel regulatory actors under HT response and CI protection.

Abstract Heat treatment (HT) prior to cold storage (CS) has been successfully applied to ameliorate fruit chilling injury (CI) disorders. Molecular studies have identified several HT-driven benefits and putative CI-protective molecules and mechanisms. However, bioinformatic tools and analyses able to integrate fruit-specific information are necessary to begin functional studies and breeding projects. In this work, a HT-responsive gene dataset (HTds) and four fruit expression datasets (FEds), containing gene-specific information from several species and postharvest conditions, were developed and characterized. FEds provided information about HT-responsive genes, not only validating their sensitivity to HT in different systems but also revealing most of them as CS-responsive. A special focus was given to peach heat treatment-sensitive transcriptional regulation by the development of a novel Perl motif analysis software (cisAnalyzer) and a curated plant cis-elements dataset (PASPds). cisAnalyzer is able to assess sequence motifs presence, localization, enrichment and discovery on biological sequences. Its implementation for the enrichment analysis of PASPds motifs on the promoters of HTds genes rendered particular cis-elements that indicate certain transcription factor (TF) families as responsible of fruit HT-sensitive transcription regulation. Phylogenetic and postharvest expression data of these TFs showed a functional diversity of TF families, with members able to fulfill roles under HT, CS and/or both treatments. All integrated datasets and cisAnalyzer tool were deposited in FruitGeneDB (https://www.cefobi-conicet.gov.ar/FruitGeneDB/search1.php), a new available database with a great potential for fruit gene functional studies, including the markers of HT and CS responses whose study will contribute to unravel HT-driven CI-protection and select tolerant cultivars.

Keywords Chilling injury · cisAnalyzer · Database · Dataset · Heat treatment · Prunus persica · Transcriptional regulation

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Abbreviations

CI Chilling injury
CS Cold storage

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The quality of fruit is compromised by internal and pre- and postharvest external factors. Cold storage (CS) extends shelf-life maintaining nutritional properties. However, it can generate chilling injury (CI), a group of undesired physiological disorders perceived at consumption (Lurie and Crisosto 2005). Many strategies prevent CI, being heat treatment (HT) prior to CS efficient in several species (Lurie and Pedreschi 2014). Additionally, natural diversity offers cultivars with contrasting tolerance to CI (Monti et al. 2016; Brizzolara et al. 2018; Bustamante et al. 2018; Nilo-Poyanco et al. 2018). To unravel mechanisms of chilling injury and tolerance, various studies were conducted on peach fruit (*Prunus persica* (L.) Batsch) after cold storage (Ogundiwin et al. 2008; Pons et al. 2014; Puig et al. 2015) or protective treatments (Tanou et al. 2017; Wang et al. 2017; Cao et al. 2018). In this sense, we characterized the transcriptome, metabolome, proteome and lipidome in HT- (Lara et al. 2009; Bustamante et al. 2012; Lauxmann et al. 2012, 2014) and CS-treated fruit (Genero et al. 2016; Bustamante et al. 2016, 2018). Even when a variety of molecules were identified as putative chilling injury protectors, the lack of Rosaceae knowledge at molecular level (Farinati et al. 2017) and integrative in silico resources makes their characterization difficult, suggesting the need to develop new tools to select tolerant cultivars.

Here, we developed a novel peach heat treatment-responsive gene dataset (HTds) with gene-specific and orthologue-related information, and also postharvest expression data from the results of our lab and other groups, including other fruit and plants. Extra expression information was obtained for each HTds gene from four fruit expression datasets (FEds) that were also created. We comprehensively searched for expression data from several articles, fruit species and postharvest conditions and integrated them at gene id level. To further characterize HTds determinants, a curated plant cis-elements dataset (PASPds) and a novel Perl program (cisAnalyzer), able to identify motif signatures in groups of biological sequences, were developed. Considering transcription is under complex cis- and trans-elements combinatorial control (Brljicajic and Grotewold 2017), the mentioned tools were able to provide a means for a first exploration of the fruit HTds cistrome. Several known cis-elements were found conserved in regulatory sequences of commonly expressed genes under heat treatment, and they have been reported as binding sites of particular families of transcription factors (TFs). Moreover, phylogenetic and postharvest expression analyses of these TFs were performed, showing a diversified postharvest presence of fruit TF family members that could account for chilling injury-preventing responses by means of the applied heat treatment. The performed analyses provided important in silico information that was included in a new and freely available database, FruitGeneDB, contributing to fruit gene functional studies through the use of the developed datasets and cisAnalyzer tool.

**Materials and methods**

**Construction of HT-responsive (HTds) and fruit expression datasets (FEds)**

Peach fruit heat treatment- and cold storage-derived proteomic and transcriptomic data previously obtained by our group were joined as the first step for HTds construction (Table 1, Fig. 1). Working sequences were obtained directly from publications or through reported gene identifiers (ids) that allowed searching the associated sequences from NCBI or Phytozome v12.1 (Goodstein et al. 2012) on-line databases. Once all HT-responsive gene-associated cDNA or protein sequences were collected, their assignments to Phytozome *P. persica* (L.) Batsch cv. Lovell 2.1 gene accessions (Ve et al. 2017) were performed by means of standalone blast strategies (Tao 2010) as indicated next. Transcripts or their protein products were subjected to blastn or blastx vs. cDNAs or protein databases, respectively. Proteins were subjected to blastp vs. protein databases. Supplementary Table S1 contains HTds assigned genomic ids, gene descriptions and details of the blast assignments.

Four fruit expression datasets (FEds) for ripening and development, cold storage, chilling injury and temperature-protective treatments were constructed using similar approaches (Fig. 1). The ids reported with a particular expression profile in a specific publication (e.g. peach fruit CI-induced proteins reported by Giraldo et al. 2012) were used to get associated sequences from NCBI, Phytozome or ChillPeach (Ogundiwin et al. 2008). The obtained sequences were utilized to perform standalone blasts against the corresponding Phytozome protein or nucleotide databases (peach fruit proteome was used for chilling injury-induced proteins reported by Giraldo et al. 2012) and assigned to genomic ids. The criteria and cut-off values used to report a molecule as induced or repressed after a particular postharvest condition were respected, without modifying or re-analysing any data but collecting the reported results at gene-specific level. Ids, methods, results and references were organized in peach fruit, tomato and miscellaneous fruit species subdatasets along with the reported expression information compiled.
from publications. The genomic ids obtained in the subdata-
sets with information of species other than *P. persica*, were
also utilized to get peach gene orthologues. They were used
as queries in BioMart mining tool, offered by Phytozome
and based in the Inparanoid program (Goodstein et al. 2012)
to search the phylogenetically related and blastp-assigned
protein orthologues from *P. persica*. Supplementary Tables
S2–S5 contain the four developed FEds with mentioned
features, including the peach fruit orthologues column for
subdatasets with data from other species.

**HTds characterization: annotations, expression data, orthologues-derived and transcriptional initiation information**

The performed steps for HTds characterization are also
detailed in Fig. 1. The genomic features of heat treatment-
responsive loci were extracted from Phytozome BioMart
mining tool, including functional annotations, exon–intron
architectures, alternative start and stop codons, and gene
context, and they were included in Supplementary Table S6
(HTds FeaturesI).

Next, HTds gene ids were used as queries to obtain their
related data in each fruit expression dataset. A grep com-
mand in linux console was applied to intersect the list of
peach heat treatment-responsive ids with each table of the
four fruit expression datasets, getting gene-specific posthar-
vest expression data from other peach fruit reports and for
HTds loci orthologues from other publications. Obtained
information is integrated in Supplementary Table S7 (HTds
FeaturesII).

Six heat treatment-responsive groups of determinants
were created based on the expression data experimentally
verified in our lab and collected in HTds FeaturesII. Induced
(I) group contains HT-induced determinants (HT vs. SL3
induced; HT, heat treatment for 3 days at 39 °C; SL3, shelf-
life for 3 days at 20 °C). Reduced (R) group contains HT-
reduced HTds loci (HT vs. SL3 repressed). Affected (A)
group harbours those HTds loci that showed HT-modified
expression but could not be grouped in I or R because
they showed extra significant changes. Induced2 (I2) and
Reduced2 (R2) are subgroups of I and R (respectively) that
were created based on extra postharvest expression behav-
iours. I2 contains up-regulated molecules in HT vs. SL3 and
HT vs. H, while they were not affected in SL3 vs. H or SLM
vs. H (H, harvest; SLM, shelf-life for a number of days at
20 °C until reaching organoleptic maturity). R2 harbours
down-regulated loci in HT vs. SL3 and HT vs. H, while
they were not affected in SL3 vs. H or SLM vs. H. Finally, U
group of 5 HT-Unaffected transcripts were included because
they are cold storage-sensitive molecules, reported in our
and other groups to be present in several fruits during post-
harvest conditions. HT-responsive groups are contained in
Supplementary Table S8 (HTds FeaturesIII). These cre-
ated gene groups allowed us to look for promoter signa-
tures that could describe a common transcriptional regula-
tion in response to heat treatment. For proximal regulatory
sequences analyses, 500 bp of upstream sequence from the
translational start site were taken for one HTds transcript per
locus from the Phytozome BioMart mining tool.

**Table 1** Selected publications for the construction of peach fruit HT-responsive gene dataset (HTds)

| References | Molecule(s)—methods | Cultivar(s) | Main findings |
|------------|----------------------|-------------|---------------|
| Lara et al. (2009) | Transcripts—RT-qPCR, proteins—Western blotting, proteins—2D-DIGE-MS/MS, metabolites, enzymatic activities | DX | HT induced stress and defence while diminished PPO proteins |
| Lauxmann et al. (2012) | Transcripts—differential display, transcripts—RT-qPCR | DX | 127 HT-responsive mRNAs and 21 of them CS-responsive |
| Bustamante et al. (2012) | Transcripts—RT-qPCR, proteins—Western blotting, proteins—2D-DIGE-MS/MS | DX | HT decreased mRNAs encoding CW enzymes and CW PpDUF642 protein |
| Lauxmann et al. (2014) | Transcripts—RT-qPCR, metabolites—GC/MS | DX | HT induced sugars and sugar alcohols and modified phenylpropanoid pathway |
| Gismondi, unpublished results | Transcripts—RT-qPCR | SL and FD | 4 HT-responsive mRNAs are differentially expressed among cultivars with contrasting susceptibility to CI |
| Genero et al. (2016) | Transcripts—RT-qPCR, proteins—Western blotting | SL, FD, and R2 | 11 CW enzymes mRNAs are CS-differentially expressed among cultivars with contrasting CI. *PpXyl* mRNA negatively correlates with woolliness |

HT, heat treatment; CS, cold storage; CW, cell wall; DX, Dixiland; SL, spring lady; FD, Flordaking; R2, Rojo2; PPO, polyphenol oxidase; RT-qPCR, quantitative reverse transcription PCR; 2D-DIGE-MS/MS, two-dimensional Difference Gel Electrophoresis-tandem mass spectrometry; GC/MS, gas chromatography–mass spectrometry
phylogenetically-related and blastp-assigned protein sequences) and their functional annotations were extracted from Phytozome, MapMan (Thimm et al. 2004) and AgriGO (Tian et al. 2017); cold regulon(s) from Boyce et al. (2003) and Pons et al. (2014), and expression data from ColdArrayDatabase (https://cold.stanford.edu/cgi-bin/data.cgi) and ExPath2.0 (Chien et al. 2015). A Singular Enrichment Analysis (SEA) was performed in AgriGO web server against A. thaliana genome (FDR ≤ 0.05). Information about the A. thaliana orthologues of peach HTds loci is contained in Supplementary Table S8 (HTds FeaturesIII).

Finally, a HTds FeaturesIII subtable was created containing transcription factor (TF) loci (Supplementary Table S9). TF-associated cis-elements were searched in PlantTFDB (Jin et al. 2017) and PlantPAN (Chow et al. 2016) databases.
based on gene ids and included in Supplementary Table S9 for peach and A. thaliana orthologues.

**Construction of plant abiotic stress- and phytohormone signalling-related cis-elements dataset (PASPds)**

PASPds was designed to include experimentally verified *cis*-elements, their references and descriptions of their molecular functions and interacting TFs (Fig. 2). All *cis*-elements from PlantPAN and footprintDB (Sebastian and Contreras-Moreira 2014) databases were collected and the associated publications were revised. The selection of *cis*-elements to be included in PASPds was based on the existence of reported results of EMSA, ChIP, in vivo studies of promoter-reporter fusions or motif enrichment analyses on promoters of commonly expressed genes. According to the reported functionality of the motifs and the interacting TFs, we were able to divide PASPds in 18 groups related to phytohormone, abiotic or biotic stimuli. PASPds is included in Supplementary Table S10.

**Development of cisAnalyzer program**

The *cis*-Analyzer software was created using different Perl (5.18.2 version) strategies, elements and operations (Tisdall 2003) that allowed the creation of a group of directories for inputs and outputs, two Perl modules with novel subroutines and two Perl scripts. The joint functioning of these files offers an interface to the user who will decide among different options of sequence analysis, processes and guarantees the quality of the inputs of the user, searches specific patterns on groups of sequences and analyses the pattern matching results to produce different outputs.

The offered interface allows the user to choose among different types of sequence analysis, insert his/her own set of amino acid or nucleotide target sequences and own motifs, and select known plant TF binding sites from PASPds. Moreover, the option of discovering novel unknown motifs could be selected after only inserting target sequences. Phytozone BioMart sequence database generations were taken into consideration for the design, specifically to allow the user to get the ready-to-use multifasta files from the online tool and avoid undesired file conversions.

A necessary step before processing sequences and motifs is the quality control, performed to find and remove sequences with mistakes and only allow proteins, peptides, DNA or RNA sequences with IUPAC characters. Handling of nucleotide sequences also includes the obtention of reverse complementary sequences in order to search for minus strand motif matches.

After controls, Perl pattern matching of selected or included motifs on the inserted target sequences is performed, automatically generating the classical search results that are shown in tables. The designed pattern matching algorithm searches globally one-dimensional motifs (substrings) in each target sequence (string), even allowing the motif to have more than one variant interpreted with IUPAC characters (e. g. TAT-STA motif is interpreted in TAT[GI]TA and when searching it, both TATGTA and TATCTTA patterns are searched and their results collected).

Finally, posterior analyses allow the user to obtain reports and graphical outputs that help extracting more information about the set of targets. Graphical outputs’ generation is based in R libraries called from Perl and uses the processed results as input. All *cis*-Analyzer features and PASPds are included in *cis*-Analyzer.zip, and extensively described in a detailed tutorial file. Additional files for different *cis*-Analyzer tests are also included and described.

**Transcription factor binding site presence and enrichment analyses on HTds targets**

*cis*-Analyzer and PASPds developed tools were implemented along with surveyed binding sites of heat treatment-responsive TFs in Supplementary Table S9 to study *cis*-elements on regulatory sequences of HTds determinants (Fig. 2). Phytozone BioMart tool allowed us to obtain 500 bp of upstream sequence from the translational start site for one HTds transcript per locus of each HTds group (I, I2, R, R2, A, U). Downloaded multifasta files were used as input in /MyTargets folder and two *cis*-Analyzer analysis options were chosen.

A PASPds-related analysis was performed in *cis*-Analyzer to search all PASPds motifs on I, I2, R, R2, A and U sets of 500 bp regulatory sequences. Then, through a second *cis*-Analyzer option, called Custom analysis, we also searched the surveyed binding sites of HT-responsive TFs not included in PASPds (Supplementary Table S9) on the same sets of regulatory sequences.

Motif enrichment analysis output files were assessed after each *cis*-Analyzer run to find *cis*-elements that could characterize I, I2, R and R2 heat treatment-responsive groups. Randomly designed reference sets (CI, CI2, CR, and CR2) and all peach primary promoters were used as references. Relative frequencies of target sequences with at least one motif match were compared with genome proportions of the motif(s) and verified in reference sets. A motif was considered enriched if frequency of matched sequences was statistically higher (*P* < 0.05) than genome wide frequency, and if the enrichment was not observed for the corresponding reference set. Chi-squared tests were performed in SigmaStat (SigmaPlot12.0), displaying absolute data on contingency tables.
Phylogenetic and expression analyses of fruit transcription factors

TF ids with verified binding sites were obtained from publications included in PASPds and associated with the found motifs (Fig. 2). Related TFs from original species and fruit orthologues were searched in PlantTFDB and Phytozome BioMart mining tool, gathering them in TF sets with potentially conserved DNA binding preferences (Supplementary Table S11). Phylogenetic analyses of AP2/ERF and ICE1/MYC TF sets of protein sequences were performed in MEGA6 (Tamura et al. 2013) and the evolutionary history was inferred by Maximum Likelihood method (bootstrapping 1000 repetitions). Postharvest expression data of TFs included in TF sets were obtained from developed FEds. Finally, an additional use of cisAnalyzer, consisting of searching for DSAW and LWSY DREB1-related (Agarwal et al. 2006a) amino acid motifs on analysed AP2/ERF protein sequences, was performed. Inputs, PASPds and custom runs on HTds groups, reference and genomic sequence sets are included in cisAnalyzer_analyses_results.zip.

Development of FruitGeneDB

HTds (Supplementary Tables S1, S6–8), FEds (Supplementary Tables S2–5), PASPds (Supplementary Table S10), TF sets with enriched binding sites in HT-responsive promoters (Supplementary Table S11) and cisAnalyzer and PASPds
results (cisAnalyzer_analyses_results.zip) generated datasets and their relationships as shown in Fig. 1, were recorded and integrated in a MySQL relational database called FruitGeneDB. Gene and motif ids are the keys to cross-correlate the datasets. The resulting database is displayed in a user-friendly PHP powered web page. Particular gene and HT-responsive group gene searches were programmed to get all related results from all datasets developed in this work, including annotations, expression, motif presence and orthologue-related information.

**Results**

**The public Fruit Expression datasets (FEds) integrate many postharvest expression data at gene id level**

A comprehensive compilation of 152 publications with gene-specific postharvest expression data of 14 plant species led to the development of four fruit expression datasets (FEds): ripening and development (Supplementary Table S2, Fig. 1), cold storage or CS (Supplementary Table S3), chilling injury or CI (Supplementary Table S4) and temperature-protective treatments (Supplementary Table S5), with 6650, 5027, 10,928, and 8349 gene accessions, respectively. Each FEds contains expression information results (taking into consideration the criteria originally used by the authors), methods and ids extracted from publications and associated with genomic identifiers of three subdatasets related to peach, tomato or a group of other different fruit species. Tomato and other fruit subdatasets also contain associated peach orthologues (obtained from Phytozome BioMart mining tool, based in Inparanoid approach with protein sequences), providing a way to find relationships between species.

**The Heat Treatment-responsive dataset (HTds) comprises peach HT-responsive genes**

A total of 153 peach HT-responsive determinants detected as differentially expressed transcripts or proteins in different publications of our lab were compiled in HTds (Table 1, Fig. 1) and assigned to genomic *P. persica* accessions through different blast strategies (Supplementary Table S1). Several steps of data acquisition enabled the characterization of HT-responsive dataset in three modules: FeaturesI to III. Performed steps for HTds characterization are shown in Fig. 1.

Phytozome-reported functional and genomic annotations, including alternative start and stop codons and gene context for each heat treatment-responsive gene, were integrated in the first module: HTds FeaturesI (Supplementary Table S6). The results of the intersections of peach HT-sensitive determinants identifiers with FEds fruit expression datasets (based on the presence of the same genes in other peach fruit reports or of their orthologues from other species) were compiled at gene specific level in HTds FeaturesII (Supplementary Table S7).

Finally, FeaturesIII (Supplementary Table S8) contains the delimitation of HTds in six groups of determinants which share common profiles after postharvest heat treatment exposure: I, Induced; I2, Induced2; R, Reduced; R2, Reduced2; A, Affected; and U, Unaffected (being I2 and R2 subsets of I and R, respectively, with additionally identified
expression behaviours under ripening conditions). Moreover, *A. thaliana* orthologues of HT-responsive genes were also included in FeaturesIII along with their annotations and expression data under heat and cold stimuli exposures.

Functional annotations analyses based on *A. thaliana* identifiers pointed out transcription and stress responses as HT-induced, and cell wall degradation and carbohydrate metabolism as HT-reduced processes (Supplementary Fig. S1). Interestingly, many RNA metabolism factors were identified as heat treatment-sensitive suggesting their implication under temperature stimuli responses and a relevant number of loci with modified expression after HT were classified as functionally unknown. This is in agreement with the current knowledge about HTds determinants, obtained after gathering information about HT-responsive loci and their orthologues from FEds (Supplementary Fig. S2).

**Several HTds genes showed conserved heat- and cold-sensitivity**

A particular analysis performed with HTds Features II, revealed interesting postharvest expression information and showed the potentiality of the created fruit expression datasets to characterize sets of fruit genes.

The sensitivity of HTds determinants to HT in peach reports and other fruit species, and the conservation of the expression behaviours between orthologues of considered species were compiled (Table 2). From them, some HTds genes have at least one fruit orthologue with conserved HT-driven expression behaviour: HT-induced *PpHSP20* (Spot 70; Heat Shock Protein 20) and *PpPEX10* (I41, Peroxin 10); HT-reduced *PpDUF642* (Protein of Unknown Function 642) and *PpACO1* (Aminocyclopropane-1-Carboxylate Oxidase 1) (Bustamante et al. 2012); and HT-affected *PpRS* (Raf-finose Synthase) (Lauxmann et al. 2014), *PpABA_WDS* (ABA/WDS-induced protein) and *PpUSP* (Universal Stress Protein). *PpTIL* (I60, Temperature-Induced Lipocalin; Lauxmann et al. 2012) is the only HT-responsive gene whose orthologues in *A. thaliana* and FEds-included species present heat-inducible expression (Chi et al. 2009).

On the other hand, the cold-sensitivity and cold regulation belonging of HTds loci and their orthologues were obtained (Table 3). As an interesting result, approximately a half of HT-responsive genes and/or their orthologues show cold-responsiveness in at least one species. Moreover, we were able to identify 9 HT-induced, 15 HT-reduced and 6 HT-affected determinants that show sensitivity to cold stimuli in all considered species. Among them, we distinguish *PpDhn3* (Spot 134, Dehydrin 3), *PpACS1* (R25, Acetyl-CoA Synthetase 1), and *PpRS* because they share common cold-driven expression patterns with their orthologues from other species.

**PASPds: a novel set of Plant abiotic stress- and phytohormone signalling-related cis-elements**

Aiming to contribute to the characterization of plant regulatory sequences, we developed a curated motif dataset that could facilitate in silico searching of TF binding sites on promoters and looking for associated TFs and related publications (Fig. 2). As the conservation of TF DNA binding properties among families and species is assumed (including *P. persica* promoters’ studies; Artlip et al. 2013; Pons et al. 2014; Wang et al. 2017), we collected in vivo/in vitro experimentally verified PlantPAN and footprintDB TF binding sites of different plant species and integrated their descriptions and references. We added a feature to PASPds dataset dividing it in 18 groups of abiotic stress- and phytohormone signalling-related motifs: light, cold, heat, oxidative stress, wounding, ABA-dependent or -independent dehydration, unfolded protein

### Table 2: Heat-sensitivity and its conservation for HTds genes and orthologues

| Group | HTds genes | Heat-responsive genes | Heat-responsive genes with conserved heat-sensitive expression profile |
|-------|------------|-----------------------|---------------------------------------------------------------|
|       |            | Ppe\(^a\) Oth Ath     | Ppe\(^a\) Oth Ath                                               |
| I     | 65         | 6 27 3                | 5 8 1                                                          |
| I2    | 45         | 16 2                  | 1 2 1                                                          |
| R     | 58         | 2 32 1                | 0 7 1                                                          |
| R2    | 41         | 21 1                  | 0 0 1                                                          |
| U     | 5          | 2 2                   | 0 0 0                                                          |
| A     | 25         | 7 14 2                | 1 6 2                                                          |

\(^a\)Indicates reports from other groups.

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response (UPR), sugar response, circadian rhythm, auxin (AUX), cytokinin (CK), ethylene (Et), jasmonic acid (JA), salycilic acid (SA), gibberellins (GA) and miscellaneous. These groups allow the user to choose particular(s) motif groups to study promoter sequences, focusing the search on a set of TF binding sites already related to the stimulus of interest. PASPds design considered multiple stimuli-sensitivity and promiscuous motif-binding of certain TFs (Sun et al. 2008; Cheng et al. 2013). As a result, PASPds is an accessible and useful curated list of 264 verified motifs from 47 plant species, and the respective 227 references (Supplementary Table S10).

**cisAnalyzer: a novel tool developed for cis-elements analyses on biological sequences**

Along with PASPds, we developed cisAnalyzer, software for cis-elements presence, enrichment, discovery, co-occurrence and position analysis on DNA, RNA and amino acid sequences. It was designed to provide the user different options of studies of reported or own, known or unknown motifs on a set of biological sequences, avoiding time-consuming filtering of found motifs and/or finding motif-based commonalities between sequences.

Requirements for cisAnalyzer utilization, directories’ structure, tests files and a detailed tutorial file are included in cisAnalyzer.zip (freely available at https://www.cefobi-conicet.gov.ar/FruitGeneDB/search1.php).

The program was designed in Perl language as a console application in which the user is able to choose among cisAnalyzer capabilities showed in Supplementary Table S12. A PASPds-assisted analysis searches all, particular group(s) of motif(s) or particular known and experimentally proven TF binding sites included in PASPdb on plant regulatory sequences. The user could also perform a Custom analysis of DNA, RNA or peptide motifs on an own set of target sequences. Finally, an Unknown motif enrichment analysis could discover novel motifs on a group of biologically-related nucleotidic sequences. Supplementary Fig. S3 summarizes cisAnalyzer main functioning steps. After user choices and sequence quality controls that remove targets with non-IUPAC characters, global pattern matching of each one-dimensional motif on each target sequence is performed and results are reported in classical tables. Next, an exhaustive sub-processing of matching results generates novel informative outputs, based on the calculation of relative frequencies of motif matches or target sequences with at least one motif match vs. different totals (matches of a motif vs. total matches of all motifs or total matches of the same motif; sequences with at least one match of a motif vs. input sequences, sequences with at least one match of the same or all analysed motifs), and the classification into presence, strand, co-ocurrence and position categories. A very useful report allows getting calculated frequencies for each motif as well as a fast and easy id-browsing of accessions under specific categories (e.g. the user could obtain subsets of sequences that contain co-occurrence of two motifs in a particular region). An enrichment report is also automatically generated, showing the frequencies of targets with at least one motif match vs. the amount of input targets of the set. Moreover, the different relative frequencies are represented in useful R-based generated graphs that offer a fast visualization of the motif matching footprints on the set of targets. As an example, one graphical output after PASPds analysis on the promoters of I group is shown (Fig. 3): 174 motifs were found, 17 of them occur at least once in half or more targets.

### Table 3 Cold-sensitivity and its conservation for HTds genes and orthologues

| Group | HTds genes | Cold-responsive genes | Genes belonging to *A. thaliana* cold regulons |
|-------|------------|-----------------------|---------------------------------------------|
|       |            | Ppe | Oth | Ath |                               |
| I     | 65         | 29  | 25  | 37  | 15 CBF DREB2 ICE1 ESK1 SRF6 ZAT12 ZFHD/NAC |
| I2    | 45         | 17  | 17  | 25  | 7 CBF DREB2 ICE1 SRF6 ZAT12 |
| R     | 58         | 38  | 36  | 34  | 13 CBF CBF4 SRF6 ZAT12 |
| R2    | 41         | 25  | 24  | 21  | 8  CBF CBF4 ICE1 ZAT12 |
| U     | 5          | 5   | 2   | 2   | 2 DREB2 SRF6 ZAT12 |
| A     | 25         | 18  | 15  | 12  | 7 CBF DREB2 DZAT12 |

**ds, dataset; HT, Heat Treatment; HTds HT-sensitive groups: I, Induced; I2, Induced2 (included in I); R, Reduced; R2, Reduced2 (included in R); A, Affected; and U, Unaffected; Ppe, *P. persica*; Oth, other fruit species; Ath, *A. thaliana*

*a* Indicates reports from our and other groups.
Exploring HT transcription regulation: identifying cis-elements with cisAnalyzer and PASPds and trans-elements that could act on HTds gene promoters

We focused on HT-driven transcriptional initiation as one of the mechanisms that could regulate heat treatment response and chilling injury-protection (Fig. 2). In this sense, HTds FeaturesI allowed us to get important details about gene contexts that could affect transcriptional start sites and TFs binding. 30 HT-responsive genes have upstream adjacent loci at 1000 bp or less apart, and 12 of them could share bidirectional promoters, including *PpESP1* (R7, Enhanced Silencing Phenotype 1; Funck et al. 2012).

I, I2, R, R2, A and U sets of HT commonly expressed determinants (HTds Features III) were created to look for particular motif signatures that could describe a common HT transcriptional regulation. We analysed proximal 500 bp
of upstream regulatory regions with cisAnalyzer, PASPds and surveyed HT-responsive TFs-related binding sites (Supplementary Table S9). Inputs, PASPds and custom runs on HTds, reference and genomic sets are included in cisAnalyzer_analyses_results.zip (https://www.cefoi-conicet.gov.ar/FruitGeneDB/search1.php). These browseable data contained in cisAnalyzer outputs allow verifying cis-elements predictions on particular HT-sensitive determinants’ promoters and integrating them with the rest of HTds gene-specific information, including FEds-derived expression reports from other groups and other fruit orthodoxes.

Then, enrichment analyses of cisAnalyzer found motifs on proximal promoter sequences of I, I2, R and R2 groups were performed. Enrichment frequencies that cisAnalyzer reports for each motif in each HTds set were statistically compared to P. persica genomic and random created sets of promoters (see “Materials and methods”). To know more about the functional roles of enriched cis-elements, a total of 74 TFs (including 29 fruit TFs) putatively able to interact with them, were obtained (Supplementary Table S11). Their postharvest expression data from FEds as well as the phylogenetic features of two selected TF sets were examined.

Table 4  cis-elements’ enrichment at I and I2 gene regulatory sequences

| Motif name | Associated gene | PASPds group(s) | Motif          | Relative frequencies of sequences with at least one motif occurrence | Chi-squared test p value |
|------------|----------------|----------------|---------------|---------------------------------------------------------------|-------------------------|
|            |                |                |               | Genome | I | I2 | I | I2 |                |
| DREB2A bs  | ERF1 bs        | No             | Heat dehydration | RCCGAC | 0.12 | 0.23 | 0.22 | 0.014 | 0.073     |
| DBF1-2 bs  |                | ABA dehydration | ACCGAC        | 0.08  | 0.18 | 0.20 | 0.003 | 0.005     |
| CBF4 bs    |                |                | CCGAC         | 0.21  | 0.35 | 0.36 | 0.009 | 0.031     |
| CRT/DRE    |                | Cold           | RYCGAC        | 0.20  | 0.34 | 0.31 | 0.012 | 0.114     |
| DRE-like   |                |                | DRCCGACNW     | 0.06  | 0.12 | 0.09 | 0.044 | 0.553     |
| DREB1/CBF2 |                |                | TTNCAGT       | 0.05  | 0.12 | 0.11 | 0.031 | 0.179     |
| Osa Cold Novel II | |                | CTGACG        | 0.03  | 0.08 | 0.04 | 0.028 | 0.753     |
| CM4        |                |                | TCCACGTC      | 0.02  | 0.06 | 0.07 | 0.016 | 0.036     |
| HSE I      |                | No             | Heat          | AGAANNTTCT | 0.02 | 0.03 | 0.02 | 0.764 | 0.725     |
| Light HSE  |                | Light          | GAANNCTC      | 0.18  | 0.26 | 0.33 | 0.101 | 0.010     |
| CARE       |                | GA             | CAACTC        | 0.26  | 0.17 | 0.11 | 0.146 | 0.040     |
| HvGAMYB bs |                |                | YAACSRHM      | 0.44  | 0.52 | 0.58 | 0.208 | 0.082     |
| AtbHLH110 G-box | I1       | ABA            | CACGTG        | 0.10  | 0.17 | 0.18 | 0.088 | 0.130     |
| Pp/AtARR-B bs | R14       | Cold          | MRDATCTH      | 0.50  | 0.62 | 0.67 | 0.096 | 0.043     |
| AtARR2 bs  |                |                | RGATT         | 0.92  | 0.95 | 0.96 | 0.403 | 0.513     |
| ARR5/7/15 bs |            |                | NGATT         | 0.99  | 0.97 | 0.98 | 0.450 | 0.929     |
| WRKY bs    | AtWRKY18 bs   | I44            | Cold          | YTGACY | 0.54 | 0.51 | 0.51 | 0.733 | 0.847     |

Bolded values indicate statistically enriched and sub-enriched frequencies against genome wide proportions, respectively, and associated p-values. IUPAC characters of motifs: R = A/G, Y = C/T, M = A/C, K = G/T, W = A/T, S = G/C, D = T/A/G, H = A/C/T, N = A/C/G/T. bs, binding site; ds, dataset; TF, transcription factor; GA, gibberellins; CK, cytokinins; ABA, abscisic acid.
and on their review on studied members, we performed a phylogenetic study of peach AP2/ERFs in comparison to *A. thaliana* TF members (Fig. 4). The obtained phylogenetic tree shows that PpCBF2* arises as AtCBF1-3/DREB1A-C’s most similar protein, suggesting a possible function in plant low temperature responses. We also performed a cisAnalyzer DREB1-related amino acid motif search on revised fruit AP2/ERF members (results are included in Supplementary Table S11), showing the conservation of DSAW and LWSY motifs in peach members of DREB subfamily and, at the same time, other possible use of cisAnalyzer. FEds-derived data revealed the induction of *PpCBF-DREB1s, PpDREB2s* and other AP2/ERF members during fruit CS conditions (Artlip et al. 2013; Pons et al. 2014; Wang et al. 2017). However, we gathered some evidences that contrast with classic cold-sensitivity of CBF/DREB1s and classic cold-insensitivity of DREB2s (Agarwal et al. 2006b; Park et al. 2015; Eremina et al. 2016). CBFs orthologues in kiwifruit, tomato, peach and soybean seedlings were found to be heat-responsive (Liang et al. 2013; Ma et al. 2014; Cruz-Mendívil et al. 2015; Kidokoro et al. 2015). Moreover, fruit DREB2s were found to respond to cold (Wang et al. 2017). Overall,
a revision of the fruit AP2/ERF set and the expression data suggests that several members could bind CRT/DRE motifs not only under CS as expected but also under HT or both cold storage and heat treatment, possibly accounting for HT-driven induction of chilling injury-protective determinants at transcriptional level. On the other hand, Table 5 summarizes R and R2 enrichment analysis (analyses on CR and CR2 reference sets are summarized in Supplementary Table S14). P-box, a GA-related motif, was overrepresented in R and R2 and reported in \textit{PpDhn} promoters for cold acclimation-photoperiodic control (Wisniewski et al. 2006). An interesting sub-enrichment of several WRKYbss was evidenced in R and R2, suggesting the independence of this functionally wide family (Chen et al. 2016; Wang et al. 2017) in the HT-repression of R and R2 determinants. TA5-box, reported in rice OsMYBS3-affected promoters (Su et al. 2010), was found enriched in R2. This could suggest a particular role in cold responses as OsMYBS3 represses cold acclimation and the fruit orthologues are CS- and HT-sensitive (Sanchez-Ballesta et al. 2003; Pons et al. 2014; Cruz-Mendivil et al. 2015). On the other hand, DREB1/CFBs-repressor \textit{MYB15} (Agarwal et al. 2006b) possess R-enriched TF binding sites and the orthologues are cold storage- and heat treatment-sensitive (Sanchez-Ballesta et al. 2003; Pons et al. 2014; Cruz-Mendivil et al. 2015). Finally, ICE1/JIN1bs was overrepresented in promoters of R group. This motif is related to \textit{MYC}-like bHLH ICE1 (\textit{Inducer of CBF Expression 1}), an important cold-inducible DREB1/CFBs regulator (Benedit et al. 2006; Chinnusamy et al. 2010). The same motif is target of JA responses-master regulator \textit{MYC2/JIN1/JAI1} (Jasmonates INSensitive 1) which plays a role in development, defense, light and phytohormone responses (Kazan and Manners 2013). Moreover, ICE1/JIN1bs reverse complement sequence corresponds to high salinity-/cold-/ABA-related NAC072 binding site (Li et al. 2016). \textit{A. thaliana}, peach and \textit{Malus domestica} \textit{ICE1/MYC2/NAC072} protein orthologues were obtained and phylogenetically assessed, revealing clear TF-specific clades (Fig. 5). Although there is no fruit \textit{MYC2}-orthologues’ information reported and included in FEds, tomato and peach NAC072-orthologues

### Table 5 cis-elements’ enrichment at R and R2 gene regulatory sequences

| Motif name | Associated gene | PASPds group(s) | Motif | Relative frequencies of sequences with at least one motif occurrence | Chi-squared test p value |
|------------|----------------|-----------------|-------|-----------------------------------------------------|------------------------|
| DREB2A bs ERF1 bs | No | Heat dehydration | RCCGAC | 0.12 0.21 0.20 | 0.082 0.244 |
| DBF1-2 bs | ABA dehydration | ACCGAC | 0.08 0.19 0.17 | **0.001** 0.053 |
| CBF4 bs | Cold | RYGCAG | 0.20 0.26 0.24 | 0.394 0.670 |
| CRT/DRE | Cold JA Biotic | CATGTC | 0.22 0.38 0.32 | **0.005** 0.179 |
| NAC072 bs | | CATGTG | | |
| AtZAT12 bs | I42 | Cold | RSAATGAG | 0.08 0.16 0.10 | **0.040** 0.804 |
| TA5 box | | No | TATCCT | 0.18 0.28 0.32 | 0.081 **0.036** |
| HOS9 bs | | VCKCGT | 0.29 0.40 0.46 | 0.092 0.021 |
| MYB15 bs | | Light JA | AMCWAMC | 0.52 0.62 0.66 | 0.145 0.096 |
| AtMYB3 bs | – | TAACTAAC | 0.02 0.05 0.07 | 0.121 **0.029** |
| HSE I | Heat | AGAANNTCT | 0.02 0.02 0.02 | 0.656 0.777 |
| NAC072 bs | | CAT GTG | | |
| AtWRKY18 bs | I44 | Cold RSAAT | GAG | 0.08 0.16 0.10 | 0.021 **0.015** |
| WRKY bs | | – | – | – | – |
| WRKY40 bs | | – | – | – | – |

Bolded values indicate statistically enriched and sub-enriched frequencies against genome wide proportions, respectively, and associated p-values. IUPAC characters of motifs: R = A/G, Y = C/T, M = A/C, K = G/T, W = A/T, S = G/C, D = T/A/G, H = A/C/T, N = A/C/G/T. bs, binding site; ds, dataset; TF, transcription factor; GA, gibberellins; JA, jasmonic acid; ABA, abscisic acid.
were detected in heated and refrigerated samples (Pons et al. 2014; Cruz-Mendívil et al. 2015), and one ICE1-orthologue (Prupe.5G035400.1), close in amino acid sequence to apple cold-tolerance-related MdbHLH1 TF (Feng et al. 2012), was found induced by cold storage (Pons et al. 2014). Mentioned TFs could have a particular impact in transcriptional control of heat treatment-repressed genes of R and R2 groups through ICE1/JIN1bs binding during HT and/or CS.

**FruitGeneDB is a novel gene database for fruit functional studies**

After obtaining different important fruit gene data, including genes, annotations, expression, motifs’ presence in promoters, orthologues and TFs, a suite that compiles and integrates all results was constructed. FruitGeneDB (https://www.cefobi-conicet.gov.ar/FruitGeneDB/search1.php) is MySQL and PHP powered and allows the user not only to retrieve information at gene id level presented and discussed in this work, but also to browse developed datasets. Links for download cisAnalyzer, PASPds and HTds-cisAnalyzer results are also included.

**Discussion**

**FruitGeneDB can boost fruit functional studies.** Peach HT-responsive dataset (HTds) could contribute to selecting and characterizing chilling injury-protective markers.

Developed FruitGeneDB is the result of an interdisciplinary effort in response to the need of modern genomic resources able to integrate the vast amount of fruit gene information generated in the wet lab. The main contribution of this resource is to concentrate multiple reports previously unrelated at gene-specific level, increasing the possibility of reaching new conclusions and facilitating the inclusion of newly generated data that could make FruitGeneDB even more valuable in the future.

FruitGeneDB includes Fruit Expression datasets (FEds) obtained from 152 publications, as useful fruit data tools to manipulate and extract interesting postharvest information of different species. The differences in the utilized experimental methods and analysis tools that the 152 publications contain made it necessary to limit the FEds included data to qualitative information, respecting the criteria of each particular publication to report a transcript/protein as induced/repressed under particular postharvest conditions. In spite of this, FEds are one of the first attempts to integrate a rapidly growing amount of fruit expression and orthologue information, providing a novel way to find relationships between species and easily gather the current knowledge about specific set of genes. Novel experimental approaches including NGS data will allow to expand the limits of these datasets and to generate normalized atlas with quantitative fruit postharvest data.

Peach HT-responsive dataset (HTds), also included in FruitGeneDB, can be used as a start point for experimental characterizations in peach and other fruit. Functional annotations revealed that HT could not only stimulate direct effectors, metabolic and cell wall re-arrangement actors but also different mechanisms of expression regulation for protecting fruit against chilling injury. Moreover, finding many functionally unknown heat treatment-sensitive molecules reveals particularities of non-model species at the molecular level.

Focusing on FEds-derived heat- and cold-sensitivity of HTds genes and orthologues, conserved fruit temperature stimuli markers were identified, including classically reported lipocalins and HSPs, as well as stress response and metabolic protein-coding genes as \( PpABA\_WDS \) (ABA/WDS-induced protein), \( PpUSP \) (Universal Stress Protein) and \( PpACS1 \) (R25, Acetyl-CoA Synthetase 1). Noticeably, HT-induced lipocalins and dehydrins prevent lipid peroxidation (Abo-Ogiala et al. 2014; Graether and Boddington, 2014), suggesting the importance of their functions to cope...
with CI-driven oxidative stress. Additionally, raffinose synthase \textit{PpRS} was induced by cold storage and HT-repressed after shelf-life, indicating a fine temperature control of antioxidant metabolism and functionalities (Lara et al. 2009; Lauxmann et al. 2014; Bustamante et al. 2016).

As a very interesting result, approximately a half of HT-responsive genes and/or their orthologues show cold-responsiveness in at least one plant species, suggesting that HT is not only affecting cold-independent mechanisms that provide positive functions against chilling injury but also cold-sensitive components that could already have a role in CS response and CI development. These results show putative links between HT and cold storage that are in agreement with the reported positive effect of this postharvest treatment (Lurie and Pedreschi 2014).

cisAnalyzer and PASPds are useful tools for the processing of sequence analysis results

Transcriptional initiation regulation involves multiple elements and mechanisms less-studied in fruit (Farinati et al. 2017), making it necessary to develop new tools that allow performing analyses on non-model species. On the other hand, although several programs are used to identify \textit{cis}-elements in sequences (Bailey et al. 2015; Chow et al. 2016), the novel features of \textit{cis}Analyzer and PASPds contribute to answer a wide variety of biological issues through the search of sequence motifs in nucleotide and amino acid sequences. It is possible to implement it for two different but close purposes when working with TFs: enrichment of TF binding sites in a set of co-regulated plant promoters and characterization of conserved peptide motifs for describing a TF family. After motif(s) searches, \textit{cis}Analyzer posterior subanalyses and outputs’ generation provide a great diversity...
of motif and target data in tabular and graphical formats (Fig. 3), including detailed reports and graphs that speed up the biological interpretation of results, PASPds motif information and bibliographic references. PASPds and cisAnalyzer are open source and included in FruitGeneDB site.

A novel set of fruit cis- and trans-elements could be involved in transcription of HTds loci

As not addressed before, we focused on transcriptional regulation as one of the regulatory mechanisms underlying HT response. The analysis of HTds cistrome was the first implementation of cisAnalyzer and PASPds, and raw as well as integrated results can be browsed at FruitGeneDB web page. Epigenetic, post-transcriptional and/or post-translational processes could also act under HT, and cisAnalyzer could be implemented to discover particular features on intronic, upstream, downstream and amino acid HT-responsive sequences.

The discovery of various enriched motifs revealed differential features of R and R2, and I and I2 groups of regulatory sequences, and along with expression data of related TFs, suggest a complex HT-driven regulation of transcription and the postharvest importance of certain fruit TFs. The enrichment of LightHSE in I/I2 and the postharvest expression of certain HSFs suggest a role in HT-induction and/or in chilling injury-protection. Particular sub-enrichment results were also found for certain TF families, suggesting a putative heat treatment-driven participation through their absence. HT-driven effects on posterior CS and/or CI were suggested by HT-responsive gene cold-sensitivity and enrichment of cis-elements with prominent cold acclimation roles. TA5-box (OsMYBS3bs) and MYB15bs were found enriched in R and R2 and have associated TFs with negative effects on cold acclimation (Agarwal et al. 2006b; Su et al. 2010). Enrichment of ICE1/MYC2/NAC072bs in R and R2 groups suggests roles of these and related TFs in HT-driven CI protection, in agreement with their detection under cold storage, the relationship between MYB15 and ICE1, and the existence of cold negatively-regulated ICE1 sub-regulons (Agarwal et al. 2006b). Finally, CRT/DREs‘ overrepresentation in I/I2, combined with phylogeny and postharvest expression data, suggests interesting roles for AP2/ERF members in HT and questions the conservation of temperature-driven expression of their TF subfamilies.

To better understand the complex interplay between HT and CS responses, the novel features identified after our genomic and expression data surveys and cis-elements’ enrichment analyses are summarized in Fig. 6. The proposed model shows how AP2/ERF, HSF and ARR-B TF family members would be able to generate novel and/or improve cold-responsive CI-protective mechanisms and suggests a role for ICE1/MYC, MYBS and MYB15 TF families as inhibitors of negative mechanisms for CI-protection and/or CI-inductive processes. It is noticeable the common heat treatment- and cold storage-sensitivity of several determinants, suggesting that HT could induce or reinforce the presence of CS-sensitive beneficial actors and/or generate the reduction/absence of CI-inducers. Other levels of regulation are certainly important under the studied processes, including the particular temperature regulation of individual members within each TF family and the combinatorial interaction of multiple cis-elements to control transcriptional initiation. Other aspects that should be taken into account are the differences that fruit cultivars show in their capability of inducing chilling injury-protective mechanisms, and their interaction with protective strategies as heat treatment.

In summary, our studies provide novel and useful tools for the study of non-model species, contribute substantially to increase the knowledge about P. persica [L.] Batsch integration responses during the HT-driven CI- protection and suggest particular cis-elements and their related and functionally diversified families of trans-elements as key players in the regulation of these processes at transcriptional level.

Author contribution statement All authors conceived and designed the research. MG and LDD designed and developed cisAnalyzer and PASPds. LLM, MVL, MFD and CAB contributed to HTds creation. MG and LDD analyzed and interpreted the data. CM and MG designed and created FruitGeneDB. MG, LDD and CAB wrote the manuscript. All authors read and approved the final version.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Abo-Ogiala A, Carsjens C, Diekmann H et al (2014) Temperature-induced lipocalin (TIL) is translocated under salt stress and protects chloroplasts from ion toxicity. J Plant Physiol 171:250–259. https://doi.org/10.1016/j.jplph.2013.08.003
Agarwal M, Hao Y, Kapoor A et al (2006a) A R2R3 type MYB transcription factor is involved in the cold regulation of CBF genes and in acquired freezing tolerance. J Biol Chem 281:37636–37645. https://doi.org/10.1074/jbc.M605895200

Agarwal PK, Agarwal P, Reddy MK, Sopory SK (2006b) Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. Plant Cell Rep 25:1263–1274. https://doi.org/10.1007/s00299-006-0204-8

Artlip TS, Wisniewski ME, Bassett CL, Norelli JL (2013) CBF gene expression in peach leaf and bark tissues is gated by a circadian clock. Tree Physiol 33:866–877. https://doi.org/10.1038/tp.2015.56

Bailey TL, Johnson J, Grant CE, Noble WS (2015) The MEME suite. Nucleic Acids Res 43:W39–W49. https://doi.org/10.1093/nar/gkv416

Benedict C, Geisler M, Trygg J et al (2006) Consensus by democ-...
