

A proteometabolomic study of *Actinidia deliciosa* fruit development

Anna Maria Salzano^{a,1}, Anatoly Sobolev^{b,1}, Virginia Carbone^{c,1}, Milena Petriccione^{d,1}, Giovanni Renzone^{a,1}, Donatella Capitani^b, Monica Vitale^{a,e}, Paola Minasi^c, Maria Silvia Pasquariello^d, Gianfranco Novi^a, Nicola Zambrano^{e,f}, Marco Scortichini^d, Luisa Mannina^{b,g,*}, Andrea Scaloni^{a,**}

^a Proteomics & Mass Spectrometry Laboratory, ISPAAM, National Research Council, 80147 Naples, Italy

^b Magnetic Resonance Laboratory “Annalaura Segre”, Institute of Chemical Methodologies, National Research Council, 00015, Monterotondo, Rome, Italy

^c Institute of Food Sciences, National Research Council, 83100 Avellino, Italy

^d Centro di Ricerca per Olivicoltura, Frutticoltura e Agrumicoltura, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, 81100 Caserta, Italy

^e Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli “Federico II”, 80131 Naples, Italy

^f CEINGE Biotecnologie Avanzate, 80145 Naples, Italy

^g Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, 00185 Rome, Italy

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ABSTRACT

Green-fleshed kiwifruit is worldwide appreciated for its flavor and macronutrients that are related to its physiological development. Fruit ripe stage for harvesting does not correspond to an optimal edible condition due to firmness/acidity pome characteristics; this is overcome with postharvest fruit processing. To describe the metabolic pathways/molecular effectors underlying *Actinidia deliciosa* cv. Hayward pome physiological development, kiwifruits were harvested at four growth stages (from fruit set to physiological maturity), and corresponding outer endocarp samples were analysed for metabolite and protein content. Combined NMR/LC-UV/ESI-IT-MSⁿ procedures quantified 46 metabolites at these developmental stages; similarly, integrated 2D-DIGE/nLC-ESI-LIT-MS/MS analyses described corresponding proteomic changes. Quantitative protein dynamics showed that components related to disease/defense, protein destination/storage, metabolism, energy and cell structure functions were highly affected at specific moments of fruit development, suggesting a rationale to pomological and metabolite content characteristics at those times. Bioinformatic interaction prediction revealed a main network of differentially represented proteins, which may control metabolic changes in developing kiwifruit. Main pome allergens were also quantified, demonstrating their highest levels at the mature stage. By aligning kiwifruit development to a proteometabolomic representation, this investigation integrates previous metabolic observations and provides a reference framework for further physiological/nutritional studies, also allowing cross comparison among crop species.

Biological significance: Compared with some other fruits, green-fleshed kiwifruit is unique for its nutrient density, health benefits, and consumer appeal; in fact, it is exceptionally rich in vitamins, carotenoids, potassium, fibre and phytochemicals acting in synergy to achieve multiple health advantages. However, kiwifruit is allergenic, and although symptoms in most susceptible individuals are mild, severe reactions have also been described. In the course of their 6-month development, kiwifruit undergoes radical changes in its morphology and chemical composition; these modifications may highly affect fruit nutraceutical and allergenic properties. To gain a better understanding of the molecular processes regulating metabolite concentration during fruit development but also affecting general pomological characteristics, a time-course metabolomic and proteomic analysis of kiwifruit flesh tissues was undertaken. Combined information on modified levels of 46 metabolites and 241 proteins showed that molecular processes underlying central and secondary metabolism, energy, cell structure, protein destination/storage, disease/defense kiwifruit functions were highly affected during fruit development, providing a rationale to the corresponding changes in organic acids, sugars, amino acids, polyphenols, fatty acids, phospholipids and allergens content, but also to the corresponding modifications in pome firmness, pulp colour,

Abbreviations: AA, ascorbic acid; C₂H₄, ethylene; FA, formic acid; HMBC, ¹H-¹³C heteronuclear multiple-bond correlation; HSQC, ¹H-¹³C heteronuclear single quantum coherence; ROS, reactive oxygen species; TOCSY, ¹H-¹H total correlation spectroscopy

* Correspondence to: L. Mannina, Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, P.le A. Moro 5, 00185 Rome, Italy.

** Correspondence to: A. Scaloni, Proteomics & Mass Spectrometry Laboratory, ISPAAM, National Research Council, via Argine 1085, 80147 Naples, Italy.

E-mail addresses: luisa.mannina@uniroma1.it (L. Mannina), andrea.scaloni@ispaam.cnr.it (A. Scaloni).

¹ These authors gave an equal contribution to this work.

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protein and total solid content. By providing original information on fruit development, this multiomic study integrates previous metabolomic/transcriptomic observations in describing molecular mechanisms associated with nutritional and agronomic traits of great interest for kiwifruit molecular breeding.

1. Introduction

Hexaploid, green-fleshed kiwifruit (*Actinidia deliciosa* cv. Hayward) is worldwide appreciated for its high content of bioactive metabolites, which also allow fruit to tolerate protracted storage [1]. It is harvested at a physiological mature stage that does not correspond to an optimal edible condition, due to firmness/acidity pome characteristics. For fruit commercialization, a postharvest treatment is generally necessary, which consists of pome exposure to ethylene (C_2H_4) and/or low temperatures for further ripening [2,3]. Depending on treatment, kiwifruit displays a climacteric behaviour at temperatures close to 20 °C (during ripening in storage/conditioning rooms, even following cold storage) and a non-climacteric behaviour at temperatures < 10 °C (during cold storage) [4]. Thus, kiwifruit can be successfully stored at 0 °C for 6 months in the absence of C_2H_4 [5]. Kiwifruit metabolism during development is different from that of fruits from other plants since carbon is mostly accumulated as starch [6]. Starch storage arises only after the fruit has completed cell division, and continues until its complete development [7]. Starch degradation occurs after kiwifruit harvest, leading to polysaccharide conversion into soluble sugars at the edible stage [8].

Since genomic information are still not available on green-fleshed kiwifruit, research activity on this organism has been greatly facilitated after characterizing the draft genome of diploid yellow-fleshed *Actinidia chinensis* [9,10]. This allowed defining a genetic map of *A. deliciosa* chromosomes [11] and assaying ESTs underlying fruit flavor, health, colour and ripening [12,13]. Various proteomic investigations were also accomplished on green-fleshed kiwifruit through the identification of corresponding proteins based on yellow-fleshed fruit homologues. They consisted of differential studies where the treatment of pomes with specific chemical additives or temperatures during postharvest storage was evaluated; in some cases, fruits were analysed after their further ripening at room temperature. Thus, the effect of the treatment with exogenous O_3 [14,15], C_2H_4 [16], sodium nitroprusside [15], of chilling [16] or of combination of them [15,16] was assessed identifying differentially represented proteins in treated kiwifruits with respect to control. The effect of the treatment of kiwi plants with

cytokinins having phytohormonal activity was similarly analysed on developing/harvested fruits eventually stored for different times under specific storage conditions [17]. These investigations reflected the attention of kiwifruit industry in evaluating potential agronomic procedures to increase pome size at harvest and technological approaches for maintaining fruit quality during its postharvest life. However, above-mentioned proteomic studies were performed using silver staining-based 2-DE procedures, which suffer of well-known limitations due to a reduced accuracy in quantitative measurements; the latter were solved with the development of 2D-DIGE [18]. Various studies were also realized on kiwifruit metabolites by NMR and chromatographic approaches with the aim of evaluating corresponding concentrations during fruit development [19,20], after knockdown of enzymes involved in C_2H_4 biosynthesis [13] in the course of comparative assays on different plant genotypes/cultivars [6,20], or in plants treated with cytokinin phytohormones [17]. Similar investigations were also realized on specific parts of fresh kiwifruit [21,22] or on fruit juices obtained with specific food processing procedures [21].

All studies reported above emphasize the lack of proteomic information on metabolic pathways and molecular processes involved in kiwifruit development from early unripe to physiological ripe stage before fruit harvest. In this context, proteomics has largely been used to describe physiological changes occurring during development of many fruits, i.e. apple [23,24], orange [25–27], apricot [28], sweet cherry [29], strawberry [30–31], mango [32–33], date palm fruit [34], grape [35–44], pear [45–46], peach [47,48] and tomato [49,50]. The results of these studies have been summarized in dedicated reviews [51,52], where climacteric and non-climacteric fruits were compared with the aim to tentatively highlight common variably represented metabolic pathways and protein effectors, and their relation with increased fruit respiration rate and C_2H_4 biosynthesis. In the present study, a proteo-metabolomic approach was used to fill this gap by comparing metabolite and protein levels in kiwifruit at four developmental stages. Results were subjected to bioinformatics analysis that suggested metabolic pathways and molecular processes/interactions important for fruit physiological development and nutritional characteristics.

Table 1

Stage parameters, and pomological and qualitative traits of kiwifruits sampled at developmental stages T0, T1, T2 and T3. Reported are data on: percentage of the fruit size with respect to the developed counterpart for commercial picking (% FS); BBCH scale; days post anthesis (DPA); fruit fresh weight (FW); fruit firmness; total soluble solid content (SSC); Chroma; Hue angle; total protein content (TP). ND, not determined. Reported values correspond to the mean $\pm$ SD. Measurements were performed as reported in Supplementary material.

Developmental stage	% FS	BBCH scale	DPA	FW (g)	Firmness (N)	SSC (°Brix)	Chroma	Hue angle	TP (mg/g FW)
 T0	20	72	40	22.7 $\pm$ 1.2	ND	3.8 $\pm$ 0.3	29.5 $\pm$ 1.3	125.1 $\pm$ 5.4	0.40 $\pm$ 0.05
 T1	50	75	72	50.9 $\pm$ 1.7	ND	4.8 $\pm$ 0.5	32.7 $\pm$ 1.8	121.3 $\pm$ 6.2	0.67 $\pm$ 0.09
 T2	80	78	135	80.3 $\pm$ 2.0	> 100	6.1 $\pm$ 0.2	41.6 $\pm$ 1.2	113.2 $\pm$ 3.7	0.63 $\pm$ 0.09
 T3	100	82	175	104.9 $\pm$ 4.0	71.2 $\pm$ 2.1	7.4 $\pm$ 0.3	50.8 $\pm$ 1.1	109.2 $\pm$ 4.6	0.59 $\pm$ 0.08

2. Materials and methods

2.1. Fruit sampling and pomological measurements

Kiwifruit (*Actinidia deliciosa* cv. Hayward) samples were harvested from a commercial orchard located in Francolise (Caserta, Italy). Fruits were randomly sampled from ten selected vines at 4 different development stages (T0–T3) (from fruit set to physiological maturation). Sampling stage parameters, and fruit pomological/compositional characteristics are summarized in Table 1 and Supplementary Fig. S1. Kiwifruit traits were evaluated as described in Supplementary material. Kiwifruits were quickly peeled and their outer pericarp was rapidly cut, frozen in liquid N₂ and stored to –80 °C, until used for further metabolomic and proteomic analyses.

2.2. NMR analysis of metabolites

Metabolites from kiwifruits sampled at T0–T3 were separately extracted according to the Bligh and Dyer's procedure [53], which generates hydroalcoholic and organic extracts (see Supplementary material for details). The latter were dried and stored at –20 °C until further analysis. Hydroalcoholic extracts were solved in 0.7 ml phosphate buffer in D₂O containing 2 mM 3-(trimethylsilyl)-propionic-2,2,3,3-d₄ acid sodium salt (TSP) as internal standard. Lipid counterparts were solved in 0.7 ml of 2:1 v/v CDCl₃/CD₃OD. NMR spectra were recorded at 27 °C on a Bruker AVANCE 600 instrument operating as previously reported [19]. ¹H spectra were referenced to CH₃ signals of TSP (= 0.00 ppm) in D₂O, and to the residual CHD₂ signal of methanol (set to 3.31 ppm) in CD₃OD/CDCl₃. ¹H spectra of aqueous extracts were acquired by coadding 256 transients with a recycle delay of 3 s. The residual HDO signal was suppressed using a standard pre-saturation pulse sequence. Experiments were carried out by using a 45° pulse of 5.0 μs and 32 K data points. ¹H spectra of CD₃OD/CDCl₃ extracts were obtained using 256 transients, 64 K data points, a recycle delay of 3 s and a 90° pulse of 10 μs. The following 2D NMR experiments were performed on organic extracts: i) ¹H–¹H Total Correlation Spectroscopy (TOCSY); ii) ¹H–¹³C Heteronuclear Single Quantum Coherence (HSQC); iii) ¹H–¹³C Heteronuclear Multiple-Bond Correlation (HMBC) [54]. The mixing time for the ¹H–¹H TOCSY was 80 ms. HSQC experiments were performed using a coupling constant ¹J_{C–H} of 150 Hz; ¹H–¹³C HMBC experiments were performed using a delay for the evolution of long-range couplings of 80 ms.

Metabolite concentrations were derived from the integral values of the corresponding resonances in ¹H NMR spectra. For aqueous extracts, the integral values were normalized to the integral of TSP methyl group and the content of selected metabolites was calculated (in mg/g or mg/kg of fresh weight - FW). For organic extracts, spectral integrals were normalized to the integral of α-CH₂ groups of all fatty acid chains that were set to 100%. Then, the total content (molar percentage, % mol) of fatty acids of four different types, *i.e.* saturated, mono-unsaturated, di-unsaturated and poly-unsaturated ones, was calculated. The integrals of selected resonances due to sterols, phospholipids and galactolipids were also normalized to the integral of α-CH₂ groups of all fatty acid chains. NMR data were subjected to PCA, which was performed using Statistica software for Windows (Statsoft, USA).

2.3. LC-UV analysis of metabolites combined with ESI-IT-MSⁿ

Polyphenols from kiwifruits sampled at T0–T3 were extracted as reported in Supplementary material. Samples were resolved by LC-UV on a HP1110 chromatographer (Agilent) equipped with a UV detector. Chromatographic analysis was carried out on a XBridge BEH C18 column (5 mm, 4.6 × 150 mm, 130 Å) (Waters), at a flow rate of 1 ml/min; solvent A and B were 0.1% v/v formic acid (FA) and 0.1% v/v FA in ACN, respectively. After a 2 min hold at 3% solvent B, the latter ramped from 3% to 40% in 45 min, and to 95% in 10 min. Polyphenols

were monitored at 280 nm. For quantitative analysis, a standard curve for each polyphenol derivative was prepared by using standard compounds over a concentration range of 0.25–5 μg/μl, by means of six different concentration levels and duplicate injections at each level. All samples had three replications and each replicate was measured twice. Results were expressed as mg/100 g of FW. Phenolic compounds present in chromatographic fractions were identified by ESI-IT-MSⁿ using a Finnigan LCQ DECA XP Max ion trap mass spectrometer equipped with Xcalibur® manager software (ThermoFisher). Mass spectra were recorded in negative ionization mode in the *m/z* range 80–1800. The capillary voltage was set at –19 V, the spray voltage was at 3 kV and the tube lens offset was at 5 V. The capillary temperature was 275 °C. Data were acquired in MS, MS/MS and MSⁿ scanning mode. Individual and total polyphenol concentration values were subjected to statistical analysis (one-way analysis of variance and multiple mean comparisons Tukey's HSD), which was performed using SYSTAT 13.0 for Windows (Systat Software, Richmond, CA, USA).

2.4. Protein 2D-DIGE analysis

Kiwifruit outer pericarp proteins sampled at T0–T3 were extracted using a modified version of the phenol/SDS-based method [55] (Supplementary material). Dried extracts from 3 biological replicates of each stage were separately solved in 2 M thiourea, 7 M urea and 4% w/v CHAPS and analysed according to 2D-DIGE procedure [18]. Fifty microgram of proteins from each biological sample were labeled with 400 pmol of Cy2-, Cy3- or Cy5-dyes (GE Healthcare) using a dye-swapping strategy to avoid a dye bias [56]. A mixture of all samples was labeled with Cy2 dye and used as the internal standard. IEF was carried out with an IPGphor II apparatus (GE Healthcare) up to 80,000 V/h at 20 °C. After IEF and *in situ* protein modification with iodoacetamide (IAA), IPG strips were transferred onto 12% T polyacrilamide gels to perform the second-dimension SDS-PAGE using an Ettan DALT six electrophoretic device (GE Healthcare). Gels image scanning and analysis were performed as reported in Supplementary material. A parallel preparative 2-DE was performed using 500 μg of unlabeled protein samples. Resulting 2-DE gel was stained with Sypro Ruby (ThermoFisher) according to the manufacturer's instructions. After spot matching with the master gel from the analytical experiment, spots of interest were picked with an Ettan spot robotic picker (GE Healthcare).

2.5. Protein identification by mass spectrometry analysis

Spots from preparative 2-DE were gel excised, reduced with DTT, S-alkylated with IAA, and digested with trypsin [28]. Resulting peptide mixtures were desalted/concentrated with μZip-TipC₁₈ devices (Millipore) and analysed by nLC-ESI-LIT-MS/MS, using an LTQ XL mass spectrometer (ThermoFisher) equipped with a Proxeon nanospray source connected to an Easy-nLC (ThermoFisher) [56]. Peptides were resolved on an Easy C18 column (100 × 0.075 mm, 3 μm) (ThermoFisher). Mobile phases were 0.1% v/v FA (solvent A) and 0.1% v/v FA in ACN (solvent B), running at a total flow rate of 300 nl/min. After a 20 min hold at 5% solvent B, the latter ramped from 5% to 35% in 45 min, to 60% in 10 min, and to 95% over 20 min. Peptide samples were analysed under CID-MS/MS data-dependent product ion scanning procedure as previously reported [56]. For protein identification, raw data from nLC-ESI-LIT-MS/MS analysis were searched using MASCOT software (v. 2.2.06, Matrix Science, UK) against the kiwifruit protein sequence database (39,040 entries) [9]. Database searching was performed using previously reported [49,56] or default MASCOT parameters. Protein candidates assigned on the basis of at least 2 sequenced peptides with an individual peptide ion score > 30 and a peptide expectation value < 0.05 (corresponding to a confidence level > 95%) were considered confidently identified. The false discovery rate for all identifications was calculated performing searches against a decoy database containing reversed protein sequences and resulted < 1%.

Definitive protein assignment was always associated with manual verification. Differentially represented protein (DRPs) were subjected to bioinformatic analysis for sequence similarity and function assignment [57] according to Bevan classification [58] as reported in Supplementary material.

3. Results and discussion

Kiwifruit was studied at principal stages (T0–T3) of its development on plants, which follow the fully opening of the flower, from the fruit set to the mature stage. The latter corresponds to the physiologically ripe fruit, which is harvested for commercial purpose and continues ripening during postharvest pome storage. Developmental stage parameters and fruit pomological/compositional characteristics are summarized in Table 1. An exponential increase of fruit size was observed during the early developmental stages (T0 and T1), which was followed by a slower trend (T2 and T3). At the final developmental stages, kiwifruit seeds hardened and changed colour from white to brown. Flesh firmness gradually decreased during fruit development from a value > 100 N (T0, T1 and T2) to 70 N (T3); dissimilarly, solid soluble content increased moving from T0 to T3 stages. During initial kiwifruit growth (T0 and T1), colour of the outer pericarp was dark green, with a Hue angle value of about 125 and 121, respectively. At T3, colour progressively changed to light green because of initial chlorophyll degradation; this corresponded to a progressive decrease of the corresponding Hue angle value. Chroma values suggested a progressive increase of flesh colour intensity during fruit development. On the other hand, protein content increased moving from T0 to T1 (where it reached an apex) and then progressively decreased (T2 and T3). Outer pericarp of fruits at T0–T3 stages was sampled, extracted for specific compounds and subjected to metabolomic and proteomic analyses. In the whole, measured developmental stage parameters and fruit pomological/compositional characteristics reflected those already described for the yellow-fleshed fruit homologues [3].

3.1. Metabolomics

Aqueous and organic extracts of outer pericarp samples were analysed by NMR experiments. Assignment of ^1H spectra of aqueous extracts has been previously reported [19,20], while that of organic extracts is originally described in this study. The latter was based on literature data [59] and 2D experiments. Technical details and results of both analyses are described in Supplementary material, Table S1 and Fig. S2. Diagnostic ^1H NMR signals identified and quantified 34 metabolites in kiwifruits at T0–T3, including organic acid, sugar, amino acid, fatty acid and lipid derivatives. To study their concentration changes during kiwifruit development (from T0 to T3), quantitative NMR data were submitted to PCA (Fig. 1). The first two principal components explained 78.2% of the total variability of data. PC scores showed that samples were grouped according to the stage of fruit development along PC1 (Fig. 1A), except for T2 and T3 that showed similar PC1 scores. Nevertheless, T2 and T3 were well separated along PC2. PC loadings showed that almost all metabolites were subject to changes during the development (Fig. 1B). Different time trends were observed. Metabolites with high positive PC1 loadings (> 0.83), i.e. Ile, Val, Thr, Ala, Gln, Trp, Phe, γ -aminobutyric acid (GABA), ascorbic acid (AA), shikimic acid (SHA), quinic acid (QA), di-unsaturated fatty acids (DUFA), phosphatidylcholine (PC), uridine (URI), choline (CHN) and β -sitosterol plus campesterol (SC), showed a progressive concentration decrease moving from T0 to T3 (Supplementary Fig. S3 and S4); in particular, Phe and SHA were present in detectable amounts only at T0 and T1. On the contrary, Asp, Glu, citric acid (CA), β -fructopyranose (BFRUPY), sucrose (SUCR), stigmast-7-en-3 β -ol (S7), poly-unsaturated fatty acids (PUFA), mono-unsaturated fatty acids (MUFA) and digalactosyl-diacylglycerol (DG) progressively increased in their amount moving from T0 to T3, and reached the highest levels at T2 and T3 (Supplementary

Fig. S3 and S4). Finally, a specific trend was observed for α - and β -glucose (AGLC, and BGLC) concentration, which decreased moving from T0 to T2, and then increased again at T3.

Identification and quantification of polyphenolic compounds present in kiwifruit outer pericarp at T0–T3 was achieved by LC-UV combined with ESI-IT-MSⁿ (Supplementary material). A LC-UV profile of fruits sampled at T0 is shown in Supplementary Fig. S5. ESI-IT-MSⁿ-based structural characterization of individual chromatographic fractions confirmed the presence of phenolic acids, flavonols, flavan-3-ols and procyanidins, in agreement with previous studies [21,22,60–62]. Diagnostic MS and MSⁿ signals identified 12 polyphenolic metabolites (Supplementary Table S2), among which (*epi*)catechin-gallate-hexose-malonate, 2-caffeoyl-3,4-dihydroxybutanoic acid (or 4-caffeoyl-2,3-dihydroxybutanoic acid) and apigenin-C-deoxyhexoside never detected in kiwifruit so far. Three additional phenolic derivatives having unknown structure were also detected (Supplementary Fig. S5 and Table S2). LC-UV analysis ascertained content of total and individual polyphenols in developing kiwifruit; a general quantitative decrement was observed moving from T0 to T3 (Fig. 2), in agreement with previous studies [63,64]. In particular, total polyphenol content was 81.67 ± 5.42 and

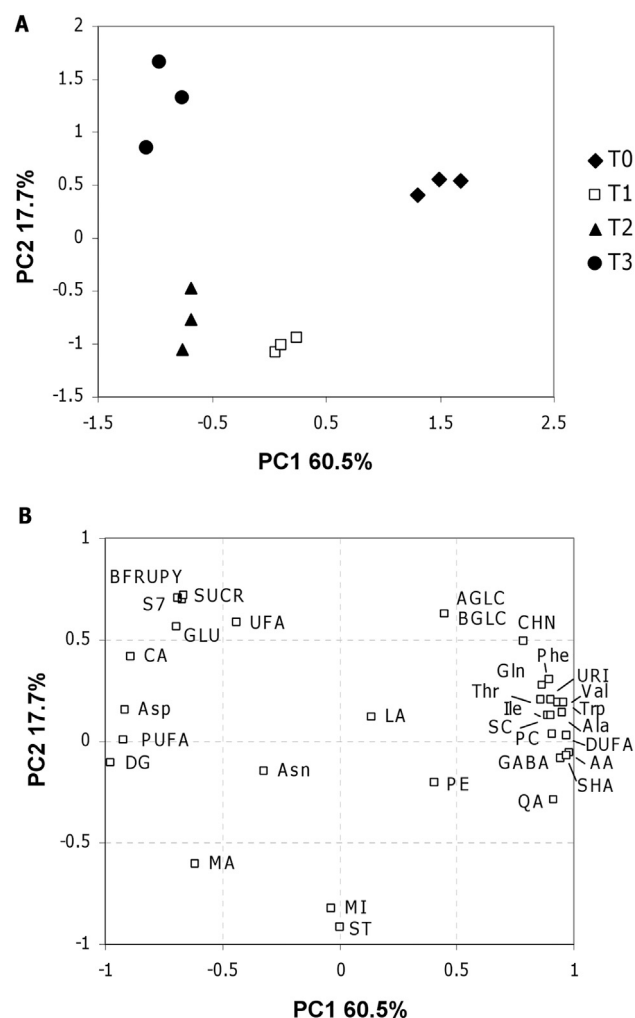


Fig. 1. PCA score (A) and loading (B) plots of metabolite profiles (34 metabolites/metabolite classes) obtained from NMR data. Data related to malic acid (MA), citric acid, ascorbic acid, lactic acid (LA), shikimic acid, quinic acid, α - and β -glucose, sucrose, β -fructopyranose, Ala, Gln, Thr, Glu, Asn, Asp, Val, Ile, Trp, Phe, γ -aminobutyric acid, choline, uridine, myo-inositol (MI), phosphatidylcholine, phosphatidylethanolamine (PE), digalactosyl-diacylglycerol, stigmast-7-en-3 β -ol, stigmasterol (ST), poly-unsaturated fatty acids, di-unsaturated fatty acids, unsaturated fatty acids (UFA), mono-unsaturated fatty acids (MUFA), saturated fatty acids (SFA), and β -sitosterol plus campesterol are shown. Other abbreviations are reported in the manuscript text.

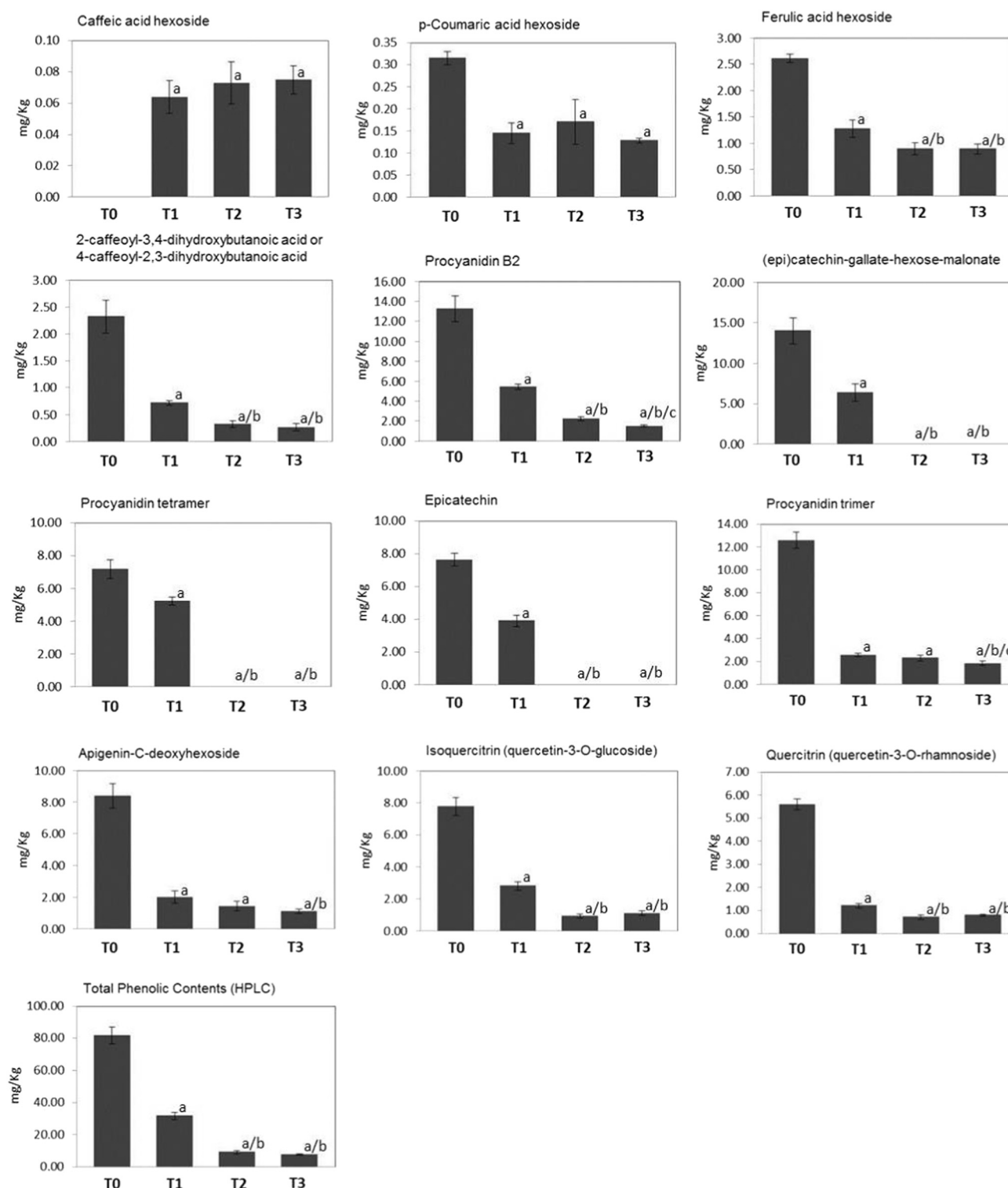


Fig. 2. Concentration (mg/kg FW) of individual and total polyphenols identified in kiwifruit extracts at different developmental stages (T0–T3). Histograms represent the mean $\pm$ SD ($n = 6$); letters on the bars indicate significant differences of polyphenol concentrations within the harvest stage (Tukey's test, $p \leq 0.05$). Results from multiple comparisons over time are indicated with specific letters; a = statistically different compared to T0; b = statistically different compared to T1; c = statistically different compared to T2.

7.71 ± 0.53 mg/kg FW at T0 and T3, respectively. Among the detected classes of polyphenols, flavan-3-ols and procyanidins were the most predominant at all stages, accounting for about 67% of total phenolic content at T0. Nevertheless, the content of these classes of molecules drastically decreased over time (from 54.68 ± 0.51 mg/kg FW at T0 to 3.34 ± 0.10 mg/kg FW at T3). On the other hand, flavonols and apigenin-C-deoxyhexoside were present in a similar amount

at T0 (13.37 ± 0.23 and 8.39 ± 0.77 mg/kg FW, respectively); their content decreased of about 87% at T3. Phenolic acids were found in minor amounts at all sampled stages (Fig. 2). Caffeic acid hexoside was the unique polyphenolic compound showing a positive quantitative trend moving from T0 to T3. Together with those on AA (Fig. 1), these results suggested a general progressive reduction of the level of antioxidant metabolites during kiwifruit development.

In the whole, this study provided original information on quantitative trends of sterol, saturated/unsaturated fatty acid, acylglycerol, phospholipid and polyphenolic derivatives during fruit development, also describing compounds never reported so far. Other metabolites showed quantitative profiles highly similar to those already reported during development of this and other green- [6,17,19] and yellow-fleshed [3,20] kiwifruit varieties. Discrepancies may depend on the specific cultivar assayed, such as in the case of: i) AA that showed decreasing levels in *A. deliciosa* cv. Hayward (this study and [17,19,20]) and *A. chinensis* cvs. Hongyang and Hort 16A [9,20], while it presented an opposite trend in *A. chinensis* cv. Lindl [6]; ii) Glu and GABA, whose quantitative trends reported here were similar to that already published [19,20] and to that of *A. chinensis* cv. Hort 16A [20], but in part differed from that already reported for *A. chinensis* cv. Lindl [6]; iii) organic acids (except CA and malic acid - MA), which showed in this study a general decrease in their concentration, similarly to what reported before [3,6,17,19,20], while in *A. chinensis* cv. Hort 16A they presented a progressive increase [3]. In the latter context, CA and MA trends seem essential in increasing kiwifruit acidity, highly contributing to the osmotic potential important for fruit growth [3,6]. In the whole, changes in the level of metabolites across developmental stages of kiwifruit were in good agreement with what reported before on their specific roles as related to fruit physiology [3,6,9]. Specific metabolites will be discussed below, together with proteins and corresponding metabolic pathways and physiological processes.

3.2. Proteomics

To describe metabolic pathways/genes deregulated during kiwifruit physiological development, outer pericarp extracts from fruits harvested at T0 (used as a reference) and T1–T3 stages were subjected to comparative proteomic analysis by 2D-DIGE (Supplementary Table S3). Corresponding 2D-DIGE maps were characterized for the occurrence of about 4000 protein spots migrating within pI 3–10 and Mr 10–120 kDa ranges, which were then filtered for fold changes > 1.5 or < 1.5 (T1–T3 vs T0) and p value ≤ 0.05 (Student's paired t -test). This revealed 418 differentially represented spots (DRSs) associated with fruit physiological development (Supplementary Fig. S6). Proteomic map at T0 presented almost all spots found as differentially represented at subsequent stages, demonstrating that most metabolic pathways/molecular effectors found as modified at T1–T3 were already active at the beginning of fruit development. Venn diagram gave a representation of unique and common DRSs at T1–T3 stages (Fig. 3). Hierarchical clustering of protein spot abundance ratios evidenced that most relevant proteomic changes in developing kiwifruit occurred at T3 (Supplementary Fig. S7).

After matching analytical and preparative gel images, DRS from the latter gel were subjected to trypsinolysis and MS analysis, which lead to protein identification in 393 cases (Supplementary Table S4). Due to the occurrence of two proteins in specific spots, DRS for final

biochemical interpretation of data were further filtered to specifically exclude: i) spots (14 over 393) that contained components not having coherent quantitative data in multiple 2D-DIGE spots; ii) co-migrating species (67 entries in number) for which a quantitative determination only in a single spot was obtained by 2D-DIGE (Supplementary Table S5). This process reduced proteomic information to 241 non-redundant sequence entries from the kiwifruit genome [9], which were associated with 206 counterparts in TAIR database. Resulting DRPs were then catalogued according to Bevan functional classification [58]. Seven proteins were not classified (3%) and 23 proteins (10%) were even not assigned to any ontology/function by the preliminary Mercator analysis. Based on their nature (and frequency in 393 DRPs), DRPs were associated with the functional categories of disease/defense (28%), protein destination and storage (16%), metabolism (16%), energy (13%), cell structure (12%), highlighting the most important processes affected during kiwifruit development; other functional categories were less represented ($\leq 4\%$) (Fig. 4A). Distribution of functional categories of DRPs at different developmental stages is shown in Fig. 4B and C. Among over-represented proteins, most significant classes were disease/defense (T1, T2 and T3), protein destination and storage (T1 and T3), energy (T3) and metabolism (T3). Down-represented proteins were generally more abundant at T3 than at previous stages, most of them belonging to metabolism, energy, protein destination and storage, cell structure, disease/defense, protein synthesis and secondary metabolism categories. A heat-map and hierarchical clustering representation of abundance ratios of the DRPs within each functional category during different kiwifruit developmental stages is reported in Supplementary Fig. S8. This figure is discussed in detail in subsequent sections dealing with most significant protein functional groups, where individual DRPs and metabolites are also discussed.

3.3. Predictive interaction network of DRPs involved in kiwifruit development

A bioinformatic analysis of protein-protein interaction networks involving DRPs predicted the involvement of 143 sequence entries (Supplementary Table S6) from 11 functional classes, which were organized in a main, highly ramified network, where 5 highly populated subnetworks were also recognized (Fig. 5); other minor networks were also identified. The groups of energy (28), protein destination and storage (27), stress/defense (25), metabolism (21) and cell structure (18) included most members (whose names are reported in parenthesis in subsequent sections). This suggested the strict relation of the different molecular pathways and effectors that underlie kiwifruit development. An analysis of the heat-map representation (Supplementary Fig. S8) of the interacting proteins within the main network showed a general common dynamic trend over time, mainly concentrated at T3. In the whole, results indicated that various defensive/stress-responsive, energetic, metabolic and structural processes represented in the network are synergistically orchestrated on a temporal basis during

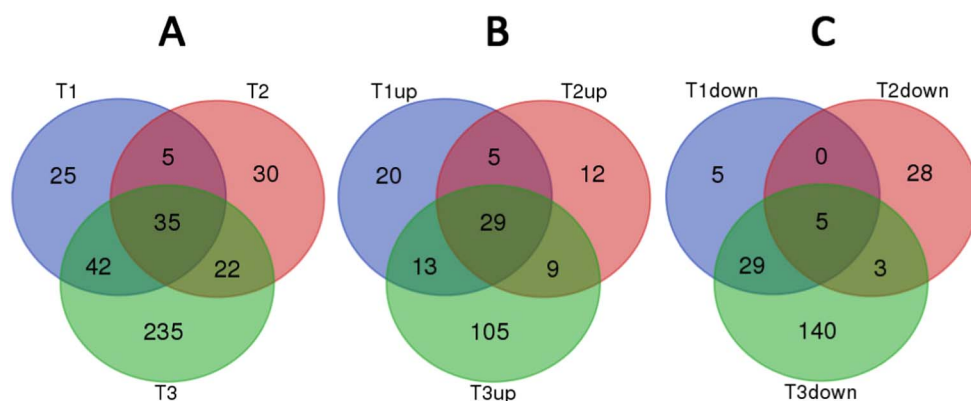
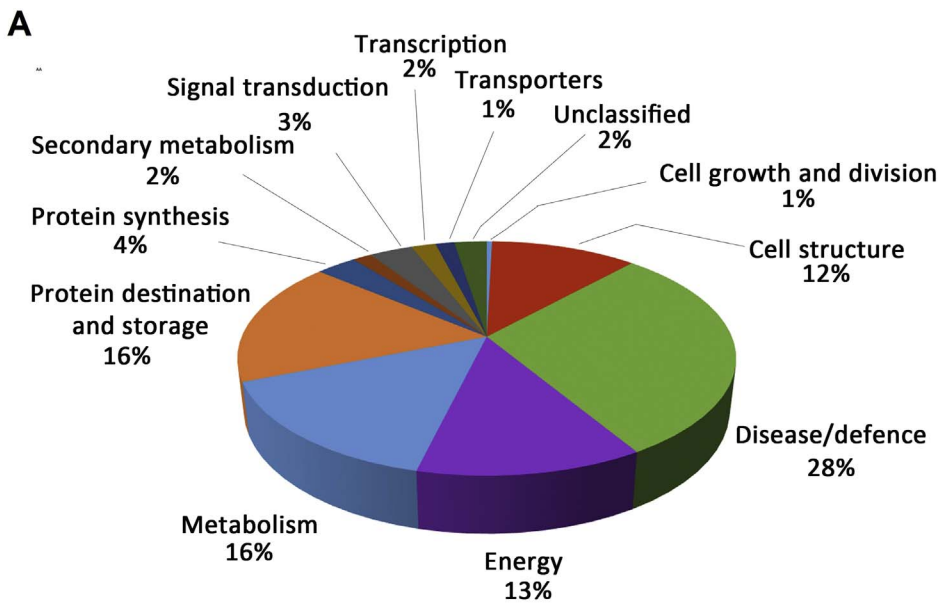
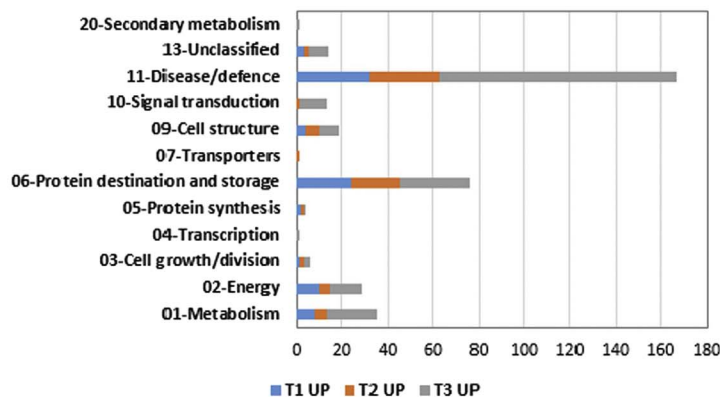


Fig. 3. Venn diagram showing differentially represented spots among the different kiwifruit developmental stages (T1, T2 and T3 in comparison with T0), which gave positive identification results. Diagrams refer to all species (A), those over- (B) and down-represented (C), respectively.



B

Over-represented proteins during fruit development



C

Down-represented proteins during fruit development

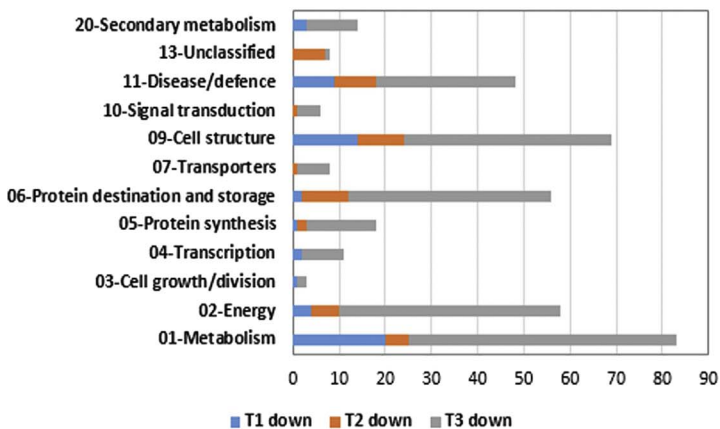


Fig. 4. General information on differentially represented proteins (T1, T2 and T3 vs T0). A) Distribution of DRPs according to Bevan classification [58]. Distribution of functional categories of DRPs during different development stages for up-represented (B) and down-represented (C) proteins.

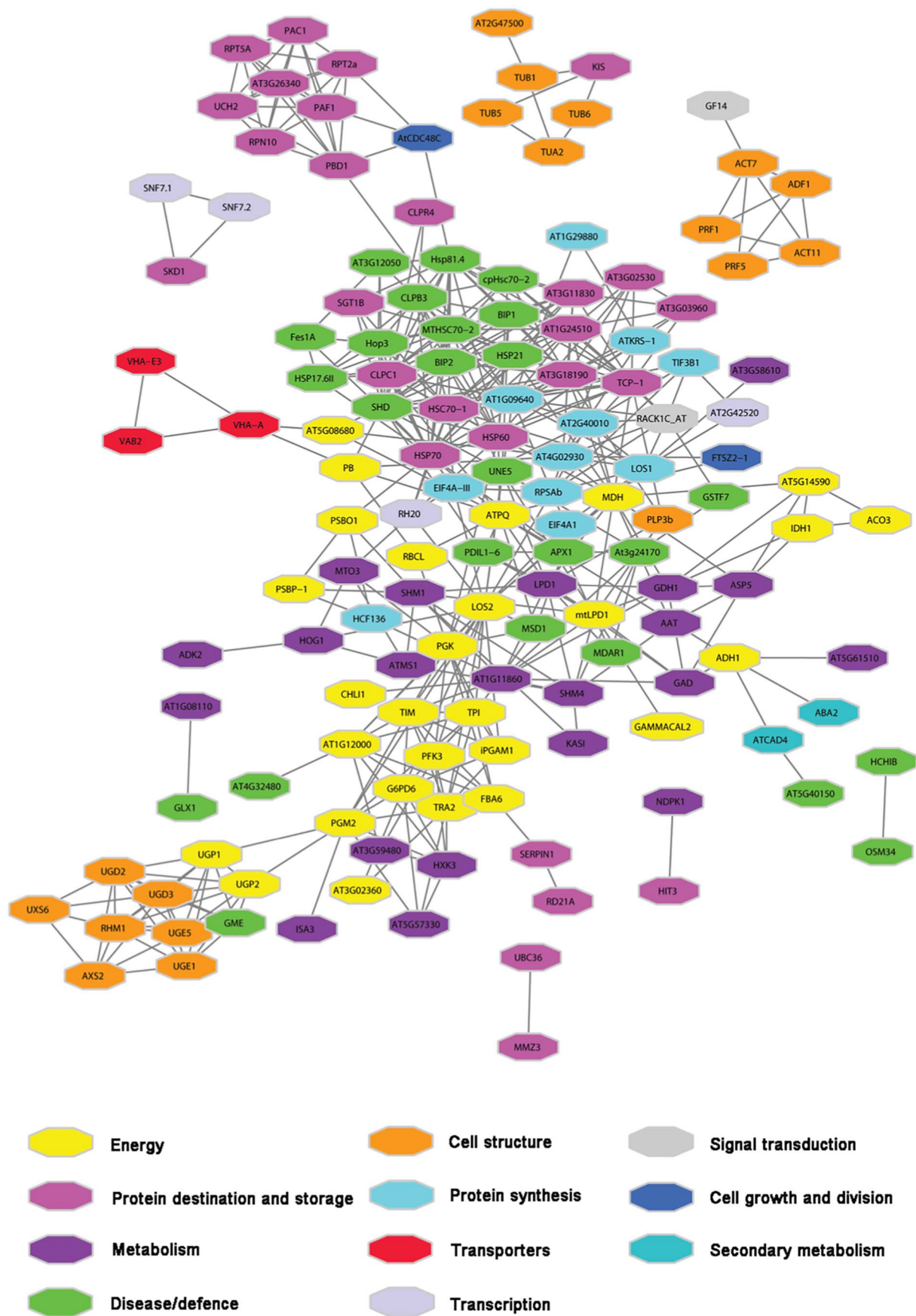


Fig. 5. STRING analysis of differentially represented proteins in kiwifruit at T1, T2 and T3 with respect to T0. Only-high confidence interactions (0.7) are evidenced. Abbreviations are reported in the manuscript text.

kiwifruit development to elicit important physiological mechanisms.

3.4. Stress/defense proteins

To protect themselves against drought, temperature, salinity, pathogen and insect challenges, plants produce various defensive proteins to face these environmental stresses. Exposure to adverse abiotic/biotic stresses often associates with increased concentration of ROS [65,66]; thus, various antioxidant enzymes are induced in plant tissues to counteract the detrimental activity of these toxic species. Indeed, ascorbate peroxidase (APX1), peroxidase 31 (AT5G40150), Mn-superoxide dismutase (MSD1), glutathione S-transferase (GSTF7), GSH reductase (AT3G24170), lactoylglutathione lyase I (GLX1) and II (AT1G08110), Se-binding protein, quinone oxidoreductases wrbA and AT5G61510, abscisic stress ripening protein and two aldo/keto reductases showed increased levels at the late stages of kiwifruit development (Supplementary Fig. S8A, C and I). Above-mentioned proteins display similar quantitative trends in other developing fruits [28–32,34–36,38,39,43,44,48–50], underlining that redox stress is a physiological part of fruit maturation [67]. Some of these enzymes are O_2^{2-} -scavenging species or are integral parts of the GSH-AA cycle [68], which detoxifies H_2O_2 and hydrogen peroxides using AA as substrate. AA has also been reported to play a vital role in controlling cell division at the early stages of fruit development [69] and to affect Met/ C_2H_4 metabolism (see below) [70], where Se-binding protein also plays a role. Similarly, lactoylglutathione lyases are involved in GSH-based detoxification of methylglyoxal, a cytotoxic product of glycolysis and amino acid metabolism [71]. GSTs are important detoxification enzymes that catalyze the conjugation of redox stress-related xenobiotics or their metabolites to GSH [65], while quinone oxidoreductase wrbA directly participates in the active response toward xenobiotics or oxidative challenge [72]. Abscisic stress ripening protein and aldo/keto reductase have been reported to reduce oxidative stress by effectively acting as a ROS scavenger [73] or by detoxifying compounds generated by the interaction between ROS and membrane fatty acids [31], respectively.

Differently from the proteins reported above but similarly to what observed in other developing fruits [30,31,41,44,47], peroxiredoxin (TPX1), monodehydroascorbate reductase (MDAR1) and GDP-mannose 3,5-epimerase (GME) were down-represented at the late stages of kiwifruit growth, emphasizing the selectivity of the corresponding redox response. Since MDAR1 catalyzes recycling of monodehydroascorbate into AA [68] and GME promotes AA biosynthesis, their decreased levels matched the progressive reduction of AA concentration measured during fruit growth (Supplementary Fig. S3), underlining the importance of high AA levels at the early but not late stages of fruit development [69]. In the whole, these proteomic results suggest that the augmented levels of various antioxidant enzymes in developing kiwifruit are probably the result of specific compensative mechanisms facing the concomitant reduction of AA and polyphenolics (Figs. 1 and 2), in an attempt to counteract corresponding redox challenge.

A number of proteins involved in defense response against pathogens/insects were also observed as over-represented at T2–T3 stages, namely kiwellin, thaumatin-like protein (OSM34), α -toxin (AT4G32480) and chitinase (HCHIB) (Supplementary Fig. S8A and G). After a quantitative decrease at T2, major latex-like protein (Bet VI class) and wound/stress protein (lipoxygenase) also showed augmented levels at T3. Their protein homologues in other fruits were already found over-represented at late developmental stages [28,30,32,35,36,38,39,43,44,50]. Most of these proteins have been classified as pathogenesis-related proteins [74], protecting fruits from microorganisms. For example, chitinases are known to exert their enzymatic activity against the cell wall of pathogens [75]. Kiwellin is a Cys-rich protein having an antimicrobial function [76]; its nicked N-terminal portion (kissper) was shown to elicit a voltage-gated pore-forming activity [77]. Various proteins reported above are among the

most effective allergenic molecules in kiwifruit (Supplementary Table S7) [78]. This suggested that the late stage of fruit physiological development is generally characterized by the highest content of allergens, with important consequences on food nutritional characteristics.

3.5. Protein synthesis, destination and storage

The inventory of stress responsive/defensive proteins showing differential representation in the developing kiwifruit was completed by various HSPs, chaperones and protein disulfide isomerases (PDIs) that, assisting polypeptide folding and correct disulfide pairing, have already been reported to face adverse environmental conditions in plants (Supplementary Fig. S8A, B and D) [79]. Depending on their nature, they showed a different quantitative trend over time. In particular, 17 kDa class II HSPs (HSP17.6II), Hsp70 nucleotide exchange factor fes1 (Fes1A), cytosolic/mitochondrial chaperone DnaK (cpHsc70-2 and MTHSC70-2) and activator of 90 kDa HSP ATPase-like (AT3G12050) were over-represented at T1–T3. Some of these HSPs are highly expressed in response to abiotic/biotic stresses, including the oxidative one [79]; for example, class II HSPs enhance cell survival to redox stress based on their capability to decrease intracellular ROS levels through a GSH-dependent mechanism [80]. Conversely, PDI UNE5 and L-3b, 70 kDa HSP (HSC70-1), 60 kDa chaperonin (HSP60), HSP20-like, 70 kDa HSP (HSP70), HSP 90-2 (HSP81.4), HSP 70 kDa protein C isoforms BIP1 and BIP2, HSP21, chaperones ClpB (CLPB3) and HtpG (SHD), and stress-induced phosphoprotein (Hop3) showed reduced levels at T2–T3. Based on these results, we may speculate that most HSPs, chaperones and PDIs assist protein synthesis and prevent misfolding during the early stages of kiwifruit growth, when novel enzymes are required for metabolism and assembling of structural components in dividing cells. Later during fruit development, augmented levels of these proteins are not necessary, and their abundance is specifically regulated to face stress conditions or decreased. Similar quantitative data for definite proteins were reported in other fruits [29–31,34,35,39,41,43,50]. Above-mentioned HSPs, chaperones and PDIs are highly represented in the main network shown in Fig. 5, where they are linked with components related to protein synthesis and destination/storage.

Considerations reported above were confirmed by the analysis of species directly involved in protein synthesis and transcription (Supplementary Fig. S8B, E and N), which were generally down-represented in kiwifruit at T2–T3. The first class included elongation factors LOS1 (LOS1) and Tu (AT4G02930), initiation factor 4A (EIF4A1), translation initiation factor 3B (TIF3B1), ATP-dependent RNA helicase (EIF4A-III), 60S acidic ribosomal protein P0 (AT2G40010), 40S ribosomal protein (RPSAb), Gly-tRNA ligase (AT1G29880), Lys-tRNA ligase (ATKRS-1), OB-fold nucleic acid binding protein and Ycf48 protein (HCF136). The second class include ATP-dependent RNA helicase DED1, charged multivesicular body proteins (SNF7.1 and SNF7.2), two RNA-binding proteins and staphylococcal nuclease domain-containing protein 1. Quantitative trend of these proteins was similar to that observed in apricot, grape, peach and tomato [28,35,36,41,43,48,50], manifesting an analogous block of protein synthesis and transcription at the late stages of kiwifruit development, in agreement with data on total protein content (Table 1). Above-mentioned components are present in main network shown in Fig. 5, but also in a distinct subnetwork.

A strict relation of the quantitative proteomic changes mentioned above was observed with the representation of components of molecular machineries involved in polypeptide degradation. In this context, it has been reported that the occurrence of a restricted protein synthesis at the late stages of grape development is concomitant with a down-representation of components (i.e. RPT2a) of the ubiquitin/26S proteasome system [43], reducing corresponding degradation efficiency. In this condition, the latter machinery should play a minor role in removing misfolded/damaged proteins or regulatory components that

otherwise are abundant when protein synthesis is augmented (at the early fruit developmental stages). In agreement with this observation, we observed decreased levels of ubiquitin C-terminal hydrolase 2 (UCH2) and 26 proteasome regulatory subunits 6A-like (RPT5A), RPT2a and non-ATPase (RPN10) at T3, to underline the reduced need of the ubiquitin/26S proteasome system when protein synthesis is reduced (Supplementary Fig. S8B). In contrast, an opposite proteomic profile was observed for subunits of the ubiquitin-independent 20S proteasome system, namely subunit α (PAC1) and α -type (PAF1), and β (PDB1) and β -type (AT3G26340), which showed increased levels at the last stages of fruit development. A similar result has been reported in grape [41], where a loss of 26S proteasome function was associated with increased 20S proteasome biogenesis. This proteomic modification should enhance the cellular capacity to degrade oxidized proteins, increasing corresponding oxidative stress tolerance [81]. Accordingly, 26S and 20S proteasome activities seem finely regulated to control fruit development and stress responses. Apart from its function in regulating the turnover of components implicated in ROS production, proteasome components have also been identified to directly contribute to ROS-mediated defense [82]. Above-reported proteasome components occur in a distinct subnetwork in Fig. 5, which is linked to main core through HSPs and chaperones.

A number of proteolytic enzymes with different specificity were also differentially represented during kiwifruit development (Supplementary Fig. S8B), in agreement with previous data on developing date palm fruit, grape, and tomato [34,35,44,50]. Among these, worth mentioning are two members of the papain (C1) family of proteases, *i.e.* cathepsin S and cathepsin B-like (RD21A), also known as actinidin and actinidin Act2a, which showed augmented levels at T1–T3. Their quantitative profile perfectly matched previous studies on the proteolytic activity of cysteine proteases in developing kiwifruit [83]. Actinidins are the most abundant proteins in the ripe kiwifruit and have already been recognized as major allergens therein (Supplementary Table S7) [78]; their role in the physiology of fruit development has not yet been defined in detail.

3.6. Cell structure and cellular wall remodelling

Various cytoskeletal proteins and subunits of machineries involved in supramolecular assembling (T-complex proteins - TCPs) or disassembling (actin depolymerization factors) of cytoskeleton were differentially represented in developing kiwifruit (Supplementary Fig. S8B and F), in agreement with firmness data reported in Table 1. They included actin 1 (ACT7) and 4 (ACT11), tubulin α (TUA2), β (TUB6) and β 2 isoforms (TUB1 and TUB5), profilin 1 (PRF1) and 5 (PRF5), kinesin, and TCP-1 subunit α (TCP-1), β , δ (AT3G18190), ϵ (AT1G24510), η (AT3G11830), θ (AT3G03960) and ζ (AT3G02530), which were down-represented at T2–T3. Conversely, actin depolymerizing factor 1 and 2 (ADF1) and tubulin-specific chaperone (KIS) showed an opposite quantitative trend at these times. Some of these proteins are directly linked to HSPs and chaperones (Fig. 5), or constitute two distinct functional subnetworks. A similar representation of specific proteins was observed during development of other fruits [25,28–31,34,35,39,41,43]. A constant expression of cytoskeletal proteins (actins, tubulins and profilins) and TCP-1 subunits is predictable during the first phases of kiwifruit development (Table 1), in which active cellular division occurs and cells require synthesis of microtubules to drive chromosome segregation during mitosis. When fruit growth is complete (T3), these proteins are not necessary, their biosynthesis is repressed, and their disassembling is driven by the induced expression of corresponding depolymerizing factors.

It is well known that fruit development is accompanied with biogenesis and remodelling of the cellular wall to accompany corresponding division/growth, which is associated with both cell wall loosening and increase in turgor pressure [84]. Accordingly, proteins involved in biosynthesis of nucleotide sugars (as universal sugar

donors) and controlling assembling/disassembling of cell wall polysaccharides/proteoglycans were deregulated in developing fruits [34,35,39,41,50,84]. In agreement with this observation and with firmness data (Table 1), kiwifruit development was associated with a differential representation of bifunctional polymixin resistance arnA protein (UDP-xylose synthase) (AXS2), GDP-mannose 3,5-epimerase (GME), UTP-glucose-1-phosphate uridylyltransferase 1 (UGP1), UTP-glucose-1-phosphate uridylyltransferase 2 (UGP2), UDP-glucose-6-dehydrogenase (UGD2), UDP-glucose-6-dehydrogenase (UGD3), UDP-glucose 4-epimerase (UGE1), UDP-glucose 4-epimerase (UGE5), UDP-glucose 4,6-dehydratase (RHM1) and putative UDP-glucose 4-epimerase (UDP-glucuronate decarboxylase) (UXS6), which initially showed constant levels and were down-represented at T2–T3 (Supplementary Fig. S8A, D and F). A similar quantitative trend over time was observed for enzymes involved in cell wall assembling/disassembling, *i.e.* α -1,4-glucan-protein synthase UDP-forming (UDP-arabinopyranose mutase) and β -xylosidase, indicating that the whole process is temporarily regulated in kiwifruit. This hypothesis found a confirmation in data reported in Fig. 5, where above-mentioned proteins constitute a specific subnetwork also linked to that of energy/metabolism. Since it has been reported that plant cell wall peroxidation (by cross-linking polymers) occurs as mechanism of termination of cellular expansion/development [85], data reported above on the over-representation of peroxidases at T3 further support the role of these enzymes in arresting kiwifruit growth.

3.7. Central carbon and energy metabolism

Previous studies showed that both central carbon and energy metabolism play key roles in developing fruits [51,52]. Metabolic/respiratory pathways of glycolysis, tricarboxylic acid (TCA) cycle and photosynthesis, together with those of anaerobic alcoholic fermentation, provide energy, cofactor regeneration, and building blocks for cell growth, metabolite interconversion and final biosynthesis of starch, sucrose, sugars and secondary metabolites, with compound concentration gradients generally acting as signals for fruit development. Coherently, we verified a significant representation of enzymes involved in starch/sucrose metabolism and glycolysis at the early stages of kiwifruit development, *e.g.* glycogen debranching enzyme (ISA3), UTP-glucose-1-phosphate uridylyltransferase 1 (UGP1), UTP-glucose-1-phosphate uridylyltransferase (UGP2), phosphoglucomutase (PGM2), hexokinase (HXK3), fructokinase 2 (AT3G59480), phosphofructokinase (PFK3), diphosphate/fructose-6-phosphate 1-phosphotransferase (AT1G12000), fructose-bisphosphate aldolase 2 (FBA6), two triose-phosphate isomerases (TIM and TPI), phosphoglycerate kinase (PGK), phosphoglycerate mutase (IPGAM1) and enolase (LOS2), which otherwise showed reduced levels at T3 (Supplementary Fig. S8C and D). Specific enzyme representation trends were in agreement with those on sucrose and fructose concentration in kiwifruit, which remained low in the T0–T2 period and then became elevated at T3 (Supplementary Fig. S3); temporal analogies were also observed for glucose levels, which progressively diminished in the T0–T2 period and then rose quickly at T3. Data reported above confirmed previous observations on enzymatic activity and metabolite content in developing green-fleshed kiwifruit regarding UGP1, UGP2, AT3G59480, HXK3, PGM2, sucrose, glucose and fructose quantitative trends [3,6,86]; these studies also suggested a similar temporal behaviour for another enzyme involved in starch metabolism not detected in this study, *i.e.* sucrose synthase, justifying the beginning of starch accumulation in coincidence with the glucose concentration minimum [7,8]. Temporal data on the concentration of glucose isomers in kiwifruit were also tentatively rationalized based on quantitative trend of aldose 1-epimerase (glucose 1-phosphate epimerase) (AT5G57330). Above-mentioned results were coherent [24,25,30–32,34,41,42,46,47,49,50] or discordant [24,25,39,42] with literature data, depending on the specific protein/plant considered.

A similar representation profile was also observed for proteins

involved in the citric acid cycle (Supplementary Fig. S8C and D), *i.e.* aconitate hydratases 2 (ACO3) and 3, isocitrate dehydrogenases 1 (IDH1) and NADP-dependent (AT5G14590), and dihydrolipoyl dehydrogenases LPD1 and mtLPD1, thus suggesting that the TCA cycle is active during early kiwifruit development but becomes less efficient at the ripe stage, leading to accumulation of citric acid at T3 (Supplementary Fig. S3). Unique temporal exceptions were uncharacterized protein 259I16.6 (succinate dehydrogenase) and malate dehydrogenase (MDH), which showed a mixed quantitative behaviour at T2–T3 or a down-representation at T1–T3, respectively. In agreement with proteomic data was the observed accumulation of malic acid in kiwifruit at T1, which then progressively decreased as result of remaining cycling activity or transformation into acid amino acids (Supplementary Fig. S3) (see below). A down-representation at T3 was also observed for alcoholic fermentation enzyme alcohol dehydrogenase (ADH1) that is involved in the detoxification of formaldehyde resulting from plant metabolism of one-carbon (C1) compounds [87,88]. A similar quantitative behaviour of proteins involved in TCA cycle and fermentation was observed during development of other fruits [24,26,28–32,34,35,39,41–43,46–49]. Most of the proteins reported above constitute a functional subnetwork in the main network shown (Fig. 5), which is highly linked to that of metabolism and cell structure.

Reduction of central carbon metabolism at the late stages of kiwifruit development corresponded to a constant over-representation of enzymes involved in photosynthesis, *i.e.* ribulose biphosphate carboxylase large chain (RBCL), oxygen-evolving enhancer protein 1 (PSBO1) and 2 (PSBP-1), protochlorophyllide reductase, magnesium chelatase subunit I (CHL1) and phosphatidylglycerol/phosphatidylinositol transfer protein (Supplementary Fig. S8D and G), which directly contribute in stabilizing/synthesizing molecules essential to provide the energetic supply and to maintain the fruit endogenous O₂ balance. These data suggest that photosynthesis is one of the earliest activated energetic processes during kiwifruit development, confirming previous observations based on gene expression data [3]. Analogous results were observed in developing strawberry, grape, peach and tomato [30,41,47,49].

Proteomic data also highlighted a variable representation over the time of enzymes involved in: i) energy production, *i.e.* mitochondrial (AT5G08680) and chloroplastic (PB) ATP synthase β and D chain (ATPQ); ii) respiration, *i.e.* carbonic anhydrase CaiE (GAMMACAL2) and electron transfer flavoprotein β chain; iii) pentose-phosphate metabolism, *i.e.* 6-phosphogluconate dehydrogenase (AT3G02360), glucose-6-phosphate 1-dehydrogenase (G6PD6) and transaldolase (TRA2) (Supplementary Fig. S8C and D). Similar quantitative profiles were observed for specific components in other fruits [28,29,31,32,34,35,39,41,47,49,50]. A reduction of central carbon/energetic metabolism at T2–T3, determining ATP reduction, corresponded to a significant down-representation of subunits of organelle transporters of protons, *i.e.* V-type ATP synthase α (VHA-A) and β chain (VBA2) (Supplementary Fig. S8H), as already observed in developing citrus, grape and tomato [25,35,41,50]. Most of these proteins occur in the central functional subnetwork shown in Fig. 5.

3.8. Signal transduction

The observed reduction of enzymes involved in energetic and central carbon metabolic processes at T3 corresponded to a concomitant differential representation of proteins involved in signal transduction (Supplementary Fig. S8D and S8M). In particular, proteins related to Ca²⁺-signaling (calcium-binding protein), guanine nucleotide-binding (G) protein signaling (G protein subunit β -RACK1C_AT) and GTP-binding protein-mediated processes (ADP-ribosylation factor - SAR2, ADP-ribosylation factor-like protein 3 - ARFA1F, and Ras-related proteins Rab-25 and Rab-2A - RAB11c and RAB11C) showed increased levels at that time. In plants, GTP-binding proteins regulate glycolytic/

TCA cycle pathways by a direct interaction with corresponding enzymes, and are also involved in modulating cell growth and sensitivity to hormones [89]. Their over-representation at T3 was suggestive of this regulative function also in kiwifruit.

Conversely, 14-3-3 GF14 and GF14-like proteins and D-*myo*-inositol-3-phosphate synthase showed reduced levels at T3 (Supplementary Fig. S8C and S8M); the latter protein regulates inositol concentration (Supplementary Fig. S3). These three components exert a marked regulatory function on hormone abscisic acid (ABA) signal transduction and expression of ABA-responsive genes [90]. In this context, over-expression of D-*myo*-inositol-3-phosphate synthase has been already reported to activate glycolysis and TCA cycle [91]. The active role of ABA as a ripening control factor also in kiwifruit was confirmed by the coherent quantitative representation of enzymes involved in its metabolism, namely short-chain dehydrogenase/reductase (ABA2) and UDP-glucosyltransferase (Supplementary Fig. S8I), confirming the importance of this phytohormone in fruit development [38,92]. In the whole, ascertained results suggest that two signaling mechanisms simultaneously exert their active control on energetic and central carbon metabolic processes. A similar representation pattern of 14-3-3 and GTP-binding proteins was already described in citrus, sweet cherry, grape, pear and peach [25,29,41–43,47,48].

3.9. Metabolism of amino acids, cofactors and secondary metabolites

Quantitative trends of amino acids differed significantly over the time (Supplementary Fig. S3) and proteomic data were often suggestive of the regulation of the corresponding metabolic pathways (Supplementary Fig. S8C and I). In this context, Val and Ile decreased in developing kiwifruit similarly to the reduced representation of Val/Leu/Ile biosynthetic enzymes ketol-acid reductoisomerase (AT3G58610) and 3-isopropylmalate dehydratase large subunit (ACO3), as already observed in citrus, grape and peach [26,41,48]. Likewise, drastic reduction over time of Phe was rationalized based on quantitative trend of its metabolite precursor shikimic acid and the augmented representation of mono- and di-phenol-degradating polyphenol oxidase at T1–T2, as reported in grape, peach and date palm fruit [34,39,47]. We also found that several enzymes involved in Met metabolism decreased during kiwifruit development, namely 5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase (ATMS1), S-adenosylmethionine synthase (MTO3), adenosylhomocysteinase (HOG1) and adenosine kinase 2 (ADK2). Down-representation of these proteins well paralleled with a concomitant reduction of AA concentration in developing fruit (Supplementary Fig. S3), further supporting the role of this secondary metabolite in controlling Met metabolism, as related to S-adenosylmethionine (SAM) consuming pathways [69,70]. In this context, SAM enters in biosynthetic pathways of C₂H₄, nicotianamine and polyamine, and provides the methyl group for most of methylation reactions required for plant growth/development. To sustain these processes, synthesis, recycling and distribution of SAM must be finely regulated [93]. In the whole, ascertained proteomic results provide a rationale to previous observations on the almost absent production of C₂H₄ in developing kiwifruit [3]; activation of C₂H₄ biosynthesis and ethylene-induced metabolic processes occur later at the fruit postharvest stage [3,14–17]. Temporal induction of enzymes involved in Met metabolism, as related to C₂H₄ production, found a counterpart in apricot, strawberry, mango, date palm fruit, grape, peach and tomato [28,31,32,34,36,39,41,48,50].

On the other side, the tentative interpretation of Asp, Asn, Ala, Glu, Gln and GABA quantitative trends during kiwifruit development (Supplementary Fig. S3) was more challenging, based on: i) the multiple (overlapping) pathways in which these amino acids are implicated; ii) asparagine synthase, L-asparaginase, aspartate aminotransferases (ASP5 and AAT), glutamate dehydrogenase (GDH1) and glutamate decarboxylase (GAD) representation trends (Supplementary Fig. S8C); iii) the role of these enzymes in various metabolic processes,

including GABA shunt, which bypasses two steps of the TCA cycle transforming α -ketoglutarate into succinate. The latter has already been reported to play an essential role in plant growth [94]. Previous studies have described the opposite relation of Glu and GABA levels in plants [95]. A recent investigation on the quantitative levels of above-mentioned amino acids have provided indications on their mutual concentrations in kiwifruit at two developmental stages similar to that reported in this study, and their regulation as result of the TCA cycle and GABA shunt [17]. Quantitative trend of specific enzymes found a counterpart in developing apple, citrus, mango and grape [24,26,32,41,42].

A number of proteins involved in vitamin/cofactor metabolism were also differentially represented in developing kiwifruit (Supplementary Fig. S8C). They included those involved in metabolism of: i) vitamin A (retinol dehydrogenase 14, which showed reduced levels at T1); ii) vitamin B6 (phosphoserine aminotransferase and pyridoxal biosynthesis lyase PdxS, which were down-represented at T3); iii) folic acid (serine hydroxymethyltransferases SHM1 and SHM4, and aminomethyltransferase AT1G11860, which increased or decreased at T3, respectively). Their differential representation may either have a strong impact on kiwifruit metabolism during development, regulating corresponding metabolic fluxes, but also on final vitamin content in the physiologically ripe product, with important nutritional consequences.

Regarding proteins involved in metabolism of kiwifruit secondary metabolites (Supplementary Fig. S8I), measured proteomic levels in some cases provided a rationale to corresponding compound concentrations (Figs. 2 and 3). In particular, augmented representation of polyphenol oxidase at T1–T2 was associated with the general trend of disappearance observed for polyphenolic compounds (Fig. 3). This was coherent with analogous observations made in apricot, strawberry, grape and peach [28,30,35,39,41,47]. On the other hand, the reduced representation at T3 of cinnamyl alcohol dehydrogenase 4 (ATCAD4), which catalyzes NADPH-dependent reduction of caffeoyl aldehyde to its respective alcohol within the phenylpropanoid biosynthetic pathway, was hypothetically related to the measured accumulation of caffeic acid hexoside during kiwifruit development (Fig. 3). Its abundance in developing kiwifruit was similar to that observed in pear [45].

4. Conclusion

In this study, combined NMR/LC-UV/ESI-IT-MSⁿ and 2D-DIGE/nLC-ESI-LIT-MS/MS procedures identified quantitative changes of 46 metabolites and 241 non-redundant proteins at various kiwifruit developmental stages. Protein species related to disease/defense, protein destination and storage, metabolism, energy and cell structure functional categories were highly affected in their representation at specific moments of fruit development. More than a half of these components were predicted to be a part of a functional interaction network, which seems to orchestrate protein representation on a temporal basis to elicit essential reactions underlying fruit growth and development until to a physiologically mature condition. Protein representation trends suggested a rationale to some pomological characteristic and metabolite concentration changes, integrating previous observations on specific metabolites, transcripts and enzymatic activities in *A. deliciosa* and *A. chinensis* cultivars [1–13,17,19–22]. By providing an original proteo-metabolomic description of kiwifruit physiological development, this investigation offers a novel reference framework for further physiological studies on this economically relevant fruit crop, and will permit to add novel insights on the comparative evaluation of the metabolic pathways and molecular effectors contributing to the fruit developmental process in important plant crop species [51,52].

Transparency document

The Transparency document associated with this article can be found, in online version.

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Conflict of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jprot.2017.11.004>.

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A PROTEOMETABOLOMIC STUDY OF *ACTINIDIA DELICIOSA* FRUIT DEVELOPMENT

Anna Maria Salzano ¹, Anatoly Sobolev ², Virginia Carbone ³, Milena Petriccione ⁴,
Giovanni Renzone ¹, Donatella Capitani ², Monica Vitale ^{1,5}, Paola Minasi ³, Maria Silvia Pasquariello ⁴,
Gianfranco Novi ¹, Nicola Zambrano ^{5,6}, Marco Scortichini ⁴, Luisa Mannina ^{2,7} and Andrea Scaloni ¹

¹ Proteomics & Mass Spectrometry Laboratory, ISPAAM, National Research Council, 80147 Naples, Italy

² Magnetic Resonance Laboratory “Annalaura Segre”, Institute of Chemical Methodologies, National Research Council, 00015 Monterotondo (RM), Italy

³ Institute of Food Sciences, National Research Council, 83100 Avellino, Italy

⁴ Centro di Ricerca per Olivicoltura, Frutticoltura e Agrumicoltura, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria, 81100 Caserta, Italy

⁵ Dipartimento di Medicina Molecolare e Biotecnologie Mediche, Università degli Studi di Napoli “Federico II”, 80131 Naples, Italy

⁶ CEINGE Biotecnologie Avanzate, 80145 Naples, Italy

⁷ Dipartimento di Chimica e Tecnologie del Farmaco, Sapienza Università di Roma, 00185 Rome, Italy

SUPPLEMENTARY MATERIAL

22 **Materials and Methods**

23 **Chemicals**

24 Reported are chemicals not described in other manuscript sections. HPLC-grade solvents were
25 from Merck (Darmstadt, Germany) or Carlo Erba (Rodano, Milan, Italy). Gallic acid, caffeic acid,
26 ferulic acid, cumaric acid and quercitrin (quercetin-3-O-rhamnoside) were purchased from Sigma
27 Chemical Company (St. Louis, USA). Procyanidin B2 and [+] catechin were obtained from
28 Extrasynthese (Genay, France). Isoquercitrin (quercetin-3-O-glucoside) and [-]-epicatechin were
29 obtained from Fluka (Buchs SG, Switzerland). HPLC grade water (18.2 MΩ) was prepared with
30 a Millipore Milli-Q purification system (Millipore Corp., Bedford, MA, USA).

31

32 **Kiwifruit sampling and analysis of corresponding pomological and qualitative traits**

33 In 2014, kiwifruit (*Actinidia deliciosa* cv. Hayward) samples were harvested from a commercial
34 orchard located in Francolise (Caserta, Italy) where, depending on climatic conditions, the flowering
35 period extends over several weeks from May to June. Fruits were randomly sampled from ten selected
36 vines at four different development stages (T0-T3) (Tab. 1 and Supplementary Fig. S1). Based on
37 BBCH scale [1], stages corresponding to 72 (20% of fruit final size - T0), 75 (50% of fruit final size
38 - T1), 78 (80% of fruit final size - T2) and 82 (100% of fruit final size for commercial harvesting -
39 T3) values were chosen for this study. For each stage, days post anthesis (DPA) were registered.
40 Kiwifruits were screened for uniformity, appearance and absence of physical defects or decay. For
41 each stage, about 60 selected fruits were harvested, which were divided in 3 biological replicates.
42 Whenever necessary, fruits were peeled and only their outer pericarp (without inner pericarp
43 containing locules and seeds) was sampled; it was frozen in liquid N₂ and stored at -80 °C, until used
44 for further metabolomic and proteomic analyses.

45 Fruit weight was measured with an electronic balance having a 0.01 g-sensitivity. Flesh firmness
46 was measured at two opposite sides of fruits using a TR digital penetrometer (Turoni, Forlì, Italy)
47 bearing a 8 mm-diameter probe; data are expressed in Newton (N). Outer pericarp colour was assessed

using a CR5M colorimeter (Minolta Camera, Japan); recorded data allowed determining chromaticity L* (lightness), a* (green to red) and b* (blue to yellow) values at the opposite side of thirty kiwifruits for each stage. Corresponding Hue angle and Chroma values were calculated from chromaticity a* and b* data, as previously reported [2]. Measurements were performed on 3 biological fruit replicates, each one consisting of 20 kiwifruits. Total soluble solid content (SSC, °Brix) was determined from flesh juice of these kiwifruits using a DBR35 digital refractometer (Sinergica Soluzioni, Pescara, Italy). Total proteins were extracted as specified in the proteomic section (see below); protein content in fruits was measured with the Bio-Rad (Heracles, CA, USA) protein assay kit, based on the Bradford quantification method [3], using bovine serum albumin as standard. In the latter cases, measurements were carried out in triplicate on 3 biological replicates, each one consisting of 10 kiwifruits.

Extraction of kiwifruit metabolites for NMR analysis

Independent kiwifruit outer pericarp samples (about 2 g) taken at four developmental stages (T0-T3) were separately placed in liquid N₂ and finely powdered in a mortar with a pestle. They were treated in parallel with 3 ml of 2:1 v/v methanol/chloroform, at 4 °C [4]. Samples were transferred in separate glass vials, where 1 ml of chloroform and 1.2 ml of distilled water were added to form an emulsion. Samples were vortexed and then kept at 4 °C for 40 min. Resulting homogenates were then centrifuged at 10,000 rpm for 20 min, at 4 °C. The upper (hydroalcoholic) and lower (organic) phases were carefully separated. Remaining pellets were re-extracted using half of the solvent volumes, under the same conditions described above; separated fractions were pooled and added to the counterparts from the first extraction. Hydroalcoholic and organic extracts from each kiwifruit sample were dried under a N₂ flow, at room temperature. Dried residues were stored at –20 °C, until subjected to NMR analysis; the latter was realized as described in the main text.

74 **Assignment of kiwifruit metabolites by NMR**

75 Hydroalcoholic and organic extracts of kiwifruits at T0 to T3 stages were analyzed by NMR
76 experiments. Assignment of ^1H spectra of aqueous extracts was realized as previously reported [5,6].
77 Diagnostic ^1H -NMR signals identified and quantified various metabolites in aqueous extracts, namely
78 malic acid (MA), citric acid (CA), ascorbic acid (AA), lactic acid (LA), shikimic acid (SHA), sugars
79 α - and β -glucose (AGLC, and BGLC), sucrose (SUCR), β -fructopyranose (BFRUPY), amino acids
80 Ala, Gln, Thr, Glu, Asn, Asp, Val, Ile, Trp, Phe and GABA, and other compounds such as choline
81 (CHN), uridine (URI) and myo-inositol (MI). Assignment of ^1H spectra of kiwifruit organic extracts
82 (Supplementary Fig. S2) was based on literature data [7] and 2D experiments (Supplementary Tab.
83 S1). Thus, poly-unsaturated fatty acids (PUFA), di-unsaturated fatty acids (DUFA), unsaturated fatty
84 acids (UFA), mono-unsaturated fatty acids (MUFA) and saturated fatty acids (SFA), together with
85 phosphatidylcholine (PC) and phosphatidylethanolamine (PE), digalactosuldiacylglycerol (DG),
86 were identified and quantified by means of their characteristic signals. Previous studies showed that
87 the most abundant sterols in the pulp of mature kiwifruit are β -sitosterol (ST), stigmast-7-en-3 β -ol
88 (S7), campesterol and stigmasterol [7]. ^1H -NMR spectra confirmed the presence of these sterols in
89 organic extracts, due to their characteristic 18-CH₃ signals observed in the peculiar high field spectral
90 region (from 0.55 to 0.8 ppm) (see Supplementary Fig. S2 and Tab. S1 for assignment).
91 Unfortunately, the ^1H -NMR signals from 18-CH₃ group of β -sitosterol and campesterol were
92 completely overlapped, and all the other sterol signals were overlapped with fatty acid chain signals;
93 thus, only the sum of β -sitosterol and campesterol (SC) was quantified. Quantification of all
94 metabolites at T0-T3 stages is shown in Supplementary Fig. S3 and S4.

95

96 **Extraction of kiwifruit polyphenolic metabolites for LC-UV combined with ESI-IT-MSⁿ** 97 **analysis**

98 Independent kiwifruit outer pericarp samples (about 2.5 g) taken at different developmental stages
99 (T0-T3) were finely chopped and treated with 6 ml of 1% v/v acetic acid (AcOH) in acetone to extract

phenolic compounds. Extraction was carried out on each sample in an ultrasonic bath for 15 min, followed by an additional step in a horizontal shaker for 20 min, in the dark. After centrifugation at 4,000 rpm for 10 min, supernatants were removed and pellets were suspended in 6 ml of 1% v/v AcOH in acetone. Extraction was repeated twice. For each sample, supernatants were pooled and dried in a LaboRota 4000/HB Efficient rotary evaporator (Heidolph, Schwabach, Germany). Sample extracts were separately reconstituted in 3% FA and then passed through a C18 Sep-Pak cartridge (Waters, Milford, MA), which was previously activated according to manufacturer instructions. After removal of sugars and other water-soluble compounds with 10 ml of 3% v/v FA, phenolics were eluted from the cartridge with 3 ml of methanol followed by additional 3 ml of 3% v/v FA in methanol. For each sample, the methanolic fractions were pooled, dried under an N₂ flow at room temperature, and reconstituted in 3% v/v FA for further analyses.

111

112 **Assignment of kiwifruit polyphenolic metabolites by LC-UV combined with ESI-IT-MSⁿ**

113 **analysis**

Identification of polyphenols present in kiwifruit outer pericarp at T0, T1, T2 and T3 was achieved through the analysis and characterization of kiwifruit pulp extracts by LC-UV and ESI-IT-MSⁿ. The LC-UV chromatogram of kiwifruit pulp extract at T0 is shown in Supplementary Fig. S5. ESI-IT-MSⁿ structural characterization of individual phenolics in the different extracts confirmed the presence of phenolic acids, flavonols, flavan-3-ols and procyanidins, in agreement with previous studies on *Actinidia deliciosa* [8-13]. Phenolic compounds identified in kiwifruit pulp extracts are reported in Supplementary Tab. S2. Identification was based on pseudomolecular [M-H]⁻ ions, together with the interpretation of their collision induced dissociation (CID) fragments.

For instance, analysis of peak 6 (Supplementary Fig. S5) showed a pseudomolecular ion [M-H]⁻ at *m/z* 689 and the following fragmentation pattern: *m/z* 537, corresponding to the loss of a galloyl unit [M-H-152]⁻; *m/z* 451, due to the subsequent release of a malonyl moiety [M-H-152-86]⁻; *m/z* 289, corresponding to the deprotonated ion of catechin (or epicatechin) after the successive loss of a hexose

moiety [M-H-152-86-162]⁻. Therefore, the compound eluted in peak 6 was tentatively identified as (epi)catechin-gallate-hexose-malonate. It was present only in fruit extracts collected at T0 and T1. Analysis of peak 4 showed a [M-H]⁻ at *m/z* 297 in the mass spectrum and fragment ions at *m/z* 179 and 135 in the MS² spectrum, suggesting the presence of a caffeoyl moiety. In the MS³ experiment on the product ion at *m/z* 135, ions at *m/z* 117 and 89 were observed, which were tentatively assigned to the consecutive loss of water and carbon monoxide from the parent ion. By comparing these data with those previously reported in the literature [14,15], the compound eluted in peak 4 was tentatively identified as 2-caffeoyl-3,4-dihydroxybutanoic acid or 4-caffeoyl-2,3-dihydroxybutanoic acid.

As regard peak 10, ESI-IT-MSⁿ analysis revealed the presence of an ion at *m/z* 415 in the mass spectrum and fragment ions at *m/z* 397 [M-H-H₂O]⁻, 341 [M-H-74]⁻, and 311 [M-H-104]⁻ in the MS² spectrum, in accordance with the main fragmentation pathway of C-glucosyl flavonoids and, in particular, of C-deoxyhexosides [16]. Based on the obtained mass spectrum and data reported in literature [17], peak 10 was tentatively identified as apigenin-C-deoxyhexoside.

The mass spectra of peaks A, B and C (Supplementary Fig. S5) displayed *m/z* values of 1039, 401 and 977 respectively. MSⁿ analyses of these compounds did not lead to a definitive identification and additional experiments are still in progress.

Protein extraction and 2D-DIGE analysis

Kiwifruit outer pericarp proteins were extracted using a modified version of the phenol/SDS-based method [18]; 3 biological replicates for each developmental stage (T0-T3) were analysed. Kiwifruit outer pericarp samples (about 5 g) were chopped and thoroughly ground in a pre-cooled mortar containing liquid N₂ to obtain a very fine powder. Tissue powder (3 g) was suspended in 12 ml of ice-cold 500 mM EDTA, 100 mM Tris-HCl pH 8.0, 30% w/v sucrose, 1% w/v polyvinylpolypyrrolidone, 100 mM KCl, 2% w/v SDS, 1 mM phenylmethanesulfonyl fluoride, 5% v/v β-mercaptoethanol, and vortexed for 30 s. Each sample was then added with 2 ml of ice-cold Tris-HCl buffered phenol, pH 8.0, and vortexed thoroughly for 30 s; samples were then centrifuged at

152 17,000 x *g* for 5 min, at 4 °C. After centrifugation, the upper phenolic phase was collected and
153 precipitated overnight with 5 vol of cold 100 mM ammonium acetate in methanol, at -20 °C. The
154 precipitated proteins were recovered by centrifugation at 17,000 x *g* for 10 min, at 4 °C. After
155 supernatant removal, the pellet was rinsed twice with ice-cold 80% v/v acetone. The pellet was air-
156 dried and resuspended in 2 M thiourea, 7 M urea and 4% w/v CHAPS. Protein concentration was
157 quantified with the Bio-Rad protein assay kit, based on the Bradford quantification method [3], using
158 bovine serum albumin as standard.

159 Fifty µg of proteins from each biological sample were labeled with 400 pmol of Cy2-, Cy3- or
160 Cy5-dyes (GE Healthcare, UK), using a dye-swapping strategy to avoid a dye bias (Supplementary
161 Tab. S3). A mixture of all biological samples (12 in number) was labeled with Cy2 dye and used as
162 internal standard, as required in 2D-DIGE protocol [19]. Each labeling reaction was performed at 0
163 °C for 30 min, in the dark, and quenched with 1 mM lysine. Appropriate Cy3- and Cy5-labeled pairs
164 and a Cy2-labeled control were used to generate mixtures. Each mixture was supplemented with 1%
165 v/v IPG buffer (an optimized mixture of carrier ampholytes), pH 3-10 NL (GE Healthcare), 1.4% v/v
166 DeStreak reagent (GE Healthcare) and 0.2% w/v DTT to reach a final volume of 450 µl in 7 M urea,
167 2 M thiourea, and 4% w/v CHAPS. The mixtures (150 µg of total protein content) were used for
168 passive hydration of immobilized pH gradient IPG gel strips (24 cm, pH 3-10 NL), in the dark, for
169 16 h, at 20 °C. Isoelectric focusing (IEF) was carried out with an IPGphor II apparatus (GE
170 Healthcare) up to 80,000 V/h at 20 °C. After IEF, each strip was equilibrated with an equilibration
171 solution composed of 6 M urea, 2% w/v SDS, 20% w/v glycerol, and 0.375 M Tris-HCl (pH 8.8), in
172 the presence of 0.5% w/v DTT, for 15 min, in the dark; then, it was equilibrated in the same buffer
173 containing 4.5% w/v iodacetamide, for another 15 min. Equilibrated IPG strips were transferred onto
174 12% polyacrilamide gels to perform the second-dimension SDS-PAGE, using an ETTAN DALT six
175 electrophoresis system (GE Healthcare).

176 Resulting gels were scanned with a Typhoon 9400 variable mode imager (GE Healthcare) using
177 proper excitation/emission wavelengths for Cy2 (488/520 nm), Cy3 (532/580 nm), and Cy5 (633/670

178 nm). Gel images were cropped and visualized within the Image-Quant software (GE Healthcare) and
179 analyzed using the DeCyder 5.0 software (GE Healthcare). A DeCyder differential In-gel-Analysis
180 (DIA) module was used for spot detection, background subtraction and pairwise comparison of each
181 sample (Cy3 and Cy5) to the Cy2 mixed standard present in each gel, in order to perform Cy5/Cy2
182 and Cy3/Cy2 normalizations. Then, the DeCyder Biological Variation Analysis (BVA) module was
183 used to simultaneously match all of the protein-spot maps from the gels, and to calculate average
184 abundance ratios and statistical parameters (Student's T-test). Differentially represented spots were
185 identified as those having a relative expression ratio >1.50 or <1.50 , with a p value ≤ 0.05 .

186 Preparative 2-DE was performed using 500 μ g of unlabeled protein samples. Resulting 2-DE gel
187 was stained with Sypro Ruby, according to the manufacturer's instructions (ThermoFisher). After
188 spot matching with the master gel from the analytical assay in the BVA module of DeCyder software,
189 a pick list was generated for spot picking by an Ettan spot robotic picker (GE Healthcare). Picked gel
190 pieces were alkylated with iodoacetamide, digested with trypsin, desalted and analyzed by nLC-ESI-
191 LIT-MS/MS for protein identification.

192

193 **Bioinformatics**

194 Identified proteins were subjected to BLAST sequence homology search against the *Arabidopsis*
195 *thaliana* protein sequence database TAIR 10 from The Arabidopsis Information Resource repository.
196 Functional categorization of differentially represented protein (DRPs) was obtained using Mercator
197 pipeline (<http://mapman.gabipd.org/web/guest/app/mercator>) for automated sequence annotation,
198 selecting TAIR 10, SwissProt-UniProt plant proteins, KOG clusters and InterPro scan, with a cut-off
199 value of 80. DRPs were assigned to Bevan functional classes [20] using classification from Mercator.
200 Hierarchical cluster analysis of DRPs in T0-T3 stages was performed using Genesis 1.7.7 platform
201 (Institute for Genomics and Bioinformatics, Graz University). Pearson correlation as distance and
202 average linkage clustering were chosen as parameters. Protein interaction networks were obtained

203 with STRING (<http://string-db.org>) using the *A. thaliana* database; they were visualized with
204 Cytoscape software (<http://www.cytoscape.org>).
205
206

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Supplementary Tab. S1. Assignment of ^1H - and ^{13}C -NMR signals from metabolites in the organic fraction from kiwifruit outer pericarp.

Kiwi organic extract			
Assignment	^1H , (ppm)	^{13}C , (ppm)	
PUFA chains			
COOH			174.31
CH ₂ -2	2.342		34.44
CH ₂ -3	1.622		25.16
CH ₂ -4-7	1.322		
CH ₂ -8	2.062		27.49
CH-9	5.348		130.32
CH-10	5.358		128.52
CH ₂ -11,14	2.814	t(6.1)	25.89
CH-12,13	5.360		128.52
CH-15	5.310		127.44
CH-16	5.389		132.18
CH ₂ -17	2.086		20.79
CH ₃ -18	0.982	t(7.6)	14.38
Free fatty acids			
CH ₂ -2	2.290		35.05
CH ₂ -3	1.620		25.15
CH ₂	1.321		29.59
DUFA chains			
CH ₂ -8,14	2.065		27.49
CH-9,13	5.358		130.32
CH-10,12	5.358		128.52
CH ₂ -11	2.812	t(6.49)	25.90
Phosphatidylcholine			
(CH ₃) ₃ N	3.269		54.37
CH ₂ OP	4.448		60.84
CH ₂ N	3.770		66.15
CH ₂ sn3	4.162		65.51
	4.380		
CH ₂ sn1	4.183		62.34
CH sn2	5.261		70.61

Phosphatidylethanolamine		
CH ₂ OP	4.238	63.4
CH ₂ N	3.230	40.3
Triacylglycerols		
CH ₂ sn1,3	4.330, 4.163	62.36
CH sn2	5.274	70.03
Digalactosyldiacylglycerol		
CH-1'	4.238	104.33
CH-2'	3.541	71.56
CH-3'	3.505	73.59
CH-4'	3.919	68.47
CH-1"	4.911	d(3.78) 99.66
CH-3",5"	3.735	70.66
CH-4"	3.965	70.12
	3.807	
CH ₂ -6"	3.826	62.12
β-Sitosterol		
	1.085	37.64
CH ₂ -1	1.856	
	1.498	31.44
CH ₂ -2	1.823	
CH-3	3.467	71.62
CH ₂ -4	2.251	42.15
CH-6	5.364	121.85
	1.524	32.25
CH ₂ -7	2.000	
CH-8	1.495	32.8
CH-9	0.942	50.61
CH ₂ -11	1.510	21.42
	1.185	40.16
CH ₂ -12	2.023	
CH-14	1.026	57.17
	1.084	24.61
CH ₂ -15	1.589	

CH ₂ -16	1.304		28.56
	1.852		
CH-17	1.129		56.40
CH ₃ -18	0.699		12.03
CH ₃ -19	1.020		19.51
CH-20	1.374		36.53
CH ₃ -21	0.936		19.06
	1.028		34.30
CH ₂ -22	1.360		
CH ₂ -23	1.184		26.41
CH-24	0.942		46.26
CH-25	1.677		29.53
CH ₃ -26	0.840		19.93
CH ₃ -27	0.829		19.24
CH ₂ -28	1.301		22.98
CH ₃ -29	0.859		12.16
Campesterol			
CH ₃ -18	0.698	s	12.03
Stigmasterol			
CH ₃ -18	0.720	s	12.27
	5.167		
CH-22,23	5.020	dd(15.1, 8.8)	
CH-20,24	2.056; 1.539		
CH ₃ -21, CH ₂ -28	0.805; 1.174		
CH-25	1.030		
CH(OH)-3	3.467		
CH ₂ -4	2.253		
CH ₂ -2,1	1.827; 1.498; 1.082		
Stigmast-7-en-3β-ol			
CH ₃ -18	0.553		12.03
	1.244		39.71
CH ₂ -12	2.038		
CH-14	1.829		55.28
CH-17	1.241		56.38

Supplementary Tab. S2. Polyphenolic compounds identified in outer pericarp extracts from kiwifruit at T0-T3. Specific quasi-molecular ions and fragment ions are reported for each compound. Identified metabolites are numbered; metabolites with an unknown structure are indicated with capital letters. Metabolite labeling corresponds to that shown in Supplementary Fig. S5. RT, retention time.

^a Tentatively identified compounds.

Peak	RT	[M-H] ⁻ (m/z)	MS ⁿ ions (m/z)	Identification
1	8.26	341	MS ² [341]: 179	Caffeic acid hexoside
2	10.58	325	MS ² [325]: 163	<i>p</i> -Coumaric acid hexoside
3	12.57	355	MS ² [355]: 193	Ferulic acid hexoside
4	12.71	297	MS ² [297]: 179, 135 MS ³ [297→135]: 117, 89	2-Caffeoyl-3,4-dihydroxybutanoic acid or 4-Caffeoyl-2,3-dihydroxybutanoic acid ^a
5	15.74	577	MS ² [577]: 559, 451, 425, 407, 289 MS ³ [577→425]: 407	Procyanidin B2
6	16.15	689	MS ² [689]: 537, 451, 289 MS ² [1153]: 865, 577, 451, 407	(Epi)catechin-gallate-hexose-malonate ^a
7	16.51	1153	MS ³ [1153→865]: 575, 451, 425, 407, 289	Procyanidin tetramer
8	17.23	289	MS ² [289]: 245, 205, 203, 179, 137, 125 MS ³ [289→245]: 203, 227, 187, 161	[-]-Epicatechin
A	18.33	1039		Unknown
9	18.55	865	MS ² [865]: 847, 739, 713, 695, 587, 577, 575, 451, 425, 407, 289 MS ³ [865→695]: 543, 525, 407, 289	Procyanidin trimer
B	19.05	401		Unknown
C	19.40	977		Unknown
10	19.74	415	MS ² [415]: 397, 341, 311	Apigenin-C-deoxyhexoside ^a
11	23.40	463	MS ² [463]: 301 MS ³ [463→301]: 179, 151	Isoquercitrin (quercetin-3- <i>O</i> -glucoside)
12	25.82	447	MS ² [447]: 301 MS ³ [447→301]: 179, 151	Quercitrin (quercetin-3- <i>O</i> -rhamnoside)

Supplementary Tab. S3. Experimental design of 2D-DIGE for the analysis of outer pericarp proteins from kiwifruit at different developmental stages (T0, T1, T2 and T3). Scheme of labeling for protein extracts. Reference mixture was a pool of all biological replicates from all developmental stages. Biological replicate number is reported in parenthesis.

Gel	Cy2	Cy3	Cy5
1	Reference mixture	T0 (1)	T1 (1)
2	Reference mixture	T2 (1)	T3 (1)
3	Reference mixture	T1 (3)	T2 (2)
4	Reference mixture	T3 (2)	T0 (2)
5	Reference mixture	T0 (3)	T2 (3)
6	Reference mixture	T1 (2)	T3 (3)

Supplementary Tab. S5. Differentially represented proteins in the outer pericarp of kiwifruits sampled at different developmental stages (T0, T1, T2 and T3) as deriving from combined 2D-DIGE/nLC-ESI-LIT-MS/MS analysis. Proteins are listed according to their functional classification. Spot number, kiwifruit database accession code, protein name, STRING gene name of the *Arabidopsis thaliana* protein homologue with the highest sequence similarity, corresponding TAIR 10 and GI accession code, corresponding sequence identity (%), Mascot score, experimental and theoretical Mr and pI values, number of peptides identified, number of unique peptides identified, and sequence coverage (%) values are listed, together the corresponding fold change values at various stages (T1, T2 and T3 with respect to T0). Asterisk and circle report data for which a relative expression ratio >1.50 or <1.50 and a *P* value ≤0.05 was not observed, respectively.

Spot	Accession - Kiwifruit database	Protein Name - Kiwifruit database	Gene name (STRING)	Accession TAIR 10	Identity (%)	GI <i>A.thaliana</i>	Mascot score	Mr _{ex} (Da)	Mr _{th} (Da)	pI _{ex}	pI _{th}	Σ Pept matches	Σ Pep unique seq	Cover (%)	T1 vs T0	T2 vs T0	T3 vs T0
Cell growth/division																	
2566	Achn068561	Cell division protein ftsZ, putative	FTSZ2-1	AT2G36250	66	18404086	448	44182	109162	5.02	6.12	9	7	12.3	3.3	3.41	2.93
1150	Achn107431	Transitional endoplasmic reticulum ATPase, putative	AtCDC48C	AT5G03340	92	18414193	602	74924	95019	5.26	5.44	12	12	18.3	-1.03**	-1.06**	-2.42
Cell structure																	
2330	Achn107181	Actin 1	ACT7	AT5G09810	99	15242516	418	48666	41865	5.38	5.31	9	8	26.3	1.16**	1.20**	-2.21
2431	Achn107181	Actin 1	ACT7	AT5G09810	99	15242516	412	47031	41865	5.20	5.31	9	8	25.5	-1.19**	-1.53	-1.77
2452	Achn107181	Actin 1	ACT7	AT5G09810	99	15242516	623	46479	41865	5.28	5.31	18	11	36.1	-1.01**	-1.38**	-1.57
2455	Achn107181	Actin 1	ACT7	AT5G09810	99	15242516	371	46589	41865	5.49	5.31	8	8	23.3	-1.01**	-1.04**	-1.58
2525	Achn107181	Actin 1	ACT7	AT5G09810	99	15242516	194	44759	41865	5.28	5.31	4	4	13.3	-1.05**	-2.06	1.17**
2707	Achn107181	Actin 1	ACT7	AT5G09810	99	15242516	434	41457	41865	5.69	5.31	8	8	26.5	-1.40*	-1.19**	-2.56
3153	Achn107181	Actin 1	ACT7	AT5G09810	99	15242516	153	33176	41865	5.47	5.31	4	3	10.3	-1.30**	-1.28**	-2.4
2431	Achn212061	Actin 4	ACT11	AT3G12110	97	15229955	299	47031	41888	5.20	5.31	7	6	19.1	-1.19**	-1.53	-1.77
2452	Achn212061	Actin 4	ACT11	AT3G12110	97	15229955	604	46479	41888	5.28	5.31	14	11	43	-1.01**	-1.38**	-1.57
3939	Achn109181	Actin depolymerizing factor 1	ADF1	AT3G46010	83	145332763	133	18795	16228	5.82	5.52	3	2	26.6	3.15°	2.93	1.39**
3927	Achn020921	Actin depolymerizing factor 2	ADF1	AT3G46010	81	145332763	289	18950	19292	6.55	6.62	6	4	43.9	1.1**	-1.29**	1.72
3939	Achn020921	Actin depolymerizing factor 2	ADF1	AT3G46010	81	145332763	227	18795	19292	5.82	6.62	5	3	29.9	3.15°	2.93	1.39**
3990	Achn020921	Actin depolymerizing factor 2	ADF1	AT3G46010	81	145332763	152	18078	19292	5.78	6.62	2	2	17.1	4.45	4.19	2.84
2580	Achn122231	Alpha-1,4-glucan-protein synthase [UDP-forming], putative	RGP3	AT3G08900	86	30680679	494	43819	41875	5.87	5.67	13	10	36	-1.11**	-1.37*	-1.62
2621	Achn138021	Alpha-1,4-glucan-protein synthase [UDP-forming], putative	RGP3	AT3G08900	85	30680679	352	43051	41775	6.05	5.66	8	8	23.6	-1.31*	-1.13*	-1.7

2678	Achn338421	Alpha-1,4-glucan-protein synthase [UDP-forming], putative	RGP3	AT3G08900	87	30680679	293	41899	40765	5.76	5.65	7	6	21.7	-2.45	-1.25**	-4.85
2706	Achn338421	Alpha-1,4-glucan-protein synthase [UDP-forming], putative	RGP3	AT3G08900	87	30680679	424	41359	40765	5.53	5.65	8	8	26.5	-1.9	-1.27*	-3.49°
2811	Achn134781	Annexin, putative	ANNAT4	AT2G38750	53	15224946	509	39547	36009	6.96	6.62	12	12	34.5	-1.56°	1.01**	3.47
2843	Achn134781	Annexin, putative	ANNAT4	AT2G38750	53	15224946	624	38854	36009	7.30	6.62	16	13	42.8	1.26**	1.46*	1.84
4023	Achn134781	Annexin, putative	ANNAT4	AT2G38750	53	15224946	90	17698	36009	7.28	6.62	2	2	6.1	5.35	2.77	2.29
1777	Achn262021	Beta-xylosidase 4	XYL4	AT5G64570	71	15237736	250	61245	83425	6.04	8.15	5	5	5.9	-1.09**	1.04**	-1.52
1779	Achn262021	Beta-xylosidase 4	XYL4	AT5G64570	71	15237736	174	61317	83425	6.09	8.15	4	4	6.1	-1.05**	-1.16**	-1.93
2455	Achn258291	Bifunctional polymyxin resistance arnA protein	AXS2	AT1G08200	89	18390863	322	46589	44959	5.49	5.67	8	7	21	-1.01**	-1.04**	-1.58
2490	Achn258291	Bifunctional polymyxin resistance arnA protein	AXS2	AT1G08200	89	18390863	223	45611	44959	5.48	5.67	5	5	16.2	-1.01**	-1.06**	-1.62
3726	Achn205971	Fasciclin-like arabinogalactan protein	FLA8	AT2G45470	66	18406799	130	22404	42648	7.13	5.3	2	2	8.9	1.64	1.49**	2.63
3815	Achn205971	Fasciclin-like arabinogalactan protein	FLA8	AT2G45470	66	18406799	187	20678	42648	7.08	5.3	3	3	11.3	N/D	1.39**	1.58
2257	Achn065131	Kinesin-related protein	AT2G47500	AT2G47500	64	145361758	294	50537	146156	7.96	6.84	5	5	4	-1.6°	-1.27**	-1.88
3265	Achn150541	Plastid-lipid-associated protein, chloroplastic	AT4G22240	AT4G22240	74	15235575	185	30874	26708	4.97	4.97	3	3	14.3	1.63°	-1.22**	-2.16
3379	Achn150541	Plastid-lipid-associated protein, chloroplastic	AT4G22240	AT4G22240	74	15235575	266	28799	26708	5.08	4.97	5	4	18.8	-1.05**	-1.94	-1.18**
4502	Achn115471	Profilin	PRF5	AT2G19770	77	15224839	134	14303	14303	5.06	5.06	3	3	26	-2.00	-1.17**	-2.08
4615	Achn069801	Profilin	PRF1	AT2G19760	77	15224838	103	12353	14166	4.58	4.71	2	2	16.8	1.29**	-2.78	-1.01**
2734	Achn383111	Putative UDP-glucose 4-epimerase	UXS6	AT2G28760	89	15226950	293	41019	38137	8.19	8.31	5	5	18.5	-1.25**	-1.06**	-1.66
2743	Achn383111	Putative UDP-glucose 4-epimerase	UXS6	AT2G28760	89	15226950	323	40730	38137	7.43	8.31	5	5	25	-1.41**	-2.36	-1.86°
2794	Achn383111	Putative UDP-glucose 4-epimerase	UXS6	AT2G28760	89	15226950	514	39828	38137	7.23	8.31	8	8	31.8	1.00**	1.17**	-1.77
1989	Achn132141	T-complex protein 1 subunit beta	sks5	AT1G76160	82	15222981	256	56261	103206	6.34	6.07	4	4	3.5	-1.20**	1.02**	-1.98
3253	Achn313901	Thioredoxin domain-containing protein, putative	PLP3b	AT5G66410	73	18425069	308	31093	26058	5.57	5.14	5	5	26.8	2.26	1.69	2.79
2172	Achn147861	Tubulin alpha chain, putative	TUA2	AT1G50010	97	15220329	532	52419	50253	5.27	4.91	11	10	28.4	-1.07**	1.01**	-1.72
2170	Achn293391	Tubulin alpha-1 chain	TUA2	AT1G50010	97	15220329	549	52542	50286	5.11	4.97	10	10	27.3	-3.12	-2.09	-4.79
2183	Achn293391	Tubulin alpha-1 chain	TUA2	AT1G50010	97	15220329	815	52357	50286	5.16	4.97	19	15	42	-2.31	-1.23**	-4.05
2047	Achn081211	Tubulin beta-2 chain	AT2G34930	AT2G34930	38	15226862	285	55536	145392	4.99	5.19	6	5	4.8	-1.33**	1.08**	-2.05
2170	Achn081211	Tubulin beta-2 chain	AT2G34930	AT2G34930	38	15226862	313	52542	145392	5.11	5.19	5	5	4.9	-3.12	-2.09	-4.79
2045	Achn328141	Tubulin beta-2 chain	TUB6	AT5G12250	98	15239914	601	55405	40376	5.02	4.51	13	10	39	-1.52°	-1.04**	-2.9
2116	Achn095821	Tubulin beta-2 chain	TUB1	AT1G75780	89	15239914	820	54050	42477	4.95	4.54	19	13	49.5	-1.69°	1.07**	-5.00

2117	Achn328141	Tubulin beta-2 chain	TUB6	AT5G12250	98	15239914	655	53796	40376	5.02	4.51	19	12	42.4	-2.26	1.08**	-7.69
2126	Achn027511	Tubulin beta-2 chain	TUB5	AT1G20010	75	15239914	586	44173	44173	4.44	4.44	15	10	32.3	-2.54	-1.10**	-5.50
2248	Achn221031	Tubulin, beta chain	TUB6	AT5G12250	79	15239914	275	50776	44966	5.03	4.58	5	5	15.9	-1.23**	1.04**	-1.69
2761	Achn002461	UDP-glucose 4-epimerase	UGE1	AT1G12780	83	15222072	242	40538	38975	7.65	6.67	5	5	18.7	-1.06**	-1.63	-1.44**
2767	Achn189071	UDP-glucose 4-epimerase	UGE5	AT4G10960	82	15236988	349	40253	38450	5.95	5.95	6	6	25.9	-1.09**	-1.04**	-1.72
2789	Achn189071	UDP-glucose 4-epimerase	UGE5	AT4G10960	82	15236988	345	40111	38450	6.74	5.95	5	5	22.2	-1.01**	1.06**	-1.85
2137	Achn161931	UDP-glucose 6-dehydrogenase	UGD2	AT3G29360	87	15242316	540	53291	31146	5.95	5.55	9	8	48.4	-2.04	1.00**	-3.05
2137	Achn256641	UDP-glucose 6-dehydrogenase	UGD3	AT5G15490	68	15242316	460	53291	40037	5.95	5.72	9	8	27.2	-2.04	1.00**	-3.05
1557	Achn139811	UDP-glucose-4,6-dehydratase	RHM1	AT1G78570	87	15218420	295	66045	76761	7.79	7.24	5	5	9	-2.95	-1.18**	-2.5
1574	Achn005541	UDP-glucose-4,6-dehydratase	RHM1	AT1G78570	87	15218420	151	66045	76788	7.66	7.24	3	3	4.9	-1.88	-1.26**	-3.18
1611	Achn139811	UDP-glucose-4,6-dehydratase	RHM1	AT1G78570	87	15218420	298	65348	76761	7.72	7.24	5	5	9	-2.76	1.02**	-2.45
Disease/defence																	
4082	Achn027451	17.5 kDa class II heat shock protein	HSP17	AT5G12020	61	15239846	152	17003	25765	6.01	7.77	3	3	15.8	1.56°	1.87	1.29**
4104	Achn027451	17.5 kDa class II heat shock protein	HSP17	AT5G12020	61	15239846	192	16705	25765	6.22	7.77	4	4	20.2	3.65	4.09	2.42
4109	Achn383281	17.6 kDa class II heat shock protein	HSP17	AT5G12020	63	15239846	108	16606	16660	6.68	6.32	2	2	18.4	2.22	2.49	1.49**
3093	Achn305291	Abscisic stress ripening protein homolog	AT1G70810	AT1G70810	38	N/D	197	34168	20048	5.80	5.88	5	3	18.1	2.14	-1.00**	3.64
3099	Achn305291	Abscisic stress ripening protein homolog	AT1G70810	AT1G70810	38	N/D	206	33649	20048	5.92	5.88	4	3	25.3	1.86°	-1.15**	2.25
3310	Achn305291	Abscisic stress ripening protein homolog	AT1G70810	AT1G70810	38	N/D	188	30084	20048	5.90	5.88	4	3	18.1	1.87°	1.14**	2.75
3404	Achn305291	Abscisic stress ripening protein homolog	AT1G70810	AT1G70810	38	N/D	155	28495	20048	4.74	5.88	3	3	15.9	1.99	-1.16**	1.21**
3457	Achn305291	Abscisic stress ripening protein homolog	AT1G70810	AT1G70810	38	N/D	149	27151	20048	6.20	5.88	3	3	22	1.36**	-1.13**	1.74
3593	Achn305291	Abscisic stress ripening protein homolog	AT1G70810	AT1G70810	38	N/D	139	24795	20048	6.23	5.88	3	2	11	1.42**	1.14**	1.89
4020	Achn305291	Abscisic stress ripening protein homolog	AT1G70810	AT1G70810	38	N/D	73	17698	20048	6.71	5.88	2	2	15.9	4.81	4.04	3.01
4025	Achn305291	Abscisic stress ripening protein homolog	AT1G70810	AT1G70810	38	N/D	148	17656	20048	6.79	5.88	4	3	15.9	5.21°	2.55	2.58
2552	Achn295561	Activator of 90 kDa heat shock protein ATPase-like protein	AT3G12050	AT3G12050	69	18399461	306	44391	39204	5.80	5.61	6	6	19	1.33**	1.00**	1.6
2150	Achn294821	Alpha-toxin	AT4G32480	AT4G32480	45	N/D	299	53732	53572	7.69	6.61	7	6	17.6	1.29**	-1.24**	1.66
2731	Achn294821	Alpha-toxin	AT4G32480	AT4G32480	45	N/D	246	40586	53572	6.56	6.61	7	5	14.4	1.01**	1.15**	1.71
2843	Achn294821	Alpha-toxin	AT4G32480	AT4G32480	45	N/D	288	38854	53572	7.30	6.61	8	6	17	1.26**	1.46*	1.84
3290	Achn294821	Alpha-toxin	AT4G32480	AT4G32480	45	N/D	197	30369	53572	6.66	6.61	5	4	10.3	1.29**	1.13**	1.62

3310	Achn315041	Ascorbate peroxidase	APX1	AT1G07890	62	15223049	127	30084	30781	5.90	5.94	3	3	10.4	1.87°	1.14**	2.75
3316	Achn082241	Ascorbate peroxidase	APX1	AT1G07890	67	15223049	141	30119	40960	5.79	6.24	3	3	10.9	1.96	1.45*	3.23
1650	Achn373451	Chaperone DnaK	cpHsc70-2	AT5G49910	80	15233779	475	64051	75119	4.98	5.29	8	8	14.7	2.05°	2.75	1.40**
1678	Achn334081	Chaperone DnaK	MTHSC70-2	AT5G09590	87	15233779	339	63600	68488	5.55	5.27	7	6	12.4	1.8	1.68	-1.19**
1307	Achn256711	Chaperone protein clpB	CLPB3	AT5G15450	87	18417676	146	72406	100532	5.93	5.58	3	3	4.2	1.00**	1.19**	-1.69
1334	Achn186491	Chaperone protein HtpG	SHD	AT4G24190	83	42573019	349	70886	93982	4.98	4.84	6	6	7.8	-1.58°	1.30**	-2.6
3335	Achn340321	Chitinase	HCHIB	AT3G12500	71	15234281	195	29591	58640	9.09	8.76	3	3	8	1.47*	1.63	2.04
3400	Achn340321	Chitinase	HCHIB	AT3G12500	71	15234281	230	28428	58640	9.08	8.76	14	4	9.7	1.77	3.63	3.86
3406	Achn340321	Chitinase	HCHIB	AT3G12500	71	15234281	357	28361	58640	8.94	8.76	9	5	16	2.28	2.84	3.1
2673	Achn101631	Disulfide-isomerase-like protein	UNE5	AT2G47470	76	15226610	417	40409	40409	5.82	5.82	8	8	27.5	-1.21**	-1.59	-1.27**
2693	Achn101631	Disulfide-isomerase-like protein	UNE5	AT2G47470	76	15226610	572	41702	40409	5.91	5.82	16	12	40	-1.00**	-1.09**	-1.5
2406	Achn054171	Epimerase	GME	AT5G28840	86	15241945	286	47364	41534	6.27	6.29	5	5	18.7	-1.32**	-1.08**	-1.94
2424	Achn054171	Epimerase	GME	AT5G28840	86	15241945	442	46754	41534	6.47	6.29	10	9	29.7	-2.78	1.30**	-4.32
2443	Achn054171	Epimerase	GME	AT5G28840	86	15241945	419	46589	41534	6.67	6.29	7	7	22.8	-2.45	1.33**	-4.7
2530	Achn054171	Epimerase	GME	AT5G28840	86	15241945	494	44864	41534	6.46	6.29	8	8	24.7	-1.51	1.38**	-1.94
2411	Achn030021	GDP-D-mannose 3',5'-epimerase	GME	AT5G28840	85	15241945	287	47253	53075	6.33	5.87	5	5	10.4	-4.38	-1.51°	-4.13
2193	Achn300731	Glutathione reductase	AT3G24170	AT3G24170	72	15230074	280	51621	74283	7.22	5.85	4	4	8.9	-1.05**	1.97	1.90°
1628	Achn181471	Heat shock 70 kDa protein C	BIP1	AT5G28540	79	15241844	389	64964	167125	5.16	6.44	8	8	5.8	-1.11**	1.09**	-2.49
1537	Achn184401	Heat shock 70 kDa protein C	BIP2	AT5G42020	92	15241844	605	66514	73655	5.17	5.08	12	11	19.2	1.09**	1.06**	-1.93
1604	Achn031911	Heat shock 70 kDa protein C	BIP2	AT5G42020	93	30693962	349	65117	80312	5.33	5.35	6	6	10.3	1.52°	1.52	-1.50
1619	Achn184401	Heat shock 70 kDa protein C	BIP2	AT5G42020	92	15241844	1064	65579	73655	5.12	5.08	26	19	27.1	-1.01**	-1.05**	-1.91
1430	Achn079561	Heat shock protein 90-2	Hsp81	AT5G56000	92	15241102	588	69152	80452	5.06	4.99	12	11	21.2	-1.12**	1.08**	-2.89
1458	Achn146451	Heat shock protein 90-2	Hsp81	AT5G56000	90	15241102	743	68261	80441	5.16	4.94	16	13	19	1.33**	-1.06**	-1.53
1604	Achn079561	Heat shock protein 90-2	Hsp81	AT5G56000	92	15241102	225	65117	80452	5.33	4.99	5	5	6.4	1.52°	1.52	-1.5
3839	Achn350901	Heat shock protein, putative	HSP21	AT4G27670	42	15234240	85	20363	21883	4.93	5.7	2	2	10.8	1.12**	-1.78	1.45*
2481	Achn366411	Hsp70 nucleotide exchange factor fes1	Fes1A	AT3G09350	74	145332008	113	46370	39206	5.03	5.01	3	2	8.9	1.87	1.89	1.86
3330	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	323	29696	23040	4.97	5.68	5	4	36.2	-2.19	-1.46*	-4.96
3398	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	288	28462	23040	4.85	5.68	4	4	36.2	2.24	-1.30**	1.21**
3420	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	304	27733	23040	5.24	5.68	7	5	36.2	1.33**	-1.52°	2.07
3421	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	420	28062	23040	5.83	5.68	8	7	55.4	1.27**	1.01**	1.66

3429	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	422	28095	23040	5.13	5.68	7	6	49.8	1.35**	-1.05**	1.72
3430	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	400	27963	23040	5.52	5.68	14	7	52.6	1.35**	1.07**	2.19
3431	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	242	27279	23040	6.68	5.68	4	4	35.7	1.40**	1.13**	1.78
3438	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	432	27538	23040	5.89	5.68	8	7	53.1	1.35**	1.21**	2.35
3441	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	277	27570	23040	5.44	5.68	4	4	34.3	1.27**	-1.06**	2.52
3457	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	361	27151	23040	6.20	5.68	8	6	41.8	1.36**	-1.13**	1.74
3499	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	426	26300	23040	5.24	5.68	9	7	48.8	1.20**	-1.51°	1.97
3510	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	417	25839	23040	5.51	5.68	8	6	48.8	1.33**	1.02**	2.11
3512	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	178	26487	23040	4.92	5.68	3	3	20.7	1.49**	1.04**	1.6
3524	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	319	26238	23040	5.88	5.68	7	5	33.8	1.39*	-1.27*	2.26
3526	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	316	25992	23040	7.04	5.68	5	5	38.5	1.32**	1.26**	2.16
3527	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	401	26146	23040	7.26	5.68	9	6	41.8	1.65°	1.51**	2.47
3531	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	410	25992	23040	6.46	5.68	8	6	48.4	1.48**	-1.05**	2.07
3532	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	336	25992	23040	6.90	5.68	6	5	39.9	1.65°	1.26**	2.26
3541	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	409	25809	23040	6.61	5.68	16	7	54.9	1.23**	-1.16**	1.94
3542	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	405	25900	23040	6.35	5.68	10	7	54.9	1.44**	1.10**	2.19
3543	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	498	25931	23040	7.14	5.68	10	7	54.9	1.70°	-1.61°	2.73
3550	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	544	25476	23040	6.56	5.68	14	8	54.9	2.08°	-1.15**	2.11
3556	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	505	25386	23040	5.24	5.68	11	8	62	1.19**	-1.57°	2.05
3598	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	431	24649	23040	5.97	5.68	6	6	48.4	1.49**	-1.10**	2.24
3612	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	317	24360	23040	6.78	5.68	7	5	41.8	1.50°	1.11**	2.20
3657	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	148	23293	23040	5.96	5.68	3	3	18.3	1.07**	-1.19**	1.66
3662	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	241	23375	23040	6.35	5.68	3	3	28.2	1.28**	-1.01**	1.8
3708	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	130	22616	23040	5.98	5.68	2	2	15	1.41**	-1.10**	2.39
3718	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	95	22483	23040	6.57	5.68	2	2	15	1.45**	1.20**	2.31
3722	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	209	22115	23040	6.77	5.68	3	3	28.2	1.32**	1.39**	1.93
3726	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	148	22404	23040	7.13	5.68	3	3	23.5	1.64	1.49**	2.63
3752	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	162	21524	23040	7.13	5.68	3	2	16.9	2.62°	1.55°	2.03
3780	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	147	21146	23040	6.00	5.68	2	2	16.9	1.65	1.27*	2.45
3786	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	178	21022	23040	7.23	5.68	2	2	21.6	N/D	1.71°	2.42

3809	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	164	20997	23040	6.57	5.68	3	3	22.5	1.16**	1.13**	1.99
3812	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	305	20678	23040	7.44	5.68	4	4	35.7	2.03°	2.41	2.35
3815	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	289	20678	23040	7.08	5.68	5	4	35.7	N/D	1.39**	1.58
3865	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	340	20054	23040	7.09	5.68	6	4	36.6	1.23**	1.23**	1.74
3872	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	400	19656	23040	6.84	5.68	5	5	42.3	1.44**	1.76°	2.34
3888	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	191	19725	23040	7.47	5.68	2	2	19.7	2.71°	2.07	2.57
3892	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	326	19448	23040	7.07	5.68	5	4	36.6	1.11**	1.38**	1.88
3923	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	238	18928	23040	6.71	5.68	4	3	28.2	3.38	3.2	2.23°
3963	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	204	18378	23040	6.88	5.68	2	2	21.6	2.99	4.08	4.78
3974	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	146	18335	23040	5.51	5.68	2	2	15	2.69	1.82	1.85
3975	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	150	18400	23040	7.22	5.68	3	2	16.9	2.86	2.57°	2.29
3997	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	157	18014	23040	5.93	5.68	4	3	20.7	3.86	1.71°	1.92°
3998	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	131	17740	23040	5.53	5.68	2	2	16.9	2.66	1.61	1.64°
4000	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	309	17635	23040	6.42	5.68	4	4	36.6	3.95	2.28	2.20
4004	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	251	17824	23040	6.22	5.68	3	3	30	6.82	5.68	3.44
4007	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	352	18035	23040	5.85	5.68	8	4	35.7	2.83°	3.29	2.57
4033	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	290	17532	23040	6.52	5.68	5	4	33.8	2.43	1.61	1.82
4044	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	141	17306	23040	5.75	5.68	2	2	14.1	2.19	2.25	1.76
4064	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	186	17224	23040	6.13	5.68	6	3	28.2	4.64°	3.87°	3.94
4148	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	248	16200	23040	5.04	5.68	4	3	20.7	2.18°	1.51°	3.70
4149	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	311	15898	23040	5.37	5.68	17	5	40.4	2.78	2.61	4.52
4161	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	307	15898	23040	4.94	5.68	6	4	33.8	1.43**	-1.06**	2.64
4237	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	191	14777	23040	5.00	5.68	4	3	20.7	1.56°	1.41**	2.28
4320	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	150	14013	23040	5.86	5.68	4	3	20.7	2.95°	1.39**	3.69
4410	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	134	13574	23040	5.95	5.68	2	2	14.1	2.03	1.35**	3.47
4479	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	203	13399	23040	5.36	5.68	3	3	25.4	3.22°	2.17	3.70
4489	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	175	12995	23040	5.87	5.68	3	2	12.2	2.02°	1.31**	2.76
4521	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	229	12782	23040	6.77	5.68	7	3	20.7	1.50°	1.24**	2.20
4668	Achn022471	Kiwellin	AT5G60615	AT5G60615	28	N/D	137	12050	23040	6.82	5.68	3	2	12.2	1.48**	1.21**	2.33
2926	Achn326191	Lactoylglutathione lyase	GLX1	AT1G11840	72	15221116	439	37108	33875	5.47	4.92	10	8	35.9	1.41**	1.27**	1.83

3914	Achn277251	Major latex-like protein	MLP28	AT1G70830	43	186494506	128	19243	17401	4.69	4.69	2	2	14.7	1.29**	-1.88°	1.87
3917	Achn277251	Major latex-like protein	MLP28	AT1G70830	43	186494506	160	19153	17401	4.79	4.69	3	3	25.3	1.52°	-2.85	1.84
4028	Achn277251	Major latex-like protein	MLP28	AT1G70830	43	186494506	169	17615	17401	4.71	4.69	3	3	28	1.07**	-1.69	1.47**
4123	Achn277251	Major latex-like protein	MLP28	AT1G70830	43	186494506	261	16470	17401	4.78	4.69	5	4	29.3	-1.16**	-2.08	1.41**
4161	Achn277251	Major latex-like protein	MLP28	AT1G70830	43	186494506	179	15898	17401	4.94	4.69	4	3	18.7	1.43**	-1.06**	2.64
4185	Achn277251	Major latex-like protein	MLP28	AT1G70830	43	186494506	225	15785	17401	4.55	4.69	4	3	25.3	N/D	-1.86°	3.67
4187	Achn277251	Major latex-like protein	MLP28	AT1G70830	43	186494506	322	15748	17401	4.69	4.69	6	5	38.7	-1.04**	-2.38	2.12
4188	Achn277251	Major latex-like protein	MLP28	AT1G70830	43	186494506	289	15711	17401	4.79	4.69	7	6	46	1.56°	-3.11	2.94
2394	Achn075231	Monodehydroascorbate reductase	MDAR1	AT3G52880	71	15231702	303	47532	60414	5.72	6.27	6	5	12	-2.11	-1.03**	-2.89
2411	Achn091771	Monodehydroascorbate reductase	MDAR1	AT3G52880	77	15231702	309	47253	67359	6.33	8.57	6	6	12.8	-4.38	-1.51°	-4.13
2498	Achn075231	Monodehydroascorbate reductase	MDAR1	AT3G52880	71	15231702	557	45503	60414	6.52	6.27	11	10	21.1	1.56	1.35*	1.12**
2945	Achn323941	Peroxidase 31, putative	AT5G40150	AT5G40150	62	15242580	156	37021	35886	8.99	8.71	3	3	10.2	1.38**	1.29*	2.67
3880	Achn050871	Peroxiredoxin, putative	TPX1	AT1G65980	84	15218877	113	19679	10054	5.21	5.04	3	2	31.9	1.01**	-1.19**	-1.80
3886	Achn050871	Peroxiredoxin, putative	TPX1	AT1G65980	84	15218877	146	19380	10054	5.34	5.04	2	2	31.9	1.04**	-1.17**	-1.71
1952	Achn113361	Protein disulfide isomerase L-3b	PDIL1-6	AT3G16110	53	42564231	366	57400	51274	5.18	4.9	7	7	18	1.19**	1.03**	-1.60
2654	Achn175381	Protein disulfide isomerase, putative	UNE5	AT2G47470	74	15226610	356	42647	42015	6.42	5.91	6	5	21.3	-1.20**	1.19**	-2.00
3587	Achn259181	Putative glutathione S-transferase	GSTF7	AT1G02920	59	15218639	179	24736	15107	7.14	5.44	5	3	26.1	1.42*	1.12**	1.68
3618	Achn259181	Putative glutathione S-transferase	GSTF7	AT1G02920	59	15218639	387	24303	15107	6.58	5.44	10	8	61.9	1.47**	1.23**	2.34
2119	Achn190971	Selenium-binding protein, putative	AT4G14030	AT4G14030	80	15236385	339	53796	53436	5.84	5.69	7	7	16.7	1.68	1.23**	1.03**
1680	Achn001561	Stress-induced-phosphoprotein	Hop3	AT4G12400	74	15221275	162	63226	67871	6.29	5.61	3	3	5.2	-1.06**	1.12**	-1.73
1698	Achn001561	Stress-induced-phosphoprotein	Hop3	AT4G12400	74	15221275	299	63226	67871	6.05	5.61	7	7	12.1	1.51°	1.03**	-1.91
3670	Achn062661	Superoxide dismutase	MSD1	AT3G10920	85	15228407	253	23348	15294	6.78	4.68	5	3	26.9	1.35**	1.22**	1.99
3106	Achn132631	Thaumatococcus-like protein	OSM34	AT4G11650	69	15234263	212	34008	25221	8.68	8.29	4	4	21.3	1.82°	1.82	2.26
3297	Achn132631	Thaumatococcus-like protein	OSM34	AT4G11650	69	15234263	212	30261	25221	8.62	8.29	3	3	17.3	2.21	1.63°	2.25°
3378	Achn132631	Thaumatococcus-like protein	OSM34	AT4G11650	69	15234263	195	28936	25221	8.12	8.29	3	3	17.3	1.92	1.35*	1.82
3573	Achn132611	Thaumatococcus-like protein	OSM34	AT4G11650	67	15234263	208	25000	27363	6.98	5.8	3	3	16.3	1.24**	-1.16**	1.69
3587	Achn132631	Thaumatococcus-like protein	OSM34	AT4G11650	69	15234263	218	24736	25221	7.14	8.29	5	4	22.7	1.42*	1.12**	1.68
3623	Achn132631	Thaumatococcus-like protein	OSM34	AT4G11650	69	15234263	240	24188	25221	8.98	8.29	4	4	23.1	2.23	1.67	3.09
3635	Achn132611	Thaumatococcus-like protein	OSM34	AT4G11650	67	15234263	289	23820	27363	7.82	5.8	5	5	22.5	1.63°	1.06**	1.98
3855	Achn132631	Thaumatococcus-like protein	OSM34	AT4G11650	69	15234263	190	20030	25221	8.17	8.29	3	3	16	1.85°	1.73°	2.57

4102	Achn132631	Thaumatococcus-like protein	OSM34	AT4G11650	69	15234263	175	16509	25221	8.25	8.29	7	3	16	1.56°	1.10*	2.41
4127	Achn132631	Thaumatococcus-like protein	OSM34	AT4G11650	69	15234263	122	16528	25221	6.90	8.29	2	2	11.6	1.99°	1.34**	2.15
4141	Achn132631	Thaumatococcus-like protein	OSM34	AT4G11650	69	15234263	121	16010	25221	6.90	8.29	2	2	11.6	1.94°	1.39*	2.3
3024	Achn072831	Universal stress protein (Usp)	AT1G11360	AT1G11360	66	30682187	190	35524	25179	3.92	5.17	3	3	17.7	-1.32**	1.16**	-1.76
3880	Achn103351	Universal stress protein	AT3G17020	AT3G17020	74	18401345	100	19679	18389	5.21	5.46	2	2	12.9	1.01**	-1.19**	-1.8
Energy																	
1231	Achn079831	3-isopropylmalate dehydratase large subunit	ACO3	AT2G05710	83	30678219	400	74134	143020	6.74	6.19	10	9	8.1	1.20**	1.00**	-1.58
2266	Achn170221	6-phosphogluconate dehydrogenase, decarboxylating	AT3G02360	AT3G02360	89	15232888	431	50418	53674	7.08	6.15	8	7	23.3	-1.05**	1.30**	-1.5
2296	Achn321141	6-phosphogluconate dehydrogenase, decarboxylating	AT3G02360	AT3G02360	89	15232888	295	49535	50902	7.37	6.37	5	5	16.2	N/D	-1.02**	-1.73
1138	Achn353581	Aconitate hydratase 2	ACO3	AT2G05710	76	18651667 3	120	73013	98084	7.28	6.37	2	2	2.5	-1.49**	-1.28*	-2.16
1164	Achn353581	Aconitate hydratase 2	ACO3	AT2G05710	76	18651667 3	242	74836	98084	7.13	6.37	5	4	6.3	1.16**	-1.15**	-1.77
1168	Achn353581	Aconitate hydratase 2	ACO3	AT2G05710	76	18651667 3	329	74660	98084	7.01	6.37	6	6	8.9	1.16**	-1.15**	-1.77
1164	Achn332951	Aconitate hydratase 3	ACO3	AT2G05710	85	30678219	224	74836	132173	7.13	5.84	4	4	4	1.16**	-1.15**	-1.77
2521	Achn388521	Alcohol dehydrogenase	ADH1	AT1G77120	83	15223838	383	45076	42273	6.90	6.29	9	7	23.2	1.04	1.26	-1.72
3700	Achn060071	ATP synthase D chain, mitochondrial, putative	ATPQ	AT3G52300	81	15231176	379	22912	19699	7.31	5.13	9	8	38.7	-1.15**	-1.61	-1.48*
2088	Achn008641	ATP synthase subunit beta, chloroplastic	PB	ATCG00480	93	22326673	432	54718	53893	5.69	5.37	9	7	17.3	1.51	1.38*	1.10**
1980	Achn282931	ATP synthase subunit beta	AT5G08680	AT5G08680	86	22326673	763	56527	59493	5.31	5.95	14	12	34.4	-1.28**	1.29**	-1.84
2013	Achn282931	ATP synthase subunit beta	AT5G08680	AT5G08680	86	22326673	1122	56128	59493	5.34	5.95	25	18	47.6	1.05**	-1.21**	-1.81
2070	Achn282931	ATP synthase subunit beta	AT5G08680	AT5G08680	86	22326673	411	54950	59493	5.38	5.95	6	6	17.6	1.06**	-1.27*	-1.68
3505	Achn114191	Carnitine operon protein CaiE	AT3G48680	AT3G48680	73	15228424	196	26456	27538	7.70	9.1	4	4	18.1	1.82	1.47*	1.67
3519	Achn114191	Carnitine operon protein CaiE	AT3G48680	AT3G48680	73	15228424	111	23268	27538	7.55	9.1	2	2	8.9	1.48**	-1.19**	1.81
1944	Achn235801	Dihydrolipoyl dehydrogenase	mtLPD1	AT1G48030	86	15221044	326	57535	60945	6.60	5.64	7	6	17.6	-1.06**	1.29**	-1.96
1931	Achn150561	Diphosphate/fructose-6-phosphate 1-phosphotransferase	AT1G12000	AT1G12000	72	15221156	265	57671	57343	6.73	6.22	6	6	14.6	-1.26**	-1.09**	-1.62
3287	Achn246801	Electron transfer flavoprotein beta-subunit, putative	ETFBETA	AT5G43430	80	15239866	100	30512	27856	7.24	6.77	2	2	12	1.03**	-1.20**	-1.70
2121	Achn354501	Enolase, putative	LOS2	AT2G36530	75	15227987	410	53986	62299	6.37	5.8	9	7	18.6	-1.19**	-1.01**	-1.94
2357	Achn354501	Enolase, putative	LOS2	AT2G36530	75	15227987	191	48437	62299	5.87	5.8	3	3	9.4	-4.58	-1.06**	-5.91
2224	Achn086741	Enolase	LOS2	AT2G36530	81	15227987	488	51257	45347	6.11	5.2	7	7	22.3	-1.06**	1.04**	-1.92

2371	Achn086741	Enolase	LOS2	AT2G36530	81	15227987	335	48096	45347	5.49	5.2	6	6	20.8	-1.24**	-1.26**	-2.03
2641	Achn044851	Fructose-bisphosphate aldolase 2	FBA6	AT2G36460	83	15227981	655	42697	39529	7.77	7.01	16	11	38.3	-1.15**	-1.02**	-1.99
2659	Achn044851	Fructose-bisphosphate aldolase 2	FBA6	AT2G36460	83	15227981	540	42496	39529	4.48	7.01	14	10	34.2	-1.18**	-1.09**	-1.88
2741	Achn044851	Fructose-bisphosphate aldolase 2	FBA6	AT2G36460	83	15227981	212	40730	39529	7.82	7.01	4	4	12.4	-1.16**	-1.31**	-1.68
1937	Achn307451	Glucose-6-phosphate 1-dehydrogenase	G6PD6	AT5G40760	86	15237485	507	57807	59253	6.89	6.17	10	10	24.4	1.09**	1.69	1.03**
2453	Achn118341	Isocitrate dehydrogenase [NADP]	AT5G14590	AT5G14590	59	22326811	253	46261	54860	7.06	9.04	4	4	10.5	-1.35**	-1.50*	-2.41
2606	Achn019991	Isocitrate dehydrogenase, putative	IDH1	AT4G35260	84	15236932	428	43254	41430	6.67	6.47	7	7	22.5	1.00**	-1.04**	-1.58
2557	Achn344641	Magnesium chelatase subunit I	CHL1	AT4G18480	79	15233888	358	44391	46435	4.89	5.62	6	6	18.9	2.13	1.17**	1.45**
2565	Achn344641	Magnesium chelatase subunit I	CHL1	AT4G18480	79	15233888	313	44130	46435	4.95	5.62	6	6	13.2	3.57	3.12	2.51
2692	Achn385881	Malate dehydrogenase, putative	MDH	AT3G47520	83	15232820	458	41702	36512	5.19	5.25	7	6	25.9	-1.71	-1.67	-2.53
3060	Achn210541	Oxygen-evolving enhancer protein 1 of photosystem II	PSBO1	AT5G66570	86	15240013	284	34655	38616	4.96	5.59	5	5	23.8	1.70	1.06**	-1.01**
3144	Achn210541	Oxygen-evolving enhancer protein 1 of photosystem II	PSBO1	AT5G66570	86	15240013	428	33254	38616	5.27	5.59	9	8	27.5	1.57	1.42*	-1.09**
3165	Achn210541	Oxygen-evolving enhancer protein 1 of photosystem II	PSBO1	AT5G66570	86	15240013	611	32981	38616	5.23	5.59	16	11	40.1	-1.04**	1.08**	-2.07
3618	Achn132971	Oxygen-evolving enhancer protein 2, chloroplastic	PSBP-1	AT1G06680	70	15222166	167	24303	26238	6.58	8.65	4	3	17.9	1.47**	1.23**	2.34
3653	Achn132971	Oxygen-evolving enhancer protein 2, chloroplastic	PSBP-1	AT1G06680	70	15222166	299	23653	26238	6.65	8.65	6	5	29.7	1.87	1.32**	1.49**
3657	Achn132971	Oxygen-evolving enhancer protein 2, chloroplastic	PSBP-1	AT1G06680	70	15222166	91	23293	26238	5.96	8.65	2	2	13.8	1.07**	-1.19**	1.66
2077	Achn130681	Phosphofructokinase, putative	PFK3	AT4G26270	77	18416717	315	545672	57524	6.66	8.2	6	6	12.1	-1.09**	1.12**	-1.85
1797	Achn284661	Phosphoglucosmutase, putative	PGM2	AT1G70730	79	15220668	175	61389	60490	6.33	5.46	4	4	10.1	-1.11**	-1.02**	-1.75
2559	Achn305841	Phosphoglycerate kinase	PGK	AT1G79550	88	15219412	597	44286	48655	6.98	6.11	14	11	30.3	-1.20**	-1.01**	-2.16
2580	Achn005301	Phosphoglycerate kinase	PGK	AT1G79550	88	15219412	741	43819	45698	5.87	5.83	15	13	40.7	-1.11**	-1.37*	-1.62
2593	Achn005301	Phosphoglycerate kinase	PGK	AT1G79550	88	15219412	690	44078	45698	6.11	5.83	17	12	37.7	-1.04**	1.23**	-1.66
2606	Achn005301	Phosphoglycerate kinase	PGK	AT1G79550	88	15219412	465	43254	45698	6.67	5.83	9	8	29.1	1.00**	-1.04**	-1.58
2621	Achn005301	Phosphoglycerate kinase	PGK	AT1G79550	88	15219412	742	43051	45698	6.05	5.83	14	12	44.7	-1.31*	-1.13*	-1.7
2653	Achn005301	Phosphoglycerate kinase	PGK	AT1G79550	88	15219412	951	42546	45698	6.11	5.83	21	14	44.9	-1.21**	1.10**	-2.41
2859	Achn325921	Protochlorophyllide reductase	PORA	AT5G54190	70	15234129	201	38353	39222	8.97	9.26	4	4	14	1.11**	1.16*	2.03
1777	Achn133211	Putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	iPGAM1	AT1G09780	84	18391066	163	61245	96323	6.04	6.44	3	3	3.8	-1.09**	1.04**	-1.52

1779	Achn133211	Putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	iPGAM1	AT1G09780	84	18391066	344	61317	96323	6.09	6.44	8	8	9.4	-1.05**	-1.16**	-1.93
1796	Achn133211	Putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase	iPGAM1	AT1G09780	84	18391066	336	60456	96323	5.55	6.44	8	7	7.8	-1.03**	1.02**	-1.70
3962	Achn316261	Putative uncharacterized protein 259I16.6	SDH5	AT1G47420	71	18401719	279	18270	19407	4.79	4.99	4	4	42.4	-1.04**	-2.21	2.22
2180	Achn008791	Ribulose biphosphate carboxylase large chain	RBCL	ATCG00490	95	7525041	279	52728	58998	6.87	6.54	10	6	8.9	1.90°	3.07	1.67°
2343	Achn220571	Transaldolase, putative	TRA2	AT5G13420	88	15240625	177	48494	58864	4.91	5.41	3	3	6.4	3.09	1.42**	2.11
2476	Achn220571	Transaldolase, putative	TRA2	AT5G13420	88	15240625	221	46261	58864	4.96	5.41	4	4	7.3	3.14	2.09	2.29
3354	Achn264191	Triosephosphate isomerase	TPI	AT3G55440	69	15233272	168	29313	29454	5.37	4.99	3	3	13.3	-1.50°	-1.20**	-3.96
3365	Achn340601	Triosephosphate isomerase	TIM	AT2G21170	78	14532920 4	247	29141	33287	5.32	7.62	4	4	17.8	-1.68	-1.99	-3.25
3483	Achn203191	Triosephosphate isomerase	TPI	AT3G55440	82	15233272	491	26864	30606	6.34	6.86	11	8	30.1	1.42**	-1.20**	2.20
2070	Achn239081	Utp-glucose-1-phosphate uridylyltransferase, putative	UGP2	AT5G17310	87	30686293	362	54950	32828	5.38	5.9	10	7	27.8	1.06**	-1.27*	-1.68
2075	Achn239081	Utp-glucose-1-phosphate uridylyltransferase, putative	UGP2	AT5G17310	87	30686293	429	54820	32828	5.50	5.9	12	7	27.8	1.08**	1.28**	-1.59
2075	Achn191791	Utp-glucose-1-phosphate uridylyltransferase, putative	UGP1	AT3G03250	69	30686293	411	54820	33904	5.50	8.24	11	7	29.3	1.08**	1.28**	-1.59
Metabolism																	
2406	Achn253271	3-ketoacyl-CoA thiolase	ACAT2	AT5G48230	85	30695411	244	47364	42089	6.27	6.14	5	5	15.2	-1.32**	-1.08**	-1.94
2443	Achn253271	3-ketoacyl-CoA thiolase	ACAT2	AT5G48230	85	30695411	402	46589	42089	6.67	6.14	7	7	20.8	-2.45	1.33**	-4.7
2291	Achn231061	3-oxoacyl-[acyl-carrier-protein] synthase	KASI	AT5G46290	69	33418822 3	344	49769	58266	6.53	6.61	8	7	13.6	1.69	1.35**	1.10**
1259	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	115	72920	84577	7.59	6.71	3	3	4	-2.06°	-1.58	-1.28**
1269	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	164	72834	84577	7.35	6.71	3	3	4.3	-1.83	-1.40**	-1.58°
1363	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	162	70886	84577	7.64	6.71	3	3	3.6	-1.84	-1.42**	-5.07
1368	Achn149271	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	88	15238686	171	70469	85087	6.79	6.06	4	4	5.5	-2.01	-1.02**	-2.15°
1377	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	199	70802	84577	7.79	6.71	3	3	4.3	-1.34**	1.05**	-2.56

1388	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	152	70386	84577	7.22	6.71	4	4	4.5	-1.67°	-1.85°	-3.84
1389	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	328	70220	84577	7.41	6.71	8	7	10.5	-1.97	-1.47*	-5.25
1390	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	230	70469	84577	7.48	6.71	5	5	8	-2.23	-1.77	-6.63
1401	Achn008171	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	89	15238686	506	70303	85140	7.68	6.68	10	10	13.6	-1.80-	-1.13**	-3.38
1407	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	126	70303	84577	7.34	6.71	3	3	4.3	-1.97	1.45*	-4.79
1414	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	606	70969	84577	7.54	6.71	13	12	17.8	-2.06	-1.38*	-5.06
1417	Achn149271	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	88	15238686	258	69972	85087	6.95	6.06	5	5	6.1	-1.45**	-1.47**	-2.18
1428	Achn149271	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	88	15238686	358	69972	85087	7.13	6.06	8	8	11.8	-1.29**	-1.14**	-2.22
1432	Achn256141	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase, putative	ATMS1	AT5G17920	87	15238686	287	69808	84577	7.28	6.71	5	5	6.7	-1.76	-1.31**	-2.42
2697	Achn194021	Adenosine kinase, putative	ADK2	AT5G03300	83	15242717	236	41555	43772	5.14	5.54	4	4	13.4	-1.15**	-1.09**	-2.26
2700	Achn194021	Adenosine kinase, putative	ADK2	AT5G03300	83	15242717	362	41604	43772	5.27	5.54	8	7	23.1	-1.49*	-1.25*	-2.36
2709	Achn194021	Adenosine kinase, putative	ADK2	AT5G03300	83	15242717	518	41407	43772	5.40	5.54	11	9	30.3	-1.29**	-1.02**	-2.08
1994	Achn190951	Adenosylhomocysteinase	HOG1	AT4G13940	92	15236376	204	56593	53890	6.19	5.72	5	5	10.7	-1.39**	1.29**	-2.74
2108	Achn190951	Adenosylhomocysteinase	HOG1	AT4G13940	92	15236376	610	53923	53890	6.18	5.72	16	12	30.3	-1.33**	1.07**	-2.56
2152	Achn190951	Adenosylhomocysteinase	HOG1	AT4G13940	92	15236376	451	53103	53890	6.04	5.72	9	8	14.8	-1.18**	-1.08**	-1.58
2811	Achn068091	Aldo-keto reductase, putative	AT2G27680	AT2G27680	73	18401495	408	39547	38320	6.96	5.74	6	6	24.1	-1.56°	1.01**	3.47
2852	Achn092201	Aldose 1-epimerase, putative	AT5G57330	AT5G57330	78	15242099	544	38900	35739	6.93	6.09	10	8	38.2	-1.01**	1.11*	3.36
2540	Achn150451	Aminomethyltransferase	AT1G11860	AT1G11860	85	15221119	380	44546	39964	8.04	8.05	7	7	23.4	1.01**	1.04**	-1.57
2628	Achn150451	Aminomethyltransferase	AT1G11860	AT1G11860	85	15221119	162	42697	39964	8.00	8.05	3	3	14	-1.29**	-1.05**	-1.63
3420	Achn186231	Asparagine synthetase [glutamine-hydrolyzing] 2	AILP1	AT5G19140	82	15239658	172	27733	25720	5.24	5.95	3	3	17.7	1.33**	-1.52°	2.07
3421	Achn186231	Asparagine synthetase [glutamine-hydrolyzing] 2	AILP1	AT5G19140	82	15239658	225	28062	25720	5.83	5.95	3	3	17.7	1.27**	1.01**	1.66

3441	Achn186231	Asparagine synthetase [glutamine-hydrolyzing] 2	AILP1	AT5G19140	82	15239658	276	27570	25720	5.44	5.95	4	4	20.7	1.27**	-1.06**	2.52
2486	Achn186891	Aspartate aminotransferase	ASP5	AT4G31990	84	15236129	199	45718	45901	7.17	6.68	3	3	10.4	-1.32*	-1.14**	-2.94
2491	Achn186891	Aspartate aminotransferase	ASP5	AT4G31990	84	15236129	249	45503	45901	7.46	6.68	4	4	11.3	-1.25*	-1.38*	-3.3
2530	Achn093921	Aspartate aminotransferase	AAT	AT2G22250	74	18399915	377	44864	42030	6.46	5.13	6	6	22.4	-1.51	1.38*	-1.94
2220	Achn260381	Dihydrolipoyl dehydrogenase	LPD1	AT3G16950	80	145332599	187	51318	60922	6.65	7.02	3	3	7.7	-1.41**	1.05**	-2.5
3573	Achn176961	Flavoprotein wrbA	AT4G27270	AT4G27270	92	30687535	119	25000	22275	6.98	5.85	2	2	13	1.24**	-1.16**	1.69
3598	Achn333161	Flavoprotein wrbA	AT4G27270	AT4G27270	91	30687535	281	24649	22352	5.97	5.83	4	4	24.6	1.49**	-1.10**	2.24
2108	Achn191731	Glutamate decarboxylase, putative	GAD	AT5G17330	84	15237949	562	53923	55584	6.18	5.68	11	9	22	-1.33**	1.07**	-2.56
2121	Achn323101	Glutamate decarboxylase, putative	GAD	AT5G17330	86	15237949	438	53986	56338	6.37	5.68	10	8	19.9	-1.19**	-1.01**	-1.94
2495	Achn184871	Glutamate dehydrogenase	GDH1	AT5G18170	90	15238762	251	45450	44619	7.30	6.09	6	5	14.6	-1.24**	1.01**	-2.2
1452	Achn348911	Glycogen debranching enzyme	ISA3	AT4G09020	56	22328517	201	68422	78517	6.61	8.57	3	3	6.1	-1.30**	-1.25**	-1.84
2191	Achn094531	Hexokinase, putative	HXK3	AT1G47840	65	30694182	387	52172	51949	5.22	5.49	7	7	19.3	-2.07	1.15*	-3.95
2039	Achn171511	Inositol-3-phosphate synthase	MIPS2	AT2G22240	87	15227169	486	55601	54615	5.75	5.52	9	8	19.1	-2.77	-1.22**	-4.56
1981	Achn367481	Ketol-acid reductoisomerase	AT3G58610	AT3G58610	89	15231092	421	56995	63901	5.78	6.54	10	8	17.1	-1.23**	1.06**	-2.14
2027	Achn367481	Ketol-acid reductoisomerase	AT3G58610	AT3G58610	89	15231092	262	55536	63901	6.12	6.54	5	5	10.7	-1.05**	1.19**	-1.66
2055	Achn367481	Ketol-acid reductoisomerase	AT3G58610	AT3G58610	89	15231092	261	55667	63901	5.93	6.54	5	5	12.9	-1.39*	-1.01**	-2.32
3615	Achn098881	Lactoylglutathione lyase, putative	AT1G08110	AT1G08110	81	79317307	114	24446	22547	6.34	6.22	3	3	14.1	1.43**	-1.08**	2.27
3571	Achn322841	L-asparaginase, putative	ASPGA1	AT5G08100	80	15241528	166	24227	34009	5.29	5.41	4	4	13.8	1.62°	1.06**	2.36
4144	Achn153111	Nucleoside diphosphate kinase 1	NDPK1	AT4G09320	84	18413214	183	15991	16486	5.96	6.92	3	3	25.7	3.27	2.94	5.1
4190	Achn026931	Nucleoside diphosphate kinase 1	NDPK1	AT4G09320	84	18413214	120	15730	16454	6.70	6.43	2	2	16.9	2.95	2.51	2.27
4368	Achn026931	Nucleoside diphosphate kinase 1	NDPK1	AT4G09320	84	18413214	94	14080	16454	6.55	6.43	2	2	16.9	2.18	1.28**	1.56°
4126	Achn116471	Nucleoside diphosphate kinase, putative	NDPK2	AT5G63310	70	30697820	93	16238	27810	7.24	8.87	2	2	9.9	1.66°	1.06**	2.83
2628	Achn145231	Phosphoserine aminotransferase	PSAT	AT4G35630	77	15237069	137	42697	46585	8.00	8.8	2	2	7.2	-1.29**	-1.05**	-1.63
2734	Achn145231	Phosphoserine aminotransferase	PSAT	AT4G35630	77	15237069	282	41019	46585	8.19	8.8	5	5	15.8	-1.25**	-1.06**	-1.66
2770	Achn336721	Putative fructokinase 2	AT3G59480	AT3G59480	86	15231692	656	40205	33682	4.92	5.03	14	11	46.1	-1.83	-1.28**	-3.38
2792	Achn336721	Putative fructokinase 2	AT3G59480	AT3G59480	86	15231692	793	39922	33682	5.05	5.03	15	13	55.5	-1.57°	-1.03**	-2.36
2938	Achn303541	Pyridoxal biosynthesis lyase PdxS	RSR4	AT5G01410	94	15240972	283	37152	33597	5.83	5.78	5	5	18.3	1.25**	-2.84	1.13**
2996	Achn303541	Pyridoxal biosynthesis lyase PdxS	RSR4	AT5G01410	94	15240972	253	35861	33597	6.06	5.78	4	4	18.3	-1.23**	1.10**	-2.33
2775	Achn080991	Quinone oxidoreductase, putative	AT5G61510	AT5G61510	81	42568692	261	40158	21397	6.52	5.56	7	6	32.8	1.52°	1.26**	1.65

2948	Achn031121	Quinone oxidoreductase, putative	AT4G21580	AT4G21580	72	15234529	240	37064	35002	6.65	6.9	5	5	16.6	1.01**	1.29**	-1.85
2794	Achn252281	Quinone oxidoreductase-like protein	AT3G03080	AT3G03080	65	15228456	726	39828	37188	7.23	6.96	14	12	48.9	1.00**	1.17**	-1.77
3880	Achn135751	Regulator of ribonuclease-like protein	AT5G16450	AT5G16450	86	15237348	111	19679	18035	5.21	5.39	3	3	18.7	1.01**	-1.19**	-1.8
3937	Achn135751	Regulator of ribonuclease-like protein	AT5G16450	AT5G16450	86	15237348	265	18950	18035	5.21	5.39	9	5	32.5	-1.09**	-1.03**	-1.75
3149	Achn081301	Retinol dehydrogenase 14	AT4G23420	AT4G23420	53	42567066	295	33215	56080	9.18	9.7	4	4	11.3	-1.84	-1.41**	-6.51°
2307	Achn276811	S-adenosylmethionine synthase	MTO3	AT3G17390	93	15229033	567	49593	43582	5.87	5.57	15	9	33.8	-6.04	1.04**	-11.82
2331	Achn276811	S-adenosylmethionine synthase	MTO3	AT3G17390	93	15229033	208	49302	43582	5.73	5.57	3	3	10.2	-3.91	-1.15**	-4.06
2357	Achn276811	S-adenosylmethionine synthase	MTO3	AT3G17390	93	15229033	281	48437	43582	5.87	5.57	6	5	19.1	-4.58	1.06**	-5.91
2380	Achn276811	S-adenosylmethionine synthase	MTO3	AT3G17390	93	15229033	262	47813	43582	6.05	5.57	4	4	14.8	-1.21**	1.17**	-2.32
2113	Achn088371	Serine hydroxymethyltransferase	SHM1	AT4G37930	78	15235745	179	54178	51435	8.09	9.18	3	3	9	1.22**	-1.08**	1.61
2150	Achn190991	Serine hydroxymethyltransferase	SHM4	AT4G13930	88	15236375	340	53732	52278	7.69	6.84	7	7	18.7	1.29**	-1.24**	1.66
2213	Achn190991	Serine hydroxymethyltransferase	SHM4	AT4G13930	88	15236375	231	51621	52278	7.69	6.84	4	4	12.1	1.32**	-1.26**	1.68
2965	Achn051021	Sulfurtransferase	MST1	AT1G79230	74	18412307	123	36457	34077	5.52	5.68	2	2	14.2	1.58	-1.08**	1.50
3124	Achn072771	Thiamine thiazole synthase	THI1	AT5G54770	80	15239735	223	33649	37415	5.53	5.33	7	4	16.7	-1.44**	1.02**	-2.14
Protein destination and storage																	
2248	Achn183941	26S protease regulatory subunit 6A-like protein	RPT5A	AT3G05530	92	15230005	778	50776	60291	5.03	6.94	18	17	36.4	-1.23**	1.04**	-1.69
2237	Achn164141	26S protease regulatory subunit 7	RPT1A	AT1G53750	97	15220930	309	51257	65695	6.38	8.32	6	6	12.5	1.37**	1.60	-1.07**
2077	Achn127491	26S protease regulatory subunit, putative	RPT2a	AT4G29040	95	15235544	185	54562	49689	6.66	5.97	4	4	8.5	-1.09**	1.12**	-1.85
3120	Achn207511	26S proteasome non-atpase regulatory subunit, putative	RPN10	AT4G38630	62	15233973	224	33768	50943	5.11	4.76	4	4	10	-1.03**	-1.11**	-1.55
3243	Achn315911	26S proteasome non-ATPase regulatory subunit	RPN10	AT4G38630	77	15233973	142	31167	30946	5.35	5.01	3	3	14.1	-1.47**	1.05**	-2.82
2936	Achn231381	3-ketoacyl-CoA synthase	AT1G07750	AT1G07750	45	15223000	201	37152	37482	5.58	6.85	5	5	15.8	1.17**	-2.12	1.02**
2968	Achn231381	3-ketoacyl-CoA synthase	AT1G07750	AT1G07750	45	15223000	600	37482	37482	6.85	6.85	13	10	30.1	1.22**	-3.30	-1.41*
1795	Achn078521	60 kDa chaperonin	HSP60	AT3G23990	90	15229559	254	60885	62798	5.50	6.01	4	4	9	-1.25**	1.14**	-2.02
1844	Achn070951	60 kDa chaperonin	HSP60	AT3G23990	90	15229559	536	59888	61476	5.25	6.01	10	10	21.4	1.08**	1.29**	-1.67
1872	Achn078521	60 kDa chaperonin	HSP60	AT3G23990	90	15229559	1334	59256	62798	5.49	6.01	34	23	45.4	1.05**	1.19**	-1.67
1598	Achn107951	70 kDa heat shock protein	HSP70	AT3G12580	92	15230534	659	65194	71417	5.42	5.17	13	12	23.6	1.76°	1.80	-1.40**
1604	Achn107951	70 kDa heat shock protein	HSP70	AT3G12580	92	15230534	773	65117	71417	5.33	5.17	15	13	25.6	1.52°	1.52	-1.50
1616	Achn107951	70 kDa heat shock protein	HSP70	AT3G12580	92	15230534	1122	65040	71417	5.23	5.17	22	19	34.7	1.57	1.11**	-1.29**
1619	Achn107951	70 kDa heat shock protein	HSP70	AT3G12580	92	15230534	1086	65579	71417	5.12	5.17	21	19	32.2	-1.01**	-1.05**	-1.91

1628	Achn107951	70 kDa heat shock protein	HSP70	AT3G12580	92	15230534	1328	64964	71417	5.16	5.17	27	22	41.1	-1.11**	-1.09**	-2.49
1655	Achn107951	70 kDa heat shock protein	HSP70	AT3G12580	92	15230534	817	63750	71417	5.34	5.17	14	14	24.5	-1.06**	1.10**	-1.94
1738	Achn107951	70 kDa heat shock protein	HSP70	AT3G12580	92	15230534	535	62044	71417	5.23	5.17	9	9	18.2	1.06**	-1.20**	-1.53
2092	Achn107951	70 kDa heat shock protein	HSP70	AT3G12580	92	15230534	413	54306	71417	6.03	5.17	8	8	16.6	-1.01**	-1.06**	-1.64
2520	Achn107951	70 kDa heat shock protein	HSP70	AT3G12580	92	15230534	445	44811	71417	6.15	5.17	10	8	19.6	-1.22**	1.11**	-1.70
1393	Achn040121	70-kDa heat shock protein	HSC70-1	AT5G02500	93	15230534	210	69397	71596	5.28	5.03	4	4	7.1	-1.09**	-1.53	-2.01°
1458	Achn040121	70-kDa heat shock protein	HSC70-1	AT5G02500	93	15230534	327	68261	71596	5.16	5.03	6	6	12.6	1.33**	-1.06**	-1.53
1537	Achn040121	70-kDa heat shock protein	HSC70-1	AT5G02500	93	15230534	611	66671	71596	4.51	5.03	11	9	20.8	1.09**	1.06**	-1.93
1636	Achn390331	70-kDa heat shock protein	HSP70	AT3G12580	88	15230534	634	64506	67963	5.38	5.28	13	12	23.3	1.47**	1.23**	-1.54
1650	Achn040121	70-kDa heat shock protein	HSC70-1	AT5G02500	93	15230534	346	64051	71596	4.98	5.03	7	6	11.9	2.05°	2.75	1.40**
1673	Achn040121	70-kDa heat shock protein	HSC70-1	AT5G02500	93	15230534	688	63600	71596	5.31	5.03	15	13	20.5	1.12**	-1.62	-1.72
1678	Achn390331	70-kDa heat shock protein	HSP70	AT3G12580	88	15230534	746	63600	67963	5.55	5.28	14	13	23	1.80	1.68	-1.19**
1686	Achn390331	70-kDa heat shock protein	HSP70	AT3G12580	88	15230534	766	63600	67963	5.47	5.28	15	15	27.7	1.75	1.48*	-1.09**
1994	Achn040121	70-kDa heat shock protein	HSC70-1	AT5G02500	93	15230534	360	56593	71596	6.19	5.03	6	6	12.6	-1.39**	1.29**	-2.74
2055	Achn040121	70-kDa heat shock protein	HSC70-1	AT5G02500	93	15230534	445	55667	71596	5.93	5.03	8	8	16.6	-1.39*	-1.01**	-2.32
3888	Achn324381	ADP-ribosylation factor, putative, expressed	ARFA1F	AT1G10630	98	30681825	104	19725	24427	7.47	6.18	2	2	10	2.71°	2.07	2.57
2891	Achn018851	Alpha-soluble nsf attachment protein, putative	ASNA2	AT3G56190	56	15228848	441	38173	25425	4.05	4.85	7	7	33.9	1.11**	-1.67	-1.78°
3429	Achn141811	ATP-dependent Clp protease proteolytic subunit	CLPR4	AT4G17040	59	18414804	198	28095	33669	5.13	8.59	3	3	14.3	1.35**	1.07**	1.72
2222	Achn305601	ATP-dependent zinc metalloprotease FtsH	SKD1	AT2G27600	88	15226199	376	51439	89265	7.15	7.3	7	6	10.2	1.89	1.53	1.88
2943	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	206	37021	42052	9.10	8.02	4	4	16.7	1.29**	-1.76	2.61
3335	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	289	29591	42052	9.09	8.02	6	6	11.9	1.47*	1.63	2.04
3400	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	371	28428	42052	9.08	8.02	8	8	21.4	1.77	3.63	3.86
3406	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	323	28361	42052	8.94	8.02	8	6	19.3	2.28	2.84	3.1
3440	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	459	26946	42052	8.44	8.02	10	8	22.2	2.29	1.65	2.11
3448	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	484	27408	42052	9.00	8.02	11	8	24.3	2.19	1.48*	1.44**
3453	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	528	27279	42052	8.93	8.02	14	10	28.8	2.56	1.92	1.70°
3492	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	426	26393	42052	8.38	8.02	8	7	15.9	2.00°	1.74	2.01
3501	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	610	26612	42052	8.24	8.02	14	11	32.5	1.65	1.75	1.68
3506	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	745	26362	42052	8.79	8.02	21	13	39.4	3.33	2.78	3.22

3523	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	464	26115	42052	8.90	8.02	14	8	26.7	4.52	3.5	3.31
3623	Achn125831	Cathepsin B-like cysteine proteinase 3	RD21A	AT1G47128	58	18422289	257	24188	42052	8.98	8.02	6	6	16.9	2.23	1.67	3.09
2350	Achn125851	Cathepsin S	RD21B	AT5G43060	58	18422289	180	48437	28620	4.97	4.79	4	4	18.4	2.73	1.51°	2.08
3913	Achn125851	Cathepsin S	RD21B	AT5G43060	58	18422289	238	19289	28620	4.23	4.79	8	3	19.6	1.09**	-1.26**	2.57
4471	Achn125851	Cathepsin S	RD21B	AT5G43060	58	18422289	174	13211	28620	6.53	4.79	2	2	10.2	3.91	2.03°	2.98
1506	Achn017401	Clp protease ATP binding subunit	CLPC1	AT5G50920	86	18423214	911	67302	109513	5.35	6.27	16	16	17.2	1.26**	1.23**	-1.50
2032	Achn370171	Coatomer subunit delta-3	AT5G05010	AT5G05010	66	18414767	322	55798	60361	5.60	8.6	7	6	15.2	-4.22	-1.87	-4.50
4286	Achn058711	Histidine triad nucleotide binding protein	HIT3	AT3G56490	76	18410510	192	14450	16860	5.93	6.51	4	4	33.3	2.37°	1.79°	1.76
3093	Achn234761	Hypersensitive-induced response protein	HIR1	AT5G62740	89	15241939	240	34168	32101	5.80	5.47	4	4	16.3	2.14	-1.00**	3.64
3126	Achn234761	Hypersensitive-induced response protein	HIR1	AT5G62740	89	15241939	150	33609	32101	5.61	5.47	4	3	11.4	1.89°	1.30**	2.21
3144	Achn234761	Hypersensitive-induced response protein	HIR1	AT5G62740	89	15241939	213	33254	32101	5.27	5.47	4	4	17	1.57	1.42*	1.09**
1947	Achn059811	Importin subunit alpha	IMPA-2	AT4G16143	82	15230778	353	57671	57821	5.27	5.19	5	5	13.8	1.02**	1.02**	-1.54
1980	Achn059811	Importin subunit alpha	IMPA-2	AT4G16143	82	15230778	354	56527	57821	5.31	5.19	8	7	18.6	-1.28**	1.29**	-1.84
1493	Achn348701	Lysosomal alpha-mannosidase, putative	AT5G13980	AT5G13980	70	22326796	191	67302	183443	7.48	7.18	4	4	2.8	-1.78	-1.54°	-2.79
4178	Achn246001	Nascent polypeptide-associated complex subunit alpha-like protein	AT3G12390	AT3G12390	88	15230476	202	15823	20217	4.60	4.31	3	3	22	-1.12**	-2.82	2.73
4187	Achn246001	Nascent polypeptide-associated complex subunit alpha-like protein	AT3G12390	AT3G12390	88	15230476	158	15748	20217	4.69	4.31	2	2	16.7	-1.04**	-2.38	2.12
2956	Achn014361	Proteasome subunit alpha type	PAF1	AT5G42790	85	15239061	251	36716	37809	5.40	6.1	4	4	15.7	1.67	1.30**	1.51°
3009	Achn014361	Proteasome subunit alpha type	PAF1	AT5G42790	85	15239061	213	35734	37809	5.32	6.1	5	5	15.7	1.75	1.08**	1.59
3503	Achn222771	Proteasome subunit alpha type	PAC1	AT3G22110	90	15233268	217	26487	40318	5.81	5.27	4	4	16.3	1.40**	-1.23**	2.35
3503	Achn349541	Proteasome subunit beta type	AT3G26340	AT3G26340	82	15231544	341	26487	29320	5.81	5.75	6	6	29.7	1.40**	-1.23**	2.35
3664	Achn130061	Proteasome subunit beta type	PBD1	AT3G22630	81	15228805	175	23265	21958	6.57	6.3	3	3	18.9	1.45**	1.43**	2.12
2587	Achn026181	Serpin-like protein	SERPIN1	AT1G47710	66	15220298	575	43922	42423	6.82	5.83	13	9	30.3	1.01**	-1.14**	-1.65
2471	Achn158261	SGT1, suppressor of G2 allele of SKP1	SGT1B	AT4G11260	64	15237122	169	46261	40621	4.90	4.97	3	3	12.9	2.88	1.38**	1.64°
2476	Achn158261	SGT1, suppressor of G2 allele of SKP1	SGT1B	AT4G11260	64	15237122	124	46261	40621	4.96	4.97	3	3	10.2	3.14	2.09	2.29
1400	Achn058851	Subtilisin-like protease	AT5G67090	AT5G67090	35	18425150	214	70055	57155	7.02	5.65	5	4	9.2	-1.34**	-1.74°	-2.38
1550	Achn243811	Surface antigen (D15) (Precursor)	TOC75-III	AT3G46740	80	15232625	645	66279	94117	7.29	8.58	12	11	20	-1.10**	-1.06**	-1.75
1929	Achn027121	T-complex protein 1 subunit alpha	TCP-1	AT3G20050	74	15231024	212	57467	59327	6.77	7.68	4	4	8.6	-1.22**	-1.11**	-1.86
1959	Achn015671	T-complex protein 1 subunit delta	AT3G18190	AT3G18190	88	15229595	514	57807	58130	7.13	6.2	7	7	20.2	2.26**	-1.97	-2.12

1872	Achn099451	T-complex protein 1 subunit epsilon	AT1G24510	AT1G24510	90	15221770	329	59256	59842	5.49	5.59	7	6	11.4	1.05**	1.19**	-1.67
1916	Achn017921	T-complex protein 1 subunit eta	AT3G11830	AT3G11830	73	15229866	255	58769	51656	7.21	6.58	6	5	11.4	-1.06**	1.18**	-1.65
1981	Achn350381	T-complex protein 1 subunit theta	AT3G03960	AT3G03960	82	18396719	581	56995	116134	5.78	5.54	10	10	10.1	-1.23**	1.06**	-2.14
1894	Achn339651	T-complex protein 1 subunit zeta	AT3G02530	AT3G02530	91	15232923	273	58700	64897	6.34	5.59	5	5	10.8	1.11**	-1.07**	-1.55
1931	Achn193181	T-complex protein 1 subunit zeta	AT3G02530	AT3G02530	92	15232923	225	57671	59308	6.73	5.65	4	4	9.6	-1.26**	-1.09**	-1.62
1942	Achn193181	T-complex protein 1 subunit zeta	AT3G02530	AT3G02530	92	15232923	518	57467	59308	6.45	5.65	9	9	24.5	-1.01**	1.09**	-1.56
2027	Achn339651	T-complex protein 1 subunit zeta	AT3G02530	AT3G02530	91	15232923	207	55536	64897	6.12	5.59	5	5	9.3	-1.05**	1.01**	-1.66
4120	Achn274431	Tubulin-specific chaperone A	KIS	AT2G30410	80	18402340	114	16665	12870	4.85	4.78	2	2	19.5	-1.23**	-1.60°	2.12
2866	Achn314771	Ubiquitin carboxyl-terminal hydrolase family 1 protein	UCH2	AT1G65650	77	18408331	299	38671	37543	6.41	8.75	6	6	23.5	1.12**	1.12**	-1.80
4186	Achn312161	Ubiquitin-conjugating enzyme E2 36	UBC36	AT1G16890	69	18412149	100	15785	28192	6.21	5.83	3	2	10.4	3.74	3.56	2.63
4018	Achn073511	Ubiquitin-conjugating enzyme, putative	MMZ3	AT2G36060	92	18404032	83	17824	20221	6.07	7.74	2	2	18.4	3.09	2.41	2.34
Protein synthesis																	
2618	Achn199581	40S ribosomal protein sa, putative	RPSAb	AT3G04770	71	79321150	205	43000	34455	5.15	5.01	3	3	16	-1.11**	-1.06**	-2.31
2823	Achn171901	60S acidic ribosomal protein P0, putative	AT2G40010	AT2G40010	85	15225613	220	39222	31914	5.78	5.06	4	4	19.1	-1.12**	-1.08**	-1.86
2251	Achn260931	Elongation factor 1 gamma-like protein	AT1G09640	AT1G09640	77	18391048	185	50716	67568	5.68	6.76	3	3	6.1	1.64	1.12**	1.35*
2515	Achn357801	Elongation factor Tu	AT4G02930	AT4G02930	88	15236220	585	45183	47286	6.71	5.93	10	10	29.6	1.15**	1.33**	-1.52
1232	Achn306341	Elongation factor	LOS1	AT1G56070	94	30696056	426	74309	96547	6.88	6	11	9	10.3	1.06**	1.19**	-1.85
2284	Achn330671	Eukaryotic initiation factor 4A (ATP-dependent RNA helicase eIF4A)	EIF4A1	AT3G13920	93	18400210	671	50063	47235	5.62	5.48	21	13	37	-1.09**	-1.23**	-1.59
2331	Achn050471	Eukaryotic initiation factor 4A (ATP-dependent RNA helicase eIF4A)	EIF4A1	AT3G13920	93	18400210	113	49302	47295	5.73	5.49	3	3	5.8	-3.91	-1.15**	-4.06
2656	Achn181621	Eukaryotic initiation factor 4A (ATP-dependent RNA helicase eIF4A)	EIF4A1	AT3G13920	87	18400210	396	42596	45089	7.58	5.28	8	8	18.4	1.07**	1.18*	-1.58
1393	Achn331821	Eukaryotic translation initiation factor 3 subunit B	TIF3B1	AT5G27640	77	18421140	176	69397	82260	5.28	5.18	3	3	5.3	-1.09**	-1.53	-2.01°
1453	Achn017141	Glycine--tRNA ligase	AT1G29880	AT1G29880	83	18397278	150	68181	71190	6.79	6.09	3	3	5	-1.16**	-1.10**	-2.32
1668	Achn271321	Glycine--tRNA ligase	AT1G29880	AT1G29880	83	18397278	498	63525	71267	6.65	6.02	10	10	18.2	-1.01**	1.06**	-1.51
1759	Achn318191	Lysine--tRNA ligase	ATKRS-1	AT3G11710	71	15229833	171	61680	74792	6.27	6.47	5	5	5.9	-1.03**	1.15**	-1.57
3946	Achn324421	OB-fold nucleic acid binding protein	AT1G23750	AT1G23750	80	18395285	190	18795	15326	7.85	8.76	3	3	32.1	1.12**	-1.21**	-1.61
2380	Achn106891	Putative atp-dependent rna helicase	EIF4A-III	AT3G19760	85	15230945	349	47813	48391	6.05	5.9	7	7	15.5	-1.21**	1.17**	-2.32
2682	Achn106861	Ycf48-like protein	HCF136	AT5G23120	85	15237225	529	41702	42604	5.10	7.03	7	7	25	-1.45**	-1.53	-1.82°
Secondary metabolism																	

2775	Achn223401	Aldo/keto reductase	ATB2	AT1G60710	80	18406861	357	40158	38245	6.52	5.98	8	6	21.6	1.52°	1.26**	1.65
2603	Achn281341	Cinnamyl alcohol dehydrogenase 1	ATCAD4	AT3G19450	68	15230382	202	43407	36927	5.64	5.15	5	4	18.2	-1.16**	-1.04**	-1.75
2491	Achn226891	Polyphenol oxidase	NdhS	AT4G23890	35	N/D	191	45503	65514	7.46	6.27	3	3	6.7	-1.25**	-1.38*	-3.30
2641	Achn226891	Polyphenol oxidase	NdhS	AT4G23890	35	N/D	510	42697	65514	7.77	6.27	10	9	19.4	-1.15**	-1.02**	-1.99
3168	Achn112561	Short-chain dehydrogenase/reductase	ABA2	AT1G52340	71	15218213	133	32865	29948	5.68	5.65	2	2	9.3	-1.64	1.13**	-3.35
2331	Achn300191	UDP-glucosyltransferase, putative	IAGLU	AT4G15550	44	18414478	171	49302	50701	5.73	5.38	3	3	8.9	-3.91	-1.15**	-4.06
2371	Achn300191	UDP-glucosyltransferase, putative	IAGLU	AT4G15550	44	18414478	262	48096	50701	5.49	5.38	4	4	12	-1.24**	-1.26**	-2.03
Signal transduction																	
3329	Achn087581	14-3-3 protein beta/alpha	F3F9	AT1G78300	76	18411901	77	29731	25201	5.68	4.49	3	2	8.1	-1.36**	1.18**	-1.61
4296	Achn087581	14-3-3 protein beta/alpha	F3F9	AT1G78300	76	18411901	116	14416	25201	5.36	4.49	2	2	11.7	2.77°	2.52	4.12
3140	Achn390871	14-3-3-like protein GF14 Epsilon	GRF12	AT1G26480	79	18395915	281	33412	31872	4.93	5.05	7	5	23.6	-1.12**	1.08**	-2.14
3664	Achn260391	ADP-ribosylation factor-like protein 3	SAR2	AT4G02080	93	15235226	132	23265	21982	6.57	6.92	3	3	19.7	1.45**	1.43**	2.12
4653	Achn262321	Calcium-binding protein, putative	AT5G47540	AT5G47540	83	15236013	139	11797	54019	7.75	8.95	3	2	5.3	1.82°	1.05**	2.51
4691	Achn262321	Calcium-binding protein, putative	AT5G47540	AT5G47540	83	15236013	136	11645	54019	7.62	8.95	3	2	5.3	1.53°	1.17**	2.21
4742	Achn262321	Calcium-binding protein, putative	AT5G47540	AT5G47540	83	15236013	130	11577	54019	7.48	8.95	2	2	5.3	1.56°	1.38**	2.88
4826	Achn262321	Calcium-binding protein, putative	AT5G47540	AT5G47540	83	15236013	127	11161	54019	7.51	8.95	2	2	5.3	1.84°	1.09**	3.18
4843	Achn262321	Calcium-binding protein, putative	AT5G47540	AT5G47540	83	15236013	136	11214	54019	7.72	8.95	9	2	5.3	1.60°	-1.70°	2.73
5001	Achn262321	Calcium-binding protein, putative	AT5G47540	AT5G47540	83	15236013	102	10314	54019	7.72	8.95	2	2	5.3	1.51°	-1.04**	2.17
2419	Achn014941	COP9 signalosome complex subunit 4, putative, expressed	COP8	AT5G42970	79	15239134	429	47309	42490	5.11	5.02	8	7	23	-1.40*	-1.83	-1.65°
3076	Achn292741	GF14 protein	AT5G16050	AT3G02520	91	18396217	425	34249	33139	4.74	4.94	10	9	23.9	-1.05**	-1.03**	-1.73
3140	Achn292741	GF14 protein	AT5G16050	AT3G02520	91	18396217	262	33412	33139	4.93	4.94	6	6	15.7	-1.12**	1.08**	-2.14
3016	Achn382901	Guanine nucleotide-binding protein subunit beta-like protein	RACK1C_AT	AT3G18130	82	15229589	269	35524	36521	7.38	6.65	5	5	23.3	1.47**	1.48**	1.94
3438	Achn260971	Ras-related protein Rab-25	RAB11c	AT1G09630	88	15217568	241	27538	23934	5.89	5.84	5	5	26	1.35**	1.21**	2.35
3658	Achn176441	Ras-related protein Rab-2-A	RABB1C	AT4G17170	93	15235981	101	23597	24001	7.25	7.05	2	2	10.6	1.53°	1.13**	2.22
Transcription																	
1557	Achn185251	ATP-dependent RNA helicase DED1	AT2G42520	AT2G42520	75	15231074	133	66045	64043	7.79	7.77	2	2	3.9	-2.95	-1.18**	-2.5
1611	Achn148851	ATP-dependent RNA helicase DED1	AT2G42520	AT2G42520	76	15231074	102	65348	66224	7.72	7.25	2	2	3.8	-2.76	1.02**	-2.45
2188	Achn050541	ATP-dependent RNA helicase	RH20	AT1G55150	79	15221998	135	52110	55902	8.70	9.26	3	3	7.2	-1.05**	-1.06**	1.51
2858	Achn310721	Charged multivesicular body protein 4b, putative	SNF7	AT2G19830	72	15224854	263	38808	27233	4.99	4.74	5	5	26	-1.31**	-1.19**	-1.70

2885	Achn069761	Charged multivesicular body protein 4b, putative	SNF7	AT4G29160	88	15224854	224	38128	18966	5.05	4.45	5	5	31.6	-1.17*	-1.29*	-1.67
2885	Achn003711	RNA-binding protein Nova-1	BTR1L	AT5G04430	59	30680512	270	38128	33392	5.05	6.19	4	4	18	-1.17*	-1.29*	-1.67
2453	Achn091301	RNA-binding protein, putative	AT4G36960	AT4G36960	62	18419943	155	46261	85060	7.06	8.78	3	3	5.2	-1.35**	-1.50°	-2.41
961	Achn017291	Staphylococcal nuclease domain-containing protein 1	Tudor1	AT5G07350	72	22326646	381	79755	85872	7.08	5.78	8	8	12.1	-1.17**	1.07**	-2.49
1388	Achn017291	Staphylococcal nuclease domain-containing protein 1	Tudor1	AT5G07350	72	22326646	102	70386	85872	7.22	5.78	2	2	3.2	-1.67°	-1.85°	-3.84
Transporters																	
1636	Achn217001	V-type ATP synthase alpha chain	VHA-A	AT1G78900	94	15219234	630	64506	69010	5.38	5.2	12	11	22.8	1.47**	1.23**	-1.54
1655	Achn217001	V-type ATP synthase alpha chain	VHA-A	AT1G78900	94	15219234	521	63750	69010	5.34	5.2	10	9	19.9	-1.06**	1.10**	-1.94
1673	Achn217001	V-type ATP synthase alpha chain	VHA-A	AT1G78900	94	15219234	775	63600	69010	5.31	5.2	14	12	25.8	1.12**	-1.62	-1.72
1738	Achn217001	V-type ATP synthase alpha chain	VHA-A	AT1G78900	94	15219234	868	62044	69010	5.23	5.2	15	13	25	1.06**	-1.20*	-1.53
2045	Achn382191	V-type ATP synthase beta chain	VAB2	AT4G38510	86	15233891	780	55405	57401	5.02	4.89	15	13	34.6	-1.52°	-1.04**	-2.9
2047	Achn019481	V-type ATP synthase beta chain	VAB2	AT4G38510	94	15233891	298	55536	62095	4.99	4.98	5	5	11.4	-1.33**	1.08**	-2.05
3065	Achn331421	V-type proton ATPase subunit E	VHA-E3	AT1G64200	73	15222641	214	34737	34015	7.43	7.25	4	3	8.5	1.52°	1.54	1.43**
Unclassified																	
827	Achn132881	Myosin-10	AT2G32240	AT2G32240	52	14532297 3	1031	81947	153845	4.79	4.88	17	17	16.2	1.60°	-2.55°	2.72
828	Achn132881	Myosin-10	AT2G32240	AT2G32240	52	14532297 3	1419	81947	153845	4.84	4.88	22	21	19.5	1.71°	-2.87	3.07
832	Achn132881	Myosin-10	AT2G32240	AT2G32240	52	14532297 3	200	81851	153845	4.75	4.88	4	4	3.9	1.50°	-2.04°	2.69
3448	Achn197381	Outer envelope pore protein 24, chloroplastic	AT1G45170	AT1G45170	70	79360075	200	27408	23484	9.00	9.21	4	4	30.2	2.19	1.48*	1.44**
3453	Achn197381	Outer envelope pore protein 24, chloroplastic	AT1G45170	AT1G45170	70	79360075	259	27279	23484	8.93	9.21	5	5	29.7	2.56	1.92	1.70°
4485	Achn286131	Phosphatidylglycerol/phosphatidylinositol transfer protein	AT3G11780	AT3G11780	52	18399355	123	13274	24675	5.71	6.58	3	3	18.3	1.93	2.2	3.37
2546	Achn159811	Putative uncharacterized protein P0046B10.2-1	AT5G11420	AT5G11420	74	15239049	465	44759	43435	5.42	6.96	12	10	27.3	1.19**	-1.91	1.77
2575	Achn159811	Putative uncharacterized protein P0046B10.2-1	AT5G11420	AT5G11420	74	15239049	508	44600	43435	5.60	6.96	21	9	24	1.33**	-1.67	1.96
2746	Achn159811	Putative uncharacterized protein P0046B10.2-1	AT5G11420	AT5G11420	74	15239049	276	40682	43435	5.57	6.96	7	6	18.4	1.70°	-1.57	1.12**
4178	Achn230841	Wound/stress protein	PLAT1	AT4G39730	72	15236014	198	15823	18512	4.60	5.89	6	3	29.6	-1.12**	-2.82	2.73
4187	Achn230841	Wound/stress protein	PLAT1	AT4G39730	72	15236014	185	15748	18512	4.69	5.89	3	2	24.3	-1.04**	-2.38	2.12
4188	Achn012051	Wound/stress protein	PLAT1	AT4G39730	68	15236014	144	15711	17237	4.79	4.82	2	2	19.4	1.56°	-3.11	2.94

Supplementary Tab. S6. Differentially represented proteins in developing kiwifruit at T0-T3 that matched with homologous counterparts from the *A. thaliana* interaction network database. Data were obtained with STRING and results were visualized with Cytoscape software (Fig. 5).

STRING identifier	Protein description
AAT	Aspartate aminotransferase
ABA2	Short-chain dehydrogenase/reductase
ACO3	Aconitate hydratase 2
ACT11	Actin 4
ACT7	Actin 1
ADF1	Actin depolymerizing factor 2
ADH1	Alcohol dehydrogenase
ADK2	Adenosine kinase, putative
APX1	Ascorbate peroxidase
ASP5	Aspartate aminotransferase
AT1G08110	Lactoylglutathione lyase, putative
AT1G09640	Elongation factor 1 gamma-like protein
AT1G11860	Aminomethyltransferase
AT1G12000	Diphosphate/fructose-6-phosphate 1-phosphotransferase
AT1G24510	T-complex protein 1 subunit epsilon
AT1G29880	Glycine--tRNA ligase
AT2G40010	60S acidic ribosomal protein P0, putative
AT2G42520	ATP-dependent RNA helicase DED1
AT2G47500	Kinesin-related protein
AT3G02360	6-phosphogluconate dehydrogenase, decarboxylating
AT3G02530	T-complex protein 1 subunit zeta
AT3G03960	T-complex protein 1 subunit theta
AT3G11830	T-complex protein 1 subunit eta
AT3G12050	Activator of 90 kDa heat shock protein ATPase-like protein
AT3G18190	T-complex protein 1 subunit delta
AT3G24170	Glutathione reductase
AT3G26340	Proteasome subunit beta type
AT3G58610	Ketol-acid reductoisomerase
AT3G59480	Putative fructokinase 2
AT4G02930	Elongation factor Tu
AT4G32480	Alpha-toxin
AT5G08680	ATP synthase subunit beta
AT5G14590	Isocitrate dehydrogenase [NADP]
AT5G40150	Peroxidase 31, putative

AT5G57330	Aldose 1-epimerase, putative
AT5G61510	Quinone oxidoreductase, putative
ATCAD4	Cinnamyl alcohol dehydrogenase 1
AtCDC48C	Transitional endoplasmic reticulum ATPase
ATKRS-1	Lysine--tRNA ligase
ATMS1	5-methyltetrahydropteroyltriglutamate--homocysteine methyltransferase, putative
ATPQ	ATP synthase D chain, mitochondrial, putative
AXS2	Bifunctional polymyxin resistance arnA protein
BIP1	Heat shock 70 kDa protein C
BIP2	Heat shock 70 kDa protein C
CHLI1	Magnesium chelatase subunit I
CLPB3	Chaperone protein clpB
CLPC1	Clp protease ATP binding subunit
CLPR4	ATP-dependent Clp protease proteolytic subunit
cpHsc70-2	Chaperone DnaK
EIF4A1	Eukaryotic initiation factor 4A (ATP-dependent)
EIF4A-III	Putative atp-dependent rna helicase
FBA6	Fructose-bisphosphate aldolase 2
Fes1A	Hsp70 nucleotide exchange factor fes1
FTSZ2-1	Cell division protein ftsZ, putative
G6PD6	Glucose-6-phosphate 1-dehydrogenase
GAD	Glutamate decarboxylase, putative
GAMMACAL2	Carnithine operon protein CaiE
GDH1	Glutamate dehydrogenase
GF14	14-3-3 protein beta/alpha
GLX1	Lactoylglutathione lyase
GME	Epimerase
GSTF7	Putative glutathione S-transferase
HCF136	Ycf48-like protein
HCHIB	Chitinase
HIT3	Histidine triad nucleotide binding protein
HOG1	Adenosylhomocysteinase
Hop3	Stress-induced-phosphoprotein
HSC70-1	70-kDa heat shock protein
HSP17.6II	17.5 kDa class II heat shock protein
HSP21	Heat shock protein, putative
HSP60	60 kDa chaperonin
HSP70	70 kDa heat shock protein
Hsp81.4	Heat shock protein 90-2
HXK3	Hexokinase, putative

IDH1	Isocitrate dehydrogenase, putative
iPGAM1	Putative 2,3-bisphosphoglycerate-independent phosphoglycerate mutase
ISA3	Glycogen debranching enzyme
KASI	3-oxoacyl-[acyl-carrier-protein] synthase
KIS	Tubulin-specific chaperone A
LOS1	Elongation factor
LOS2	Enolase, putative
LPD1	Dihydrolipoyl dehydrogenase
MDAR1	Monodehydroascorbate reductase
MDH	Malate dehydrogenase, putative
MMZ3	Ubiquitin-conjugating enzyme, putative
MSD1	Superoxide dismutase
MTHSC70-2	Chaperone DnaK
mtLPD1	Dihydrolipoyl dehydrogenase
MTO3	S-adenosylmethionine synthase
NDPK1	Nucleoside diphosphate kinase 1
OSM34	Thaumatococcus-like protein
PAC1	Proteasome subunit alpha type
PAF1	Proteasome subunit alpha type
PB	ATP synthase subunit beta, chloroplastic
PBD1	Proteasome subunit beta type
PDIL1-6	Protein disulfide isomerase L-3b
PFK3	Phosphofructokinase, putative
PGK	Phosphoglycerate kinase
PGM2	Phosphoglucomutase, putative
PLP3b	Thioredoxin domain-containing protein, putative
PRF1	Profilin
PRF5	Profilin
PSBO1	Oxygen-evolving enhancer protein 1 of photosystem II
PSBP-1	Oxygen-evolving enhancer protein 2, chloroplastic
RACK1C_AT	Guanine nucleotide-binding protein subunit beta-like protein
RBCL	Ribulose biphosphate carboxylase large chain
RD21A	Cathepsin B-like cysteine proteinase 3
RH20	ATP-dependent RNA helicase
RHM1	UDP-glucose-4,6-dehydratase
RPN10	26S proteasome non-atpase regulatory subunit, putative
RPSAb	40S ribosomal protein sa, putative
RPT2a	26S protease regulatory subunit, putative
RPT5A	26S protease regulatory subunit 6A-like protein
SERPIN1	Serpin-like protein

SGT1B	SGT1, suppressor of G2 allele of SKP1
SHD	Chaperone protein HtpG
SHM1	Serine hydroxymethyltransferase
SHM4	Serine hydroxymethyltransferase
SKD1	ATP-dependent zinc metalloprotease FtsH
SNF7.1	Charged multivesicular body protein 4b, putative
SNF7.2	Charged multivesicular body protein 4b, putative
TCP-1	T-complex protein 1 subunit alpha
TIF3B1	Eukaryotic translation initiation factor 3 subunit B
TIM	Triosephosphate isomerase
TPI	Triosephosphate isomerase
TRA2	Transaldolase, putative
TUA2	Tubulin alpha-1 chain
TUB1	Tubulin beta-2 chain
TUB5	Tubulin beta-2 chain
TUB6	Tubulin beta-2 chain
UBC36	Ubiquitin-conjugating enzyme E2 36
UCH2	Ubiquitin carboxyl-terminal hydrolase family 1 protein
UGD2	UDP-glucose 6-dehydrogenase
UGD3	UDP-glucose 6-dehydrogenase
UGE1	UDP-glucose 4-epimerase
UGE5	UDP-glucose 4-epimerase
UGP1	Utp-glucose-1-phosphate uridylyltransferase, putative
UGP2	Utp-glucose-1-phosphate uridylyltransferase, putative
UNE5	Protein disulfide isomerase, putative
UXS6	Putative UDP-glucose 4-epimerase
VAB2	V-type ATP synthase beta chain
VHA-A	V-type ATP synthase alpha chain
VHA-E3	V-type proton ATPase subunit E

Supplementary Tab. S7. Kiwifruit allergens identified in this study as differentially represented during fruit development. Proteins were associated with specific entries in the Allergome database (<http://www.allergome.org>) or from literature data [21,22].

Protein	Allergene name	Allergome code	Reference
Cathepsin S - actinidin	Act d1	1	[45]
Cathepsin B-like - actinidin Act2a	Act d1	1	[45]
Major latex-like protein Bet VI class	Act d11	5903	[45]
Thaumatin-like protein	Act d2	747	[45]
Kiwellin	Act d5	2821	[45]
Profilin	Act d9	5740	[45]
Chitinase	Act d chitinase	1271	[46]

Legend to Supplementary Figures

Supplementary Fig. S1. Kiwifruits at the developmental stages T0, T1, T2 and T3. Sampling stage parameters and corresponding fruit pomological and compositional characteristics are summarized in Tab. 1 in the main text.

Supplementary Fig. S2. ^1H -NMR spectrum of an organic extract of kiwifruits in 2:1 v/v $\text{CDCl}_3/\text{CD}_3\text{OD}$.

Supplementary Fig. S3. Content of identified metabolites in kiwifruit during development (T0, T1, T2 and T3). Reported are metabolites identified in the corresponding hydroalcoholic extracts. Abbreviations are reported within the manuscript text.

Supplementary Fig. S4. Content of identified metabolites in kiwifruits during development (T0, T1, T2 and T3). Reported are metabolites identified in the corresponding organic extracts. Abbreviations: PUFA, poly-unsaturated fatty acids; DUFA, di-unsaturated fatty acids; UFA, unsaturated fatty acids; MUFA, mono-unsaturated fatty acids; SFA, saturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; DG, digalactosyl-diacyl-glycerol.

Supplementary Fig. S5. LC chromatogram recorded at 280 nm of the polyphenolic extract from kiwifruit at T0. Peaks are labeled according to Supplementary Tab. S2. Identified metabolites are numbered; metabolites with an unknown structure are indicated with capital letters.

Supplementary Fig. S6. Preparative two-dimensional gel electrophoresis of the outer pericarp proteins extracted from kiwifruit. The figure reports the results of a Sypro Ruby-stained gel loaded with a sample containing equimolar amounts of protein extracts from kiwifruit at T0, T1, T2 and T3. Preparative and analytical 2D-DIGE was performed as reported in the experimental section. Differentially represented spots are numbered on the gel image; they were picked automatically for

protein identification and analyzed for their tryptic digests by nLC-ESI-LIT-MS/MS. Protein identification results are reported in Supplementary Tables S4 and S5.

Supplementary Fig. S7. Heat-map representation and hierarchical cluster analysis of differentially represented spots (DRSs) after 2D-DIGE analysis of protein extracts from kiwifruit outer pericarp samples taken at T0-T3 stages. Hierarchical cluster analysis of DRSs was performed using Genesis 1.7.7 platform (Institute for Genomics and Bioinformatics, Graz University of Technology).

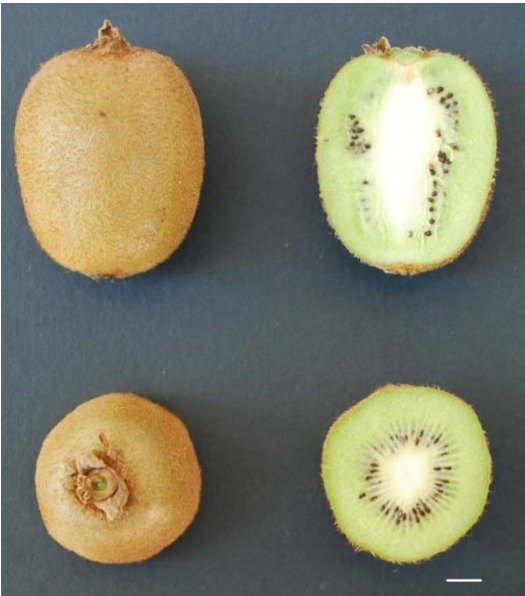
Supplementary Fig. S8. Heat-map representation and hierarchical cluster analysis of differentially represented proteins (DRPs) after combined 2D-DIGE/nLC-ESI-LIT-MS/MS analysis of protein extracts from kiwifruit outer pericarp samples taken at T0-T3 stages. Panels A to N report proteins organized according to their functional category grouping. Hierarchical cluster analysis of DRPs was performed using Genesis 1.7.7 platform (Institute for Genomics and Bioinformatics, Graz University of Technology).



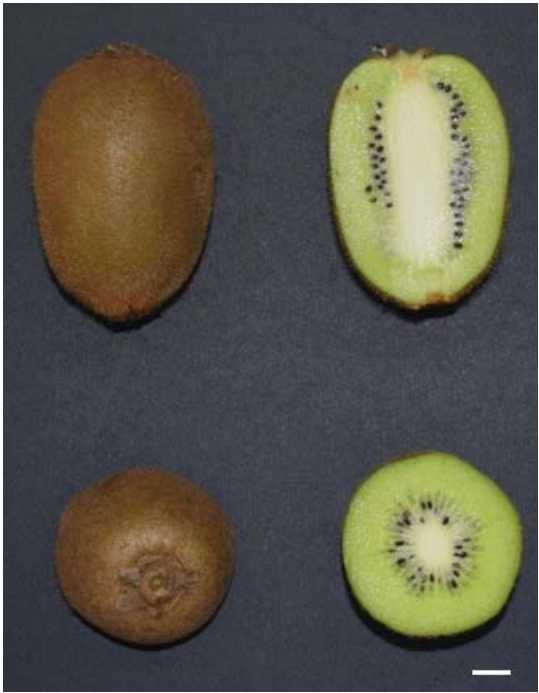
T0



T1

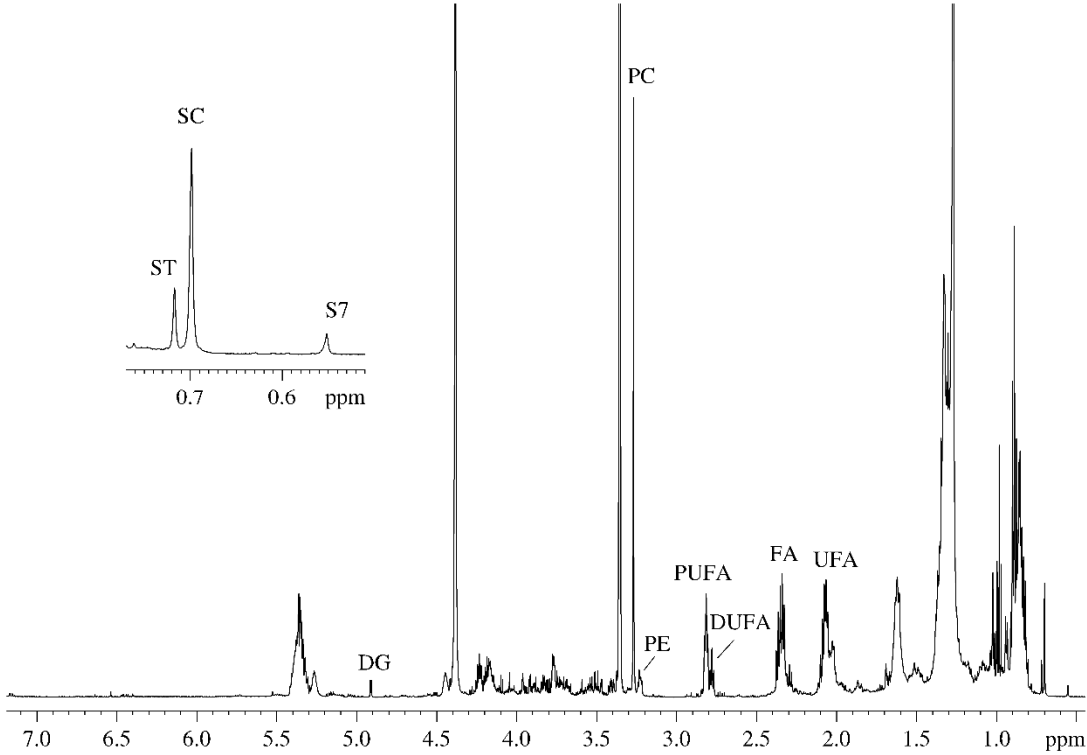


T2

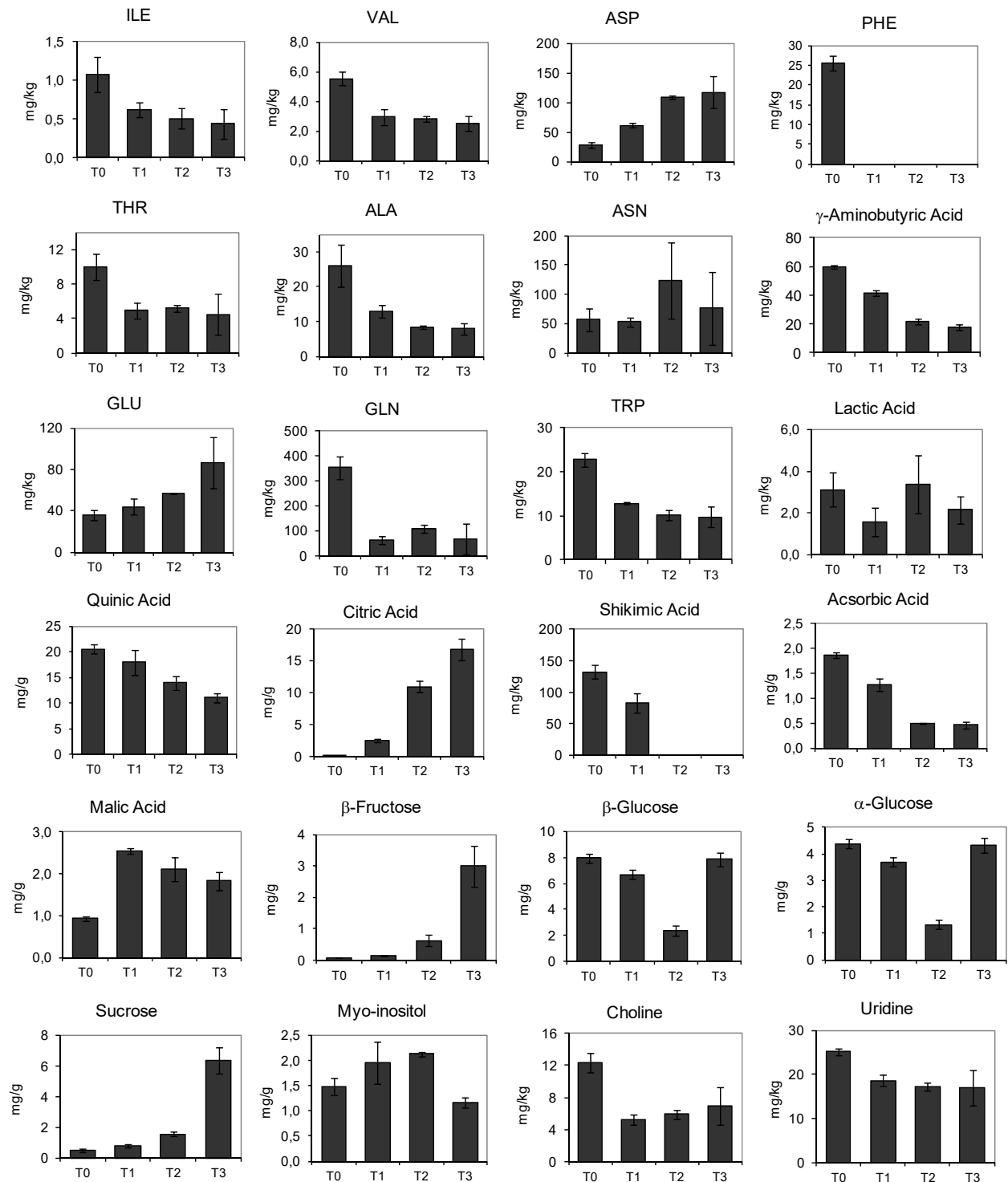


T3

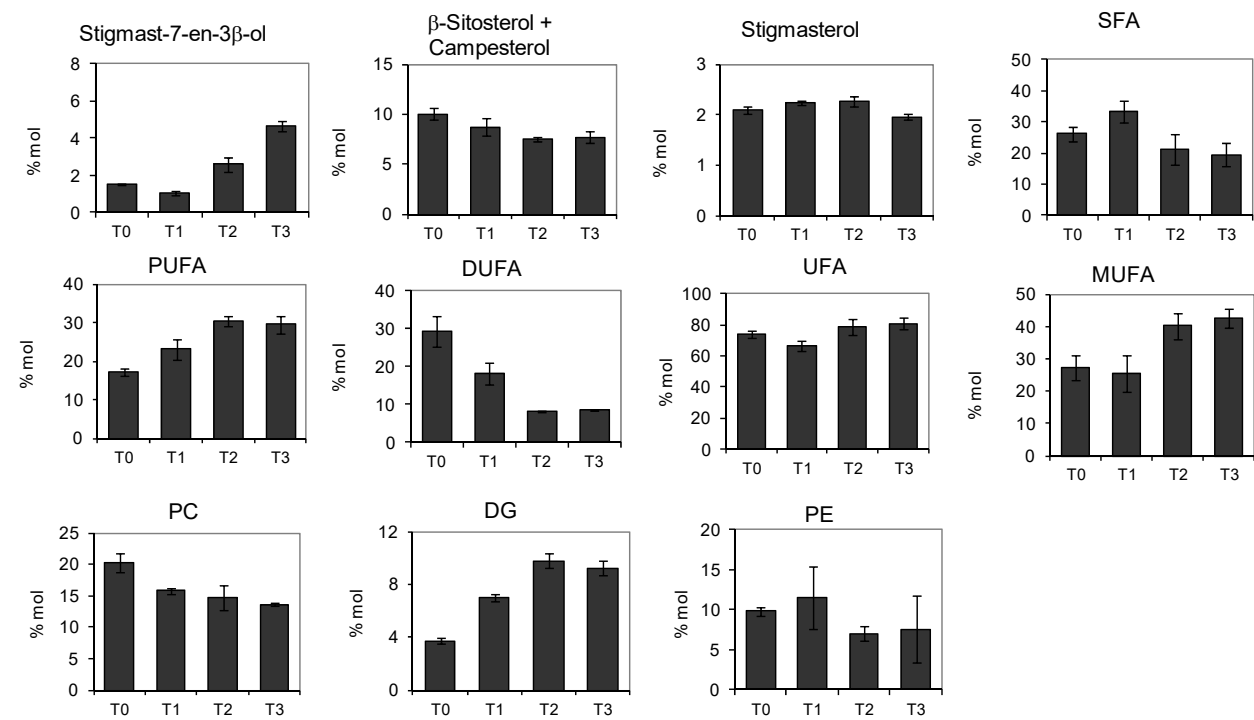
Salzano et al., Supplementary Fig. S2

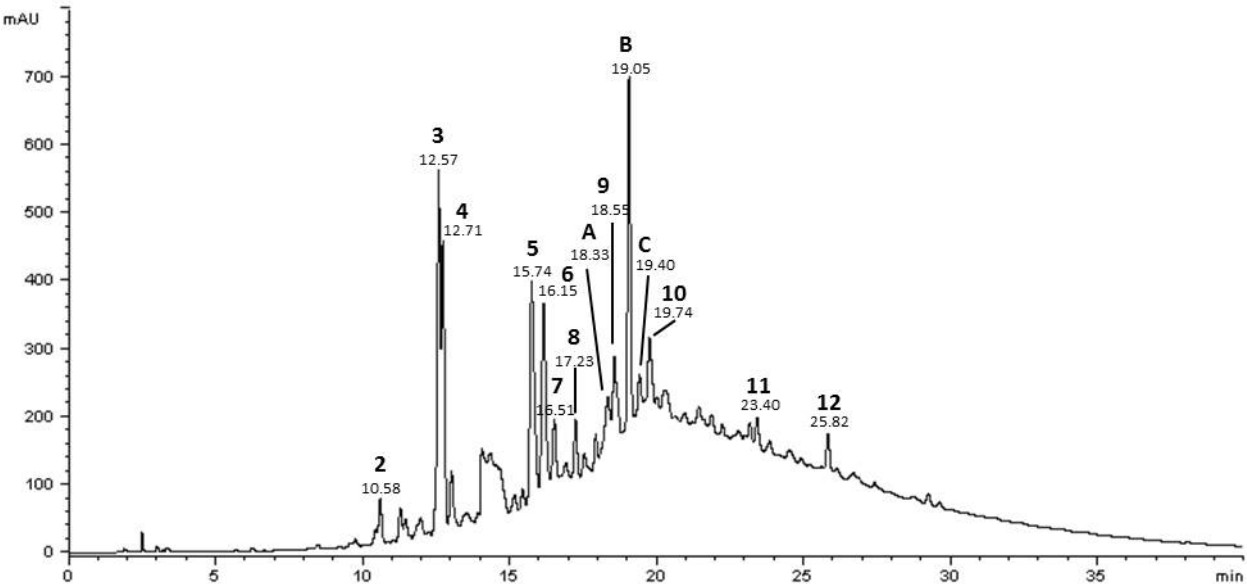


Salzano et al., Supplementary Fig. S3

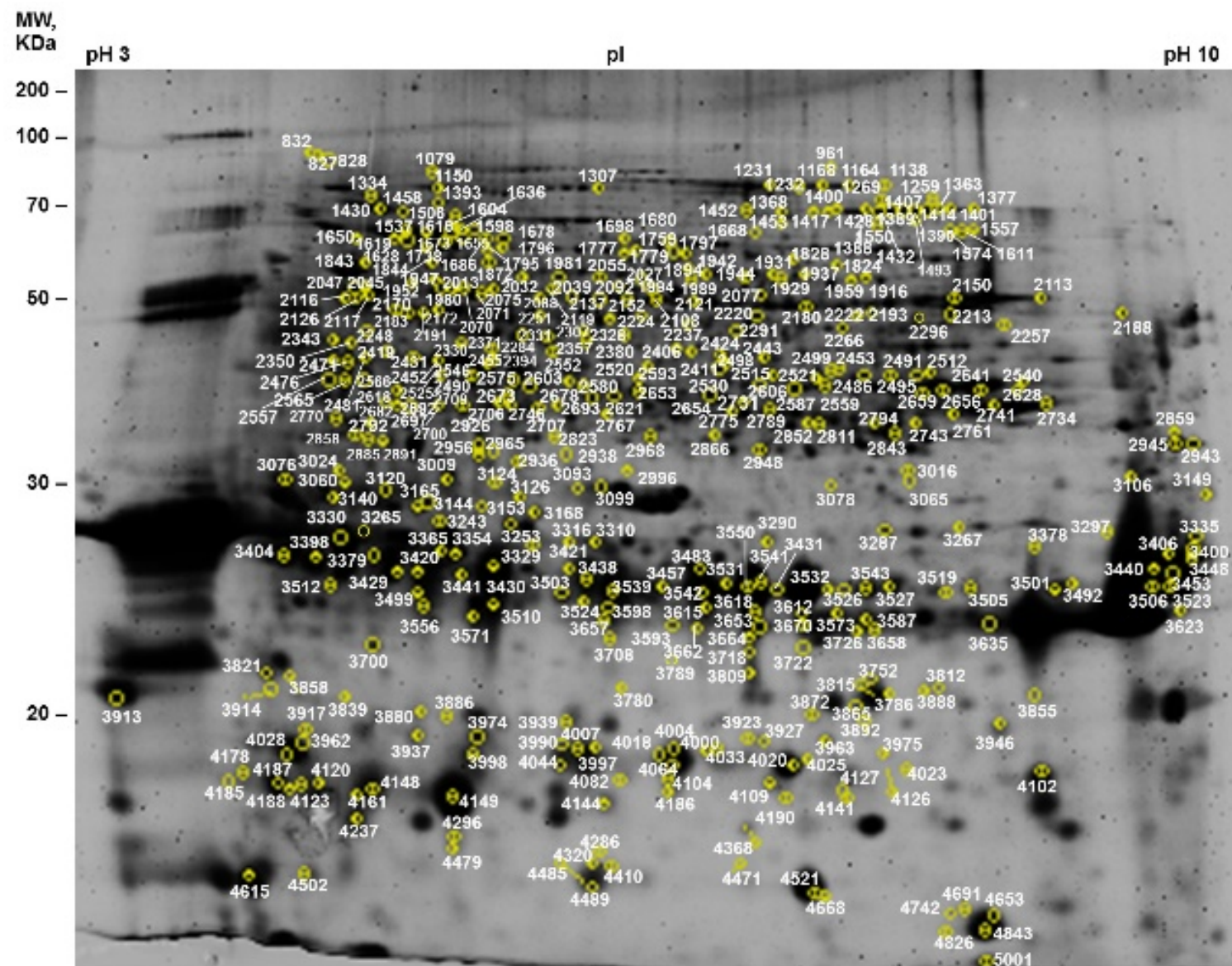


Salzano et al., Supplementary Fig. S4

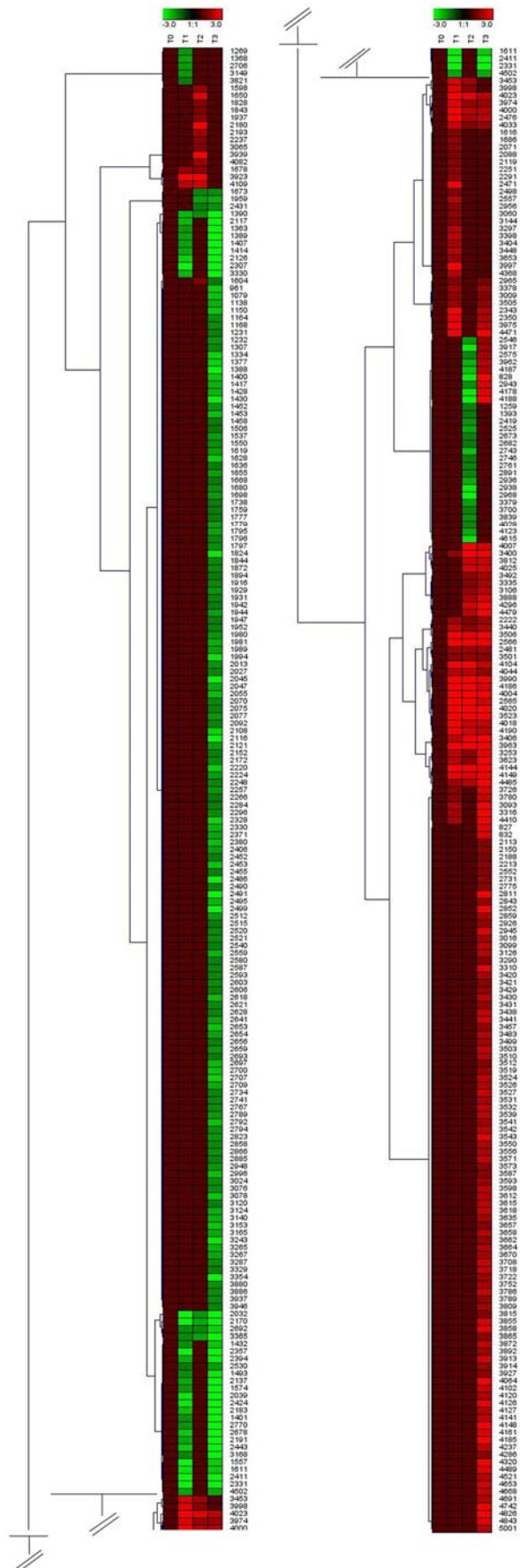


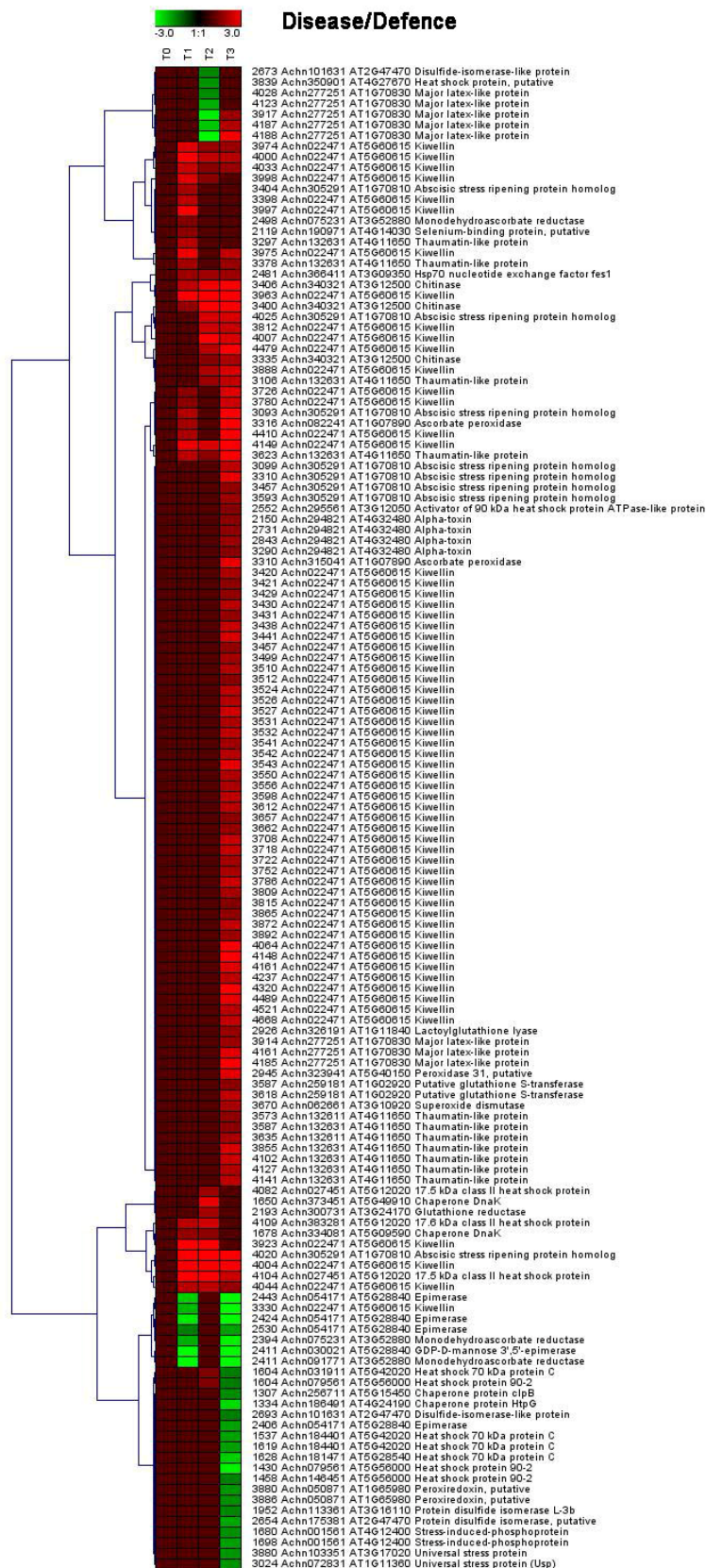


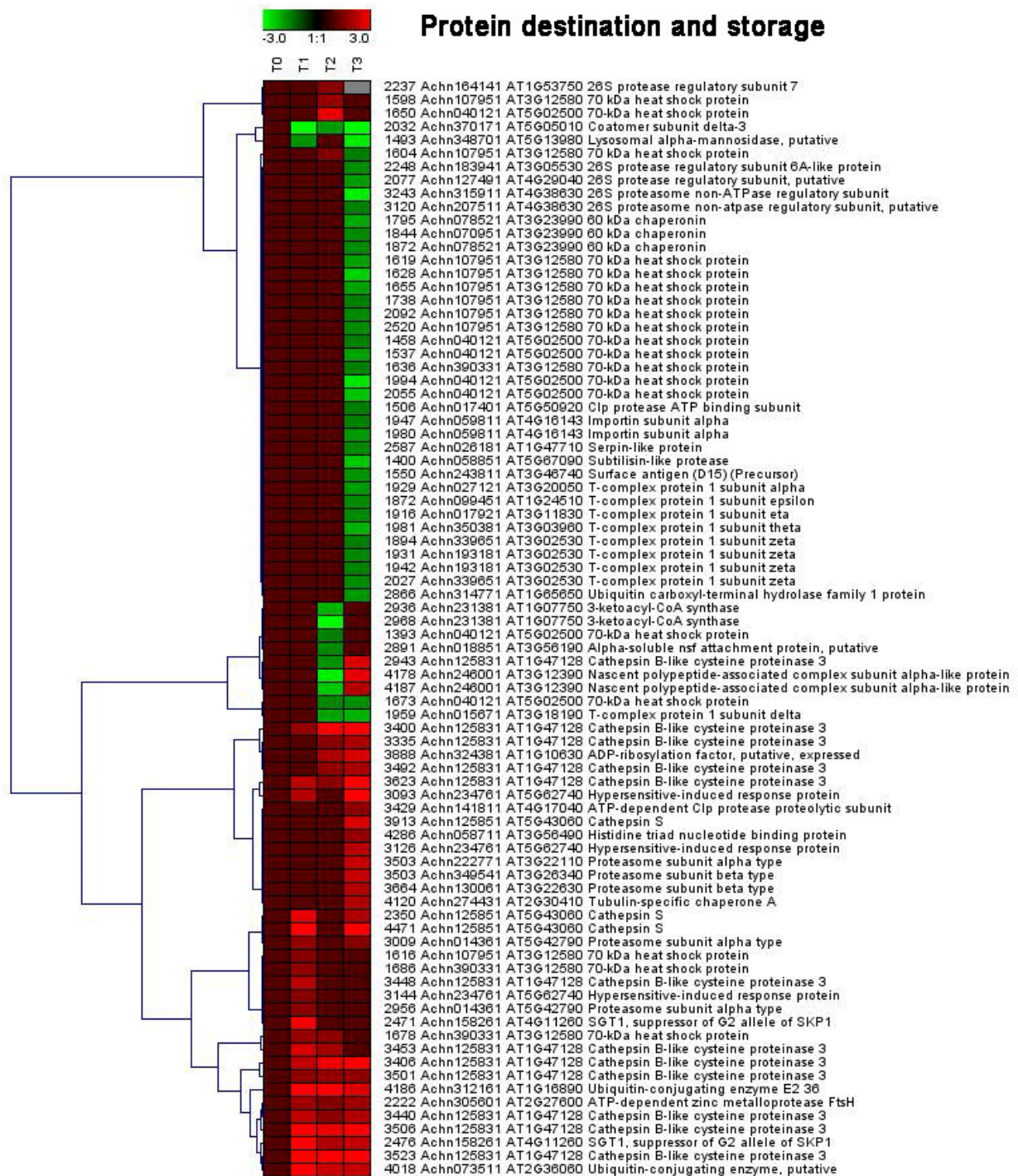
Salzano et al., Supplementary Fig. S6



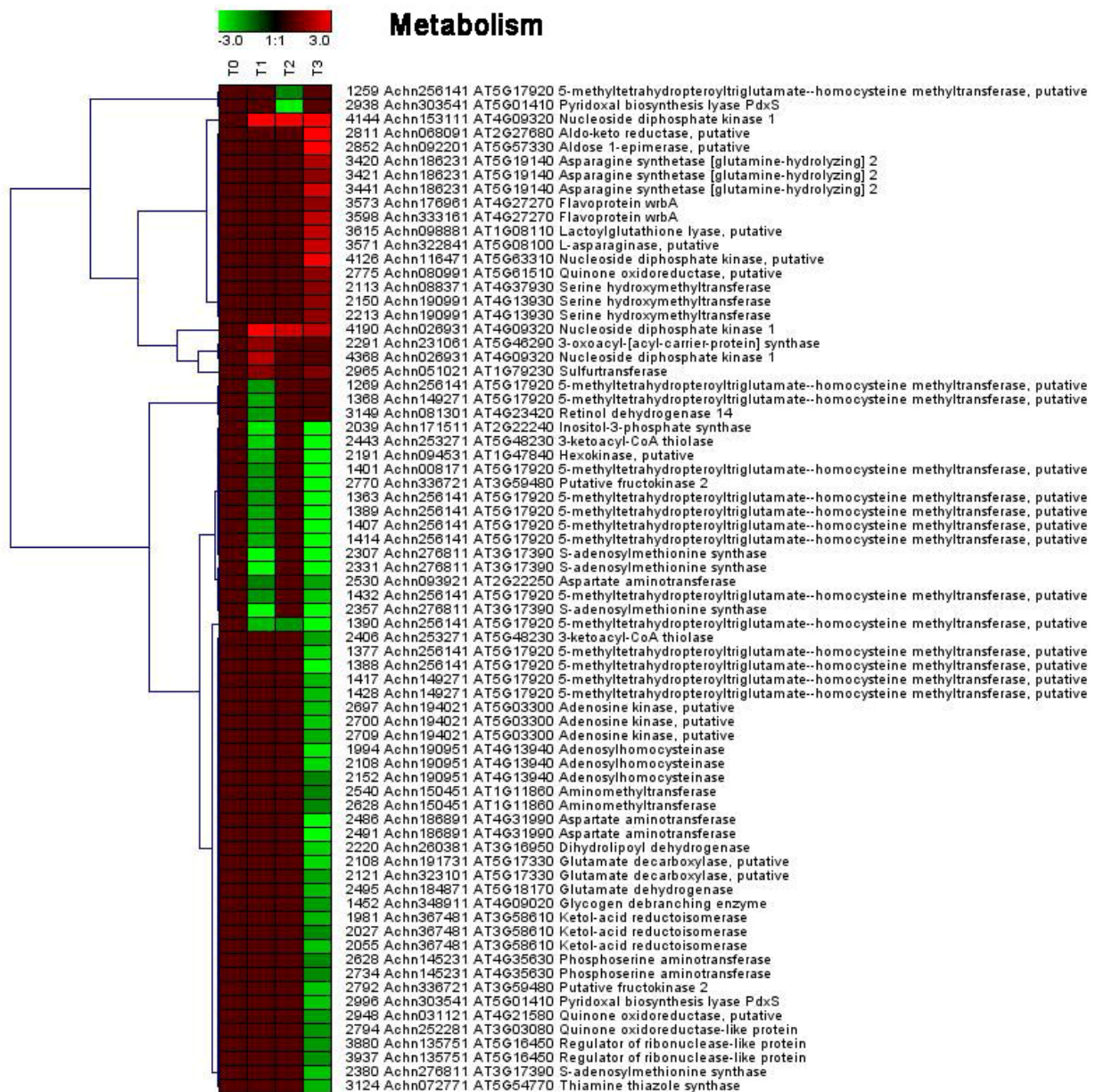
Salzano et al., Supplementary Fig. S7



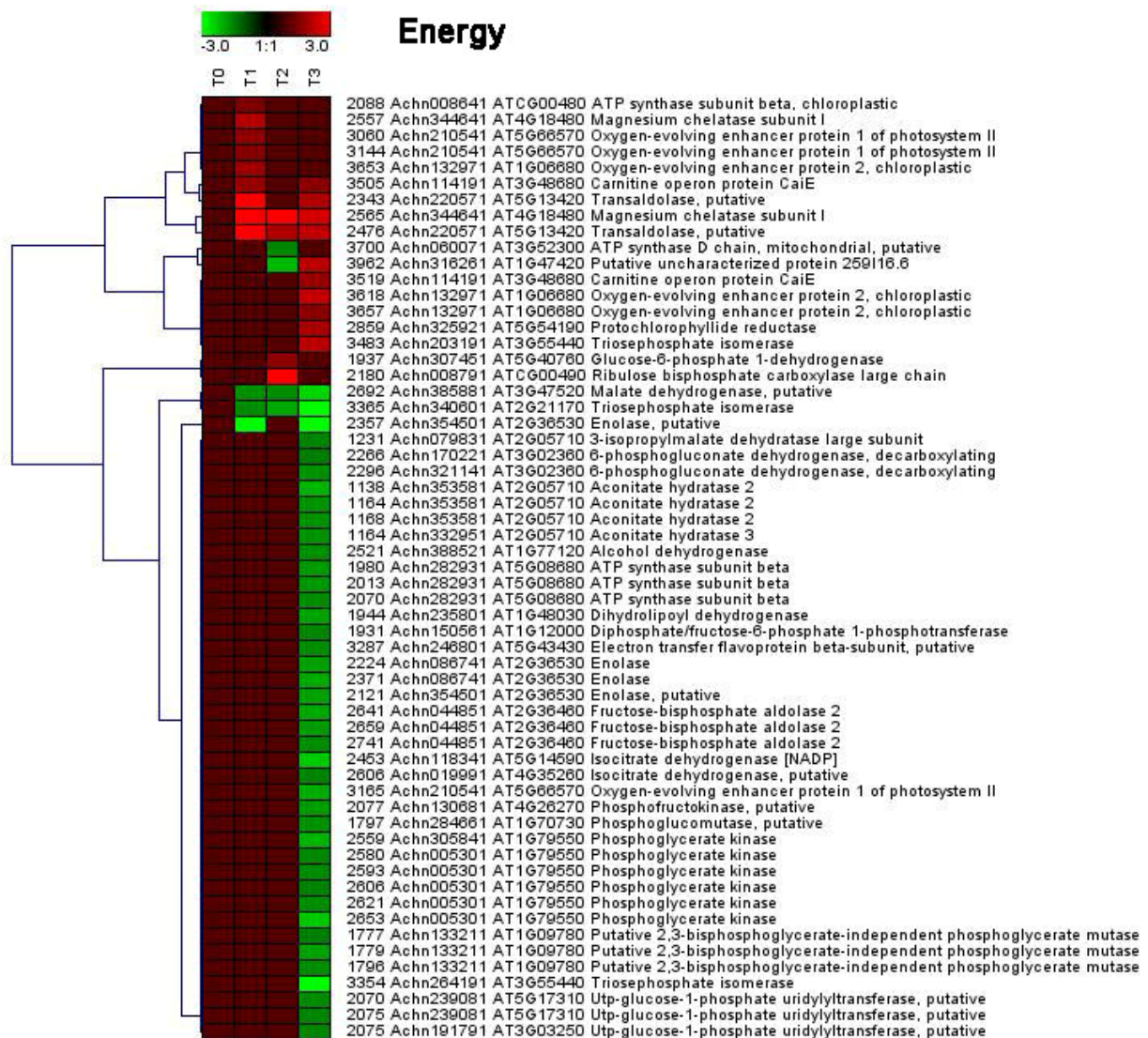




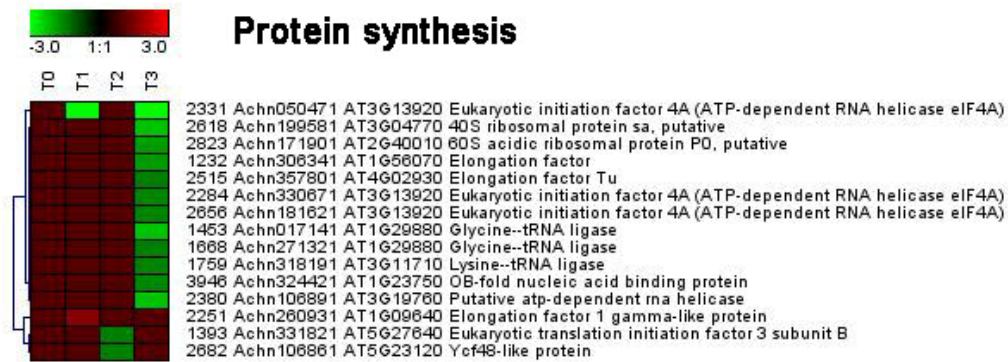
Salzano et al., Supplementary Fig. S8C

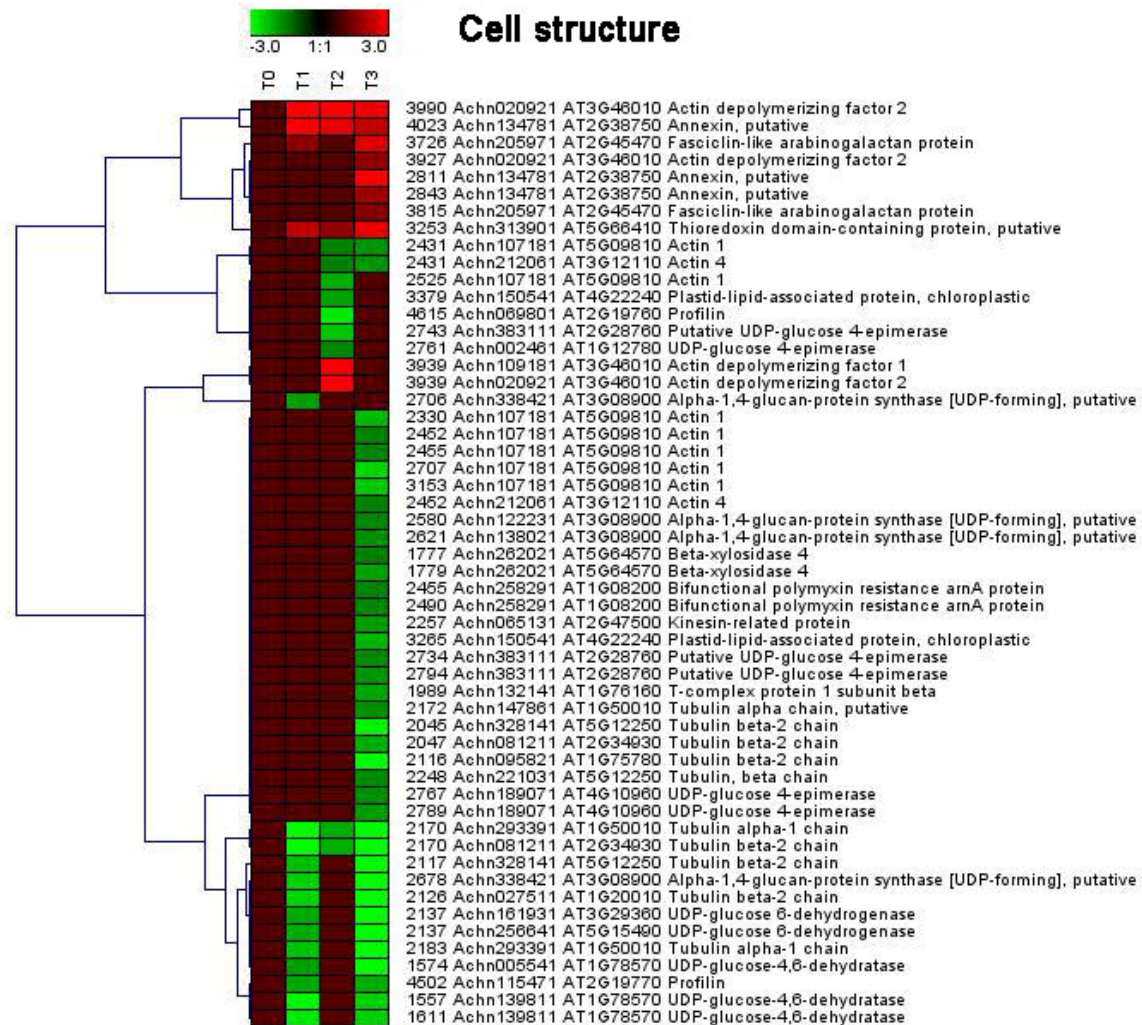


Salzano et al., Supplementary Fig. S8D



Salzano et al., Supplementary Fig. S8E





Salzano et al., Supplementary Fig. S8G, 8H and 8I

