ACÁCIO RODRIGUES SALVADOR

METABOLIC AND TRANSCRIPTIONAL ASPECTS OF *Capsicum chinense* FRUITS IN DIFFERENT DEVELOPMENTAL STAGES

Thesis submitted to the Plant Physiology Graduate Program of the Universidade Federal de Viçosa in partial fulfillment of the requirements for the degree of *Doctor Scientiae*.

Adviser: Adriano Nunes Nesi

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Assent:

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Acácio Rodrigues Salvador
Author

_____
Adriano Nunes Nesi
Adviser

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ABSTRACT

SALVADOR, Acácio Rodrigues, D.Sc., Universidade Federal de Viçosa, February, 2021. **Metabolic aspects of fruits from contrasting *Capsicum chinense* accessions throughout development.** Advisor: Adriano Nunes Nesi.

The *Capsicum* genus is highly phenotypically diverse and considered nonclimacteric. Although some studies with nonclimacteric fruits have already been carried out, knowledge about the regulation between transcripts and metabolites is still scarce. We selected phenotypically contrasting *Capsicum chinense* accessions and analyzed in detail the metabolism in placenta and pericarp fruit and performed a transcripts profile to identify possible relationships between metabolism and gene expression. We quantified and analyzed primary metabolites, capsaicin, phenols, proteins, total amino acids, sugars and starch in three stages of development (20, 45 and 60 days after anthesis) and identified 16803 genes expressed from different functional classes and correlated with 69 metabolites quantified in two stages of development (20 and 60 days after anthesis). Our results suggest that most of the observed metabolic variability is between the stages of fruit development and not between accessions. In addition, we show that pungency, determined by the metabolism of the placenta, significantly interferes with the metabolism of the fruit. The integration of the transcript and metabolite profile through multivariate analysis provides several correlations between the metabolic and transcript classes that can be studied in detail in future research. Our results suggest that there is a complex control during fruit development that determines phenotypic characteristics, in addition, our data increase our knowledge about the regulation of TSS accumulation in nonclimacteric fruits.

Keywords: Capsaicin. Primary metabolites. Fruits. Transcriptome.

RESUMO

SALVADOR, Acácio Rodrigues, D.Sc., Universidade Federal de Viçosa, fevereiro de 2021. **Aspectos moleculares e metabólicos de frutos de *Capsicum chinense* em diferentes fases de desenvolvimento.** Orientador: Adriano Nunes Nesi.

O gênero *Capsicum* é altamente diversificado fenotipicamente e considerado não climatérico. Embora alguns estudos com frutos não climatéricos já foram realizados, o conhecimento a respeito da regulação entre transcritos e metabólitos ainda é escasso. Nós selecionamos acessos de *Capsicum chinense* contrastantes fenotipicamente e analisamos de forma detalhada o metabolismo em placenta e pericarpo de frutos e realizamos um perfil de transcritos para identificar possíveis relações entre o metabolismo e a expressão gênica. Quantificamos e analisamos metabólitos primários, capsaicina, fenóis, proteínas, aminoácidos totais, açúcares e amido em três estágios de desenvolvimento (20, 45 e 60 dias após a antese) e identificamos 16803 genes expressos de diferentes classes funcionais e correlacionamos com 69 metabólitos/ classes de metabolitos quantificados. Nossos resultados sugerem que a maior parte da variabilidade metabólica observada está entre os estágios de desenvolvimento do fruto e não entre os acessos. Além disso, evidenciamos que a pungência, determinada pelo metabolismo da placenta, interfere significativamente no metabolismo do fruto. A integração do perfil de transcritos e metabólitos através de análises multivariadas fornece várias correlações entre as classes metabólicas e de transcritos que poderão ser estudas detalhadamente em pesquisas futuras. Nossos resultados sugerem que há um complexo controle durante o desenvolvimento dos frutos que determinam as características fenotípicas, além disso, nosso dados aumentam nosso conhecimento a respeito da regulação do acúmulo de TSS em frutos não climatéricos.

Palavras-chave: Capsaicina. Metabólitos primários. Frutos. Transcriptoma.

SUMMARY

GENERAL INTRODUCTION

The *Capsicum* genus comprises a highly diverse group of sweet and spicy peppers. Interestingly, the fruits of *C. chinense* have variability in terms of size, shape, color and chemical composition (Lannes et al., 2007). In addition to the typical pungency characteristic of the fruits of this genus, resulting from the presence of capsaicinoids (Kim et al., 2014), fruits of C. *chinense* are also recognized as excellent sources of carotenoids, ascorbic acid, tocopherols and flavonoids. The fruits of this species are used mainly as fresh or cooked food, condiment or seasoning and as a coloring agent in the food industry (Lannes et al., 2007).

Despite the great potential for use in breeding and biotechnological programs, only morphological characterization has been used to study and explore the genetic variability of pepper populations. Thus, it is necessary to know how the genotypes of a population are divergent in terms of biochemistry and molecular biology so that they can be used in the development of new populations, strains and cultivars with agronomic and industrial characteristics superior to those already existing in the market, in order to meet the current demands (Barbazuk et al., 2007; Trick et al., 2009). Transcriptome data can provide important insights into the genes and families of genes involved in important biological processes, as well as an excellent platform for exploring polymorphic molecular markers (Wang et al., 2009).

Through the metabolic profile it is possible to detect and subsequently identify compounds that correlate with characteristics of interest, such as the chemical composition of the fruits (Fernie and Schauer, 2009; Wahyuni et al., 2011). Thus, analyzing together the transcriptome and metabolite profiling of fruits of Brazilian accessions of pepper (*Capsicum chinense*) with contrasting characteristics, allow a wide genetic and metabolic characterization of these fruits.

Therefore, the present work aimed the understanding of the biochemical and molecular mechanisms involved in the determination of characteristics of economic interest in *C. chinense* fruits. It is believed that the results obtained from this study will provide new information for breeding programs, indicating metabolic and molecular information associated with characteristics related to fruit quality.

CHAPTER 1

Metabolic aspects of fruits from contrasting *Capsicum chinense* accessions throughout development

ABSTRACT

Capsicum chinense fruits vary greatly in pungency, from sweet to some of the hottest known chili peppers. Fruit pungency is caused by accumulation of capsaicinoids, secondary metabolites whose relation to primary metabolism remains unclear. We selected ten diverse accessions of *C. chinense* with different fruit size, shape and color, and a wide range of pungency levels and conducted a deep characterization of their metabolic profile in the fruit placenta and pericarp across fruit development. We quantified and analysed primary metabolites, capsaicin, phenols, protein, total amino acids, sugars and starch at three developmental stages (20, 45 and 60 days after anthesis). Despite considerable variation in fruit pungency among the ten accessions, the results showed that composition and metabolite levels in both placenta and pericarp are generally uniformly stable across accessions. Most of the metabolite variability was observed between the fruit developmental stages rather than among the accessions. Interestingly, differences in metabolite adjustment in the placenta were observed among the pungent and non-pungent accessions, which seem be related to differences in the genetic background and their respective geographic origin. Our results provide novel insights on the high coordination between primary metabolism and capsaicin production in *C. chinense* fruit with some evidence that pungency, determined by placenta metabolism, interferes significantly on fruit metabolism in general.

1. INTRODUCTION

Capsicum species are members of the *Solanaceae* family, along with other major crops such as tomato and potato. Unlike tomatoes, pepper fruits are generally considered non-climacteric (Klie et al., 2014), but growing evidence suggests their ripening dynamics to be genotype-dependent, with intermediate or climacteric types (Paul et al., 2012; Hou et al., 2018). The *Capsicum* genus comprises a highly diverse group of hot (pungent) and sweet (non-pungent) peppers, with ca. 40 species described to date, originating from the Americas (Jarret et al., 2019), of which five are considered domesticated. Although most of the research and breeding efforts have been carried out in C. annuum (Pickersgill, 1997), the greatest variation in fruit size, shape and pungency is found in the species C. *chinense*, which includes the famous Habanero and Carolina Reaper peppers, among more than 700 other known varieties (DeWitt and Bosland, 2009).

Pungency in *Capsicum* fruits is caused by capsaicinoids, a family of alkaloids unique to the genus (Bennett and Kirby, 1968). Capsaicinoids are a group of compounds, with capsaicin and dihydrocapsaicin representing approximately 90% of the total fruit capsaicinoid content (Wahyuni et al., 2013b). Capsaicinoids display a wide range of biochemical and physiological properties that make them attractive to the food and pharmaceutical industries (Anand and Bley, 2011; Chapa-Oliver and Mejía-Teniente, 2016; Varghese et al., 2017). They are further interesting from an evolutionary ecology perspective, as it has been suggested that pungency in chilies may be an adaptive response to selective pressure by microbial pathogens (Tewksbury et al., 2008). Moreover, in C. chacoense pungency appears to be functionally integrated with traits influencing water-use efficiency (Haak et al., 2012). Considerable interest exists, therefore, in unveiling the regulation of capsaicinoid biosynthesis in pepper fruits (Stewart et al., 2007; Mazourek et al., 2009; Stellari et al., 2010; Aza-González et al., 2011; Haak et al., 2012). In Cayenne pepper (C. annuum L.) capsaicinoid levels fluctuate throughout fruit development, reaching a maximum at 40 days after anthesis and then steadily declining (Barbero et al., 2014). Capsaicinoids synthesis is usually limited to the epidermal cells of the placenta, and afterwards are exported and stored in vesicles ("blisters") located on the placenta surface (Mazourek et al., 2009). However, in some extremely highly pungent cultivars of C. *chinense*, some of the genes involved in the capsaicinoid biosynthesis can also be expressed in

the pericarp and, together with the formation of ectopic secreting vesicles in the pericarp, they may contribute to capsaicinoid accumulation in the whole fruit (Bosland et al., 2015; Tanaka et al., 2017). In addition, capsaicinoids may also be found in other parts of the fruit pepper such as the seeds (Conforti et al., 2007).

Processes such as the biosynthesis of carotenoids, degradation of chlorophylls and the differentiation of chloroplasts to chromoplasts take place during fruit maturation (Bouvier et al., 1998; Martí et al., 2009). The levels of glucose, fructose and sucrose, the major sugars present in Habanero (*C. chinense*) fruits, increase during fruit development up to 52 days after anthesis (DAA), declining afterwards; starch levels, on the other hand, decrease during fruit development (Bouvier et al., 1998; Martí et al., 2009; Osorio et al., 2012a). Starch degradation remobilizes carbon skeletons required for the synthesis of soluble sugars such as glucose and fructose. In addition, sucrose content increases at the initial stages of development by translocation from photosynthetic tissues (Beckles, 2012; Aizat et al., 2014). This is consistent with the increase in total soluble solids (TSS) content observed in non-climacteric fruit during ripening (Niklis et al., 2002; Martínez et al., 2007), similar to the increase in soluble sugars that occurs in climacteric fruits (Baxter et al., 2005; Beckles et al., 2012). In tomato fruits, sugars represent around 55% of TSS (Helyes et al., 2006) and, noteworthy, other metabolites such as malate and citrate also contribute to the increase in TSS at advanced stages of development (Mounet et al., 2009). Organic acids contribute to flavor and fruit quality, and their content varies depending on tissue type, developmental stage, and species (Batista-Silva et al., 2018).

Pepper fruits are also recognized as valuable sources of important compounds for human health such as carotenoids (provitamin A), ascorbic acid (vitamin C), tocopherol (vitamin E), and flavonoids (Topuz and Ozdemir, 2007; Wahyuni et al., 2013a). These compounds are widely used in the food, medical and pharmaceutical industries (Lee et al., 1996; Caterina et al., 2000; Ochoa-Alejo and Ramirez-Malagon, 2001; Daood et al., 2014). Other secondary metabolites, like polyphenols derivatives from cinnamic acid, are also of interest due to their antioxidant activity and as intermediates in the biosynthesis of capsaicinoids and flavonoids (Materska et al., 2003; Broderick and Cooke, 2009; Wahyuni et al., 2013a). However, it remains unclear how exactly the biosynthesis and accumulation of these compounds is regulated during ripening, and in particular how primary metabolism feed into their biosynthesis

(Wahyuni et al., 2013a). To date, a thorough characterization is still lacking of the dynamic metabolic changes that occur in different compartments of the chili pepper fruit during ripening.

Here, we used a panel of ten Brazilian *C. chinense* accessions of varying degrees of pungency, from sweet to extremely hot, and with contrasting fruit size, shape and color (Lannes et al., 2007) to investigate the metabolic changes in the placenta and pericarp during fruit development and their potential connection to capsaicinoid metabolism. For this, an analysis of primary and secondary metabolites, as well as multivariate analyzes were performed on the placenta and pericarp of pepper fruits pungent and non-pungent during three stages of development, 20, 45, and 60 days after anthesis. In general, the metabolism in the placent differentially regulated as compared to pericarp tissue during the pepper fruit development and these changes may be yet verified between the contrasting accessions of *C. chinense*.

2. MATERIAL AND METHODS

2.1 Plant material and experimental conditions

Among a population of 49 Brazilian *C. chinense* accessions described previously (Lannes et al., 2007; Finger et al., 2010; Rosado-Souza et al., 2015), we selected eight accessions of different fruit shape (round or elongated), color (red to yellow) and pericarp capsaicinoid content. These eight accessions did not differ significantly in leaf physiological parameters (Rosado-Souza et al., 2015). Two commercial varieties, Habanero (pungent cultivar of Mexican origin) and Biquinho (non-pungent cultivar from Brazil), were used as contrasting controls (Fig. 1).

Seeds were bulked for two generations, avoiding cross-pollination (Bosland, 1993). They were germinated on commercial substrate (Tropstrato HT hortaliças) and the seedlings were transplanted 25 days after sowing into 5-liter pots with a mixture of soil and substrate (1:1 w/w) fertilized with fertilizer 20-5-20 (N-P2O5-K2O Heringer). Plants were grown in a greenhouse located at Viçosa (642 m asl, 20o45’ S; 42o51’ W), Minas Gerais, Brazil. Plants were watered regularly and fertilized weekly with 40 mL of a solution containing 5.0 g of (NH4)2SO4 and 2.5 g of KCl per liter.

2.2 Fruit harvests

For accurate determination of the fruit physiological age, flowers were tagged at anthesis as shown in (Osorio et al., 2012b) and samples of fruit pericarp and placenta were collected at midday at 20, 45 and 60 days after anthesis (DAA). Samples were immediately frozen in liquid nitrogen and stored at -80 °C. Six fruits per accession and development stages were collected from different plants and used for the analyses.

2.3 Determination of capsaicin content

Fruit capsaicin content in the pericarp and placenta tissues was evaluated by high performance liquid chromatography (HPLC) according to Maillard et al., 1997 with modifications. Approximately 15 mg of freeze-dried were suspended in 2 mL of methanol: water (60:40, v/v) and sonicated for 15 min at room temperature. The mixture was centrifuged at 1.600 g for 15 min and the supernatant filtrated through a Millipore membrane of 0.22 µM. 30 µL from the filtrate was injected on a HPLC coupled with a UV detector at 229 nm and 281 nm and equipped with Agilent ZORBAX Eclipse Plus C18 column (150 mm, particle size 3 µM in diameter). Capsaicin were separated isocratically with a mobile phase composed of methanol: water: acetic acid (70: 28: 2, v/ v/ v) and a flow rate of 1.0 mL min-1 at 20°C. Absolute quantification of capsaicin in the samples was determined based upon calibration curves using authentic standards (Sigma- Aldrich).

2.4 Determination of primary metabolites and total soluble phenols

Metabolite extraction was performed by grinding pericarp and placenta samples in liquid nitrogen followed by ethanolic extraction as described by (Cross et al., 2006b). The levels of starch, sucrose, fructose, and glucose in the tissues were determined as previously described (Fernie et al., 2001). Total protein and total amino acid contents were quantified as previously reported (Cross et al., 2006a). Malate and fumarate levels were determined according to Nunes-Nesi et al., 2007.

2.5 Experimental design and statistical analyses

The experiment was performed in randomized block design, using six replicates per accession, a replicate analyzed consisted of several fruits from the same plant that grown in an individual 5 L pot. Statistical analyses were performed using the GENES program (Cruz, 2006). All data were submitted to analysis of variance (ANOVA) followed by Tukey's (HSD) test at 5% probability. The multivariate analysis by principal component analysis (PCA) and clustering thorough Pearson distance method were performed using Minitab 17 software. The Pearson's correlations were performed using R script provided by the Rbio software (www.biometria.ufv.br) and we applied false discovery rate (FDR)-controlling method using the same script and software (Benjamini and Hochberg, 1995). The significance of Pearson's correlation coefficient was assessed by the Student´s t-test for examining the relationships among variables.

3. RESULTS

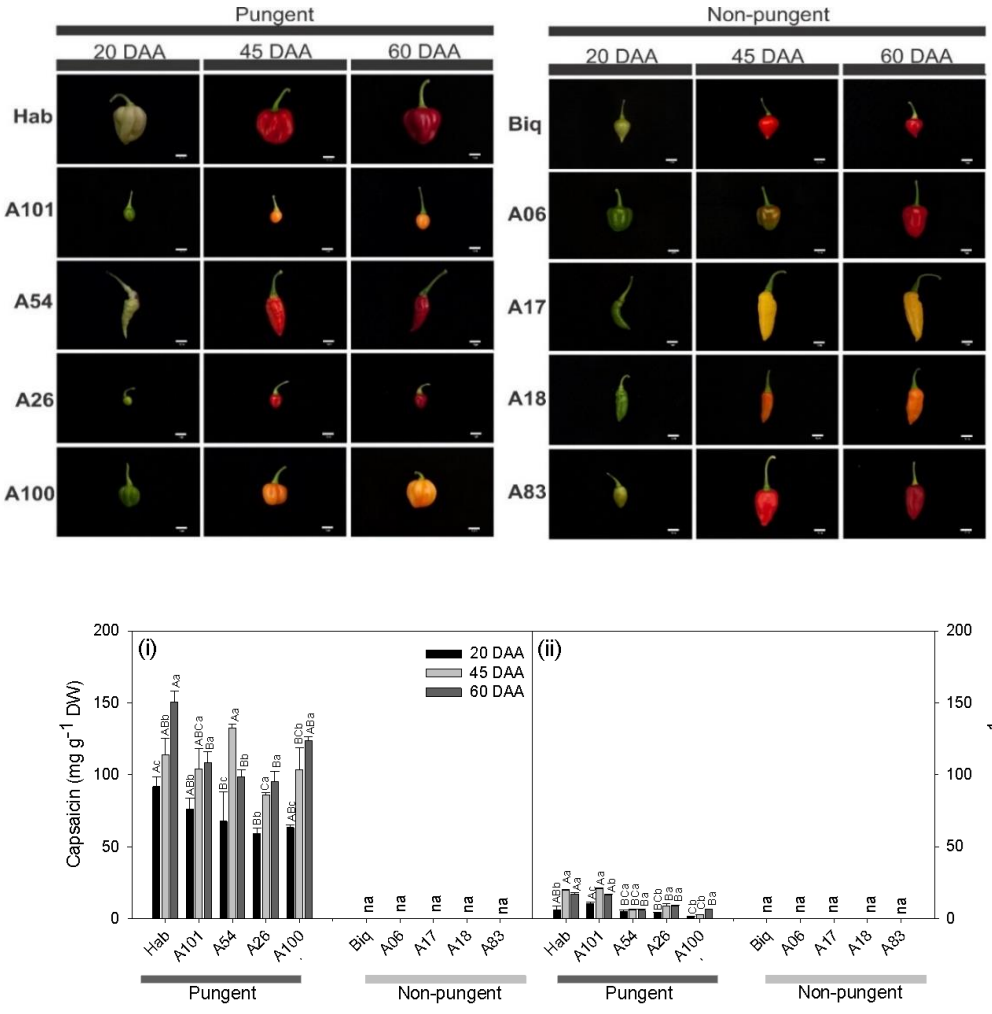
3.1 Phenotypic variation in the fruit pericarp of ten accessions of *C. chinense* during fruit development

To characterize the variability in color, shape, flavor and biochemical components of fruits within the studied population, we sampled fruits from each of the ten accessions of *C. chinense* and evaluated them at three stages post-anthesis (20, 45 and 60 DAA) in the placenta and pericarp tissues. Variation in shape, size and color between the selected accessions throughout development was evident (Fig. 1A). The fruits from accessions A17, A18, A54 and A83 have elongated shape, in contrast with the round shape of the others (accessions A06, A26, A100 and A101). Regarding fruit color, the accessions A17, A18, A100 and A101 were yellow, while A54, A83, A06, A26, AC1 and AC2 had red fruits at full ripe stage. Except for accession A06, which was still transitioning from green to red at 45 DAA, all remaining accessions were uniform at 45 DAA, having acquired their final fruit color (Fig. 1A).

Quantification of capsaicin levels in pericarp and placenta demonstrated the variability in fruit pungency of the genotypes (Fig. 1B). As expected, capsaicin was more abundant in placenta than pericarp tissues. The commercial cultivar Habanero (Hab) showed the highest level of capsaicin (~150 mg g-1 DW at 60 DAA; Fig. 1B-i).

Capsaicin content increased with fruit maturity in almost all accessions, with the maximum levels observed in fully mature red fruits. Large variations in capsaicin levels were also observed in placenta and pericarp during fruit development (Fig. 1B). Hab (placenta) and A100 (placenta and pericarp) showed increased capsaicin levels from green (20 DAA) to mature fruits (60 DAA). The accessions A101 (placenta), A26 (placenta), Hab (pericarp), A54 (pericarp) and A26 (pericarp) maintained the pungency levels from 45 to 60 DAA. Interestingly, A54 (placenta) and A101 (pericarp) peaked at 45 DAA and their content then decreased at 60 DAA. The amounts of capsaicin in non-pungent genotypes Biquinho (Biq) and accessions A06, A17, A18 and A83 were below the detection limits.

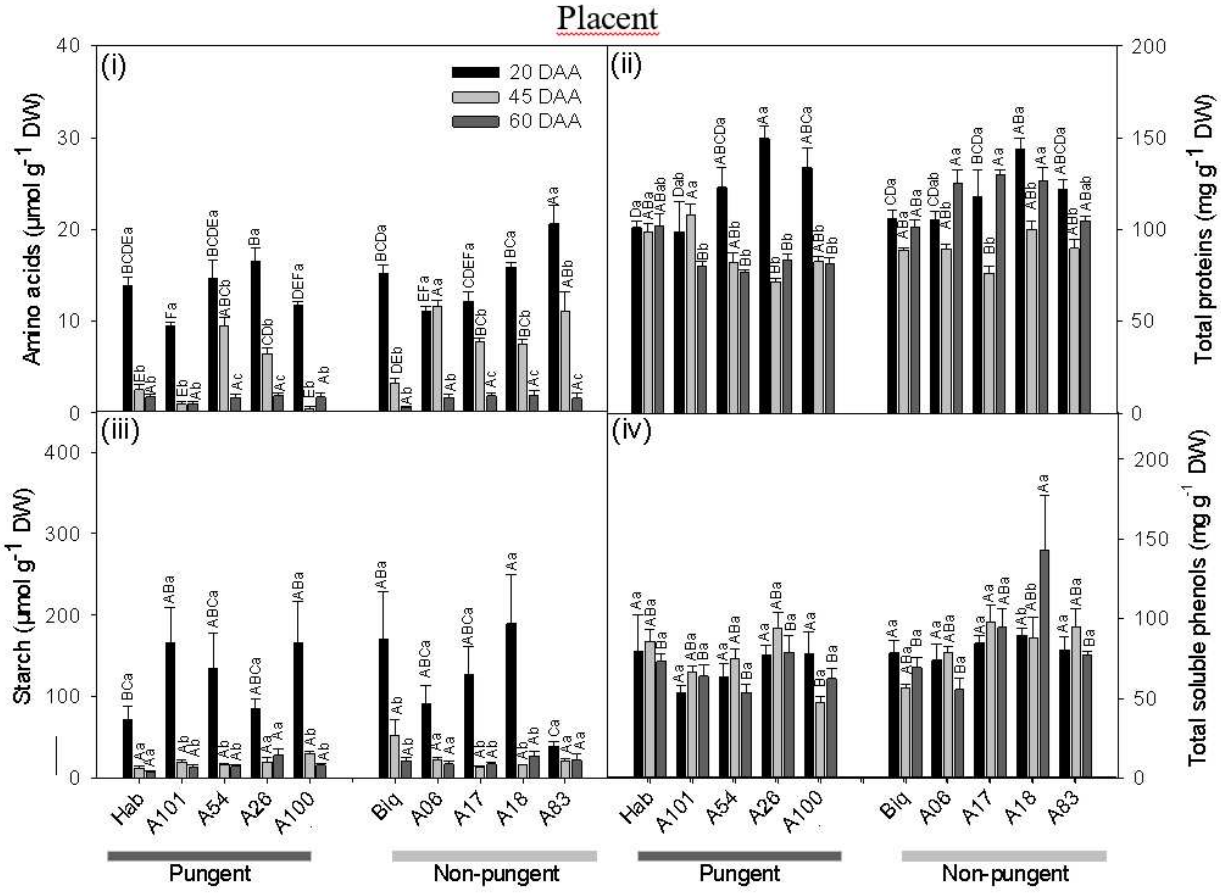
Figure 1. (A) Phenotypic variability of 10 *C. chinense* accessions indicated in numbers, in three stages of development- 20, 45 and 60 days after anthesis (DAA), scale 1cm. (B) Capsaicin from placenta (i), Capsaicin from pericarp (ii) along developmental period of pepper fruits (20 – 60 DAA). Black, light and dark gray represent 20, 45 and 60 DAA respectively. Bars represent average ± standard error (n=6). Capital letters indicate difference between accessions and small letters indicate difference over timer of development after anthesis. Separation of means by Tukey test (p≤0,05).



3.2 Primary metabolite profiling during fruit development

To place the differences in fruit phenotype between the accessions into a broader metabolic context, a detailed metabolic characterization of the fruit placenta and pericarp was performed. First, we turned our attention to the shifts in contents of total amino acids and proteins, starch and total soluble phenols in placenta (Fig. 2A) and pericarp (Fig. 2B). The results showed a large reduction in total amino acids and starch contents during fruit development, independently of pungency levels (Fig. 2A-i and iii and 2B-i and iii, respectively).

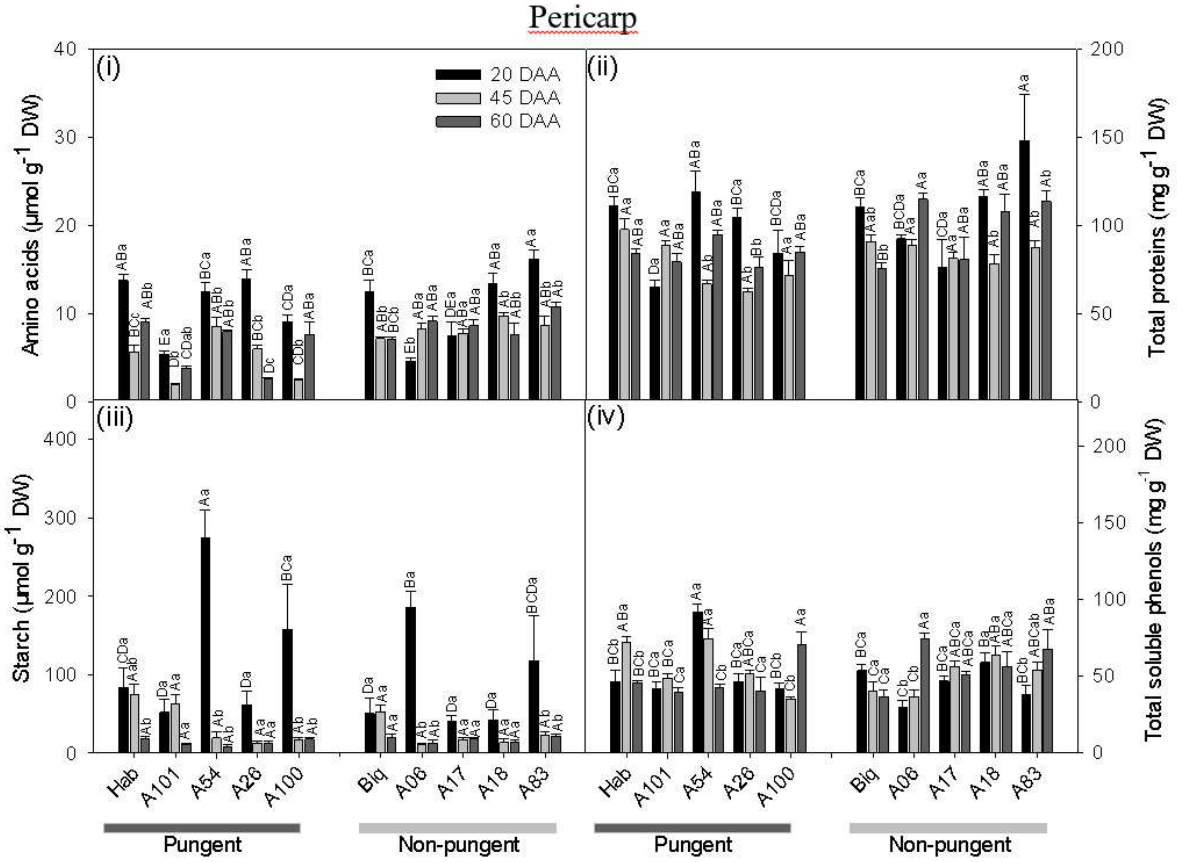
Figure 2A. (i) Amino acids, (ii) total protein, (iii) starch, (iv) soluble phenols along developmental period from placenta of pepper fruits (20 – 60 days after anthesis (DAA)) and comparing pungent and non-pungent peppers. Black, light and dark gray represent 20, 45 and 60 DAA respectively. Bars represent average ± standard error (n=6). Capital letters indicate difference between accessions and small letters indicate difference over timer of development after anthesis. Separation of means by Tukey test (p≤0,05).



In both the placenta and pericarp, the amino acid content globally decreased progressively during fruit maturation, while starch content sharply decreased after 20 DAA (Fig. 2). Protein content remaining stable in most of the accession, except for the placenta of three pungent accessions: A54, A26 and A100 at 20 DAA (Fig. 2A-ii). Total phenols content was unaltered in most cases, except access A18 (pericarp) which peaked at 60 DAA (Fig. 2A-iv and 2B-iv).

We next quantified by GC-TOF-MS 43 and 45 metabolites of known chemical structure for placenta and pericarp, respectively. These compounds include mostly plant sugars, amino acids and organic acids. The full data sets from the metabolite profiling study are shown in a heat map (Fig. 3A and 3B; the full data set is additionally provided in Table S1 and S2). Figure 3 shows, in a false color scale, increases or decreases in relative metabolite content. In both the placenta and pericarp, the ripening phases 45 and 60 DAA showed similar results when compared to 20 DAA.

Figure 2B. (i) Amino acids, (ii) total protein, (iii) starch, (iv) soluble phenols along developmental period from pericarp of pepper fruits (20 – 60 days after anthesis (DAA)) and comparing pungent and non-pungent peppers. Black, light and dark gray represent 20, 45 and 60 DAA respectively. Bars represent average ± standard error (n=6). Capital letters indicate difference between accessions and small letters indicate difference over timer of development after anthesis. Separation of means by Tukey test (p≤0,05).



We analyzed 19 amino acids in the placenta tissues of all accessions during fruit maturation. Amino acids levels varied during fruit development with three patterns in the placenta: (i) increasing from 20 DAA to 45 and 60 DAA (methionine, ornithine and phenylalanine); (ii) remaining constant (asparagine, glutamine and serine); or (iii) decreasing from 20 DAA to 45 and 60 DAA (alanine, aspartate, beta-alanine, GABA, glutamate, glycerate, glycine, hydroxyproline, leucine, isoleucine, valine, tryptophan and threonine). For the pericarp tissue, 17 amino acids were analyzed. Likewise, three patterns along fruit development were observed: (i) increasing from 20 DAA to 45 and 60 DAA (asparagine, methionine, ornithine and phenylalanine); (ii) remaining constant (alanine, GABA, glutamine, glycerate, glycine, hydroxyproline, serine and threonine); or (iii) decreasing from 20 DAA to 45 and 60 DAA (aspartate, beta-alanine, isoleucine, leucine and valine). Fruits from non-pungent accessions (A06, A17, A17 and A83) accumulated amino acids (serine, aspartate, methionine, ornithine and asparagine) at 45 DAA in placenta as well some accessions accumulated metabolites as phenylalanine and asparagine at 60 DAA in pericarp.

We measured ten organic acids in the placenta and twelve in the pericarp during maturation of the fruits (Fig. 3A and 3B). Most accessions showed the largest variations in their organic acid contents from 20 DAA to 45 DAA in both tissues. In the placenta, 2-oxoglutarate, ascorbate, dehydroascorbate and pyruvate increased during fruit development. Nevertheless, citrate, malonate, malate, fumarate and threonate decreased from 20 to 60 DAA in almost all accessions, whereas benzoic acid showed no considerable changes during fruit development (Fig. 3A). The pericarp tissue showed a different pattern from the placenta. Most accessions showed globally a decrease in all organic acid levels throughout fruit development, except dehydroascorbate content that increased over time (Fig. 3B).

We next turned our attention to the variation in sugar content. For the placenta, the levels of sugars, including fructose, glucose, maltose and mannose, increased during developmental stages. In contrast, the levels of arabinose, myo-inositol and sucrose decreased. The levels of erythritol, glycerol, raffinose and cellobiose did not change during development, although small differences among the accessions were observed (Fig. 3A). The accumulation of some sugars in the pericarp was similar to placenta in most of the genotypes along fruit development, except for glucose-6-phosphate (reduce in pericarp from 20 DAA to 60 DAA), maltose (reduce in pericarp

from 20 DAA to 60 DAA) and sucrose (increase in pericarp from 20 DAA to 60 DAA; Fig. 3B).

Figure 3A. Heat map of primary metabolic profile in placenta of fruits from 10 *C. chinense* accessions at 20 (A), 45 (B) and 60 (C) days after anthesis (DAA). A color-coded matrix represents the mean values of the metabolite intensity in six biological replicates of placenta from pepper accessions. Blue and red square represent negative and positive values respectively. The values of the genotypes were normalized by their own accessions at 20 DAA. The complete metabolite data set is presented in the Supplemental Tables 1.

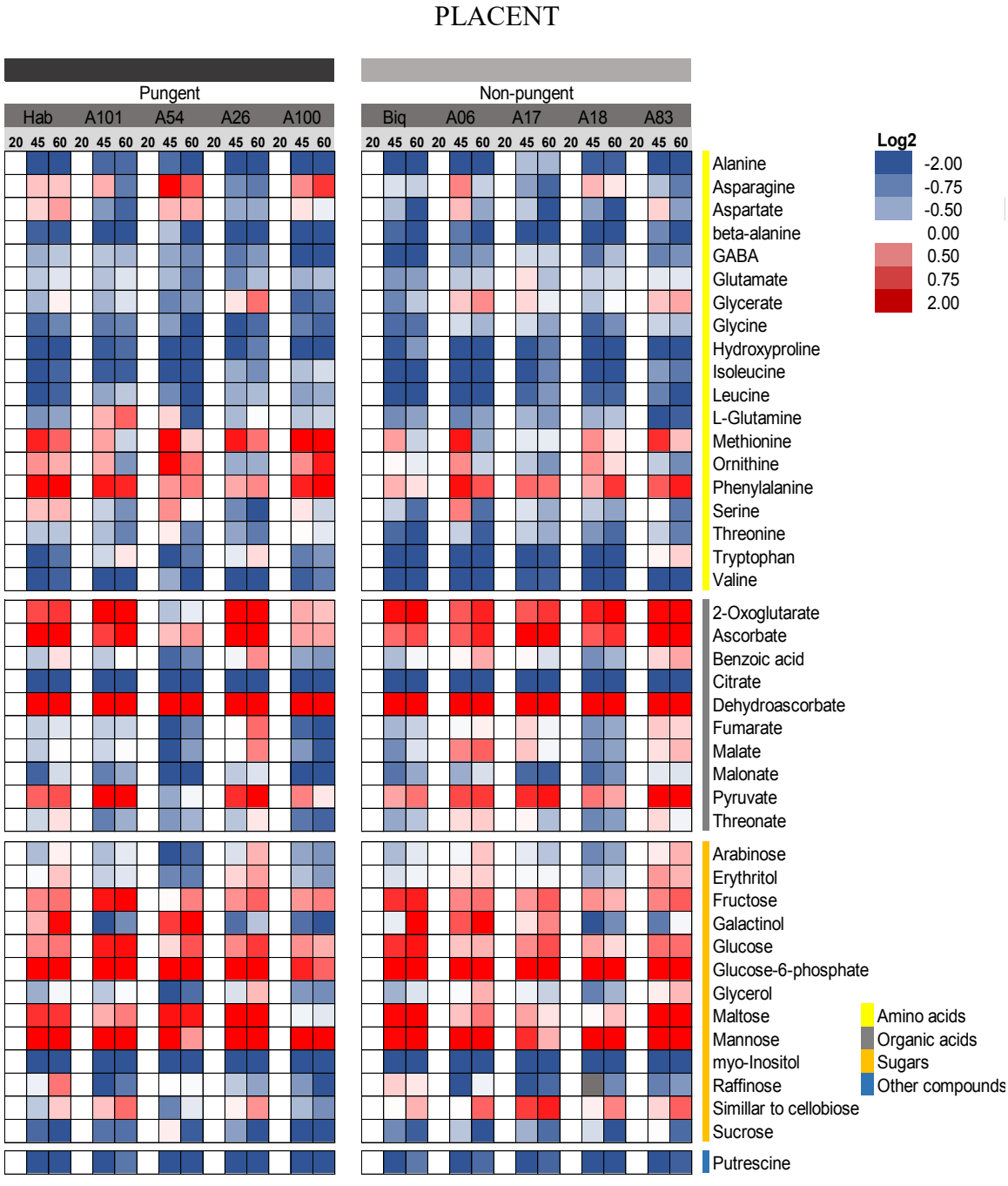
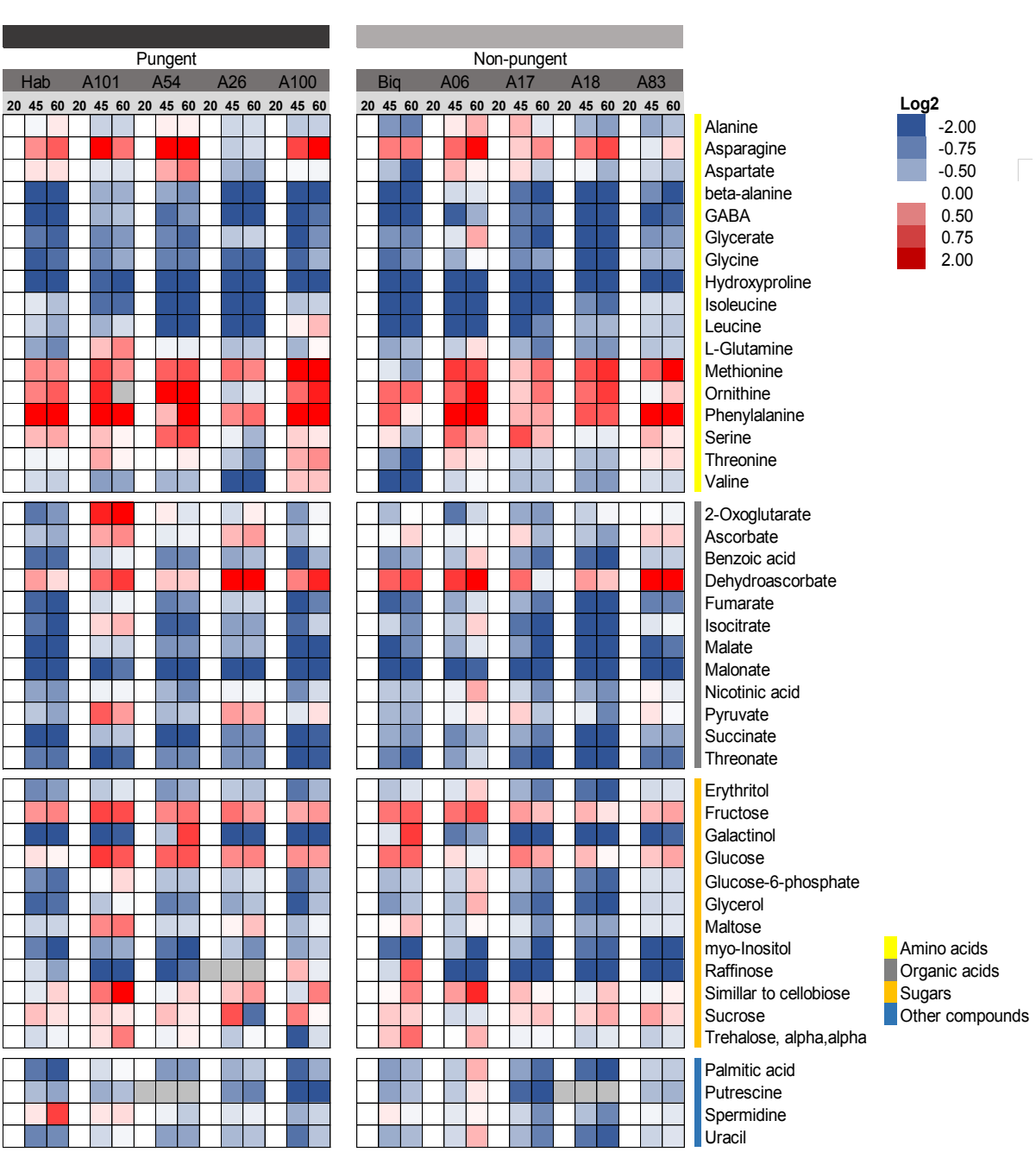


Figure 3B. Heat map of primary metabolic profile in pericarp of fruits from 10 *C. chinense* accessions at 20 (A), 45 (B) and 60 (C) days after anthesis (DAA). A color-coded matrix represents the mean values of the metabolite intensity in six biological replicates of placenta from pepper accessions. Blue and red square represent negative and positive values respectively. The values of the genotypes were normalized by their own accessions at 20 DAA. The complete metabolite data set is presented in the Supplemental Tables 2.



The levels of organic acids and sugars in the *C. chinense* accessions are much higher in placenta compared to pericarp tissue (Table S1 and S2). All accessions displayed strong reduction in putrescine levels throughout fruit development in

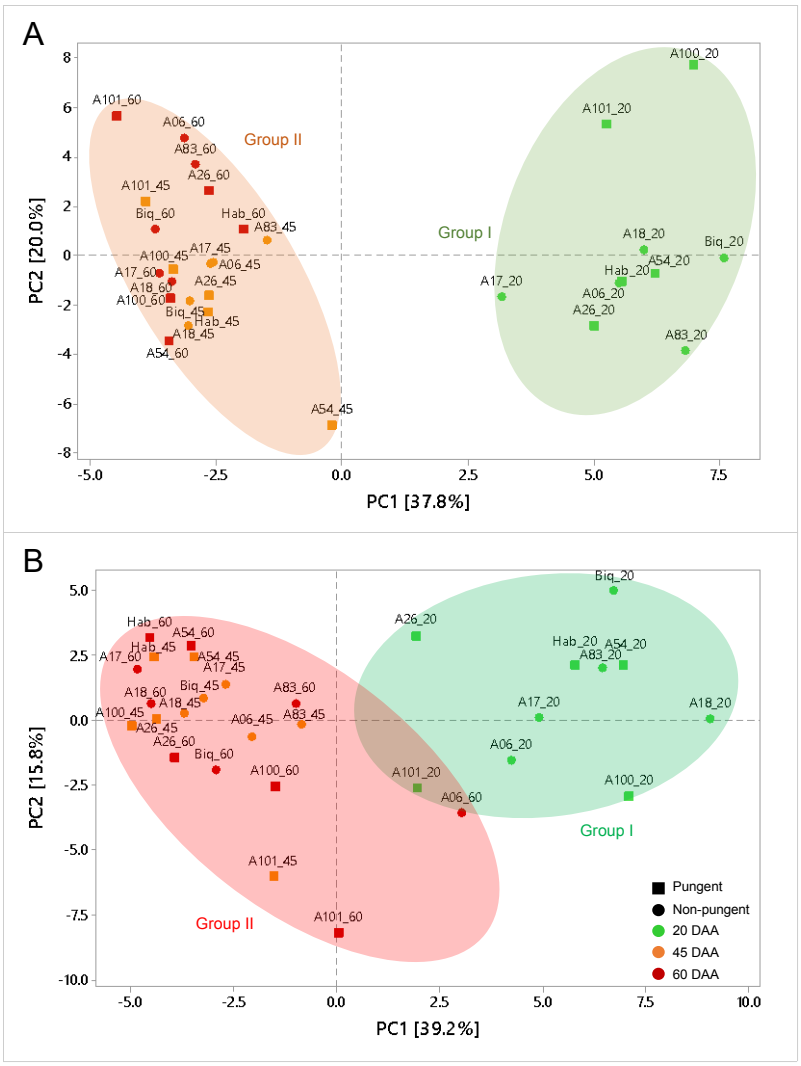
placenta and pericarp. Palmitic acid and uracil also tended to decrease in most of accessions with exception of A101 and A06 that increased at 60 DAA in pericarp (Table S2). On the other hand, the content of spermidine did not change over time, although small differences among the accessions were observed in pericarp.

3.3 Multivariate analysis

To reduce the dimensionality of the data set and identify the variables that explain the separation between the accessions and the development stages of fruit pepper, a principal component analysis (PCA) was performed. The PCA showed separation according to developmental stages, based on primary metabolite profile, macromolecules, total soluble phenols and capsaicin content in pericarp and placenta (Fig. 4). In the placenta, the first component (PC1) explained 37.8 % of the variation and separated the fruits harvested 20 DAA from the fruits harvested 45 and 60 DAA (Fig. 4A). The separation along PC1 was due mainly to the contribution of myo-inositol, beta-alanine, valine, alanine, total amino acids, glycine, sucrose, and GABA which contribute for cluster I (blue), composed of 20 DAA fruits (Fig. 4A, B, and Table S3).

On the other hand, glucose, fructose, ascorbate, and dehydroascorbate contributed for cluster II (orange), which consists of fruits of 45 and 60 DAA (Fig. 4A, B, and Table S3). Looking at the second component (PC2) in the placenta, which explains 20.0 % of the variation, accessions were not separated according to their pungency, but the A100 at 20 DAA and A101 at 20 and 60 DAA (two pungent accessions) are highlighted in their groups by benzoic acid, glycerate, glycerol, erythritol, and malate levels (Fig. 4A, B, and Table S3). A54, another pungent accession, was separated below others at 45 DAA mainly due to ornithine, asparagine, serine and methionine (Fig. 4A, B, and Table S3).

Figure 4. Principal component analysis (PCA) of 10 *C. chinense* accessions at 20 (Green), 45 (Orange) and 60 (Red) days after anthesis (DAA) based on primary metabolites, soluble phenois, and capsaicinoids content. (A) Score plot of placenta and (B) pericarp data set. The large circles represent the clusters formed by the Pearson distance method. PC1, principal component 1; PC2, principal component 2.



Similar to the placenta, when analyzing the pericarp by PCA, the PC1 separated fruits harvested 20 DAA from the fruits harvested 45 and 60 DAA while the PC2 separated the accessions (Fig. 4B). The PC1 explained 39.2 % of the variation whilst PC2 explained just 15.8 %. The cluster I (green) comprised the accessions at 20 DAA which were separated of the others development stages along the axis 1 (PC1) mainly by fumarate, malate, benzoic acid, glycine, and threonate levels (Fig. 4C, D, and Table S4). The glucose, methionine, fructose, and phenylalanine contributed for cluster II (red), which consists of fruits of 45 and 60 DAA (Fig. 4C, D, and Table S4). In general, the main compounds explaining the separation in PC2 were threonine, serine, alanine, valine, and total amino acids (Fig. 4D). In addition to these metabolites, maltose,

cellobiose, ascorbate, and dehydroascrobate also contributed to the separation of accessions along axis 2 (PC2), especially for the separation of A101 pungent accession at 45 and 60 DAA (Fig. 4C, D, and Supplemental table S4). The individual contribution of each compound and their eigenvectors for placenta and pericarp PCA can be observed in supplemental data (Table S3 and S4).

The results allowed a quantitative description of the patterns for the metabolite levels during *Capsicum* fruit growth and ripening. In addition, we assessed the metabolic network in placenta and pericarp fruit of pungent and non-pungent accessions. A combinatorial analysis of metabolites was performed by running all data points through pairwise correlation analysis (Fig. 5 and 6). For a better visualization of correlations and to simplify the interpretation, placenta and pericarp metabolites were grouped into six classes: secondary compounds, macromolecules (starch and total protein content), amino acids (including total and free amino acids), organic acids, sugars and sugars alcohols, and others (Fig. 5 and 6). When these correlations were scrutinized, several trends became apparent in both tissues. First, all measured metabolic classes presented significant correlations to compounds outside of their compound class in placenta and pericarp (Fig. 5 and 6). Curiously, pericarp exhibited a lower correlation number than placenta for both pungent and non-pungent accessions (Fig. 5 and 6). Unlike the placenta, the capsaicin content did not display correlation in the pericarp of pungent accessions (Fig. 5A and 6A). Phenylalanine, the amino acid precursor of the secondary metabolites biosynthetic pathway, positively correlated with capsaicin only in the placenta of pungent accessions (Fig. 5A). In addition, the fructose, glucose, and glucose-6-phosphate were positively correlated with the capsaicin content, whilst starch, total amino acids, hydroxyproline, malonate, myo-inositol, and sucrose were negatively correlated in placenta of pungent accessions (Fig. 5A). Curiously, a large number of correlations were observed for starch with all metabolite classes, at the exception of the pericarp of non-pungent accessions (Fig. 6B). Interestingly, in both tissues of pungent accessions, total protein positively correlated with amino acids class (total amino acids, GABA, glycine, hydroxyproline, valine) (Fig. 5A and 6A) while little or no correlation was observed in non-pungent accessions (Fig. 5B and 6B). These differences between pungent and non-pungent accessions, as well as a larger number of significant correlations (positive and negative) in the placenta of pungent and non-pungent accessions compared to

those observed in the pericarp (Fig. 5A and 5B), suggest that especially the placenta do present variations into their metabolic network.

In general, in placenta, the organic acids positively correlated with other organic acids and sugars in both pungent and non-pungent accessions (Fig. 5A and 5B). On the other hand, while the amino acids positively correlated with other amino acids in placenta of non-pungent accessions, some negative correlations were observed in pungent accessions (Fig. 5A and 5B). The main negative correlations were observed between amino acids and sugars and organic acids in placenta of the two group of accessions (Fig. 5A and B). In this regard, the placenta development appears to involve a higher number of metabolites and pathways, such as secondary metabolites and capsaicinoids biosynthesis.

In the pericarp, more significant and positive correlations were observed than negative correlations between metabolites in both pungent and non-pungent accessions (Fig. 6A and B). Amino acids correlated mostly with other amino acids and organic acids, indicating an important role of these metabolites along fruit development, markedly in non-pungent accessions (Fig. 6A and B). Moreover, organic acids were positively correlated with other organic acids and sugars in the pericarp of pungent accessions (Fig. 6A). In addition, the sugars positively correlated with amino acids and organic acids in the pericarp of non-pungent accessions (Fig. 6B). These results suggest that these three metabolite classes are strongly involved in the maturation of the pericarp, with some differences between pungent and non-pungent accessions.

Figure 5. Heat map of metabolite-metabolite correlations from placenta of pungent and non-pungent pepper fruits. Metabolites were grouped by compound class, and each square represents the correlation between the metabolite heading the column with the metabolite heading the row. Correlation coefficients and significances were calculated by Pearson correlations (P<0.05) and presented in colors. Blue and red square represent positive and negative correlation, respectively. The asterisk represent significances with base in FDR correction (Benjamini and Hochberg, 1995).

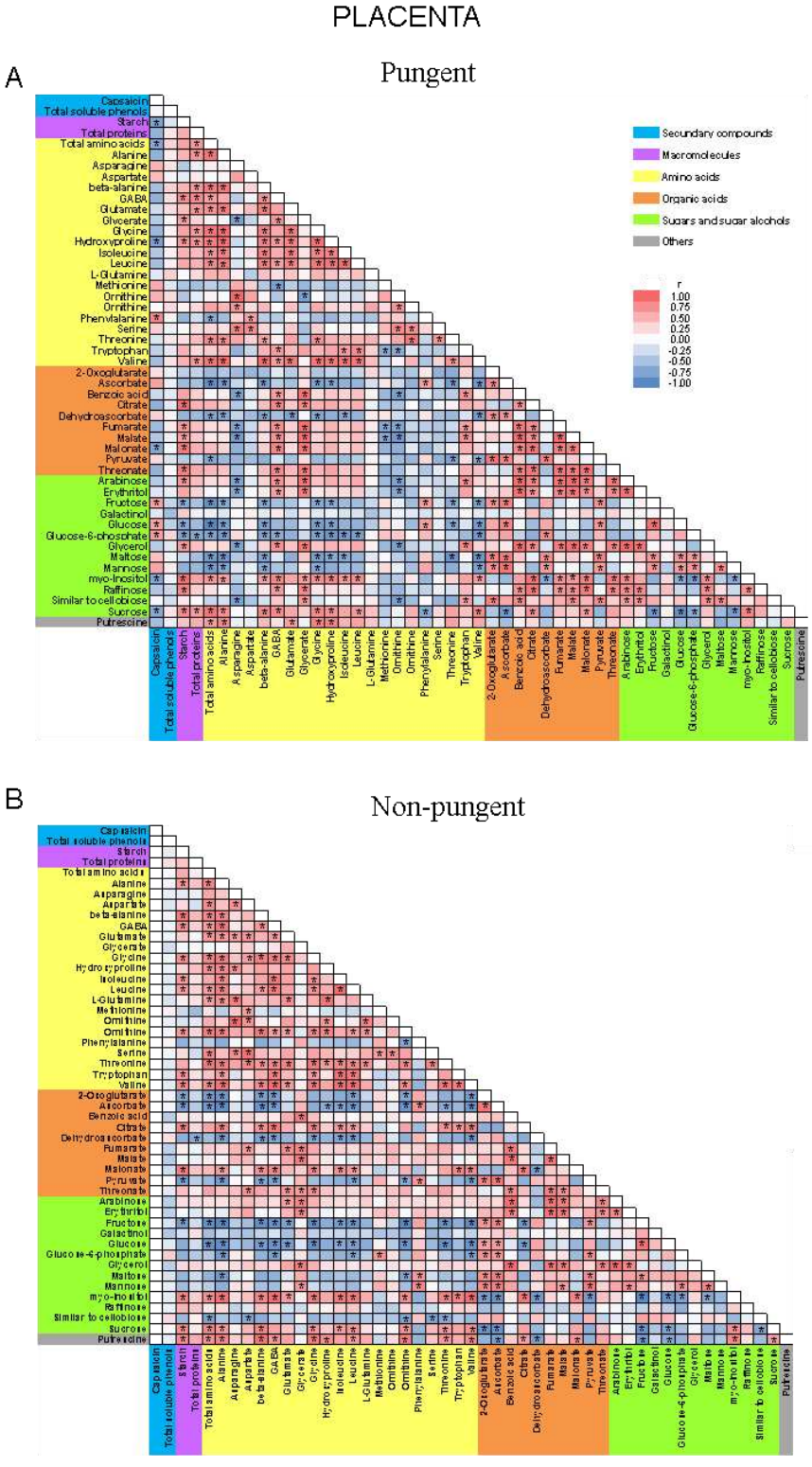
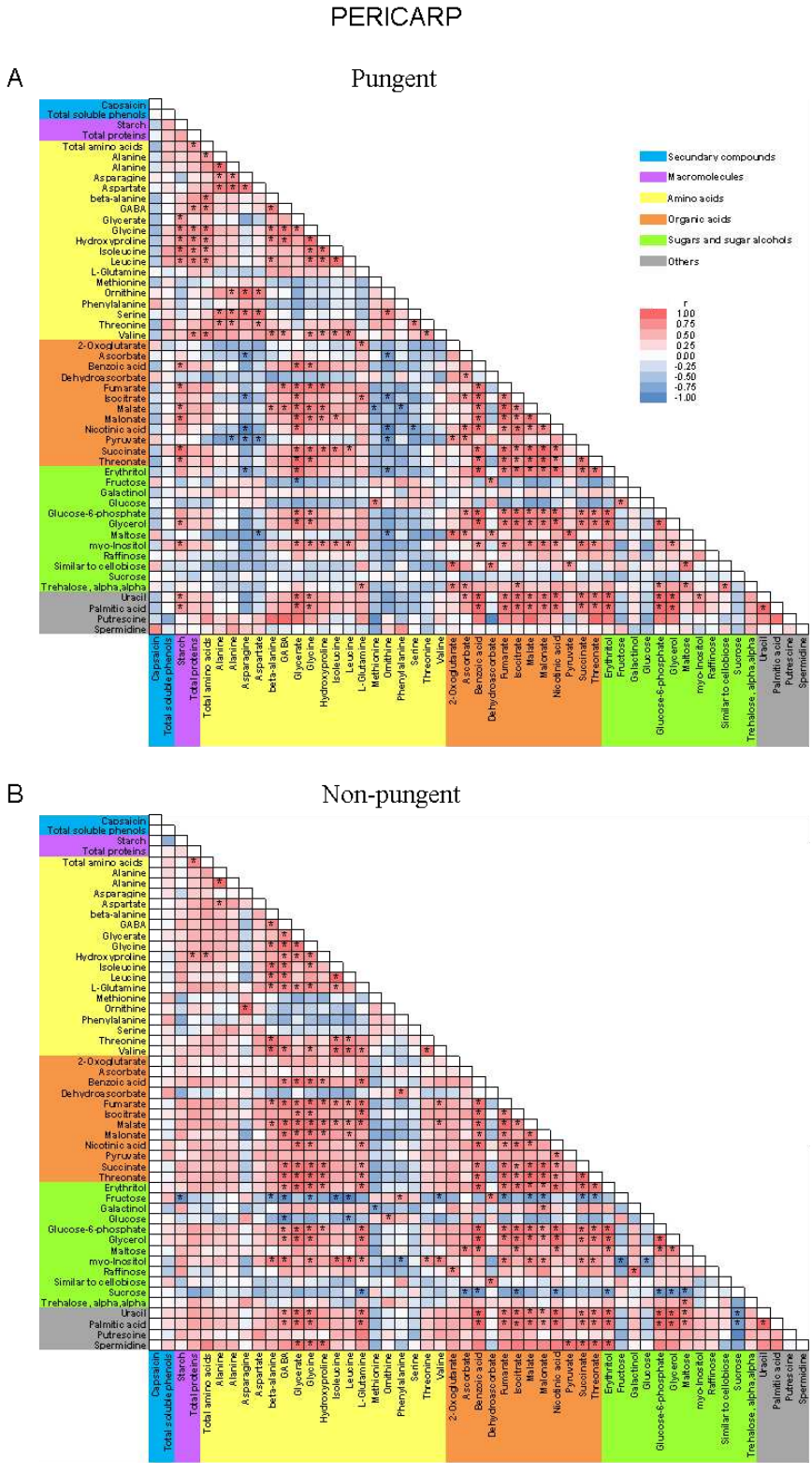


Figure 6. Heat map of metabolite-metabolite correlations from pericarp of pungent and non-pungent pepper fruits. Metabolites were grouped by compound class, and each square represents the correlation between the metabolite heading the column with the metabolite heading the row. Correlation coefficients and significances were calculated by Pearson correlations ($p \leq 0.05$) and presented in colors. Blue and red square represent positive and negative correlation respectively. The asterisk represent significances with base in FDR correction (Benjamini and Hochberg, 1995).



4. DISCUSSION

The study of natural genetic variation has provided numerous insights on plant development and physiology, with particular emphasis on plant growth and architecture, crop domestication, flowering time and primary metabolism (Alonso-Blanco et al., 2009; Nunes-Nesi et al., 2016). Natural variation has also been used to detect correlations between metabolic parameters providing information about the regulation of important biochemical pathways (Cross et al., 2006a; Rosado-Souza et al., 2015). However, even though metabolic changes are primary drivers of crop domestication and improvement, natural variation for fruit metabolism remains poorly explored in the literature. Few studies exist demonstrating the temporal behavior of primary metabolism and their correlations with characteristics of economic interest in cash crops (Carrari and Fernie, 2006; Osorio et al., 2012a).

Here, we explored metabolic variation in a panel of ten cultivars peppers (*C. chinense*). Our results indicate that the levels of primary metabolites largely vary between the fruits of different accessions and their developmental stage. This demonstrates the potential of tapping into large germplasm collections for genetic improvement of metabolic traits. In addition, despite large variability in placenta and pericarp capsaicin levels, minor variations in the levels of metabolites were observed between pungent and non-pungent accessions. Thus, large variation for capsaicin content is not associated with large variations in the metabolite profile, both in placenta and pericarp tissues.

4.1 Metabolic and transcript changes along fruit development

A previous work has shown that expression of genes related to photosynthetic light reactions is up-regulated during early stages of fruit ripening followed by down-regulation at later stages (Osorio et al., 2012a). In addition, down-regulation in genes for starch biosynthesis is coupled to up-regulation of sucrose degradation at the onset of ripening (Osorio et al., 2012a). Similarly, the starch turnover and the export of sucrose are considered crucial players for fruit set (de Ávila Silva et al., 2019). Thus, it has been suggested that photosynthesis is activated together with carbon translocation during early stages of fruit development in *C. chinense*. In our study, we confirmed this fact, as all accessions showed gradual increase in the accumulation of

glucose and fructose during fruit ripening in placenta and pericarp (Fig. 3A and 3B). Sucrose, in contrast, showed reduction in placenta and remain constant in pericarp over time (Fig. 3A and 3B). Starch levels were highest in nine out of ten accessions at 20 DAA, and then sharply decreased at 45 DAA and remained unaltered at 60 DAA in placenta (Fig. 2A-iii). Similar results were obtained for pericarp, but only for four of the ten accessions. These are consistent with the expression of starch synthesis genes being down regulated as fruit ripening progressed (Osorio et al., 2012a).

Organic acids play many roles in plant cell metabolism and the accumulation of these metabolites is highly variable across species (Araújo et al., 2011). In tomato, they are essential for fruit ripening and flavor, and they correlate with the expression of genes associated with ethylene and cell wall metabolism-related pathways (Carrari and Fernie, 2006; Centeno et al., 2011). In the present study, we showed that organic acids levels, including the four intermediates of tricarboxylic acid (TCA) cycle, malate, fumarate, citrate, and isocitrate, are strongly influenced by fruit ripening in placenta and pericarp. Similarly, in tomato fruits the levels of malate and fumarate, two organic acids related to starch metabolism in fruits, decrease significantly during fruit development (Centeno et al., 2011). However, the levels of fumarate in the *C. chinense* accessions are much lower in comparison to malate (Table S1 and S2). Malate metabolism has been described as important for starch metabolism during tomato and *C. chinense* fruit development (Centeno et al., 2011; Osorio et al., 2012a). A strong positive correlation between malate, fumarate, and citrate with starch levels, especially in pungent fruits, was observed in placenta and pericarp (Fig. 5A and 6A). This result supports the hypothesis that malate metabolism is important for transitory starch metabolism in pepper fruits (Centeno et al., 2011), in both placenta and pericarp tissues, possibly related to the redox activation of ADP-glucose pyrophosphorylase, the key enzyme of starch metabolism (Tiessen et al., 2002; Centeno et al., 2011). In agreement, a positive correlation between malate levels and several genes involved in starch biosynthesis has been observed in *C. chinense* fruits (Osorio et al., 2012a). The levels of ascorbate (vitamin C) and its oxidized form (dehydroascorbate), increase during pepper ripening (Casson and Gray, 2008; Keat et al., 2012; Osorio et al., 2012a). Similarly, we observed an increase in dehydroascorbate in all accessions in both tissues, suggesting high redox activity in later stages of fruit development, mostly in placenta.

As observed previously in pepper and tomato fruits (Osorio et al., 2012), the changes in amino acid profiles observed during ripening from different *C. chinense* accessions (Fig. 3A and 3B, and table S1 and S2) indicate distinct metabolic regulation programs. Besides, previous study already highlighted the importance of amino acids for *C. chinense* fruit setting (de Ávila Silva et al., 2019). In our study, some amino acids increased while others decreased through ripening with genotype dependent patterns. Phenylalanine, an aromatic amino acid, accumulated during pepper ripening in the two tissues. This amino acid is precursor for various branches of the phenylpropanoids pathway and the biosynthetic plant phenolics 2-phenylethanol and 2-phenylacetaldehyde (Tieman et al., 2006; Liu et al., 2013). Capsaicinoids biosynthesis occurs via two pathways, which converge at the last step, and whose precursors are either phenylalanine or valine (Bennett and Kirby, 1968; Leete and Louden, 1968; Aza-González et al., 2011; Naves et al., 2019). The pericarp levels of these two amino acids did not correlate with capsacin levels, suggesting a more complex regulation underlying metabolism in pericarp and biosynthesis and accumulation of capsaicinoids in fruit placenta. Interestingly, the phenylalanine positively correlated with capsaicin in placenta of pungent accessions (Fig. 5A). In *C. chinense*, phenylalanine and valine produced in situ in the placenta are essential for the capsaicinoids biosynthesis in Habanero fruit peppers (Baas-Espinola et al., 2016). Indeed, it has been proposed a regulatory mechanism that integrates the synthesis of phenylalanine and its direction for the synthesis of capsaicinoids during fruit development (Baas-Espinola et al., 2016). In *C. chinense* accessions, this regulatory mechanism appears to be strongly associated with the placenta development. However, it is important to note that, in general, the levels of amino acids in the accessions of *C. chinense* were similar between placenta and pericarp contrasting with (Ananthan et al., 2018) who found a higher amino acid content in placenta than in pericarp in *C. chinense* Jacq.

4.2 Capsaicin levels and pepper fruit development

It has been proposed that phenylpropanoid and branched-chain fatty acid pathways are interconnected with other metabolic pathways, such as amino acid metabolism, and that it can largely affect capsaicinoid accumulation (Mazourek et al., 2009). In pungent accessions, capsaicin is the most abundant capsacinoids and

recently the high complexity of the regulatory network involved in capsaicinoid biosynthesis has been described (Zhang et al., 2016). In addition, the sites of the accumulation and synthesis of capsaicinoids are still a matter of debate (Stewart et al., 2007; Mazourek et al., 2009; Tanaka et al., 2017). Previous microscopic analysis indicated that capsaicin is synthesized in the epidermis of the placenta and is stored in vesicles on the surface of this tissue (Suzuki et al., 1980). As previously reported, we found considerable amounts of capsaicin in the placenta of accessions in all the fruit developmental stages when compared to pericarp (Aza-González et al., 2011; Gamboa-Becerra et al., 2015)(Fig. 1B). Moreover, accumulation of capsaicin increases during the fruit maturation from 20 DAA to 60 DAA in almost all accessions with highest content in the fully mature fruits. Similar observations have been reported in other pepper fruits (Mueller-Seitz et al., 2008; Castro-Concha et al., 2016; Ananthan et al., 2018). Some positive and negative correlations were observed between capsaicin content and other metabolites in the placenta of pungent accessions (Fig. 5A). Interestingly, we did not observe any correlation between capsaicin and other metabolites in pericarp of pungent accessions (Fig. 6A). This could be explained by the absence of capsaicinoids synthesis in this tissue as suggested by the absent expression of genes involved in capsaicinoids synthesis (Stewart et al., 2007; Mazourek et al., 2009). Indeed, Liu et al., 2012 demonstrated that 61 genes exhibited specific expression in the placenta compared to those in the pericarp according to the results of RNA-sequencing.

Interestingly, clear differences in the correlation between metabolites in the placenta and pericarp from pungent and non-pungent accessions were observed (Fig. 5 and 6).

Collectively, this suggests that these two groups of accessions vary in their metabolic adjustments during fruit ripening in both tissues. Because capsaicinoids do not correlate with any other metabolite we analysed in pericarp, it is not clear whether these networks are mechanistically influenced by capsaicinoid contents, or if it is a confounding effect due to a strong population structure present in *C. chinense* population. Contrariwise, in the present study, a large number of correlations was noted between capsaicin and primary metabolites in placenta (Fig. 5). The fructose, glucose, and glucose-6-phosphate sugars were positively correlated with the capsaicin content, whilst starch, total amino acids, hydroxyproline, malonate, myo-inositol, and

sucrose were negatively correlated in placenta of pungent accessions. Given that capsaicin is a type of secondary metabolite exclusively produced in the pungent fruits of the *Capsicum*, these results suggest that many of the metabolites recognized here might be involved in the biosynthesis and/or regulation of capsaicinoids. Indeed, it has been proposed that pungency might be related to environmental pressure (Tewksbury et al., 2006; Naves et al., 2019).

5. CONCLUSSION

In summary, the results presented here demonstrated that variation on capsaicin detected in the placenta is closely connected with fluctuation in primary metabolism during fruit development in *C. chinense* accessions. There is considerable metabolite variability between different fruit developmental stages, yet not among accessions with different levels of capsaicin. Correlation results revealed different metabolite changes in the tissues of pungent and non-pungent fruits and primary metabolism appears to be altered between accessions of each group pungent or non-pungent peppers for the two tissues analyzed. Regardless of the pungency of the accessions analyzed here the metabolic variation observed is seemingly explained by environmental pressure that most likely is driving an extensive metabolic adjustment following fruit development. While the observations of this study clearly have implications for the understanding of primary metabolism in *Capsicum* fruits, questions concerning the exact mechanism governing such adjustment remains to be addressed in future studies.

Supplementary information

Table S1. Metabolites from placenta from pepper fruits collected from 10 accessions of *C. chinense* during three different development ages (20, 45 and 60 days after anthesis (DAA)). The mean values of the metabolite intensity ± standard error (n=6). Capital letters indicate differences between accessions and small letters indicate differences between fruit ages. Differences between means are given by Tukey test ($p<0.05$).

	DAA	Alanine	Valine	Leucine	Isoleucine	Glycine	Serine	Threonine	Alanine, beta-
Hab	20	16281.6±1477.0 Aa	3883.0±223.3 Ba	525.8±19.9 BCa	755.5±50.3 Ca	53.5±4.2 ABa	182.5±14.6 Ba	768.8±41.8 DEa	274.4±30.5 Aa
	45	2301.4±258.3 Ab	825.1±44.8 ABb	128.0±6.4 Ab	162.5±17.1 Ab	15.4±1.6 ABb	257.2±70.5 Aa	482.8±35.0 CDb	76.7±8.7 ABb
	60	3310.6±299.7 Ab	1094.3±73.8 Ab	148.6±9.4 Ab	218.5±23.4 Ab	20.1±2.2 Ab	271.3±92.9 Aa	477.8±39.4 Ab	72.1±4.7 Ab
A101	20	6808.4±504.2 Da	1713.7±129.80.0002 Da	275.0±27.4 EFa	356.9±46.6 DEa	33.5±4.6 CDa	138.2±27.6 Ba	456.5±46.1 Fa	104.6±6.0 DEa
	45	2030.1±111.6 Ab	294.1±20.2 Bb	134.0±4.6 Ab	96.6±5.9 Ab	11.4±0.7 Bb	95.0±24.1 Ba	273.6±28.9 Dab	19.8±1.2 Cb
	60	2065.9±138.5 Ab	282.3±23.4 Ab	177.2±21.8 Aab	98.2±8.3 Ab	12.6±1.4 Ab	56.4±14.2 Aa	166.6±29.7 Bb	19.7±2.3 Bb
A54	20	17662.1±4388.0 Aa	2646.0±451.5 CDa	403.0±41.2 CDEa	471.6±43.4 DEa	71.1±14.2 Aa	208.9±40.2 ABb	1007.7±122.6 ABCDa	141.2±17.8 CDa
	45	5461.8±1038.7 Ab	1348.1±195.0 Ab	156.8±16.0 Ab	108.9±8.1 Ab	32.4±12.4 Ab	388.0±115.1 Aa	1119.3±185.1 Aa	85.3±10.7 Ab
	60	2147.5±149.2 Ab	442.7±16.3 Ac	66.8±3.2 Ab	76.0±2.8 Ab	8.4±0.2 Ac	211.8±38.2 Ab	377.6±27.8 ABb	18.3±1.6 Bc
A26	20	14692.6±2375.1 ABa	2989.6±323.8 BCa	231.6±31.0 Fa	308.2±36.6 Ea	42.8±5.2 BCa	208.8±44.0 ABa	914.2±113.3 BCDa	193.4±6.7 Ba
	45	1749.9±182.9 Ab	494.0±35.3 ABb	118.6±8.4 Ab	157.7±15.0 Aab	9.8±1.2 Bb	80.7±12.6 Ba	441.4±35.9 CDb	38.4±1.9 BCb
	60	2583.5±325.1 Ab	471.3±28.8 Ab	134.6±17.0 Aab	123.4±10.2 Ab	13.1±1.2 Ab	50.7±8.0 Aa	314.0±52.0 ABb	25.3±2.7 Bb
A100	20	8265.2±596.0 CDa	2068.2±136.7 CDa	329.0±21.1 DEFa	330.4±32.1 Ea	43.1±3.5 BCa	223.5±54.1 ABa	538.0±36.8 EFa	153.6±12.8 BCa
	45	1824.3±227.6 Ab	566.8±40.1 ABb	154.1±6.8 Ab	198.6±9.3 Aa	15.2±1.4 ABb	258.9±66.7 ABa	549.6±22.6 BCDa	25.6±1.0 Cb
	60	1695.5±174.0 Ab	729.5±54.7 Ab	169.3±14.4 Ab	249.3±17.6 Aa	12.6±1.5 Ab	157.0±24.8 Aa	449.0±39.2 ABa	12.7±1.1 Bb
Biq	20	13142.7±1501.0 ABCa	5278.2±654.6 Aa	551.8±66.7 Ba	1082.4±150.8 Ba	41.4±3.5 BCDa	322.3±27.9 ABa	1238.2±102.8 Aa	166.9±16.7 BCa
	45	1970.8±222.1 Ab	574.1±63.2 ABb	134.5±10.5 Ab	171.2±12.5 Ab	12.6±0.7 ABb	218.9±54.2 ABab	370.5±14.8 Db	49.6±9.2 ABCb
	60	1631.5±97.1 Ab	261.3±26.7 Ab	80.6±10.0 Ab	96.4±11.6 Ab	13.2±2.2 Ab	100.4±28.1 Ab	207.1±18.4 ABb	13.8±1.1 Bc
A06	20	9979.2±843.0 BCDa	5147.7±370.8 Aa	949.3±86.6 Aa	1673.3±160.0 Aa	29.0±3.4 CDa	204.8±57.2 ABb	1079.4±77.8 ABCa	84.0±4.2 Ea
	45	1957.2±180.1 Ab	845.0±90.9 ABb	111.7±10.0 Ab	157.5±15.7 Ab	21.9±3.4 ABa	414.8±80.6 Aa	738.6±61.2 BCb	28.6±2.6 Cb
	60	2187.8±390.4 Ab	602.0±100.1 Ab	103.2±19.2 Ab	123.4±13.3 Ab	16.1±3.3 Aa	64.2±13.5 Ab	294.4±47.1 ABc	17.3±1.9 Bb
A17	20	6755.4±684.3 Da	2579.3±158.4 CDa	430.9±68.0 BCDa	519.9±102.6 CDEa	22.4±2.3 Da	201.4±30.1 Aba	603.0±36.9 EFa	149.0±8.4 BCDa
	45	4013.1±302.8 Aa	692.1±44.8 ABb	125.4±4.0 Ab	131.4±8.0 Ab	17.0±1.9 ABa	159.6±28.5 Ba	411.2±30.1 Dab	20.1±2.5 Cb
	60	3766.5±232.7 Aa	721.6±58.5 Ab	184.2±10.7 Ab	194.8±18.1 Ab	10.8±0.7 Aa	104.8±17.5 Aa	309.0±27.2 ABb	9.9±0.7 Bb
A18	20	9911.1±1565.7 BCDa	3597.3±382.2 Ba	460.2±55.0 BCDa	718.8±89.8 Ca	38.5±6.2 BCDa	250.2±53.5 ABa	811.5±77.5 CDEa	257.8±28.4 Aa
	45	2718.0±173.4 Ab	694.9±41.2 ABb	132.2±7.7Ab	141.4±7.2 Ab	10.9±1.1 Bb	163.0±27.3 Ba	345.3±13.8 Db	43.6±5.2 ABCb
	60	2769.0±181.2 Ab	548.1±35.9 Ab	133.8±18.3 Ab	144.3±11.8 Ab	15.4±1.4 Ab	118.9±22.8 Aa	244.5±16.2 Ab	16.0±1.8 Bb
A83	20	13696.9±1647.9 ABa	3702.1±317.2 Ba	518.1±37.0 BCa	587.1±58.2 CDa	33.2±4.2 CDa	416.2±64.0 Aa	1217.7±113.6 ABa	149.3±9.8 BCDa
	45	2070.6±230.6 Ab	881.2±52.4 ABb	187.4±15.4 Ab	260.4±17.5 Ab	23.4±1.9 ABa	414.2±74.1 Aa	829.5±109.3 ABb	56.6±4.0 ABCb
	60	1927.4±154.8 Ab	637.2±35.5 Ab	111.5±4.7 Ab	198.5±10.3 Ab	19.5±2.1 Aa	140.1±41.1 Ab	431.3±47.7 ABc	23.0±1.6 Bb

Continuation of Table S1

	DAA	Hydroxyproline	GABA	Aspartic acid	Tryptophan	Methionine	Ornithine	Glutamine
Hab	20	101.1±7.7 BCDa	3927.1±220.3 BCDa	5495.9±459.6 CDb	987.4±125.8 CDa	59.6±7.6 Ab	197.7±23.3 BCa	29.2±5.6 BCa
	45	16.2±1.8 Ab	2043.0±177.5 ABb	7044.0±340.9 Cb	140.1±13.3 Ab	201.3±7.6 Ba	363.4±31.1 BCDa	12.1±1.3 Bb
	60	14.9±1.1 Ab	2485.7±149.3 Ab	9324.3±1152.4 Aa	320.9±29.6 Aa	140.3±18.6 Bab	310.6±71.6 ABa	13.9±1.0 BCDb
A101	20	63.1±7.9 DEa	4341.2±373.7 Ba	2951.8±316.1 Ea	705.0±260.9 Da	39.0±4.0 Aa	32.2±4.7 Ca	13.4±1.6 Db
	45	16.9±1.8 Ab	2694.9±157.5 Ab	1280.8±84.2 Fb	510.0±48.0 Aa	64.7±6.1 Ca	51.2±12.2 Ea	20.3±1.7 Bab
	60	19.2±2.2 Ab	2411.9±59.1 Ab	847.7±150.2 Eb	805.4±54.4 Aa	27.6±3.5 Ba	13.7±1.6 Ca	31.4±1.7 Aa
A54	20	135.8±41.2 Ba	2245.5±209.5 Ea	3252.3±304.3 Eb	548.8±121.3 Da	92.7±18.0 Ab	151.3±29.0 BCb	28.6±6.2 BCDa
	45	7.1±0.5 Ab	1117.3±188.2 BCb	4831.8±184.4 CDab	109.4±1.7 Aa	362.8±61.2 Aa	597.5±64.0 ABa	36.3±9.3 Aa
	60	7.3±0.7 Ab	872.6±110.6 Bb	5088.6±281.7 BA	190.6±22.2 Aa	121.4±14.5 Bb	314.5±32.5 ABb	7.7±0.9 Db
A26	20	80.4±14.4 CDEa	4255.4±563.2 Ba	4176.9±118.3 DEa	237.1±18.7 Da	51.0±6.5 Ab	283.6±42.3 Ba	29.0±5.9 BCa
	45	17.4±1.6 Ab	1444.4±118.2 BCb	2091.7±100.3 EFb	201.1±14.7 Aa	180.6±11.4 BCa	147.0±11.0 CDEa	16.6±1.3 Bb
	60	28.3±4.2 Ab	1851.1±215.2 ABb	2086.3±243.8 CDEb	288.6±19.2 Aa	109.2±12.2 Bab	148.1±9.6 BCa	28.4±3.5 ABa
A100	20	82.3±4.7 CDEa	5617.6±379.1 Aa	4618.2±244.1 DEa	668.0±210.2 Da	48.0±6.5 Ab	80.5±32.7 BCb	20.0±1.9 BCDa
	45	11.9±1.1 Ab	816.6±111.3 Cb	5422.4±302.0 CDa	233.6±18.6 Aa	375.5±14.0 Aa	150.3±13.0 CDEab	12.6±1.3 Ba
	60	9.1±1.4 Ab	951.8±105.3 Bb	4041.5±396.3 BCa	285.1±25.2 Aa	408.2±89.4 Aa	278.9±48.4 ABa	13.9±1.6 BCDa
Biq	20	44.0±3.5 Ea	4888.1±425.0 ABa	8150.6±597.8 Ba	2492.5±593.6 Ba	89.8±8.4 Aa	255.6±12.8 BCa	30.5±5.7 BCa
	45	11.6±0.8 Aa	1237.6±97.5 BCb	4722.5±252.7 Db	248.4±23.9 Ab	152.4±42.2 BCa	265.1±59.4 CDEa	11.9±0.7 Bb
	60	19.2±1.9 Aa	981.3±78.6 Bb	1900.2±133.0 CDEc	510.8±13.5 Ab	62.6±7.4 Ba	220.3±39.9 ABCa	14.3±1.2 BCDb
A06	20	67.4±4.6 CDEa	4104.8±276.2 BCa	7039.4±244.8 BCb	3815.4±393.4 Aa	93.9±10.9 Ab	203.5±24.3 BCb	32.7±3.2 Ba
	45	9.9±0.8 Ab	1542.3±112.4 BCb	10144.0±388.8 Ba	287.9±15.2 Ab	335.9±30.0 Aa	382.4±59.9 BCa	12.2±0.9 Bb
	60	15.9±3.3 Ab	1786.1±158.9 ABb	3437.2±765.3 BCDc	492.5±59.3 Ab	47.6±4.1 Bb	139.2±16.0 BCb	15.3±2.8 BCDb
A17	20	44.8±3.4 Ea	2348.5±247.0 Ea	5596.2±391.4 CDa	1007.0±265.5 CDa	114.1±21.1 Aa	221.3±15.0 BCa	16.2±2.3 CDa
	45	11.5±1.5 Aa	1743.0±125.5 ABCa	3638.5±593.2 DEb	247.5±35.6 Ab	96.5±16.0 BCa	142.8±45.6 DEa	9.0±0.9 Ba
	60	15.8±1.0 Aa	1635.8±96.6 ABa	1289.4±99.4 DEc	268.2±16.5 Ab	97.5±4.5 Ba	93.8±11.1 BCa	7.1±0.5 Da
A18	20	111.6±23.9 BCa	3004.5±570.5 DEa	7885.2±770.4 BCa	1809.9±586.6 BCa	92.8±6.7 Aa	121.3±26.0 BCa	15.3±2.5 CDa
	45	8.5±0.6 Ab	995.1±48.8 BCb	3404.7±372.2 DEFb	205.3±25.7 Ab	172.3±14.0 BCa	220.7±17.7 CDEa	8.2±0.4 Ba
	60	9.0±0.9 Ab	1767.8±209.5 ABb	1191.7±140.0 Ec	389.0±54.8 Ab	106.6±11.0 Ba	149.0±31.5 BCa	9.3±0.7 CDa
A83	20	270.3±20.6 Aa	3094.5±134.5 CDEa	10678.1±387.9 Ab	238.3±50.3 Da	106.1±10.1 Ab	1057.2±120.8 Aa	88.7±7.9 Aa
	45	19.5±2.2 Ab	1132.5±161.5 BCb	13644.9±1248.2 Aa	252.1±13.3 Aa	332.9±70.5 Aa	699.7±125.0 Ab	19.1±1.3 Bb
	60	18.7±1.8 Ab	1268.4±56.6 Bb	4886.9±822.7 Bc	306.8±14.0 Aa	151.6±16.1 Bb	413.3±139.2 Ac	24.5±1.6 ABCb

Continuation of Table S1

	DAA	Glutamic acid	Phenylalanine	Asparagine	Ascorbic acid	Fumaric acid	Pyruvic acid	Malonic acid
Hab	20	4531.2±499.7 Aa	180.5±4.6 Ab	935.3±152.7 Ba	17.3±2.6 Bc	116.7±17.6 BCa	18.7±2.9 Ab	9.2±0.9 CDa
	45	2912.3±358.6 ABb	691.9±37.3 Aa	1297.7±302.8 BCDa	75.9±6.8 ABCb	78.7±8.7 ABCa	44.5±3.7 CDa	2.6±0.3 Aa
	60	3666.3±269.3 ABab	789.8±78.9 Aa	1290.8±460.8 ABa	126.7±9.5 ABa	94.2±9.1 BCDa	48.5±2.9 CDEFa	6.9±1.3 ABCa
A101	20	2708.7±388.7 Ba	94.2±8.2 Ab	132.2±30.7 Ba	35.5±3.9 ABc	166.3±17.6 ABa	26.4±2.9 Ab	34.3±5.2 Ba
	45	1651.5±144.6 Ba	333.7±58.8 BCDa	204.7±68.6 Da	101.0±9.9 ABb	110.5±14.1 ABb	113.5±14.4 Aa	12.1±1.0 Ab
	60	2179.6±307.7 BCa	308.6±64.5 DEFa	45.7±11.2 Ca	137.6±7.2 Aa	117.2±15.6 ABCab	119.8±8.0 Aa	17.4±1.9 Ab
A54	20	4364.8±911.4 ABa	123.2±11.5 Ab	562.6±105.5 Bb	27.6±4.1 ABa	114.7±22.2 BCa	35.1±9.5 Aa	14.7±3.0 CDa
	45	2498.6±188.4 Bb	219.5±15.0 BCDEab	2392.5±228.2 ABa	39.5±1.8 Da	28.4±5.8 Cb	17.6±1.5 Ea	2.9±0.3 Ab
	60	1546.1±135.1 Cb	250.3±12.2 DEFa	1381.1±208.7 ABb	48.3±6.8 Ea	43.5±5.3 Db	32.7±3.7 Fa	3.5±0.5 Cb
A26	20	3634.6±394.2 ABa	100.3±19.9 Aa	1226.6±244.7 Ba	8.7±1.0 Bc	76.4±6.1 Cb	13.1±2.3 Ac	18.4±3.3 Ca
	45	1425.1±113.9 Bb	160.9±20.0 Ea	486.9±70.4 CDa	67.1±3.7 CDb	77.7±9.5 ABCb	41.1±5.4 CDEb	12.0±0.8 Aa
	60	2124.6±239.2 BCb	192.4±7.7 EFa	421.8±93.4 ABCa	99.8±11.2 BCDa	171.9±47.7 Aa	72.2±11.5 BCa	14.7±2.2 ABa
A100	20	3450.7±435.6 ABa	108.0±6.8 Ab	354.3±157.9 Ba	54.7±1.8 Ab	218.2±24.5 Aa	32.7±3.7 Ab	45.2±8.2 Aa
	45	1845.6±171.7 Bb	365.5±62.3 BCa	664.1±101.7 CDa	90.1±4.2 ABCa	63.2±8.7 ABCb	64.9±3.1 BCa	6.8±0.5 Ab
	60	2049.7±236.3 BCb	462.2±27.2 BCa	1059.3±154.4 ABCa	88.8±13.1 CDa	49.7±8.5 CDb	37.3±4.3 EFb	5.7±0.4 BCb
Biq	20	4889.7±469.2 Aa	138.3±9.9 Aa	1311.0±88.5 Ba	32.9±6.0 ABb	103.2±14.4 BCa	20.9±3.4 Ab	16.5±4.6 CDa
	45	2078.1±220.4 Bb	209.9±14.2 DEa	1027.4±289.4 CDa	74.3±6.2 BCa	57.5±12.9 BCa	34.4±2.2 DEab	5.3±0.6 Ab
	60	2221.9±95.4 BCb	164.8±25.3 BCa	893.3±181.9 ABCa	87.4±8.7 CDa	71.5±6.5 BCDa	45.0±3.2 DEFa	8.3±0.8 ABCb
A06	20	4548.2±530.9 Aa	194.6±23.7 Ac	777.7±163.8 Bab	34.8±1.6 ABc	95.7±9.1 Ca	22.1±1.5 Ab	11.8±1.6 CDa
	45	2926.6±250.4 ABb	721.8±46.3 Aa	1531.0±435.6 BCa	83.7±4.6 ABCa	91.5±12.3 ABCa	60.0±6.8 Ca	6.1±1.0 Aa
	60	3015.1±480.4 ABCb	500.5±60.4 Bb	531.9±124.0 ABCb	117.6±15.1 ABCa	104.8±13.0 ABCDa	65.2±7.8 BCDa	9.2±1.7 ABCa
A17	20	2617.0±272.7 Bab	148.2±17.6 Ab	941.3±131.7 Ba	17.8±2.1 Bb	54.5±7.6 Ca	16.7±3.2 Ab	9.3±1.4 CDa
	45	3113.7±297.6 ABa	335.4±19.0 BCDa	442.3±153.5 CDa	89.0±9.8 ABCa	68.8±12.9 ABCa	53.1±7.6 CDa	2.7±0.3 Aa
	60	1574.0±142.4 Cb	320.3±24.3 CDEa	274.6±28.2 BCa	69.3±3.6 DEa	50.3±4.9 CDa	60.3±3.9 CDEa	2.6±0.3 Ca
A18	20	3796.8±608.6 ABa	133.4±14.7 Ab	492.1±153.1 Ba	26.3±3.5 ABb	96.0±20.5 Ca	23.3±4.0 Ab	14.4±2.2 CDa
	45	2600.4±167.2 ABa	212.7±16.6 CDEb	735.6±71.0 CDa	65.0±3.3 CDa	39.9±5.0 Cb	49.4±3.2 CDa	4.3±0.5 Ab
	60	2825.0±186.1 ABCa	401.6±14.7 BCDa	563.9±133.5 ABCa	80.8±6.9 Da	47.9±5.0 CDab	38.1±4.4 EFab	6.0±1.4 BCb
A83	20	5232.2±745.1 Aa	150.6±19.7 Ac	4566.5±435.6 Aa	16.6±1.8 Bc	95.3±17.6 Ca	21.2±3.2 Ab	6.2±0.4 Da
	45	4362.9±550.4 Aa	368.9±52.0 Bb	2761.2±811.6 Ab	105.4±3.4 Ab	127.9±14.3 Aa	89.2±4.8 ABa	5.2±0.3 Aa
	60	4305.3±581.5 Aa	523.0±30.9 Ba	1584.0±581.5 Ac	130.9±10.2 Aa	121.4±13.9 ABa	88.3±5.7 Ba	5.0±0.5 BCa

Continuation of Table S1

	DAA	Malic acid	2-Oxoglutaric acid	Benzoic acid	Glyceric acid	Citric acid	Raffinose	Maltose	Glucose-6-phosphate
Hab	**20**	9485.5±1124.8 BCa	11.5±3.3 Aa	1120.0±96.6 BCDa	22.8±2.2 BCa	2320.6±181 Ba	15.1±3.6 Da	53.1±10.2 BCDb	87.9±10.2 Ab
	45	6272.6±320.0 BCa	31.2±4.6 Ca	709.1±64.1 BCb	12.3±1.0 BCDb	68.5±11.6 Aa	13.5±1.4 Aa	164.0±16.1 BCDa	865.1±95.9 Ba
	60	9621.6±497.9 BCDa	34.5±3.9 EFa	1339.8±83.4 ABa	24.6±1.0 BCDa	191.6±28.7 Aa	32.1±5.7 Aa	159.4±8.3 DEa	581.6±65.0 BCa
A101	**20**	13466.1±3587.5 ABa	13.6±3.0 Ac	1631.4±154.6 ABa	44.9±6.2 Aa	10940.5±1879.0 Aa	154.7±19.1 Aa	136.2±21.1 ABc	125.1±8.4 Ab
	45	9614.5±708.5 ABa	85.3±9.6 Ab	1057.8±85.6 ABb	24.4±0.8 ABb	380.4±41.7 Ab	36.9±4.6 Ab	212.4±21.8 BCb	632.8±92.0 BCa
	60	13206.1±985.9 ABa	188.2±26.3 Aa	1630.5±207.6 Aa	35.7±2.7 ABa	647.6±58.5 Ab	51.9±6.0 Ab	276.3±21.1 ABa	744.8±215.2 ABCa
A54	**20**	10245.1±2043.4 BCa	25.5±10.8 Aa	1115.8±150.5 BCDa	18.1±2.1 BCa	3543.6±738.4 Ba	12.9±2.4 Da	33.9±5.6 Db	97.1±11.4 Ab
	45	2430.3±332.2 Cb	15.6±2.3 Ca	327.8±19.0 Cb	6.6±0.7 Db	88.6±14.1 Ab	13.1±2.2 Aa	123.3±13.6 Da	956.8±93.9 Ba
	60	4622.7±721.7 CDb	21.8±4.0 Fa	427.0±58.8 Eb	7.7±0.8 Eb	224.6±29.7 Ab	12.5±2.8 Aa	118.9±10.7 Ea	470.4±124.8 BCb
A26	**20**	5908.9±628.5 Cb	6.9±2.4 Ac	691.0±39.9 Db	17.5±0.6 BCb	1750.6±208.6 Ba	36.2±5.2 CDa	31.9±6.2 Db	63.6±4.8 Ab
	45	5941.6±361.0 BCb	42.7±4.5 BCb	634.2±36.2 BCb	20.4±1.0 ABCb	34.9±8.7 Aa	23.5±1.7 Aa	153.9±10.6 CDa	706.3±87.7 BCa
	60	11603.4±1725.5 ABa	68.8±11.5 BCDa	1288.5±175.0 ABCa	38.3±4.2 Aa	184.0±27.5 Aa	17.3±3.8 Aa	182.5±19.8 CDEa	932.0±189.1 ABa
A100	**20**	17687.6±3353.0 Aa	22.8±5.5 Aa	1979.1±94.0 Aa	55.3±7.6 Aa	12547.5±3668.8 Aa	139.2±42.6 ABa	163.3±9.1 Aa	164.1±6.8 Aa
	45	7639.8±281.9ABCb	36.1±1.6 BCa	968.0±46.4 ABb	16.1±0.5 ABCDb	200.0±24.9 Ab	60.0±10.4 Ab	148.7±6.1 CDa	546.1±57.9 BCa
	60	4655.0±673.5 CDb	32.3±3.8 EFa	845.8±109.9 BCDEb	18.6±1.2 CDEb	218.0±43.6 Ab	10.0±1.9 Ac	135.5±10.1 Ea	382.8±40.4 Ca
Biq	**20**	12420.9±1535.4 ABa	11.8±2.8 Ac	1236.6±136.4 BCDa	29.4±2.1 Ba	10274.2±1278.5 Aa	34.6±8.1 CDa	41.1±8.1 CDc	87.0±9.2 Ab
	45	4874.0±521.3 BCb	44.3±2.1 BCb	718.9±30.2 BCb	11.0±0.2 CDb	171.9±39.4 Ab	44.9±13.4 Aa	160.7±7.6 BCDb	616.6±45.5 BCa
	60	9874.5±433.0 BCa	76.0±7.0 BCa	1154.6±55.7 ABCDa	18.7±1.6 CDEb	370.5±49.9 Ab	39.4±4.5 Aa	228.2±17.4 BCDa	771.9±160.7 ABCa
A06	**20**	5299.5±317.7 Cb	17.3±2.9 Ab	1029.2±68.4 CDb	18.6±1.7 BCb	2635.3±201.5 Ba	77.1±9.6 Ca	170.6±10.8 Ac	89.4±5.9 Ab
	45	10233.7±840.1 ABa	43.1±5.0 BCa	1085.9±93.3 ABb	25.3±0.8 Ab	110.1±17.7 Aa	17.1±2.3 Ab	238.9±15.5 ABb	1047.1±156.1 Ba
	60	12550.6±1297.0 ABa	58.4±10.0 CDEa	1638.9±287.8 Aa	34.8±7.8 ABa	544.4±109.2 Aa	69.6±21.2 Aa	358.3±61.2 Aa	402.8±48.7 Cb
A17	**20**	5083.1±506.2 Ca	12.6±3.7 Ab	868.6±100.0 CDa	14.4±1.5 Ca	2816.6±267.1 Ba	197.2±33.0 Aa	109.1±14.3 ABCDb	66.6±6.7 Aa
	45	7033.1±1099.9 ABCa	31.6±5.4 Cab	906.3±107.9 ABa	18.0±2.4 ABCDa	469.1±97.7 Aa	50.4±9.3 Ab	179.9±33.1 BCDa	314.7±42.0 Ca
	60	4757.2±381.6 CDa	38.0±2.5 DEFa	676.5±50.1 DEa	12.6±0.8 DEa	291.4±32.0 Aa	59.3±11.2 Ab	127.9±9.7 Eab	353.3±63.4 Ca
A18	**20**	8638.9±1720.2 BCa	10.6±2.1 Ab	1407.6±310.1 BCa	19.3±2.8 BCa	3866.8±558.7 Ba	81.8±25.9 BCa	124.2±9.6 ABCa	105.6±16.2 Ab
	45	3337.2±159.9 Cb	35.6±3.1 BCa	565.3±17.7 BCb	12.0±0.6 BCDa	203.3±31.6 Ab	NA	128.2±8.1 Da	933.1±156.2 Ba
	60	4072.1±373.3 Db	52.7±7.3 CDEFa	767.7±51.3 CDEb	18.8±0.6 CDEa	411.7±68.2 Ab	31.7±6.0 Ab	174.4±9.9 CDEa	1183.6±86.9 Aa
A83	**20**	10612.2±966.5 BCb	17.0±4.0 Ac	1035.5±130.7 CDb	18.9±1.5 BCb	2574.2±302.1 Ba	26.2±4.9 CDa	48.1±4.9 CDb	73.2±9.3aA Ac
	45	12688.0±586.5 Aab	66.2±5.8 ABb	1302.1±57.4 Aab	26.2±1.3 Aab	112.3±16.6 Aa	9.3±1.5 Aa	305.8±26.2 Aa	1695.9±355.3 Aa
	60	15727.7±1305.4 Aa	97.5±7.5 Ba	1690.8±72.8 Aa	31.0±1.4 ABCa	378.5±41.6 Aa	11.3±1.7 Aa	251.4±21.8 BCa	953.1±204.5 ABb

Continuation of Table S1

	DAA	Glucose*	Fructose*	Sucrose*	Cellobiose	Mannose	Arabinose	Putrescine	Dehydroascorbic acid
Hab	20	219.0±16.8 Ab	279.9±19.7 Ab	84.8±7.4 ABa	124.9±11.2 BCab	941.6±62.8 Ab	467.0±37.2 BCDa	265.5±38.3 Ca	7.4±1.3 Ab
	45	402.9±27.3 ABa	540.2±32.8 ABa	25.3±2.1 Cb	80.8±5.1 BCb	8990.6±955.2 BCa	273.1±26.4 BCb	59.5±7.5 Ab	2041.0±268.6 CDa
	60	462.7±47.4 ABa	602.6±66.2 ABCDa	16.4±2.3 Ab	166.8±25.9 EFa	13726.5±884.0 BCDa	504.6±26.3 BCDa	64.1±4.9 Ab	512.6±41.6 Fb
A101	20	137.6±28.0 Ab	176.1±28.4 Ab	89.7±12.0 ABa	194.5±24.0 ABc	1159.9±118.0 Ab	669.7±61.9 ABa	202.5±18.6 Ca	4.4±0.5 Ab
	45	480.6±35.3 Aa	638.6±48.7 Aa	29.0±2.0 Cb	273.7±22.8 Ab	6279.8±1648.1 Cb	369.5±35.1 ABb	50.3±4.4 Aa	4596.7±421.4 Aa
	60	517.6±50.5 ABa	819.9±88.5 ABa	28.5±3.6 Ab	432.8±20.1 ABa	17437.4±5438.2 Ba	562.9±50.2 ABCa	67.3±8.4 Aa	4133.0±394.8 Aa
A54	20	196.9±28.7 Ab	283.2±44.1 Ab	68.4±6.1 Ba	99.1±16.6 BCa	739.5±124.7 Aa	550.4±74.7 BCa	1437.4±281.5 Aa	26.5±6.4 Ab
	45	244.5±29.8 Bb	290.3±36.8 Bb	76.1±4.0 Aa	36.3±2.6 Ca	4155.3±243.0 Ca	133.1±8.8 Cb	148.4±30.5 Ab	1928.4±85.8 Da
	60	504.1±45.1 ABa	567.9±58.8 BCDa	18.5±1.6 Ab	82.6±11.3 Fa	1306.1±540 Ea	166.5±21.4 Fb	33.3±5.0 Ab	622.7±28.9 EFb
A26	20	124.1±16.6 Ab	158.1±14.2 Ab	99.0±9.3 ABa	78.0±5.2 Ca	425.9±41.6 Ab	320.4±21.2 Db	377.4±43.2 BCa	8.5±1.8 Ab
	45	236.3±26.8 Bab	289.0±29.5 Bab	45.7±3.2 ABCb	84.3±5.8 BCa	9040.8±606.1 BCa	252.3±22.3 BCb	38.1±2.9 Ab	3140.9±241.7 BCa
	60	367.5±43.2 Ba	372.7±55.1 Da	14.1±1.4 Ac	141.4±11.5 EFa	15593.6±2491.4 BCa	483.5±67.8 BCDa	58.8±6.6 Ab	3846.7±729.4 ABa
A100	20	269.2±45.2 Ab	305.3±32.4 Ab	111.4±25.5 Aa	258.9±20.9 Aa	1466.3±119.7 Ab	849.8±28.1 Aa	214.2±39.4 Ca	6.6±1.4 Ac
	45	493.0±57.6 Aa	541.7±54.0 ABa	27.6±3.1 Cb	147.4±15.0 Bb	8244.5±2395.6 BCa	413.7±34.3 ABb	41.9±2.8 Aa	3455.9±242.6 ABa
	60	418.6±55.4 Bab	618.7±75.2 ABCDa	12.5±0.8 Ab	104.1±18.1 Fb	11991.9±420.4 BCDa	360.2±39.1 CDEFb	35.1±3.4 Aa	961.1±264.8 DEFb
Biq	20	176.6±35.2 Ab	226.0±37.9 Ab	96.9±10.8 ABa	147.8±17.1 BCa	911.7±111.7 Aa	502.5±40.6 BCDa	294.6±22.1 BCa	8.4±0.5 Ac
	45	546.2±19.9 Aa	698.1±34.0 Aa	37.5±2.4 BCb	150.9±10.3 Ba	3551.8±229.4 Ca	290.7±20.4 BCb	69.7±4.8 Ab	3876.6±264.0 ABa
	60	638.2±23.9 Aa	771.1±43.3 ABCa	26.5±1.4 Ab	224.7±7.8 DEa	4810.6±233.9 DEa	420.0±19.6 CDEab	99.6±9.8 Ab	1683.7±104.1 CDEb
A06	20	331.8±24.4 Ab	387.8±22.8 Ab	90.4±4.1 ABa	144.9±13.5 BCb	1072.4±88.6 Ab	474.2±31.4 BCDb	234.2±13.9 Ca	6.1±1.5 Ab
	45	457.3±65.0 Aab	750.4±91.7 Aa	56.7±6.5 ABCb	146.7±11.6 Bb	16317.2±2381.6 ABa	442.6±28.4 ABb	51.6±5.7 Aa	3316.5±141.5 Ba
	60	505.3±85.0 ABa	848.6±84.6 Aa	21.5±2.5 Ac	338.8±50.7 BCa	19329.5±7610.6 ABa	650.4±109.5 ABa	70.6±14.8 Aa	840.9±104.9 EFb
A17	20	215.9±14.3 Ac	340.6±14.7 Ac	109.7±6.3 Aa	132.0±11.9 BCb	694.0±68.5 Aa	388.9±38.7 CDa	148.8±10.4 Ca	17.7±5.8 Ac
	45	404.2±48.7 ABb	596.3±97.7 Ab	58.1±7.1 ABCb	380.2±74.4 Aa	2197.3±741.8 Ca	329.7±35.3 BCa	34.2±3.4 Aa	4180.6±566.2 ABa
	60	567.5±63.8 ABa	824.1±80.6 ABa	33.8±4.2 Ab	452.6±42.4 Aa	1070.4±109.5 Ea	242.1±18.7 EFa	43.1±3.7 Aa	2013.4±184.3 CDb
A18	20	329.5±43.1 Ab	357.9±58.5 Ab	109.8±20.7 Aa	148.1±13.6 BCb	932.3±124.0 Ab	619.0±115.6 Ba	549.9±104.9 Ba	29.1±9.8 Ab
	45	536.7±75.1 Aa	656.7±91.7 Aa	82.9±12.3 Aa	163.1±10.7 Bb	7224.2±299.8 Cab	235.6±13.4 BCb	47.8±2.7 Ab	1930.7±237.5 Da
	60	405.1±46.4 Bab	543.0±70.5 CDab	23.2±3.6 Ab	291.6±20.1 CDa	7828.5±1437.6 CDEa	299.4±9.0 DEFb	44.0±4.1 Ab	2701.8±384.5 BCa
A83	20	190.1±38.3 Ab	285.3±53.4 Ab	67.4±8.5 Ba	127.7±12.3 BCb	734.2±97.2 Ab	494.9±82.4 BCDb	376.1±59.6 BCa	28.7±3.9 Ac
	45	417.4±51.6 ABa	562.7±60.2 Aa	66.7±7.6 ABa	161.4±16.4 Bb	22054.2±1224.3 Aa	552.5±37.4 Ab	85.8±8.3 Ab	2118.4±329.7 CDa
	60	424.6±89.9 Ba	698.9±99.7 ABCa	20.3±2.4 Aa	305.0±29.0 CDa	28211.8±2507.0 Aa	755.5±50.2 Aa	104.1±7.2 Ab	1239.3±197.8 DEFb

Continuation of Table S1

	DAA	Inositol, myo-	Galactinol	Erythritol	Threonic acid	Glycerol
Hab	20	5480.3±503.8 DEFa	322.3±57.6 Aa	59.2±7.6 Bb	201.7±10.1 CDab	18.4±1.5 BCDa
	45	16.4±1.3 Ab	488.2±86.8 ABCa	57.2±7.2 BCb	144.8±7.9 Bb	9.5±1.1 ABCb
	60	31.7±4.0 Ab	1250.1±216.7 Ca	81.4±3.1 Aa	241.0±25.4 ABa	17.2±0.8 ABCDa
A101	20	9803.7±1044.3 ABa	3594.5±722.9 Aa	93.4±10.6 Aa	376.4058.5 ABa	24.4±2.2 ABa
	45	40.3±4.1 Ab	874.8±91.4 ABCb	64.1±6.0 ABb	133.1±8.9 Bb	15.5±1.2 ABb
	60	51.6±2.1 Ab	1407.1±70.6 Cab	76.2±6.0 ABab	197.5±18.8 ABCDb	23.7±3.5 ABCa
A54	20	7925.8±1066.8 BCDa	359.4±55.1 Ab	53.2±7.1 Ba	266.4±39.7 CDa	18.9±2.6 BCDa
	45	9.5±0.4 Ab	1054.2±184.1 ABCb	20.1±1.8 Db	114.7±9.2 Bb	4.3±0.1 Cb
	60	20.3±3.1 Ab	6052.0±1077.4 Ba	18.7±2.0 Eb	143.5±8.9 BCDb	5.8±0.6 Eb
A26	20	2810.6±130.2 Fa	866.6±63.0 Aa	34.4±3.3 Bb	246.0±21.3 CDa	10.7±0.6 Dab
	45	14.2±1.1 Ab	270.7±10.3 BCa	43.6±3.5 BCDab	154.2±11.9 Bb	8.3±0.4 BCb
	60	28.2±3.4 Ab	534.7±37.8 Ca	58.1±8.0 ABCDa	280.3±39.3 Aa	15.6±1.8 BCDa
A100	20	12041.5±1912.2 Aa	2894.5±434.5 Aa	110.4±11.1 Aa	450.7±60.3 Aa	32.9±1.6 Aa
	45	37.0±2.4 Ab	913.0±211.9 ABCab	66.7±5.9 ABb	149.3±9.6 Bb	14.4±1.3 ABb
	60	29.0±5.8 Ab	347.6±73.3 Cb	49.9±5.8 BCDb	128.8±4.2 CDb	13.1±1.8 DEb
Biq	20	9539.8±1822.5 ABCa	1927.5±62.5 Ab	59.6±7.5 Ba	249.6±11.2 CDa	19.1±2.2 BCDa
	45	31.1±0.9 Ab	1657.6±55.3 ABCb	46.8±4.3 BCDa	123.4±6.3 Bb	10.2±0.4 ABCb
	60	51.6±2.7 Ab	9275.0±852.8 Ba	54.1±4.1 ABCDa	155.9±5.3 BCDb	15.2±0.8 CDab
A06	20	6915.2±436.6 CDa	1415.7±80.7 Ab	52.2±5.6 Ba	176.0±10.0 Da	16.03±0.9 BCDb
	45	18.9±1.7 Ab	3602.9±210.1 ABb	61.0±3.6 ABa	213.4±3.8 ABa	15.6±1.2 ABb
	60	37.3±4.5 Ab	13764.0±3562.4 Aa	67.9±9.0 ABCa	234.4±36.1 ABCa	24.6±5.5 Aa
A17	20	4146.2±408.2 EFa	3277.8±251.7 Ab	46.7±4.3 Ba	188.5±20.9 CDab	14.1±1.6 CDa
	45	38.0±6.8 Ab	3841.4±662.0 Aab	44.6±5.3 BCDa	199.7±42.5 ABa	12.7±1.4 ABCa
	60	30.0±1.9 Ab	6332.2±745.9 Ba	43.7±2.5 CDEa	112.1±7.3 Db	9.7±0.7 DEa
A18	20	5386.3±586.3 DEFa	2427.1±448.8 Aa	55.3±9.0 Ba	295.5±28.7 BCa	21.5±4.4 BCa
	45	18.6±2.0 Ab	311.1±47.6 BCa	29.6±2.4 CDb	111.9±5.1 Bb	7.7±0.4 BCb
	60	26.1±3.2 Ab	956.4±237.2 Ca	36.4±4.5 DEab	133.7±7.9 BCDb	11.6±1.0 DEb
A83	20	5501.0±586.4 DEa	512.7±72.3 Aa	49.5±10.1 Bb	232.5±8.8 CDa	16.5±2.2 BCDb
	45	19.7±4.1 Ab	176.6±30.3 Ca	87.0±8.5 Aa	287.4±19.6 Aa	18.3±1.2 Aab
	60	37.2±3.8 Ab	476.9±67.4 Ca	74.9±6.6 ABa	212.3±12.0 ABCDa	24.5±1.5 ABa

(*)Asterisks indicate metabolic obtained by enzymatic assays, NA, not detected and bars in vertical dark gray indicates the pungent accesses, while light gray not pungent accesses.

Table S2. Metabolites from semi-polar phase of pericarp from pepper fruits collected from 10 accessions of *C. chinense* during three different development ages (20, 45 and 60 days after anthesis (DAA)). The mean values of the metabolite intensity ± standard error (n=6). Capital letters indicate differences between accessions and small letters indicate differences between fruit ages. Differences between means are given by Tukey test ($p<0.05$).

	DAA	Alanine	Valine	Leucine	Isoleucine	Glycine	Serine	Threonine	Alanine, beta-
Hab	20	5479.5±456.5 ABCa	2420.6±160.8 Ca	334.7±34.1 Da	767.4±68.0 DEa	43.8±2.4 Aa	289.9±49.5 Aa	696.4±52.9 CDa	341.0±30.5 ABa
	45	5147.3±523.0 BCa	1812.8±131.8 Ab	231.7±21.4 ABb	628.9±60.3 Aab	11.7±1.1 Aa	422.9±55.8 Aa	633.8±29.8 BCa	53.6±3.3 Ab
	60	6198.7±637.1 ABa	1643.2±89.9 Ac	173.3±14.5 BCDb	461.5±51.2 ABb	13.7±2.7 Aa	466.6±148.8 Aa	653.4±48.9 BCDa	43.7±2.4 Ab
A101	20	2852.1±484.4 Da	733.2±109.4 Há	170.3±22.5 Ea	319.4±37.6 Fa	20.9±0.6 Aa	97.1±11.9 Aa	203.8±20.1 Fa	49.2±4.9 Ba
	45	1991.3±317.2 Da	335.4±31.1 Gb	91.7±9.6 Db	99.4±18.2 Fb	7.8±1.0 Aa	137.9±20.7 Aa	325.8±46.0 Da	25.6±3.5 Aa
	60	2015.9±235.8 Da	353.5±38.3 Fb	127.7±13.2 CDab	96.2±9.5 Eb	10.4±1.3 Aa	103.3±14.2 Aa	217.2±23.6 Fa	25.9±4.5 Aa
A54	20	6093.7±429.1 ABa	2412.6±442.4 Ca	721.5±196.2 Ba	2220.1±810.0 Ba	44.7±7.3 Aa	241.0±19.2 Aa	996.1±176.8 Ba	171.7±15.9 ABa
	45	6650.0±577.3 ABa	1334.6±90.9 Cb	166.8±13.5 BCDb	218.9±9.3 DEFb	16.1±2.1 Aa	562.5±121.8 Aa	997.2±65.4 Aa	86.1±6.8 Aa
	60	6583.7±456.3 Aa	1408.2±54.6 Bb	141.1±7.1 BCDb	264.7±12.7 BCDEb	15.9±2.0 Aa	652.4±131.4 Aa	1099.3±53.0 Aa	71.4±4.4 Aa
A26	20	3817.7±528.6 CDa	2855.2±194.3 Ba	487.5±37.4 Ca	810.1±132.7 DEa	25.8±1.8 Aa	290.9±33.0 Aa	935.9±100.4 BCa	427.8±64.9 Aa
	45	2761.2±206.1 Da	633.6±34.7 Fb	84.2±3.7 Db	166.3±12.3 EFb	7.6±0.8 Aa	256.8±30.8 Aa	604.0±75.1 BCb	41.9±4.6 Ab
	60	2875.2±330.6 CDa	627.8±70.5 Eb	72.5±6.0 Db	123.4±19.0 Eb	7.3±0.8 Aa	160.7±26.1 Aa	407.2±50.6 DEFc	23.3±1.6 Ab
A100	20	4211.3±482.8 BCDa	978.0±109.9 Gb	163.1±13.9 Ea	655.7±134.3 Ea	29.9±2.5 Aa	203.0±37.8 Aa	410.7±31.0 EFb	153.6±22.4 ABa
	45	2724.9±265.0 Da	1347.2±77.8 Ca	176.1±6.9 BCDa	406.1±17.1 ABCDb	8.7±0.4 Aa	261.2±43.0 Aa	641.3±37.8 BCa	26.2±1.4 Aa
	60	2872.8±319.0 CDa	1361.0±93.4 Ba	236.6±18.1 ABa	451.0±34.3 ABCb	15.9±1.9 Aa	232.2±36.1 Aa	765.3±78.3 BCa	19.6±1.3 Aa
Biq	20	5819.9±388.8 ABCa	5224.4±349.4 Aa	1127.8±49.1 Aa	2560.3±123.4 Aa	35.4±3.4 Aa	360.2±24.8 Aa	1557.0±69.3 Aa	450.9±34.4 Aa
	45	2504.5±190.9 Db	861.9±32.5 Eb	201.9±14.0 ABCb	373.8±25.2 BCDEb	11.0±1.1 Aa	421.3±110.7 Aa	716.8±30.5 BCb	47.3±4.5 Ab
	60	2078.4±110.5 Db	450.0±30.9 Fc	111.7±17.9 CDc	173.3±18.5 DEc	15.0±1.8 Aa	199.5±48.3 Aa	372.7±23.0 EFc	21.6±1.2 Ab
A06	20	2840.1±843.0 Da	1810.2±161.8 Fa	738.5±46.4 Ba	1420.9±97.9 Ca	25.1±6.4 Aa	146.8±16.8 Aa	434.9±32.0 EFa	46.0±5.8 Ba
	45	3190.3±353.6 CDa	1307.6±100.5 Cc	113.9±14.4 CDb	284.4±36.2 CDEFb	12.9±1.7 Aa	331.9±46.6 Aa	564.7±81.1 BCDa	34.1±2.4 Aa
	60	4343.5±374.0 BCa	1719.4±118.4 Ab	142.2±12.9 BCDb	233.2±18.6 BCDEb	24.3±3.3 Aa	219.0±39.5 Aa	483.3±31.1 DEa	37.5±2.2 Aa
A17	20	5277.5±808.8 ABCb	1871.0±194.5 EFa	538.6±50.6 Ca	994.2±80.6 Da	34.7±4.3 Aa	254.1±49.2 Aa	783.5±88.8 BCDa	75.7±12.1 Ba
	45	7929.7±808.8 Aa	1096.8±64.0 Db	128.4±10.6 CDc	161.9±17.8 EFb	13.6±1.7 Aa	669.3±149.7 Aa	551.2±39.6 BCDb	24.4±1.3 Aa
	60	4402.0±657.3 BCb	1096.1±107.6 Cb	205.9±26.9 ABCb	221.8±23.7 CDEb	16.0±5.4 Aa	372.8±78.6 Aa	546.6±54.6 CDEb	16.0±1.5 Aa
A18	20	5566.1±722.1 ABCa	2127.6±224.6 Da	533.1±81.3 Ca	1257.3±133.6 Ca	48.7±5.8 Aa	285.1±36.7 Aa	817.3±76.8 BCDa	255.4±33.8 ABa
	45	3083.5±588.4 CDb	1023.5±163.7 Db	291.1±47.4 Ab	507.9±68.6 ABCb	8.7±0.6 Aa	255.2±27.7 Aa	490.9±79.3 CDb	30.7±4.4 Aab
	60	2574.6±316.6 CDb	940.8±35.9 Db	296.7±43.1 Ab	389.0±68.7 ABCDb	10.6±1.1 Aa	243.7±23.8 Aa	474.5.5±43.6 DEb	13.2±1.0 Ab
A83	20	7145.6±379.1 Aa	1958.0±135.2 Ea	299.9±17.7 Da	783.4±60.0 DEa	32.1±2.8 Aa	324.7±36.7 Aa	658.6±20.2 ABa DEa	154.6±5.2 ABa
	45	3565.6±321.4 CDb	1478.2±176.4 Bb	205.9±23.0 ABCb	581.8±84.9 ABb	17.7±1.6 Aa	480.7±123.8 Aa	752.6±86.9 ABa	61.2±10.1 Aa
	60	4321.1±474.8 BCb	1422.3±79.3 Bb	192.6±14.9 BCb	575.5±31.7 Ab	18.0±2.2 Aa	371.6±46.5 Aa	797.3±70.7 Ba	38.5±3.7 Aa

Continuation of Table S2

	DAA	Hydroxyproline	GABA	Aspartic acid	Methionine	Ornithine	Glutamine	Phenylalanine	Asparagine
	20	1140.8±90.4 Aa	8406.8±1021.2 Aa	9068.4±1057.7 Db	34.7±4.6 Aa	213.3±60.7 Aa	43.7±3.8 Aa	75.3±14.0 CDEc	970.9±286.9 Bb
Hab	45	14.1±0.9 Aa	968.9±52.5 Ab	10621.1±469.2 Ca	65.6±5.3 Aa	423.8±62.2 Aa	21.3±1.4 Aa	1217.6±72.0 Aa	1797.4±344.8 ABab
	60	9.7±1.3 Aa	1404.4±153.5 Aab	10733.3±1095.3 Ca	63.9±5.9 Aa	518.5±116.9 Aa	16.6±1.2 Aa	1122.5±114.5 Ab	2359.8±583.2 ABa
	20	83.5±8.1 Aa	1690.3±180.3 Aa	2144.9±106.2 Ia	6.9±0.7 Aa	12.8±1.8 Aa	22.9±0.7 Aa	64.8±6.5 DEc	36.6±5.3 Ba
A101	45	23.7±2.4 Aa	907.4±107.2 Aa	1748.5±131.0 Ib	18.2±2.5 Aa	41.6±4.1 Aa	32.2±2.6 Aa	391.9±92.7 Ea	167.0±15.2 Ca
	60	20.7±1.5 Aa	1014.4±135.5 Aa	1688.4±115.2 Jb	12.7±1.3 Aa	NA	44.5±4.7 Aa	262.1±47.7 Fb	78.2±17.2 Da
	20	1190.4±221.3 Aa	3245.4±222.0 Aa	5263.8±567.1 Hc	55.5±16.2 Aa	53.9±9.9 Aa	26.1±1.6 Aa	88.4±17.8 CDEb	244.2±46.9 Bb
A54	45	25.2±2.4 Aa	1008.9±48.5 Aa	8332.4±1267.4 Eb	132.6±20.8 Aa	307.8±59.5 Aa	23.8±2.2 Aa	130.1±8.4 Gb	1335.7±266.1 ABCab
	60	17.1±3.0 Aa	1408.3±60.3 Aa	11003.1±786.5 Ba	143.9±11.8 Aa	458.5±49.7 Aa	22.6±2.8 Aa	378.9±20.9 Ea	2076.1±242.9 ABCa
	20	400.3±50.9 Aa	3975.2±384.9 Aa	5987.5±740.4 Ga	48.8±4.6 Aa	312.5±49.8 Aa	39.4±4.8 Aa	88.5±7.5 CDEb	1399.5±247.9 ABa
A26	45	26.1±2.2 Aa	653.3±31.1 Aa	3337.0±152.9 Hb	106.5±9.6 Aa	212.5±26.9 Aa	23.6±2.1 Aa	167.6±8.5 Ga	934.8±148.7 ABCa
	60	23.3±2.7 Aa	817.4±63.0 Aa	2916.7±135.9 Ic	94.6±11.8 Aa	258.7±40.3 Aa	25.4±2.8 Aa	196.3±13.7 FGa	1052.7±169.6 BCDa
	20	336.7±28.3 Aa	2859.0±237.4 Aa	5404.1±767.7 Há	29.0±5.6 Aa	40.3±6.7 Aa	29.3±2.6 Aa	44.5±5.4 Ec	150.3±40.8 Ba
A100	45	10.8±1.3 Aa	537.9±28.4 Aa	5184.5±319.4 Gb	160.8±12.9 Aa	90.6±14.1 Aa	16.1±1.0 Aa	465.1±20.5 Db	414.5±67.7 BCa
	60	15.0±1.0 Aa	927.6±104.0 Aa	4987.7±365.5 Fc	204.6±22.1 Aa	138.0±13.0 Aa	30.5±2.1 Aa	1029.2±87.2 Ba	620.4±54.7 CDa
	20	250.3±10.1 Aa	5297.5±320.0 Aa	16038.2±950.0 Ba	131.9±14.1 Aa	225.3±24.5 Aa	52.2±3.1 Aa	134.7±10.9 BCb	1037.3±119.1 Ba
Biq	45	25.0±2.5 Aa	752.5±51.8 Aa	9700.6±470.1 Db	107.8±19.8 Aa	510.0±82.6 Aa	25.7±2.0 Aa	322.3±23.0 Fa	2122.9±390.5 Aa
	60	17.6±1.9 Aa	443.5±29.6 Aa	3914.8±286.6 Hc	62.2±1.4 Aa	520.1±67.2 Aa	30.3±2.0 Aa	147.6±10.4 Gb	2110.7±401.0 ABCa
	20	332.4±25.9 Aa	3230.1±340.1 Aa	7288.1±602.0 Fc	27.0±2.3 Aa	98.9±19.2 Aa	33.8±1.3 Aa	113.9±6.4 BCDc	478.3±98.1 Bb
A06	45	14.0±1.7 Aa	886.7±76.6 Aa	10611.6±732.1 Ca	79.4±6.7 Aa	235.6±18.0 Aa	22.8±2.9 Aa	693.2±65.0 Bb	1091.5±97.3 ABCab
	60	24.0±3.1 Aa	1702.2±120.0 Aa	7887.1±439.5 Db	70.6±5.0 Aa	381.7±61.9 Aa	40.2±1.9 Aa	801.6±52.3 Ca	1866.9±229.3 BCa
	20	94.9±7.1 Aa	4449.1±538.5 Aa	9995.7±698.7 Cb	67.6±8.2 Aa	198.4±37.1 Aa	40.9±2.4 Aa	203.9±8.8 Ab	953.6±197.9 Ba
A17	45	16.4±2.0 Aa	1559.4±205.8 Aa	12134.8±931.2 Ba	94.2±19.3 Aa	264.2±42.5 Aa	21.8±1.9 Aa	303.0±14.0 Fa	1252.2±217.9 ABCa
	60	13.9±0.9 Aa	1364.1±142.1 Aa	6792.2±761.4 Ec	147.1±21.6 Aa	418.2±47.5 Aa	14.4±1.0 Aa	332.2±25.1 Ea	1778.6±262.9 BCa
	20	938.3±145.5 Aa	5185.7±561.5 Aa	7809.7±1327.1 Ea	41.0±3.2 Aa	119.0±12.0 Aa	38.8±4.0 Aa	159.8±12.9 ABb	560.2±87.6 Ba
A18	45	17.9±1.3 Aa	782.6±112.1 Aa	7224.8±1303.7 Fb	103.6±20.3 Aa	263.3±22.9 Aa	18.0±1.6 Aa	401.4±46.3 DEa	1149.6±105.3 ABCa
	60	24.7±7.9 Aa	1328.2±168.8 Aa	4191.6±531.7 Gc	127.9±15.3 Aa	347.1±64.8 Aa	16.6±1.4 Aa	393.5±62.9 Ea	1508.5±262.6 BCDa
	20	892.5±31.6 Aa	3652.8±225.7 Aa	18909.3±1035.8 Aa	39.7±6.7 Aa	644.4±101.7 Aa	49.1±3.0 Aa	101.1±10.0 BCDEc	2858.2±499.4 Aab
A83	45	28.8±2.9 Aa	921.6±95.8 Aa	13555.8±1789.5 Ab	91.7±16.3 Aa	600.8±94.6 Aa	30.5±4.5 Aa	532.3±64.2 Cb	2391.5±416.8 Ab
	60	20.6±3.2 Aa	1127.5±88.0 Aa	11385.5±480.0 Ac	187.4±34.8 Aa	868.6±104.3 Aa	33.3±4.2 Aa	649.5±46.1 Da	3520.7±322.6 Aa

Continuation of Table S2

	DAA	Ascorbic acid	Fumaric acid	Nicotinic acid	Pyruvic acid	Malonic acid	Malic acid	Succinic acid	2-Oxoglutaric acid
Hab	20	65.7±12.0 Aa	281.9±21.4 Aa	520.7±58.5 Ca	84.9±15.4 Aa	62.9±13.7 Aa	30761.6±5407.1 Da	77.9±8.7 Aa	24.3±3.0 Aa
	45	40.3±2.4 Aa	81.4±17.2 Aa	247.7±19.1 Fb	54.1±4.2 Aa	7.8±1.5 Aa	7162.2±632.6 Hb	14.0±2.0 Aa	8.1±0.4 Aa
	60	33.7±2.2 Aa	71.8±13.0 Aa	218.7±30.3 Eb	40.7±5.1 Aa	7.6±0.9 Aa	7230.0±735.9 Fb	14.1±2.3 Aa	10.5±1.6 Ba
A101	20	62.4±2.8 Aa	175.8±20.5 Aa	629.8±54.5 Ba	72.0±15.1 Aa	99.6±15.0 Aa	21321.5±734.5 Ia	75.4±3.8 Aa	14.2±1.4 Ab
	45	101.4±6.2 Aa	129.6±14.3 Aa	564.5±62.3 ABa	176.0±19.6 Aa	25.0±3.6 Aa	15745.0±1557.3 Ab	44.1±4.6 Aa	47.8±3.7 Aab
	60	118.33±16.9 Aa	157.8±24.6 Aa	578.0±48.6 BCa	123.9±28.6 Aa	33.1±6.2 Aa	14665.2±1487.1 Bc	47.8±4.0 Aa	79.4±16.2 Aa
A54	20	76.7±7.0 Aa	272.4±26.1 Aa	642.9±75.4 Ba	85.5±17.9 Aa	142.3±28.4 Aa	26951.1±2560.0 Ga	149.1±19.4 Aa	17.0±3.6 Aa
	45	65.3±10.0 Aa	96.5±21.6 Aa	357.2±47.7 DEb	49.1±4.8 Aa	14.7±1.3 Aa	11370.9±1769.5 Db	28.1±4.7 Aa	18.8±1.5 Aa
	60	69.0±7.5 Aa	114.2±24.0 Aa	257.2±32.5 Ec	53.8±3.6 Aa	12.5±0.6 Aa	11455.9±1404.0 Db	21.2±2.9 Aa	13.8±1.5 Ba
A26	20	30.5±4.3 Aa	163.2±21.4 Aa	390.2±42.7 Da	48.9±6.4 Aa	68.4±13.5 Aa	19971.0±2419.5 Já	47.1±4.8 Aa	25.5±5.2 Aa
	45	44.6±4.2 Aa	108.1±16.5 Aa	356.0±28.1 DEa	83.4±11.7 Aa	16.5±3.3 Aa	10122.7±1235.6 Ec	17.9±2.7 Aa	19.1±2.7 Aa
	60	52.3±7.6 Aa	114.9±17.5 Aa	359.9±33.3 Da	74.7±8.7 Aa	15.9±3.4 Aa	10866.9±1221.1 Eb	19.0±2.1 Aa	28.0±4.6 ABa
A100	20	124.9±11.6 Aa	256.8±21.4 Aa	845.7±102.0 Aa	88.3±18.4 Aa	137.6±23.6 Aa	28997.6±3560.8 Ea	108.0±12.4 Aa	26.3±5.1 Aa
	45	70.7±5.3 Aa	53.1±4.1 Aa	337.2±18.8 EFc	72.9±2.6 Aa	11.0±2.2 Aa	4836.3±311.2 Ic	18.9±2.5 Aa	11.6±1.7 Aa
	60	123.1±10.0 Aa	92.5±7.9 Aa	635.8±49.2 Bb	104.7±19.8 Aa	20.6±3.9 Aa	5183.7±686.7 Ib	30.0±3.4 Aa	24.6±3.2 Ba
Biq	20	74.4±8.8 Aa	273.9±33.6 Aa	643.8±80.4 Ba	113.4±13.4 Aa	45.3±6.3 Aa	33269.1±1826.4 Ca	50.0±2.9 Aa	29.9±2.4 Aa
	45	72.1±3.3 Aa	75.7±11.7 Aa	414.5±43.3 CDEb	64.1±4.9 Aa	8.6±0.7 Aa	8283.3±779.2 Gc	25.5±4.4 Aa	17.4±1.4 Aa
	60	93.9±4.4 Aa	92.4±8.4 Aa	379.2±23.2 Db	59.7±11.8 Aa	10.5±0.9 Aa	13103.8±643.1 Cb	21.5±2.0 Aa	29.9±2.3 ABa
A06	20	94.4±5.3 Aa	225.3±20.3 Aa	554.4±86.3 BCb	86.4±12.9 Aa	82.6±20.3 Aa	26660.9±2522.2 Há	72.5±6.7 Aa	41.8±8.3 Aa
	45	85.2±7.5 Aa	112.7±19.9 Aa	486.7±71.2 BCc	76.9±11.0 Aa	15.4±1.6 Aa	13308.7±1996.2 Bc	27.9±5.6 Aa	13.8±1.8 Aa
	60	93.2±3.9 Aa	175.6±20.6 Aa	876.8±49.9 Aa	97.3±14.5 Aa	23.6±2.3 Aa	21945.5±1126.3 Ab	39.2±2.9 Aa	30.4±2.4 ABa
A17	20	60.9±5.8 Aa	209.5±29.5 Aa	617.2±38.4 Ba	78.4±13.3 Aa	76.2±21.5 Aa	27670.7±2963.0 Fa	66.7±5.8 Aa	49.4±10.0 Aa
	45	75.6±5.0 Aa	111.2±15.5 Aa	436.4±32.8 CDb	101.4±11.1 Aa	12.1±2.1 Aa	12652.5±1047.8 Cb	33.5±4.2 Aa	24.9±3.2 Aa
	60	34.5±4.1 Aa	70.6±7.7 Aa	250.3±20.4 Ec	49.7±6.1 Aa	7.2±1.3 Aa	6952.4±618.8 Gc	23.2±2.3 Aa	21.7±1.8 Ba
A18	20	88.5±10.3 Aa	285.8±26.5 Aa	867.4±105.5 Aa	129.3±25.4 Aa	112.1±25.8 Aa	40401.7±3910.9 Aa	103.9±10.8 Aa	32.4±12.2 Aa
	45	55.6±5.4 Aa	60.6±5.3 Aa	447.0±49.5 CDb	111.8±15.1 Aa	18.7±4.1 Aa	6942.2±821.7 Hb	17.7±2.9 Aa	22.1±2.4 Aa
	60	40.9±4.5 Aa	53.2±4.9 Aa	351.7±36.5 Dc	48.6±5.7 Aa	16.9±2.5 Aa	5976.1±963.3 Hc	20.3±3.1 Aa	30.1±3.5 ABa
A83	20	74.4±7.6 Aa	301.5±28.2 Aa	601.3±96.2 BCa	92.8±9.1 Aa	41.0±10.2 Aa	34483.8±3816.1 Ba	66.3±6.8 Aa	36.6±2.8 Aa
	45	96.9±13.7 Aa	107.7±13.2 Aa	641.2±88.2 Aa	107.7±19.6 Aa	9.2±2.2 Aa	9139.5±780.4 Fc	34.4±6.2 Aa	36.2±7.0 Aa
	60	96.9±10.9 Aa	112.7±15.0 Aa	521.4±56.7 Cb	88.4±14.0 Aa	7.2±0.8 Aa	11462.3±1202.2 Db	32.0±5.3 Aa	33.3±3.6 ABa

Continuation of Table S2

	DAA	Benzoic acid	Glyceric acid	Isocitric acid	Threonic acid	Raffinose	Maltose	Glucose-6-phosphate	Glucose*
	20	3302.4±517.2 Ca	74.7±8.4 Aa	1112.0±88.1 Ca	1015.3±79.6 Aa	14.1±2.7 Da	211.0±36.9 Aa	284.6±67.9 CDa	377.0±43.0 ABCa
Hab	**45**	1017.7±101.2 Fb	25.0±0.9 Aa	364.6±65.4 DEb	350.0±19.0 Aa	10.6±1.9 Aa	149.9±12.9 Aa	112.3±11.0 Db	444.4±19.1 CDa
	60	1006.1±92.4 Fb	21.4±1.8 Aa	231.7±16.5 Fc	324.1±37.5 Aa	6.7±1.0 Aa	148.7±12.4 Aa	89.1±8.5 Db	401.6±18.6 Ca
	20	2600.9±116.9 Da	94.4±5.8 Aa	708.6±25.0 Fc	1294.3±111.6 Aa	242.9±54.2 BCa	258.4±31.9 Aa	188.8±14.7 Eb	175.3±8.3 Db
A101	**45**	1833.9±191.2 Bc	35.6±1.9 Aa	889.7±150.5 Bb	293.8±24.5 Ab	31.5±6.2 Ab	491.9±53.2 Aa	187.7±12.3 ABb	515.5±37.7 BCDa
	60	2286.6±249.6 Bb	41.3±3.3 Aa	1049.0±343.4 Ba	383.7±31.2 Aab	43.8±10.9 Ab	548.4±83.7 Aa	232.1±38.2 Ba	438.2±43.8 BCa
	20	3324.1±263.3 Ca	77.8±8.0 Aa	894.7±64.5 Ea	1169.7±195.0 Aa	95.0±29.7 CDa	234.7±31.3 Aa	240.7±25.2 Da	268.4±18.4 BCDb
A54	**45**	1243.0±173.0 DEFb	29.4±1.9 Aa	247.5±37.8 Fb	452.3±15.5 Aa	13.7±1.8 Aa	170.3±20.0 Aa	148.3±14.5 BCDb	633.9±37.0 ABa
	60	1316.9±170.2 Db	24.2±2.7 Aa	251.0±32.1 Fb	457.1±43.5 Aa	31.0±2.1 Aa	171.5±11.2 Aa	145.9±13.7 Cb	683.5±17.1 Aa
	20	2202.0±240.3 Ea	59.2±5.8 Aa	853.0±93.0 Ea	889.2±158.5 Aa	NA	167.5±30.4 Aa	160.0±21.8 Ea	217.1±15.9 CDb
A26	**45**	1076.9±116.7 EFc	37.9±4.5 Aa	395.3±82.7 DEb	379.6±41.9 Aa	NA	178.5±26.5 Aa	104.5±13.6 Db	410.2±40.2 Da
	60	1277.9±109.5 DEb	41.0±5.6 Aa	403.8±79.5 Eb	376.5±54.8 Aa	NA	235.1±34.4 Aa	122.7±12.7 CDb	426.2±40.1 BCa
	20	4584.1±507.8 Aa	111.2±17.7 Aa	1362.9±154.7 Aa	1622.7±290.7 ABa	28.3±4.7 Da	277.5±30.5 Aa	386.1±51.3 Aa	411.5±13.5 ABb
A100	**45**	1227.8±71.2 DEFc	23.8±0.6 Aa	413.2±49.6 Dc	241.7±11.4 Ab	41.6±11.8 Aa	160.6±18.6 Aa	121.4±10.6 Dc	753.1±108.4 Aa
	60	2525.1±258.7 Bb	45.5±4.5 Aa	943.8±87.6 Cb	436.2±47.2 Ab	24.4±4.0 Aa	256.5±29.0 Aa	223.8±25.7 Bb	715.5±45.4 Aa
	20	3362.2±188.5 Ca	56.2±2.1 Aa	1249.4±89.1 Ba	919.4±63.4 Aa	26.7±4.0 Da	146.4±12.3 Aa	245.9±31.8 CDa	247.5±22.8 BCDb
Biq	**45**	1443.9±78.1 CDc	23.6±1.0 Aa	893.2±202.9 Bb	346.8±18.6 Aa	19.6±3.4 Aa	150.0±11.1 Aa	140.1±3.9 CDb	535.9±32.4 BCDa
	60	1704.0±72.7 Cb	21.3±1.2 Aa	508.6±134.7 Dc	256.1±8.7 Aa	62.2±3.7 Aa	211.1±5.1 Aa	161.1±3.1 Cb	570.5±26.2 ABa
	20	3110.8±320.3 Cb	63.8±6.3 Aa	922.7±69.2 Eb	1002.9±116.8 Aa	382.6±98.3 ABa	295.9±45.9 Aa	246.6±41.0 CDb	434.0±30.6 Aab
A06	**45**	1884.8±258.7 Bc	51.6±6.7 Aa	603.6±136.5 Cc	455.9±51.8 Aa	16.7±3.6 Ab	199.2±13.1 Aa	170.8±13.4 BCc	524.3±42.6 BCDa
	60	4056.7±249.2 Aa	101.3±7.5 Aa	1182.0±174.0 Aa	733.2±36.4 Aa	36.2±5.4 Ab	307.0±20.4 Aa	330.1±25.6 Aa	398.6±18.1 Cb
	20	3255.7±476.2 Ca	103.3±15.6 Aa	1024.4±108.4 Da	1420.5±164.2 Aa	448.0±134.9 Aa	210.3±37.3 Aa	244.4±42.8 CDa	234.9±6.6 CDb
A17	**45**	1537.9±103.4 Cb	35.6±2.7 Aa	332.1±22.4 Eb	440.1±41.3 Ab	55.4±11.4 Ab	171.7±19.8 Aa	147.6±13.4 BCDb	478.8±26.5 BCDa
	60	1007.1±128.4 Fc	20.8±1.3 Aa	184.4±22.4 Fc	298.6±24.9 Ab	62.7±18.2 Ab	90.4±15.4 Aa	92.8±13.5 Dc	383.0±28.9 Ca
	20	4451.0±473.3 Aa	145.3±22.6 Aa	1368.9±141.3 Aa	1834.2±289.1 Aa	124.6±44.3 CDa	220.6±33.0 Aa	339.7±53.0 Ba	379.1±20.4 ABCb
A18	**45**	1327.2±130.5 CDEb	29.8±2.4 Aa	334.5±61.6 Eb	285.1±30.6 Ab	18.8±3.3 Aa	113.0±18.4 Aa	120.9±15.0 Db	554.2±29.8 BCDa
	60	1024.0±105.5 EFc	21.0±2.3 Aa	253.9±12.7 Fc	231.7±21.4 Ab	40.8±7.4 Aa	105.6±17.9 Aa	97.5±12.3 Db	395.2±29.9 Cb
	20	3634.3±355.4 Ba	94.8±5.0 Aa	1239.1±117.3 Ba	1350.5±136.1 Aa	100.9±32.8 CDa	273.2±34.1 Aa	285.2±26.3 Ca	434.5±27.2 Ab
A83	**45**	2378.4±301.4 Ab	39.7±3.7 Aa	1007.6±186.1 Ac	438.8±32.7 Aa	15.1±3.6 Aa	223.7±35.4 Aa	219.0±22.0 Ab	594.5±39.2 BCDa
	60	2426.5±238.6 Bb	43.2±3.2 Aa	1142.9±308.4 Ab	432.4±18.7 Aa	11.2±1.3 Aa	224.5±15.5 Aa	216.2±18.1 Bb	708.9±54.0 Aa

Continuation of Table S2

	DAA	Fructose*	Sucrose*	Cellobiose	Trehalose	Putrescine	Dehydroascorbic acid	Inositol, myo-	Spermidine
Hab	**20**	277.7±33.7 BCb	23.4±2.8 ABa	280.8±33.8 Aa	124.6±5.8 Aa	194.9±23.8 ABa	664.8±171.0 Cc	10787.8±1588.5 Há	8.2±1.1 Aa
	45	497.4±18.8 CDa	33.1±2.3 Ba	235.5±16.8 Aa	93.4±6.8 Aa	113.4±10.9 Aa	1131.2±78.8 Ea	3883.4±385.3 Gb	9.5±1.8 Aa
	60	547.5±24.9 BCa	27.6±2.1 Aa	359.6±43.7 Aa	113.7±12.2 Aa	94.5±5.4 Aa	816.3±39.3 Gb	981.9±329.2 Ic	23.2±5.8 Aa
A101	**20**	201.0±13.6 Cb	30.8±2.9 ABa	361.7±20.0 Aa	126.9±16.8 Aa	130.8±8.3 ABa	872.0±87.7 Bc	14624.3±751.2 Ca	10.1±1.4 Aa
	45	556.3±67.3 BCD	39.0±2.9 Ba	773.6±49.8 Aa	150.3±8.0 Aa	67.5±9.2 Aa	1987.8±150.8 Ab	6634.4±497.9 CDc	11.7±1.9 Aa
	60	533.7±36.4 Ca	35.6±2.1 Aa	1521.9±154.1 Aa	259.0±35.6 Aa	77.6±11.1 Aa	2570.9±424.2 Ba	7345.8±1065.8 Ab	12.3±1.4 Aa
A54	**20**	275.5±14.0 BCb	29.2±3.4 ABa	277.2±30.7 Aa	120.6±25.1 Aa	NA	1098.6±287.0 Ab	19564.5±1604.0 Ba	9.9±1.6 Aa
	45	531.1±16.2 CDa	41.4±3.7 Ba	250.1±23.1 Aa	109.2±19.4 Aa	97.7±4.9 Aa	1496.2±216.0 Ba	6428.8±1037.6 Db	8.9±1.4 Aa
	60	594.2±17.7 BCa	33.3±1.2 Aa	352.4±33.9 Aa	131.1±9.2 Aa	80.8±8.3 Aa	1456.2±118.4 Da	1503.2±226.3 Hc	6.6±0.4 Aa
A26	**20**	211.4±13.1 Cb	61.9±5.5 Ab	227.7±19.1 Aa	131.3±17.3 Aa	154.5±16.4 ABa	177.8±31.9 Ec	8838.2±1106.7 Ia	8.8±1.5 Aa
	45	458.2±31.2 Da	160.3±44.4 Aa	312.0±28.1 Aa	85.1±11.5 Aa	63.4±6.6 Aa	853.6±86.8 Fb	5571.4±883.8 Eb	7.1±1.0 Aa
	60	361.3±32.3 Da	19.4±1.3 Ac	396.8±41.2 Aa	124.5±14.7 Aa	56.3±3.6 Aa	1007.6±161.6 Fa	3553.2±345.3 CDc	7.8±1.2 Aa
A100	**20**	325.3±16.0 ABCb	13.9±2.4 Ba	352.3±35.6 Aa	205.9±17.0 Aa	219.3±19.8 ABa	668.7±49.5 Cc	8455.9±944.6 Ja	13.2±3.3 Aa
	45	521.5±53.0 CDa	28.0±4.5 Ba	271.3±35.7 Aa	53.1±3.1 Aa	35.9±1.4 Ab	1338.6±123.2 BCDb	4117.8±407.9 Fc	6.9±0.4 Aa
	60	579.2±54.1 BCa	14.5±1.8 Aa	713.5±96.5 Aa	158.0±13.5 Aa	43.2±4.9 Aab	2212.3±238.2 Ca	5632.4±511.3 Bb	9.3±0.9 Aa
Biq	**20**	287.1±17.5 BCb	25.6±1.2 ABa	292.4±20.7 Aa	79.4±3.5 Aa	248.9±13.9 Aa	491.2±75.4 Db	21972.2±1607.7 Aa	10.8±1.4 Aa
	45	609.5±30.5 ABCa	34.0±1.6 Ba	302.2±29.1 Aa	107.5±3.2 Aa	117.7±7.7 Aa	1182.6±122.2 DEa	6727.4±524.3 Cb	12.1±1.3 Aa
	60	685.9±25.4 ABa	33.2±1.8 Aa	578.6±37.7 Aa	178.3±5.4 Aa	146.8±6.8 Aa	1278.8±17.7 Ea	3417.9±155.9 Dc	10.0±1.0 Aa
A06	**20**	289.1±18.3 BCc	28.0±2.0 ABa	331.3±32.2 Aa	156.6±9.9 Aa	190.0±20.5 ABa	471.3±62.4 Dc	13552.9±1560.7 Da	9.6±2.0 Aa
	45	627.1±40.7 ABCb	20.7±1.8 Ba	571.8±31.8 Aa	156.3±21.7 Aa	123.0±16.7 Aa	1387.9±182.2 BCb	8136.3±829.5 Ab	8.1±1.1 Aa
	60	746.3±39.4 Aa	22.7±3.4 Aa	1072.3±58.9 Aa	234.2±15.6 Aa	217.6±14.0 Aa	3100.0±307.7 Aa	2023.1±139.6 Fc	10.2±1.1 Aa
A17	**20**	437.0±10.0 Ac	33.1±2.0 ABa	345.1±44.8 Aa	127.2±13.9 Aa	218.3±33.8 ABa	853.7±155.5 Bb	12499.4±1875.2 Fa	15.7±2.4 Aa
	45	752.0±33.2 Aa	39.8±2.7 Ba	495.5±32.1 Aa	115.3±10.3 Aa	63.3±5.6 Aa	1913.6±200.2 Aa	7209.6±554.1 Bb	11.3±1.5 Aa
	60	620.4±39.3 ABCb	45.9±4.1 Aa	361.3±49.1 Aa	119.2±13.0 Aa	54.4±8.7 Aa	750.3±98.5 Gb	3108.5±380.2 Ec	6.3±0.7 Aa
A18	**20**	456.2±33.6 Ab	26.3±2.5 ABa	413.3±70.8 Aa	130.6±36.6 Aa	NA	715.2±134.2 BCc	13062.8±2280.2 Ea	23.4±2.1 Aa
	45	688.2±28.6 ABa	33.6±4.6 Ba	353.1±42.1 Aa	91.7±13.0 Aa	194.0±29.9 Aa	1237.1±173.6 CDEa	4280.1±453.0 Fb	14.0±1.3 Aa
	60	531.3±17.5 Cb	41.4±1.5 Aa	564.1±75.4 Aa	108.5±11.8 Aa	86.5±8.0 Aab	988.0±122.9 Fb	3746.9±451.7 Cc	8.8±1.4 Aa
A83	**20**	397.1±14.0 ABb	18.0±1.5 Ba	355.5±27.1 Aa	268.0±55.7 Aa	271.9±24.0 Aa	277.8±11.4 Ec	12065.7±940.5 Ga	17.1±2.0 Aa
	45	579.5±32.0 BCDa	30.2±2.2 Ba	327.1±42.6 Aa	181.4±34.2 Aa	158.1±21.9 Aa	1365.0±275.9 BCb	2847.7±354.8 Hb	15.9±2.9 Aa
	60	659.5±42.1 ABCa	22.4±2.8 Aa	384.8±38.7 Aa	213.8±7.5 Aa	146.0±11.5 Aa	1574.4±232.8 Da	1791.3±239.0 Gc	14.2±1.2 Aa

Continuation of Table S2

	DAA	Galactinol	Uracil	Erythitol	Glycerol	Palmitic acid
Hab	20	3149.5±229.4 Ha	120.0±16.3 Aa	137.9±12.0 Aa	48.9±9.3 Aa	41622.6±10422.7 Ba
	45	572.5±130.9 FGHb	44.4±7.0 Aa	53.0±5.0 Aa	13.9±1.5 Aa	13888.5±1813.6 CDb
	60	727.6±94.3 Gb	46.9±6.2 Aa	62.4±8.0 Aa	15.4±1.5 Aa	10915.1±1300.7 Db
A101	20	8911.9±1493.9 Da	107.2±17.3 Aa	156.8±4.7 Aa	40.1±1.8 Aa	29049.9±2124.3 Ea
	45	1520.2±98.0 Ec	79.3±12.0 Aa	101.3±7.2 Aa	25.1±2.3 Aa	22251.8±1704.8 Bb
	60	2427.0±467.5 Eb	99.2±8.7 Aa	126.3±9.6 Aa	37.9±5.1 Aa	27788.8±5160.0 Ba
A54	20	6616.2±651.2 Eb	129.1±21.9 Aa	133.9±11.3 Aa	47.7±3.7 Aa	35121.5±3925.2 CDa
	45	4048.2±739.1 Bc	60.1±14.9 Aa	56.9±8.3 Aa	16.8±2.3 Aa	15455.2±1582.1 CDb
	60	18969.0±1967.8 Aa	62.2±12.4 Aa	70.6±12.2 Aa	19.1±2.7 Aa	15583.7±2018.3 Db
A26	20	3462.0±180.5 Ga	83.8±15.7 Aa	103.9±11.1 Aa	30.8±2.2 Aa	23405.3±2740.6 Fa
	45	372.2±36.6 Hc	47.7±8.9 Aa	66.6±5.0 Aa	15.0±2.2 Aa	12400.0±1531.8 Db
	60	897.9±66.5 Gb	51.4±4.0 Aa	62.1±6.2 Aa	18.8±2.2 Aa	14906.7±1604.4 Db
A100	20	6660.6±831.8 Ea	173.5±28.3 Aa	212.2±19.3 Aa	67.1±6.0 Aa	52232.3±7219.8 Aa
	45	436.2±41.9 GHc	55.4±7.2 Aa	69.5±5.4 Aa	17.5±1.1 Aa	15081.0±1160.6 CDc
	60	957.2±108.0 Gb	108.2±11.9 Aa	118.2±9.8 Aa	39.9±4.5 Aa	27077.7±3172.9 Bb
Biq	20	3651.8±1070.6 Gb	127.5±23.1 Aa	120.9±12.3 Aa	48.4±3.4 Aa	39454.4±4571.5 BCa
	45	2947.5±463.8 Cc	65.7±9.7 Aa	74.7±5.9 Aa	20.8±1.5 Aa	17921.9±650.1 ABCc
	60	10673.9±436.5 Ba	73.2±10.1 Aa	95.9±8.2 Aa	29.2±1.9 Aa	21610.4±1056.7 Cb
A06	20	18955.2±2585.9 Ba	119.8±23.2 Aa	114.3±6.2 Aa	46.8±6.7 Aa	34040.3±5564.3 Db
	45	6406.2±410.2 Ac	88.7±20.7 Aa	91.4±12.5 Aa	27.9±4.3 Aa	22172.4±2801.8 Bc
	60	8857.8±547.4 Cb	178.3±27.6 Aa	150.0±13.7 Aa	70.8±7.2 Aa	512947.3±4464.1 Aa
A17	20	19349.0±2684.5 Aa	121.4±18.1 Aa	144.7±6.2 Aa	51.5±5.7 Aa	39360.2±7481.3 BCa
	45	2603.7±374.7 Dc	68.3±9.7 Aa	77.8±6.7 Aa	21.8±2.0 Aa	18546.9±2188.0 BCb
	60	3907.7±436.2 Db	43.5±4.3 Aa	50.6±5.2 Aa	14.9±1.9 Aa	11953.9±2321.6 Dc
A18	20	9273.5±2471.6 Ca	173.7±28.5 Aa	202.1±23.9 Aa	66.0±7.2 Aa	50389.2±7952.4 Aa
	45	699.8±69.8 Fc	57.0±8.6 Aa	64.0±5.0 Aa	18.8±1.6 Aa	15078.1±1962.5 CDb
	60	1274.8±185.2 Fb	47.1±4.5 Aa	53.6±5.4 Aa	16.2±1.6 Aa	11867.8±1683.5 Db
A83	20	4905.1±962.3 Fa	138.7±24.5 Aa	143.8±6.4 Aa	52.6±5.7 Aa	44106.3±4442.3 Ba
	45	621.4±131.8 FGc	98.2±14.4 Aa	107.5±10.1 Aa	37.6±4.8 Aa	29960.2±3797.9 Ab
	60	1429.9±111.4 Fb	111.4±16.5 Aa	112.5±10.5 Aa	39.4±3.9 Aa	29084.1±2529.3 Bb

(*)Asterisks indicate metabolic obtained by enzymatic assays, NA, not detected and bars in vertical dark gray indicates the pungent accesses, while light gray not pungent accesses.

Table S3. Contribution of the placenta eigenvectors to separation of 10 *C. chinense* accessions at 20, 45, and 60 days after anthesis (DAA) obtained from the principal components analysis (PCA). PC1, principal component 1; PC2, principal component 2.

Variable	PC1	PC2
Capsaicin	-0.036	-0.006
Phenois	-0.006	-0.061
Proteins	0.124	0.042
Starch	0.184	0.106
Glucose	-0.19	0.041
Fructose	-0.187	0.067
Sucrose	0.192	-0.024
Amino acids	0.202	-0.078
Alanine	0.209	-0.053
Pyruvic acid	-0.149	0.16
Glycine	0.199	-0.001
Valine	0.209	-0.053
Glycerol	0.092	0.275
Malonic acid	0.129	0.208
Leucine	0.186	-0.026
Isoleucine	0.169	-0.029
Glyceric acid	0.056	0.287
Benzoic acid	0.062	0.288
Threonine	0.175	-0.137
Fumaric acid	0.093	0.252
beta-alanine	0.202	-0.035
Erythritol	0.043	0.269
Malic acid	0.061	0.264
Hydroxyproline	0.176	-0.029
GABA	0.19	0.111
Aspartic acid	0.076	-0.072
Threonic acid	0.142	0.202
Serine	0.084	-0.141
Asparagine	0.045	-0.154
Methionine	-0.08	-0.138
Ornithine	0.03	-0.157
Arabinose	0.096	0.259
Glutamic acid	0.165	0.003
L-Glutamine	0.117	-0.055
Putrescine	0.147	-0.022
2-Oxoglutaric acid	-0.135	0.158
Phenylalanine	-0.132	0.021
Ornithine_1	0.13	-0.174
Mannose	-0.133	0.12
Citric acid	0.174	0.14
Dehydroascorbic acid	-0.174	0.017
myo-Inositol	0.213	0.087
Ascorbic acid	-0.183	0.151
Glucose-6-phosphate	-0.16	-0.007
Tryptophan	0.127	0.03
Similar to cellobiose	-0.08	0.189
Maltose	-0.144	0.169
Galactinol	-0.045	0.089
Raffinose	0.089	0.131

Table S4. Contribution of the pericarp eigenvectors to separation of 10 *C. chinense* accessions at 20, 45, and 60 days after anthesis (DAA) obtained from the principal components analysis (PCA). PC1, principal component 1; PC2, principal component 2.

Variable	PC1	PC2
Capsaicin	-0.061	-0.101
Phenois	0.017	0.071
Proteins	0.127	0.106
Starch	0.132	0.02
Glucose	-0.119	-0.045
Fructose	-0.107	-0.055
Sucrose	-0.066	0.028
Amino acids	0.128	0.205
Alanine	0.069	0.229
Pyruvic acid	0.083	-0.189
Glycine	0.205	0.099
Valine	0.125	0.22
Glycerol	0.203	-0.081
Malonic acid	0.184	-0.025
Leucine	0.142	0.146
Isoleucine	0.155	0.15
Glyceric acid	0.197	-0.05
Benzoic acid	0.212	-0.06
Alanine_1	0.054	0.199
Succinic acid	0.195	-0.024
Threonine	0.055	0.259
Fumaric acid	0.216	0.018
Nicotinic acid	0.179	-0.144
Uracil	0.202	-0.08
beta-alanine	0.145	0.166
Erythritol	0.198	-0.11
Malic acid	0.214	0.021
Hydroxyproline	0.17	0.091
GABA	0.178	0.118
Aspartic acid	0.05	0.22
Threonic acid	0.205	0.018
Serine	-0.068	0.229
Asparagine	-0.063	0.159
Methionine	-0.111	0.122
Ornithine	-0.066	0.152
L-Glutamine	0.167	-0.039
Putrescine	0.093	0.038
2-Oxoglutaric acid	0.07	-0.213
Phenylalanine	-0.103	0.001
Isocitric acid	0.188	-0.091
Dehydroascorbic acid	-0.066	-0.207
myo-Inositol	0.172	0.056
Ascorbic acid	0.081	-0.226
Palmitic acid	0.207	-0.064
Spermidine	0.079	0.006
Glucose-6-phosphate	0.198	-0.098
Similar to cellobiose	-0.007	-0.273
Trehalose,alpha,alpha	0.077	-0.181
Maltose	0.065	-0.289
Galactinol	0.09	-0.01
Raffinose	0.095	-0.045

CHAPTER 2

Integrative analysis of transcriptome and metabolome from contrasting *Capsicum chinense* accessions along fruit development

ABSTRACT

The *Capsicum* genus is highly phenotypically diverse and the fruits are generally considered as nonclimacteric. Although some studies with nonclimacteric fruits have already been carried out, knowledge about the regulation between transcripts and metabolites is still scarce. We selected four phenotypically contrasting *Capsicum chinense* accessions and performed detailed analyzes of the transcriptome and metabolome to generate information on the regulation of processes, in particular, the accumulation of total soluble solids (TSS) that depends on the activity of enzymes such as invertases, sucrose synthase and others of carbohydrate metabolism and different metabolite levels, including sugars, organic acids and secondary metabolites. We identified 16,803 genes expressed from different functional classes and correlated them with 69 quantified metabolites / metabolite classes, including: TSS, starch, amino acids, proteins, sugars, organic acids, pigments, capsaicin and other secondary metabolites. Looking at the transcriptome data, 2747 were negatively regulated and 1121 genes were positively regulated between accessions, and the most influential factor in this difference was the age of the fruits. In addition, we demonstrated several correlations between the metabolic and transcript classes that might be importante in future research studies. Except for the metabolic classes that have their behavior marked over time, such as: chlorophylls (decreased over time), carotenoids (increased over time), starch (decreased dramatically over time), TSS (increased over time) over time) and sugars (increased over time), the other classes had varied behaviors over time and between accessions. Together, our results suggest that there is a complex coordination at the level of transcriptome and metabolome during fruit development that determine phenotypic characteristics. Furthermore, the results presented here improve our knowledge about the regulation of TSS accumulation in a nonclimacteric fruit.

1. INTRODUCTION

The species of the *Capsicum* genus are members of the Solanaceae family, including other species of economic importance, such as tomato (*Solanum lycopersicum*), potato (*Solanum tuberosum*), eggplant (*Solanum melongena*), tobacco (Nicotiana tabacum) and ornamentals like petunia spp. Currently, ca. 40 species of the genus *Capsicum* from American origin are described, being highly diversified in leaf architecture, shape, color and chemical composition of fruits (Rosado-Souza et al., 2015; Jarret et al., 2019). Unlike tomato, a climacteric fruit model, the fruits of *C. chinense* are classified as non-climacteric demonstrating a differentiated maturing pattern (Klie et al., 2014).

Pepper fruits are known for their attractive chemical composition for human health including compounds, such as capsaicinoids, carotenoids, ascorbic acid, flavonoids and tocopherols. There is a complex metabolic and transcriptional interaction to determine the final chemical composition of the fruit, processes such as biosynthesis of carotenoids, degradation of chlorophylls, formation of chromoplasts and alterations in sugar levels occur during fruit maturation (Hubbard and Pharr, 1992; Bouvier et al., 1998; Martí et al., 2009). Previous studies demonstrated that sucrose, glucose and fructose levels in pericarp of *C. chinense* (cultivar Habanero) fruits increase during fruit development up to around 50 days and declining afterwards (Osorio et al., 2012a). Consequently, the starch levels decrease during fruit development (Hubbard and Pharr, 1992; Osorio et al., 2012a), remobilizing carbon skeletons for the synthesis of soluble sugars. In higher plants the sink organs accumulate high levels of sugars, and this depends on the expression of several genes associated with the transport, accumulation and metabolism of these compounds. The sucrose synthase (SuSy) and vacuolar acid invertase (AINV), involved in the cleavage of sucrose in glucose, fructose or UDP-glucose, are associated with the accumulation of soluble sugars in fruits (Qin et al., 2016). The increase of soluble sugars during the fruit development is the result of the import of sucrose in initial stages of fruit, as a result of the translocation of sucrose from photosynthetic to sink organs (Beckles et al., 2012). This is consistent with the total soluble solids (TSS) increment in non-climacteric and climateric fruits during ripening (Niklis et al., 2002; Baxter et al., 2005; Martínez et al., 2007; Beckles et al., 2012). Although other metabolites such as organic acids (malate and citrate) and starch metabolism contribute to the content of soluble

solids (Tiessen et al., 2002; Mounet et al., 2009; Centeno et al., 2011a), the sugars represent around 55% of that (Helyes et al., 2006). Furthermore, organic acids are important for flavor and quality of fruits (Kader, 2008) and their levels are tissue dependent, altered by developmental stage and species analyzed. As indicated in the Chapter 1 of this Thesis and other previous studies, pepper fruits have highly altered contents of organic acids during the fruit development (Flores et al., 2012; Osorio et al., 2012b; Aizat et al., 2014; Jang et al., 2015).

In this work, we selected two accessions from the UFV germplasm bank and two commercial cultivars of *C. chinense*, contrasting in fruit pungency and color and performed a detailed transcriptome and metabolome analyzes to identify transcriptional and metabolic traits related with soluble solids contents in mature fruits. Furthermore, we investigated the correlations between metabolites and several genes of different functional categories in different fruit ages. By doing this we were able to identify possible players and suggest the mechanisms determining the soluble solids contents in *C. chinense* fruits.

2. MATERIAL AND METHODS

2.1 Plant material and experimental conditions

We have selected two contrasting accessions of different geographic origin and these accessions do not differ significantly in the physiological parameters of the leaf (Rosado-Souza et al., 2015). In addition, two commercial genotypes were added to the experiment, Habanero (pungent cultivar of Mexican origin) and Biquinho (non-pungent cultivar from Brazil) (Fig. 1). To avoid cross-pollination, seeds were joined for two generations, (Bosland, 1993. Seeds were germinated on commercial substrate (Tropstrato HT hortaliças). Twenty five days after, seedlings were transplanted into 5-liter pots with a mixture of soil and substrate (1:1 w/w) fertilized with 20N - 5P2O5 - 20K2O fertilizer (Heringer). Plants were grown in a greenhouse located at the Federal University of Viçosa (Viçosa-MG, Brazil; 642 m of altitude, 20°45 'S; 42°51' W). Plants were watered regularly and fertilized weekly with 40 mL of a solution containing 5.0 g of (NH4)2SO4 and 2.5 g of KCl per liter.

2.2 Fruit harvests

To set the precise age of the fruits, the flowers were marked in the anthesis period after initiating flowering as previously described (Osorio et al., 2012). Pericarp samples of six fruits from four accessions were collected at middle of the light period at 20 and 60 days after anthesis (DAA). Samples were snap frozen in liquid nitrogen and stored at -80 °C until analyses.

2.3 Determination of capsaicinoids level

High performance liquid chromatography (HPLC) was used for evaluated the fruit capsaicinoid content in the pericarp tissues according to Maillard et al., 1997 with modifications. About 15 mg of freeze-dried fruit pericarp were suspended in 2 mL of methanol: water (60:40, v/v) and sonicated for 15 min at room temperature. The mixture was centrifuged at 1.600 g for 15 min and filtrated with a Milipore membrane (0.22 µM). Subsequently, 30 ul from the filtrate was inject on a HPLC equipped with Agilent ZORBAX Eclipse Plus C18 column (150 mm, particle size 3 µM in diameter) and UV detector at 229 nm and 281 nm. The isocratic mobile phase was composed of methanol: water: acetic acid (70: 28: 2, v/ v/ v) and a flow rate of 1.0 mL min-1 at 20°C. The authentic standards (Sigma- Aldrich) were used for the absolute quantification of capsaicin and dehydrocapsaicin.

2.4 Determination of primary and secondary metabolites

Metabolite extraction, derivatization, standard addition, and sample injection for gas chromatography-time of flight-mass spectrometry (GC-TOF-MS: Agilent 7890 A GC system coupled to a Pegasus HT high throughput TOF/MS (LECO)) were performed according to (Osorio et al., 2012a). The mass spectra were cross-referenced with those in the Golm Metabolome Database (Kopka et al., 2005). The levels of starch, sucrose, fructose, and glucose in the pericarp tissue were determined as previously described (Fernie et al., 2001). Total protein and total amino acid contents were quantified as previously reported (Cross et al., 2006b). Malate and fumarate levels were determined according to (Alhagdow et al., 2007). Total soluble

solids (TSS) were quantified by refractometry using a portable digital refractometer (ATC 106-D Biobrix) expressed in °Brix (Lannes et al., 2007).

The pigments levels (chlorophyll a, chlorophyll b and carotenoids) were determined as described by Wellburn, 1994 with minor modifications. About 40 mg of fresh weight (FW) pericarp powder were suspended in 2 mL of acetone 80% and incubated in darkness for 30 minutes and centrifuged at 15,000 g for 10 min at 4 ° C. Absorbance of the supernatant was determined at 470, 646 and 663 nm using a spectrophotometer (VERSA max microplate reader).

Total soluble phenols were quantified using the Folin-Ciocalteu method (Sun et al., 2007) and the quantification of individual secondary metabolites performed on HPLC as described by Keinanen et al., 2001 with modifications. The ethanolic extraction was performed using 50 mg of freeze-dried pericarp sample (Cross et al., 2006a). After filtration on a Milipore filter of 0.22 µM, aliquots of 50 µL were injected into HPLC. Absorbances were determined at 210, 254, 320 and 365 nm for the determination of secondary metabolites. The biphasic elution gradient was made of Phase A-0.25% of H3PO4 in ultrapure H2O (pH 2.2); phase B-100% acetonitrile, and the program running: 0-6 min (0-12% B), 6-10 min (12-18% B) and 10-30 min (18-58% B) with a flow 1 mL min-1 and the column was Agilent ZORBAX Eclipse Plus C18 column (150 mm, particle size 3 µM in diameter). Authentic standards were used for identification and quantification by use of calibration curve of metabolites.

2.5 RNA extraction

The total RNA was extracted from the pericarp fruits collected at 20 and 60 DAA using the TRIzol reagent (Invitrogen, USA) according to the manufacturer's instructions. Each RNA sample was subjected to DNase digestion (Promega, Madison, WI) to remove any remaining DNA. The quality and integrity of the RNA were monitored by spectrophotometer and electrophoresis on agarose gel. Total RNA concentrations were determined using a spectrophotometer (Nano-Drop, Wilmington, DE, USA). RNA with an OD 260/280 between 1.8 and 2.2 and an OD 260/230 ≥ 1.8 was used for the construction of cDNA libraries.

2.6 RNAseq analysis

The reads were mapped to the hot pepper genome (Mexican landrace of *Capsicum annuum* cv. CM334) (Kim et al., 2014) using TopHat version 2.1.1. All analysis to identify the differentially expressed genes (DEGs) were performed using R version 3.5.1 (R Core Team, 2018). The read counts were normalized using the trimmed mean of M-values (TMM) method and DEGs were identified using edgeR version 3.8.5 (Robinson et al., 2010; McCarthy et al., 2012).

The identified DEGs were classified into MapMan functional plant categories using Mercator4 version 1.0 (http://www.plabipd.de/portal/web/guest/mercator4). In addition, their sequences were also aligned with UniRef Enriched KEGG Orthology (UEKO) database (http://maxixe.icb.ufmg.br/ueko/) using BLAST version 2.8.1 (Altschul et al., 1990) to assign KO identifiers. The KO identifiers were selected as input for pathway analysis using Interactive Pathways Explorer (iPath) version 3.0 (Darzi et al., 2018).

2.7 Experimental design and statistical analyses

Three replicates per accession comprising one plant in each 5 liter pot were used in the experiment performed in randomized block design. Statistical from metabolite analysis were performed using the GENES program (Cruz, 2006). The data were submitted to analysis of variance (ANOVA) and Tukey's (HSD) test at 5% probability.

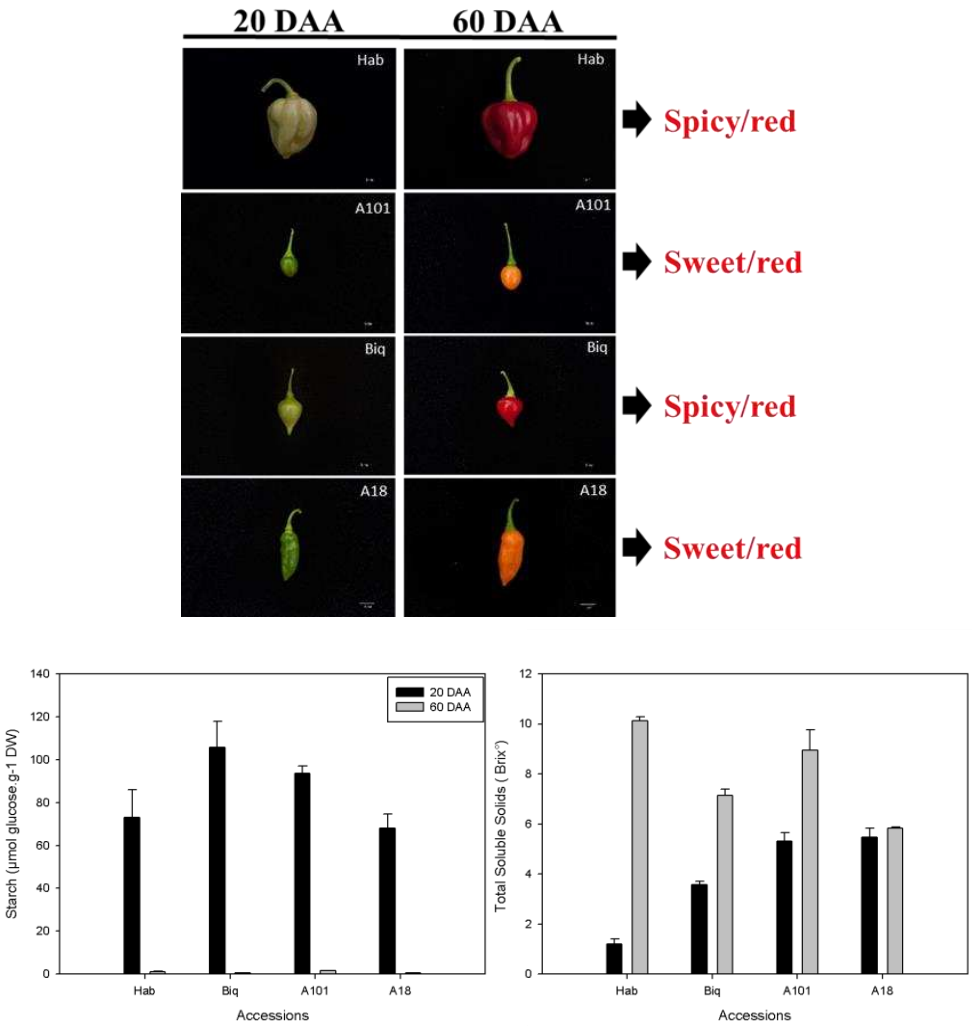
Metabolite-Transcript (MT) correlations were calculated using R version 3.5.1 (R Core Team, 2018). A table with metabolites and transcripts in the rows and samples in the columns was loaded and analyzed using a correlation function (Supplementary Material Table S1 and S2). Then, the calculated MT correlations were selected in the pairwise correlation matrix to produce a table with transcripts in the rows and metabolites in the columns. Only the significant MT correlations ($P \leq 0.01$) were considered and the non-significant values were replaced by zero in the MT correlation table. The genes were ordered according its functional classification and the MT correlation heatmap was plotted using the heatmap.2 function of gplots package (Warnes et al., 2016).

3. RESULTS

3.1 Morphological and metabolic characterization of fruits from *C. chinense* accessions in two developmental stages

To evaluate the phenotypic variability, fruits of the four accessions were sampled in two post-anthesis stages, 20 and 60 DAA. The fruits of the accessions a18 and a101 showed yellow color and the Hab and Biq accessions exhibit red fruits in full ripeness (Fig. 1A). In addition, the four accessions showed different levels of capsaicinoids, the a101 and Hab accessions have high levels at the two stages of development, while the a18 and Biq accessions have only traces of capsaicinoids (Fig. 1A).

Figure 1. A- Phenotypic variability of 4 *C. chinense* accessions indicated in numbers, in three stages of development- 20, 45 and 60 days after anthesis (DAA), scale 1cm. B- Total soluble solids and starch levels from fruits of *Capsicum chinense* accessions at 20 and 60 days after anthesis. Grey and black bars represent the stage of development (20DAA and 60 DAA).



Currently it is well understood how changes in primary metabolism occur in climacteric fruits such as tomatoes (Carrari et al., 2006). However, the dynamics of metabolism in non-climacteric fruits is still poorly understood. In this work, we seek to define the main relationships with the levels of starch and the content of soluble solids in the fruits. It is evident that the content of TSS increases during the development of the fruits (Fig. 1B) in all accessions. Surprisingly, less variation was observed in a18 accession. Furthermore, the cultivar Habanero exhibited the lowest content of TSS at 20 DAA and values increased dramatically compared to other accessions at 60 DAA (Fig. 1B). As expected, a massive decrease in the starch levels was observed from 20 to 60DAA fruits, suggesting an inversion with the total soluble solids content (Fig. 1 B). Regarding the differences between the accessions, the yellow accessions a101 and a18 had the high content at 20 DAA, while at 60 DAA the pungent Hab and a101 accessions had the highest TSS content, interestingly, although Hab accession showed the reduced value to 20 DAA, presented high content at 60 DAA, in addition, the high TSS content of accession a18 at 20 DAA did not reflect in high content at 60 DAA (Fig. 1 B).

We next turned our attention to differences in the contents of total amino acids, proteins and main pigments between accessions between the four genotypes and the two fruit ages. We observed variation depending on the fruit age in total amino acids and pigments, while protein levels remained stable (Fig. 2). The levels of total amino acids increase slightly over time. Carotenoids increased in red fruits and remained stable in yellow fruits during fruit ripening. Chlorophyll levels, however, decreased during fruit development.

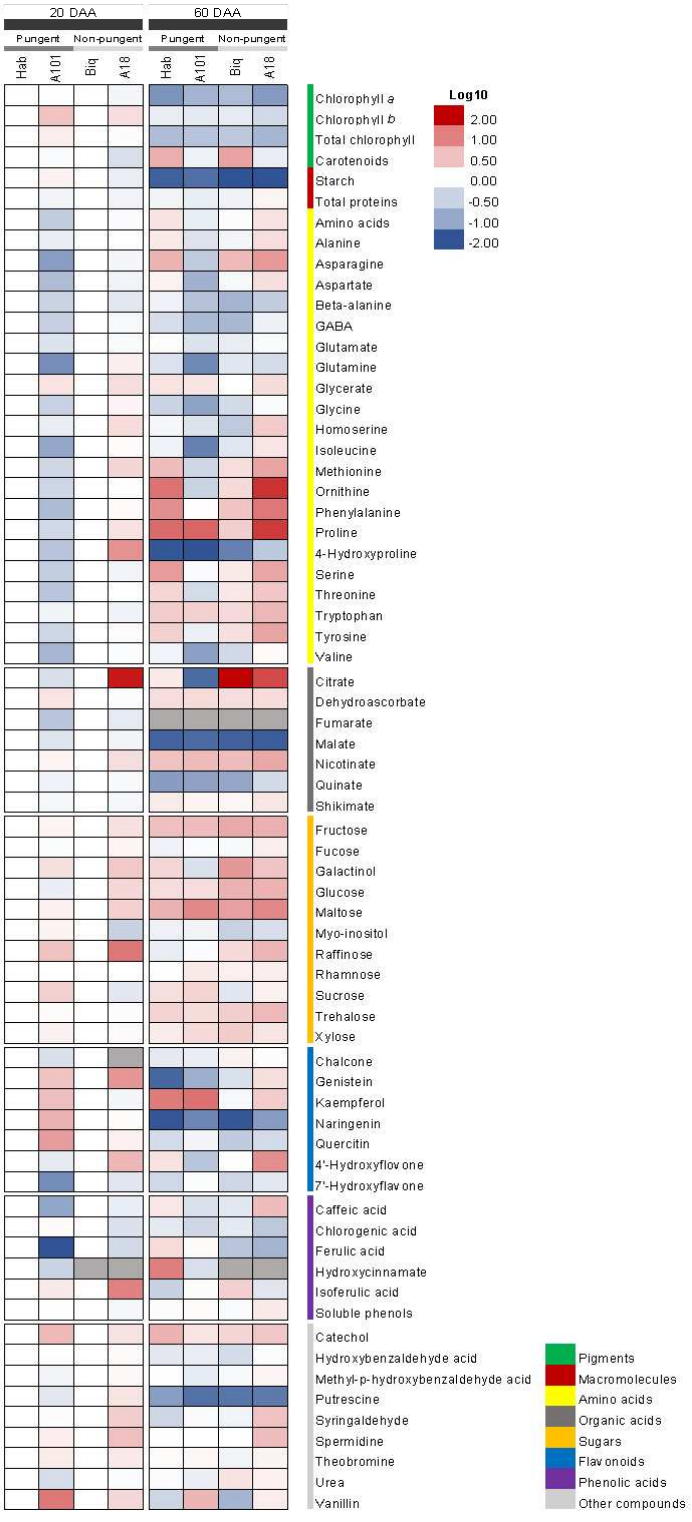
The metabolic profiles were obtained through quantification by GC-tof-MS of 42 metabolites of known chemical structure. For simplicity, the metabolite profile study is presented as a heat map and the complete data sets are presented as supplementary data (table S1). On a false color scale, increases or decreases in the relative content of metabolites (Fig. 2). A total of 21 amino acids were analyzed and varied along the fruit development: (i) increase (asparagine, methionine, ornithine, phenylalanine, proline, serine, threonine, tyrosine and tryptophan); (ii) remaining constant (Alanine, aspartate, glutamate, glycerate, homoserine, isoleucine and valine); or (iii) decreasing (beta-alanine, GABA, glutamine, glycine and 4-hydroxy-proline). Seven organic acids were measured in pericarp of fruits at two developmental stages (Fig. 2). Nicotinic acid,

citric acid and dehydroascorbate increased during fruit development. Malic acid and quinic acid decreased from 20 to 60 DAA whereas shikimate showed no changes during fruit development. The levels of sugars in general showed a tendency to increase 20 DAA to 60 DAA, except for fucose myo-inositolm rhamnose and sucrose that tended to remain constant (Fig. 2).

Subsequently, we turned our attention to the variation observed in secondary metabolites in different fruit ages and between accessions. Twenty-one metabolites were quantified including flavonoids (e.g., flavones, chalcones, flavanones, flavonols and hydroxyflavones) phenolic acids and other phenols (Fig. 2; Table S2).

Flavonoids showed compound specific patterns. Chalcone and 4'-hydroxyflavone contents varied differently between accessions. Kaempferol content increased, while genistein content globally decreased from green to mature fruit. The accessions generally exhibited a reduction in levels of quercetin and 7'-hydroxyflavone. Nevertheless, all accessions displayed strong reduction in naringenin levels between green and mature fruits. Analysis of phenolic acids highlighted instead a more diverse pattern of accumulation from the flavonoids discussed so far: Hydroxycinnamic acid and caffeic acid, for example, increased during fruit development. On the other hand, The content of chlorogenic acid, ferulic acid and isoferulic acid tended to decrease, while Hydroxycinnamic acid and caffeic acid no showed pattern. The other as hydroxybenzaldehyde acid, putrescine and vanillin decreased over time. The levels of theobromine did not change during development, although small differences among the accessions were observed. Syringaldehyde and catechol behaved differently. Syringaldehyde levels in general did not change during fruit development with exception of Hab that decreased during development (Fig. 2;Table S2). Catechol levels in general increased during development in the accessions Hab, A101, Biq and A18.

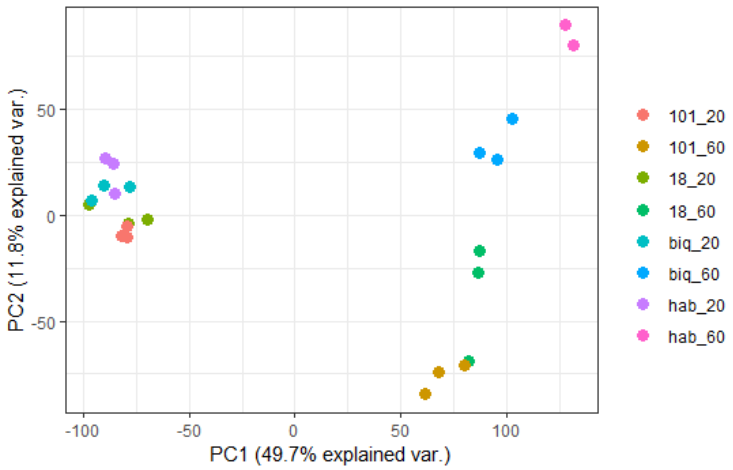
Figure 2. Heat map of primary metabolic profile in pericarp of fruits from 4 *C. chinense* accessions at 20 and 60 days after anthesis (DAA). A color-coded matrix represents the mean values of the metabolite intensity in six biological replicates of pericarp from pepper accessions. Blue and red square represent negative and positive values respectively. The values of the pungent genotypes were normalized by habanero at 20 DAA and non-pungent by biquinho at 20 DAA. The complete metabolite data set is presented in the Supplemental Tables 1 and 2.



3.2 Transcripts analyses in pericarp of *Capsicum chinense* accessions in fruits of two developmental stages

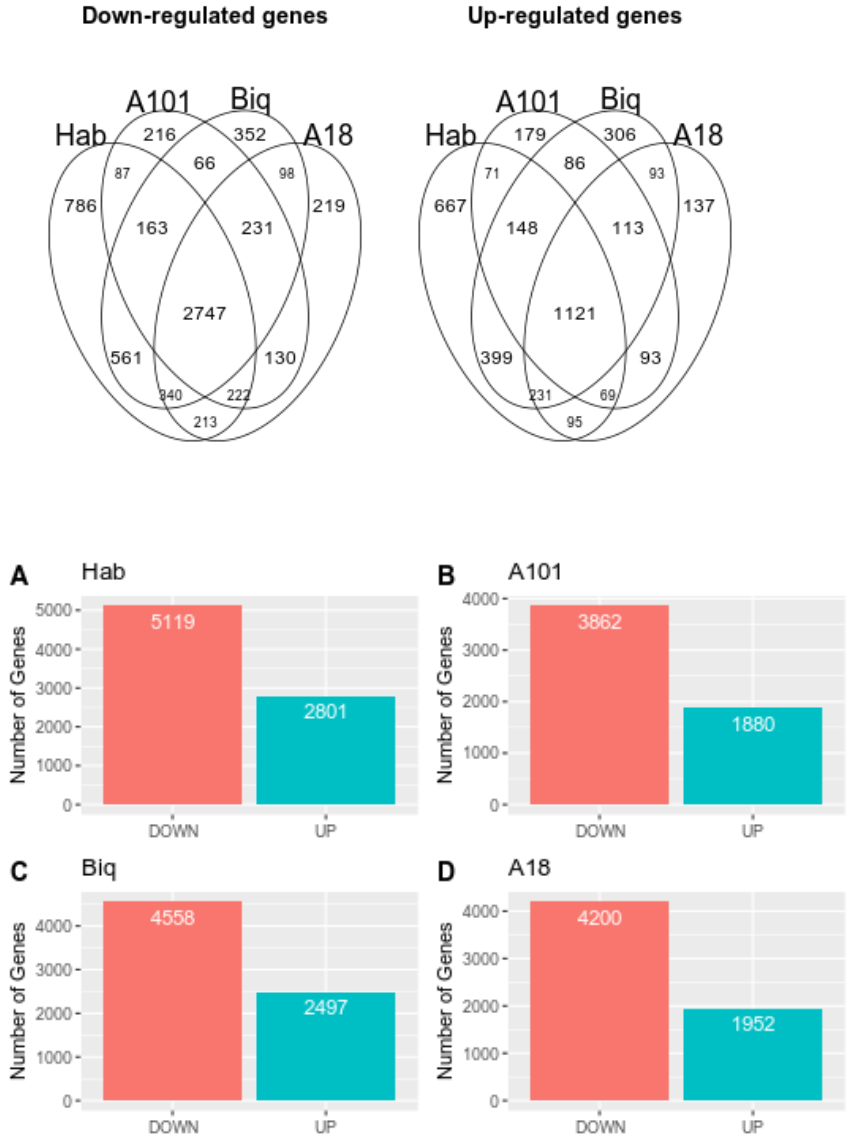
The RNA-seq analysis identifies 34898 unique genes and the calculation of FPKM for each gene resulted in a total of 16803 genes that were used for all statistical analysis.

Figure 3. Principal component analysis (PCA) resulting from the expression values of 16803 genes in each one of the three biological replicates for A101, A18, Biq and Hab genotypes at 20 and 60 days after anthesis stage from RNA-Seq data set. Scores of principal component 1 (PC1) and 2 (PC2) explained 49.7% and 11.8% of total variance, respectively.



The analysis of PCA with the expression value of 16803 genes demonstrated that the first two components could explain 61.5% of the total transcript expression level variance in the score plot (Fig. 3). Each accession was plotted on the graph, presenting low variance at 20 DAA in relation to the 60 DAA stage, demonstrating that the highest transcriptional variation among the genotypes occurred at 60 DAA, in addition, all accessions with age 20 DAA were separated from those of 60 DAA, showing great difference in the transcript profile influenced by the developmental stage. Three biological replicates show relatively low variation, demonstrating the reliability and reproducibility of the gene expression data sets. When comparing the number of differentially expressed genes (DEGs) in each accession throughout development, we counted 5119, 2862, 4558 and 4200 down-regulated genes and 2801, 1880, 2497 and 1952 up-regulated genes for Hab, A101, Biq and A18 respectively (Fig. 4).

Figure 4. Venn diagram and summary of DEGs from RNASeq data obtained from *Capsicum chinense* genotypes at two developmental stages (20 and 60 days ater anthesis). Analyses of the differentially expressed genes (DEGs), comparing the number of upregulated and downregulated genes within the same genotypes in different developmetal stages (20 DAA and 60 DAA).

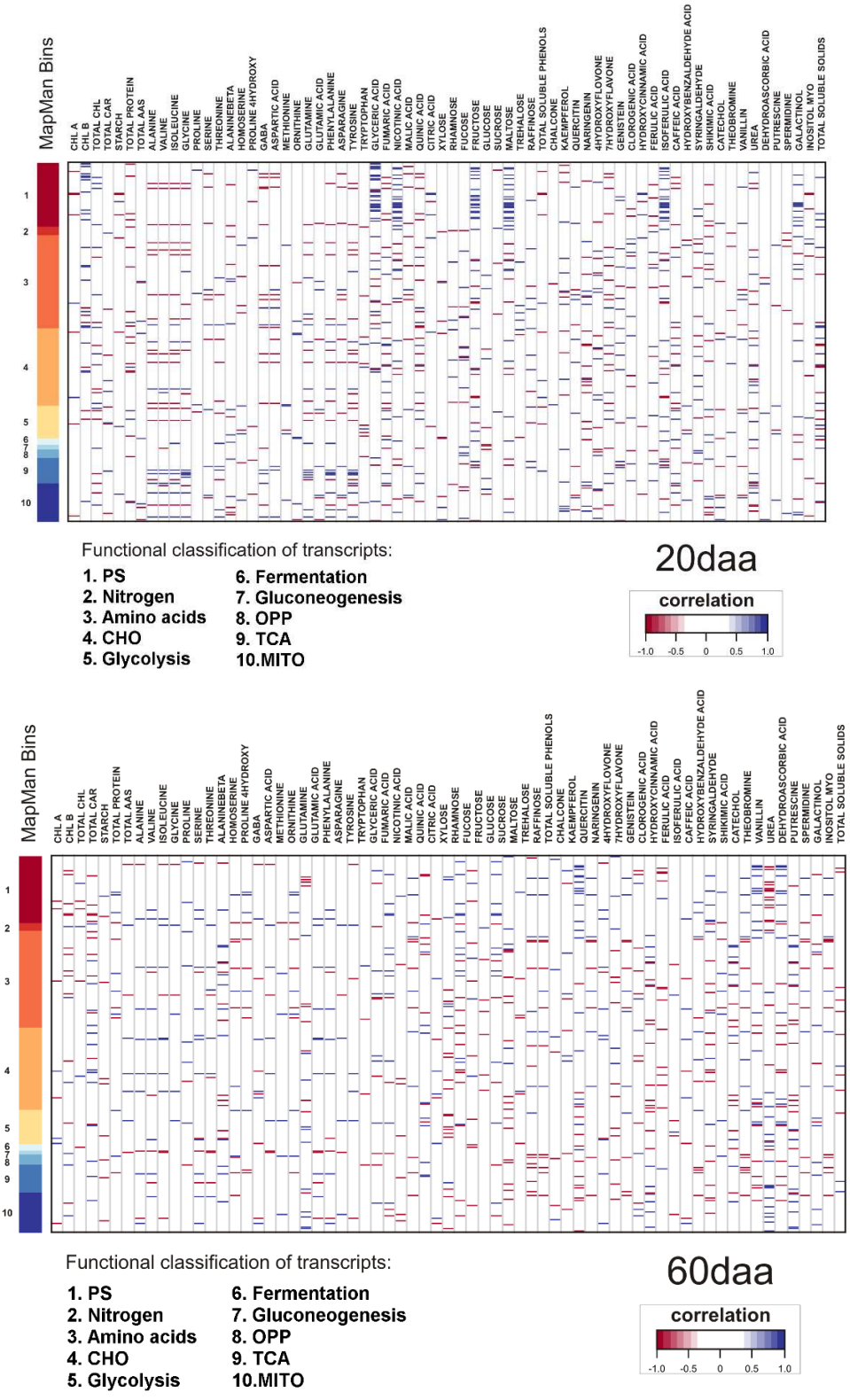


The Venn diagram was used to demonstrate the relationship between these DEGs from genotypes. According to figure 3, 2747 down-regulated genes and 1121 up-regulated genes were shared between accessions. Coincidentally, red fruits (Hab and Biq) had a higher number of DEGs, both down and up-regulated genes (Fig. 4).

3.3 Identification of key metabolism-associated genes and their correlation with total soluble solids content

Person correlation analyzes were performed between the transcript profile and the metabolite profile at 20 and 60 DAA in order to understand the relationship between the transcriptome and the metabolic abundance in the fruits, and thus have a overview of which gene classes are related to specific metabolites (Fig. 5). 15854 and 15180 genes were used for 20 and 60 DAA respectively, correlating pair with 68 metabolites or class of metabolites. 15109 and 14183 genes at 20 and 60 DAA respectively correlated with at least 1 metabolite. At 20 DAA, among 1.078.072 possible combinations, 12437 positive and 12465 negative correlations were generated. In the 60 DAA stage, 1.032.240 combinations were possible, which generated 9491 positive and 10738 negative correlations. Overview of Person correlation analysis calculated between the metabolite profile and transcript profile demonstrated variation in the number of transcribed / metabolite interactions between 20 DAA and 60 DAA, after selecting 10 functional classifications (the correlation matrix with all functional classifications is shown in table S3 and S4). In general, the 20DAA stage presented 15.58% (3198 correlations) significant additional correlations in relation to the 60 DAA stage (2700 correlations). Except for the oxidative pentoses phosphate pathway (OPPP), all nine other functional classifications of the selected genes had more significant interactions at 20 DAA, the detailed numbers are shown below: PS: 624 and 485 significant correlations at 20 DAA and 60 DAA respectively, NITROGEN: 65 and 65, AMINO ACIDS: 831 and 786, CHO: 648 and 510, GLYCOLYSIS: 317 and 239, FERMENTATION: 53 and 41, GLUCONEOGENESIS: 42 and 40, OPP: 70 and 91, TCA: 210 and 169 and finally MITO: 338 and 277. From this overview, we selected all genes significantly correlated with total soluble solids content, shown in table 1 and table 2. The correlation was considered significant if, PCC was > 0.90 (positive) or <-0.90 (negative), at a P-value <0. 05 and we found 62 genes for 20 DAA and 17 for 60 DAA which are part of different functional classifications and encode enzymes.

Figure 5. Overview of Person correlation analysis calculated between the metabolite profile and transcript profile obtained from RNA-seq analysis from fruits of *Capsicum chinense* accessions sampled at 20 and 60 days after anthesis, in pairwise comparisons. The correlation was considered significant if, PCC was > 0.90 (positive) or <-0.90 (negative), at a P-value <0.05. The White square represent the correlations non-significant and <0.90 and >-0.90 and the blue and red square represent the significant positive and negative correlations, respectively.



The genes positively correlated with the total soluble solids content at 20 DAA were: CA01g31780 (2Fe-2S ferredoxin-like), CA02g05860 (fructose-bisphosphate aldolase chloroplastic), CA09g05540 (ribulose bisphosphate carboxylase oxygenase), CA10g10930 (fructose-1,6- chloroplastic), CA11g04070 (ribulose-1,5 bisphosphate carboxylase oxygenase large subunit), CA01g10910 (nitroreductase family), CA01g24340 (glutamine synthetase), CA05g12940 (glutamine synthetase), CA01g29060 (2-isopropylmalate synthase), CA02g00970 (cystathionine gamma synthase), CA09g16620 (serine acetyltransferase 5), CA10g19810 (cystathionine beta- chloroplastic isoform X1) CA12g20610 (S-adenosylhomocysteine hydrolase),CA12g20620 (S-adenosyl-L-homocysteine hydrolase),CA12g21760 (serine--glyoxylate aminotransferase),CA03g02770 (beta-amylase chloroplastic),CA09g00240 (apoplastic invertase),CA01g01470 (glucose-6-phosphate 1-epimerase), CA01g22690 (galactinol synthase), CA07g14540 (alpha,alpha-trehalose-phosphate synthase), CA12g04290 (phosphoglycolate phosphatase chloroplastic), CA12g09510 (inositol-3-phosphate synthase), CA01g02250 (phosphoglycerate mutase), CA05g01330 (6-phosphofructokinase 2), CA05g04290 (phosphoenolpyruvate carboxylase kinase), CA07g02180 (NADP-dependent glyceraldehyde-3-phosphate dehydrogenase), CA07g14650 (phosphoenolpyruvate carboxylase), CA07g17160 (phosphoenolpyruvate carboxylase), CA12g03130 (ATP-dependent 6-phosphofructokinase chloroplastic), CA11g20330 (alcohol dehydrogenase-like isoform X1), CA01g23570 (phosphate dikinase chloroplastic), CA09g13800 (glyoxysomal citrate synthase), CA12g10550 (cytochrome c oxidase subunit 6b-1-like). In addition to these, some enzymes correlated negatively at that same stage of development: CA03g19580 (glutamate synthase 1 chloroplastic isoform X1), CA03g07080 (homocysteine S-methyltransferase 2), CA04g06500 (4-hydroxy-tetrahydrodipicolinate chloroplastic),CA05g01570 (fumarylacetoacetase), CA11g14850 (homoserine dehydrogenase isoform X1), CA12g09890 (asparagine synthetase 2), CA12g10090 (alanine--glyoxylate aminotransferase 2), CA02g24560 (probable hexokinase), CA04g18770 (alpha-amylase), CA04g21750 (hexokinase-chloroplastic), CA04g21760 (hexokinase- chloroplastic), CA04g21800 (alkaline neutral invertase CINV2), CA01g05740 (pfkB-like carbohydrate kinase family), CA02g13420 (alpha,alpha-trehalose-phosphate synthase), CA05g13870 (myo-inositol-1-phosphate

synthase), CA03g06560 (pyruvate kinase isozyme chloroplastic), CA06g04400 (triosephosphate cytosolic), CA06g25020 (cytosolic enolase 3), CA08g16520 (pyruvate kinase isozyme chloroplastic), CA02g21900 (aldehyde dehydrogenase family 3), CA11g07710 (aldehyde dehydrogenase family 2 mitochondrial), CA07g12030 (isocitrate lyase), CA07g12040 (isocitrate lyase), CA12g13590 (6-phosphogluconate decarboxylating chloroplastic), CA01g16000 (ATP-citrate synthase beta chain 2), CA01g03460 (NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8-B), CA03g21560 (NADH dehydrogenase [ubiquinone] iron-sulfur mitochondrial), CA06g24420 (NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2), CA08g08770 (NAD(P)H:quinone oxidoreductase) (Table 1).

We also verified the number of correlations at 60 DAA, which was interestingly less than at 20 DAA. Positive correlations: CA10g07200 (nitrite reductase), CA01g05740 (pfkB-like carbohydrate kinase), CA03g24310 (glyceraldehyde-3-phosphate cytosolic), CA08g05200 (NADP-dependent malic enzyme isoform X2), CA12g01280 (isocitrate dehydrogenase [NADP]). Negative correlations: CA01g14810 (ATP synthase CF0 subunit IV (chloroplast)), CA02g24690 (branched-chain-amino-acid aminotransferase chloroplastic), CA07g20250 (serine acetyltransferase chloroplastic), CA09g08100 (3-hydroxyisobutyryl- hydrolase 5), CA11g00650 (3-isopropylmalate dehydrogenase chloroplastic), CA02g24490 (starch synthase chloroplastic amyloplastic isoform X1), CA07g09080 (sucrose synthase), CA02g22200 (galactinol synthase), CA06g10330 (aldo keto reductase), CA03g06550 (pyruvate kinase isozyme chloroplastic), CA04g23530 (pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha), CA04g21820 (NADH dehydrogenase [ubiquinone] 1 mitochondrial) (Table 2).

Table 1. List of the genes significantly correlated with total soluble solids contents at 20 DAA. The correlation was considered significant if, PCC was > 0.90 (positive) or <-0.90 (negative), at a P-value <0.05.

***Capsicum annum* gene IDs**	**Description of related genes**	**Total Soluble Solids**		
		Abbreviation	**PCC**	***P*-value**
CA01g31780	2Fe-2S ferredoxin-like superfamily		0.99	0.001
CA02g05860	fructose-bisphosphate aldolase chloroplastic-like		0.98	0.023
CA04g10570	psbP domain-containing chloroplastic		0.98	0.020
CA09g05540	ribulose bisphosphate carboxylase oxygenase activase chloroplastic isoform X2		0.99	0.011
CA10g10930	fructose-1,6- chloroplastic-like		0.99	0.008
CA11g04070	ribulose-1,5 bisphosphate carboxylase oxygenase large subunit N-chloroplastic-like		0.96	0.039
CA01g10910	nitroreductase family		0.96	0.040
CA01g24340	glutamine synthetase		0.99	0.003
CA03g19580	glutamate synthase 1 [NADH] chloroplastic isoform X1		-0.96	0.037
CA05g12940	glutamine synthetase		0.99	0.001
CA01g17930	amino acid binding		-0.96	0.041
CA01g29060	2-isopropylmalate synthase		0.97	0.030
CA02g00970	cystathionine gamma synthase		0.99	0.006
CA02g25470	amino acid binding		0.99	0.011
CA03g07080	homocysteine S-methyltransferase 2-like		-0.98	0.024
CA04g06500	4-hydroxy-tetrahydrodipicolinate chloroplastic-like		-0.97	0.034
CA04g08530	bifunctional aspartokinase homoserine dehydrogenase chloroplastic-like		-0.98	0.016
CA05g01570	fumarylacetoacetase-like		-0.96	0.042
CA08g06890	multiple C2 and transmembrane domain-containing 2-like		0.97	0.028
CA09g16620	serine acetyltransferase 5-like		0.98	0.018
CA10g19810	cystathionine beta- chloroplastic isoform X1		0.95	0.047
CA11g14850	homoserine dehydrogenase isoform X1		-0.97	0.032
CA12g09890	asparagine synthetase [glutamine-hydrolyzing] 2		-0.99	0.014
CA12g10090	alanine--glyoxylate aminotransferase 2 homolog mitochondrial		-0.99	9.48E+09
CA12g20610	S-adenosylhomocysteine hydrolase		0.96	0.041
CA12g20620	S-adenosyl-L-homocysteine hydrolase		0.99	0.008
CA12g21760	serine--glyoxylate aminotransferase		0.95	0.045
CA02g24560	probable hexokinase-like 2		-0.98	0.019
CA03g02770	beta-amylase chloroplastic-like		0.99	0.006
CA04g18770	alpha-amylase		-0.97	0.026
CA04g21750	hexokinase- chloroplastic		-0.98	0.016
CA04g21760	hexokinase- chloroplastic		-0.98	0.016
CA04g21800	alkaline neutral invertase CINV2		-0.98	0.019
CA09g00240	apoplastic invertase		0.95	0.047
CA11g11520	alpha-glucan water chloroplastic isoform X1		0.95	0.049
CA01g01470	glucose-6-phosphate 1-epimerase		0.99	0.003
CA01g05740	pfkB-like carbohydrate kinase family		-0.98	0.023
CA01g11290	callose synthase 9		-0.97	0.033
CA01g11300	callose synthase 9		-0.96	0.044
CA01g22690	galactinol synthase		0.98	0.021
CA02g13420	probable alpha,alpha-trehalose-phosphate synthase [UDP-forming] 7		-0.96	0.037
CA03g25180	callose synthase 10-like		-0.98	0.022
CA05g13870	myo-inositol-1-phosphate synthase		-0.97	0.027
CA07g13560	callose synthase 12-like		-0.97	0.033
CA07g14540	probable alpha,alpha-trehalose-phosphate synthase [UDP-forming] 7		0.96	0.037
CA12g04290	phosphoglycolate phosphatase chloroplastic-like		0.98	0.024
CA12g09510	inositol-3-phosphate synthase		0.96	0.037
CA01g02250	phosphoglycerate mutase AT74		0.97	0.031
CA03g06560	pyruvate kinase isozyme chloroplastic		-0.99	0.012
CA03g16630	calcium-dependent kinase 26-like		-0.97	0.026
CA05g01330	6-phosphofructokinase 2-like		0.97	0.027
CA05g04290	phosphoenolpyruvate carboxylase kinase		0.97	0.027
CA06g04400	triosephosphate cytosolic		-0.99	0.008
CA06g25020	cytosolic enolase 3		-0.97	0.027
CA07g02180	NADP-dependent glyceraldehyde-3-phosphate dehydrogenase		0.99	0.006
CA07g14650	phosphoenolpyruvate carboxylase		0.97	0.030
CA07g17160	phosphoenolpyruvate carboxylase		0.97	0.031
CA08g16520	pyruvate kinase isozyme chloroplastic-like		-0.95	0.046
CA12g03130	ATP-dependent 6-phosphofructokinase chloroplastic		0.98	0.019
CA02g21900	aldehyde dehydrogenase family 3 member F1		-0.95	0.048
CA11g07710	aldehyde dehydrogenase family 2 member mitochondrial-like		-0.99	0.009
CA11g20330	alcohol dehydrogenase-like isoform X1		0.97	0.034
CA01g23570	phosphate chloroplastic		0.98	0.018
CA07g12030	isocitrate lyase		-0.97	0.033
CA07g12040	isocitrate lyase		-0.99	0.013
CA09g13800	citrate glyoxysomal-like		0.98	0.018
CA12g13590	6-phosphogluconate decarboxylating chloroplastic		-0.99	0.005
CA01g16000	ATP-citrate synthase beta chain 2		-0.99	0.010

CA01g03460	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 8-B-like	-0.97	0.030
CA03g21560	NADH dehydrogenase [ubiquinone] iron-sulfur mitochondrial	-0.96	0.035
CA06g16960	cytochrome c1- heme mitochondrial	0.95	0.048
CA06g24420	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2-like	-0.98	0.023
CA08g08770	NAD(P)H:quinone oxidoreductase	-0.97	0.028
CA12g10550	cytochrome c oxidase subunit 6b-1-like	0.96	0.043

Table 2. List of the genes significantly correlated with total soluble solids contents at 60 DAA. The correlation was considered significant if, PCC was > 0.90 (positive) or <-0.90 (negative), at a P-value <0.05.

***Capsicum annum* gene IDs**	**Description of related genes**	**Total Soluble Solids**	
	Abbreviation	**PCC**	***P*-value**
CA01g14810	ATP synthase CF0 subunit IV (chloroplast)	-0.96	0.036
CA02g00810	photosystem I reaction center subunit chloroplastic-like	-0.98	0.019
CA02g12050	chlorophyll a-b binding chloroplastic-like	-0.96	0.044
CA10g07200	nitrite reductase	0.99	0.008
CA02g24690	branched-chain-amino-acid aminotransferase chloroplastic-like	-0.97	0.034
CA07g20250	serine acetyltransferase chloroplastic-like	-0.97	0.028
CA09g08100	3-hydroxyisobutyryl- hydrolase 5	-0.99	0.009
CA11g00650	3-isopropylmalate dehydrogenase chloroplastic-like	-0.98	0.019
CA02g24490	probable starch synthase chloroplastic amyloplastic isoform X1	-0.99	0.011
CA07g09080	sucrose synthase	-0.97	0.028
CA01g05740	pfkB-like carbohydrate kinase family	0.97	0.034
CA01g21000	conserved oligomeric Golgi complex subunit 8	0.99	0.011
CA02g22200	galactinol synthase	-0.99	0.010
CA06g10330	aldo keto reducatase family	-0.99	0.013
CA03g06550	pyruvate kinase isozyme chloroplastic	-0.96	0.035
CA03g24310	glyceraldehyde-3-phosphate cytosolic	0.99	0.011
CA04g23530	pyrophosphate--fructose 6-phosphate 1-phosphotransferase subunit alpha	-0.95	0.050
CA08g05200	NADP-dependent malic enzyme-like isoform X2	0.98	0.019
CA12g01280	isocitrate dehydrogenase [NADP]	0.98	0.021
CA01g20230	oligomycin sensitivity conferring	0.96	0.037
CA04g21820	probable NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit mitochondrial	-0.97	0.025

4. DISCUSSION

The number of studies to understand the mechanisms involved in fruit development and ripening has been increasing (Alba et al., 2004; Fait et al., 2008; Steinhauser et al., 2010; Lombardo et al., 2011; Osorio et al., 2012a; Klie et al., 2014; Zhu et al., 2017; Fuentes et al., 2019). Several of these studies have been related the transcriptome to the downstream metabolic modulation. However, research efforts using metabolomics and transcriptomics in *C. chinense*, species described as nonclimacteric fruit are still scarce. Here, we combined transcriptome and metabolome analyzes in order to understand the complex interactions that occur throughout the development of this pepper species.

4.1 Metabolic and transcript changes along fruit development

The comparative transcriptome and metabolome analyzes demonstrated differences in the pattern of gene expression and metabolite behavior between 20 and 60 DAA. These results demonstrated that although there is no direct relationship

between transcription and metabolism, these two processes exhibit behaviors of alterations that can explain biological processes. The analyzes of DEGs and principal components analyzes suggest that the fruit developmental stage was the factor that most influenced the transcriptional and metabolic pattern variations, suggesting that although the accessions have different phenotypic characteristics and different evolutionary history, these phenotypes are the result of specific changes at the transcriptional and metabolic level. We analyzed the correlation between transcripts and metabolomics data that will direct possible future research based on the found associations. We next discuss the behavior of some important key metabolites and transcripts in fruits.

Organic acids are crucial to many aspects of tomato fruit biology, maturation and plant cell metabolism (Carrari and Fernie, 2006; Araújo et al., 2011; Centeno et al., 2011b). In tomato, they are essential for fruit ripening and flavor, and they correlate with the expression of genes associated with ripening (Carrari and Fernie, 2006; Centeno et al., 2011b; Osorio et al., 2012a). In strawberry a nonclimateric fruit, only some TCA cycle intermediates such as succinate, fumarate, and 2-oxoglutarate are altered throughout development, associated with a heavy demand for carbon skeleton components (Fait et al., 2008). We demonstrated that organic acids levels are strongly influenced by fruit ripening (Fig. 2). Differently, in tomato, malate and fumarate, two important organic acids, decrease significantly during fruit development (Oa et al., 2013). However, the levels of fumarate in the *C. chinense* accessions are much lower in comparison to malate. This decrease was coupled with the tendency to increase the expression of malate dehydrogenase and fumarase related genes from the 20 DAA stage to the 60 DAA (Table S6). In Osorio et al., 2012, the authors revealed a positive correlation between malate level and genes involved in the synthesis of starch. However we did not find strong correlation between them, suggesting that relationship between malate and starch synthesis, previously described in tomato (Centeno et al., 2011a), might be at post-transcriptional level and occur in peppers.

During earlier and mature fruit stages, we observed large variations in the metabolism of sugars and starch (Figures 1A and 2). Genes encoding proteins involved photosynthetic light reactions were up-regulation at 20 DAA followed by down-regulation at 60 DAA, as observed in Osorio et al., 2012. In mature fruits, a strong down-regulation of genes involved in the synthesis of starch such as ADP-glucose

pyrophosphorylase (AGPase) and starch synthase (SS), was observed along with the down-regulation of genes related to sucrose degradation at 60 DAA (Table S6). These results suggest an activated photosynthesis and carbon assimilation processes during early fruit stages followed by reduction in mature stages. Coupled with this, we observed a drastic reduction in the content of chlorophylls and an increase in the content of carotenoids, due to biosynthesis of carotenoids, degradation of chlorophylls and differentiation of chloroplasts in chromoplasts that occur during fruit ripening (Sadali et al., 2019). In plants, sucrose, the main product of photosynthesis, which provides carbon skeletons for fruit development, is hydrolyzed by two enzymes, invertase and Susy (Beckles et al., 2012). Consistently, we demonstrate an increase in glucose, fructose and maltose from 20 DAA to 60 DAA stage, coupled with a dramatic decrease in starch content only at 60 DAA. Additionally, in tomato fruit, hexoses derived from the degradation of sucrose are described as key metabolites to starch synthesis during early development and at the onset of ripening (Carrari and Fernie, 2006; Centeno et al., 2011). We observed a reduction in the expression of genes related to Susy and invertase at 60 DAA (Table S6), suggesting less activity of these enzymes in mature fruits, as well as an up-regulation of alpha and beta amylase genes. These results suggest that the carbon demand by mature fruits is probably supplied by starch degradation in *Capsicum chinense*.

The total soluble solids (TSS) is a variable that is generally associated with fruit quality and flavor. In tomato, TSS is composed of around 55% of sugars and other metabolites (Helyes et al., 2006). Research studies have shown the importance of organic acids for determination of TSS (Tiessen et al., 2002; Mounet et al., 2009; Centeno et al., 2011b). In addition, starch degradation and increased sucrose content in stages of development by phloem translocation contribute to the increase of TSS content (Martínez et al., 2007; Beckles et al., 2012; Aizat et al., 2014). In this work, we demonstrate that between the 20 DAA and 60 DAA development stages there is an inversion between starch levels and TSS content (Fig. 1B). While the starch levels decreased dramatically over time, the TSS content increased in all accessions. Furthermore, there was an increase in the soluble sugar content, as previously described. The behavior of drastic reduction of starch over time and accumulation of sugars, the metabolism of sugars in *C. chinense*, appears to be similar to that of tomato (Osorio et al., 2012). However, the percentage between sugars and other metabolic

classes related to TSS content may be different, since TSS levels are correlated with some genes of carbohydrate metabolism (Table 1). However, the number of correlations found between TSS and expression of genes related functional categories (Figure 5) was expressive, although not yet described in the literature. Most associations have not been described in the literature, while others have caught our attention. Therefore, this work provides information that must be deepened investigated and validated by future research efforts.

Recently, the importance of alkaline / neutral invertases for the sucrose metabolism in pepper fruits and their collaboration for the ideal fruit development has been described (Shen et al., 2018). In this work, a chloroplastic INV and apoplastic INV correlated negatively and positively respectively with TSS content in young fruits (Table 1). These results clearly suggests that the soluble sugars content are the result of sucrose hydrolysis from source leaves in the apoplast. In addtion to INV, the SuSy related gene was down-regulated in mature fruits and correlated negatively with high TSS content, suggesting a more significant apoplastic import of hexoses, as described in Beckles, 2012. The Hexokinases regulate the hexoses pool, in addition, function as sugar sensors and are central to sugar signalling in plants (Halford et al., 1999). Although hexokinases related genes correlated negatively with brix at 20 DAA (Table 1), we found high expression at this stage followed by a drastic reduction at 60 DAA, suggesting less activity in glucose metabolism. These results suggest a complex interaction between INV, Susy, and other enzymes of starch biosynthesis and degradation that regulate the TSS levels in nonclimateric fruits. In addition to the levels of sucrose, soluble sugars and starch, other metabolites might contribute for the TSS content in *C. chinense* fruits.

5. CONCLUSION

In summary, the comparative transcript and metabolite analyses at 20 and 60 DAA suggest a complex molecular and metabolic regulation during fruit development, demonstrating highly coordinated and complex process. Taken together, our data improve our understanding of *C. chinense* fruit development, typically considered as nonclimateric. In addition, our data provide new insights into the regulation of TSS content in mature fruits and also identified unexpected relationships that should be considered in future research.

Supplementary information

Table S1. Metabolites from semi-polar phase of pericarp from pepper fruits collected from 10 accessions of *C. chinense* during three different development ages (20, 45 and 60 days after anthesis (DAA)). The mean values of the metabolite intensity ± standard error (n=6). Capital letters indicate differences between fruit ages and small letters indicate differences between accessions. Differences between means are given by Tukey test ($p<0.05$).

	DAA	Alanine	Valine	Isoleucine	Glycine	Proline	Serine	Threonine	Alanine, beta-
	20	0.0078±0.0017 bB	0.0176±0.0070 aA	0.1152±0.0434 aA	0.0198±0.0047 abA	0.1085±0.0218 aB	0.0747±0.0231 abB	0.0402±0.0072 aA	0.0080±0.0007 bcA
Hab	**45**	0.0043±0.0006 cdeB	0.0072±0.0008 abcB	0.0265±0.0030 abB	0.0037±0.0004 bB	0.0771±0.0054 bB	0.1547±0.0116 cdeB	0.0440±0.0033 dA	0.0039±0.0008 bcB
	60	0.0116±0.0003 bA	0.0153±0.0008 abA	0.0815±0.0042 aA	0.0063±0.0006 bcB	1.5684±0.1218 cA	0.5441±0.0223 bA	0.0873±0.0021 dA	0.0056±0.0007 bAB
	20	0.0049±0.0004 bA	0.0029±0.0002 cA	0.0109±0.0010 cA	0.0058±0.0006 cA	0.0398±0.0063 aB	0.0245±0.0023 aA	0.0085±0.0008 aA	0.0025±0.0001 dA
A101	**45**	0.0036±0.0002 deA	0.0021±8.87E-05 cA	0.0079±0.0007 bA	0.0014±9.05E-05 bA	1.4327±0.3080 aA	0.1192±0.0116 deA	0.0256±0.0024 dA	0.0026±0.0001 cA
	60	0.0036±0.0001 cA	0.0016±0.0001 dA	0.0039±0.0007 cA	0.0016±0.0001 cA	1.7536±0.1440 bcA	0.0822±0.0175 dAB	0.0155±0.0021 eA	0.0015±0.0003 cA
	20	0.0172±0.0009 aC	0.0065±0.0002 bcC	0.0117±0.0002 cB	0.0224±0.0009 aC	0.4708±0.0164 aAB	0.1605±0.0104 aC	0.0321±0.0014 aC	0.0081±0.0002 bC
A54	**45**	0.0383±0.0031 aA	0.0137±0.0007 aB	0.0385±0.0012 abAB	0.0380±0.0030 aA	0.0601±0.0026 bB	0.5499±0.0211 aB	0.1092±0.0032 aB	0.0158±0.0006 aB
	60	0.0252±0.0026 aB	0.0203±0.0022 aA	0.0543±0.0070 abA	0.0309±0.0041 aB	0.6269±0.0727dA	0.8987±0.0741 aA	0.2075±0.0169 aA	0.0207±0.0028 aA
	20	0.0048±0.0002 bA	0.0083±0.0006 bcA	0.0553±0.0072 abcA	0.0070±0.0002 cA	0.4240±0.0880 aB	0.0413±0.0021 abB	0.0251±0.0013 aB	0.0082±0.0003 bA
A26	**45**	0.0030±0.0003 deA	0.0048±0.0002 bcA	0.0119±0.0004 bB	0.0033±0.0003 bA	0.0510±0.0025 bB	0.0644±0.0085 eAB	0.0294±0.0019 dB	0.0062±0.0006 bAB
	60	0.0049±0.0002 cA	0.0039±8.04E-05cdA	0.0166±0.0012 bcB	0.0032±0.0001 bcA	1.8130±0.1080 bcA	0.1436±0.0054 dA	0.0690±0.0025 cdA	0.0039±0.0005 bcB
	20	0.0055±0.0006 bAB	0.0047±0.0004 bcB	0.0530±0.0074 abcA	0.0118±0.0015 bcA	0.0469±0.0072 aB	0.0525±0.0091 abB	0.0222±0.0017 aA	0.0077±0.0013 bA
A100	**45**	0.0091±0.0010 bcA	0.0145±0.0012 aA	0.0451±0.0016 abA	0.0082±0.0011 bAB	0.0768±0.0032 bB	0.1944±0.0296 cdA	0.0590±0.0084 bcA	0.0033±0.0006 bcB
	60	0.0039±0.0002 cB	0.0082±0.0008 bcdB	0.0315±0.0078 bcA	0.0048±0.0007 bcB	1.2287±0.4278 cA	0.2001±0.0469 dA	0.0618±0.0143 cdA	0.0011±0.0003 cB
	20	0.0061±0.0012 bA	0.0102±0.0016 bA	0.0532±0.0076 abcA	0.0098±0.0014 bcA	0.0784±0.0131 aA	0.0829±0.0126 abA	0.0291±0.0037 aA	0.0122±0.0014 aA
Biq	**45**	0.0034±0.0002 deA	0.0038±0.0004 bcB	0.0256±0.0024 abA	0.0028±0.0003 bB	0.0820±0.0143 bA	0.1022±0.0109 deA	0.0295±0.0037 bcdB	0.0017±0.0003 cB
	60	0.0047±0.0003 cA	0.0037±0.0001 cbB	0.0269±0.0011 bcA	0.0037±0.0001 bcB	0.1900±0.0323 dA	0.1239±0.0093 dA	0.0449±0.0019 cA	0.0017±0.0001 cB
	20	0.0060±0.0009 bA	0.0040±0.0006 bcB	0.0166±0.0033 bcB	0.0065±0.0013 cA	0.2059±0.0384 aB	0.0445±0.0030 abB	0.0195±0.0022 aB	0.0033±0.0005 cdA
A06	**45**	0.0026±0.0002 **eA**	0.0029±0.0001 cB	0.0135±0.0004 bB	0.0040±0.0003 bA	0.0461±0.0063 bB	0.1114±0.0079 deB	0.0336±0.0019 cdB	0.0025±0.0001 cA
	60	0.0045±0.0004 cA	0.0147±0.0017 abA	0.0776±0.0120 aA	0.0079±0.0007 bcA	2.1908±0.3348 abA	0.4374±0.0619 bcA	0.1396±0.0109 bA	0.0029±0.0004 bcA
	20	0.0052±0.0013 bB	0.0071±0.0008 bcA	0.0398±0.0053 bcA	0.0073±0.0019 cA	0.2123±0.1063 aC	0.0438±0.0096 abB	0.0249±0.0031 aB	0.0037±0.0003 cdA
A17	**45**	0.0117±0.0009 bA	0.0103±0.0005 abA	0.0639±0.0094 aA	0.0072±0.0008 bA	1.6939±0.0890 aB	0.3732±0.0223 bA	0.0689±0.0072 bA	0.0046±0.0006 bcA
	60	0.0113±0.0006 bA	0.0115±0.0009 bA	0.0587±0.0083 abA	0.0096±0.0008 bA	2.5045±0.1372 aA	0.3352±0.0291 cA	0.0799±0.0079 cA	0.0026±0.0003 bcA
	20	0.0061±0.0008 bB	0.0095±0.0003 bcA	0.0572±0.0020 abAB	0.0119±0.0013 bcA	0.1340±0.0434 aC	0.0615±0.0082 abC	0.0295±0.0026 aB	0.0064±0.0005 bcA
A18	**45**	0.0078±0.0007bcdAB	0.0065±0.0006 bcA	0.0424±0.0028 abB	0.0046±0.0004 bB	0.5859±0.1376 bB	0.2403±0.0246 cB	0.0495±0.0047 bcdB	0.0044±0.0004 bcAB
	60	0.0114±0.0007 bA	0.0117±0.0008 bA	0.0863±0.0112 aA	0.0089±0.0007 bcAB	2.7043±0.0908 aA	0.4141±0.0407 cA	0.0802±0.0093 cA	0.0032±0.0004 bcB
	20	0.0060±0.0005 bA	0.0067±0.0004 bcA	0.0441±0.0103 bcA	0.0064±0.0006 cA	0.1802±0.0296 aA	0.0587±0.0051 abB	0.0192±0.0014 aB	0.0035±0.0003 cdA
A83	**45**	0.0052±0.0004 cdeA	0.0076±0.0011 abcA	0.0476±0.0054 abA	0.0059±0.0005 bA	0.0797±0.0073 bA	0.1317±0.0239 aceAB	0.0418±0.0051 bcdA	0.0047±0.0005 bcA
	60	0.0082±0.0009 **bcA**	0.0110±0.0016 bcA	0.0510±0.0111 abcA	0.0065±0.0008 bcA	0.1700±0.0159 dA	0.2196±0.0271 dA	0.0493±0.0065 deA	0.0035±0.0003 bcA

Continuation of Table S1

	DAA	Homoserine	Proline, 4-hydroxy-	GABA	Aspartic acid	Methionine	Ornithine	Glutamine
Hab	20	0.0014±0.0002 abcA	0.1596±0.0501 bcA	0.0162±0.0042 aA	0.0660±0.0051 abB	0.0050±0.0011 aB	0.0129±0.0012 aB	0.060±0.010 aA
	45	0.0011±0.0001 bcA	0.0166±0.0041 aB	0.0065±0.0011 bB	0.0479±0.0025 bB	0.0040±0.0008 bB	0.0484±0.0139 bB	0.041±0.007 aAB
	60	0.0011±0.0001 bA	0.0018±0.0002 aB	0.0081±0.0009 bcdeB	0.0837±0.0057 bA	0.0168±0.0004 bcA	0.1635±0.0097 cdA	0.029±0.002 abB
A101	20	0.0009±0.0001 cA	0.0325±0.0047 dA	0.0055±0.0003 dA	0.0113±0.0011 cA	0.0017±0.0002 aA	0.0043±0.0004 aA	0.003±0.000 cA
	45	0.0004±0.0000 cA	0.0011±0.0001 aA	0.0025±0.0002 bA	0.0098±0.0004 cA	0.0015±0.0001 BA	0.0017±0.0004 bA	0.001±0.000 cA
	60	0.0006±0.0001 bA	0.0013±0.0001 aA	0.0030±0.0002 efA	0.0082±0.0006 cA	0.0017±0.0004 bcA	0.0040±0.0013 eA	0.003±0.001 bcA
A54	20	0.0022±0.0001 abcA	0.0768±0.0029 cdA	0.0139±0.0006 abA	0.0431±0.0025dA	0.0027±0.0001 aB	0.0120±0.0008 aB	0.017±0.003 bcB
	45	0.0015±0.0001 bcA	0.0354±0.0017 aAB	0.0138±0.0003 aA	0.0489±0.0018 abcB	0.0071±0.0004 bB	0.0843±0.0025 bB	0.012±0.002 bcB
	60	0.0010±0.0002 bA	0.0037±0.0006 aB	0.0163±0.0024 aA	0.1291±0.0134 bB	0.0425±0.0056 aA	0.3961±0.0399 bA	0.053±0.014 aA
A26	20	0.0028±0.0003 abcA	0.0464±0.0081 dA	0.0104±0.0008 bcdA	0.0258±0.0014 aA	0.0063±0.0004 aA	0.0086±0.0006 aA	0.037±0.006 abA
	45	0.0011±0.0001 bcA	0.0242±0.0039 aAB	0.0066±0.0008 bAB	0.0361±0.0029 bcA	0.0033±0.0002 bA	0.0073±0.0009 bA	0.023±0.003 abcAB
	60	0.0006±0.0001 bA	0.0038±0.0006 aB	0.0034±0.0001 defB	0.0270±0.0011 bcA	0.0082±0.0008 cA	0.0103±0.0018 eA	0.012±0.001 bcB
A100	20	0.0052±0.0017 abA	0.0279±0.0075 dA	0.0127±0.0024 abA	0.0282±0.0031 dA	0.0070±0.0020 aB	0.0032±0.0003 aA	0.006±0.002 cA
	45	0.0009±0.0001 bcB	0.0007±0.0001 aA	0.0027±0.0003 bB	0.0304±0.0020 bcA	0.0155±0.0012 abB	0.0059±0.0012 bA	0.003±0.001 cA
	60	0.0011±0.0003 bB	0.0009±0.0002 aA	0.0035±0.0005 def	0.0205±0.0012 dA	0.0408±0.0130 aA	0.0098±0.0061 eA	0.002±0.000 cA
Biq	20	0.0033±0.0009 abcA	0.0241±0.0042 dA	0.0131±0.0021 abA	0.0482±0.0068 abA	0.0066±0.0016 aA	0.0173±0.0013 aA	0.029±0.007 abcA
	45	0.0007±0.0001 bcA	0.0015±0.0003 aA	0.0021±0.0004 bB	0.0320±0.0079 bcA	0.0063±0.0008 bA	0.0284±0.0099 bA	0.014±0.004 abcA
	60	0.0008±0.0000 bA	0.0008±0.0001 aA	0.0020±0.0002 fB	0.0404±0.0060 cdA	0.0123±0.0006 cA	0.0340±0.0078 deA	0.015±0.002 bcA
A06	20	0.0028±0.0005 abcA	0.2199±0.0304 aA	0.0117±0.0010 abcA	0.0393±0.0036 bcAB	0.0054±0.0010 aB	0.0088±0.0004 aA	0.048±0.007 aA
	45	0.0010±0.0001 bcA	0.0078±0.0026 aB	0.0052±0.0003 bB	0.0346±0.0016 bcB	0.0025±0.0002 bB	0.0066±0.0007 bA	0.008±0.002 abA
	60	0.0006±0.0001 bA	0.0029±0.0003 aB	0.0095±0.0007 bcAB	0.0634±0.0067 bcA	0.0362±0.0033 aA	0.0393±0.0056 deA	0.023±0.004 abcAB
A17	20	0.0040±0.0015 abcB	0.0522±0.0251 dA	0.0052±0.0015 dB	0.0440±0.0034 abcB	0.0103±0.0022 aB	0.0130±0.0034 aB	0.035±0.008 abA
	45	0.0074±0.0031 aA	0.0114±0.0024 aAB	0.0074±0.0010 bB	0.1037±0.0112 aA	0.0266±0.0063 aA	0.3158±0.0391 aA	0.018±0.006 abcAB
	60	0.0036±0.0005 bB	0.0049±0.0006 aB	0.0125±0.0016 abA	0.1255±0.0226 aA	0.0288±0.0026 abA	0.2790±0.0249 bcA	0.011±0.002 bcB
A18	20	0.0062±0.0020 aAB	0.1741±0.0155 abA	0.0108±0.0010 abcdA	0.0358±0.0021 bcB	0.0139±0.0038 aB	0.0169±0.0040 aC	0.040±0.009 abA
	45	0.0047±0.0007 abB	0.0125±0.0017 aB	0.0049±0.0002 bB	0.0445±0.0043 bB	0.0159±0.0014 abB	0.3815±0.0780 aB	0.009±0.002 bcB
	60	0.0084±0.0021 aA	0.0057±0.0008 aB	0.0090±0.0010 bcdAB	0.0871±0.0092 bA	0.0342±0.0054 aA	0.7092±0.1457 aA	0.012±0.001 bcB
A83	20	0.0013±0.0002 bcA	0.0362±0.0024 dA	0.0068±0.0004 cdA	0.0753±0.0061 aA	0.0041±0.0006 aB	0.0100±0.0014 aA	0.028±0.007 abcA
	45	0.0010±0.0002 bcA	0.0054±0.0007 aA	0.0056±0.0008 bA	0.0892±0.0150 aA	0.0091±0.0018 bAB	0.0561±0.0162 bA	0.034±0.014 abA
	60	0.0006±0.0000 bA	0.0009±0.0000 aA	0.0048±0.0005 cdefA	0.0462±0.0050 cdB	0.0200±0.0034 bcA	0.0227±0.0051 deA	0.009±0.001 bcB

Continuation of Table S1

	DAA	Glutamic acid	Phenylalanine	Asparagine	Tyrosine	Tryptophan	Glyceric acid	Fumaric acid*
Hab	20	0.2348±0.0254 bcA	0.0248±0.0077 aB	0.0813±0.0104 abB	0.0182±0.0086 aB	0.0095±0.0018 aB	0.0024±0.0002 dB	56.07±7.69 aA
	45	0.1943±0.0211 abA	0.0219±0.0023 bcdB	0.0983±0.0097 bcdA	0.0128±0.0014 dB	0.0110±0.0008 aB	0.0019±0.0003 dB	22.86±7.30 aB
	60	0.2222±0.0150 abA	0.1863±0.0074 cbA	0.3152±0.0143 cA	0.0500±0.0038 abcA	0.0243±0.0008 abA	0.0039±0.0002 aA	NA
A101	20	0.1121±0.0107 dA	0.0041±0.0003 aB	0.0060±0.0013 bA	0.0075±0.0006 aA	0.0069±0.0003 aB	0.0039±0.0001 abA	24.16±2.01 bA
	45	0.0736±0.0037 dB	0.0569±0.0057 bA	0.0079±0.0012 dA	0.0176±0.0015 cdA	0.0231±0.0023 bB	0.0036±0.0002 abcA	33.85±3.21 aA
	60	0.1106±0.0062 dA	0.0258±0.0025 eB	0.0203±0.0058 cA	0.0149±0.0018 eA	0.0226±0.0011 bcdA	0.0039±0.0004 aA	NA
A54	20	0.2286±0.0207 abcA	0.0054±0.0004 aB	0.0379±0.0049 bC	0.0059±0.0002 aB	0.0075±0.0005 aB	0.0033±0.0003 abcdA	12.85±1.38 bB
	45	0.2708±0.0120 aA	0.0162±0.0002 cdB	0.1914±0.0127 abcB	0.0111±0.0003 dB	0.0074±0.0003 bcB	0.0014±0.0001 dB	47.85±8.05 aA
	60	0.2650±0.0275 aA	0.0745±0.0110 cdA	0.3833±0.0274 aA	0.0446±0.0065 bcA	0.0192±0.0021 bcdA	0.0034±0.0003 abA	NA
A26	20	0.2237±0.0096 abcA	0.0104±0.0007 aA	0.0602±0.0051 abB	0.0063±0.0004 aA	0.0062±0.0004 aB	0.0029±0.0002 bcdA	23.56±5.32 bB
	45	0.2133±0.0172 abA	0.0084±0.0007 dA	0.1998±0.0250 abA	0.0052±0.0004 dA	0.0081±0.0003 bcB	0.0029±0.0002 bcdA	44.65±5.57 aA
	60	0.1418±0.0053 bcdB	0.0250±0.0020 eA	0.2031±0.0159 bA	0.0164±0.0005 eA	0.0192±0.0004 bcdA	0.0034±0.0002 abA	NA
A100	20	0.1513±0.0156 cdA	0.0038±0.0005 aC	0.0104±0.0022 bA	0.0085±0.0004 aB	0.0045±0.0002 aC	0.0045±0.0006 aA	35.56±2.81 bA
	45	0.0666±0.0045 dB	0.0535±0.0071 bcB	0.0156±0.0022 dA	0.0373±0.0025 bA	0.0134±0.0016 bB	0.0020±0.0001 dC	42.07±9.31 aA
	60	0.0905±0.0095 dB	0.1062±0.0279 cA	0.0404±0.0135 cA	0.0359±0.0097 cdA	0.0206±0.0037 bcdA	0.0031±0.0003 abB	NA
Biq	20	0.2338±0.0279 abA	0.0129±0.0022 aA	0.0649±0.0096 abB	0.0126±0.0021 aA	0.0075±0.0009 aB	0.0021±0.0005 cdA	50.97±25.45 bA
	45	0.1026±0.0097 cdB	0.0296±0.0021 bcdA	0.0744±0.0126 bcdB	0.0187±0.0013 cdA	0.0123±0.0012 bB	0.0020±0.0003 dA	30.72±2.26 bA
	60	0.1402±0.0136 cdB	0.0380±0.0019 deA	0.2285±0.0401 abA	0.0222±0.0022 deA	0.0151±0.0011 dA	0.0021±0.0001 bA	NA
A06	20	0.1918±0.0169 bcdA	0.0079±0.0007 aB	0.0439±0.0028 bB	0.0058±0.0006 aB	0.0046±0.0007 aB	0.0027±0.0005 bcdA	35.37±4.97 bA
	45	0.1691±0.0087 bcAB	0.0149±0.0008 cdB	0.1036±0.0092 bcdB	0.0085±0.0006 dB	0.0052±0.0003 cB	0.0018±0.0002 dA	26.57±2.48 aA
	60	0.1213±0.0125 dB	0.2130±0.0206 aA	0.3099±0.0526 abA	0.0656±0.0066 aA	0.0231±0.0022 abA	0.0027±0.0003 abA	NA
A17	20	0.1807±0.0280 bcdA	0.0127±0.0019 aB	0.0706±0.0096 abB	0.0156±0.0037 aB	0.0057±0.0006 aB	0.0035±0.0005 abcA	37.56±6.16 bA
	45	0.1781±0.0111 bcA	0.1290±0.0096 aA	0.3099±0.0177 aA	0.0667±0.0094 aA	0.0208±0.0008 aA	0.0045±0.0002 aA	37.13±7.12 aA
	60	0.1339±0.0129 cdA	0.1483±0.0134 bA	0.2902±0.0311 abA	0.0599±0.0052 abA	0.0239±0.0015 abA	0.0041±0.0002 aA	NA
A18	20	0.2116±0.0132 abcA	0.0148±0.0006 aC	0.0516±0.0077 bC	0.0136±0.0004 aC	0.0053±0.0002 aC	0.0039±0.0003 abA	29.45±9.15 bA
	45	0.1607±0.0133 bcA	0.0598±0.0036 bB	0.2487±0.0454 aB	0.0314±0.0013 bcB	0.0121±0.0005 aB	0.0023±0.0002 cdB	30.75±7.64 bA
	60	0.2108±0.0160 abcA	0.1500±0.0132 bA	0.4045±0.0421 aA	0.0655±0.0047 aA	0.0279±0.0020 aA	0.0040±0.0004 aA	NA
A83	20	0.2795±0.0173 aA	0.0129±0.0012 aB	0.1794±0.0192 aA	0.0128±0.0007 aA	0.0085±0.0004 aB	0.0029±0.0002 bcdB	24.39±10.94 bB
	45	0.1651±0.0197 bcB	0.0270±0.0061 bcdB	0.2619±0.0804 aA	0.0185±0.0028 cdA	0.0106±0.0008 bcB	0.0040±0.0005 abA	56.95±10.50 aA
	60	0.1571±0.0116 cdB	0.0801±0.0125 cdA	0.2492±0.0466 bA	0.0259±0.0032 deA	0.0195±0.0012 cdA	0.0028±0.0003 bB	NA

Continuation of Table S1

	DAA	Nicotinic acid	Malic acid*	Quinic acid	Citric acid	Xylose	Rhamnose	Fucose	Glucose, 1,6-anhydro
Hab	20	0.0027±0.0002 aB	2095.531±159.82 aA	0.0300±0.0026 bA	0.073±0.01 abC	0.0797±0.013 cB	0.0041±0.0009 aA	0.0347±0.0040 abA	0.0021±0.0001 aB
	45	0.0067±0.0005 deA	489.006±93.92 cB	0.0067±0.0005 bcdB	4.085±0.48 dB	0.0581±0.004 deB	0.0046±0.0003 bcA	0.0231±0.0018 abA	0.0036±0.0005bcA
	60	0.0083±0.0005 cdA	33.064±4.59 aC	0.0022±0.0002 aB	6.811±0.46 bA	0.1136±0.006 cdA	0.0050±0.0001 bA	0.0250±0.0008 aA	0.0048±0.0003aA
A101	20	0.0033±0.0002 aC	1022.274±41.83 cA	0.0212±0.0009 bA	0.590±0.03 cC	0.1041±0.004 bcB	0.0049±0.0001 aB	0.0331±0.0008 abA	0.0021±0.0001aB
	45	0.0120±0.0006 aA	800.966±33.57 bcA	0.0024±0.0001 dB	2.973±0.22 aB	0.1510±0.005 abcA	0.0054±0.0002 abcB	0.0264±0.0009 abA	0.004±0.000abcA
	60	0.0094±0.0007 abB	39.186±8.23 aB	0.0025±0.0003 aB	6.786±0.83 eA	0.1589±0.008 bcA	0.0072±0.0002 aA	0.0317±0.0006 aA	0.0044±0.0002aA
A54	20	0.0019±0.0001 aB	1580.135±62.93 bA	0.0236±0.0014 bA	0.085±0.004 cC	0.0562±0.006 cB	0.0036±0.0001 aB	0.0252±0.0009 abA	0.0020±0.0001aB
	45	0.0031±0.0004 eAB	1259.210±38.92 aB	0.0145±0.0008 abB	2.132±0.102 aB	0.0500±0.003 eB	0.0040±0.0002 cB	0.0207±0.0011 abA	0.0031±0.0003cB
	60	0.0053±0.0003 dA	65.254±29.96 aC	0.0056±0.0003 aC	7.255±0.403 eA	0.1306±0.011 bcdA	0.0054±0.0005 bA	0.0200±0.0021 bcA	0.0062±0.0017aA
A26	20	0.0029±0.0001 aB	1112.019±57.16 cB	0.0213±0.0009 bA	0.034±0.004 dC	0.0878±0.006 bcAB	0.0046±0.0002 aB	0.0303±0.0009 abA	0.001±0.0001 aB
	45	0.0029±0.0002 eB	1450.617±86.53 aA	0.0206±0.0015 aA	1.002±0.062 aB	0.0489±0.004 eB	0.0055±0.0004 abcAB	0.0297±0.0018 aA	0.002±0.0002cB
	60	0.0082±0.0008 bcdA	23.269±2.22 aC	0.0063±0.0005 aB	7.413±0.59 bA	0.1290±0.009 bcdA	0.0063±0.0002 abA	0.0254±0.0009 abcA	0.0047±0.0003aA
A100	20	0.0035±0.0003 aC	922.342±47.39 cA	0.0275±0.0029 abA	0.064±0.012 dB	0.1809±0.006 aB	0.0054±0.0003 aB	0.0311±0.0005 abA	0.0018±0.0001aB
	45	0.0074±0.0003 cdB	518.925±63.16 cB	0.0042±0.0010 cdB	3.944±0.155 dB	0.1692±0.010 aB	0.0068±0.0002 aA	0.0270±0.0006 abA	0.003±0.0001bcB
	60	0.0118±0.0014 aA	39.833±3.48 aC	0.0032±0.0004 aB	4.12±0.441 cA	0.2803±0.021 aA	0.0060±0.0004 abAB	0.0289±0.0034 abA	0.0050±0.0006aA
Biq	20	0.0019±0.0003 aB	2052.813±175.24 aA	0.0256±0.0016 abA	0.136±0.032 dC	0.0677±0.006 cB	0.0044±0.0005 aB	0.0233±0.0020 bA	0.0020±0.0004aB
	45	0.0053±0.0005 cdA	671.475±79.71 bcB	0.0028±0.0005 bcdB	10.084±1.092 cdB	0.1650±0.017 abA	0.0053±0.0004 abcAB	0.0189±0.0013 bA	0.0034±0.0002bcAB
	60	0.0062±0.0004 bcdA	29.724±8.02 aC	0.0024±0.0004 aB	13.647±0.598 aA	0.1711±0.006 bA	0.0060±0.0004 abA	0.0202±0.0010 bcA	0.0042±0.0002aA
A06	20	0.0024±0.0004 aB	1439.758±75.40 bA	0.0234±0.0012 bA	0.089±0.007 dC	0.0555±0.002 cB	0.0040±0.0002 aA	0.0307±0.0028 abA	0.0018±0.0003aB
	45	0.0044±0.0004 deB	612.742±48.53 bcB	0.0110±0.0017 bcB	5.089±0.440 bB	0.0612±0.004 deB	0.0047±0.0002 bcA	0.0221±0.0021 abB	0.0029±0.0003cAB
	60	0.0069±0.0008 bcdA	24.852±4.44 aC	0.0051±0.0015 aC	8.816±1.196 cA	0.1328±0.014 bcdA	0.0048±0.0003 bA	0.0197±0.0012 bcB	0.0042±0.0004aA
A17	20	0.0032±0.0004 aC	1581.815±45.95 bA	0.0216±0.0012 bA	0.140±0.038 eB	0.0506±0.002 cB	0.0043±0.0003 aA	0.0263±0.0022 abA	0.0021±0.0002aC
	45	0.0114±0.0019 abA	912.524±80.34 bB	0.0052±0.0004 cdB	5.171±0.213 bcA	0.1067±0.008 cdA	0.0053±0.0002 abcA	0.0286±0.0008 aA	0.0065±0.0005aA
	60	0.0073±0.0007 bcdB	29.503±6.81 aC	0.0061±0.0006 aB	3.690±0.208 bcA	0.0822±0.004 dAB	0.0051±0.0003 bA	0.0295±0.0014 aA	0.0047±0.0003aB
A18	20	0.0034±0.0001 aB	1540.053±85.59 bA	0.0222±0.0006 bA	0.273±0.016 aC	0.0745±0.002 bcA	0.0045±0.0002 aB	0.0288±0.0008 abA	0.0023±0.0001aB
	45	0.0088±0.0005 bcA	614.093±32.42 bcB	0.0092±0.0013 bcdB	7.385±0.951 dA	0.1123±0.007 bcdA	0.0059±0.0002 abA	0.0279±0.0012 abA	0.0054±0.0006abA
	60	0.0088±0.0005 abcA	27.094±2.31 aA	0.0098±0.0009 aB	7.654±0.451 dA	0.1113±0.004 cdA	0.0060±0.0002 abA	0.0317±0.0012 aA	0.0054±0.0004aA
A83	20	0.0029±0.0005 aB	1979.780±123.91 aA	0.0335±0.0054 aA	0.164±0.013 aC	0.1390±0.011 abB	0.0049±0.0005 aA	0.0294±0.0023 abA	0.0030±0.0003aA
	45	0.0066±0.0006 cdA	1425.6208±93.06 aB	0.0082±0.0008 bcdB	3.7507±0.662 dB	0.1384±0.005 abcB	0.0062±0.0004 abA	0.0241±0.0014 abAB	0.0042±0.0004bcA
	60	0.0098±0.0006 bcdA	56.8188±12.6771 aC	0.0043±0.0003 aB	10.813±0.694 dA	0.2770±0.014 aA	0.0071±0.0004 abA	0.0233±0.0008 cB	0.0053±0.0002aA

Continuation of Table S1

	DAA	Fructose*	Glucose*	Sucrose*	Maltose	Trehalose	Raffinose	Urea	Putrescine
Hab	20	265.6406±59.008 bcB	411.509±61.50 bB	23.662±3.75 aB	0.0044±0.0004 aA	0.0032±0.0003 bcdB	0.0013±0.0003 cA	0.0027±0.0003 aA	0.1476±0.0456 deA
	45	450.2898±48.037 cdeB	445.195±41.323cdeB	104.229±14.95cdeA	0.0103±0.0014 cA	0.0032±0.0003 bB	0.0050±0.0008 deA	0.0013±0.0004 aA	0.0116±0.0007 aB
	60	846.4850±17.636 abA	769.966±44.41 bcA	43.102±3.66 aAB	0.0181±0.0009 abA	0.0073±0.0006 abcA	0.0010±0.0001 aA	0.0025±0.0004 aA	0.0109±0.0003 aB
A101	20	325.9106±34.838 abcB	266.977±24.47 bB	54.340±6.958 aA	0.0058±0.0003 aC	0.0035±0.0004 abcdB	0.0047±0.0005 bcA	0.0011±0.0003 aA	0.0793±0.0124 efA
	45	907.4283±37.355 aA	953.410±50.32 aA	97.714±14.292 deA	0.2168±0.0197 aA	0.0067±0.0006 aA	0.0041±0.0003 deA	0.0017±0.0003 aA	0.0033±0.0001 aB
	60	884.3062±53.432 abA	761.862±72.70 bcA	52.690±6.974 aA	0.0383±0.0022 abB	0.0062±0.0003 abcA	0.0014±0.0001 aA	0.0018±0.0004 aA	0.0034±0.0005 aB
A54	20	214.1644±29.646 bcB	191.628±21.25 bB	52.222±5.684 aB	0.0129±0.0088 aA	0.0035±0.0004 abcdB	0.0017±0.0001 cB	0.0040±0.0028 aA	0.2341±0.0137 bcA
	45	264.2843±15.282 deB	259.623±11.67 eB	187.367±18.67abcA	0.0058±0.0003 cA	0.0022±0.0001 bB	0.0135±0.0009 bA	0.0010±0.0001 aB	0.0245±0.0007 aA
	60	786.4426±64.749 bcA	776.137±70.61 bA	28.020±6.467 aB	0.0168±0.0017 abA	0.0080±0.0003 abA	0.0010±0.0002 aB	0.0031±0.0012 aAB	0.0065±0.0003 aB
A26	20	115.3593±13.789 cB	263.868±6.33 bB	26.857±2.881 aB	0.0041±0.0004 aA	0.0058±0.0005 abA	0.0025±0.0001 cA	0.0020±0.0003 aA	0.1393±0.0186 deA
	45	196.0129±85.479 eB	239.053±77.25 eB	163.516±22.28bcdA	0.0038±0.0003 cA	0.0020±0.0002 bB	0.0063±0.0004 cdeA	0.0009±0.0001 aA	0.0166±0.0011 aB
	60	498.1651±42.680 cA	534.111±42.65 cA	34.793±3.462 aB	0.0126±0.0005 bA	0.0064±0.0011 abcA	0.0024±0.0005 aA	0.0016±0.0002 aA	0.0035±0.0001 aB
A100	20	612.1743±84.426 aB	716.342±79.71 aB	20.534±9.337 aB	0.0051±0.0004 aC	0.0036±0.0008 abcdB	0.0008±0.0002 cB	0.0023±0.0006 aA	0.0152±0.0027 fA
	45	548.016±119.77 bcdB	573.514±138.19 bcdB	225.638±87.246abA	0.1659±0.0298 bA	0.0034±0.0007 bB	0.0233±0.0062 aA	0.0011±0.0001 aA	0.0030±0.0001 aA
	60	1120.0458±67.401 aA	1213.419±110.41 aA	24.095±8.831 aB	0.0486±0.0105 aB	0.0059±0.0012 bcA	0.0031±0.0010 aB	0.0019±0.0003 aA	0.0016±0.0003 aA
Biq	20	211.9711±17.081 bcB	170.115±19.70 bB	41.909±3.350 aA	0.0025±0.0004aA	0.0023±0.0006 cdB	0.0008±0.0002 cA	0.0013±0.0002 aA	0.1902±0.0403 cdA
	45	794.9037±62.74 abA	601.462±39.69 bcdA	25.117±8.719 eA	0.0112±0.0006 cA	0.0031±0.0004 bB	0.0014±0.0003 eA	0.0018±0.0004 aA	0.0034±0.0004 aA
	60	990.3936±177.78 abA	704.175±128.43 bcA	21.836±4.337 aA	0.0140±0.0011 bA	0.0057±0.0007 bcA	0.0015±0.0001 aA	0.0022±0.0004 aA	0.0048±0.0002 aB
A06	20	209.8661±20.047 bcC	331.764±9.19 bB	36.011±3.049 aB	0.0042±0.0005 aA	0.0059±0.0011 aB	0.0059±0.0016 abcB	0.0016±0.0007 aA	0.4189±0.0854 aA
	45	433.7835±71.494 cdeB	389.645±71.08 deB	256.002±34.351 aA	0.0081±0.0006 cA	0.0028±0.0002 bC	0.0112±0.0006 bcA	0.0014±0.0005 aA	0.0097±0.0036 aB
	60	796.6296±54.905 bA	853.542±82.52 bA	51.736±11.417aB	0.0245±0.0022 abA	0.0092±0.0013 aA	0.0015±0.0003 aC	0.0013±0.0001 aA	0.0048±0.0006 aB
A17	20	451.8195±36.799 abB	357.578±16.27 bB	29.606±6.983 aA	0.0041±0.0005 aB	0.0024±0.0006 cdC	0.0063±0.0006 abcA	0.0019±0.0003 aA	0.0876±0.0273 efA
	45	867.4268±38.063 aA	710.939±36.79 abcA	65.148±15.860eA	0.0319±0.0057 cA	0.0049±0.0003 abB	0.0047±0.0016 deA	0.0020±0.0002 aA	0.0050±0.0009 aB
	60	1003.9637±48.07 abA	709.714±54.54 bcA	55.100±5.632 aA	0.0267±0.0017 abAB	0.0092±0.0010 aA	0.0050±0.0005 aA	0.0025±0.0005 aA	0.0030±0.0001 aB
A18	20	370.5750±18.900 abcC	348.051±19.64 bB	22.966±3.093 aB	0.0058±0.0004 aA	0.0023±0.0002 dB	0.0089±0.0006 abA	0.0012±0.0001 aA	0.3104±0.0157 bA
	45	622.1628±43.329 abcB	435.886±95.44 cdeB	100.067±23.672cdeA	0.0149±0.0029 cA	0.0030±0.0001 bB	0.0077±0.0008 bcdA	0.0012±0.0002 aA	0.0282±0.0015 aB
	60	893.2258±29.269 abA	677.893±37.85 bcA	54.731±6.939 aAB	0.0214±0.0010 abA	0.0083±0.0007 abA	0.0029±0.0003 aB	0.0017±0.0003 aA	0.0054±0.0008 aB
A83	20	433.3178±90.886 abC	782.310±78.68 aA	18.322±5.831 aA	0.0103±0.0006 aA	0.0054±0.0007 abcA	0.0114±0.0009 aA	0.0034±0.0007 aA	0.0759±0.0126 efA
	45	681.5712±82.868 abcB	809.101±78.95 abA	46.031±8.736 eA	0.0156±0.0017 cA	0.0047±0.0007 abA	0.0033±0.0005 deB	0.0019±0.0003 aA	0.0189±0.0029 aAB
	60	983.7625±66.347 abA	923.407±69.39 abA	19.502±2.016 aA	0.0186±0.0008 bA	0.0055±0.0005 cA	0.0005±0.0000 aB	0.0021±0.0002 aA	0.0062±0.0004 aB

Continuation of Table S1

		DAA	Dehydroascorbic acid	Inositol, myo-	Spermidine	Galactinol
		20	0.1743±0.0098 abB	0.2111±0.0359 cdefB	0.0028±0.0005 cdA	0.0242±0.0052 abB
	Hab	45	0.4322±0.0700 bA	0.2616±0.0460 abcdA	0.0016±0.0002 eA	0.0431±0.0067 bcdAB
		60	0.3270±0.0301 bA	0.1483±0.0099 abcB	0.0027±0.0002 bA	0.0510±0.0099 bcdA
		20	0.2904±0.0321 aA	0.2669±0.0097 bcA	0.0039±0.0006 bcA	0.0422±0.0028 abA
	A101	45	0.3613±0.0349 bcA	0.2226±0.0143 bcdeAB	0.0023±0.0002 cdeA	0.0112±0.0009 cdB
		60	0.3372±0.0360 bA	0.1552±0.0060 abcB	0.0028±0.0005 bA	0.0110±0.0009 dB
		20	0.2344±0.0255 aB	0.2579±0.0088 bcdB	0.0029±0.0003 bcdA	0.0147±0.0009 abC
	A54	45	0.4901±0.0440 abA	0.3324±0.0124 aA	0.0020±0.0002 deA	0.1025±0.0039 aB
		60	0.4305±0.0587 abA	0.1407±0.0110 abcC	0.0024±0.0002 bA	0.1789±0.0389 aA
		20	0.0399±0.0088 bB	0.1898±0.0105 cdefA	0.0028±0.0002 bcdA	0.0240±0.0013 abB
	A26	45	0.2177±0.0134 cA	0.1757±0.0107 deA	0.0039±0.0004 bcdA	0.0615±0.0035 abA
		60	0.2633±0.0292 bA	0.2070±0.0190 abA	0.0032±0.0002 bA	0.0099±0.0016 dB
		20	0.2554±0.0341 aB	0.1353±0.0112 efB	0.0012±0.0001 dA	0.0218±0.0021 abA
	A100	45	0.6129±0.0944 aA	0.2984±0.0096 abA	0.0016±0.0001 eA	0.0063±0.0004 dA
		60	0.5312±0.0320 aA	0.2025±0.0369 abB	0.0016±0.0002 bA	0.0279±0.0119 dA
		20	0.1775±0.0156 aA	0.5202±0.0495 aA	0.0030±0.0008 bcdA	0.0122±0.0024 bB
	AC2	45	0.2370±0.0145 bcA	0.1906±0.0118 cdeB	0.0026±0.0002 cdeA	0.0508±0.0074 bcA
		60	0.3259±0.0293 bA	0.1516±0.0067 abcB	0.0031±0.0003 bA	0.0785±0.0111 bA
		20	0.1234±0.0128 abB	0.0967±0.0047 fB	0.0041±0.0004 bcA	0.0261±0.0026 abB
	A06	45	0.2275±0.0299 cAB	0.1360±0.0090 eAb	0.0012±0.0001 eB	0.0502±0.0049 bcAB
		60	0.2891±0.0285 bA	0.2013±0.0216 abA	0.0026±0.0004 bAB	0.0723±0.0138 bcA
		20	0.1764±0.0317 abB	0.2345±0.0505 bcdeA	0.0051±0.0008 bA	0.0423±0.0067 abB
	A17	45	0.4660±0.0385 abA	0.2818±0.0099 abcA	0.0044±0.0005 bcAB	0.0086±0.0010 dC
		60	0.3900±0.0296 abA	0.1172±0.0091 bcB	0.0030±0.0003 bB	0.1415±0.0067 aA
		20	0.1706±0.0198 abB	0.1587±0.0074 defB	0.0095±0.0005 aA	0.0333±0.0023 abA
	A18	45	0.3661±0.0217 bcA	0.2612±0.0125 abcdA	0.0102±0.0011 aA	0.0171±0.0022 cdA
		60	0.3358±0.0300 bA	0.2192±0.0195 aAB	0.0101±0.0012 aA	0.0353±0.0034 cdA
		20	0.2161±0.0233 aB	0.2983±0.0278 bA	0.0042±0.0004 bcB	0.0553±0.0069 aA
	A83	45	0.3573±0.0167 bcA	0.2664±0.0098 abcdA	0.0061±0.0009 bA	0.0344±0.0051 bcdAB
		60	0.3986±0.0286 aAb	0.0710±0.0109 cB	0.0030±0.0002 bC	0.0209±0.0015 dB

The data were submitted to normality test by test Kolmogorov-Smirnov, and once met the requirements, was made the separation of means by Tukey test ($p<0,05$). Average ± error (n=6). Capital Letters indicate difference over timer of development after anthesis and small letters indicate difference between accesses. (*)Asterisks indicate metabolic obtained by enzymatic assays, NA, not detected and bars in vertical dark gray indicates the pungent accesses, while light gray not pungent accesses.

Table S2: Secondary metabolic profile of chili pepper fruit from different accesses (*C. chinense*) over three different time of the development (20, 45 and 60 days after anthesis (DAA)). Values are presented as means ± SE (n=6) obtained in one dependent assays.

	DAA	Chalcone	Kaempferol	Quercitin	Naringenin	4'-Hydroxyflavone	7'-Hydroxyflavone	Genistein	Clorogenic acid
Hab	**20**	0.0576 ± 0.0067 abA	0.0137 ± 0.0044 aB	0.0202 ± 0.0043 cA	0.0804 ± 0.0168 dA	0.0287 ± 0.0051 aC	0.0085 ± 0.0017 bA	0.0119 ± 0.0039 dA	0.0419 ± 0.0086 cA
	45	0.0398 ± 0.0065 aA	0.0738 ± 0.0074 bcAB	0.0198 ± 0.0050 cdA	0.0015 ± 0.0000 aB	0.0614 ± 0.0069 aA	0.0012 ± 0.0003 aB	0.0037 ± 0.0008 bB	0.0269 ± 0.0032 abA
	60	0.0323 ± 0.0013 bA	0.1177 ± 0.0333 bcA	0.0081 ± 0.0011 abA	0.0009 ± 0.0000 aB	0.0486 ± 0.0059 abB	0.0030 ± 0.0005 aB	0.0006 ± 0.00006 bC	0.0238 ± 0.0023 abA
A101	**20**	0.0249 ± 0.0022 bA	0.0447 ± 0.0017 aC	0.1191 ± 0.0087 aA	0.3345 ± 0.0003 bcA	0.0166 ± 0.0011 abcA	0.0004 ± 0.0001 cB	0.0351 ± 0.0011 bcA	0.0456 ± 0.0043 bcA
	45	0.0663 ± 0.0131 aA	0.2558 ± 0.0161 aA	0.0211 ± 0.0046 cdB	0.0033 ± 0.0012 aB	0.0091 ± 0.0036 deAB	0.0023 ± 0.0004 aB	0.0057 ± 0.0009 bB	0.0361 ± 0.0072 bA
	60	0.0355 ± 0.0197 bA	0.1474 ± 0.0491 bB	0.0156 ± 0.0025 abB	0.0032 ± 0.0001 aB	0.0041 ± 0.0035 eB	0.0081 ± 0.0018 aA	0.0014 ± 0.0003 abB	0.0145 ± 0.0023 bB
A54	**20**	0.0354 ± 0.0015 bA	0.0088 ± 0.0005 aA	0.0185 ± 0.0026 cA	0.0726 ± 0.0001 dA	0.0041 ± 0.0005 cC	0.0074 ± 0.0004 bcA	0.0084 ± 0.0016 dA	0.1315 ± 0.0073 aA
	45	0.0669 ± 0.0212 aA	0.0219 ± 0.0056 bcA	0.0063 ± 0.0011 cAB	0.0003 ± 0.0000 aA	0.0625 ± 0.0161 aA	0.0013 ± 0.0005 aB	0.0086 ± 0.0017 bA	0.0641 ± 0.0091 aB
	60	0.0511 ± 0.0223 abA	0.0528 ± 0.0082 bcA	0.0008 ± 0.0002 bB	0.0023 ± 0.0004 aA	0.0417 ± 0.0035 aB	0.0017 ± 0.0003 aB	0.0023 ± 0.0004 abB	0.0281 ± 0.0040 abC
A26	**20**	0.0679 ± 0.0205 abA	0.0141 ± 0.0022 aB	0.0272 ± 0.0060 cA	0.1762 ± 0.0259 cdA	0.0062 ± 0.0011 bcA	0.0049 ± 0.0005 bcA	0.0052 ± 0.0027 eA	0.0757 ± 0.0073 bcA
	45	0.0447 ± 0.0024 aA	0.0059 ± 0.0026 cB	0.0281 ± 0.0071 bcA	0.0022 ± 0.0006 aB	0.0081 ± 0.0021 deA	0.0014 ± 0.0008 aB	0.0113 ± 0.0015 bA	0.0348 ± 0.0047 abcB
	60	0.0223 ± 0.0025 bA	0.3425 ± 0.0555 aA	0.0105 ± 0.0048 abA	0.0022 ± 0.0007 aB	0.0073 ± 0.0023 deA	0.0044 ± 0.0009 aB	0.0041 ± 0.0014 abA	0.0307 ± 0.0090 abB
A100	**20**	0.0219 ± 0.005 bA	0.0129 ± 0.0022 aB	0.0367 ± 0.0117 bcA	0.0364 ± 0.0023 dA	0.0228 ± 0.0032 bcB	0.0179 ± 0.0052 aA	0.0176 ± 0.0024 cdA	0.0458 ± 0.0084 cA
	45	0.0116 ± 0.0023 aA	0.0115 ± 0.0023 cB	0.0241 ± 0.00 bcdAB	0.0022 ± 0.0009 aA	0.0498 ± 0.0066 deA	0.0046 ± 0.0016 aB	0.0138 ± 0.0045 bAB	0.0306 ± 0.0034 abAB
	60	0.0442 ± 0.0256 bA	0.0937 ± 0.0255 bcA	0.0166 ± 0.0062 abB	0.0102 ± 0.0024 aA	0.0263 ± 0.0076 cdB	0.0045 ± 0.0031 aB	0.0022 ± 0.0009 aB	0.0165 ± 0.0008 bB
Biq	**20**	0.051 ± 0.0061 abA	0.0546 ± 0.0126 aA	0.0381 ± 0.0049 bcA	0.3547 ± 0.0600 bA	0.0048 ± 0.0004 cA	0.0046 ± 0.0005 bcA	0.0081 ± 0.0014 dA	0.0994 ± 0.0174 aA
	45	0.0647 ± 0.0276 aA	0.0224 ± 0.0079 cA	0.0166 ± 0.0031 cdB	0.0046 ± 0.0010 aB	0.0077 ± 0.0010 eA	0.0026 ± 0.0005 aA	0.0063 ± 0.0005 bA	0.0602 ± 0.0069 aB
	60	0.0667 ± 0.0353 abA	0.0459 ± 0.0127 cA	0.0094 ± 0.0029 abB	0.0041 ± 0.0008 aB	0.0051 ± 0.0010 eA	0.0015 ± 0.0009 aA	0.0011 ± 0.0010 abB	0.0562 ± 0.0107 aB
A06	**20**	0.1245 ± 0.0188 aA	0.0225 ± 0.0044 aA	0.0251 ± 0.0018 cAB	0.2472 ± 0.0308 bA	0.0057 ± 0.0006 cB	0.0052 ± 0.0013 bcA	0.0076 ± 0.0008 dA	0.0566 ± 0.0058 bcA
	45	0.0221 ± 0.0027 aB	0.0181 ± 0.0011 cA	0.0509 ± 0.0055 abA	0.0024 ± 0.0010 aB	0.0214 ± 0.0032 deA	0.0020 ± 0.0005 aAB	0.0181 ± 0.0025 bA	0.0104 ± 0.0045 bB
	60	0.0459 ± 0.0261 abB	0.0492 ± 0.0113 cA	0.0201 ± 0.0045 aB	0.0039 ± 0.0009 aB	0.0096 ± 0.0029 deB	0.0019 ± 0.0009 aB	0.0053 ± 0.0033 abA	0.0122 ± 0.0048 bB
A17	**20**	0.1304 ± 0.0217 aA	0.0272 ± 0.0081 aB	0.0265 ± 0.0059 cA	0.0899 ± 0.0485 dA	0.0199 ± 0.0061 abB	0.0048 ± 0.0010 bcA	0.0296 ± 0.0093 bcA	0.0476 ± 0.0084 bcA
	45	0.0533 ± 0.0081 aB	0.0762 ± 0.0048 bcAB	0.0179 ± 0.0052 cdA	0.0041 ± 0.0009 aA	0.0398 ± 0.0019 cA	0.0015 ± 0.0006 aB	0.0071 ± 0.0006 bB	0.0461 ± 0.0087 abA
	60	0.1353 ± 0.0261 aA	0.0995 ± 0.0060 bcA	0.0015 ± 0.0004 bB	0.0032 ± 0.0008 aA	0.0484 ± 0.0026 cA	0.0021 ± 0.0005 aB	0.0049 ± 0.0015 abB	0.0114 ± 0.0013 bB
A18	**20**	0.0735 ± 0.0161 abA	0.0439 ± 0.0057 aB	0.0501 ± 0.0045 aA	0.3793 ± 0.0561 abA	0.0172 ± 0.0022 abcB	0.0024 ± 0.0005 cdA	0.0536 ± 0.0049 aA	0.0456 ± 0.0047 cA
	45	0.0411 ± 0.0025 aA	0.1264 ± 0.0062 bA	0.0434 ± 0.0029 abA	0.0056 ± 0.0007 aB	0.0222 ± 0.0021 dAB	0.0024 ± 0.0005 aA	0.0131 ± 0.0008 bB	0.0518 ± 0.0088 abA
	60	0.0544 ± 0.0044 abA	0.1374 ± 0.0092 bcA	0.0148 ± 0.0036 abB	0.0255 ± 0.0085 aB	0.0361 ± 0.0053 abA	0.0025 ± 0.0007 aA	0.0146 ± 0.0038 aB	0.0231 ± 0.0057 abA
A83	**20**	0.0359 ± 0.0058 abA	0.0406 ± 0.0103 aA	0.0329 ± 0.0077 bcA	0.4887 ± 0.1131 aA	0.0075 ± 0.0016 bcB	0.0019 ± 0.0009 bcA	0.0456 ± 0.0111 abA	0.0584 ± 0.0053 abcA
	45	0.0397 ± 0.0085 aA	0.0146 ± 0.0045 cA	0.0381 ± 0.0034 abcA	0.0327 ± 0.0085 aB	0.0202 ± 0.0014 deA	0.0009 ± 0.0003 aA	0.0687 ± 0.0063 aA	0.0279 ± 0.0038 abB
	60	0.0168 ± 0.0034 bA	0.0783 ± 0.0059 bcA	0.0245 ± 0.0027 aA	0.0318 ± 0.0022 aB	0.0194 ± 0.0015 cdB	0.0021 ± 0.0012 aA	0.0012 ± 0.0002 abB	0.0231 ± 0.0028 abB

Continuation of Table S2

	DAA	2-Hydroxycinnamic acid	Ferulic acid	Isoferulic acid	Caffeic acid	Hydroxybenzaldehyde acid	Methyl-p- hydroxybenzaldehyde acid
Hab	20	0.0031 ± 0.0010 aB	0.0033 ± 0.0008 cdA	0.0085 ± 0.0019 bcdA	0.1851 ± 0.1058 aA	0.1125 ± 0.0253 bcdA	0.4951 ± 0.0519 abA
	45	0.0002 ± 0.0001 aB	0.0036 ± 0.0016 aA	0.0006 ± 0.0002 bB	0.0459 ± 0.0082 bB	0.0875 ± 0.0224 abA	0.5893 ± 0.0640 aA
	60	0.0304 ± 0.0069 aA	0.0038 ± 0.0016 abA	0.0015 ± 0.0006 bAB	0.2411 ± 0.0629 abcA	0.0611 ± 0.0045 bcA	0.4897 ± 0.0220 abA
A101	20	0.0009 ± 0.0001 aA	0.00003 ± 0.00000 aB	0.0127 ± 0.0012 bA	0.0176 ± 0.0042 bB	0.1109 ± 0.0082 abcA	0.3711 ± 0.0171 abA
	45	0.00001 ± 0.000004 aB	0.0018 ± 0.0011 aA	0.0001 ± 0.00008 dB	0.1281 ± 0.0069 abA	0.0848 ± 0.0067 abA	0.3686 ± 0.0245 aA
	60	0.0011 ± 0.0004 cA	0.0024 ± 0.0010 bA	0.0069 ± 0.0040 bAB	0.0857 ± 0.0157dAB	0.0709 ± 0.0084 bcA	0.2881 ± 0.0379 bA
A54	20	0.0006 ± 0.0003 aB	0.0010 ± 0.00008 cA	0.0003 ± 0.00006 dA	0.0081 ± 0.0024 bB	0.0571 ± 0.0173 dA	0.4361 ± 0.0431 bA
	45	0.0019 ± 0.0005 aB	0.00034 ± 0.00010 aA	0.0004 ± 0.0004 bA	0.0164 ± 0.0046 bAB	0.0766 ± 0.0278 abA	0.2919 ± 0.0776 aA
	60	0.0108 ± 0.0026 bA	0.0052 ± 0.0013 abA	0.0018 ± 0.0005 bA	0.1108 ± 0.0207 cdA	0.0409 ± 0.0096 cA	0.3795 ± 0.0215 abA
A26	20	0.0032 ± 0.0007 aA	0.0308 ± 0.0015 aA	0.0643 ± 0.0054 aA	0.0196 ± 0.0049 bA	0.0676 ± 0.0089 cdA	0.4851 ± 0.0332 abA
	45	0.0021 ± 0.0012 aA	0.0044 ± 0.0019 aC	0.0127 ± 0.0035 aC	0.0127 ± 0.0048 bA	0.0796 ± 0.0106 abA	0.2961 ± 0.0755 bcA
	60	0.0077 ± 0.0003 bcA	0.0090 ± 0.0029 aB	0.0212 ± 0.0019 aB	0.0738 ± 0.0124 dA	0.0509 ± 0.0047 bcA	0.2891 ± 0.0293 bB
A100	20	0.0032 ± 0.0007 aAB	0.0020 ± 0.0006 cA	0.0128 ± 0.0030 bA	0.0090 ± 0.0022 bB	0.1597 ± 0.0222 aA	0.5862 ± 0.0208 aA
	45	0.0003 ± 0.00007 aB	0.0038 ± 0.0017 aA	0.0017 ± 0.0008 bB	0.0711 ± 0.0294 abAB	0.0523 ± 0.0061 abB	0.5148 ± 0.0725 aA
	60	0.0083 ± 0.0024 bA	0.0038 ± 0.0015 abA	0.0002 ± 0.0002 bB	0.1581 ± 0.0424 bcdA	0.0682 ± 0.0151 bcB	0.3261 ± 0.0269 bB
Biq	20	NA	0.0107 ± 0.0032 bA	0.0015 ± 0.0003 dA	0.0861 ± 0.0267 abA	0.1189 ± 0.0187 abcA	0.5346 ± 0.0442 abA
	45	NA	0.0014 ± 0.0006 aB	0.0008 ± 0.0003 bA	0.0518 ± 0.0059 bA	0.0325 ± 0.0033 bB	0.5074 ± 0.0491 abcA
	60	0.0030 ± 0.0016 bA	0.0018 ± 0.0008 bB	0.0029 ± 0.0021 bA	0.0464 ± 0.0103 dA	0.0471 ± 0.0091 cB	0.4583 ± 0.0896 abA
A06	20	0.0022 ± 0.0003 aAB	0.0062 ± 0.0014 bcA	0.0136 ± 0.0019 bcA	0.0042 ± 0.0007 bB	0.1211 ± 0.0088 abcA	0.5017 ± 0.0397 abA
	45	0.0002 ± 0.00007 aB	0.0012 ± 0.0005 aA	0.0040 ± 0.0025 abB	0.0363 ± 0.0323 bB	0.0599 ± 0.0080 abB	0.3561 ± 0.0173 cA
	60	0.0093 ± 0.0017 bA	0.0014 ± 0.0007 bA	0.0026 ± 0.0006 bB	0.3376 ± 0.0356 aA	0.0517 ± 0.0065 cB	0.3771 ± 0.0354 bA
A17	20	0.0024 ± 0.0007 aAB	0.0046 ± 0.0013 bcA	0.0114 ± 0.0032 bcA	0.0651 ± 0.0191 abB	0.1459 ± 0.0228 abA	0.5866 ± 0.1034 abA
	45	0.00003 ± 0.0000 cC	0.0009 ± 0.0003 aA	0.0002 ± 0.0002 bB	0.1993 ± 0.0080 aA	0.0991 ± 0.0095 aA	0.3351 ± 0.0336 bB
	60	0.0291 ± 0.0078 aA	0.00006 ± 0.00005 bA	0.0003 ± 0.00008 bB	0.2778 ± 0.0273 aA	0.1442 ± 0.0122 aA	0.3078 ± 0.0246 bB
A18	20	0.0029 ± 0.0009 aA	0.0048 ± 0.0019 bcA	0.0145 ± 0.0044 bA	0.0541 ± 0.0066 abB	0.1322 ± 0.0162 abA	0.6279 ± 0.0412 aA
	45	0.0002 ± 0.0001 aA	0.0006 ± 0.0002 aA	0.0001 ± 0.0001 bB	0.1024 ± 0.0053 abB	0.0889 ± 0.0154 abA	0.5778 ± 0.0257 aA
	60	0.0016 ± 0.0006 bA	0.0006 ± 0.0004 bA	0.0006 ± 0.0002 bB	0.2864 ± 0.0138 aA	0.1123 ± 0.0106 abA	0.6462 ± 0.0381 aA
A83	20	0.0024 ± 0.0003 aB	0.00216 ± 0.0003 cA	0.0032 ± 0.0005 bcA	0.1133 ± 0.0204 aB	0.1069 ± 0.0089 abcA	0.5741 ± 0.1106 abA
	45	0.00009 ± 0.0001 cC	0.0007 ± 0.0003 aA	0.0018 ± 0.0011 aA	0.0722 ± 0.0238 bB	0.0506 ± 0.0056 abB	0.6958 ± 0.0792 aA
	60	0.0108 ± 0.0019 bA	0.0007 ± 0.0007 bA	0.0018 ± 0.0012 bA	0.2561 ± 0.0305 abcA	0.0446 ± 0.00503 cB	0.6351 ± 0.0441 abA

Continuation of Table S2

	DAA	Syringaldehyde	Shikimic acid	Catechol	Theobromine	Vanillin
Hab	20	0.0821 ± 0.0263 aA	6.0143 ± 0.6466 abA	0.0282 ± 0.0033 bC	0.1334 ± 0.0219 bA	0.0032 ± 0.0010 bA
	45	0.0515 ± 0.0079 abAB	6.7309 ± 0.7311 abA	0.0547 ± 0.0051 bcB	0.1334 ± 0.0161 bcA	0.0042 ± 0.0010 aA
	60	0.0286 ± 0.0033 cB	8.6162 ± 0.2736 bcdA	0.1129 ± 0.0142 bA	0.1410 ± 0.0060 aA	0.0010 ± 0.0005 aA
A101	20	0.0798 ± 0.0044 aA	4.9449 ± 0.1574 abA	0.0999 ± 0.0090 aA	0.1907 ± 0.0098 abA	0.0358 ± 0.0048 aA
	45	0.0677 ± 0.0103 aA	6.9803 ± 0.4174 abA	0.0839 ± 0.0045 abA	0.1805 ± 0.0194 abA	0.0144 ± 0.0040 aB
	60	0.0725 ± 0.0151 abA	7.4319 ± 0.7400 cdA	0.0464 ± 0.0126 cdeB	0.1553 ± 0.0184 aA	0.0079 ± 0.0036 aB
A54	20	0.0303 ± 0.0065 cA	6.1222 ± 0.3699 aB	0.0190 ± 0.0042 bA	0.1876 ± 0.0152 bA	0.0170 ± 0.0096 bA
	45	0.0457 ± 0.0148 aA	9.1035 ± 1.2302 abA	0.0459 ± 0.0041 cA	0.2255 ± 0.0159 abA	0.0071 ± 0.0035 aA
	60	0.0295 ± 0.0060 bcA	10.1775 ± 1.3119 abA	0.0198 ± 0.0089 eA	0.1814 ± 0.0238 aA	0.0081 ± 0.0029 aA
A26	20	0.0870 ± 0.0145 aA	5.1117 ± 0.1482 abB	0.0125 ± 0.0008 bB	0.1755 ± 0.0171 bA	0.0054 ± 0.0013 bA
	45	0.0572 ± 0.0114 abAB	6.6551 ± 0.7228 abAB	0.0286 ± 0.0052 cB	0.2404 ± 0.0258 abA	0.0083 ± 0.0011 aA
	60	0.0246 ± 0.0035 cB	8.0966 ± 0.5875 bcA	0.0585 ± 0.0042 bcdeA	0.1775 ± 0.0190 aA	0.0072 ± 0.0021 aA
A100	20	0.0692 ± 0.0055 abA	4.4572 ± 0.4028 abB	0.0380 ± 0.0044 bB	0.1853 ± 0.0312 abA	0.0070 ± 0.0039 bA
	45	0.0497 ± 0.0035 abA	4.6037 ± 0.7817 bB	0.0906 ± 0.0287 abA	0.0955 ± 0.0138 bB	0.0056 ± 0.0015 aA
	60	0.0731 ± 0.0200 abcA	10.1804 ± 0.2733 bcA	0.0614 ± 0.0126 bcdB	0.1399 ± 0.0241 aAB	0.0028 ± 0.0017 aA
Biq	20	0.0288 ± 0.0043 bcA	6.7951 ± 0.6794 aA	0.0226 ± 0.0024 bA	0.1775 ± 0.0213 abA	0.0069 ± 0.0018 bA
	45	0.0258 ± 0.0034 aA	7.1011 ± 0.8260 abA	0.0490 ± 0.0048 bcA	0.1629 ± 0.0137 abA	0.0021 ± 0.0004 aA
	60	0.0210 ± 0.0048 cA	8.1124 ± 1.6077 bcA	0.0487 ± 0.0088 cdeA	0.1341 ± 0.0280 aA	0.0010 ± 0.0003 aA
A06	20	0.0467 ± 0.0071 abcA	3.6805 ± 0.4297 bA	0.0185 ± 0.0027 bA	0.1780 ± 0.0138 abA	0.0068 ± 0.0007 bA
	45	0.0242 ± 0.0034 bA	4.6406 ± 0.3841 bA	0.0426 ± 0.0042 cA	0.1271 ± 0.0101 abA	0.0060 ± 0.0024 aA
	60	0.0406 ± 0.0095 abcA	6.9080 ± 0.7663 cA	0.0362 ± 0.0071 deA	0.1558 ± 0.0560 aA	0.0017 ± 0.0004 aA
A17	20	0.0601 ± 0.0148 abcA	5.7325 ± 0.8030 aB	0.0423 ± 0.0076 bC	0.1930 ± 0.0268 abA	0.0070 ± 0.0016 bAB
	45	0.0368 ± 0.0061 abA	8.6093 ± 0.7297 aB	0.1146 ± 0.0052 aB	0.2151 ± 0.0299 aA	0.0133 ± 0.0054 aA
	60	0.0436 ± 0.0093 abcA	13.6758 ± 0.4187 aA	0.1549 ± 0.0112 aA	0.2186 ± 0.0071 aA	0.0036 ± 0.0009 aB
A18	20	0.0705 ± 0.0091 abA	5.5676 ± 0.5179 aB	0.0380 ± 0.0036 bA	0.2634 ± 0.0289 aA	0.0140 ± 0.0018 bA
	45	0.0555 ± 0.0066 aA	7.5036 ± 0.3555 abAB	0.0357 ± 0.0017 cA	0.1870 ± 0.0121 abA	0.0132 ± 0.0059 aA
	60	0.0871 ± 0.0056 aA	10.6332 ± 0.4145 abA	0.0627 ± 0.0025 cdA	0.2240 ± 0.0067 aA	0.0097 ± 0.0026 aA
A83	20	0.0528 ± 0.0116 abcA	5.0755 ± 0.2923 aB	0.0212 ± 0.0015 bB	0.1640 ± 0.0095 abA	0.0042 ± 0.0016 bA
	45	0.0498 ± 0.0076 aA	5.5684 ± 0.2614 abB	0.0219 ± 0.0025 cB	0.1103 ± 0.0038 bA	0.0038 ± 0.0008 aA
	60	0.0396 ± 0.0041 abcA	8.5426 ± 0.6800 bcA	0.0741 ± 0.0032 bcA	0.1338 ± 0.0187 aA	0.0038 ± 0.0027 aA

The data were submitted to normality test by test Kolmogorov-Smirnov, and once met the requirements, was made the separation of means by Tukey test ($p<0,05$). Average ± error (n=6). Capital Letters indicate difference over timer of development after anthesis and small letters indicate difference between accesses. (*)Asterisks indicate metabolic obtained by enzymatic assays, NA, not detected and bars in vertical dark gray indicates the pungent accesses, while light gray not pungent accesses.

GENERAL CONCLUSION

Despite the breadth of our results, they suggest great complexity in determining the phenotypic variability, specifically the metabolic variability between the accessions and the studied stages of fruit development. We see here great phenotypic variability explained by the environmental pressure that led to these differences. However, this variability is not linked to capsaicin levels, although it is closely related to some metabolites in the fruit placenta. When we shift our attention to the transcriptional characteristics, we observe smaller variations between genotypes and strong differences over time, resulting from the natural growth and development of the fruits. Although transcription is not directly linked to metabolism, we suggest that there may be relationships between them and that they need to be investigated in detail. In addition, our data improve our understanding of *C. chinense* fruit development and provides information that should be considered in future research.

REFERENCES

Aizat WM, Dias DA, Stangoulis JCR, Able JA, Roessner U, Able AJ (2014) Metabolomics of *Capsicum* ripening reveals modification of the ethylene related-pathway and carbon metabolism. Postharvest Biol Technol 89: 19–31

Alba R, Fei Z, Payton P, Liu Y, Moore SL, Debbie P, Cohn J, D'Ascenzo M, Gordon JS, Rose JKC (2004) ESTs, cDNA microarrays, and gene expression profiling: tools for dissecting plant physiology and development. Plant J 39: 697–714

Alhagdow M, Mounet F, Gilbert L, Nunes-Nesi A, Garcia V, Just D, Petit J, Beauvoit B, Fernie AR, Rothan C, et al (2007) Silencing of the mitochondrial ascorbate synthesizing enzyme L-galactono-1,4-lactone dehydrogenase affects plant and fruit development in tomato. Plant Physiol 145: 1408–22

Alonso-Blanco C, Aarts MGM, Bentsink L, Keurentjes JJB, Reymond M, Vreugdenhil D, Koornneef M (2009) What has natural variation taught us about plant development, physiology, and adaptation? Plant Cell 21: 1877–96

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215: 403–410

Anand P, Bley K (2011) Topical capsaicin for pain management: therapeutic potential and mechanisms of action of the new high-concentration capsaicin 8% patch. Br J Anaesth 107: 490–502

Ananthan R, Subhash K, Longvah T (2018) Capsaicinoids, amino acid and fatty acid profiles in different fruit components of the world hottest Naga king chilli (*Capsicum chinense* Jacq). Food Chem 238: 51–57

Araújo WL, Nunes-Nesi A, Fernie AR (2011) Fumarate: Multiple functions of a simple metabolite. Phytochemistry 72: 838–843

de Ávila Silva L, Condori-Apfata JA, Marcelino MM, Tavares ACA, Raimundi SCJ, Martino PB, Araújo WL, Zsögön A, Sulpice R, Nunes-Nesi A (2019) Nitrogen differentially modulates photosynthesis, carbon allocation and yield related traits in two contrasting *Capsicum chinense* cultivars. Plant Sci 283: 224–237

Aza-González C, Núñez-Palenius HG, Ochoa-Alejo N (2011) Molecular biology of capsaicinoid biosynthesis in chili pepper (*Capsicum* spp.). Plant Cell Rep 30: 695–706

Baas-Espinola FM, Castro-Concha LA, Vázquez-Flota FA, Miranda-Ham ML (2016) Capsaicin synthesis requires in situ phenylalanine and valine formation in in vitro maintained placentas from *Capsicum chinense*. Molecules 21: 799

Barbazuk WB, Emrich SJ, Chen HD, Li L, Schnable PS (2007) SNP discovery via 454 transcriptome sequencing. plant J 51: 910–918

Barbero GF, Ruiz AG, Liazid A, Palma M, Vera JC, Barroso CG (2014) Evolution of total and individual capsaicinoids in peppers during ripening of the Cayenne pepper plant (*Capsicum annuum* L.). Food Chem 153: 200–206

Batista-Silva W, Nascimento VL, Medeiros DB, Nunes-Nesi A, Ribeiro DM, Zsögön A, Araújo WL (2018) Modifications in organic acid profiles during fruit development and ripening: correlation or causation? Front Plant Sci 9: 1689

Baxter CJ, Carrari F, Bauke A, Overy S, Hill SA, Quick PW, Fernie AR, Sweetlove LJ (2005) Fruit carbohydrate metabolism in an introgression line of tomato with increased fruit soluble solids. Plant Cell Physiol 46: 425–437

Beckles DM (2012) Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) fruit. Postharvest Biol Technol 63: 129–140

Beckles DM, Hong N, Stamova L, Luengwilai K (2012) Biochemical factors contributing to tomato fruit sugar content: a review. Fruits 67: 49–64

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc Ser B 289–300

Bennett DJ, Kirby GW (1968) Constitution and biosynthesis of capsaicin. J Chem Soc C Org 442

Bosland PW (1993) An effective plant field cage to increase the production of genetically pure Chile (*Capsicum* spp.) seed. HortScience 28: 1053

Bosland PW, Coon D, Cooke PH (2015) Novel formation of ectopic (nonplacental) capsaicinoid secreting vesicles on fruit walls explains the morphological mechanism for super-hot chile peppers. J Am Soc Hortic Sci 140: 253–256

Bouvier F, Backhaus RA, Camara B (1998) Induction and Control of Chromoplast-specific Carotenoid Genes by Oxidative Stress. J Biol Chem 273: 30651–30659

Broderick CE, Cooke PH (2009) Fruit composition, tissues, and localization of antioxidants and capsaicinoids in *Capsicum* peppers by fluorescence microscopy. Acta Hortic 841: 85–90

Carrari F, Baxter C, Usadel B, Urbanczyk-Wochniak E, Zanor M-I, Nunes-Nesi A, Nikiforova V, Centero D, Ratzka A, Pauly M, et al (2006) Integrated Analysis of Metabolite and Transcript Levels Reveals the Metabolic Shifts That Underlie Tomato Fruit Development and Highlight Regulatory Aspects of Metabolic Network Behavior. Plant Physiol 142: 1380–1396

Carrari F, Fernie AR (2006) Metabolic regulation underlying tomato fruit development. J Exp Bot 57: 1883–1897

Casson S, Gray JE (2008) Tansley review Influence of environmental factors on stomatal development. New Phytol 178: 9–23

Castro-Concha LA, Baas-Espinola FM, Ancona-Escalante WR, Vázquez-Flota FA, Miranda-Ham ML (2016) Phenylalanine biosynthesis and its relationship to accumulation of capsaicinoids during *Capsicum chinense* fruit development. Biol Plant 60: 579–584

Caterina MJ, Leffler A, Malmberg AB, Martin WJ, Trafton J, Petersen-Zeitz KR, Koltzenburg M, Basbaum AI, Julius D (2000) Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science (80-) 288: 306–313

Centeno DC, Osorio S, Nunes-Nesi A, Bertolo ALF, Carneiro RT, Araújo WL, Steinhauser M-CM-C, Michalska J, Rohrmann J, Geigenberger P, et al (2011a) Malate plays a crucial role in starch metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest softening. Plant Cell 23: 162–84

Chapa-Oliver AM, Mejía-Teniente L (2016) Capsaicin: From Plants to a Cancer-Suppressing Agent. Molecules 21: 931

Conforti F, Statti GA, Menichini F (2007) Chemical and biological variability of hot pepper fruits (*Capsicum annuum var. acuminatum* L.) in relation to maturity stage. Food Chem 102: 1096–1104

Cross JM, von Korff M, Altmann T, Bartzetko L, Sulpice R, Gibon Y, Palacios N, Stitt M (2006a) Variation of enzyme activities and metabolite levels in 24 Arabidopsis accessions growing in carbon-limited conditions. Plant Physiol 142: 1574–1588

Cruz CD (2006) Programa Genes: estatística experimental e matrizes. UFV

Daood HG, Halasz G, Palotás GG, Palotás GG, Bodai Z, Helyes L (2014) HPLC Determination of Capsaicinoids with Cross-Linked C18 Column and Buffer-Free Eluent. J Chromatogr Sci 1–9

Darzi Y, Letunic I, Bork P, Yamada T (2018) iPath3. 0: interactive pathways explorer v3. Nucleic Acids Res 46: W510–W513

DeWitt D, Bosland PW (2009) The complete chile pepper book: A gardener's guide to choosing, growing, preserving, and cooking. Timber Press

Fait A, Hanhineva K, Beleggia R, Dai N, Rogachev I, Nikiforova VJ, Fernie AR, Aharoni A (2008) Reconfiguration of the achene and receptacle metabolic networks during strawberry fruit development. Plant Physiol 148: 730–750

Fernie AR, Roscher A, Ratcliffe RG, Kruger NJ (2001) Fructose 2,6-bisphosphate activates pyrophosphate: Fructose-6-phosphate 1-phosphotransferase and increases triose phosphate to hexose phosphate cycling heterotrophic cells. Planta 212: 250–263

Fernie AR, Schauer N (2009) Metabolomics-assisted breeding: a viable option for crop improvement? Trends Genet 25: 39–48

Finger FL, Lannes SD, Schuelter AR, Doege J, Comerlato AP, Gonçalves LS, Ferreira FR, Clovis LR, Scapim CA (2010) Genetic diversity of *Capsicum chinensis* (*Solanaceae*) accessions based on molecular markers and morphological and agronomic traits. Genet Mol Res 9: 1852–1864

Flores P, Hellín P, Fenoll J (2012) Determination of organic acids in fruits and vegetables by liquid chromatography with tandem-mass spectrometry. Food Chem 132: 1049–1054

Fuentes L, Figueroa CR, Valdenegro M (2019) Recent advances in hormonal regulation and cross-talk during non-climacteric fruit development and ripening. Horticulturae 5: 45

Gamboa-Becerra R, Ramírez-Chávez E, Molina-Torres J, Winkler R (2015) MSI. R scripts reveal volatile and semi-volatile features in low-temperature plasma mass spectrometry imaging (LTP-MSI) of chilli (*Capsicum annuum*). Anal Bioanal Chem 407: 5673–5684

Haak DC, McGinnis L a., Levey DJ, Tewksbury JJ (2012) Why are not all chilies hot? A trade-off limits pungency. Proc R Soc B Biol Sci 279: 2012–2017

Halford NG, Purcell PC, Hardie DG (1999) Is hexokinase really a sugar sensor in plants? Trends Plant Sci 4: 117–120

Helyes L, Pék Z, Lugasi A (2006) Tomato fruit quality and content depend on stage of maturity. HortScience 41: 1400–1401

Hou B-Z, Li C-L, Han Y-Y, Shen Y-Y (2018) Characterization of the hot pepper (*Capsicum frutescens*) fruit ripening regulated by ethylene and ABA. BMC Plant Biol 18: 1–12

Hubbard NL, Pharr DM (1992) Developmental changes in carbohydrate concentration and activities of sucrose metabolizing enzymes in fruits of two *Capsicum annuum* L. genotypes. Plant Sci Limerick 86: 1: 33–39

Jang YK, Jung ES, Lee HA, Choi D, Lee CH (2015) Metabolomic Characterization of Hot Pepper (*Capsicum annuum* “cM334”) during Fruit Development. J Agric Food Chem 63: 9452–9460

Jarret RL, Barboza GE, da Costa Batista FR, Berke T, Chou Y-Y, Hulse-Kemp A, Ochoa-Alejo N, Tripodi P, Veres A, Garcia CC (2019) *Capsicum*—An abbreviated compendium. J Am Soc Hortic Sci 144: 3–22

Kader AA (2008) Flavor quality of fruits and vegetables. J Sci Food Agric 88: 1863–1868

Keat C, Mohd Z, Zainal Z (2012) Scientia Horticulturae Changes in ethylene production , carbohydrase activity and antioxidant status in pepper fruits during ripening. Sci Hortic (Amsterdam) 142: 23–31

Keinanen M, Oldham NJ, Baldwin IT (2001) Rapid HPLC screening of jasmonate-induced increases in tobacco alkaloids, phenolics, and diterpene glycosides in *Nicotiana attenuata*. J Agric Food Chem 49: 3553–3558

Kim S, Park M, Yeom S-I, Kim Y-M, Lee JM, Lee H-A, Seo E, Choi J, Cheong K, Kim K-T, et al (2014) Genome sequence of the hot pepper provides insights into the evolution of pungency in *Capsicum* species. Nat Genet 46: 270–278

Klie S, Osorio S, Tohge T, Drincovich MF, Fait A, Giovannoni JJ, Fernie AR, Nikoloski Z (2014) Conserved changes in the dynamics of metabolic processes during fruit development and ripening across species. Plant Physiol 164: 55–68

Kopka J, Schauer N, Krueger S, Birkemeyer C, Usadel B, Bergmüller E, Dörmann P, Weckwerth W, Gibon Y, Stitt M, et al (2005) Systems biology GMD@CSB.DB : the Golm Metabolome Database. 21: 1635–1638

Lannes SD, Finger FL, Schuelter AR, Casali VWD (2007) Growth and quality of Brazilian accessions of *Capsicum chinense* fruits. Sci Hortic (Amsterdam) 112: 266–270

Lee RJ, Yolton RL, Yolton DP, Schnider C, Janin ML (1996) Personal defense sprays: effects and management of exposure. J Am Optom Assoc 67: 548–560

Leete E, Louden MC (1968) Biosynthesis of capsaicin and dihydrocapsaicin in *Capsicum frutescens*. J Am Chem Soc 90: 6837–6841

Liu S, Chen C, Chen G, Cao B, Chen Q, Lei J (2012) RNA-sequencing tag profiling of the placenta and pericarp of pungent pepper provides robust candidates contributing to capsaicinoid biosynthesis. Plant Cell, Tissue Organ Cult 110: 111–121

Liu S, Li W, Wu Y, Chen C, Lei J (2013) De Novo Transcriptome Assembly in Chili Pepper (*Capsicum frutescens*) to Identify Genes Involved in the Biosynthesis of Capsaicinoids. PLoS One. doi: 10.1371/journal.pone.0048156

Lombardo V a., Osorio S, Borsani J, Lauxmann M a., Bustamante C a., Budde CO, Andreo CS, Lara M V., Fernie a. R, Drincovich MF (2011) Metabolic Profiling during Peach Fruit Development and Ripening Reveals the Metabolic Networks That Underpin Each Developmental Stage. Plant Physiol 157: 1696–1710

Maillard M-N, Giampaoli P, Richard H (1997) Analysis of eleven capsaicinoids by reversed-phase high performance liquid chromatography. Flavour Fragr J 12: 409–413

Martí MC, Camejo D, Olmos E, Sandalio LM, Fernández-García N, Jiménez A, Sevilla F (2009) Characterisation and changes in the antioxidant system of chloroplasts and chromoplasts isolated from green and mature pepper fruits. Plant Biol 11: 613–624

Martínez S, Curros A, Bermúdez J, Carballo J, Franco I (2007) The composition of Arnoia peppers (*Capsicum annuum* L.) at different stages of maturity. Int J Food Sci Nutr 58: 150–161

Materska M, Piacente S, Stochmal A, Pizza C, Oleszekc W, Perucka I (2003) Isolation and structure elucidation of flavonoid and phenolic acid glycosides from pericarp of hot pepper fruit *Capsicum annuum* L. Phytochemistry 63: 893–898

Mazourek M, Pujar A, Borovsky Y, Paran I, Mueller L, Jahn MM (2009) A Dynamic Interface for Capsaicinoid Systems Biology. Plant Physiol 150: 1806–1821

McCarthy DJ, Chen Y, Smyth GK (2012) Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res 40: 4288–4297

Mounet F, Moing A, Garcia V, Petit J, Maucourt M, Deborde C, Bernillon S, Le Gall G, Colquhoun I, Defernez M, et al (2009) Gene and metabolite regulatory network analysis of early developing fruit tissues highlights new candidate genes for the control of tomato fruit composition and development. Plant Physiol 149: 1505–28

Mueller-Seitz E, Hiepler C, Petz M (2008) Chili pepper fruits: content and pattern of capsaicinoids in single fruits of different ages. J Agric Food Chem 56: 12114–12121

Naves ER, de Ávila Silva L, Sulpice R, Araújo WL, Nunes-Nesi A, Peres LEP, Zsögön A (2019) Capsaicinoids: pungency beyond *Capsicum*. Trends Plant Sci 24: 109–120

Niklis ND, Siomos AS, Sfakiotakis EM (2002) Ascorbic acid, soluble solids and dry matter content in sweet pepper fruit: change during ripening. J Veg Crop Prod 8: 41–51

Nunes-Nesi A, Carrari F, Gibon Y, Sulpice R, Lytovchenko A, Fisahn J, Graham J, Ratcliffe RG, Sweetlove LJ, Fernie AR (2007) Deficiency of mitochondrial

fumarase activity in tomato plants impairs photosynthesis via an effect on stomatal function. Plant J 50: 1093–1106

Nunes-Nesi A, Nascimento VDL, De Oliveira Silva FM, Araujo WL, Sulpice R (2016) Natural genetic variation for morphological and molecular determinants of plant growth and yield. J Exp Bot 67: 2989–3001

Oa ILW, Morgan MJ, Osorio S, Gehl B, Baxter CJ, Kruger NJ, Ratcliffe RG, Fernie AR, Sweetlove LJ (2013) Metabolic Engineering of Tomato Fruit Organic Acid Content Guided by Biochemical Analysis of an. Plant Physiol 161: 397–407

Ochoa-Alejo N, Ramirez-Malagon R (2001) In vitro chili pepper biotechnology. Vitr Cell Dev Biol 37: 701–729

Osorio S, Alba R, Nikoloski Z, Kochevenko A, Fernie AR, Giovannoni JJ (2012a) Integrative Comparative Analyses of Transcript and Metabolite Profiles from Pepper and Tomato Ripening and Development Stages Uncovers Species-Specific Patterns of Network Regulatory Behavior. Plant Physiol 159: 1713–1729

Paul V, Pandey R, Srivastava GC (2012) The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene—an overview. J Food Sci Technol 49: 1–21

Pickersgill B (1997) Genetic resources and breeding of *Capsicum* spp. Euphytica 96: 129–133

Qin G, Zhu Z, Wang W, Cai J, Chen Y, Li L, Tian S (2016) A tomato vacuolar invertase inhibitor mediates sucrose metabolism and influences fruit ripening. Plant Physiol 172: 1596–1611

R Core Team R (2018) R: A Language and Environment for Statistical Computing. R Found. Stat. Comput.

Robinson MD, McCarthy DJ, Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics 26: 139–140

Rosado-Souza L, Scossa F, Chaves IS, Kleessen S, Salvador LFD, Milagre JC, Finger F, Bhering LL, Sulpice R, Araújo WL, et al (2015) Exploring natural variation of photosynthetic, primary metabolism and growth parameters in a large panel of *Capsicum chinense* accessions. Planta 242: 677–691

Sadali NM, Sowden RG, Ling Q, Jarvis RP (2019) Differentiation of chromoplasts and other plastids in plants. Plant Cell Rep 38: 803–818

Shen L-B, Yao Y, He H, Qin Y-L, Liu Z-J, Liu W-X, Qi Z-Q, Yang L-J, Cao Z-M, Yang Y (2018) Genome-wide identification, expression, and functional analysis of the alkaline/neutral invertase gene family in pepper. Int J Mol Sci 19: 224

Steinhauser M-C, Steinhauser D, Koehl K, Carrari F, Gibon Y, Fernie AR, Stitt M (2010) Enzyme activity profiles during fruit development in tomato cultivars and *Solanum pennellii*. Plant Physiol 153: 80–98

Stellari GM, Mazourek M, Jahn MM (2010) Contrasting modes for loss of pungency between cultivated and wild species of *Capsicum*. Heredity (Edinb) 104: 460–71

Stewart C, Mazourek M, Stellari GM, O'Connell M, Jahn M (2007) Genetic control of pungency in *C. chinense* via the Pun1 locus. J Exp Bot 58: 979–991

Sun T, Xu Z, Wu CT, Janes M, Prinyawiwatkul W, No HK (2007) Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). J Food Sci 72: 98–102

Suzuki T, Fujiwake H, Iwai K (1980) Intracellular localization of capsaicin and its analogues, capsaicinoid, in *Capsicum* fruit I. Microscopic investigation of the structure of the placenta of *Capsicum annuum* var. annuum cv. Karayatsubusa. Plant Cell Physiol 21: 839–853

Tanaka Y, Nakashima F, Kirii E, Goto T, Yoshida Y, Yasuba K (2017) Difference in capsaicinoid biosynthesis gene expression in the pericarp reveals elevation of capsaicinoid contents in chili peppers (*Capsicum chinense*). Plant Cell Rep 36: 267–279

Tewksbury JJ, Levey DJ, Huizinga M, Haak DC, Traveset A (2008) Costs and benefits of capsaicin-mediated control of gut retention in dispersers of wild chilies. Ecology 89: 107–17

Tewksbury JJ, Manchego C, Haak DC, Levey DJ (2006) Where did the chili get its spice? Biogeography of capsaicinoid production in ancestral wild chili species. J Chem Ecol 32: 547–564

Tieman D, Taylor M, Schauer N, Fernie AR, Hanson AD, Klee HJ (2006) Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. Proc Natl Acad Sci 103: 8287–8292

Tiessen A, Hendriks JHM, Stitt M, Branscheid A, Gibon Y, Farré EM, Geigenberger P (2002) Starch synthesis in potato tubers is regulated by post-translational redox modification of ADP-glucose pyrophosphorylase. Am Soc Plant Biol 14: 2191–2213

Topuz A, Ozdemir F (2007) Assessment of carotenoids, capsaicinoids and ascorbic acid composition of some selected pepper cultivars (*Capsicum annuum* L.) grown in Turkey. J Food Compos Anal 20: 596–602

Trick M, Long Y, Meng J, Bancroft I (2009) Single nucleotide polymorphism (SNP) discovery in the polyploid Brassica napus using Solexa transcriptome sequencing. Plant Biotechnol J 7: 334–346

Varghese S, Kubatka P, Rodrigo L, Gazdikova K, Caprnda M, Fedotova J, Zulli A, Kruzliak P, Büsselberg D (2017) Chili pepper as a body weight-loss food. Int J Food Sci Nutr 68: 392–401

Wahyuni Y, Ballester A-RR, Sudarmonowati E, Bino RJ, Bovy AG (2013a) Secondary Metabolites of *Capsicum* Species and Their Importance in the Human Diet. J Nat Prod 76: 783–793

Wahyuni Y, Ballester AR, Sudarmonowati E, Bino RJ, Bovy AG (2011) Metabolite biodiversity in pepper (*Capsicum*) fruits of thirty-two diverse accessions: Variation in health-related compounds and implications for breeding. Phytochemistry 72: 1358–1370

Wahyuni Y, Ballester AR, Tikunov Y, de Vos RCH, Pelgrom KTB, Maharijaya A, Sudarmonowati E, Bino RJ, Bovy AG (2013b) Metabolomics and molecular marker analysis to explore pepper (*Capsicum* sp.) biodiversity. Metabolomics 9: 130–144

Wang Z, Gerstein M, Snyder M (2009) RNA-Seq: a revolutionary tool for transcriptomics. Nat Rev Genet 10: 57–63

Warnes MGR, Bolker B, Bonebakker L, Gentleman R, Huber W (2016) Package 'gplots.' Var. R Program. tools plotting data

Wellburn AR (1994) The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Plant Physiol 144: 307–313

Zhang Z, Zhao S, Liu G-F, Huang Z, Cao Z-M, Cheng S-H, Lin S-S (2016) Discovery of putative capsaicin biosynthetic genes by RNA-Seq and digital gene expression analysis of pepper. Sci Rep 6: 34121

Zhu Q, Gao P, Liu S, Zhu Z, Amanullah S, Davis AR, Luan F (2017) Comparative transcriptome analysis of two contrasting watermelon genotypes during fruit development and ripening. BMC Genomics 18: 1–2