

Biochemical variances through metabolomic profile analysis of *Capsicum chinense* Jacq. during fruit development

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ABSTRACT

Capsicum chinense Jacq. is classified under the Solanaceae family, which is an extensively consumed spice and vegetable globally. Therefore, to gain more knowledge and insight into the diversity of *Capsicum chinense* Jacq. metabolites, a total of 18 placental tissues from various development stages were collected and untargeted metabolomics was conducted by means of ultra-performance liquid chromatography (UPLC) and mass spectrometry (MS). Principal component analysis (PCA) analysis established the existence of distinct metabolite distribution patterns as observed at 16 days post anthesis (DPA), compared with the metabolites at 36 and 48 DPA groups, whereas there was a difference in metabolites between the orange ripening period (B) and the red ripening period (C), which intersected with each other. Furthermore, several pathways including metabolic pathways, biosynthesis of phenylpropanoids, ABC transporters, alanine, aspartate and glutamate metabolism, fatty acid biosynthesis, pentose and glucuronate pathways, secondary metabolites biosynthesis, cutin, biosynthesis of suberine and wax were significantly enriched across the fruit ripening stages. The capsaicin content was observed to be less in the early ripening stages, but gradually increased to a high concentration during the late ripening stages. In conclusion, our study findings submit a suitable approach for interpreting the biochemical variances of non-targeted metabolomics in hot pepper developmental stages, as well as offer new findings that can be applied in the development strategies in breeding of *Capsicum chinense* Jacq.

Keywords: *Capsicum chinense* Jacq., chili pepper, GC-MS, LC-MS, pepper fruit diversity, pepper fruit morphology, untargeted metabolomics

INTRODUCTION

Pepper fruits (family Solanaceae) are widely diverse, representing more than 200 species that vary according to size, colour, shape and chemical composition. They are important nutritional and economical fruits which can be consumed fresh as vegetables, and serve as spices when dried because of their pungent and unique flavor. Additionally, it is an important pharmaceutical resource in topical ointments and pain-relievers, serving

as ingredients and patches, respectively, and also aids in digestion in the form of condiments. Hot peppers contain broad biological compounds like carotenoids (provitamin A), capsaicinoids, flavonoids, vitamins (vitamins C and E), minerals, essential oils and the aroma of the fruits (Aniel et al., 2009; Purkayastha et al., 2012; Howard et al., 2016). These compounds have exhibited anticancer (Oyagbemi et al., 2010;

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Anandakumar et al., 2013), anti-inflammatory (Spiller et al., 2008), antimicrobial (Careaga et al., 2003) and antioxidant (Alvarez-Parrilla et al., 2011) properties.

Previously conducted studies on pepper fruits have majorly focused on the antioxidant activity (Tan et al., 2012), nutritive components (Serrano et al., 2010) and phenolic contents (Tan et al., 2012) across various fruit developmental stages. Moreover, the majority of these metabolomic studies focused only on the targeted metabolite analysis that evaluated capsaicinoids, carotenoids, flavonoids and ascorbic acid. Thus, there exists a research gap in extensive non-targeted metabolomic studies on pepper. Indeed, non-targeted metabolomic approaches can elucidate the plant responses to various environmental situations, as displayed by the changes in metabolites seen in its application in various plant species such as *Oryza sativa* (Jung et al., 2013; Kim et al., 2014), blueberries (Lee et al., 2014a) and pitayas (Lee et al., 2014b). In pepper fruits, the phytochemicals generally undergo various changes all through fruit development, which could impact on significant dietary aspects concerning the consumption of pepper (Ghasemnezhad et al., 2012).

Equally, capsaicinoid compounds are found among the members in the genus *Capsicum*. Capsaicinoid is the group of alkaloids which gives pungency or heat to hot peppers. Its biosynthesis entails a combination of pathways, namely the phenylpropanoid and branched chain fatty acid pathways. The precursors for the formation of vanillyl aminophenylalanine are generated by the phenylpropanoid pathway while the branched chain fatty acid pathway provides valine or leucine for 8-methyl-6-nonenoylCoA precursors. Capsaicinoids are known to accumulate in the dissepiment of the placental tissue of the epidermal layer. Its synthesis starts after 20–30 days of plant pollination and lasts up to the ripening stage of the fruit (Stewart et al., 2005). Thus far, about 23 analogues of capsaicinoids have been described. Capsaicin and dihydrocapsaicin (trans-8-methyl-N-vanillyl-6-nonenamide, 8-methyl-N-vanillylnonanamide, respectively) form the highest capsaicinoid constituents of up to 98% in *Capsicum* (Zewdie et al., 2001). Other less major capsaicinoids in capsicum fruits include nonivamide, homocapsaicin, homodihydrocapsaicin and nordihydrocapsaicin (Huang et al., 2013).

Therefore, ultra-performance liquid chromatography (UPLC) and mass spectrometry (MS) were used to examine the metabolite profile of *Capsicum chinense* Jacq. during the development stages of the pepper fruit. The identified metabolites could assist in defining the diversity of pepper metabolites and the molecular basis of pepper fruit pungency.

MATERIALS AND METHODS

Sample preparation and collection

The plants *Capsicum chinense* Jacq. (chili pepper) were grown in an open field, in the year 2017, at Xinjiang

Academy of Agricultural Sciences in Xinjiang. In March, the seeds were first sown in a greenhouse under typical conditions (27°C day/19°C night; 16 h light/8 h dark), which facilitated their germination, and thereafter, in mid-May the seedlings were transferred to an open field. According to normal agronomy practices in the region, the open field was properly managed. Irrigation using drips was employed to water the seedlings. Fungicides were applied six to eight times during the growing season.

Extraction and measurement of capsaicinoid and other metabolites in fruit placenta

To study the various metabolites in the *Capsicum chinense* Jacq. pepper fruit, 18 placental tissues were randomly collected during various stages of development, ranging between 16, 36 and 48 days post anthesis (DPA). Thereafter, the placental tissue samples were dried at 50°C overnight, and then grounded using a mortar and pestle. During capsaicinoid extraction, 0.2 g of dried pepper samples were mixed with ethanol (70%, 2.0 ml) in a 10 ml glass bottle covered with a Teflon-lined lid. For 4 h, capped bottles with the extract contents were run at 80°C in a water bath. Thereafter, at a frequency of 35 kHz, the samples were sonicated for 50 min. The extract was then centrifuged at 12,000×g for 15 min, and the supernatants were filtered using a 0.45 µm syringe-mounted membrane filter. Metabolite analyses were conducted using UPLC and MS. Wavelength detection was optimized at 280 nm with an injection volume of 10 µL. Tests for each ripening stage were done in six repetitions. The ProteoWizard software (version 3.0) was used to convert the original MS data to mzXML, and then XCMS was employed for retention time correction, peak identification, extraction, integration, as well as alignment, and metabolite profiling and annotation was conducted using the MWDB database (Metware biotechnology Co., Ltd. Wuhan, China – <http://www.metware.cn/>), as described elsewhere (Zhu et al., 2018).

Statistical analysis

Principal component analysis (PCA) and the *Capsicum* plant metabolites statistics were generated on the mixOmics package using R environment for statistical computation (version 3.2.3). Mean, standard deviation, analysis of variance (ANOVA) at 95% confidence interval and the *F*-value ($p \leq 0.001$) significant level constituted the summarized statistics done. Pearson's correlation method was employed to generate correlation analysis. Generally, when conducting multiple analyses on the same dependent variable, the chance of committing a Type I error increases, thus increasing the likelihood of reaching at a significant result by pure chance. To correct this under this study, multiple testing was adjusted by use of Bonferroni's correction for accurate inference. The qRT-PCR results were evaluated using Student's *t*-test method.

RESULTS

Placental metabolite quantity during fruit development

The pericarp colours of the pepper fruits transformed as fruit maturation progressed: A = green (16 DPA), B = orange (36 DPA) and C = red (48 DPA) (Figure 1). Multivariate analysis was made on the MS spectrum data to identify the differences in primary metabolites. In total, 370 metabolites were identified from the *Capsicum chinense* Jacq. pepper fruit (Supplementary Table 1).

PCA of metabolic groups

As per PCA analysis, our study findings revealed that the metabolites present at the 16 DPA (green ripening period) were highly distinct with a clear cluster distribution, compared with the metabolites of the 36 and 48 DPA groups (orange and red ripening periods), whereas the difference in metabolites between the orange ripening period (B) and the red ripening period (C) intersected with each other (Figure 2).

For differential multiple analysis of metabolites, a total of 145 up-regulated metabolites were identified, while 320 metabolites were down-regulated during

the green ripening period. During the colour transition period of the pepper fruit, 205 metabolites were up-regulated and 405 down-regulated, whereas 57 metabolites were up-regulated and 45 down-regulated

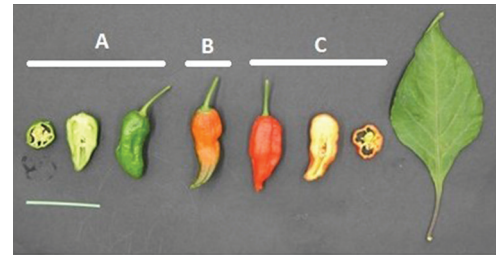


Figure 1. *Capsicum chinense* Jacq pepper fruit developmental stages; green ripening stage (A), colour transit stage (B), and the red ripening stage (C).

Table 1. Results of the differential metabolites (A represents 16 DPA, B represents 36 DPA and C represents 48 DPA).

Group name	All diff	Down-regulated	Up-regulated
A vs. B	465	320	145
A vs. C	610	405	205
B vs. C	102	45	57

DPA, days post anthesis.

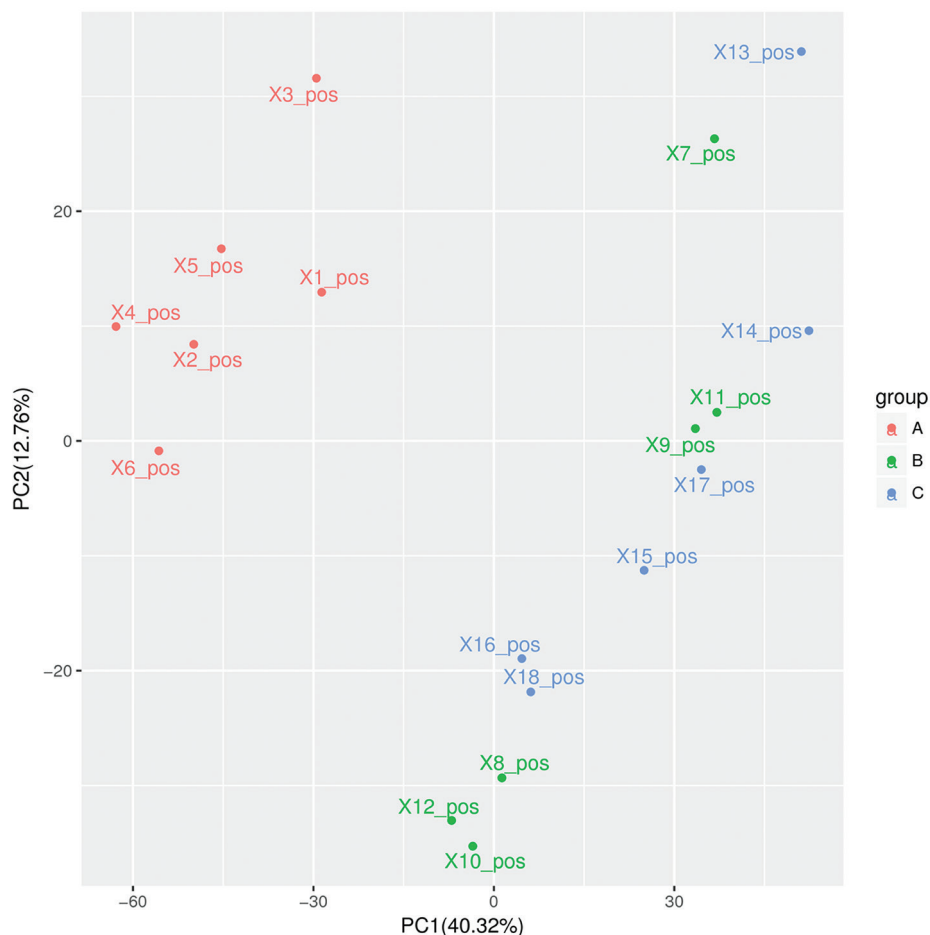


Figure 2. PCA analysis of the three fruit developmental stages (green ripening period (A), colour transit period (B), and the red ripening stage (C)). PCA, principal component analysis.

during the red ripening stage of the fruit (Table 1 and Figure 3A–C).

Differential subset gene analysis

Using Venn diagram, the identified 726 expressed genes detected at each developmental stage (A = green stage,

B = yellow stage and C = red stage) were analysed (Figure 4). 14 of the total 726 genes were commonly differentially expressed among the three groups while 82, 13 and 194 genes were uniquely expressed at A, B and C, respectively.

Differential metabolites (Kyoto Encyclopedia of Genes and Genomes) functional annotation and enrichment analysis

In studying the *in vivo* differential metabolites interaction in forming distinct pathways, the obtained differing metabolites were annotated using the KEGG database. Some of the results are presented in Supplementary Table 2. Table 2 shows the statistics of the KEGG annotation results of different metabolites in each of the three study groups.

The annotation results for the differential metabolite KEGG were classified according to their respective pathway type in the KEGG database. The differential metabolites of the three groups have a varied influence on all stages of fruit ripening. Several pathways, including metabolic pathways, biosynthesis of phenylpropanoids, ABC transporters, alanine, aspartate and glutamate metabolism, fatty acid biosynthesis, pentose and glucuronate pathways, secondary metabolites biosynthesis, cutin, biosynthesis of suberine and wax, biosynthesis of antibiotics, amino and nucleotide metabolism of sugars, biosynthesis of glucosinolate, biosynthesis of plant hormones, and microbial metabolism in diverse environments were

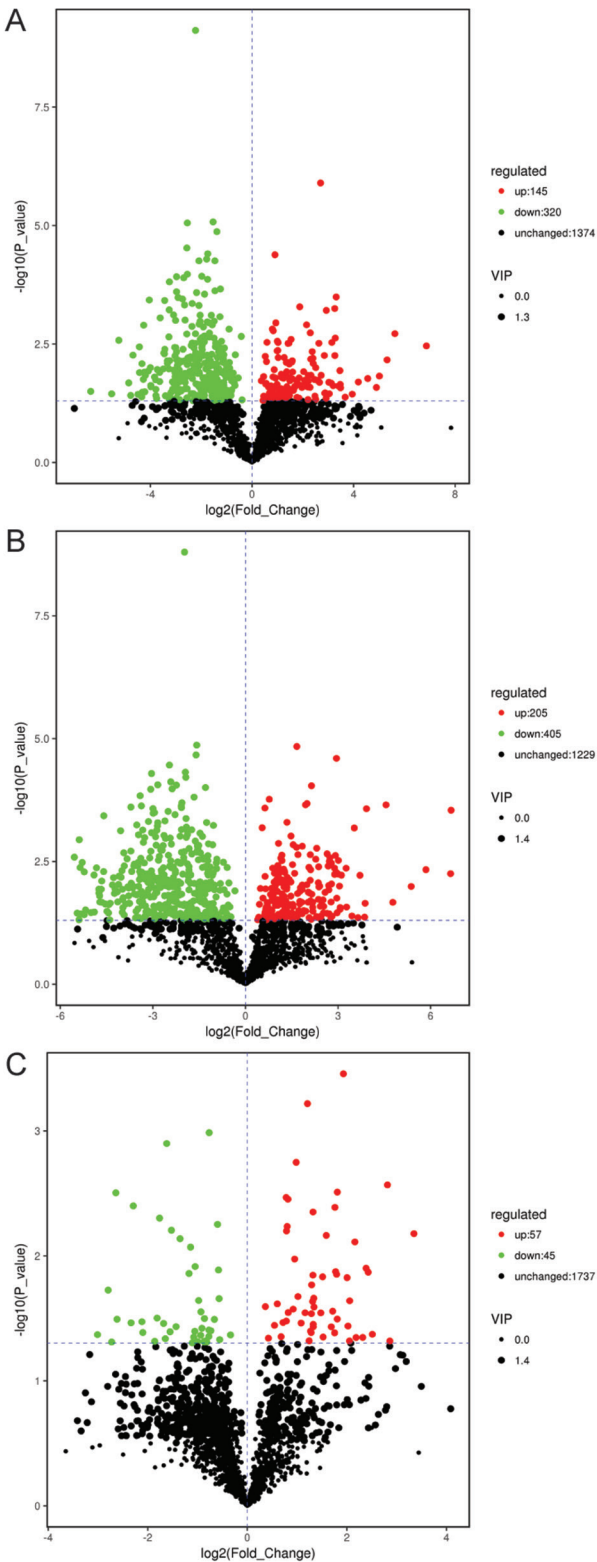


Figure 3. Volcano diagram illustrating the difference in the expression levels of metabolites in the study groups.

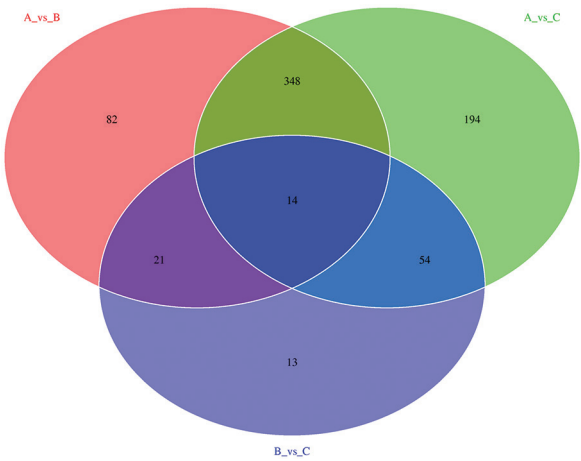


Figure 4. Venn diagram for expressed genes detected at each developmental stage.

Table 2. KEGG annotation results of different metabolites in each group (A represents 16 DPA, B represents 36 DPA and C represents 48 DPA).

Group	Diff number	Diff KEGG Annotation
A vs. B	465	27
A vs. C	610	29
B vs. C	102	6

DPA, days post anthesis.

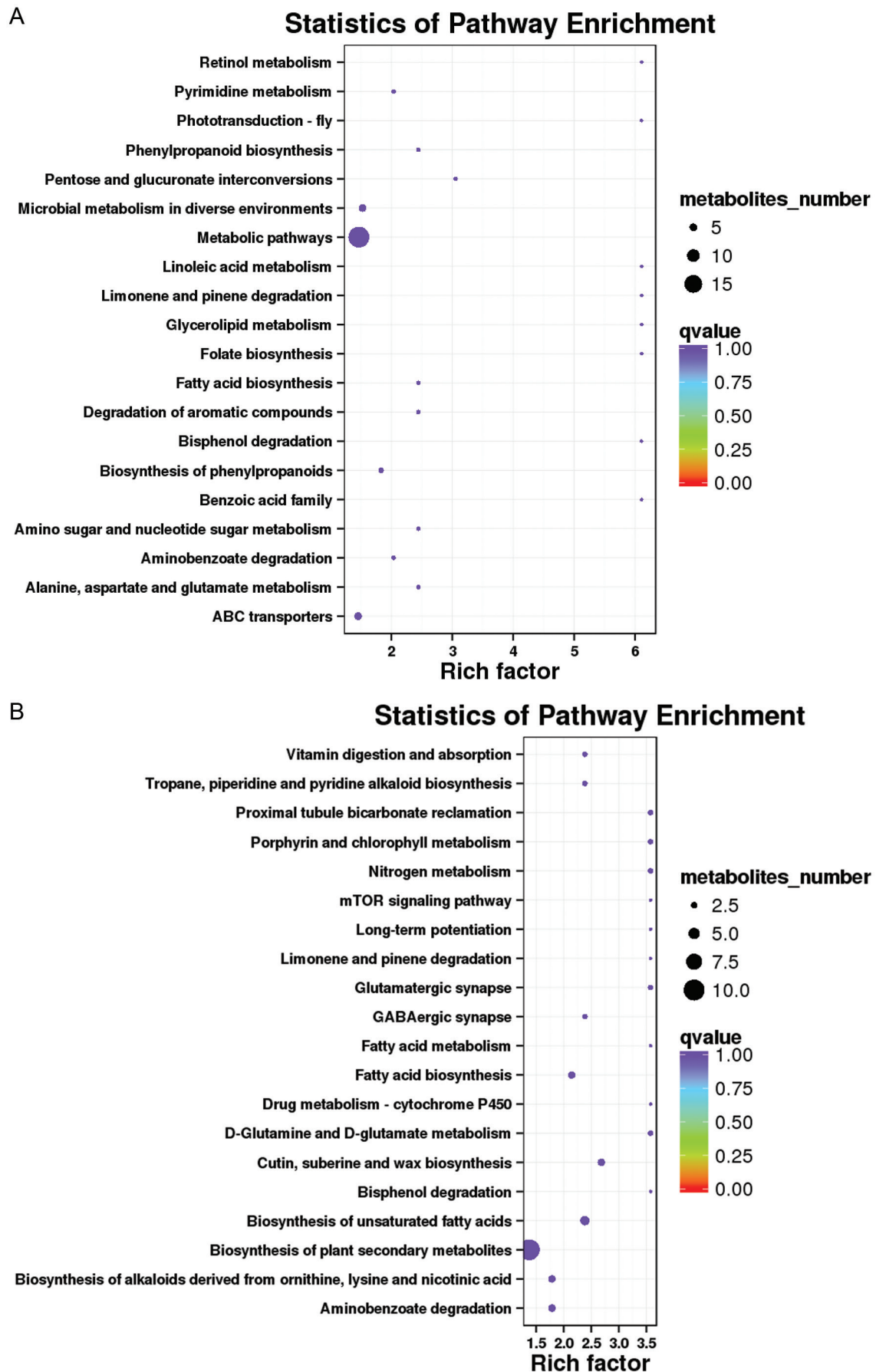


Figure 5. KEGG enrichment pathway analysis of DEGs between the three study groups (Figure A: A vs. B, Figure B: A vs. C and Figure C: B vs. C). The KEGG pathway is shown by the left Y-axis while the X-axis shows the Rich factor. A high q -value is represented by blue and a low q -value is represented by red ($q < 0.05$).

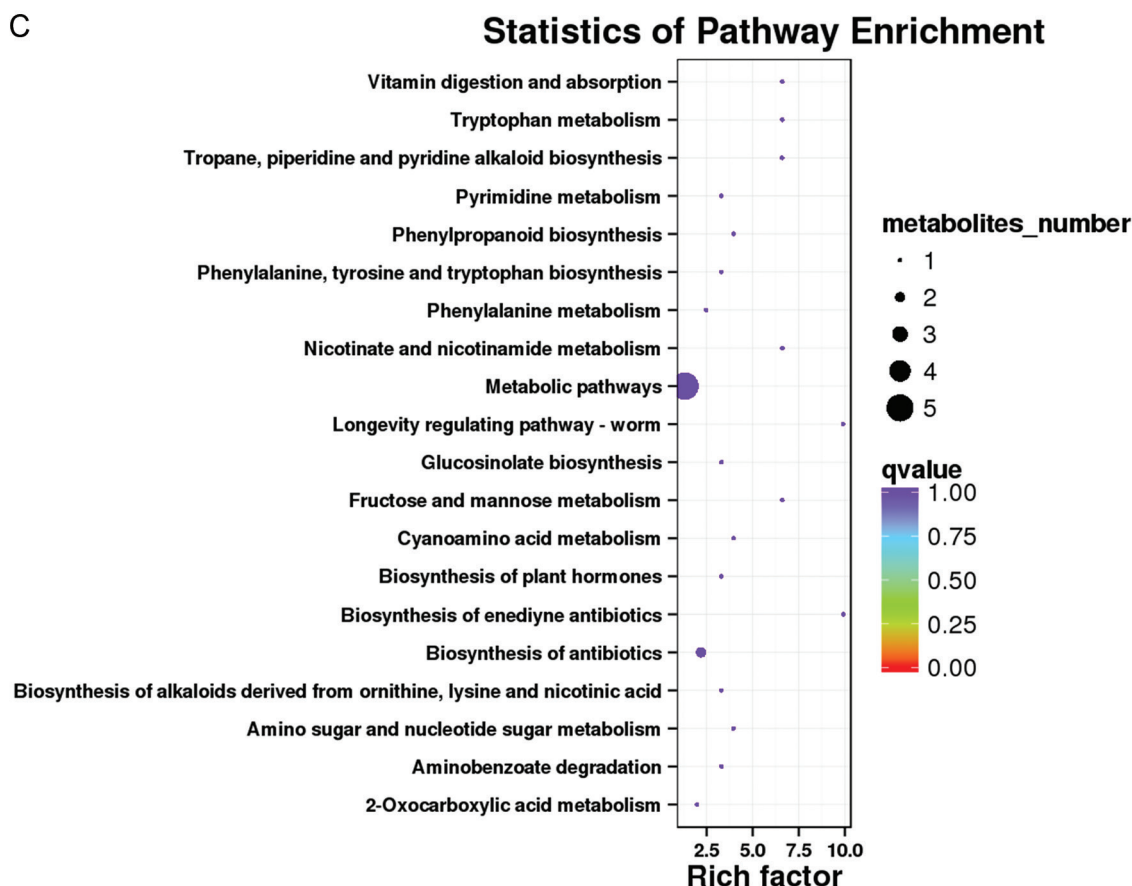


Figure 5. Continued.

significantly enriched across the fruit ripening stages ($q < 0.05$; Figure 5A–C).

Canonical correlation analysis of pepper fruit metabolites

Through typical correlation analysis (canonical correlation analysis [CCA]), we found that metabolite numbers 6,257, 10,464, 5,100, 16,349, 304 and 809 were synthesized from fatty acids associated with the synthesis of horseradish (fatty acid biosynthesis) pathway partial gene, propyl coenzyme A synthesis (malonyl-CoA biosynthesis) pathway partial gene and benzene propane metabolism (phenylpropanoid and benzenoid metabolism). Some genes in the pathway were typically related (Figure 6).

Analysis of identified potential capsaicinoid biosynthetic pathway-related genes

Placental capsaicin concentration for each respective developmental stage was also measured. From the obtained results, it was found that there was less capsaicin ($631.00 \pm 84.15 \text{ mg} \cdot \text{kg}^{-1}$) accumulation in green ripening stage A and slightly more capsaicin ($633.46 \pm 86.96 \text{ mg} \cdot \text{kg}^{-1}$) accumulation in the red ripening stage C. The peak capsaicin accumulation was at $846.64 \pm 157.48 \text{ mg} \cdot \text{kg}^{-1}$ at the colour transition stage C.

DISCUSSION

Fruits are a significant part of the dietary needs in humans due to their fiber, vitamins, minerals and flavour (Giovannoni, 2004). During their development and ripening, fruits undergo various changes associated with their nutrient composition, colour, aroma and even texture. These developmental changes occur due to various alterations associated with the biochemical and physiological processes that involve enzymatic activity, gene expression and formation of metabolites, in response to environmental perturbations (Osorio et al., 2012). Numerous scientific studies have aimed to investigate the developmental, maturation, ripening stages and organogenesis of fruits (Giovannoni, 2007). Herein, we applied the metabolomics approach in assessing the various cascade profile changes of *Capsicum chinense* Jacq. pepper fruit during its development stages. From our study findings, we established that variation in the pericarp colours and metabolite content were diverse and distinct for each developmental stage (16, 36 and 48 DPA).

During the three fruit developmental phases, the pericarp colours of the pepper fruits transformed as shown in Figure 1A–C. In pepper fruit development, carotenoids are known as the visual markers in maturation. This is attributed to the change in colour

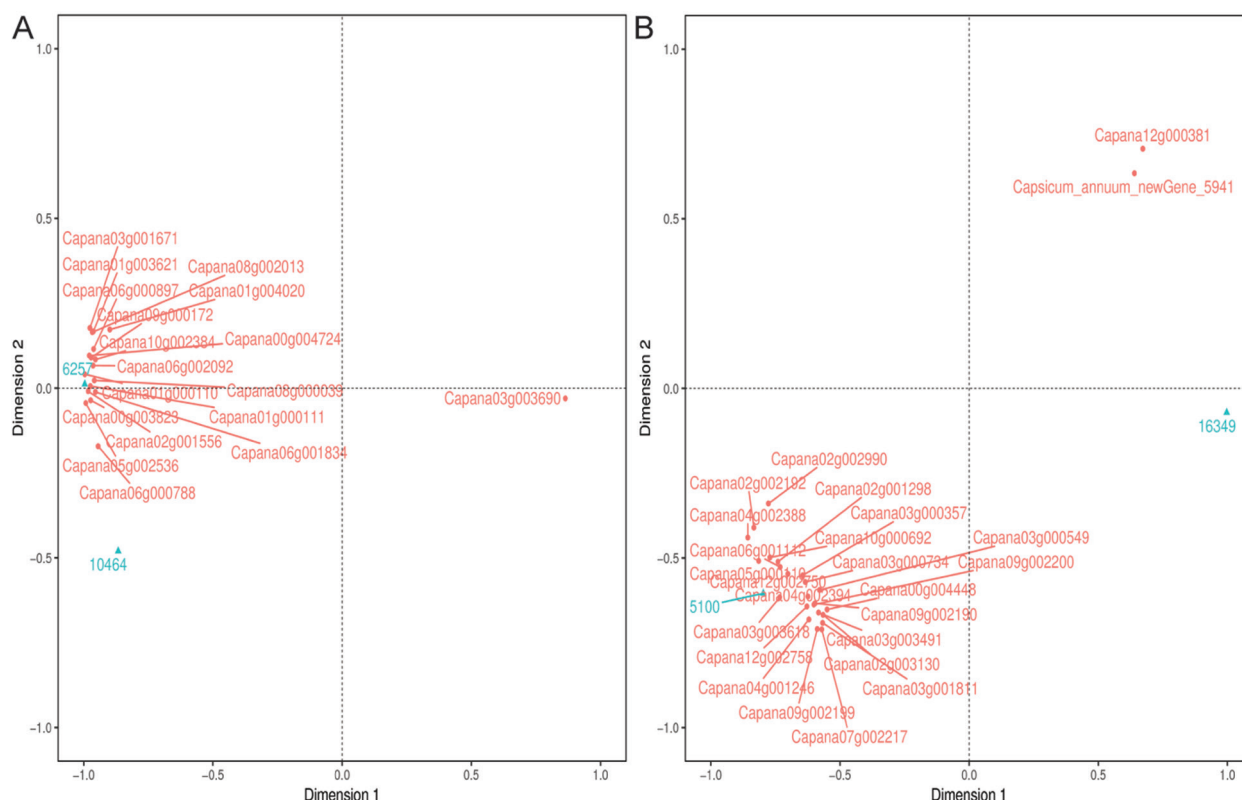


Figure 6. CCA analysis diagram of green ripening period (A) and colour conversion period (B). CCA, canonical correlation analysis.

of the green pepper fruit to yellow and finally red or yellow, depending on the carotenoids synthesized and accumulated by the fruits (Gómez-García et al., 2013). PCA established that the metabolites at 16 DPA were highly distinct, compared with the 36 and 48 DPA developmental phases. Equally, the metabolites in the last two developmental phases exhibited a high correlation between them (Figure 2). This phenomenon can be attributed to the diverse gene transcriptomic and metabolite variations of the fruit during its development.

During the early developmental stages (16 DPA), the levels of most organic acids, such as dicaffeoylputrescine and maltose, which were initially high, gradually decreased. Generally, organic acids are known to play a crucial role in contributing to the flavour, taste and quality of the fruits (Shin et al., 2015). However, organic acid constituents are diverse, depending on the species of the plant, its developmental stage and tissue type (López-Bucio et al., 2000). A major polyamine, putrescine, is directly produced from ornithine through the action of ornithine decarboxylase enzymes, and the ornithine levels expressed showed consistency with the metabolic levels of dicaffeoylputrescine. Our findings were in agreement with those of other researchers (Aizat et al., 2014), where their teams had studied putrescine compounds extensively, because it is linked to biotic and abiotic stressors, ethylene production, plant growth, flowering and fruit development (Malik and Singh,

2004; Choi et al., 2012). Most flavonoid compounds like apigenin 7-O-(6''-O-acetylglucoside) also showed high levels at 16 and 28 DPA.

During development, the colour of the pepper fruit generally changes from green to red, while an orange colour signifies the breaker stage or changing point (Sun et al., 2006). The amino acids, compounds that are naturally found in fruits and vegetables, play an important role in the maintenance of quality and nutritional value of the fruits (Glew et al., 2003). The precursor L-valine amino acid, found at one end of the capsaicin chain structure, through its pathway, plays an essential role in the biosynthesis of capsaicin (Keum et al., 2012).

At the later stages (48 DPA), the hydrophobic amino acids, L-leucine, L-phenylalanine L-aspartic acid and dihydrocapsaicin levels were higher significantly than at the earlier stages. The precursor of the phenylpropanoid biosynthetic pathway, L-phenylalanine, is associated with the production of secondary metabolites in plant species. In addition, PAL (CA09g02410) is a major enzyme during biosynthesis of phenolic compounds, which is derived by converting phenylalanine to trans-cinnamic acid in the initial stages of the phenylpropanoid pathway; it is also a precursor in both flavonoid pathways and biosynthesis of capsaicinoid (Sutoh et al., 2006).

During pepper fruit maturation, the contents of capsaicinoid compounds change with each developmental stage (Howard et al., 2000). Capsaicinoid

metabolites undergo biosynthesis through condensing of vanillylamine, a derivative of the phenylpropanoid pathway, and a series of branched-chain fatty acid moieties, which originated from the branched-chain fatty acid pathway (Zhang et al., 2016). The precursors of these aforementioned pathways include Phenylalanine, and valine or leucine. In the pepper fruit, capsaicinoids are biosynthesized and accumulate to the maximum value during the ripening period in the placental tissue. In the present study, the capsaicinoid levels gradually increased from 16 DPA up to 48 DPA, while the peak accumulation was observed at $846.64 \pm 157.48 \text{ mg} \cdot \text{g}^{-1}$ at the colour transition stage, which is developmental phase C. These findings are in agreement with a previously conducted study by Bae et al. (2014), which established an increase of capsaicin and dihydrocapsaicin levels significantly in Cayenne pepper developmental stages. Furthermore, an analogous reflection was observed in Shimmatarashi peppers, where there was an increase in the levels of capsaicin from $1071 \pm 16.7 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ during the immature stage, to $4363 \pm 23.1 \text{ } \mu\text{g} \cdot \text{g}^{-1}$ during the fruit maturation stage (Menichini et al., 2008).

In conclusion, we established that the metabolite distribution, expressed genes and activity of antioxidant were differential in the early stage (16 DPA), but more correlated in the breaker and later (28 and 48 DPA) stages. Thus, our findings submit that a suitable approach in interpreting biochemical variances is a non-targeted metabolomics in hot pepper developmental stages.

FUNDING

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AUTHOR CONTRIBUTIONS

Yaping Tang and Shengbao Yang conceived and designed the experiments. Yaping Tang and Guoru Zhang performed the experiments. Yaping Tang, Shengbao Yang, Tao Yang and Guoru Zhang analyzed the data. Patiguli Aisimutuola, Baïke Wang, Ning Li, Juan Wang and Qinghui Yu contributed reagents/materials/analysis tools. Yaping Tang, Guoru Zhang and Tao Yang wrote the paper.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES

AIZAT, W. M., DIAS, D. A., STANGOULIS, J. C. R., ABLE, J. A., ROESSNER, U. AND ABLE, A. J. (2014). Metabolomics of capsicum ripening reveals modification of the

ethylene related-pathway and carbon metabolism. *Postharvest Biology and Technology*, 89, 19–31, doi: 10.1016/j.postharvbio.2013.11.004.

ALVAREZ-PARRILLA, E., DE LA ROSA, L. A., AMAROWICZ, R. AND SHAHIDI, F. (2011). Antioxidant activity of fresh and processed Jalapeño and Serrano peppers. *Journal of Agricultural and Food Chemistry*, 59(1), 163–173, doi: 10.1021/jf103434u.

ANANDAKUMAR, P., KAMARAJ, S., JAGAN, S., RAMAKRISHNAN, G. AND DEVAKI, T. (2013). Capsaicin provokes apoptosis and restricts benzo(a)pyrene induced lung tumorigenesis in Swiss albino mice. *International Immunopharmacology*, 17(2), 254–259, doi: 10.1016/j.intimp.2013.05.015.

ANIEL, K. O., AND SUBBA, T. S. (2009). Ascorbic acid contents in chili peppers (*Capsicum* L.). *Notulae Scientia Biologicae*, 1, 50–56, doi: 10.15835/nsb113445.

BAE, H., JAYAPRAKASHA, G. K., CROSBY, K., YOO, K. S., LESKOVAR, D. I., JIFON, J. AND PATIL, B. S. (2014). Ascorbic acid, capsaicinoid, and flavonoid aglycone concentrations as a function of fruit maturity stage in greenhouse-grown peppers. *Journal of Food Composition and Analysis*, 33(2), 195–202, doi: 10.1016/j.jfca.2013.11.009.

CAREAGA, M., FERNÁNDEZ, E., DORANTES, L., MOTA, L., JARAMILLO, M. E. AND HERNANDEZ-SANCHEZ, H. (2003). Antibacterial activity of *Capsicum* extract against *Salmonella typhimurium* and *Pseudomonas aeruginosa* inoculated in raw beef meat. *International Journal of Food Microbiology*, 83(3), 331–335, doi: 10.1016/s0168-1605(02)00382-3.

CHOI, S. H. AHN, J. B., KIM, H. J., IM, N. K., KOZUKUE, N., LEVIN, C. E. AND FRIEDMAN, M. (2012). Changes in free amino acid, protein, and flavonoid content in jujube (*Ziziphus jujube*) fruit during eight stages of growth and antioxidative and cancer cell inhibitory effects by extracts. *Journal of Agricultural and Food Chemistry*, 60(41), 10245–10255, doi: 10.1021/jf302848u.

GHASEMNEZHAD, M., SHERAFATI, M. AND PAYVAST, G. A. (2012). Variation in phenolic compounds, ascorbic acid and antioxidant activity of five coloured bell pepper (*Capsicum annum*) fruits at two different harvest times. *Journal of Functional Foods*, 3(1), 44–49, doi: 10.1016/j.jff.2011.02.002.

GIOVANNONI, J. J. (2004). Genetic regulation of fruit development and ripening. *The Plant Cell*, 16, S170–S180, doi: 10.1105/tpc.019158.

GIOVANNONI, J. J. (2007). Fruit ripening mutants yield insights into ripening control. *Current Opinion in Plant Biology*, 10(3), 283–289, doi: 10.1016/j.pbi.2007.04.008.

GLEW, R. H., AYAZ, F. A., SANZ, C., VANDERJAGT, D. J., HUANG, H. S., CHUANG, L. T. AND STRNAD, M. (2003). Changes in sugars, organic acids and amino acids in medlar (*Mespilus germanica* L.) during fruit development and maturation. *Food*

- Chemistry*, 83(3), 363–369, doi: 10.1016/S0308-8146(03)00097-9.
- GÓMEZ-GARCÍA, M. D. R. AND OCHOA-ALEJO, N. (2013). Biochemistry and molecular biology of carotenoid biosynthesis in chili peppers (*Capsicum* spp.). *International Journal of Molecular Sciences*, 14(9), 19025–19053, doi: 10.3390/ijms140919025.
- HOWARD, L. R. AND WILDMAN, R. E. C. (2016). Antioxidant vitamin and phytochemical content of fresh and processed pepper fruit (*Capsicum annuum*). In R. E. C. Wildman, R. Wildman, and Wallace, T. C. (Eds), *Handbook of Nutraceuticals and Functional Foods*. Boca Raton, USA: CRC Press.
- HOWARD, L. R., TALCOTT, S. T., BRENES, C. H. AND VILLALON, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *Journal of Agricultural and Food Chemistry*, 48(5), 1713–1720, doi: 10.1021/jf990916t.
- HUANG, X. F., XUE, J. Y., JIANG, A. Q. AND ZHU, H. L. (2013). Capsaicin and its analogues: Structure-activity relationship study. *Current Medicinal Chemistry*, 20(21), 2661–2672, doi: 10.2174/0929867311320210004.
- JUNG, E. S., LEE, S., LIM, S. H., HA, S. H., LIU, K. H. AND LEE, C. H. (2013). Metabolite profiling of the short-term responses of rice leaves (*Oryza sativa* cv. ilmi) cultivated under different LED lights and its correlations with antioxidant activities. *Plant Science*, 210, 61–69, doi: 10.1016/j.plantsci.2013.05.004.
- KEUM, Y. S., PARK, H. W., SONG, H. H., KIM, B. D., KANG, B. C. AND KIM, J. H. (2012). Metabolite analysis of long chain branched fatty acids and capsaicin biosynthesis in capsicum annuum placenta. *Journal of Korean Society for Applied Biological Chemistry*, 55(2), 189–195, doi: 10.1007/s13765-012-1163-0.
- KIM, G. R., JUNG, E. S., LEE, S., LIM, S. H., HA, S. H. AND LEE, C. H. (2014). Combined mass spectrometry-based metabolite profiling of different pigmented rice (*Oryza sativa* L.) seeds and correlation with antioxidant activities. *Molecules*, 19(10), 15673–15686, doi: 10.3390/molecules191015673.
- LEE, S., JUNG, E. S., DO, S.-G., JUNG, G. Y., SONG, G., SONG, J. AND LEE, C. H. (2014a). Correlation between species-specific metabolite profiles and bioactivities of blueberries (*Vaccinium* spp.). *Journal of Agricultural and Food Chemistry*, 62(9), 2126–2133, doi: 10.1021/jf405272b.
- LEE, S., SUH, D. H., LEE, S., HEO, D. Y., KIM, Y. S., CHO, S. K. AND LEE, C. H. (2014b). Metabolite profiling of red and white pitayas (*Hylocereus polyrhizus* and *Hylocereus undatus*) for comparing betalain biosynthesis and antioxidant activity. *Journal of Agricultural and Food Chemistry*, 62(34), 8764–8771, doi: 10.1021/jf5020704.
- LÓPEZ-BUCIO, J., NIETO-JACOBO, M. F., RAMÍREZ-RODRÍGUEZ, V. AND HERRERA-ESTRELLA, L. (2000). Organic acid metabolism in plants: From adaptive physiology to transgenic varieties for cultivation in extreme soils. *Plant Science*, 160(1), 1–13, doi: 10.1016/S0168-9452(00)00347-2.
- MALIK, A. U. AND SINGH, Z. (2004). Endogenous free polyamines of mangos in relation to development and ripening. *Journal of American Society for Horticultural Science*, 129(3), 280–286, doi: 10.21273/JASHS.129.3.0280.
- MENICHINI, F., TUNDIS, R., BONESI, M., LOIZZO, M. R., CONFORTI, F., STATTI, G., DE CINDIO, B., HOUGHTON, P. J. AND MENICHINI, F. (2008). The influence of fruit ripening on the phytochemical content and biological activity of *Capsicum chinense* Jacq. cv Habanero. *Food Chemistry*, 114(2), 553–560, doi: 10.1016/j.foodchem.2008.09.086.
- OSORIO, S., ALBA, R., NIKOLOSKI, Z., KOICHEVENKO, A., FERNIE, A. R. AND GIOVANNONI, J. J. (2012). 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 Physiology*, 159(4), 1713–1729, doi: 10.1104/pp.112.199711.
- OYAGBEMI, A. A., SABA, A. B. AND AZEEZ, O. I. (2010). Capsaicin: A novel chemopreventive molecule and its underlying molecular mechanisms of action. *Indian Journal of Cancer*, 47(1), 53, doi: 10.4103/0019-509X.58860.
- PURKAYASTHA, J., ALAM, S. I., GOGOI, H. K., SINGH, L. AND VEER, V. (2012). Molecular characterization of ‘Bhut Jolokia’ the hottest chilli. *Journal of Biosciences*, 37(4), 757–768, doi: 10.1007/s12038-012-9249-8.
- SERRANO, M., ZAPATA, P. J., CASTILLO, S., GUILLÉN, F., MARTÍNEZ-ROMERO, D. AND VALERO, D. (2010). Antioxidant and nutritive constituents during sweet pepper development and ripening are enhanced by nitrophenolate treatments. *Food Chemistry*, 118(3), 497–503, doi: 10.1016/j.foodchem.2009.05.006.
- SHIN, G. R., LEE, S., LEE, S., DO, S. G., SHIN, E. AND LEE, C. H. (2015). Maturity stage-specific metabolite profiling of *Cudrania tricuspidata* and its correlation with antioxidant activity. *Industrial Crops and Products*, 70, 322–331, doi: 10.1016/j.indcrop.2015.01.048.
- SPILLER, F., ALVES, M. K., VIEIRA, S. M., CARVALHO, T. A., LEITE, C. E., LUNARDELLI, A., POLONI, J. A., CUNHA, F. Q. AND DE OLIVEIRA, J. R. (2008). Anti-inflammatory effects of red pepper (*Capsicum baccatum*) on carrageenan- and antigen-induced inflammation. *Journal of Pharmacy and Pharmacology*, 60(4), 473–478, doi: 10.1211/jpp.60.4.0010.
- STEWART, C., KANG, B. C., LIU, K., MAZOUREK, M., MOORE, S. L., YOO, E. Y., KIM, B. D., PARAN, I. AND JAHN, M. M. (2005). The Pun1 gene for pungency in pepper encodes a putative acyltransferase. *The Plant Journal*, 42(5), 675–688, doi: 10.1111/j.1365-313X.2005.02410.x.

- SUN, T., XU, Z., WU, C. T., JANES, M., PRINYAWIWATKUL, W. AND NO, H. K. (2006). Antioxidant activities of different colored sweet bell peppers (*Capsicum annuum* L.). *Journal of Food Science*, 72(2), doi: 10.1111/j.1750-3841.2006.00245.x.
- SUTOH, K., KOBATA, K., YAZAWA, S. AND WATANABE, T. (2006). Capsinoid is biosynthesized from phenylalanine and valine in a non-pungent pepper, *Capsicum annuum* L. cv. CH-19 Sweet. *Bioscience, Biotechnology, and Biochemistry*, 70(6), 1513–1516, doi: 10.1271/bbb.50665.
- TAN, C. K., ALI, Z. M. AND ZAINAL, Z. (2012). Changes in ethylene production, carbohydrase activity and antioxidant status in pepper fruits during ripening. *Scientia Horticulturae*, 142, 23–31, doi: 10.1016/j.scienta.2012.04.030.
- ZEWDIE, Y., AND BOSLAND, P. W. (2001). Capsaicinoid profiles are not good chemotaxonomic indicators for *Capsicum* species. *Biochemical Systematics and Ecology*, 29(2), 161–169, doi: 10.1016/S0305-1978(00)00041-7.
- ZHANG, Z. X., ZHAO, S. N., LIU, G. F., HUANG, Z. M., CAO, Z. M., CHENG, S. H. AND LIN, S. S. (2016). Discovery of putative capsaicin biosynthetic genes by RNA-Seq and digital gene expression analysis of pepper. *Scientific Reports*, 6, 34121, doi: 10.1038/srep34121.
- ZHU, G., WANG, S., HUANG, Z., ZHANG, S., LIAO, Q., ZHANG, C., LIN, T., QIN, M., PENG, M., YANG, C., CAO, X., HAN, X., WANG, X., VAN DER KNAAP, E., ZHANG, Z., CUI, X., KLEE, H., FERNIE, A. R., LUO, J. AND HUANG, S. (2018). Rewiring of the fruit metabolome in tomato breeding. *Cell*, 172(1–2), 249–261.e12, doi: 10.1016/j.cell.2017.12.019.

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