Comparison of the Metabolites and Carotenoids between a Graft Chimera ‘Hongrou Huyou’ (Citrus unshiu + C. changshan-huyou) and Two Donors

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Abstract
Background: Graft chimeras can be synthesized out, which is crucial to cultivar breeding. A new graft chimera named ‘Hongrou Huyou’, simply as OCC (the cell layers constitutions were ‘O’ for L1 and ‘C’ for L2 & L3, determined by ‘Owari’ satsuma mandarin and ‘Changshan Huyou’, respectively) was found at the junction where a scion C top-grafted onto a stock O, in an orchard in Changshan county, Quzhou city of Zhejiang province. This study investigated the primary metabolites, volatiles and carotenoids of OCC, aiming to figure out which substances are derived from layer source donors and which are newly generated by genetic regulation and interaction of two donors. Results: Based on statistical similarity analysis, the main results indicated that some substances in peels of OCC, such as 4-aminobutanoic acid, and palmitic acid were derived from C, while others in juice sacs, such as 4-aminobutanoic acid and palmitic acid were produced by O. Among those identical compounds observed in three cultivars, the concentrations of 13 and 7 compounds in OCC were significantly higher and lower than those in any of the donors, respectively. Especially, Germacrene D even exceeded 6-17 times than two donors in juice sacs. Interestingly, α-ylangene was herein exclusively observed in OCC and were rarely reported in any other citrus species, this was probably due to the interactions of cell layers from different genotypic donor parents and then lead to addictive effect in chimeras; the presence and absence of some profiles, such as the disappearance of α-carotene in C, can be used for genotype differentiation and citrus breeding assessment. Conclusion: Our results first systematically provide a chemical characterization of a citrus chimera, and found the genetic regularity of chemical substances and put forward some predictions on donor-controlled and autonomous metabolic patterns in plant chimeras. This work provide a theory guidance for synthetic chimera and diversity of certain chemical-preserved cultivars can try to be obtained in this way. Key words: Citrus, Periclinal chimera, Metabolites, Volatiles, Carotenoids

Background
Plant chimeras refer to those plants composed of more than two genotype cells. According to the theory of ‘Tunica-Corpus’, the shoot apical meristem (SAM) of dicotyledonous plants are composed of three cell layers, L1, L2 and L3, from the outermost layer [1]. For citrus fruits, the juice sacs and
epidermal pericarps are derived from cell layer L1; rind color and aroma, seeds and segment walls, are developed from L2; L3 produces vascular bundles, and fruit shape was determined by L2 and/or L3 [2]. Because of the characteristic cell constitutions in the meristem, plant chimeras are unique, and those valuable metabolites and carotenoids profiles controlled and modified by poly-genes in different cell layers definitely worth exploring.

It is possible that the chimera OCC arose because was excessively planted in 1980s, so that the selling period became too concentrated; thus, we speculated that farmers top-grafted C onto O to meet the market demand, but because of freezing injury, the scion (C) was died, an adventitious bud grew from the calli formed by scion (C) and stock (O). And then we called the fruit that branch bears ‘Hongrou Huyou’ (simply as OCC). OCC possessed the yellow-skinned and strong aroma traits like C, a dark orange juice sac and easy-peeling traits like O (Table 1, Figure 1), moreover, its juice sac also has the mixed flavour and texture from O and C. Up to now, there have been some reports on citrus chimeras, for example, Zhang and his colleagues investigated two citrus chimeras named ‘Zaohong’ navel orange and ‘Hongrou Taoye’ orange, both two chimeras are from the same donor plants sweet orange (C. sinensis) and satsuma mandarin (C. unshiu), and evaluated the morphology, cytology and molecular markers of two chimeras [3, 4]. However, through the observation, we found that citrus chimeras not only possess the independent characteristics from specific layer source donor, but also possess mixing characteristics from two donors in one cell layer. For example, in this work, the juice sac of OCC had the mixed flavour and texture of O and C, and even some new characteristics have been observed here. Therefore, with this as the starting point of the paper, we are trying to explain the heredity rules of primary metabolites, volatiles and carotenoids in OCC.

Citrus fruits are in great value of their nutrient components, such as metabolites and carotenoids. Primary metabolites are a diverse class of organic compounds which are essential for plant growth and internal quality [5]. Volatiles, as one of the most important secondary metabolites has received extensively attention, due to their massive healthiness and commodity value. Carotenoids are particularly rich in citrus fruits, and they used as nutraceuticals has considerably increased these years [6]. There have been many studies concerning the metabolites in oranges, mandarins,
pummelos and grapefruits [7-10]. For example, the highest levels of amino acids may be the part of reason that lemon possess a longer shelf life than other citrus [11]. d-limonene, as the dominant volatile in citrus, has special efficacy with clinicopathologic significance that contributes to breast and rectal cancer [12]; linalool and a relevant acetate, linalyl acetate, have been reported as anti-inflammatory agents [13]; and the rearrangements of Germacrene D can eventually form some natural compounds [14]. Some carotenoids containing β-ring moieties are precursors of vitamin A are highly beneficial to chronic disease and cancer prevention [15]. The carotenoids biosynthesis and their regulation in citrus fruits have been extensively reported [16-18], which were helpful for our analysis on carotenoids expression of citrus chimeras.

Therefore, it is very necessary to study how these valuable chemical substances distribute in the different tissues of citrus chimeras and how related-genes regulate profiles expressions. To date, our research group has demonstrated that OCC is a periclinal chimera by SSR (simple sequence repeat) analysis. Polyacrylamide gel electrophoresis showed that OCC possessed the specific DNA bands from two donors in nuclear, chloroplast and mitochondria genome, respectively, and we confirmed that L1 of OCC was derived from O and L2 & L3 were from C (Zhang M, unpublished results). Combining the existing results with our observations that although OCC possessed the yellow peel like C and dark orange juice sac like O, its juice sac has the mixed flavour and texture from O and C, it has become our great interest to investigate the inheritance pattern and genetic regulations of primary metabolites, volatiles and carotenoids in the different tissues of OCC, and provide a theoretical basis for the donor selection in synthetic chimeras.

Methods

**Plant Materials**

The chimera OCC was discovered in an orchard in Changshan county of Zhejiang province during a bud mutation investigation in 2001. Under regular management, three cultivars were separately grafted onto the *Poncirus trifoliata* in 2005, and remained stable for 12 years. Three cultivars were grown on different trees, every 3 trees were set as one cultivar group, that is 9 trees in total. Every 10 ripe fruits were picked from each tree at the optimal maturity, with three replications was used, for
a total of 90 fruits.

The picking standard was based on the uniform size, skin colour and tree location. Harvest fruits were picked and washed with tap water. Peels (including epidermis, flavedo and albedo) were cut carefully along the longitudinal axis by girdling; the fruit tissues (peel and juice sac) were separated quickly. Then the peels and juice sacs of every 10 fruits from one tree were separately blended and ground into powder in liquid nitrogen, respectively. Finally, the samples were preserved at -80°C for later research.

**Primary metabolites and volatiles extraction**

The primary and volatile substances were determined using a modification of the procedure originally developed [38]. To determine the primary contents, we first ground 0.2 g tissue into powder using liquid nitrogen, and then added 2.7 ml of pure, precooled (-20°C) pure methanol. These components were mixed and 0.3 ml ribitol (0.2 g/ml) was added as the internal standards. The procedure was later applied to the volatiles samples.

For volatiles, samples were freeze-dried with a vacuum freeze-drier (Labconco FreeZoneR, USA) and fully ground in liquid nitrogen. A 0.2 g sample of powder was poured into the centrifuge tube (2 ml volume), which was homogenized with 500 μl double distilled water (DDW) and 500 μl MTBE (containing 0.02 μl/ml methyl pelargonate), followed by gentle shaking. Samples were vibrated using an ultrasonic bath (model FS60, Fisher Scientific, Pittsburgh, PA) maintained at 4°C for 40 min and were centrifuged at 12000 g for 10 min at 4°C. The 200 μl supernatants were then transferred into another tube. Finally, 1 μl sample was injected with a syringe and filtered through a 0.22 μm membrane (SCAA-104, ANPEL, Shanghai, China) for Gas Chromatography-Mass Spectrometry (GS-MS).

**Primary metabolites and volatiles analysis**

The compounds were determined by use of TRACE GC Ultra GC coupled with a DSQ II mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) with a TRACE TR-5 MS column (30 m ×
0.25 mm × 0.25µm; Thermo Scientific, Bellefonte, PA, USA). With pure helium as a carrier gas, the peels (flavedo and albedo) and juice sacs of samples were identified at 1.0 ml/min with a split ratio of 50:1 and 1:1, respectively. The concentrations of the primary and volatile substances were calculated in µg/g FW. Three replications were used for each sample.

The public database Massbank (http://www.massbank.jp/) and Metlin (https://metlin.scripps.edu/index.pCF) supported to identify tentative metabolite substances; for some other compounds, we obtained information from the published literatures.

**Carotenoids extraction**

The total carotenoids of OCC and its donor parents were extracted according to a previously described method [40] with some modification. Juice sac powder (1 g) and peel powder (0.5-1g) were homogenized in a 50 ml centrifuge tube after lyophilization using a lyophilizer (LABCONCO FreeZone®). Next, 15 ml pigment extraction solvent (n-hexane/acetone/anhydrous ethanol, 2:1:1, v/v/v, containing 0.1‰ BHT) was added. Samples subjected to ultrasonic vibration for 30 min and centrifuged for 10 min at 4000 g at 4°C. Supernatants were transferred to another 50 ml centrifuge tube, and sediment was extracted using 15 ml pigment solvent until it was colourless. Supernatants were combined in 50 ml separating funnel and washed 3 times using a saturated 10% of NaCl solution until neutral, and the underlayer was discarded. Supernatants were separated into a 10 ml centrifuge tube and then concentrated under the vacuum condition. The samples were redissolved with 2 ml of methyl tert-butyl ether (MTBE) and 2 ml of 10% KOH (containing 0.1‰ BHT), and the residue was dried under nitrogen. Samples were kept in the dark for 10 hours of saponification. Then, 4 ml saturated NaCl and 2 ml MTBE (containing 0.1‰ BHT) were added to better separate layers and wash away the water, and then 5 ml NaCl was added 3 times to wash the solution to neutral. Meanwhile, the supernatant was concentrated by the vacuum concentration and was diluted with 0.6–1 ml MTBE (containing 0.1‰ BHT). The samples were centrifuged at 12000 rpm for 30 min at 4 °C for later determination.
Carotenoids analysis

A gradient elution method of OCPLC, composed of A (acetonitrile/methanol, 3:1, v/v, containing 0.1‰ BHT, 0.05% TEA) and B (100% MTBE, containing 0.1‰ BHT) as the mobile phase, was used to determine the carotenoid contents. The flow rate was fixed at 1 ml/min. The gradients were used as followed, 0 min: (95: 5); 0–10 min: A-B (95: 5); 10–19 min: A-B (86: 14); 19–29 min: A-B (75: 25); 29–54 min: A-B (50: 50); 54–66 min: A-B (26: 74); 67 min: A-B (95: 5). The volume of the above gradient solvent was 20 µl and the test adopted an external standard method for quantitation. All carotenoids extraction, saponification and other determinations identified above were conducted under low light levels or in the dark.

Statistical analysis

The concentration of each chemical compound was expressed as the means ± standard deviation of three replicates. A statistical analysis was performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Significant differences were calculated using one-way analysis of variance (ANOVA) followed by Duncan’s multiple-range test at the 5% level (p < 0.05).

Results

Primary metabolites in OCC and its donors

As shown in Table 2, 21 primary metabolites were identified in peels. Based on the significant analysis of statistics, OCP shared more similarities with CP in these profiles. Among them, 4 acids, namely: 4-aminobutanoic acid, shikimic acid, palmitic acid and L-serine were exclusively detected in OCP and CP, suggesting that these 4 compounds of OCP were only produced by CP. In the contrary, sorbose was specific to OP, with no detectable evidence in OCP and CP. Furthermore, in all of the acids (except 2-ketoglutaric acid) and alcohols, OCP and CP presented higher values than those in OP; but in sugars (except fucose), OCP and OP presented significantly lower values than those in CP. Notably, some particular chemical characteristics were observed in OCP. Among those identical profiles detected in three samples, carbamic acid and fucose exhibited the highest level in OCP; but in
total metabolites, OCP showed a significantly lower value than that in any of the donors.

For the juice sacs, Table 3 listed 18 primary substances among OJ, OCJ and CJ. In the present study, there was no significant difference being observed between OCJ and two donors in total primary metabolites, acids and sugars. But OCJ still showed some genetic similarities with OJ. On the one hand, 4-aminobutanoic acid, palmitic acid and allose were common to OCJ and OJ, but were not presented in CJ. That is to say, these three profiles in OCJ were completely derived from OJ. Conversely, sorbose was only specifically existed in CJ, indicating that OCJ and OJ may lack a common biosynthesis gene of sorbose. On the other hand, in terms of the similar dark orange color and sweetness (Figure 1, Table 3), OCJ phenotypically inclined to OJ.

Interestingly, there were also some hereditary differences appeared in OCJ. Two compounds (oxalic acid and rhamnose), were only undetectable in OCJ, which caused the obvious discrepancies between OCJ and its donors. For another, in both citric acid and arabinose, OCJ had the largest amount among three samples, and were significantly higher than that in two donor parents.

**Volatile compositions between OCC and its donors**

With regards to the volatiles in the peels of three cultivars, Table 4 listed 36 substances, including monoterpenes, sesquiterpenes, alcohols, aldehydes, phenol and others. The monoterpenes were the most abundant of profiles quantified, with d-limonene as the dominant compound, accounting for 88.65%, 81.23% and 80.77% of the total volatiles in OP, OCP and CP, respectively. After d-limonene, followed by γ-Terpinene, β-Myrcene and α-Pinene, they were the main compounds for three samples had in common.

The result showed that OCP had a stronger correlation with CP than with OP. Firstly, according to the significance analysis, 14 volatiles had no significant difference between OCP and CP, but only 3 volatiles between that of OCP and OP. This indicated, CP had the dominant position in the regulation of chemical profiles in OCP and more chemical traits in OCP were inherited from CP. Secondly, the main volatiles of OCP were completely consistent with that of CP, including d-limonene, γ-terpinene, Germacrene D, β-myrcene and α-pinene (sort from high to low concentration), but the main volatiles
order in OP were divergent (d-limonene, γ-terpinene, β-myrcene, α-pinene, β-elemene). This was mainly because the Germacrene D was significantly higher in OCP and CP than OP, and so strongly suggest that Germacrene D was mainly originated from CP and OP had less impact on the development of OCP. Thirdly, it is worth noting that 2,4-di-t-butylphenol was really unique, which was only detected in OP, and this is the only one volatile that OCC and C exclusively possessed in common.

In addition, in OCP, most of volatiles concentrations were either inclined to specific donors, OP or OCP, or stay intermediate between them. However, only (E)-3-Hexen-1-ol and 3-Hexenal, they were significantly higher in OCP than both of two donors.

For the edible juice sacs, the volatiles containing up to 19 constituents (Table 5). OCJ was highly correlated with OJ in total volatiles and monoterpenes (the leading volatiles). Especially in dominant substances, d-limonene, its concentrations of OJ and OCJ were significantly higher than CJ, occupied 78.07%, 72.64% of the total volatiles in OJ and OCJ, respectively, but only 60.03% in that of CJ.

Meanwhile, besides d-limonene, there were also significant similarities between OJ and OCJ in methyl nonanoate, copaene and octanal, and we considered that all these compounds in OCJ were originated from O to a great extent.

In addition, typical volatiles quantitative inheritance traits were observed in OCJ. For example, nootkatone and pentadecanal presented the largest amount in OCJ, those were significantly higher than that in both of donors. Instead, γ-terpinene in OCJ was significantly lower than that in any of the donors. Furthermore, what we were particularly interested in was α-ylangene, which was only detected in OCJ but not in two donors, and this volatile was hardly ever been reported in any citrus species.

**Carotenoid constituents between OCC and its donors**

A total of 9 carotenoids were detected (Table 6). Violaxanthin and β-cryptoxanthin were the dominant profiles in peels and juice sacs, respectively. In peels, the total amount of carotenoids in OCP was significantly close to CP, but in juice sacs, the total carotenoids were significantly different among all
three samples. In terms of the main carotenoids (β-cryptoxanthin, phytoene and phytofluene), they were varied in three cultivars, which were significantly higher in O than in the other two cultivars, in both of peels and juice sacs. It is worth noting that violaxanthin dominated in peels, but stayed at particularly low level in juice sacs, therefore it was not the main carotenoids in both peels and juice sacs.

Although there were some discrepancies existed, the main results still showed close heredity relationships between OCP and CP. At first, OCP had no significant difference with CP in most of carotenoids, the most representative example was α-carotene, which was commonly undetectable in OCP and CP. But when it comes to the juice sacs, α-carotene was commonly appeared in OJ and OCJ, with no sign in CJ. Similarly, phytoene was a missing compound in CJ but were relatively high in OJ and OCJ. Furthermore, all of the carotenoids herein detected in OJ and OCJ were particularly higher than those in CJ. That means, the contents of carotenoids were the lowest in C, but the highest in O. OCC generally stayed at a lower level but inclined to C in peels, and stayed between two donors but inclined to O in juice sacs.

Discussion
As regards plant chimeras, some researchers have studied their phenotypes, fruit qualities and genome compositions [2-4], but they rarely studied the genetic regularity and variant expressions of biochemical substances. In this work, we firstly systematically compared the primary metabolites, volatiles and carotenoids between a novel citrus chimera OCC and its donor plants, identifying that the genetic contributions and inheritance patterns from two donors are not equal in plant chimeras. Some substances content herein detected in OCC were inclined to specific layer source donors; some were intermediate between two donors; but some were deviated from both of two donors, presenting the significantly higher or lower level than that of any of the donor plants. Besides, there were some characteristic substances were exclusively presented in OCC or in two donor plants.

**Metabolic Interrelationships between OCC and its layer source donor**

In citrus chimeras, the juice sacs were developed from L1 cell layer and the peels were from L2 cell
layers, the expression of genetic substances in different tissues of OCC showed transgressive (i.e., not intermediate) with its layer source donors. According to the significant difference analysis, some compounds, such as myo-Inositol (Table 3) and d-limonene (Table 5), in OCJ and OJ had no significant difference but were commonly significantly higher than that in CJ; by contrast, some compounds, such as quinic acid (Table 2) and Germacrene D (Table 4), had no significant difference in OCP and CP but significantly higher than that in OP. The results indicated that there were a number of specific chemical-related genes in cell layer L1 (mainly controlled juice sacs) of OCJ were inherited from O, and others in L2 (mainly controlled peels) were inherited from C. This observation was consistent with the study that the juice sacs of a citrus periclinal chimera Ekuliku, obtained by grafting, were developed from L1 donor Nankan (C. unshiu), while the epicarp and mesocarp were both developed from L2&L3 donor Hamlin (C. sinensis) [19]. Moreover, our results also attested to the genetic regularity previously described in the leaf morphology variation of Brassica chimeras, that the variation was only controlled by the cells of the donor red cabbage and was reproducible and directional in progenies [20]. Furthermore, in the present study, we also found that the contents of the same chemical substances in different tissues of OCC were seperately apt to donor O or donor C. For example, in peels (Table 2), 4-aminobutanoic acid and palmitic acid were only occurred in OCP and CP, but in juice sacs (Table 3), 4-aminobutanoic acid and palmitic acid were only occurred in OCJ and OJ. That is to say, the appearance of 4-aminobutanoic acid and palmitic acid in the peels and juice sacs of the chimera OCC were largely depended on each layer-determined donor C and O, respectively. Similar study also noted that among the 8 high reproducibility primers, STS primers and OPH20 primers amplified 600 bp bands only appeared in chimera Ekuliku and donor Nankan, but not in another donor Hamlin, that is, these two primers were specific to Ekuliku and Nankan [19]. This specific inheritance pattern in chimera was proved at the metabolic level in this research. Therefore, the part of substances herein investigated were genotype-specific and largely controlled by independent layer source donor.

**Significant deviations in metabolites between OCC and both donors**
However, the expression quantities of a number of chemicals (including primary metabolites and volatiles) in OCC were not “loyalty” to layer source donor, but deviated far from both two donors (i.e., significantly higher or lower than donors). Among them, 13 compounds and 7 compounds in OCC showed significantly higher and significantly lower level than those in any of the donors, respectively. This observation was similar to two citrus hybrids, that 56 of the 113 volatile profiles in the hybrids were significantly higher or lower than in parents [21]. Looking back to this study, for instance, Germacone D (Table 5) in OCJ was 6-17 times higher than that in CJ and OJ, respectively. The quantities of arabinose was over 3-9 times higher than in CJ and OJ (Table 3), and this profile have been reported as a good source of dietary fiber and could be available for juice production [22]. And as reported, scyllo-Inositol (Table 2, 3) could be responsible for the tolerance of citrus destructive disease HLB [23], even though its concentration was not the highest in OCC, it still had a relatively higher level. For these substances with a significantly higher or lower content than two donors, we predicted it was possible related to gene expression that regulates the metabolic status of those substances. In citrus, CsMYBF1 is an important transcription factor of R2R3-MYB, the expression pattern of CsMYBF1 in the citrus fruit may be related to the content of metabolites. In recent study through isolation of R2R3-MYB transcription factor CsMYBF1 from citrus, and analysed CsMYBF1 overexpression lines in transgenic tomato and the RNAi (RNA interference) lines in citrus, it was found that CsMYBF1 induced an up-regulation of the primary metabolites and phenylpropanoic acid pathway in the tomato, on the contrary, the RNAi of CsMYBF1 in the citrus callus resulted in down-regulation of many of the phenylpropanoic acid pathway genes and reduced the content of hydroxycinnamic acid and flavonol [24]. Accordingly, we speculated that because of the interaction between genetic materials from different genetic backgrounds in chimeras, the expression of these deviated chemical-related genes may be overexpressed, and some may be surpressed. Therefore, these genes could have been expressed normally in a single chimeric parent, O or C, but when these genes are simultaneously went into the chimera OCC, they would co-expressed and interacted in the same cell layer of OCC, at which time the role of relevant genes will be strengthened, and of course, the role of them may also be diminished, so that the substance content would show significantly higher or lower
in OCC than that of two O and C. In terms of this kind of peculiar expression of genes in chimera, Fernandez and his colleagues [25] investigated the weight reduction in the berry of a grape chimera which was caused by unusual Vvpl gene expression in L1, L2 or in both cell layers, which lead to phenotypic variations (fleshless) in progeny. This study can also prove that the overexpression or surpression of the gene expressions may exist in cell layers of OCC, resulting in the significantly different content of the relevant metabolic substance from O and C.

**Characteristic metabolite in OCC**

The interaction between tissues with different backgrounds in chimeras may be a new source of genetic variation has been previously recognized [26-27]. In this study, a novel substances α-ylangene was only exclusively detected in the chimera OCC. α-ylangene was a unique compound, which was hardly ever been reported in any citrus volatiles, but was a main sesquiterpenoid at the post-maturation stage in grapes [28]. Zhou and her colleagues investigated two citrus chimeras (L1-L2-L3=N-F-F and F-N-N; N represents ‘Natsudaidai’, represents ‘Fukuhara’), they did found some variations that the chimeras NFF and FNN not only had the specific bands of two donor plants, but also had their own unique new bands by RAPD analysis [2]. It is suggested that the chimeras interacted at DNA level. Therefore, at metabolic level, α-ylangene was never detected in two donors but presented in the chimera OCC could be another evidence for the genetic variation during the chimeras’ development, and intercellular movements may be the reason for it.

Furthermore, the finding of this variation raised the question of what circumstances happened during so-called interactions. By using artificial plant chimeras, it has been found that transcription factors can move from one cell to another in a plant, and maintain biological activity [29, 30]. For example, the floral transcription factors LFY and AP1 have been demonstrated can participate in cell-to-cell signal transmission between and within different layers of the meristem. These two transcription factors could activate homeotic genes and then led to phenotypic variation in flower structure [30]. In recent years, the heritable variations caused by gene mutations and intercellular trafficking were extensively studied in chimeras. A grape periclinal chimera ‘Malian’, whose flesh is bronze, a
spontaneous mutation appeared in L2 cell layer, and thereafter this mutant invaded L1 cell layer to give rise to a new phenotype, white flesh [31]. And there were many studies have been reported that berry color variants in grape Pinot can be mapped back to the mutation on a single locus named “berry color locus”, which is consist of four tandem MYB transcription factors on chromosome 2 [32–34]. Meanwhile, a peach mutant, where the mutation carried a PRUPE.6G281100 allele entered L2 cell layer, then led to a phenotype change of peach from flat to round [35]. In this study, according to previous studies on the metabolic pathway of α-ylangene[36], it is possible that due to the interaction between genetic materials from different backgrounds, which led to an up-regulation of a series of genes involved in sesquiterpene metabolism and the α-ylangene pathway, and induced a strong accumulation of α-ylangene, or it may be due to the lack of a series of genes consuming α-ylangene accumulation.

**The presence and absence of some typical metabolites**

In the context of this study, notably, the presence and absence of some typical chemical substances were also observed in OCC and two donor plants. In carotenoids, for example, α-carotene was commonly undetectable in OCP and CP, but specific to OJ and OCJ. And phytoene was only exclusively undetectable in CJ. A related study also in agreement with that α-carotene was only detected in the juice sac of ‘Rio Red’ among six grapefruits [37]. 2,4-di-t-butylphenol, an phenol which uncommonly appeared in many citrus species, was herein detected in OCP and CP, and this profile was only reported in harvested ‘Huanong’ red pomelo [38]. Observations here also consistent with the study that two DNA bands of 930bp and 1,500 bp were specifically presented in chimeric donor N but not in donor F, and were hereditarily stable in two citrus chimeras NFF and FNN [2]. In this work, it is possible that the gene for the synthesis of α-carotene in the chimera OCC peel was completely dependent on the L2 source donor C. Even if the other donor O has this gene for synthesizing the α-carotene, the OCC cannot inherited from it or the relevant gene had successfully passed to OCC, but it just expressed in other tissues instead of peels, so that the substance cannot be synthesized in OCP. On the contrary, in juice sacs, α-carotene appeared in OCJ, this may because OCJ independently
inherited the relevant gene from L1 source donor O. This independent inheritance was agreement
with a citrus chimera Ekuliku, by comparing the certain quantitative traits with its donor plants, the
color of the skin and flesh of the chimera was more independent because of its “loyalty” to the traits
of their layer source donors [19]. These observations were consistent with the conclusion that juice
sacs originate from L1 and peels originate from L2 [1–4, 19]. Additionally, in response to the absence
and presence of these typical chemicals, such as α-carotene, it is suggested that which can be used
as a characteristic pigment and a biochemical marker for genotype differentiation and assessment of
citrus breeding programs.

Speculation of genetic laws in chimeras’ metabolites
Up to now, there is limited knowledge available with regards to the inheritance regularity of chemical
compounds in plant chimeras. While small RNAs expressions and DNA methylation have recently been
considered to be involved in stock-scion to describe genetic variations in graft chimeras. For instance,
researchers have found some conserved miRNAs were differentially expressed in graft chimera
(Brassica juncea + B. oleracea) progeny rTTT (sexual self-crossing of the chimera) and donor plant
TTT (B. juncea), which may contributed to the changes in expression of their target genes [20]. In this
work, the content of some chemicals in OCC were between two donors but some were deviated far
from both of donor parents. These phenomenons might be related to the gene expressions of these
chemicals. Then, we are wondering whether the genes from O and C are co-expressed in OCC, or only
expressed one gene from one donor. Furthermore, in the graft chimeras Brassica juncea and
B.oleracea, sequence analysis revealed that DNA methylation will affect the flowering time-and
gibberellin response-related genes expressions, and may lead to the phenotypic variations in
progenies [39]. According to the studies discussed above, chemical substances variations herein
observed in OCC also suggested that whether the gene sequences of same gene in two donors were
consistent, if not, will DNA methylation exist to influence chemical-related gene expression in OCC,
and how these genes coordinate to regulate substances quantities are valuable to study more.
Conclusions
The genetic pattern and pathway of metabolites and carotenoids in plant chimeras is a complicated phenomenon, yet to be fully understood. In order to investigate the genetic regularity of biochemical substances in plant chimeras, we systematically compared the metabolites and carotenoids between a novel citrus chimera OCC and its donor plants.

Firstly, obvious inheritance correlation was noted between OCC and two donors, indicating by significant similarities in peels and juice sacs. The results identified a certain number of compounds, which were only existed in specific tissue of OCC and a donor, suggesting that those compounds were independently controlled by a single donor. Secondly, some profiles herein detected in OCC were intermediate between donors, suggesting that the expression levels of chemical-related genes in OCC were commonly regulate by two donors. For instance, it was speculated that a highly-expressed gene in one donor could drive the same gene that was expressed lower in another donor to work together in OCC to regulate the substances expression quantity in the middle. Thirdly, some specifically expressed substances were obtained in OCC, including 13 compounds and 7 compounds, those were significantly higher and lower than that in any of the donors, respectively. Interestingly, a novel profile (α-ylangene) was only exclusively observed in OCC. These conditions may because the interactions between different genotypic donors activate the movements of some transcription factors and then changed the gene expression, finally a higher or lower accumulation of relevant substances appeared in a specific cell layer of OCC. Notably, our results also found some profiles, those were present or absent in specific cultivars’ tissues. These profiles can well be used as chemical markers for genotype differentiation and citrus breeding assessment, more importantly, were helpful for donor selection during chimera synthesis.

Abbreviations

OP: the peel of ‘Owari’ satsuma mandarin; OCP: the peel of OCC; CP: the peel of ‘Changshan Huyou’;

OJ: the juice sac of ‘Owari’ satsuma mandarin; OCJ: the juice sac of OCC; CJ: the juice sac of ‘Changshan Huyou’

Declarations

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**Availability of data and materials**

All data generated and analysed in this study is presented in this published article.

**Authors’ contributions**

MZ and CZ contributed to the experimental design. LJ contributed to the data analysis and wrote the manuscript. QW provided experimental materials. KZ performed the experiments. FK and JX provided financial support. SZ and GW contributed to experimental material planting management. All authors read and approved the final manuscript.

**Ethics approval and consent to participate**

Not applicable.

**Consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.
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Tables

Table 1

| No. | Cultivars                          | Scientific name                        | Abbreviation | Peel color |
|-----|------------------------------------|----------------------------------------|--------------|------------|
| 1   | 'Owari' satsuma mandarin           | C. unshiu                              | O            | Orange     |
| 2   | ‘Hongrou Huyou’                    | C. unshiu + C. changshan-huyou         | OCC          | Yellow     |
| 3   | ‘Changshan Huyou’                  | C. changshan-huyou                     | C            | Yellow     |

a,b Peel color and juice sac color were taken in fully mature period (collection period December 2017), see Figure 1 for details

Table 2

Primary metabolite profiles (μg g⁻¹ FW) in the peel of OCC and its donor plants

22
| No. | Primary content (μg g⁻¹) | OP       | OCP       | CP       |
|-----|--------------------------|----------|-----------|----------|
|     |                          |          |           |          |
| 1   | Carbamic acid            | 11.32±2.40^c   | 97.79±16.79^a | 73.42±(  |
| 2   | Cyclohexaneacetic acid   | 8.23±0.45c^b   | 205.82±14.44a| 173.78±  |
| 3   | Malic acid               | 0.56±0.09c     | 2.28±0.41b   | 3.35±0.  |
| 4   | Quinic acid              | 92.87±9.42c    | 211.84±8.73b | 280.92±  |
| 5   | 2-Ketoglutaric acid      | 273.14±11.78b  | 341.09±25.51a| 354.57±  |
| 6   | 4-Aminobutanoic acid     | 64.77±6.22a    | 35.08±2.73b  | 41.24±   |
| 7   | Shikimic acid            | 4.29±3.71a    |           | 24.26±   |
| 8   | Palmitic acid            | 11.21±1.44a    |           | 11.16±   |
| 9   | L-Serine                 | nd^c           |           |          |
|     | Sum                      | 439.57±18.76b  | 878.94±28.67a| 921.27±  |
|     |                          |           |           |          |
|     | Sugars                   |           |           |          |
| 10  | Xylose                   | 215.09±3.73a  | 79.35±6.14c | 112.00±  |
| 11  | Mannose                  | 24997.79±1538.92a| 11139.04±654.61c| 14008.2 |
| 12  | Galactose                | 6961.91±512.69a| 2910.33±191.21c| 3885.98 |
| 13  | Fucose                   | 5.83±0.60c    | 12.82±1.55a | 9.50±0   |
| 14  | Fructose                 | 20950.50±1276.05a| 9533.23±501.14b| 10982.4 |
| 15  | d-Psicose                | 246.44±11.15a | 47.54±9.82c | 88.83±   |
| 16  | Turanose                 | 91.76±9.73a   | 25.64±2.07b | 33.00±   |
| 17  | Sucrose                  | 16576.74±471.54a| 7352.21±162.93c| 8777.85 |
| 18  | Myo-Inositol             | 1102.16±76.96a| 1035.31±59.25ab| 965.58± |
| 19  | Sorbose                  | 160.32±4.82a  | nd         | nd       |
|     | Sum                      | 71308.53±3772.64a| 32135.47±1437.52b| 38863.3 |
|     | Alcohols                 |           |           |          |
| 20  | Glycerol                 | 125.80±13.79c | 247.62±32.8b | 303.96±  |
| 21  | Scyollo-Inositol         | 54.75±4.92c   | 169.21±4.08b| 291.89±  |
|     | Sum                      | 180.56±18.63c | 416.84±29.40b| 595.85±  |
|     | Total                    | 71928.66±3809.02a| 33431.25±1454.01c| 40380.4 |

^a Data expressed as means±standard deviation of triplicate samples’ fresh weight in their maturity

^b Different lowercase within a line represent significant differences between cultivars (p<0.05)

^c nd, the substance was not detectable

**Table 3**
Primary metabolite profiles (μg g\(^{-1}\) FW) in the juice sac of OCC and its donor plants\(^a\)

| No. | Primary content (μg g\(^{-1}\)) | OJ       | OCJ      | CJ       |
|-----|---------------------------------|----------|----------|----------|
|     |                                 | OJ       | OCJ      | CJ       |
|     |                                 | 134.46±111.75\(^c\) | 352.70±31.67\(^b\) | 569.63±19.16\(^a\) |
| 1   | Oxalic acid                     | 22.67±0.90\(^a\) | nd\(^c\) | 19.16±19.16\(^a\) |
| 2   | Malic acid                      | 206.33±21.44\(^b\) | 172.40±8.68\(^b\) | 363.95±19.16\(^a\) |
| 3   | 4-Aminobutanoic acid            | 10.36±1.00b | 16.01±4.16a | nd        |
| 4   | Citric acid                     | 1131.33±9.58ab | 1213.74±59.98a | 965.81±49.98ab |
| 5   | Quinic acid                     | 37.49±4.79b | 22.24±1.77c | 50.68±4.79b |
| 6   | Palmitic acid                   | 38.62±2.57a | 20.23±0.16b | nd        |
|     | Sum                             | 1446.81±18.13a | 1444.61±48.07a | 1399.60±22.71a |
|     |                                 | OJ       | OCJ      | CJ       |
|     |                                 | 17227.71±687.96a | 17073.88±1494.37a | 15731.5±49.98ab |
| 7   | Xylose                          | 57.18±0.58a | 16.76±1.28b | 4.47±0.47 |
| 8   | Arabinose                       | 5.39±0.10b | 15.11±0.81a | 2.36±0.36 |
| 9   | Fructose                        | 14259.18±237.19a | 12389.95±1357.50b | 11731.4±237.19a |
| 10  | Mannose                         | 155.25±17.50a | 139.88±11.24a | 162.82±17.50a |
| 11  | Sorbose                         | nd       | nd       | 54.88±5.48 |
| 12  | Glucose 2,3,4,5,6-pentakis-O-(trimethylsilyl)-, o-methyloxyme, (1Z)- | 17227.71±687.96a | 17073.88±1494.37a | 15731.5±49.98ab |
| 13  | Rhamnose                        | 17.51±1.60a | nd       | 10.55±1.60 |
| 14  | Myo-Inositol                    | 1508.79±45.70a | 1503.50±31.59a | 240.54±45.70a |
| 15  | Allose                          | 2.69±0.26a | 2.10±0.28b | nd        |
| 16  | Sucrose                         | 21362.29±1880.50a | 21537.81±1705.67a | 22017.8±1880.50a |
| 17  | Turanose                        | 223.49±34.64a | 160.54±14.71b | 17.18±34.64a |
| 18  | Sum                             | 54819.49±2889.60a | 52839.53±3493.60a | 49973.6±2889.60a |
|     |                                 | OJ       | OCJ      | CJ       |
|     |                                 | 56330.92±2910.91a | 54400.95±3463.03a | 51564.1±2910.91a |

\(^a\) Data expressed as means±standard deviation of triplicate samples’ fresh weight in their maturity

\(^b\) Different lowercase within a line represent significant differences between cultivars (p<0.05)

\(^c\) nd, the substance was not detectable

**Table 4**
Volatiles profiles (µg g⁻¹ FW) in the peel of OCC and its donor plants

| No. | Volatiles (µg g⁻¹) | OP       | OCP       | Cl |
|-----|--------------------|----------|-----------|----|
|     |                    |          |           |    |
|     | Monoterpene        |          |           |    |
| 1   | α-Thujene          | 144.35±1.27b | 867.17±23.49a | 9! |
| 2   | α-Pinene           | 804.57±7.00b | 207.23±1.41a | 2i |
| 3   | Sabinene           | 128.77±1.12a | 842.83±5.74ab | 1! |
| 4   | β-Pinene           | 236.92±2.06b | 140.56±1.01a | 1   |
| 5   | β-Myrcene          | 1262.26±11.19a | 429.98±3.04a | 5i |
| 6   | α-Phellandrene     | 33.08±0.34c | 118.21±0.78a | 1i |
| 7   | α-Terpinene        | 73.05±0.61b | 55.34±0.30b | 7i |
| 9   | d-limonene         | 60800.80±328.50a | 59857.96±265.43a | 6i |
| 9   | β-cis-Ocimene      | 46.03±0.37b | 49.36±0.37b | 6i |
| 10  | γ-Terpinene        | 3213.21±26.66b | 118.21±0.78a | 1i |
| 11  | Terpinolene        | 149.98±1.34b | 246.07±1.77a | 2i |
|     | Monoterpene alcohols |        |           |    |
| 12  | Linalool           | 152.41±1.01a | 55.37±0.24b | 5i |
| 13  | α-Terpineol        | 76.01±0.36a | 64.6±0.26a | 4i |
|     | Monoterpene aldehydes |        |           |    |
| 14  | Citronellal        | 28.25±0.23a | 19.82±0.15b | 1i |
|     | Monoterpene esters |          |           |    |
| 15  | Methyl 2-methyloctanoate | 227.59±0.04a | 225.77±0.11a | 2i |
| 16  | Citronellol acetate | 5.56±0.02b | 16.83±0.14a | 1i |
| 17  | (R)-lavandulyl acetate | 16.55±0.15c | 68.04±0.47b | 9i |
|     | Sum                | 67399.39±382.27a | 69032.36±328.01a | 7i |
|     | Sesquiterpene      |          |           |    |
| 18  | Copaene            | 44.00±0.41b | 60.57±0.48ab | 6i |
| 19  | β-Cubebene         | 35.07±0.34b | 45.92±0.34ab | 5i |
| 20  | β-Elemene          | 21.77±0.17a | 18.42±0.16a | 2i |
| 21  | Caryophyllene      | 22.68±0.17b | 58.03±0.44a | 6i |
| 22  | (E)-β-Famesene     | 37.4±0.34b | 117.74±1.05a | 1i |
|   | Compounds                                      | Mean ± Standard Deviation | Mean ± Standard Deviation | Mean ± Standard Deviation |
|---|-----------------------------------------------|---------------------------|---------------------------|---------------------------|
| 23| Germacrene D                                  | 123.72±1.22b              | 2772.96±22.34a            | 2'                        |
| 24| γ-Elemene                                     | 17.19±0.12b               | 196.80±1.61a              | 1'                        |
| 25| (-)-β-Elemene                                 | 440.45±4.29a              | 138.93±1.14b              | 1'                        |
| 26| δ-Cadinene                                    | 57.02±0.56a               | 69.14±0.57a               | 7'                        |
| 27| δ-Elemene                                     | 33.08±0.29c               | 155.35±1.22b              | 1'                        |
|   | Sesquiterpene alcohols                        |                           |                           |                           |
| 28| Nootkatone                                    | 2.79±0.04c                | 28.59±0.17b               | 6'                        |
|    | Sum                                           | 857.75±8.16b              | 4232.06±34.13a            | 4'                        |
|   | Alcohol                                        |                           |                           |                           |
| 29| (E)-3-Hexen-1-ol                              | 8.30±0.05c                | 34.14±0.10a               | 1'                        |
|   | Aldehydes                                      |                           |                           |                           |
| 30| 3-Hexenal                                     | 51.52±0.22c               | 72.66±0.05a               | 6'                        |
| 31| Hexanal                                       | 25.21±0.11a               | 25.38±0.01a               | 1'                        |
| 32| (E)-2-Hexenal                                 | 5.49±0.05ab               | 7.48±0.07a                | 4'                        |
| 33| Decanal                                       | 72.59±0.63a               | 80.43±0.70a               | 9'                        |
|    | Sum                                           | 154.81±1.01a              | 185.95±0.83a              | 1'                        |
|   | Phenol                                         |                           |                           |                           |
| 34| 2,4-di-t-butylphenol                          | ndc                       | 42.13±0.31a               | 3'                        |
|   | Others                                         |                           |                           |                           |
| 35| o-Cymene                                      | 103.06±0.90b              | 91.13±0.57b               | 1'                        |
| 36| n-Hexadecanoic acid                           | 58.71±0.73b               | 115.47±1.80b              | 2'                        |
|    | Sum                                           | 161.77±1.63b              | 206.6±2.37b               | 3'                        |
|    | Total                                         | 68582.02±393.12a          | 73691.11±365.44a          | 8'                        |

*Data expressed as means±standard deviation of triplicate samples’ fresh weight in their maturity*

*b Different lowercase within a line represent significant differences between cultivars (p<0.05)*

*c nd, the substance was not detectable*

**Table 5**
Volatiles profiles (μg g⁻¹ FW) in juice sac of OCC and its donor plants

| No. | Volatiles (μg g⁻¹) | OJ          | OCJ         | CJ          |
|-----|--------------------|-------------|-------------|-------------|
|     |                    |             |             |             |
| **Monoterpane** |                |             |             |             |
| 1   | Linalool           | 0.675±0.013ᵃ | 0.357±0.021ᶜ | 0.41        |
|     |                    | 46.22±0.38ᵃᵇ | 33.91±0.24ᵇ | 20.5        |
| 2   | γ-Terpinene        | 20.90±0.17ᵇ | 16.01±0.32ᶜ | 28.0        |
| 3   | d-Limonene         | 383.05±5.92ᵃ | 402.18±4.53ᵃ | 298         |
| 4   | β-Myrcene          | 44.59±0.31ᵃ | 36.43±0.36ᵇ | 29.5        |
| 5   | β-Elemene          | 1.96±0.03ᶜ  | 2.48±0.01ᵇ  | 3.09        |
| **Monoterpane esters** |            |             |             |             |
| 6   | Methyl 2-methyloctanoate | 11.01±0.10ᵇ | 18.12±0.10ᵃ | 1.80        |
| 7   | Methyl nonanoate   | 1.85±0.01ᵇ  | 1.87±0.01ᵇ  | 3.65        |
|     | Sum                | 801.93±3.33ᵃ | 633.21±2.97ᵃ | 341.        |
| **Sesquiterpane** |                |             |             |             |
| 8   | Germacrene D       | 0.17±0.01ᵇ  | 2.81±0.09ᵃ  | 0.46        |
| 9   | Copaene            | 14.31±0.51ᵇ | 19.92±0.36ᵇ | 49.6        |
| 10  | α-ylangene         | ndᶜ         | 16.05±0.10ᵃ | nd          |
| 11  | Germacrene B       | 11.45±0.39ᵃ | 6.99±0.36ᵃᵇ | 0.74        |
| **Sesquiterpane alcohols** |          |             |             |             |
| 12  | Nootkatone         | 1.13±0.04ᵇ  | 3.62±0.07ᵃ  | 0.45        |
|     | Sum                | 27.06±0.95ᶜ | 43.14±0.62ᵇ | 57.5        |
| **Aldehydes** |                |             |             |             |
| 13  | Decanal            | 1.47±0.01ᶜ  | 2.47±0.14ᵇ  | 3.59        |
| 14  | Dodecanal          | 2.25±0.01ᵃᵇ | 2.83±0.12ᵃ | 1.72        |
| 15  | Pentadecanal       | 2.28±0.03ᵇ  | 3.63±0.14ᵃ | 2.53        |
| 16  | Octanal            | 1.06±0.01ᵃ | 1.13±0.03ᵃ | 0.69        |
|     | Sum                | 7.06±0.06ᵇ  | 10.06±0.43ᵃ | 8.53        |
| **Phenol** |                |             |             |             |
| 17  | 2,4-di-t-Butylphenol | 3.53±0.05ᵇ | 6.24±0.02ᵃ | 5.42        |
| 18  | n-Tridecan-1-ol    | 23.51±1.39ᵃ | 16.12±0.33ᵇ | 12.6        |
|     | Sum                | 27.04±1.44ᵃ | 22.36±0.35ᵃ | 18.1        |
| **Others** |                |             |             |             |
| 19  | n-Hexadecanoic acid | 1.39±0.03ᵃ | 0.83±0.08ᵇ | 1.52        |
Total 

864.48±5.81a 709.60±4.45a 427. 

\(^a\) Data expressed as means±standard deviation of triplicate samples’ fresh weight in their maturity

\(^b\) Different lowercase within a line represent significant differences between cultivars \((p<0.05)\)

\(^c\) nd, the substance was not detectable

### Table 6

| No. | Carotenoid content \((\mu \text{g g}^{-1} \text{DW})\) | OP       | OCP       | CP       | OJ       | OCJ      |
|-----|---------------------------------------------|---------|-----------|---------|---------|---------|
| 1   | Violaxanthin                                | 941.53±42.97\(^a\)  |
|     |                                             | 772.11±54.36\(^b\)  |
|     |                                             | 911.83±37.03\(^a\)  |
|     |                                             | 5.02±0.75\(^a\)  |
|     |                                             | 5.20±1.2\(^a\)  |
| 2   | Luteoxanthin                                | 67.33±8.93\(^a\)  |
|     |                                             | 33.24±3.57\(^b\)  |
|     |                                             | 29.94±5.62\(^b\)  |
|     |                                             | 18.04±3.64\(^a\)  |
|     |                                             | 21.79±3.\(^b\)  |
| 3   | Lutein                                     | 110.04±8.67\(^a\)  |
|     |                                             | 44.54±5.56\(^b\)  |
|     |                                             | 24.98±1.27\(^c\)  |
|     |                                             | 5.67±1.94\(^a\)  |
|     |                                             | 4.97±1.5\(^b\)  |
| 4   | Zeaxanthin                                  | 61.40±5.73\(^a\)  |
|     |                                             | 3.36±0.84\(^b\)  |
|     |                                             | 4.63±1.21\(^c\)  |
|     |                                             | 34.39±1.18\(^a\)  |
|     |                                             | 19.97±2.\(^b\)  |
| 5   | \(\beta\)-cryptoxanthin                     | 356.81±8.04\(^a\)  |
|     |                                             | 9.07±1.66\(^b\)  |
|     |                                             | 14.65±0.96\(^b\)  |
|     |                                             | 290.73±4.00\(^a\)  |
|     |                                             | 132.74±\(^\text{f}\)\(^c\) |
| 6   | \(\alpha\)-carotene                         | 12.13±1.52\(^a\)  |
|     |                                             | nd\(^c\)  |
|     |                                             | nd  |
|     |                                             | 5.27±0.10\(^a\)  |
|     |                                             | 1.46±0.0\(^b\)  |
| 7   | \(\beta\)-carotene                         | 15.37±3.01\(^a\)  |
|     |                                             | 5.19±1.23\(^b\)  |
|     |                                             | 1.26±0.18\(^a\)  |
|     |                                             | 14.31±1.81\(^a\)  |
|     |                                             | 4.25±0.3\(^b\)  |
| 8   | Phytoene                                    | 383.58±9.23\(^a\)  |
|     |                                             | 44.71±3.41\(^b\)  |
|     |                                             | 71.34±6.41\(^b\)  |
|     |                                             | 123.30±6.02\(^a\)  |
|     |                                             | 42.01±1.\(^b\)  |
| 9   | Phytofluene                                 | 243.24±13.28\(^a\)  |
|     |                                             | 26.80±3.15\(^b\)  |
|     |                                             | 49.58±3.76\(^b\)  |
|     |                                             | 106.59±0.80\(^a\)  |
|     |                                             | 18.43±1.\(^b\)  |
| Total|                                           | 2191.43±101.38\(^a\)  |
|      |                                             | 939.02±73.78\(^b\)  |
|      |                                             | 1108.21±56.44\(^a\)  |
|      |                                             | 603.32±20.24\(^a\)  |
|      |                                             | 250.82±1\(^b\)  |

\(^a\) Data expressed as means±standard deviation of triplicate samples’ dry weight in their maturity

\(^b\) Different lowercase within a line represent significant differences between cultivars \((p<0.05)\)

\(^c\) nd, the substance was not detectable

**Figures**
Fruit morphology of ‘Hongrou Huyou’ (is simply as OCC) and its donor plants were taken in fully mature period. External appearance (A) and transverse section appearance (B) of ‘Owari’ satsuma mandarin (O), OCC (chimera) and ‘Changshan Huyou’ (C) were exhibited from left to right. Bars of external and transverse sections are both 5.0 cm.
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