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Content evaluation of 4 furanocoumarin monomers in various citrus germplasms

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ABSTRACT

Due to the furanocoumarin compounds in the fruit, the production and consumption of grapefruit have been affected in the past decades since the 'grapefruit juice effect' was declared. To provide elite germplasm and obtain knowledge for future citrus breeding programs, the contents of 4 furanocoumarin monomers (FCMs) in the juice sacs from 73 citrus germplasms were evaluated using ultra-performance liquid chromatography. 6′,7′-Dihydroxybergamottin and bergamottin were dominant in all the tested grapefruits, while there were some pomelos with dominant epoxybergamottin, and some with dominant 6′,7′-dihydroxybergamottin and bergamottin. The contents of FCMs were low or below detection in sweet oranges, mandarins, lemons and trifoliate oranges. The results also show that the dominant patterns of FCMs are genotype-related, and crossing and selection are effective approaches to alter FCM profiles in citrus breeding. Furthermore, the contribution of pomelo as a parent to grapefruit regarding their FCM profiles was discussed.

1. Introduction

Furanocoumarins are a group of coumarin derivatives with furan rings, and are considered as a group of phytoalexins, including linear furanocoumarins (such as psoralen, methoxypsoralen, xanthotoxin and bergapten) and angular furanocoumarins (such as angelicin and pimpinellin). In plants of the umbelliferae, the prenyl part of furanocoumarins is added onto simple coumarins (such as umbelliferone) under the enzymatic functions of isoprenyltransferase located in plastids and a series of cytochrome P450 enzymes. Furan rings from the 1-deoxy-D-xylulose-5-phosphate or 2-C-methyl-D-erythritol 4-phosphate (DOX or MEP) pathway are added onto the coumarins to form furanocoumarins, which may be stimulated by the addition of jasmonic acid (Stanjek, Piel, & Boland, 1999). Angular furanocoumarins are always present together with linear furanocoumarins, but in lower contents.

A group of naturally-occurring toxins in food (Dolan, Matulka, & Burdock, 2010), furanocoumarins are found in plants belonging to umbelliferae, sankoh, legumes, rutaceae, asteraceae, etc., possessing extensive phototoxic and photo-genotoxic activities on organisms ranging from bacteria to mammals and causing skin damage or skin diseases. However, in some traditional Chinese herbs, such as *Angelica dahurica*, furanocoumarins were found to have multifaceted anti-tumour, anti-inflammatory and analgesic and antidepressant therapeutic properties (Karamat et al., 2012). Although much lower contents of furanocoumarins than the assumed pathogenic dosage were detected in various citrus juices (Gorgus, Lohr, Raquet, Guth, & Schrenk, 2010), citrus breeding programs aiming at lower furanocoumarin contents in the fruits are underway (Chen, Cancalon, Haun, & Gmitter, 2011; Greenblatt et al., 2012).

Grapefruit (*Citrus paradisi*) originated from the hybrid crossing between pomelo and sweet orange (Gmitter, 1995; Scora, 1975). Bailey, Spence, Edgar, Bayliff, and Arnold (1989) firstly reported that grapefruit juice could significantly improve the oral bioavailability of a prescription medicine felodipine, which was called 'grapefruit juice effect' (GJE) subsequently. GJE is mainly attributed

Abbreviations: 6,7-DHB, 6′,7′-dihydroxybergamottin; EBM, epoxybergamottin; FCMs, furanocoumarin monomers; FW, fresh weight; UPLC, ultra performance liquid chromatograph.

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to furanocoumarins, which inhibit the metabolism and transport of felodipine, lovastatin and cyclosporine in the intestine by curbing the activities of the enzymes related to cardiovascular drug metabolism in the small intestine, especially cytochrome P450. The curbing of enzyme activities then increases the concentration of drug in the blood, leading to severe side-effects in human bodies (Seden, Dickinson, Khoo, & Back, 2010; Won, Oberlies, & Paine, 2012).

Cytochrome P450 enzymes belong to an enzyme family that are important in drug metabolism. Among the members CYP3A4, CYP1B1, 2D6, 2C9, 2B1 and so on, CYP3A4 plays a major role in grapefruit juice-mediated inhibition (Dresser, Spence, & Bailey, 2000; Girennavar, Poulose, Jayaprakasha, Bhat, & Patil, 2006; Guo, Fukuda, Ohta, & Yamazoe, 2000). Early studies proposed that the flavonoids in grapefruit, such as naringin and quercetin, are the main causative agents of GJE, while subsequent studies showed that naringin could actually inhibit CYP3A4 *in vitro* to some extent, but little inhibition was found *in vivo* (Bailey, Spence, Munoz, & Arnold, 1991; Bailey et al., 1989; Edwards, Bellevue, & Woster, 1996). However, 6′,7′-dihydroxybergamottin (6,7-DHB) is a furanocoumarin that was discovered to inhibit CYP3A4 *in vivo* (Edwards & Bernier, 1996; Edwards et al., 1996). Subsequent studies revealed that some other monomers and dimers of furanocoumarins also inhibit CYP3A4 (Dresser et al., 2000; Kane & Lipsky, 2000), with the dimers exhibiting stronger inhibition effects (Bourgaud et al., 2006; Cancalon & Haun, 2006). Accordingly, small amounts of dimers are always detected in the grapefruit juices (Widmer & Haun 2005). So far, a variety of furanocoumarins has been found in citrus, all of which are linear furanocoumarins (Messer, Nieborowski, Strasser, Lohr, & Schrenk, 2011), and those with inhibitory effects on CYP3A4 are listed in Fig. S1.

As the effect of improving oral bioavailability of drugs was firstly discovered in grapefruit juice, early GJE studies were thus mainly focused on grapefruit and its juice, and other citrus species were gradually included later. Fukuda et al. (1997) suggested that 6,7-DHB and the dimer compounds in grapefruit juice have strong inhibitory effects on CYP3A4, at about 10 and 200 times stronger than that of bergamottin, respectively. Guo et al. (2000) and Ohta et al. (2002) evaluated the inhibitory effects of 6 furanocoumarins on CYP3A4 in grapefruits, pomelos and tangerines. As a result, the furanocoumarins with the strongest to the weakest inhibitory effects were GF-I-1 (paradisin A), GF-I-4 (paradisin B), GF-I-6 (paradisin C or orthospiroester, OSE), 6,7-DHB, GF-I-2 (bergamottin) and bergapten. Ohnishi et al. (2000) and Row, Brown, Stachulski, and Lennard (2006) reported the order as paradisin C, 6,7-DHB, bergamottin, isoimperatorin, bergapten and bergaptol. Likewise, the order of paradisin A > 6,7-DHB > bergamottin was reported by Girennavar et al. (2006). Interestingly, Chen et al. (2011) determined furanocoumarins in the juice of 48 crossing combinations of grapefruits and pomelos. Strong correlations among the contents of 6,7-DHB, bergamottin and paradisin C were found in combination with the 1:1 co-segregation of the three furanocoumarins in the tested hybrids, suggesting that a single enzymatic or regulatory gene in the pathway is dominant in controlling the biosynthesis of furanocoumarins.

For methodologies to determine furanocoumarins, Fŕerot and Decorzant (2004) determined 15 furanocoumarins in the peel oil of bergamot, grapefruit and mandarins using HPLC–MS coupled with UV detector and fluorescence spectroscopy. By using ultra-performance liquid chromatography (UPLC), Vandermolen, Cech, Paine, and Oberlies (2013) simultaneously determined bergamottin, 6,7-DHB and 4 flavonoids within 5 min. Furthermore, Dugrand et al. (2013) separated and quantified 6 coumarins and 21 furanocoumarins in the peel (including flavedo and albedo) of 6 citrus germplasms with UPLC-MS. Recent research of Stohs, Miller, and Romano (2014) employed liquid chromatography–mass spectroscopy in analysing the contents of furanocoumarins such as bergamottin and 6,7-DHB in bitter orange (*Citrus aurantium*) extract.

The contents of furanocoumarins were found to be vary greatly in commercial grapefruit juices. From 58 commercial grapefruit juices of 2 seasons, Widmer and Haun (2005) found that bergamottin contents in white grapefruit juice were higher than that in red grapefruit juice, and shelf-stable juice contained less 6,7-DHB and OSE1 & 2 dimer but had higher levels of bergamottin and OSE3 compared with refrigerated juice. Fukuda, Guo, Ohashi, Yoshikawa, and Yamazoe (2000) also reported that there were lower levels of GF-I-1, GF-I-2 and GF-I-4 in red grapefruit juice compared with white grapefruit juice; and the accumulations of the 3 compounds were high in the fruit tissues of grapefruit, low in lemon juice, while undetectable in orange and tangerine juice. Cancalon, Barros, Haun, and Widmer (2011) reported that heating and storage temperature have greater impacts on the furanocoumarin contents in both the fruit and juice of grapefruit, and the contents change obviously with the prolonging of storage time.

In this study, the contents of 4 furanocoumarin monomers, namely bergapten, bergamottin, 6,7-DHB and epoxybergamottin (EBM), were evaluated in various citrus germplasms/accessions using UPLC. Special germplasms were then defined based on the furanocoumarin contents, which might be taken as promising cultivars in the citrus industry, or candidate parents and wild type in future breeding and selection. In addition, these germplasms might be precious materials for researches on the biosynthesis and regulation mechanism of furanocoumarins.

2. Materials and methods

2.1. Materials

Seventy-three citrus germplasms/accessions were collected from 8 citrus production areas of China, with 33 (45.2%) from the National Citrus Breeding Center in Huazhong Agricultural University (Wuhan, Hubei), 25 (34.2%, 4 were duplicate genotypes of that in Wuhan, Hubei) from the Citrus Research Institute, Chinese Academy of Agricultural Sciences, Chongqing (Beibei, Chongqing), and 16 from 6 other areas. There were in total 11 mandarins (*Camellia reticulata*), 14 sweet oranges (*Camellia sinensis*), 8 grapefruits (*C. paradisi*, including 1 citrumelo of Xiaoyuanzhiyou, a crossing hybrid between a grapefruit and trifoliate orange), 32 pomelos (*Calomyscus grandis*), 5 pomelo hybrids or cybrids, 2 lemons (*Citrus limon*) and 1 trifoliate orange (*Poncirus trifoliata*) harvested in the years of 2011 and/or 2012 from the peripheral part of the canopy of adult and healthy trees in various orchards under normal management (Table S1). Among them, 45 germplasms were investigated for 2 years. Average-sized healthy fruits at commercial maturity with typical traits true to their genotypes were collected; 12–24 fruits per germplasm were randomly divided into 3 repetitions. After surface sterilisation, juice sacs were dissected from other fruit tissues and then ground to coarse powders in liquid nitrogen and stored at −20 °C for further analysis.

2.2. Reagents

Acetonitrile (HPLC grade) was obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Authentic standards of bergamottin, bergapten, 6,7-DHB (HPLC grade) were obtained from Sigma Co. Ltd. (St. Louis, MO, USA), while that of EBM (HPLC grade) was purchased from Chromdex Inc. (Irvine, CA, USA). Ethyl acetate (AR grade) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China).