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Profiling of polyphenols for in-depth understanding of Tartary buckwheat sprouts: Correlation between cultivars and active components, dynamic changes and the effects of ultraviolet B stress

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ABSTRACT

Tartary buckwheat sprouts have a high nutritional value and are gluten-free, and polyphenols are their main active constituents. However, information regarding the active constituents' difference of Tartary buckwheat sprouts grown from seeds with different morphology, at different developmental stages and environments is limited. Here, we developed a LC–MS-based targeted metabolomics approach to analyze polyphenols (46 flavonoids and 6 anthraquinones) in 40 Tartary buckwheat sprouts varieties. Both flavonoids and anthraquinones contributed to significant differences in sprouts grown from seed with different color or shape. Twenty-seven differential compounds were all at a higher level in 3-day-old sprouts, and the fold change from 3-day-old to 8-day-old sprouts was 1.42–6.64. A total of 25 differential compounds were all significantly upregulated upon UV-B radiation, especially for epicatechin. This study is valuable not only for better breeding cultivars of Tartary buckwheat sprouts, but also assessing their metabolic quality.

1. Introduction

Tartary buckwheat (*Fagopyrum tataricum* L. Gaertn.) sprouts have spread across the world, including to eastern Asia, Europe, Australia, and the United States, due to their high nutritional value and their use as a gluten-free food for patients with coeliac disease (Ruan et al., 2020). As a raw material for healthcare products, Tartary buckwheat sprouts are listed in the New Resource Food Catalog of the People's Republic of China. Polyphenols (flavonoids and anthraquinones) are the main active constituents of Tartary buckwheat. Flavonoids exhibit antioxidant, antitumor, antihypertensive, and anti-inflammatory activities, and about seventeen flavonoids have been isolated and identified from Tartary buckwheat sprouts (Fahmy, Al-Sayed, El-Shazly, & Singab, 2018; Zhu, 2016). Natural anthraquinones have diverse biological activities, such as antioxidant, antibacterial, and antifungal properties (Friedman et al., 2020; Masi, & Evidente, 2020), and several anthraquinones have also been identified in Tartary buckwheat seeds and sprouts (Watanabe, 1998; Kim et al., 2008; Ren, Wu, Ren, & Zhang, 2013).

Levels of plant metabolites are strongly affected by variety characteristics, growth status as well as environmental factors (Hounsome, Hounsome, Tomos, & Edwards-Jones, 2008; Zou et al., 2021). It is reported that color and shape of Tartary buckwheat seeds could be differentiated based on secondary metabolites, such as flavonoids and

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anthraquinones (Yang et al., 2020). However, relatively few studies have examined the secondary metabolites profiles of sprouts from different varieties of Tartary buckwheat. Additionally, limited studies have addressed the optimal timing for harvesting in order to yield high-content components. The levels of some flavonoids including four C-glycosylflavones (vitexin, isovitexin, orientin, and isoorientin), cyanidin-3-O-rutinoside, cyanidin-3-O-glucoside, and rutin during the development of Tartary buckwheat sprouts have been studied (Kim et al., 2007; Kim et al., 2008; Li et al., 2012). There is also a lack of information regarding methods of growing sprouts to increase the content of effective components through the adjustment of light sources. To data, it has been demonstrated that the levels of rutin, quercetin, and total anthocyanin in Tartary buckwheat leaves were increased by UV-B radiation (Yao et al., 2006; Suzuki, Honda, & Mukasa, 2005; Huang et al., 2016). In accordance with previous study, we hypothesized that flavonoids and anthraquinones profiling of Tartary buckwheat sprots could be related with their seed morphology, developmental stages and environment.

Metabolomic method have been used to discriminate various cultivars and provide a holistic overview of the global changes that occur after stress in plants (Herman et al., 2017; Seyler et al., 2020). Ultra-High Performance liquid chromatography coupled with triple quadrupole mass spectrometry (UHPLC–QqQ–MS/MS) usually was used for the simultaneous analysis of multiple components in complex matrix (Chen et al., 2013; Sawada et al., 2009). Therefore, UHPLC–QqQ–MS/MS-based targeted metabolomics could be appropriate to identify the correlations between cultivars and chemical components, the suitable collection timing, and the effective approaches for increasing the active compound content.

In the present study, first, a metabolomics approach was developed and used for the chemical profiling of polyphenols (46 flavonoids and 6 anthraquinones) in Tartary buckwheat sprouts using UHPLC–MS/MS. Second, forty cultivars of Tartary buckwheat sprouts were analyzed to trace the correlation between cultivars and active components (flavonoids and anthraquinones). Third, forty Tartary buckwheat sprouts with different harvesting times were analyzed to explore the optimal time to collect sprouts. Fourth, a batch of Tartary buckwheat sprouts was irradiated with UV-B stress to explore a viable approach to increase the content of active compounds.

2. Materials and methods

2.1. Chemicals and reagents

Kaempferol, isoquercitrin, ω-hydroxyemodin, quercetin, quercitrin, rutin, luteolin, emodin-8-O-β-D-glucoside, epicatechin, catechin, and emodin were purchased from Shanghai Yuanye Biotechnology Co., Ltd. The purities of these authentic standards were >98%. HPLC-grade acetonitrile was acquired from Honeywell (Morris, NJ, USA). Acetic acid and ammonium acetate (HPLC-grade) were obtained from Sigma-Aldrich (Steinheim, Germany). Pure distilled water from Watsons water (Hong Kong, China) was used.

2.2. Sample preparation and extraction

A total of 40 varieties of Tartary buckwheat seeds were harvested in 2017 from 6 provinces of China, including Gansu, Hunan, Jiangxi, Sichuan, Guizhou, and Yunnan. These seeds were stored at the Traditional Chinese Medicine Medica Resource Center. Seed color, seed length and width were measured using a colorimeter (CR-10 Plus, Konica Minolta, Japan) and digital micrometer (DL312300, Deli, China), respectively. For excluding the influence of cultivation environment, seeds with full grains, no damage, and no mildew were cultivated under constant enviroment with 24 h light at 25 ℃ in the Institute of Chinese Materia Medica. Tartary buckwheat sprouts were harvested at two time points: the 3rd day after sowing and the 8th day after sowing, then they were frozen at −80 ℃.

Three varieties of Tartary buckwheat seeds were selected and grown in the plant cultivation room for 8 days in the dark at 25 °C with a relative humidity of 60%. After 8 days, part of each seedling was transferred to a custom-made controlled environment chamber (25 °C) and radiated using UV-B (275–320 nm, 2 W/m^2) for 6 h. Meanwhile, a control sample was kept in the dark. Three biological replicates sprouts were collected and stored at −80 °C.

Tartary buckwheat sprouts were ground with liquid nitrogen in a mortar. A 1.0 g sample of sprouts was extracted using 5.0 mL of 70% aqueous methanol for 30 min in an ultrasonic ice bath, centrifuged at 6800 g for 5 min at 4 °C, and filtered with a 0.22 μm millipore filter.

2.3. Preparation of standard solutions

We weighed the standards, dissolved them in methanol, and prepared individual 1 mg/mL solutions. In order to generate a quality control (QC), a sample with kaempferol, quercetin, quercitrin, irutin, soquercitrin, catechin, epicatechin, luteolin, emodin, emodin-8-O-β-D-glucoside, and ω-hydroxyemodin, the 11 authentic standards were mixed and diluted to 100 ng/mL. For monitoring the stability and reproducibility of LC–MS system, every 10 experimental samples were followed by a QC sample.

2.4. LC-MS/MS analysis

The UHPLC Agilent 1290II system coupled to a quadrupole time-of-flight mass spectrometer (Q-Tof) Agilent G6500 was used to perform full scans (Agilent Technologies, Santa Clara, CA, USA). The Q-Tof operating parameters were set as follows: electrospray ionization source (ESI); sheath gas temperature 350 ℃; gas temperature 250℃; nozzle voltage 20 V; nebulizer pressure 35 psi; and capillary voltage 4000 V. An UHPLC Agilent 1290II system combined with triple quadrupole mass spectrometer (QqQ) Agilent G6400 was used to perform the product ion (PI) scans and multiple reaction monitor (MRM). The QqQ operating parameters were set as follows: electrospray ionization source (ESI); sheath gas temperature 320 °C; gas temperature 250 °C; nebulizer pressure 30 psi; capillary voltages 3500 V.

UHPLC equipped with an auto-sampler, a column compartment, and a binary solvent delivery system was used with the following operating parameters: column temperature 35 °C; Agilent Eclipse Plus C_{18} column (2.1×100 mm, 1.8 μm); mobile phase consisted of water containing 0.5% acetic acid, 5 mM ammonium acetate (A), and acetonitrile (B); flow rate 0.3 mL/min; and sample injection volume 1 μL. Agilent MassHunter was used for the control of UHPLC solvent gradients and MS scan functions.

2.5. Statistical analysis

As a pattern recognition approach, orthogonal partial-least squares discrimination analysis (OPLS–DA) model of SIMCA-P (version 14.0, Umetrics, Umeå, Sweden) was used to screen markers. The filter criterion was variable importance for projection (VIP) >1. Then, a *t*-test with a value of $p < 0.05$ was utilized for discriminating significant differences.

3. Results and discussion

3.1. Chemical profiling of polyphenols in Tartary buckwheat sprouts

The molecular formulas and structures of these compounds were deduced taking into account their accurate mass of adduct ions and fragmentation ions profiling of MS^2, respectively. The polarity of the compounds was indicated by their retention time. Accurate mass was provided by a high-resolution full scan of Q-Tof, and total ion chromatograms of Tartary buckwheat sprouts is shown in Fig. S1. The MS/

Table 1
Detail information of constituents identified in Tartary buckwheat sprouts.

No.	R_t (min)	Adduct ion[a] $[M+H]^+/[M-H]^-$	Error (ppm)	Molecular formula	Molecular Mass (dalton)	Profiling of fragment ion (relative abundance %)	Identification	Type
1	9.90	287.0551	0.35	$C_{15}H_{10}O_6$	286.0477	213.1, 153.1, 121.1 (100%)	Kaempferol	Subclass of flavonoid: flavonol
2	8.29	303.0501	0.33	$C_{15}H_{10}O_7$	302.0427	257.1, 229.1, 151.1 (100%), 121.0	Quercetin	
3	8.84	317.0652	−1.26	$C_{16}H_{12}O_7$	316.0583	302.1 (100%), 301.1, 273.1, 153.0, 123.1	3-O-Methylquercetin	
4	10.87	331.0810	−0.91	$C_{17}H_{14}O_7$	330.0740	316.1, 315.1, 301.1 (100%), 273.1, 217.0, 151.1	3,5-Dimethylquercetin	
5	3.83	449.1081	0.45	$C_{21}H_{20}O_{11}$	448.1006	287.0 (100%), 269.0	Kaempferol-O-hexose	
6	4.35	449.1085	1.34	$C_{21}H_{20}O_{11}$	448.1006	287.0 (100%), 269.0	Same as No. 5	
7	5.29	449.1088	2.00	$C_{21}H_{20}O_{11}$	448.1006	287.0 (100%)	Same as No. 5	
8	5.85	449.1089	2.23	$C_{21}H_{20}O_{11}$	448.1006	287.0 (100%), 96.8	Kaempferol-3-O-glucoside	
9	5.92	449.1079	0.00	$C_{21}H_{20}O_{11}$	448.1006	303.1 (100%)	Quercitrin	
10	5.19	465.1035	1.51	$C_{21}H_{20}O_{12}$	464.0955	303.0 (100%), 285.0, 257.1, 229.2, 165.0, 137.0	Isoquercetrin	
11	6.52	465.1042	3.01	$C_{21}H_{20}O_{12}$	464.0955	303.1 (100%), 185.0, 114.1	Quercetin-O-glucoside	
12	4.85	479.1175	−1.88	$C_{22}H_{22}O_{12}$	478.1111	317.1 (100%), 191.0, 174.0	Methylquercetin-O-hexose	
13	5.76	479.1176	−1.88	$C_{22}H_{22}O_{12}$	478.1111	317.1 (100%), 302.1, 123.1	Same as No. 12	
14	6.53	479.1178	−1.25	$C_{22}H_{22}O_{12}$	478.1111	317.1 (100%)	Same as No. 12	
15	7.03	479.1174	−2.10	$C_{22}H_{22}O_{12}$	478.1111	317.1 (100%), 302.1	Same as No. 12	
16	3.85	493.1339	−0.41	$C_{23}H_{24}O_{12}$	492.1268	331.1 (100%), 316.1, 301.0, 185.1	Dimethylquercetin-O-hexose	
17	3.94	493.1342	0.20	$C_{23}H_{24}O_{12}$	492.1268	331.1 (100%), 316.1, 301.0, 185.1	Same as No. 16	
18	7.03	493.1341	0.00	$C_{23}H_{24}O_{12}$	492.1268	331.1 (100%), 316.1, 301.0	Same as No. 16	
19	7.37	493.1345	0.81	$C_{23}H_{24}O_{12}$	492.1268	331.1 (100%), 121.1	Same as No. 16	
20	5.50	595.1668	1.68	$C_{27}H_{30}O_{15}$	594.1585	449.1, 287.0 (100%)	Kaempferol-3-O-rutinoside	
21	4.95	611.1623	2.62	$C_{27}H_{30}O_{16}$	610.1534	465.0, 303.0 (100%)	Rutin	
22	5.62	625.1773	1.60	$C_{28}H_{32}O_{16}$	624.1690	479.1, 317.1 (100%), 301.1	Methylquercetin-O-rutinoside	
23	8.30	623.1617*	−0.16	$C_{28}H_{32}O_{16}$	624.1690	299.1 (100%)	Isokaempferide-O-glucoside-glucoside	
24	3.99	757.2181	−0.66	$C_{33}H_{40}O_{20}$	756.2113	611.0, 449.0 (100%), 287.0	Kaempferol-O-rutinoside-O-glucoside	
25	5.21	757.2177	−1.19	$C_{33}H_{40}O_{20}$	756.2113	449.0 (100%), 287.0	Same as No. 24	
26	3.85	773.2141	0.78	$C_{33}H_{40}O_{21}$	772.2062	465.0 (100%), 303.0	Quercetin-O-rutinoside-O-glucoside	
27	4.27	773.2136	0.13	$C_{33}H_{40}O_{21}$	772.2062	465.1, 449.1, 303.0 (100%)	Same as No. 26	
28	4.43	773.2138	0.39	$C_{33}H_{40}O_{21}$	772.2062	303.0 (100%)	Same as No. 26	
29	4.73	773.2141	0.78	$C_{33}H_{40}O_{21}$	772.2062	611.0, 465.1, 303.0 (100%)	Same as No. 26	
30	5.15	893.2565	0.78	$C_{37}H_{48}O_{25}$	892.2485	585.1, 303.0 (100%), 121.1	Quercetin-O-rutinoside-O-xylobiose	
31	3.79	935.2669	0.64	$C_{39}H_{50}O_{26}$	934.2590	773.0, 627.0, 611.0, 465.0, 449.0, 303.0 (100%)	Quercetin-O-rutinoside-O-glucoside-O-glucoside	
32	3.96	935.2673	1.07	$C_{39}H_{50}O_{26}$	934.2590	627.0, 303.0 (100%)	Same as No. 31	
33	4.99	935.2655	−0.86	$C_{39}H_{50}O_{26}$	934.2590	303.0 (100%)	Same as No. 31	
34	4.11	291.0865	0.69	$C_{15}H_{14}O_6$	290.0790	273.1, 161.1, 147.1, 139.1, 123.1 (100%)	Epicatechin	Subclass of flavonoid: flavanol
35	4.39	291.0863	0.00	$C_{15}H_{14}O_6$	290.0790	273.1, 165.1, 161.0, 147.0, 139.1, 123.0 (100%)	Catechin	
36	3.92	453.1390	−0.44	$C_{21}H_{24}O_{11}$	452.1319	291.2 (100%), 165.1, 139.1, 123.0	Epicatechin-7-O-glucoside	
37	4.09	453.1385	−1.54	$C_{21}H_{24}O_{11}$	452.1319	291.2 (100%), 273.1, 139.1, 123.0	Catechin-7-O-glucoside	
38	8.21	287.0547	−1.05	$C_{15}H_{10}O_6$	286.0477	153.0 (100%)	Luteolin	Subclass of flavonoid: flavone
39	5.08	431.0990*	1.39	C21H20O10	432.1056	311.1(100%), 283.1, 269.0	Vitexin/isovitexin	
40	4.50	449.1080	0.22	$C_{21}H_{20}O_{11}$	448.1006	395.0, 353.0, 329.1, 299.0 (100%), 287.0	Orientin	
41	4.72	449.1079	0.00	$C_{21}H_{20}O_{11}$	448.1006	413.1, 383.2, 329.1, 299.1 (100%), 287.0	Isoorientin	

(*continued on next page*)

Table 1 (*continued*)

No.	R_t (min)	Adduct ion[a] $[M+H]^+/[M-H]^-$	Error (ppm)	Molecular formula	Molecular Mass (dalton)	Profiling of fragment ion (relative abundance %)	Identification	Type
42	8.20	273.0753	−1.83	$C_{15}H_{12}O_5$	272.0685	255.0, 137.0 (100%)	Naringenin	Subclass of flavonoid: dihydroflavone
43	6.14	433.1148*	1.85	$C_{21}H_{22}O_{10}$	434.1213	271.1(100%)	Naringenin-O-glucoside	
44	6.96	433.1141*	0.23	$C_{21}H_{22}O_{10}$	434.1213	271.1(100%)	Same as No. 43	
45	6.04	449.1100	4.68	$C_{21}H_{20}O_{11}$	448.1006	287.0 (100%), 137.1	Cyanidin-O-glucoside	Subclass of flavonoid: anthocyanidin
46	6.44	449.1099	4.45	$C_{21}H_{20}O_{11}$	448.1006	287.1 (100%), 121.0	Same as No. 45	
47	12.75	269.0452*	−1.12	$C_{15}H_{10}O_5$	270.0528	241.1, 225.1 (100%)	Emodin	Anthraquinone
48	9.95	285.0407*	0.70	$C_{15}H_{10}O_6$	286.0477	267.0, 257.0, 229.0, 211.1 (100%)	ω-Hydroxyemodin	
49	5.73	431.0983*	−0.23	$C_{21}H_{20}O_{10}$	432.1056	269.1 (100%)	Emodin/aloe-emodin-O-glucoside	
50	6.63	431.0979*	−1.16	$C_{21}H_{20}O_{10}$	432.1056	269.2 (100%)	Same as No. 49	
51	8.38	431.0981*	−0.70	$C_{21}H_{20}O_{10}$	432.1056	269.1 (100%)	Emodin-8-O-glucoside	
52	9.66	431.0980*	−0.93	$C_{21}H_{20}O_{10}$	432.1056	269.1 (100%)	Same as No. 49	

a: $[M+H]^+$ for all the compounds except for compounds 23, 39, 43, 44, 47–52. The compounds with $[M-H]^-$ are marked with*.

MS spectra was provided by product ion (PI) scan of QqQ. Eventually, 52 compounds were characterized or tentatively identified in sprouts, including 46 flavonoids (33 flavonols, 4 flavanols, 4 flavones, 3 dihydroflavones, and 2 anthocyanidins), and 6 anthraquinones, whose retention times, accurate mass of adduct ions, and fragmentation ions profiling of MS^2 are listed in Table 1.

3.1.1. Identification of flavonoids

Here, four flavonol aglycones (compound 1–4) were identified, and compounds 1 and 2 were clearly identified as kaempferol and quercetin on comparing with reference standards. Kaempferol (286 Da) and quercetin (302 Da) were the major flavonol aglycones of Tartary buckwheat sprouts. Compound 3 was tentatively identified as 3-O-methylquercetin based on its molecular formula ($C_{16}H_{12}O_7$), a fragment ion of Retro Diels-Alder reaction (RDA) cleavage *m/z* 153.0, and a characteristic fragment ion $[M+H\text{-}CH_3]^+$ *m/z* 302.1. Due to $[M+H]^+$ *m/z* 331.0810, and a series of fragment ions $[M+H\text{-}CH_3]^+$ *m/z* 316.1, $[M+H\text{-}CH_4]^+$ *m/z* 315.1, $[M+H\text{-}2CH_3]^+$ *m/z* 301.1, compound 4 was tentatively characterized as 3,5-dimethylquercetin. 3-O-Methylquercetin and 3,5-dimethylquercetin have been identified from Tartary buckwheat and *Fagopyrum dibotrys* (D. Don) Hara (Wang, Zhang, & Yang, 2005 ; Jing et al., 2016; Yang et al., 2020).

Twenty-nine flavonol glycosides (compound 5–33) were determined. Rhamnose (146 Da), glucoside (162 Da), and rutinoside (308 Da) were the major sugar moieties, which mostly cleaved from flavonol glycosides of Tartary buckwheat sprouts. Comparing with authentic standards, compounds 9, 10, and 21 were conclusively characterized as quercitrin, isoquercetrin and rutin. Compounds 5–8 with molecular formula ($C_{21}H_{20}O_{11}$) and a characteristic fragment ion $[M+H\text{-}162\ Da]^+$ *m/z* 287.0 were tentatively characterized as kaempferol-O-hexose. Moreover, compound 8 created the largest peak, thus it could be kaempferol-3-O- glucoside. Compound 11 was tentatively characterized as quercetin-O-glucoside in agreement with characteristic ions $[M+H\text{-}162\ Da]^+$ *m/z* 303.0 and $[M+H]^+$ *m/z* 465.1042. Compounds 12–15 with the characteristic ions $[M+H\text{-}162\ Da]^+$ *m/z* 317.1 and $[M+H\text{-}162\ Da\text{-}CH_3]^+$ *m/z* 302.1 were considered to be methylquercetin-O-hexose. Compounds 16–19 were tentatively identified as dimethylquercetin-O-hexose due to the same molecular formula ($C_{23}H_{24}O_{12}$), a series of fragment ions from neutral losses of 162 Da, (162 Da + CH_3), (162 Da + $2CH_3$). Based on characteristic ions such as $[M+H\text{-}146\ Da]^+$ *m/z* 449.1 and $[M+H\text{-}146\ Da\text{-}162\ Da]^+$ *m/z* 287.0, compound 20 was tentatively characterized as kaempferol-3-O-rutinoside. Based on the molecular formula $C_{28}H_{32}O_{16}$ and fragment ions from neutral losses of 146 Da or (146 Da + 162 Da), compound 22 was deduced to be methylquercetin-O-rutinoside. Considering the molecular formula $C_{28}H_{32}O_{16}$ and major fragment ions from neutral losses of (162 Da + 162 Da), compound 23 was tentatively designated as isokaempferide-O-glucoside-glucoside. In agreement with the same fragment ions from neutral losses of 308 Da or (308 Da + 162 Da), compounds 24 and 25 were tentatively determined to be kaempferol-O-rutinoside-O-glucoside. Compounds 26–29 with the same molecular formula ($C_{33}H_{40}O_{21}$) were characterized as quercetin-O-rutinoside-O-glucoside due to a major neutral loss (308 Da + 162 Da). Based on two fragment ions *m/z* 585.1 (loss of 308 Da) and *m/z* 303.0 (loss of 308 Da + 282 Da), compound 30 ($C_{37}H_{48}O_{25}$) was temporarily identified as quercetin-O-rutinoside-O-xylobiose. Compounds 31–33 were tentatively characterized as quercetin-O-rutinoside-O-glucoside-O-glucoside based on the same neutral losses of 308 Da or (308 Da + 162 Da + 162 Da). Kaempferol-3-O-glucoside, kaempferol-3-O-rutinoside, and quercetin-3-O-rutinoside-3′-O-glucopyranoside have been reported previously in Tartary buckwheat grains (Jiang et al., 2015).

Here, 2 flavanol aglycones (compound 34–35) and 2 flavanol glycosides (compound 36–37) were characterized in Tartary buckwheat sprouts. Compound 34–35 were unequivocally identified as epicatechin and catechin on comparison with authentic standards. Based on the major neutral losses of 162 Da and a characteristic ion *m/z* 123.0 ($C_6H_3O_3^+$), compounds 36–37 were temporarily recognized as epicatechin-7-O-glucoside and catechin-7-O-glucoside, respectively, which have been previously reported in Tartary buckwheat (Watanabe, 1998; Ren et al., 2013).

Four flavones were characterized, including 1 flavone aglycone (compound 38) and 3 flavone glycosides (compound 39–41). Compound 38 was unequivocally identified as luteolin by comparing with authentic standards. Compound 39 (molecular formula $C_{21}H_{20}O_{10}$) was tentatively identified as vitexin or isovitexin, with the characteristic neutral losses of 120 Da and 162 Da. Compounds 40 and 41 (molecular formula $C_{21}H_{20}O_{11}$) were tentatively identified as orientin and isoorientin, respectively, as they shared the same characteristic neutral losses of 120 Da and 162 Da. Orientin, vitexin, isovitexin, and isoorientin have been detected previously in Tartary buckwheat sprouts (Nam et al., 2015).

Three dihydroflavones were tentatively identified, including 1 dihydroflavones aglycones (compound 42) and 2 dihydroflavones glycosides (compound 43–44). Compound 42 was tentatively classified as naringenin due to the RDA cleavage fragment ion *m/z* 137.0 and $[M+H\text{-}H_2O]^+$ *m/z* 255.0. Naringenin has been isolated and identified from *Polygonum cuspidatum* (Ma et al., 2009). Based on the same characteristic neutral losses of 162 Da, both compounds 43 and 44 were determined as naringenin-O-glucoside.

Two anthocyanidins were tentatively identified. Compounds 45 and 46 ($C_{21}H_{20}O_{11}$) were tentatively distinguished as cyanidin-O-glucoside due to a neutral loss of 162 Da and characteristic fragment ions *m/z* 137.0 and 121.0. It is reported that cyanidin-3-O-glucoside has been isolated from sprouts of buckwheat (Kim et al., 2007).

3.1.2. Identification of anthraquinones

On comparison with authentic standards, emodin (compound 47), ω-hydroxyemodin (compound 48), and emodin-8-O-glucosid (compound 51) were unambiguously identified. Due to the same

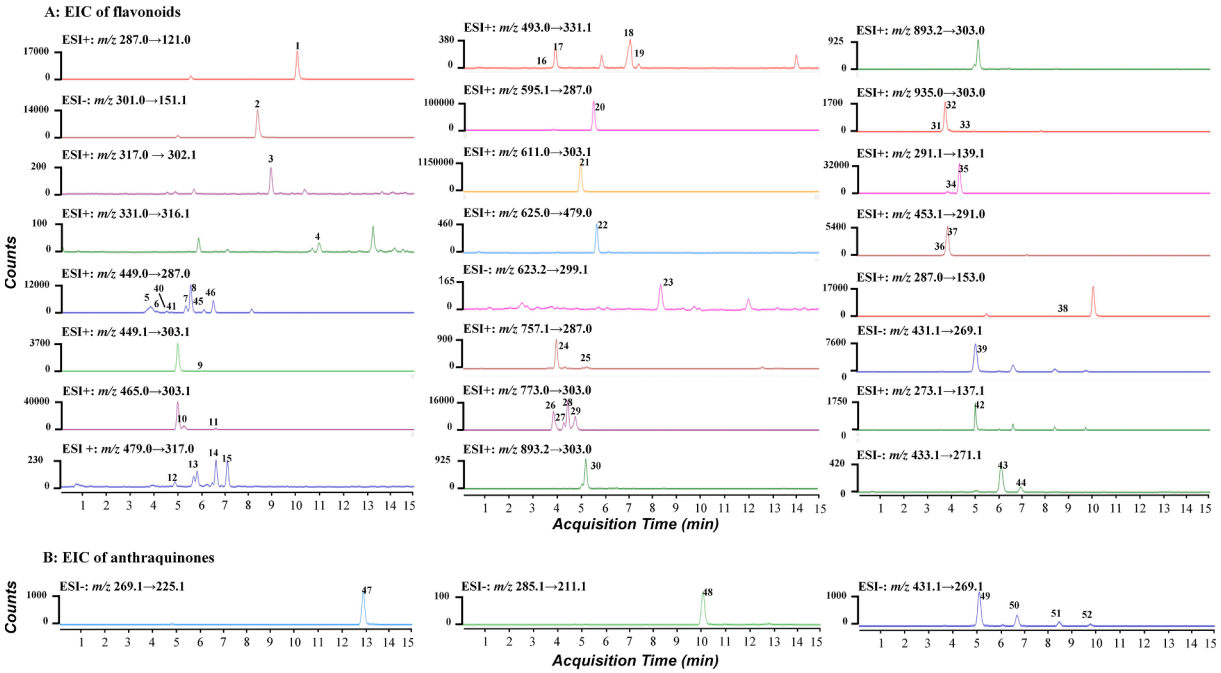

Fig. 1. Extracted ion chromatograms of 52 compounds identified in Tartary buckwheat sprouts, including 46 flavonoids (A) and 6 anthraquinones (B). These compounds were all studied in the positive ion mode (ESI +), with the exception of compounds 2, 23, 43, 44, and 47–52. Compound number is in agreement with those in Table 1.

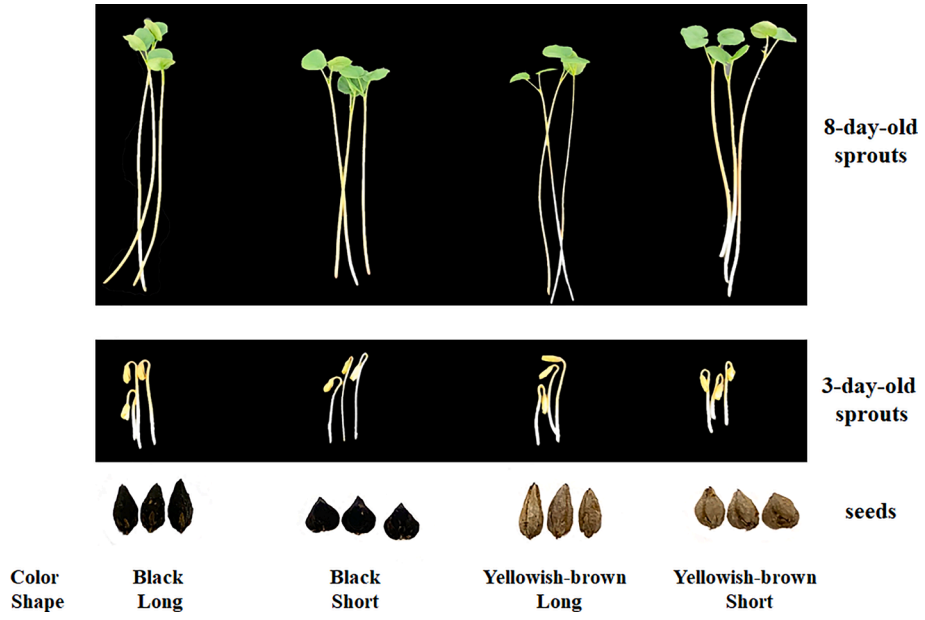

Fig. 2. Four Tartary buckwheat sprouts (3-day-old and 8-day-old) grown from seeds with different color and shape (TB063, TB177, TB054, and TB275).

characteristic neutral losses of 162 Da, compounds 49, 50, and 52 were tentatively characterized as emodin/aloe-emodin-O-glucoside.

3.2. Relative quantification of polyphenols in Tartary buckwheat sprouts

The 52 components in Tartary buckwheat sprouts were quantitatively analyzed, and their optimized extract ion chromatograms are shown in Fig. 1. The adduct ion and characteristic fragment ion were selected as MRM transitions. The MS condition of these MRM transitions was optimized, including collision energy and ion scanning model. Positive ionization mode showed a higher ion response than negative ionization mode, except for the flavonoid (compounds 2, 23, 39, 43, 44) and all the anthraquinones. The detail information of the 52 compounds MRM scan, such as collision energy, fragmentor and ion scanning model, is shown in Table S1.

3.3. Targeted metabolomics analysis of Tartary buckwheat sprouts

Long seed with yellowish-brown or black hull, and short seed with yellowish-brown or black hull were collected and germinated (Fig. 2), which allowed us to investigate the correlation between sprouts grown from seed with different morphology and metabolites (Yao et al., 2017). Sprouts with two key morphological characteristics were collected, which were sprouts with a curved hook at the top (3-old-day sprouts) or

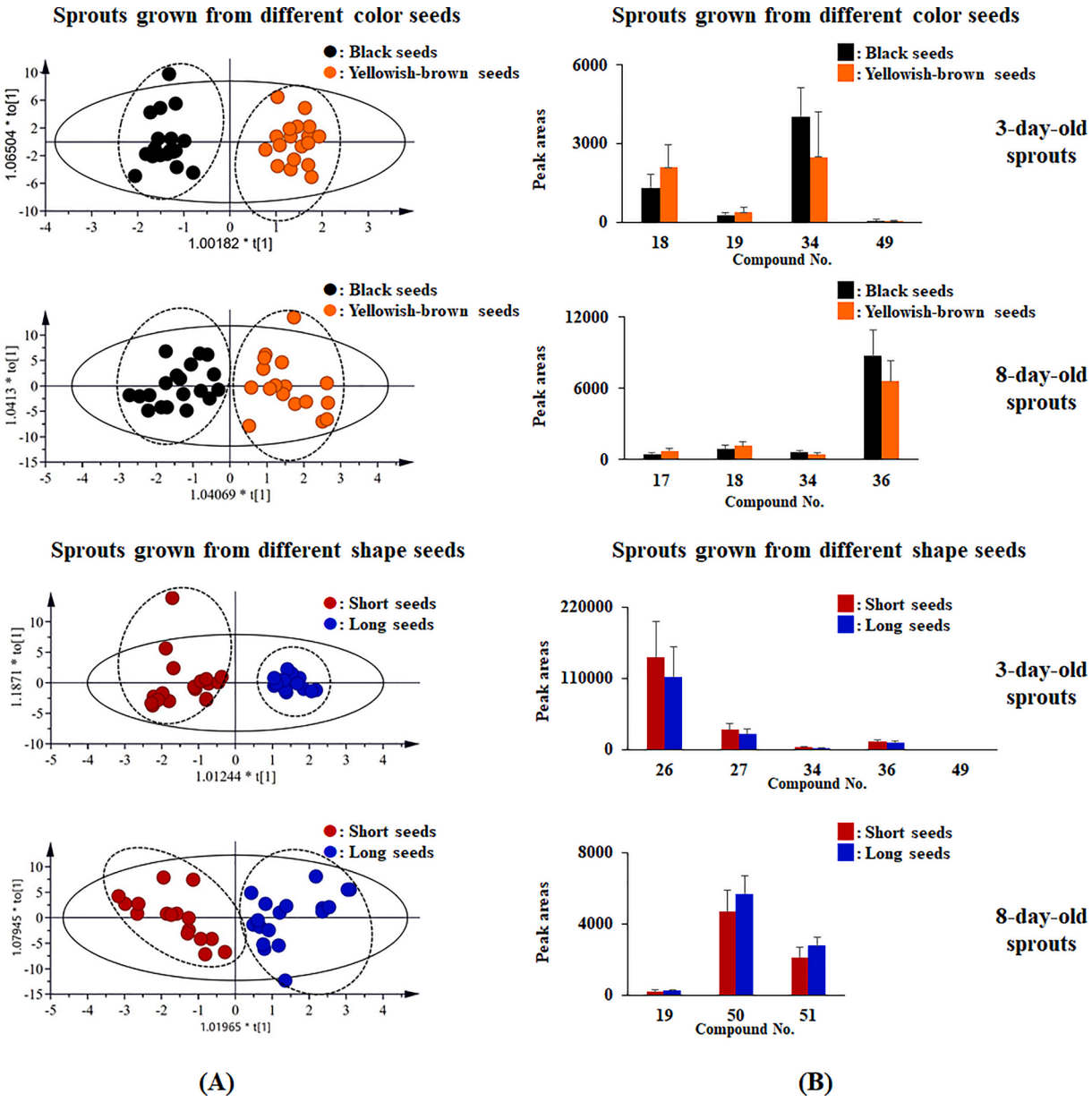

Fig. 3. Correlation between active components and sprouts grown from seeds with different color or shape. (A) OPLS–DA plot of Tartary buckwheat sprouts; (B) histogram of differential metabolites peak areas.

sprouts with two fully open cotyledons (8-old-day sprouts), as shown in Fig. 2. Their hypocotyls were 3.63–4.87 cm and 9.30–12.40 cm, respectively, and commercially available sprouts are similar to 8-old-day sprouts. These samples of Tartary buckwheat sprouts were analyzed by the relative quantification method. The line plots of QC samples were between 2SD and −2SD, which ensured the reliable and high-quality data (Fig. S2).

3.3.1. Correlation between sprouts grown from various color seed and metabolites

In our study, we collected various Tartary buckwheat sprouts grown from black seeds (n = 20) and yellowish-brown seeds (n = 20), as shown in Table S2. The lightness values of black and yellowish-brown seeds were 15.29 ± 2.18 and 34.26 ± 2.56, respectively. Tartary buckwheat sprouts from these seeds were used to explore their metabolic variation.

OPLS–DA was applied to reveal the representative differential compounds from the 3-day-old sprouts with different seed color ($Q_2 = 0.454$, $R_2X = 0.523$, $R_2Y = 0.818$). As shown in Fig. 3A, the sprouts grown from black and yellowish-brown seeds were sorted into two groups. Based on VIP > 1 and $p < 0.05$ of *t*-test, three flavonoids (compounds 18, 19, 34) and one anthraquinones (compound 49) could be used to distinguish the 3-day-old sprouts with different seed color. The 8-day-old sprouts with different seed color also presented clear separation on the OPLS–DA plot ($Q_2 = 0.626$, $R_2X = 0.636$, $R_2Y = 0.954$). Four flavonoids (compounds 17, 18, 34, and 36) were screened using VIP > 1 and $p < 0.05$.

Peak areas histogram of differential compounds is presented in Fig. 3B. For the 3-day-old sprouts, the concentrations of compound 18 and 19 in sprouts grown from yellowish-brown seeds were higher than in those from black seeds. The content ratio between sprouts grown from yellowish-brown and black seeds was 1.52–1.59. Conversely, the concentrations of compounds 34 and 49 in sprouts grown from yellowish-brown seeds were lower than in those from black seeds. The fold change between sprouts with yellowish-brown and black seeds was 0.62–0.67. For the 8-day-old sprouts, the higher concentrations of compounds 17 and 18 in sprouts grown from yellowish-brown seeds compared with those from black seeds, and the fold change between

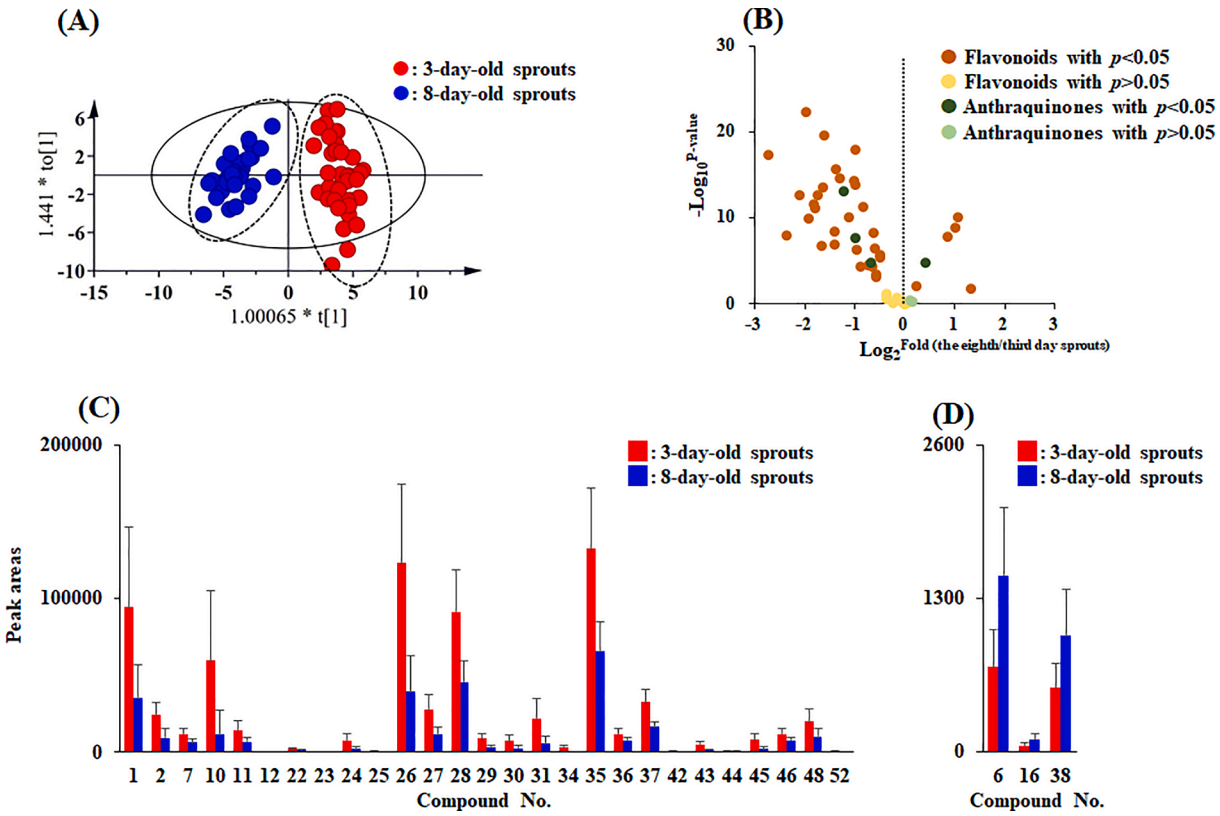

Fig. 4. Chemical differences between 3-day-old and 8-day-old sprouts. (A) OPLS–DA score plots; (B) volcano plots; (C) twenty-seven metabolites were more abundant in 3-day-old sprouts; (D) three metabolites were more abundant in 8-day-old sprouts.

sprouts with yellowish-brown and black seeds was 1.27–1.62. Conversely, the fold change of compounds 34 and 36 between sprouts with yellowish-brown and black seeds was 0.70–0.75. In both 3-day-old and 8-day-old sprouts, compounds 18 (dimethylquercetin-O-hexose) and 34 (epicatechin) could be used to discriminate the sprouts from black and yellowish-brown seeds. The concentration of compound 18 was higher in sprouts grown from yellowish-brown seeds, while the concentration of compound 34 was higher in sprouts grown from black seeds.

3.3.2. *Correlation between sprouts grown from various shape seed and metabolites*

We collected various Tartary buckwheat sprouts from short (n = 20) and long (n = 20) seeds, as shown in Table S2. The length/width ratio values of long and short seeds were approximately 1.78–2.11 and 1.15–1.51, respectively.

As shown in Fig. 3A, there was a clear separation in the OPLS–DA plot of 3-day-old sprouts grown from long and short seeds, which indicated significant differences in the 3-day-old sprouts grown from seed with different shapes ($Q_2 = 0.460$, $R_2X = 0.597$, $R_2Y = 0.768$). A total of four flavonoids (compounds 26, 27, 34, and 35) and one anthraquinone (compounds 49) were marked with $p < 0.05$ and VIP > 1. For the 8-day-old sprouts, the segregation between sprouts grown from short and long seeds was clearly separated on the OPLS–DA plot ($Q_2 = 0.527$, $R_2X = 0.533$, $R_2Y = 0.884$), and one flavonoid (compound 19) and two anthraquinones (compounds 50 and 51) with statistical significance were identified (Fig. 3A).

Differential compounds to distinguish Tartary buckwheat sprouts grown from different shape seeds were presented in Fig. 3B. For 3-day-old sprouts from seeds with different shapes, the concentrations of five compounds in the sprouts grown from short seeds were higher than those in the sprouts grown from long seeds, and the fold change was 1.19–1.50. Conversely, the concentrations of all the metabolites in the 8-day-old sprouts grown from long seeds were higher than those from short seeds, and the fold change was 1.22–1.45.

3.3.3. *Exploring a suitable collecting time for sprouts*

The data of 52 compounds in the 80 sprouts harvested on the 3rd day after sowing (n = 40) and 8th day after sowing (n = 40) were used to identify the differential metabolites between the sprouts harvested at different times. Regardless of the morphological variations (shape and color), the 3-day-old and 8-day-old sprouts were subjected to the OPLS–DA analysis. They were obviously distinguished on the OPLS–DA plot ($Q_2 = 0.589$, $R_2X = 0.934$, $R_2Y = 0.903$; Fig. 4A). As presented in the volcano plots, fifty-two compounds were assigned into two groups due to their fold change (Fig. 4B), and 42 compounds have a negative impact based on the $Log_2^{fold\ change}$. A total of 30 different metabolites (28 flavonoids and 2 anthraquinones) were identified. We found that concentrations of 27 compounds (25 flavonoids and 2 anthraquinones) were at a higher level in 3-day-old sprouts (Fig. 4C). The fold change between 3-day-old and 8-day-old sprouts was 1.42–6.64, and compound 34 (epicatechin) was heavily affected. Only three flavonoids were present at higher concentrations in the 8-day-old sprouts (Fig. 4D), and the fold change between the 8-day-old and 3-day-old sprouts was 1.82–2.08. Fig. 5.

During sprouting, carbohydrates, lipids and proteins stored in the seeds are broken down to provide energy and synthesize substrates for the early stages of seed germination (Ikram et al., 2021). Meanwhile, an increased level of secondary metabolites with various health benefits is often observed during germination in various sprouts, such as Alfalfa and Buckwheat (Aloo, Ofosu, & Oh, 2021). In this study, 30 differential compounds except for compound 6, 16 and 38 were all at a higher level in 3-day-old sprouts, comparing to 8-day-old sprouts. It suggested that most of the flavonoids and anthraquinones could first increase and then decrease with increase in growing time. The length of Tartary buckwheat sprouts increases with time, and Tartary buckwheat sprouts are

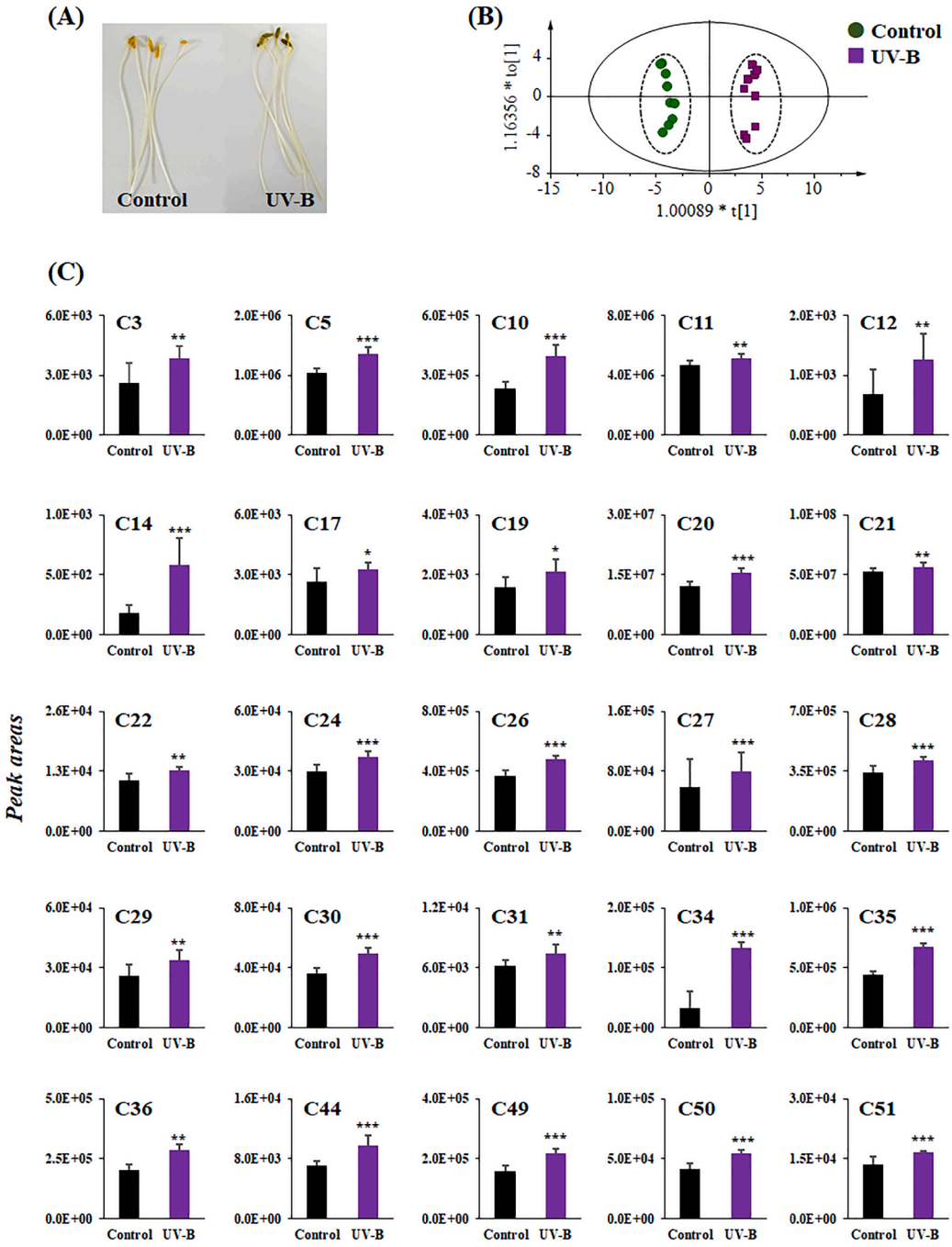

Fig. 5. UV-B radiation used for increasing the content of active compounds. (A) sprouts of Tartary buckwheat cultivars (1508-TB005) treated with UV-B; (B) OPLS–DA score plots of control and UV-B group; (C) peak areas of 25 differential metabolites significantly regulated upon UV-B radiation. Compound number is in accord with those in Table 1. The *t*-test of compounds with $0.01 \leq p < 0.05$, $0.001 \leq p < 0.01$, and $p < 0.001$ were marked with *, **, ***, respectively.

usually collected 8–12 days after sowing (Tuan et al., 2013). However, the 3rd day after sowing could be a favorable collecting time compared to the 8th day to acquire more flavonoids per unit weight.

3.3.4. *Exploring a viable approach to increase the content of active compounds*

Following the treatment with UV-B stress for 6 h, the levels of flavonoids and anthraquinone were evaluated. Sprouts of Tartary buckwheat cultivars treated with UV-B are shown in Fig. 5A. OPLS–DA displayed two well-separated clusters corresponding to the control and UV-B-treated sprouts (Q2 = 0.952, R2X = 0.488, R2Y = 0.987), as shown in Fig. 5B. This result indicated that sprouts react strongly to UV-B treatment at the metabolome level. Then, significant features were subjected to an unpaired *t*-test. As shown in Fig. 5C, a total of 25 compounds (22 flavonoids and 3 anthraquinone) were screened with $p < 0.05$ and VIP > 1, which all were significantly upregulated upon UV-B radiation. Comparing previous studies, the change of anthraquinones and more flavonoids in Tartary buckwheat sprouts were studied (Yao et al., 2006; Suzuki et al., 2005; Huang et al., 2016). Flavonoids are produced as protective substances against UV-B radiation (Liu et al., 2018). Here, we found both anthraquinones and flavonoids have the same change trend in response to UV-B irradiation. The fold changes of

compounds 14 and 34 were >2 between control and UV-B treated sprouts. It is worth mentioning that the levels of epicatechin increased by 4 times compared with the no UV-B radiation group, which was the most strongly induced during the UV-B treatment. UV-B treatment is a form of energy produced by sound waves at specific frequencies, which has several advantages, including low cost, improved quality, and reduced damages (Tsurunaga et al., 2013). Therefore, a suitable UV-B treatment is a simple and convenient way to increase the content of most anthraquinones and flavonoids in Tartary buckwheat sprouts.

4. Conclusions

We developed a targeted metabolomics approach to analyze polyphenols (46 flavonoids and 6 anthraquinones) in cultivars of Tartary buckwheat sprouts using UHPLC–MS/MS. Then the metabolomics method was applied to analyze tartary buckwheat sprout grown from seed with different morphology, at different developmental stages, or stressed by UV-B radiation. Representative flavonoids and anthraquinones of sprouts could be correlated for discriminating the Tartary buckwheat sprouts varieties grown from different shape and color seeds. The vast majority of compounds, including flavonoids and anthraquinones, were present at higher concentrations in 3-day-old sprouts compared to 8-day-old sprouts, which suggested that the 3rd day after sowing could be a suitable time for harvesting to acquire more flavonoids and anthraquinone per unit weight. Following the treatment of UV-B stress for 6 h, the flavonoid and anthraquinone contents in sprouts were significantly upregulated. UV-B is a simple and convenient treatment method that can be utilized to increase the contents of active components in Tartary buckwheat sprouts. It was concluded that compound 34 (epicatechin) was sensitive, whose level could be influenced by a variety of factors including seed morphological variations, collection time, and UV-B stress. This study gives evidence for the selection of Tartary buckwheat sprouts, optimal collecting time, and cultivation of Tartary buckwheat sprouts.

Supplementary material

The detailed information of 52-compounds MRM scan (Table S1), Tartary buckwheat sprout information (Table S2), total ion chromatograms of sprouts (Fig. S1), and line plots of PCA from the QC samples (Fig. S2), have been provided in the supplementary material.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2022.100295.

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