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Phenolic compounds in different organs of tartary buckwheat (*Fagopyrum tataricum* Gaertn.) cultivars

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Abstract

Tartary buckwheat contains variety of secondary metabolites. The present study investigates the variation of phenolic compounds among tartary buckwheat cultivars of different geographical origin. On analyzing the different parts of tartary buckwheat at flowering stage under greenhouse condition, 9 different phenolic compounds, viz. rutin, 4-hydroxy benzoic acid, epicatechin, chlorogenic acid, catechin, p-coumaric acid, 4-hydroxy-3-methoxy benzoic acid, quercetin, and kaemferol were identified. The levels of these phenolic compounds among the cultivars varied significantly. The amount of phenolic compounds in the flower was much higher than that in the leaf, stem, and root of tartary buckwheat, irrespective of cultivars. The Chinese cultivar C 2097 had the highest quercetin contents in both leaf and stems displaying 13- and 877-fold higher than the lowest quercetin producing cultivar N7608 from Nepal, respectively. Both highest (C 8920) and lowest (C 05) rutin contents in flowers obtained in the Chinese cultivars and the difference between them was 3-fold. Rutin content of the flower was 6.3- and 29.3-fold higher than that in the leaf and stem, respectively. Our results by HPLC analyses indicate that phenolic compounds in tartary buckwheat significantly varied among cultivars from different origins and the highest amount of phenolic compounds contained in the floral organs

Keywords: Phenolic compounds; Different organs; Tartary buckwheat; Cultivars.
Abbreviations: ANOVA_Analysis of variance, DMRT_Duncan Multiple Range Test, DW_Dry weight, HPLC_High Performance Liquid Chromatography, SAS_Statistical Analysis Systems.

Introduction

Plants of genus *Fagopyrum* produce a wide array of biologically active compounds. *F. esculentum* (common buckwheat) and *F. tataricum* (tartary buckwheat) have long been used as food worldwide. It is believed that the origin of common buckwheat was in Southwest China and latter has spread to other continents, but tartary buckwheat is grown only in the hilly areas of Southwest China, some part of Northern India, Nepal and Bhutan. It was reported (Bonafaccia et al., 2003; Xuan and Tsuzuki, 2004) that tartary buckwheat is only grown in a small part of Northwest Europe. Antioxidants in dietary plants have attracted attention because they protect the human body from oxidative damage caused by free radicals. Buckwheat is known to contain various antioxidative compounds, such as vitamins BI, B2, and E, flavonoid, and phenolic compounds, such as rutin, quercetin, and proanthocyanidines (condensed tannins), which are found in different organs of buckwheat (Watanabe *et al*., 1995; Watanabe *et al*., 1997; Watanabe, 1998; Kim et al., 2008; Zielinska et al., 2012). A plenty of research have been done in the past for intensive investigation of common buckwheat, whereas, a few research has been undertaken about tartary buckwheat. Recently, it has been reported that tartary buckwheat has many medicinal values especially for antidiabetic (Yao et al., 2008), anticancer (Guo et al., 2007), and antioxidant activities (Liu et al., 2008). Important pharmacological ingredient like rutin has been found as 3% dry weight in the herb of tartary buckwheat and has a small amount of quercitrin. Seeds of tartary buckwheat contain more rutin than common buckwheat seeds (Fabjan *et al.*, 2003). Tartary buckwheat seeds even contain traces of quercitrin and quercetin, whereas these compounds are absent in common buckwheat seeds (Kitabayashi *et al*., 1995). Different parts of buckwheat i.e. leaf, stem, root and inflorescence contain various amounts of phenolic and others compounds. Foods that contained phenolic compounds belong to one of the main classes of secondary metabolites and are widely distributed in higher plants. Much emphasis is given on the antioxidant activity of polyphenolic compounds and associated pathologies such as cancers, coronary heart disease, and inflammation (Croft, 1998; Karakaya, 2004; Tapiero *et al.*, 2002; Linseisen and Rohrmann, 2008). Little information is available on occurrence of phenolics found in different plant parts of Tartary buckwheat cultivars. However, no quantitative evaluation of phenolic compounds in tartary buckwheat has so far been reported. Therefore, this study aimed to quantify phenolic compounds in different plant organs of tartary buckwheat cultivars by HPLC analyses.

Table 1. Variation of phenolic compounds in the leaf of different cultivars of tartary buckwheat.

Cultivar	Phenolic compounds (µg/mg DW) in leaf								
	4-hydroxy benzoic acid	Catechin	Chlorogenic acid	4-hydroxy-3-methoxy benzoic acid	Epicatechin	p-coumaric acid	Rutin	Quercetin	Kaemferol
LX 0101 (Luxemburg)	0.16 d	0.1 e	0.57 e	0.18 d	0.4 cd	0.04 ab	33.61 c	0.07±0.04	0.01±0
C 8920 (China)	0.21 a	0.1 e	0.73 c	0.12 f	0.28 e	0.02 cd	30.46 d	0.01±0	-
C 05 (China)	0.09 g	0.07 f	0.33 g	0.08 g	0.19 f	0.02 cd	13.67 g	0.01±0	-
C 0116 (China)	0.07 h	0.06 f	0.5 f	0.14 e	0.27 e	0.01 d	14.46 g	-	-
C 2097 (China)	0.19 b	0.12 cd	0.66 d	0.14 e	0.27 e	0.01 d	36.09 b	0.01±0	-
C 8919 (China)	0.18 c	0.13 bc	0.58 e	0.15 e	0.22 f	0.02 cd	31.06 d	-	-
C 9045 (China)	0.19 b	0.13 bc	0.65 d	0.23 b	0.42 bc	0.02 cd	38.25 a	-	-
BT 10 (Bhutan)	0.13 e	0.14 ab	0.8 b	0.30 a	0.45 b	0.03 bc	29.82 de	-	-
B 9134 (Bhutan)	0.19 b	0.15 a	0.9 a	0.20 c	0.56 a	0.05 a	33.79 c	0.01±0	-
I 8622 (India)	0.10 f	0.12 cd	0.53 ef	0.18 d	0.38 d	0.03 bc	22.79 f	-	-
N 7608 (Nepal)	0.18 c	0.11 de	0.37 g	0.24 b	0.41 cd	0.02 cd	28.5 e	-	-

Mean values (mean of three replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Duncan Multiple Range Test)

Table 2. Variation of phenolic compounds in the stem of different cultivars of tartary buckwheat.

Cultivar	Phenolic compounds (µg/mg DW) in Stem								
Tartary	4-hydroxy benzoic acid	Catechin	Chlorogenic acid	4-hydroxy-3-methoxy benzoic acid	Epicatechin	p-coumaric acid	Rutin	Qucetin	Kaemferol
LX 0101 (Luxemburg)	0.17 b	0.06 d	2.01 ab	0.06 d	0.76 b	0.06 b	6.93 de	-	-
C 8920 (China)	0.12 c	0.09 b	1.82 bc	0.08 d	0.52 e	0.07 b	7.65 cd	0.01±0	0.01±0
C 05 (China)	0.07 e	0.06 d	0.59 g	0.06 d	0.57 de	0.04 bc	2.92 h	0.02±0	0.01±0
C 0116 (China)	0.12 c	0.07 cd	2.0 ab	0.17 ab	0.72 bc	0.02 c	6.89 de	0.06±0.03	0.01±0.01
C 2097 (China)	0.08 de	0.06 d	1.58 d	0.08±0	0.35 f	0.06 b	4.32 g	-	0.01±0
C 8919 (China)	0.07 e	0.08 bc	1.29 e	0.16 bc	0.17 g	0.11 a	4.05 g	-	-
C 9045 (China)	0.05 f	0.06 d	1.39 e	0.17 ab	0.33 f	0.04 bc	8.21 c	-	-
BT 10 (Bhutan)	0.09 d	0.07 cd	1.73 cd	0.20 a	0.41 f	0.06 b	6.46 ef	0.06±0.03	0.01±0
B 9134 (Bhutan)	0.07 e	0.11 a	2.19 a	0.17 ab	0.94 a	0.07 b	12.09 a	-	-
I 8622 (India)	0.08 de	0.08 bc	0.86 f	0.13 c	0.52 e	0.05 bc	5.5 f	-	0.01±0
N 7608 (Nepal)	0.22 a	0.08 bc	0.76 fg	0.20 a	0.64 cd	0.06 b	9.51 b	-	0.01±0

Mean values (mean of three replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Duncan Multiple Range Test)

Results

Phenolic compounds in leaf

From the analysis of tartary buckwheat leaf, 9 different phenolic compounds were recorded (Table 1). Among the 9 phenolic compounds, the levels of rutin, epicatechin, and chlorogenic acid varied widely among the cultivars. The rutin content in 11 tartary buckwheat cultivars ranged from 13.67 to 38.25 µg/mg DW. This showed a 2.8 times difference from C 9045 (China) to C 05 (China) cultivars, which contained the highest and lowest rutin, respectively. The content of epicatechin and chlorogenic acid ranged from 0.56 to 0.19, and 0.9 to 0.33 µg/mg DW, respectively, among the studied cultivars; the cultivar from Butan (B 9134) contained the highest quantity of both compounds, measuring 2.9- and 2.72- fold higher, respectively, than the lowest content cultivar C 05 (China). Other compounds like 4-hydroxy benzoic acid, catechin, 4-hydroxy-3-methoxy benzoic acid, and p-coumaric acid showed little variation among the cultivars. The phenolic compound quercetin was observed in only a few cultivars, and kaemferol was found only in the cultivar LX 0101 (Luxemburg).

Phenolic compounds in Stem

The 9 phenolic compounds were also identified during the analysis of tartary buckwheat stem (Table 2). It was found that the amount of chlorogenic acid and epicatechin content was higher in the stem than in the leaf, whereas, the rutin content was lower in the stem than in the leaf, and the others compounds did not vary greatly between leaf and stem. The trend of phenolic compound content was similar in stem and leaf, but the variation was higher in stem than in leaf among the cultivars. The rutin content in different tartary buckwheat cultivars ranged from 2.92 to 12.09 µg/mg DW. This displays a 4.14 times more between B 9134 (Bhutan) and C 05 (China), had the highest and lowest rutin, respectively. The content of chlorogenic acid and epicatechin ranged from 0.59 to 2.19, and 0.17 to 0.94 µg/mg DW, respectively, among the studied cultivars. The cultivar from Bhutan (B 9134) contained the highest levels of chlorogenic acid and epicatechin, at 3.71- and 5.53- fold higher, respectively, than the lowest content cultivar C 05 (China) and C 8919 (China), respectively. Other compounds like 4-hydroxy benzoic acid, catechin, 4-hydroxy-3-methoxy benzoic acid, p-coumaric acid, quercetin, and kaemferol varied similarly among the cultivars. The Nepalese cultivar N 7608 contained the highest amount of 4-hydroxy benzoic acid and vanillic acid (4-hydroxy-3-methoxybenzoic acid) among the cultivars.

Phenolic compounds in the flower

From the analysis of different parts of tartary buckwheat, it was observed that flower contained higher levels of phenolic compounds than other parts of tartary buckwheat (Table 3). A significantly higher amount of quercetin was observed in the flower than in other parts of the plant. The content of quercetin in the flower ranged from 0.69 to 8.77 µg/mg DW

Table 3. Variation of phenolic compounds in the flower of different cultivars of tartary buckwheat.

Cultivar	Phenolic compounds (µg/mg dw) in Flower								
	4-hydroxy benzoic acid	Catechin	Chlorogenic acid	4-hydroxy-3-methoxybenzoic acid	Epicatechin	p-coumaric acid	Rutin	Qucetin	Kaemferol
LX 0101 (Luxemburg)	-	0.44 e	5.46 e	0.58 g	4.04 gh	0.17 cd	52.76 e	1.49 f	0.01±0
C 8920 (China)	-	0.70 cd	10.8 a	0.76 ef	3.94 gh	0.17 cd	85.53 a	2.74 e	0.02±0
C 05 (China)	-	0.99 a	4.21 h	1.18 b	5.93 c	0.37 a	27.88 g	3.47 d	0.03±0
C 0116 (China)	-	0.66 d	10.02 b	1.69 a	9.03 a	0.35 a	55.57 e	3.06 de	0.02±0
C 2097 (China)	-	0.77 bc	7.88 d	0.82 de	3.66 hi	0.20 c	56.51 e	8.77 a	0.05±0
C 8919 (China)	-	0.73 cd	4.92 fg	0.63 g	3.34 i	0.17 cd	47.91 f	5.81 c	0.05±0
C 9045 (China)	-	0.51 e	8.37 c	0.86 d	4.11 fg	0.21 c	81.26 b	0.79 g	-
BT 10 (Bhutan)	-	0.81 b	8.48 c	0.93 c	5.06 d	0.26 b	76.79 c	2.01 f	-
B 9134 (Bhutan)	-	0.47 e	5.2 ef	1.12 b	4.61 e	0.04 e	62.03 d	7.01 b	0.04±.01
I 8622 (India)	-	0.82 b	4.77 g	1.18 b	6.3 b	0.29 b	53.60 e	1.47 f	0.02±.0
N 7608 (Nepal)	-	0.46 e	2.19 i	0.71 f	4.5 ef	0.14 d	83.07 ab	0.69 g	-

Mean values (mean of three replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Duncan Multiple Range Test)

Table 4. Variation of Phenolic compounds in the root of different cultivars of tartary buckwheat.

Cultivar	Phenolic compounds (µg/mg dw) in root								
Tartary	4-hydroxy benzoic acid	Catechin	Chlorogenic acid	4-hydroxy-3-methoxy benzoic acid	Epicatechin	p-coumaric acid	Rutin	Qucetin	Kaemferol
LX 0101 (Luxemburg)	-	0.09 c	0.07 b	0.26 c	1.66 a	0.05 cd	0.09 c	-	0.01 d
C 8920 (China)	-	0.1 b	0.07 b	0.28 b	0.95 d	0.06 bc	0.02 f	-	0.01 d
C 05 (China)	-	0.03 g	0.07 b	0.07 h	0.25 f	0.03 e	0.03 f	-	0.02 c
C 0116 (China)	-	0.03 g	0.13 a	0.09 g	0.19 f	0.03 e	0.08 cd	-	0.05 a
C 2097 (China)	-	0.07 d	0.07 b	0.17 e	0.59 e	0.08 a	0.04 ef	-	0.03 b
C 8919 (China)	-	0.13 a	0.07 b	0.30 a	1.36 b	0.07 ab	0.06 de	-	0.03 b
C 9045 (China)	-	0.07 d	0.07 b	0.30 a	1.24 b	0.06 bc	0.08 cd	-	0.01 d
BT 10 (Bhutan)	-	0.09 c	0.07 b	0.23 d	0.84 d	0.03 e	0.06 de	-	0.01 d
B 9134 (Bhutan)	-	0.1 b	0.07 b	0.30 a	1.09 c	0.06 bc	0.08 cd	-	0.05 a
I 8622 (India)	-	0.06 e	0.07 b	0.17 e	0.56 e	0.04 de	0.14 b	-	0.01 d
N 7608 (Nepal)	-	0.04 f	0.07 b	0.15 f	1.72 a	0.07 ab	0.38 a	-	0.02 c

Mean values (mean of three replicates with three samples from each replicate) indicated by the same letter in a column do not differ significantly at 5% level (Duncan Multiple Range Test)

among the studied cultivars. In the highest quercetin content cultivar C 2097 (China), the quercetin level in the flower was 12.7- fold higher than in the lowest content cultivar N 7608 (Nepal) and 877-fold higher than that in leaf and stem. The rutin content in the flowers of different tartary buckwheat cultivars ranged from 27.88 to 85.53 µg/mg DW. This shows a 3.1 times more between C 8920 (China) and C 05 (China) cultivars, which had the highest and lowest rutin content, respectively. The rutin content in the flower was 6.3- and 29.3-fold higher than that in the lowest rutin content cultivars of leaf and stem, respectively. The content of chlorogenic acid in the flower was also significantly higher than in the leaf and stem. The range of chlorogenic acid content in the flower of tartary buckwheat cultivars was 2.19 to 10.8 µg/mg DW. The cultivar C 8920 (China) contained the highest amount of chlorogenic acid, which was 5- fold higher than that of the lowest content cultivar N 7608 (Nepal). The content of chlorogenic acid in the flower was 32.7- and 18.3-fold higher than that in the leaf and stem, respectively. The content of p-coumaric acid was also much higher in the flower than in the leaf and stem. The range of p-coumaric acid content in the flower of tartary buckwheat cultivars ranged from 0.04 to 0.37 µg/mg DW. The cultivar C 05 (China) contained the highest amount of p-coumaric acid, which was 9.3- fold higher than the lowest content cultivar B 9134 (Bhutan). The content of p-coumaric acid in the flower of cultivar C 05 (China) was 37 and 18.5 times higher than that in leaf and stem of the lowest p-cumaric acid content cultivar, respectively. The epicatechin content in different tartary buckwheat cultivars in the flower ranged from 3.34 to 9.03 µg/mg DW representing a 2.7 time more between the C 0116 (China) and C 8919 (China) cultivars, which had the highest and lowest amount of rutin, respectively. The epicatechin content in flower was 47.5- and 53.1-fold higher than that in the lowest epicatechin content cultivars of leaf and stem, respectively. The content of 4-hydroxy-3-methoxy benzoic acid in the flower was higher than that in the leaf and stem of tartary buckwheat cultivars. The cultivar C 0116 (China) contained the highest amount of 4-hydroxy-3-methoxy benzoic acid, and the cultivar C 8919 (China) contained the lowest amount of 4-hydroxy-3-methoxy benzoic acid. The 4-hydroxy-3-methoxy benzoic content in flower was 12- and 28-fold higher than that in the lowest 4-hydroxy-3-methoxy benzoic content cultivars of leaf and stem, respectively. The content of catechin was also higher in the flower than leaf and stem. The cultivar C 05 (China) contained the highest amount of catechin, which was 16.5-fold than that in the leaf and stem of the lowest content cultivar.

Phenolic compounds in root

The level of phenolic compounds was much lower in the root of tartary buckwheat compared to the leaf, stem, and flowers. There were 7 phenolic compounds observed in the root. No 4-hydroxy benzoic acid and quercetin was detected in the root. The amount of epicatechin was higher than that of other phenolic compounds in the root. The epicatechin content in different tartary buckwheat cultivars in the root ranged from 0.19 to 1.72 µg/mg DW. This shows 9.1 time higher difference between N 7608 (Nepal) and C 0116 (China) cultivars, which had the highest and lowest epicatechin

content, respectively. The epicatechin content in root was 9.1- and 10.1-fold higher than that in the lowest epicatechin content cultivars of leaf and stem, respectively.

Discussion

This study for the first time quantitatively analyzed phenolic compounds in different plant organs of tartary buckwheat from varying geographic origins. Our results show that the flower of tartary buckwheat contained highest phenolic compounds than the other organs of the plant. Contents of phenolic compounds varied among different cultivars and even among the cultivars from same origins. It is notable that same cultivars do not follow the same trend for phenolic compound content in different parts. For example, the cultivar B 9134 (Bhutan) contained a higher amount of phenolic compounds in the leaf and stem, but contained lesser amount in the other parts of this cultivar. On the other hand, the cultivars C 05 and C 0116 (China) contained a large quantity of phenolic compounds in the flower, and these 2 cultivars have a higher phenolic content compared to others. There have been some previous reports of varietal differences in the antioxidative components in buckwheat, including differences in the rutin content (Kitabayashi *et al.*, 1995; Ohsawa and Tsutsumi, 1995), while varietal and environmental differences in vitamin E (Honda, 1995) and phenolic acid (Oomah *et al.*, 1996 a) have also been described. Moreover, the antioxidative activity of ethanol extract from buckwheat seeds has been reported to vary according to the cultivar and correlated with the polyphenol content (Zielinski and Kozlowska, 2000). Flavonoid content varied among the cultivars (Milbury *et al*., 2006). Oomah *et al.* (1996 b) reported a large variation of flavonoid content among the cultivars of flaxseed (*Linum usitatissimum*). The levels of flavonoid and antioxidant in almonds depended more on cultivar than on differences of seasons (Bolling *et al*., 2010). From previous studies it was reported that Portuguese almond cultivars found 4 and 18 times higher flavonoids and total phenols content among the cultivars, respectively (Barreira *et al.*, 2008). A 4.6-fold variation in the phenolic content of hulls and a similar variability in the antioxidant activity of extracts were also observed from Iranian almonds (Sfahlan *et al.*, 2009). Similar results were observed in the variation of phenolic content in the cultivars of tartary buckwheat originating from different regions. The levels of phenolic compounds in different parts of tartary buckwheat varied widely. Flower was found to have the highest levels of all the phenolic compounds analyzed in the analysis of the samples. Cultivars also had a great influence on the content of phenolic compounds. Cultivar-specific phenolic compound profiles might be helpful for commercial use or production of phenolic compounds.

Materials and Methods

Plant materials

Seeds of *F. tataricum* were collected from the Institute of Plant Germ-Plasm, Kyoto University, Japan and ten was stored at 4 °C. Seeds were germinated in a growth chamber, and then, the seedlings were transferred to the experimental farm at Chungnam National University, Daejeon, Korea. Roots, stems, leaves, and flowers of different cultivars of *F. tataricum* were collected at 3 months old plants for chemical analysis of phenolic compounds.

Sample preparation

Four organs (leaf, stem, root and inflorescence) of *F. tataricum* were collected, and fresh samples were dried in a freeze-dryer at -80^{o}C for at least 48 h. For getting fine powder, dried samples were ground using a mortar and pestle. Samples were extracted according to the procedure described by Kim et al., 2009. Fine ground samples were immersed in pure methanol (1:30 w/v) at 60 ^{o}C for 30 min for rutin and quercetin, and extracted twice with 80 % methanol for 1h at room temperature for the rest of the compounds. The solution was filtered through a poly filter (pore size, 0.45-μm) and then diluted 2-fold with methanol prior to analysis in high performance liquid chromatography (HPLC).

HPLC analysis

Quantification of phenolic compounds by HPLC was done with a Futecs model NS-4000 HPLC apparatus using a C18 column (250 mm × 4.6mm, 5 μm; RStech, Daejeon, Korea). The mobile phase was prepared from mixtures of acetonitrile and 0.15% acetic acid, and the column was maintained at 30℃. Phenolic compounds were detected at 280 nm with a Waters tunable absorbance detector after injection of 20 μl of the methanol solubilized extract sample. The column flow rate was 1 ml/ min with a 40 min total run time for each sample. The results were calculated using a standard curve. All samples were run or replicated thrice.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) with sums of squares partitioned to reflect trial effects using the SAS Software release 9.2 (SAS, 2010) and means were separated via Duncan Multiple Range Test (DMRT) at P = 0.05. The study concluded that the levels of phenolic compounds in different parts of tartary buckwheat varied widely. Flowers contained the highest levels of all the phenolic compounds. Such contents also varied greatly amongst the cultivars. Cultivar-specific phenolic compound profiles might be helpful for commercial use or production of phenolic compounds.

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