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Research paper

Variation of rutin and quercetin contents in Tartary buckwheat germplasm

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ABSTRACT

This study was carried out to investigate the regional variation and effects of shape and color of seeds and sprouts on the content of rutin and quercetin in Tartary buckwheat germplasm. A total of 44 foreign Tartary buckwheat germplasms were examined and compared their rutin and quercetin contents based on the collected countries, seed shape and seed color using high-performance liquid chromatography and spectrophotometry. The results revealed that rutin and quercetin content varied at different regions. Rutin content in seed (1326.5 mg/100 g) and sprouts (5440.4 mg/100 g) of the accession collected from Nepal area was higher than any accession collected from other regions. In seeds, the quercetin content showed the highest value (22.5 mg/100 g) from Pakistan whereas sprouts showed the highest quercetin content (392.0 mg/100 g) from China. However, the quercetin content in sprout was 4~90 times higher than that of seeds. Taken together, the present study suggests that sprouts could be used more effectively than seeds in the case of quercetin, and strains from Nepal, Bhutan, China, and Japan have a high potential material to use seed and sprouts for buckwheat industry in making functional food and medicine.

INTRODUCTION

Buckwheat, which belongs to the family Polygonaceae, genus Fagopyrum, has been a popular health food in Asian and European countries for a long time (Kreft, 2003). It has been widely cultivated in Korea since it has abundant nutrients and medicinal efficacy. Buckwheat sprouts are higher in nutritional value than other crops regarding fiber, anthocyanin, and rutin contents (Shin, 2010). Unlike traditional buckwheat, Tartary buckwheat remains in the wild and is most widely grown buckwheat species in the Himalaya region (Golob et al., 2015). The main cultivation areas are Tibet or Chinese mountainous regions, such as India, Bhutan and Nepal (Kreft et al., 2003). However, they are abundant in various nutrients and high in protein (12%) and fat (3.9%) content. Also, oleic acid and linoleic acid account for 80% of total fatty acids (Park et al., 2004).

Buckwheat contains many important bioactive compounds especially flavonoids that have been shown to exert excessive stress via activating antioxidants such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT) and glutathione reductase, (Park et al., 2000; Zhu et al., 1999). These compounds are complementary to the lack of enough activity due to environmental factors, eating habits, smoking and lifestyle habits, and by reactive oxygen species (ROS), and DNA damage (Lee et al., 2011; Park et al., 2011), organ and tissue damage (Shin et al., 1990; Graf et al., 2005). Flavonoids found in buckwheat, include rutin, c-glycosylflavones (orientin, isoorientin, vitexin, isovitexin), quercetin, and phenolic acid as chlorogenic acid (Margna et al., 1978; Watanabe et al., 2002), however, the content of rutin in Tartary buckwheat is much higher than that of common buckwheat and other crops (Wang et al., 1995; Park et al., 2005a; Park et al., 2005b).

However, it has been only a few years since the cultivation of buckwheat in Korea has started, and basic ecological studies have not been systematically established yet (Park et al., 2004; Lim et al., 2009). With growing demand for food and functional foods, interest in sprouting vegetables is increasing. In the United States, Europe and Australia, sprouting vegetables account for about 30% of the vegetable stores. In Asia, interest in sprouting vegetables is increasing, mainly in Japan. Sprouting vegetables account for 10 to 20% of the market. It is estimated that the sprout vegetable market in Korea is about 2 billion won in 2005 (Lee, 2007). In recent years, buckwheat sprouts have been developed in Korea and Japan (Kim et al., 2004; Kim et al., 2007), and sprouting vegetables (Hokkai T9, Hokkai T10) were registered especially in Japan in 2007 (Suzuki, 2008). In addition, the content of quercetin, as well as rutin, was also higher in Tartary buckwheat sprouts than in common buckwheat sprouts. Thus, the consumption of buckwheat sprouts as a source of rutin is increasing (Jeon, 2012).

Rutin, which is more abundant in sprouts than seeds in Tartary buckwheat, and compared than common buckwheat and has been reported to be effective against various diseases, including antioxidant activity (Lee et al., 2000), diabetes (Lee et al., 1994), antioxidative effects (Kwon et al., 1995) and the prevention of cardiovascular disease (He et al., 1995; Wojcicki et al., 1995). Previous study was conducted with buckwheat genetic resources for rutin, the comparison was made by the color of seed coat, seed type, and country of collection (Park et al., 2005).

Quercetin is a flavonoid substance belonging to the flavonol family. Quercetin is mainly found in fruits and minerals, especially in onions (Formical et al., 1995). In the United States, quercetin is also known to be a representative flavonoid (Park et al., 1991), as it is known to consume 25 mg per person per day (Lamson et al., 2000). The pharmacological effects of quercetin have been extensively studied both *in vivo* and *in vitro* models and have been shown to be associated with reduced lipid peroxidation (Cavallini et al., 1978) and decreased activity of carcinogens (Edenhader et al., 1996), hypotension, (Daniel et al., 2003) and antimicrobial effects (Kimura et al., 1984).

In recent years, attempts to search for natural antioxidants harmless to the human body have been studied in various ways (Halliwell et al., 1992; Lee, 2007). Recently, Korean people have much interest in using of Tartary buckwheat because of its higher rutin content and bio-active compounds than common buckwheat that has been traditionally utilized in Korea. However, there are no cultivars of Tartary buckwheat in Korea. So, it is urgent to develop the promising cultivars with high yield of seeds and herbs and good quality with high rutin concentration.

MATERIALS AND METHODS

1. Materials collection and plant growth condition

The experimental materials were collected from 7 countries, including China, Nepal, Bhutan, India, Japan,

Table 1. *Country-wise number of Tartary buckwheat germplasm based on seed shape and seed color used in evaluation of rutin and quercetin contents*

Origin	Seed shape				Seed color				
	Notched	*Round*	*Slender*	*Total*	*Brown*	*Dark-brown*	*Dark-gray*	*Gray-brown*	*Total*
China	5	14	9	28	5	7	8	8	28
Nepal	N/A	4	1	5	N/A	2	3	N/A	5
Bhutan	N/A	N/A	3	3	2	N/A	1	N/A	3
India	N/A	1	1	2	N/A	1	N/A	1	2
Japan	N/A	2	N/A	2	N/A	N/A	2	N/A	2
Pakistan	1	N/A	1	2	N/A	1	1	N/A	2
Slovenia	N/A	1	1	2	1	1	N/A	N/A	2
Total	**6**	**22**	**16**	**44**	**8**	**12**	**15**	**9**	**44**

**N/A=Not applicable*

Pakistan and Slovenia. A total of 44 kinds of Tartary buckwheat genetic resources were stored at Chungbuk National University (4°C, 30-40% RH). The genotypes used in the present study were 28 specimens from China, 5 specimens from Nepal, 3 specimens from Bhutan, 2 specimens from India, 2 specimens from Japan, 2 specimens from Pakistan, 2 specimens from Slovenia. However, the seed size of the genotypes were 6 specimens of notched, 22 specimens of round, 16 specimens of slender, and seed color were identified as 8 brown, 12 dark-brown, 15 dark-gray, and 9 gray-brown (Table 1).

The above-mentioned genetic resources were cultivated in Chungbuk National University Farm in 2015 and planted on July 31, 2015, maintaining planting density at 30×10 cm. Other cultivation management was provided in accordance with the crop cultivation guideline from Rural Development Administration. A total of 44 seedlings were transferred to a seedling tray (5.1 cm × 4.7 cm), and grown for 7-days in a controlled (25°C, 14 h day/10 h night, 150 $\mu mol.m^{-2}.s^{-1}$ light intensity) growth chamber (GC-300 TLH, JEIO TECH). Shoot cotyledons and hypocotyls of buds which were watered twice daily and grown under conditions of 10 hours of dark conditions. Prior to analysis, the collected buds were drying using freeze-drier (FDU-1200, EYELA). After freeze-drying for 3 days, the samples were ground into fine powder with a pestle in liquid nitrogen, and stored in a cryogenic freezer (DF8517S, Ilshinbiobase, 4°C, 30-40% RH) for rutin and quercetin analysis.

2. Rutin and quercetin analysis methods

2.1. Pretreatment for rutin and quercetin analysis

A portion (0.5 g) of Tartary buckwheat seeds and buds powder stored in a cryogenic freezer (DF8517S, Ilshinbiobase) was weighed into 15 ml tubes (BD, Falcon™). 10 ml of ethanol (96% Germany, MERCK) was added to each of the weighed tubes, and vortexed vigorously. The tubes were sonicated using an ultrasonic clearer (SD-D300H, Seongdong) for 60 minutes at 80°C and then cooled in a refrigerated shell (IBK-1400RFD, Infobiotech) for 60 minutes at 4°C. The mixture was centrifuged at 5000 rpm for 5 min at 4ºC. 2 ml of the extracted sample was filtered using a 0.45 μm PVDF membrane syringe filter (Whatman, USA) and analyzed by HPLC (Agilent 1200 series) instrument with 3 repetitions. The contents of rutin and quercetin were expressed on dry weight basis. The content of both investigated compounds was expressed in mg/100 g DW.

2.2. HPLC analysis method

Methanol, acetonitrile, and water (Honeywell B & J) used in HPLC analysis were HPLC grade. For the solvent A, 0.05% TFA buffer (Sigma) was added to a total volume of 100% water. The solvent B was prepared with 60% of methanol, 40% of acetonitrile, 0.05% of TFA buffer respectively.

All analyses for rutin and quercetin were performed on a HPLC system (HPLC 1200 series manufactured

Table 2. *Analytical conditions of HPLC*

Gradient Time (min)	Mobile phase condition	
	A (%): water + TFA (0.05%)	B (%): MeOH (60%) + ACN (40%) + TFA (0.05%)
0	90.0	10.0
5	85.0	15.0
50	60.0	40.0
60	50.0	50.0
61	90.0	10.0

by Agilent), equipped with a column used YMC-pack OSD-AC18 (4.6 mm ID × 250 mm, S-5 μm, YMC Co., LTD., Japan). The flow rate was 1.0 ml/min, the column temperature was 25°C, the injection volume was 10 μl, and the detection wavelength was set at 359 nm. The detailed HPLC analysis conditions are shown in Table 2. For the quantitative analysis of rutin and quercetin, the standards were made according to the protocol of Sigma, and the concentrations used in this analysis were 1, 2, 5, 10, 20, 50, 100 and 200 ppm. The pattern and retention time of rutin and quercetin observed in HPLC apparatus are shown in Fig. 1, and the equation of linear regression of rutin and quercetin y=14.993x-62.119 y=32.943x-19.09 respectively while the coefficient of determination (R^2) was 0.9989 and 0.9999 in rutin and quercetin respectively.

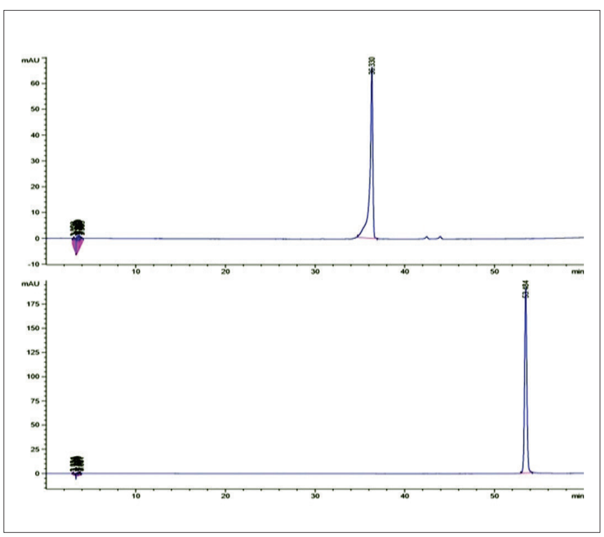

Figure 1. *HPLC chromatogram patterns and retention time of rutin & quercetin*

3. Statistical processing analysis

Analysis of variance was performed using SAS software (SAS Institute Inc., ver. 9.2). The significance test for the rutin and quercetin content of each country, seed shape, and seed color was performed using Duncan's multiple range tests. Also, the correlation between rutin and quercetin content was investigated within the seeds, within the shoots and between the seeds and sprouts.

RESULTS AND DISCUSSION

4. Variation of rutin and quercetin content in seed

4.1. Content, distribution and resource selection of rutin and quercetin in seed

Among the 44 kinds of Tartary buckwheat genetic analyses, the average content of the two components (sum of rutin and quercetin) was 815.4 mg/100 g, and the all investigated genetic samples ranged from 308.3 to 1337.0 mg /100 g. In terms of each component analysis, the average content of rutin was 808.4 mg /100 g, the in all genetic samples ranged from 304.5 to 1326.5 mg/100 g. On the other hand, the average content of quercetin was 7.0 mg /100 g, and in all genetic samples ranged from 2.0 to 22.5 mg /100 g. However, the results obtained from the present study revealed that the content of rutin was higher compared to quercetin regarding all investigated samples.

The distribution of rutin and quercetin contents in the tested buckwheat samples is shown in Fig. 2. The highest distribution of rutin (16 germplasms) was observed in the range of 600~800 mg/100 g followed by 12 germplasms in the range of 800~1000 mg/100 g, 7 germplasms in the range of 600 mg/100 g and 6 ger-

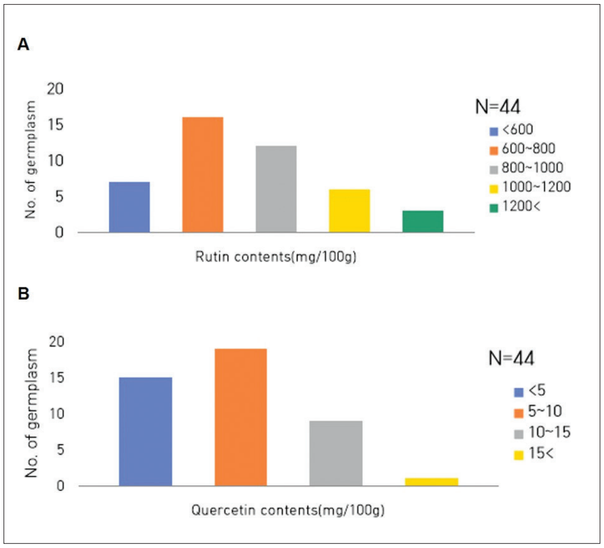

Figure 2. *Frequency distributions of rutin (A) and quercetin (B) contents in seed of Tartary buckwheat germplasm*

mplasms in the range of 1000~1200 mg/100 g respectively. However, the lowest distribution (3 germplasms) of rutin was observed in the range of 1200 mg/100 g. In the case of quercetin, the highest (19 germplasms) was observed in the range of 5~10 mg / 100 g, followed by 15 germplasms with less than 5 mg/100 g, and 9 germplasms with the range of 10~15 mg/100 g.

Table 3 summarizes the rutin and quercetin content, seed shape and seed color properties among the tested resources. The highest content of rutin (1326.0 mg/ 100 g) was found in CBU408 (Collected from Nepal), whereas the highest content of quercetin (22.5 mg/ 100 g) was observed in CBU456 (Collected from Pakistan). Taken together, the results obtained from the present study revealed that the quercetin exhibited the highest CV among the component tested in this study.

4.2. Country-wise variation of rutin and quercetin content of seeds

The total amount of the two components in seeds was measured and compared in this study. The highest total amount of the tested components (sum of rutin and quercetin) was 1002.6 mg / 100 g, followed by 854.0 mg/100 g, 838.9 mg/100 g, 813.6 mg/100 g, 802.6 mg/100 g, 715.0 mg in the order of the following manner Nepal> Bhutan> Japan> China> Pakistan> Slovenia> India. But no significant difference was observed among the germplasms (Table 4). Rutin was also found to be in the same order as the total amount of the two components. However, the total amount of rutin was in the order of 995.1 mg/100 g in Nepal, 845.2 mg / 100 g in Bhutan, 835.7 mg/100 g in Japan, 806.9 mg/100 g in China, 788.3 mg/100 g in Pakistan, 396.0 mg/100g in India. The data shown in Table 4 showed that there was no significant difference among the data examined. Quercetin showed different results compared than the rutin findings; however, the quercetin contents were 14.3 mg/100 g, 8.7 mg/100g, 7.5 mg/100 g, 7.4 mg/100 g, 6.7 mg/100 g, 3.12 mg/100 g in the order of the following manner; Pakistan> Bhutan> Nepal> India> China> Japan> Slovenia (Table 5). However, the quercetin exhibited the highest CV among the components tested in this study.

Park et al. (2005) reported that rutin content was in the order of Bhutan> Slovenia> China> Pakistan> Nepal> Japan> India. Taken together, the obtained results suggest that the various concentrations of components may provide important insights regarding the development of functional components in buckwheat.

4.3. The content of rutin and quercetin in seeds regarding seed shape

Table 5 summarizes the rutin and quercetin contents in seeds regarding seed shape. The highest total amount

Table 3. *Statistical analysis of rutin and quercetin content in seed of Tartary buckwheat germplasm*

	Statistics	Rutin (mg/100g)	Quercetin (mg/100g)	Total (mg/100g)
Tartary buckwheat germplasm (Seed)	Max.	1326.5	22.5	1337
	Min.	304.5	2	308.3
	Mean	808.4	7	815.4
	±SD	243.4	3.9	245.2
	CV (%)	30.1	55.9	30.1

Table 4. *Statistical analysis of rutin and quercetin content in seed of Tartary buckwheat germplasm based on country of origin*

Location	Statistics	Rutin (mg/100g)	Quercetin (mg/100g)	Total (mg/100g)
Bhutan (n=3)	Max.	911.5	10.2	920.3
	Min.	753.8	7.2	764
	Mean	845.2	8.7	854
	±SD	81.8	1.5	80.8
	CV (%)	9.7	17.2	9.5
China (n=28)	Max.	1247.5	13.7	1259
	Min.	364.2	2	374.3
	Mean	806.9	6.7	813.6
	±SD	236.9	3	238.6
	CV (%)	29.4	45.2	29.3
India (n=2)	Max.	487.5	10.8	498.4
	Min.	304.5	3.9	308.3
	Mean	396	7.4	403.4
	±SD	129.4	4.9	134.4
	CV (%)	32.7	67.2	33.3
Japan (n=2)	Max.	902.4	3.77	906.2
	Min.	769.1	2.47	771.5
	Mean	835.7	3.12	838.9
	±SD	94.3	0.92	95.2
	CV (%)	11.3	29.4	11.3
Nepal (n=5)	Max.	1326.5	10.5	1337
	Min.	583	3	586.3
	Mean	995.1	7.5	1002.6
	±SD	321.5	4	325.1
	CV (%)	32.3	53	32.4
Pakistan (n=2)	Max.	901.3	22.5	923.8
	Min.	675.2	6.2	681.4
	Mean	788.3	14.3	802.6
	±SD	159.9	11.5	171.4
	CV (%)	20.3	80.3	21.4
Slovenia (n=2)	Max.	798.6	3.5	802.1
	Min.	625.4	2.6	628
	Mean	712	3.1	715
	±SD	122.5	0.6	123.1
	CV (%)	17.2	20.7	17.2
***p*-value**		**0.1593**	**0.0508**	**0.1659**

Table 5. *Statistical analysis of rutin and quercetin content in seed of Tartary buckwheat germplasm based on seed shape*

Seed shape	Statistics	Rutin (mg/100g)	Quercetin (mg/100g)	Total (mg/100g)
Notched (n=6)	Max.	1028.7	22.5	1038.7
	Min.	420.1	2.6	422.7
	Mean	736.8	9.1	745.9
	±SD	216	7	220.6
	CV (%)	29.3	76.8	29.6
Round (n=22)	Max.	1326.5	13.7	1337
	Min.	364.2	2.5	374.3
	Mean	816	6.8	822.8
	±SD	258.9	3.3	260.4
	CV (%)	31.7	48.2	31.6
Slender (n=16)	Max.	1247.5	11.5	1259
	Min.	304.5	2	308.3
	Mean	824.8	6.4	831.2
	±SD	240.8	3.2	242.8
	CV (%)	29.2	49.8	29.2
***p*-value**		**0.7447**	**0.3587**	**0.7608**

of the sum of these two components were 831.2 mg/100 g, followed by 822.8 mg/100 g, and 745.9 mg/100 g in the order of Slender> Round> Notched. The rutin contents were the similar as the total amount. However, the highest rutin (824.8 mg/100 g) was observed from slender shape seed followed by round shape (816.0 mg/100 g), and notched shape (736.8 mg/100 g). In the case of quercetin, pronounced results were observed from notched shaped seed (9.1 mg/100 g) followed by round (6.8 mg/100 g), and slender (6.4 mg/100 g) respectively in the order of Notched> Round> Slender. However, the quercetin exhibited the highest CV among the component tested in this study.

A previous study reported that seed shape played a crucial role in the variation of rutin content in the Tartary buckwheat genetic resources (Park et al., 2005). However, they found that the rutin contents were in the order of Slender> Notched> Round, wherein the results showed differences compared than that of the present study.

4.4. Variation of rutin and quercetin content regarding seed color

Table 6 summarizes the rutin, quercetin and total amount of these two components in grain seeds considering seed color. The total contents of the two components were measured according to their seed color. However, the highest content was exhibited from the dark-gray (865.3 mg/100 g), followed by dark-brown (806.3 mg/100 g), gray-brown (778.7 mg/100 g), and brown (776.5 mg/100 g) respectively. Rutin content showed the similar results as the total amount of the two components. However, the pronounced rutin content was observed from the dark-gray (858.6 mg /100 g), followed by dark-brown (799.0 mg/100 g), gray-brown (771.5 mg/100 g) and brown (769.7 mg/100 g). The highest content of quercetin (7.4 mg/100 g) was observed from dark-brown, followed by gray-brown (7.2 mg/100 g), gray-brown and brown (6.7 mg/100 g) respectively. However, the quercetin exhibited the highest CV among the components tested in this study.

Table 6. *Statistical analysis of rutin and quercetin content in seed of Tartary buckwheat germplasm based on seed color*

Seed color	Statistics	Rutin (mg/100g)	Quercetin (mg/100g)	Total (mg/100g)
Brown (n=8)	Max.	1184.9	11.3	1196.2
	Min.	364.2	2.6	374.3
	Mean	769.7	6.7	776.5
	±SD	308.4	3.3	309.4
	CV (%)	40.1	49	39.8
Dark-brown (n=12)	Max.	1326.5	22.5	1337
	Min.	304.5	2	308.3
	Mean	799	7.4	806.3
	±SD	239.6	5.5	242.1
	CV (%)	30	75.1	30
Dark-gray (n=15)	Max.	1275.1	13.7	1285.3
	Min.	583	2.5	586.3
	Mean	858.6	6.7	865.3
	±SD	180.1	3.1	181.9
	CV (%)	21	46.8	21
Gray-brown (n=9)	Max.	1247.5	11.5	1259
	Min.	419	2.3	421.5
	Mean	771.5	7.2	778.7
	±SD	302.6	3.6	305.1
	CV (%)	39.2	49.5	39.2
***p*-value**		**0.7974**	**0.9704**	**0.8029**

A previous study reported that seed color played an essential role in the variation of rutin and quercetin content in the Tartary buckwheat genetic resources (Park et al., 2005). Taken together, the results obtained from the present study revealed that seed color may provide insights in the variation of rutin and quercetin content in Tartary buckwheat. The variation of the content of rutin in Tartary buckwheat samples showed in the order of Dark-gray> Dark-brown (Dark)> Gray-brown> Brown.

5. Variation of rutin and quercetin content in sprout

5.1. Contents, distribution and resource selection of rutin and quercetin in sprout

The amount of rutin (3362.9 mg/100 g) was observed from the sprout with the total genetic resources ranges from 328.8 to 5440.4 mg/100 g whereas the average content of quercetin was 143.2 mg/100 g with the total genetic resources ranged from 53.5 to 392.0 mg/100 g. The results revealed that the content of rutin was overwhelmingly higher than that of quercetin. Considering the genetic resources between seed and sprout, rutin was increased by 0.5~10.5 times and quercetin was increased by 3.7~90.7 times.

The distribution of rutin and quercetin content in the sprouts of 44 buckwheat genetic resources is shown in Fig. 3. The highest distribution of rutin (16 germplasms) was observed in the range of 3000~4000 mg/100 g fol-

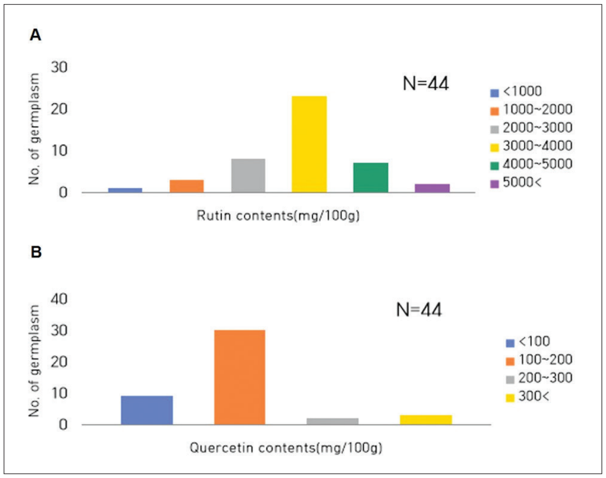

Figure 3. *Frequency distributions of rutin (A) and quercetin (B) contents in sprout of Tartary buckwheat germplasm*

lowed by 8 germplasms in the range of 2000~3000 mg/100 g, 7 germplasms in the range of 4000~5000 mg/100 g and 6 germplasms in the range of 1000~1200 mg/100 g, and 3 germplasm in the range of 1000~2000 mg/100 g respectively. However, the lowest rutin frequency distribution (1 germplasm) was observed from less than 1000 mg/100 g. In the case of quercetin, the range of 100 ~ 200 mg/100 g showed the highest frequency (30 germplasms), followed by less than 100 mg/100 g (9 germplasms), and more than 300 mg/100 g (3 germplasms) respectively, while the lowest frequency (2 germplasms) was observed in the range of 200~300 mg/100 g.

Table 7 summarizes the rutin quercetin contents of the sprouts regarding location, seed shape and seed color. The highest rutin amount (5440.4 mg/100 g) was obtained from CBU460 in Nepal with round shaped and dark-gray color seeds while the lowest amount (1462.4 mg/100 g) was obtained from CBU302 in China with round shaped brown color seeds. On the other hand, the highest quercetin contents (392.0 mg/100 g) was obtained from CBU302 in China with round shaped brown color seeds CBU302 and the lowest amount of quercetin (268.0 mg/100 g) was obtained from CBU460 in Nepal.

5.2. Country-wise variation of rutin and quercetin content in sprouts

Table 8 shows the rutin and quercetin amount in sprouts and their statistical analysis regarding different countries of the world. Nepal showed the highest amount of rutin (4205.0 mg/100 g), followed by Slovenia (3684.3 mg/100 g), Japan (3662.9 mg/100 g), Pakistan (3402.3 mg/100 g), China (3337.7 mg/100 g), Bhutan (3108.8 mg/1682.3 mg/100 g). In the case of quercetin content, Slovenia showed the highest amount (191.1 mg/100 g), followed by Nepal (165.4 mg/100 g), Japan (161.5 mg/100 g), India (145.1 mg/100 g), Bhutan (130.9 mg/100 g), Pakistan (130.9 mg/100 g) in the order of Nepal> Japan> India> China> Bhutan> Pakistan. However, no significant differences were observed among the components.

5.3. Variation of rutin and quercetin contents in sprouts regarding seed shape

Table 9 shows the rutin and quercetin contents from sprouts according to their various seed shape. The slender shaped seeds showed the highest amount of rutin (3890.2 mg/100 g), followed by round (3399.8 mg/100 g), and notched (2871.9 mg/100 g) in the order of Slender> Round> Notched. In the case of quercetin, the highest amount of quercetin was observed from the round shape seed (158.1 mg/100 g), whereas the lowest amount of quercetin (125.8 mg/100 g) was observed from the slender shaped seed. However, the amount of quercetin was in the order of Round> Notched> Slender.

5.4. Variation of rutin and quercetin contents in sprouts regarding seed color

Table 10 shows the rutin and quercetin contents of the seeds in the sprouts according to seed color. The highest rutin content (3565.0 mg/100 g) was obtained from the dark-gray color seed, followed by dark-brown

Table 7. *Accessions with highest total content of rutin and quercetin in sprout*

Line number	Location	Seed shape	Seed color	Rutin (mg/100g)	Quercetin (mg/100g)	Total (mg/100g)
CBU 460	Nepal	Round	Dark-gray	5440.4	268	5708.4
CBU 302	China	Round	Brown	1462.4	392	1854.4

Table 8. *Statistical analysis of rutin and quercetin content in sprout of Tartary buckwheat germplasm according to country of origin*

Location	Statistics	Rutin (mg/100g)	Quercetin (mg/100g)	Total (mg/100g)
Bhutan (n=3)	Max.	3160.2	140.3	3300.5
	Min.	3039.3	121.6	3170.1
	Mean	3108.8	130.9	3239.8
	±SD	62.5	9.3	65.6
	CV (%)	2	7.1	2
China (n=28)	Max.	5214	392	5273.5
	Min.	328.8	53.5	633.9
	Mean	3337.7	139.3	3477
	±SD	1048.5	70.1	998.2
	CV (%)	31.4	50.3	28.7
India (n=2)	Max.	1822.7	176	1998.7
	Min.	1542	114.1	1656.1
	Mean	1682.3	145.1	1827.4
	±SD	198.5	43.8	242.3
	CV (%)	11.8	30.2	13.3
Japan (n=2)	Max.	3825.7	228.7	3919.9
	Min.	3177.2	94.2	3405.9
	Mean	3501.4	161.5	3662.9
	±SD	458.5	95.1	363.5
	CV (%)	13.1	58.9	9.9
Nepal (n=5)	Max.	5440.4	268	5708.4
	Min.	3313	122.8	3435.9
	Mean	4205	165.4	4370.4
	±SD	841.2	59.6	893
	CV (%)	20	36.1	20.4
Pakistan (n=2)	Max.	4088.6	102.6	4191.3
	Min.	2716.1	83.6	2799.7
	Mean	3402.3	93.1	3495.5
	±SD	970.6	13.4	984
	CV (%)	28.5	14.4	28.2
Slovenia (n=2)	Max.	3603.8	317.9	3700.5
	Min.	3382.6	64.3	3668.1
	Mean	3493.2	191.1	3684.3
	±SD	156.4	179.3	22.9
	CV (%)	4.5	93.9	0.6
***p*-value**		**0.1406**	**0.8467**	**0.1038**

Table 9. *Statistical analysis of rutin and quercetin content in sprout of Tartary buckwheat germplasm based on seed shape*

Seed shape	Statistics	Rutin (mg/100g)	Quercetin (mg/100g)	Total (mg/100g)
Notched (n=6)	Max.	3781.6	179.4	3919.4
	Min.	2242.1	83.6	2378.5
	Mean	2736.6	135.2	2871.9
	±SD	537.6	37.2	536.5
	CV (%)	19.6	27.5	18.7
Round (n=22)	Max.	5440.4	392	5708.4
	Min.	328.8	64.3	633.9
	Mean	3241.7	158.1	3399.8
	±SD	1058.7	79.2	1024.1
	CV (%)	32.7	50.1	30.1
Slender (n=16)	Max.	5214	317.9	5273.5
	Min.	1542	53.5	1656.1
	Mean	3764.4	125.8	3890.2
	±SD	930.2	60.7	912.7
	CV (%)	24.7	48.3	23.5
***p*-value**		**0.0701**	**0.3532**	**0.0686**

(3558.1 mg/100 g), brown (3141.6 mg/100 g) and gray-brown (2962.3 mg/100 g) in the order of Dark-gray> Dark-brown (Dark)> Brown> Gray-brown. On the other hand, brown color seeds exhibited the highest amount of quercetin (156.0 mg/100 g), followed by gray-brown (147.4 mg/100 g), dark-gray (140.9 mg/100 g), and dark-brown (134.5 mg/100 g) in the order of Brown> Gray-brown> Dark-gray> Dark-brown.

The correlation between rutin and quercetin in seeds is shown in Fig. 4. The obtained results demonstrated that the content of quercetin was positively correlated (r = 0.4530, *p*-value = 0.002) with the content of rutin. As a result, the results suggest that both rutin and quercetin selection criteria may provide crucial insights for developing varieties. Fig. 5 shows the correlation between rutin and quercetin in sprouts. The content of quercetin

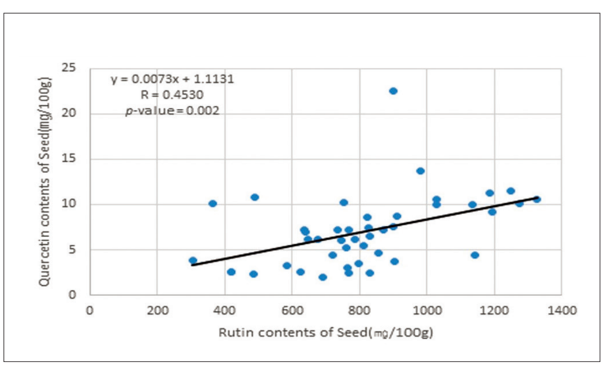

Figure 4. *Correlation between rutin and quercetin contents in seed of Tartary buckwheat germplasm*

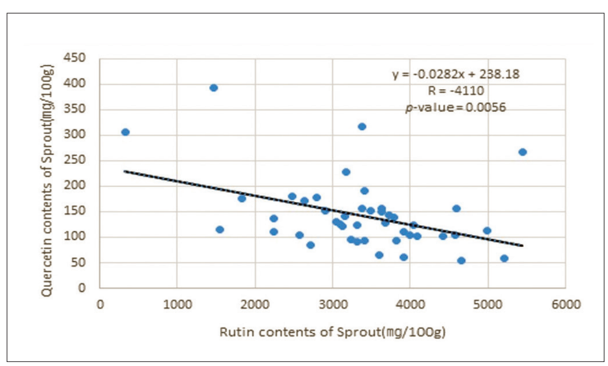

Figure 5. *Correlation between rutin and quercetin contents in sprout of Tartary buckwheat germplasm*

Table 10. Statistical analysis of rutin and quercetin content in sprout of Tartary buckwheat germplasm based on seed color

Seed color	Statistics	Rutin (mg/100g)	Quercetin (mg/100g)	Total (mg/100g)
Brown (n=8)	Max.	4660.4	392	4714
	Min.	1462.4	53.5	1854.4
	Mean	3141.6	156	3297.6
	±SD	925.4	105.2	830.1
	CV (%)	29.5	67.5	25.2
Dark-brown (n=12)	Max.	5214	317.9	5273.5
	Min.	1542	59.5	1656.1
	Mean	3558.1	134.5	3692.7
	±SD	1118.4	65	1112.1
	CV (%)	31.4	48.3	30.1
Dark-gray (n=15)	Max.	5440.4	268	5708.4
	Min.	2245.9	91.7	2355.5
	Mean	3565	140.9	3705.9
	±SD	708.1	51.7	730.9
	CV (%)	19.9	36.7	19.7
Gray-brown (n=9)	Max.	4414.9	305.1	4517.2
	Min.	328.8	60.9	633.9
	Mean	2962.3	147.4	3109.6
	±SD	1300.9	70.8	1239.8
	CV (%)	43.9	48.1	39.9
***p*-value**		**0.4204**	**0.9213**	**0.4165**

in the sprout was high (r = -4110, *p*-value = 0.0056) compared to rutin. The amount of quercetin is decreased as the amount of rutin produced when the seeds germinate, and sprouts come out as a typical quercetin glycoside (Lee et al., 2013). As a result, it would be useful to develop a variety containing high content of each component based on the criteria of rutin or quercetin using sprouts. To this end, the present study postulated that sprout would be a great choice to develop a cultivar with a high rutin and quercetin content.

Fig. 6 shows the correlation between rutin content in seed and sprouts of Tartary buckwheat germplasm. The rutin content in the seeds was found to be increased with increasing the rutin content in the sprouts (R = 0.3552, *p*-value = 0.018). However, the content of quercetin in the seeds and the sprouts showed no correlation (R = 0.0169, *p*-value = 0.9134). However, the use of quercetin would be a potential choice for utilization because it increases 3.7~90.7 times in sprout compared to seeds (Fig. 7).

CONCLUSION

The present study was carried out to investigate the variation of rutin and quercetin contents in seeds and sprouts of 44 buckwheat genetic resources for the development of high-quality Tartary buckwheat. Rutin and quercetin content varied at different regions from which each accession was collected. Rutin content in seed and sprouts of the accession collected from Nepal area was higher than any accession collected from other regions.

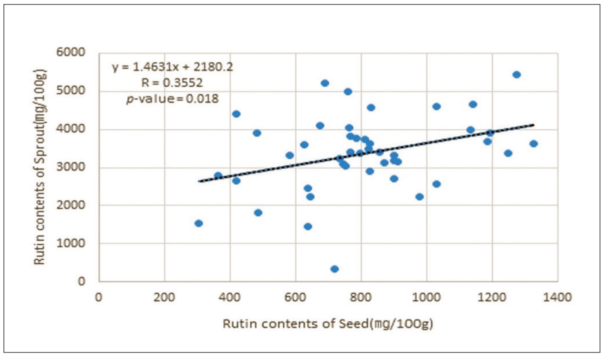

Figure 6. *Correlation between rutin contents in seed and sprout of Tartary buckwheat germplasm*

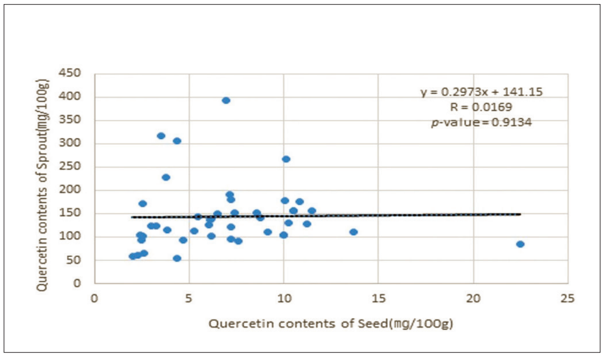

Figure 7. *Correlation between quercetin contents in seed and sprout of Tartary buckwheat germplasm*

Accession collected from Pakistan showed lower rutin content in seeds compared to the accession collected from other regions. On the other hand, quercetin content showed the opposite results in the seeds. In the case of sprout, accession collected from Nepal showed highest rutin content compared than that of accession collected from other regions. Interestingly, the China showed the highest quercetin contents in sprouts. High rutin in seeds and sprouts of Tartary buckwheat indicates that these two components would be great materials for buckwheat industry in making functional food and medicine.

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IZVLEČEK

Namen raziskave je bil proučiti geografsko pogojeno raznolikost in povezavo oblike in barve zrn in kalic z vsebnostjo rutina in kvercetina, z uporabo zbirke semen tatarske ajde. Raziskano je bilo 44 tujih vzorcev glede na obliko in barvo zrn in in glede na rezultate analiz s HPLC. Vsebnost rutina in kvercetina je bila različna pri vzorcih z različnih območij. Pri vzorcih iz Nepala sta bili najvišji vsebnosti rutina v zrnih (1326,5 mg/100 g) in v kalicah (5440,4 mg/100 g). Pri vzorcih zrn iz Pakistana je bilo rutina največ 22,5 mg/100 g in v kalicah iz Kitajske največ kvercetina (392,0 mg/100 g). V primerjavi z zrni je bilo v kalicah 4~90-krat več kvercetina. Ta raziskava kaže, da so kalice v primerjavi z zrni lahko pomembnejši vir kvercetina, vzorci iz Nepala, Butana, Kitajske in Japonske so obetavni vir pri pridelavi ajde za funkcijsko hrano in zdravila.