RESEARCH ARTICLE

Evaluation of *Fagopyrum esculentum* Moench germplasm based on agro-morphological traits and the rutin and quercetin content of seeds under spring cultivation

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Abstract Buckwheat is an important grain crop known for its nutritional value and content of bioactive compounds, particularly rutin. In this study, we characterized diverse *Fagopyrum esculentum* Moench germplasm for the diversity of agro-morphological characteristics and the rutin and quercetin content in seeds under the spring cultivation. Of the 251 germplasm accessions assessed, 193 had red stems, 182 had a pale brown seed coat color, and 238 had ovate seeds. The times taken to reach 50% flowering and 80% maturity ranged from 38–45 to 73–95 days, respectively. The 1000-seed weight (TSW) varied from 21 to 42 g. Overall, the content of rutin and quercetin ranged from 7.22–47.86 to 0–1.22 mg/100 g DW, respectively. The number of days to flowering and maturity showed negative correlations with TSW and rutin and quercetin content. However, we found that at 73–80 days after sowing, early-maturing germplasm had significantly higher mean rutin content than either intermediate-or late-maturing germplasm.

The TSW weight showed a positive correlation with the content of rutin and quercetin. We identified promising accessions based early maturity (< 80 days), higher seed weight (≥ 35 g) and higher content of rutin (≥ 35 mg/100 g DW) and quercetin (≥ 1 mg/100 g DW). These accessions will help to enhance grain yield and the rutin and quercetin content in existing buckwheat cultivars for spring cultivation.

Keywords Agro-morphological traits · Evaluation · *Fagopyrum esculentum* germplasm · Quercetin · Rutin

Introduction

Buckwheat is a dicotyledonous grain crop plant in the family Polygonaceae, which is known for its high nutritional value and bioactive components, particularly the content of rutin and quercetin. Two commonly grown buckwheat species are *Fagopyrum esculentum* Moench (common buckwheat) and *Fagopyrum tataricum* Gaertn (tartary buckwheat). Interestingly, although its common name includes the word “wheat,” these plants are taxonomically unrelated to the traditionally cultivated wheat and are generally considered pseudo-cereals (Joshi and Rana 1995). Buckwheat has a comparatively rapid life cycle and reaches maturity within 3–4 months (Arduini et al. 2016). Moreover, it shows considerable adaptability to diverse ecological environments and can be

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grown on a wide range of soils under various climatic conditions (Li and Zhang 2001). It is grown worldwide throughout Asia, Europe, the United States (USA), Brazil, Canada, Australia, and South Africa (Kiproviski et al. 2015), although the main areas of cultivation are in East Asian countries (Wiczowski et al. 2014). Tartary buckwheat is mainly cultivated in Asia, whereas common buckwheat is grown more extensively in virtually all grain crop-producing countries (Chen et al. 2018).

Buckwheat is a gluten-free grain crop that can be used in the diets of patients with celiac disease (Mazza and Oomah 2005; Sytar et al. 2016). In addition, compared with other cereals, buckwheat is characterized by a relatively higher nutritional value. Biochemical analyses of buckwheat germplasm have indicated that seeds of these plants are an excellent source of starch, proteins, fats, dietary fiber, vitamins, minerals, and phenolic components (Bobkov 2016). The protein content in buckwheat is higher than other cereals (Sytar et al. 2018), and buckwheat proteins are particularly rich in lysine, arginine, and aspartic acid (Bobkov 2016). Lysine plays an important role in protein biogenesis but cannot be synthesized by mammals (Tome and Bos 2007), and therefore must be obtained from alternative dietary sources (Yu and Tian 2018). Although cereals are typically deficient in lysine (Ferreira et al. 2005), the groats of buckwheat contain reasonable amounts of lysine (Rout and Chrungoo 1999; Sytar et al. 2016). Moreover, in comparison with the major cereal crops, buckwheat also contains a considerable quantity of flavonoids. Six flavonoids have been identified in buckwheat, namely, rutin, orientin, vitexin, quercetin, isovitexin, and isoorientin, of which rutin, also known as vitamin P, is the major flavonoid (Park et al. 2000, 2004; Ghiasi et al. 2012; Bobkov 2016; Sytar et al. 2016; Ganeshpurkar and Saluja 2017). Rutin has beneficial effects on cardiovascular diseases, and notably reduces the risk of arteriosclerosis (Kreft et al. 2003). Of the two most widely cultivated buckwheats, tartary buckwheat has higher rutin content than common buckwheat (Chauhan et al. 2010; Gupta et al. 2012); however, on account of its bitter taste, tartary buckwheat is rarely consumed. Korea, China, and Japan are the main growing areas of common buckwheat due to its use as a functional food (Kim et al. 2001; Jung et al. 2015).

Flavonoids are secondary metabolites that comprise a large group among the polyphenolic components of plants (Mathesius 2018) and are known to play important roles in the growth and development of plants and in protection against UV light and diseases (Panche et al. 2016; Vicente and Boscaiu 2018). Differences in the morphology and coloration of plant parts, such as the color of flowers, stems, leaves, and seeds and the shape of seeds, can serve as valuable diagnostic traits for the selection of genotypes with higher levels of phenolic components (Klykov et al. 2016). Research of various buckwheat species have shown that color visual assessment of the vegetative parts is a marker for selection of buckwheat genotypes with high anthocyanin and content of rutin (Sytar et al. 2016). However, variations in the color of plant vegetative organs and content of flavonoids often depend upon variety, area of collection, and ecological conditions (Fabjan et al. 2003; Podolska 2016). Successful crop improvement requires a continuous supply of genetic diversity that includes novel or enhanced variability (Dwivedi et al. 2016). In this regard, germplasm collections and the genetic diversity available in various buckwheat germplasms could represent valuable sources of material for altering certain specific aspects of plant metabolic chemistry (Matsuri et al. 2008).

Despite the high nutritional value of buckwheat, there has to date been limited research on the morphological diversity of common buckwheat germplasm associated with flavonoid content. Buckwheat shows a wide diversity in growth traits related to seed size and shape, seed coat and flower color, grain yield, and the content of phenolic components. Flavonoid contents could vary based on varietal variability (Oomah et al. 1996; Kalinová and Dadáková 2006; Brunori et al. 2009; Qin et al. 2010; Kiproviski et al. 2015; Raina and Gupta 2015), sowing time (Omidbaigi and Mastro 2004; Jinfeng et al. 2010; Hyun et al. 2018), location (Bai et al. 2015), growth stages (Bystricka et al. 2014), environmental factors such as UV radiation, light (Suzuki et al. 2005; Kim et al. 2006; Nam et al. 2018) and area of collection (Seo et al. 2013; Bai et al. 2015; Yu et al. 2019). Oomah et al. (1996) reported that variation in phenolic acids in buckwheat is mainly due to cultivar, seasonal effects, and their interaction, whereas growing location has no effect. Cultivation season is an important factor determining dry matter production, as different

buckwheat genotypes show differing responses to environmental change, and shift their growth from vegetative to reproductive in response to specific environmental cues. In Korea, most of the cultivated buckwheat varieties are of the fall type and do not set seed when planted in the spring season. Given that grain yield is generally lower in the spring season than in fall cultivation, Hyun et al. (2018) reported a 26% reduction in seed weight and a 51% reduction in rutin content under spring cultivation as compared to fall cultivation. Despite the numerous studies on buckwheat, there is still a lack of information on the comparison of morphological traits and flavonoid content of common buckwheat from a different origin. Therefore, it would be desirable to determine whether diverse germplasm sources could be exploited to enhance cultivated buckwheat grain yields and flavonoid contents in the spring season. Thus, in this study, we aimed to characterize diverse common buckwheat germplasm with respect to agro-morphological traits and the major flavonoids components (rutin and quercetin) during the spring season and to study the relationships between agro-morphological traits and the content of rutin and quercetin in seeds.

Materials and methods

In this study, we evaluated 251 common buckwheat germplasm accessions held in the collection of the National Genetic Resource Center (the RDA gene bank), Rural Development Administration (RDA), Jeonju, The Republic of Korea. The germplasm collection includes 208 accessions from Korea, 10 from Japan, 9 from Russia, 6 from China, 4 from both Pakistan and Ukraine, 3 from both the Czech Republic and India, and 1 from each of Belarus, Bhutan, Canada, and Nepal (Table 1). Two Korean commercial common buckwheat varieties “Yangjeol-2” and “Dawon” were included as a control. During the spring season, 30 seeds per accessions were sown using drill planting methodology in the field of the National Institute of Agricultural Sciences, RDA, Jeonju, maintaining the crop density at 20 cm × 40 cm. The field was fertilized with N: P: K @ 21: 17: 17 kg 10 a⁻¹, respectively. For other cultural managements practices, the recommended cultivation method of RDA was followed (RDA 2012a, b). At the time of flowering, a net-house was installed for

each accession and 3 bumblebees per net-house were release to facilitate pollination. The germplasm was evaluated in terms of agro-morphological traits and the content of rutin and quercetin of seeds.

Evaluation of agro-morphological traits

Morphological characterization of germplasm was performed based on stem color, seed coat color, and seed shape. Stem color was recorded for green, pale green, pale red, red and dark red. Seed coat color was recorded for white, grey, pale brown, dark brown and black. Seed shape was recorded for Oval, egg-shaped (Ovate), triangular-type and winged-form (RDA 2012a, b). Agronomic characterization was performed based on the number of days to flowering, number of days to maturity, and 1000-seed weight (TSW). Days to flowering were counted from the date of sowing to the stage where over 50% of flowers had opened, whereas days to maturity were counted from the date of sowing to 80% seed pods turned into black color. One hundred seeds were randomly selected from bulked seeds of each plot, weighed after drying and multiplied by 10 to calculate TSW (ISTA 2019). The TSW was measured in three biological replicates.

Evaluation of the rutin and quercetin content of seeds

Reagents

All chemicals used in this study were of HPLC grade. Methanol (MeOH), ethanol (EtOH), and acetonitrile (CH₃CN) were purchased from Thermo Fisher Scientific (Seoul, South Korea), HPLC-Grade water was purchased from Avantor Performance Materials (Suwon-Si, South Korea). Flavonoid standards (rutin and quercetin) at purity of > 99% HPLC grade were purchased from Extra Synthase (Genay, France).

Extraction of flavonoid compounds

We prepared 2 samples per genotype for HPLC analysis. For each sample, seeds from 10 randomly selected plants were ground into a fine powder, of which 100 mg sample was added to 2-mL microcentrifuge tubes and extracted with 1 mL of MeOH containing 10% phosphoric acid [0.1% (v/v)]. The mixture was vigorously vortexed for 5 min at room

Table 1 List of common buckwheat (*Fagopyrum esculentum*) accessions used in this study

Country ^a	No. of accessions	Accession number
KOR	208	100801, 100891, 100906, 100973, 101006, 101022, 101091, 101120, 101271, 101282, 101389, 101391, 101403, 101431, 102359, 102780, 102854, 103026, 103069, 103093, 103119, 103136, 103351, 103569, 103582, 103633, 103710, 103731, 103836, 103881, 104139, 104236, 104328, 104461, 104464, 104526, 104551, 104769, 105304, 105325, 105398, 105453, 105506, 105543, 105649, 105699, 105731, 105742, 105912, 105954, 105968, 105997, 108702, 108752, 108757, 108852, 108885, 108889, 108892, 108968, 109063, 109095, 109106, 109155, 109162, 109175, 109564, 109601, 110978, 111016, 111123, 112800, 112812, 112911, 112949, 113084, 113087, 113123, 113126, 113296, 113420, 113458, 113582, 115191, 118993, 118994, 119936, 119990, 121461, 134978, 135788, 138108, 138139, 138140, 138143, 138145, 141444, 141446, 141447, 141448, 141449, 141450, 141451, 141452, 141453, 141454, 141459, 148422, 148424, 148426, 148427, 148428, 155169, 158263, 160614, 160629, 161044, 162791, 162837, 162882, 175841, 175870, 175899, 175920, 175975, 178417, 180500, 180606, 180612, 180927, 180928, 180931, 181971, 181972, 181973, 185684, 185685, 185686, 185687, 185689, 185691, 185693, 185694, 185695, 185699, 185701, 185702, 185703, 185704, 185705, 185707, 185708, 185709, 185711, 185713, 185714, 185717, 185718, 185720, 185721, 185722, 185723, 185724, 189663, 191108, 191119, 191153, 191639, 194513, 195208, 195500, 195501, 208544, 208550, 208554, 208555, 208556, 208852, 209882, 209883, 209885, 210198, 212211, 212213, 212214, 214694, 220417, 220418, 220473, 220486, 228839, 228840, 235375, 250614, 250619, 250921, 261927, 264172, 264173, 264175, 289261, 301216, 301217, 301218, 301223, 301225, 301231, 301232
JPN	10	28843, 28844, 221968, 250613, 250617, 250626, 261921, 261923, 302328, 302329
RUS	9	179841, 179843, 179844, 199282, 199286, 200686, 225054, 278144, 278145
CHN	6	187869, 187871, 250624, 261925, 289118, 310514
PAK	4	301212, 301213, 310518, 310519
UKN	4	199279, 199280, 209496, 225055
CZE	3	250622, 288928, 288929
IND	3	301211, 310472, 310517
BLR	1	179846
BTN	1	261914
CAN	1	302264
NPL	1	310516
Control	2	Dawon, Yangjeol-2

^aBLR, Belarus; BTN, Bhutan; CAN, Canada; CHN, China; CZE, Czech Republic; IND, India; JPN, Japan; KOR, Korea; NPL, Nepal; PAK, Pakistan; RUS, Russia; UKN, Ukraine

temperature and then incubated at 37 °C for 3 h, with vortexing for 5 min at hourly intervals. Following incubation, the extracts were centrifugation at 12,000 × g for 10 min and the resulting supernatants were filtered using disposable syringe filters (PTFE, 0.45 µm, hydrophilic; Advantec, Tokyo, Japan). Flavonoid standards were prepared as 100 ppm (0.1 mg/mL).

HPLC analysis

Identification and quantification of flavonoid components (rutin and quercetin) were based on the methods described by Kim et al. (2008). The extracts obtained

were analyzed using an Agilent 1200 series HPLC system (Agilent Technologies, USA). Flavonoids were separated using a Capcell PAK C18 column (4.6 × 250, 5 µm; Shiseido, Tokyo, Japan). The temperature of the oven was set at 40 °C, the injection volume used was 10 µL, and absorbance was monitored at 350 nm. The mobile phase, consisting of a mixture of solvent A (MeOH:water:acetic acid [5:92.5:2.5, (v/v/v)]) and solvent B (MeOH:water:acetic acid [92.5:2.5:2.5, (v/v/v)]), was delivered at a rate of 1.0 mL/min. The mobile phase initially comprised 10% solvent B (0 min), and thereafter was altered via linear gradient of 36% solvent B for 27 min, 60% solvent B for 32 min, 60% solvent B for

35 min, 10% solvent B for 35.1 min and 10% solvent B for 40 min. The analysis was replicated two times and the content of rutin and quercetin was quantified using the following formula based on the peak areas and concentration of samples and representative standard compounds (Hyun et al. 2018)

$$x = \frac{\text{sample area}}{\text{STD area}} \times \frac{0.1 \text{ mg}}{1 \text{ mL}} \times \frac{1 \text{ mL}}{0.1 \text{ g}}.$$

All contents were expressed in terms of milligrams per 100 g dry weight (mg/100 g DW). The analysis of flavonoid was conducted in two biological replicates.

Statistical analysis

Replicated data were subjected to statistical analysis by the application of a *t* test and one-way analysis of variance (ANOVA) at the 5% probability level, using Statistix ver. 8.1 software (McGraw-Hill 2008). Principal component (PCA) and correlation analyses were performed in RStudio version 1.2.1335 (RStudio Team 2019).

Results and discussion

Evaluation of agro-morphological traits

The results of agro-morphological characterization and the corresponding frequency distributions are presented in Table 2. In the case of stem color, we found that red stem color was predominant in 76.89% (193) of the accessions assessed, whereas 23.11% (58) of accessions had a pale green stem color. Recently, Kapoor et al. (2018) reported red and pink stem color in buckwheat germplasm, which can probably be attributed to the accumulation of anthocyanins, an assumption supported by the findings of Fang et al. (2019), who reported differences in anthocyanin pathways in the cotyledons and flowers of red- and green-stemmed buckwheat. With regards to seed coat color, we found that 72.51% (182) of accessions had a pale brown color, whereas dark brown seed coats were observed in 27.49% of (69) of accessions. In contrast, Raina and Gupta (2015) have previously found brownish yellow, brown, dark brown, gray, and black seed coat colors among common buckwheat germplasms. Similarly, in the common buckwheat

germplasm examined by Baniya et al. (1995), the seeds of more than 50% of accessions were grayish-black, 36% were brown, and the remainders were either black or gray. Furthermore, we observed egg-shaped (ovate) seeds in 94.83% (238) of accessions, triangular seeds in 2.79% (7), and winged-form seeds in 2.39% (6). Our results in this regard are consistent with the findings of Kapoor et al. (2018), who reported triangular- and ovate-type seeds in their study. In contrast, Raina and Gupta (2015) observed only triangular-type seeds in their evaluation of 68 common buckwheat germplasm accessions for seed rutin content. In a further study reported by Baniya et al. (1995), 92% of the germplasm assessed had smooth-type seeds, whereas 8% had winged-form seeds. Collectively, these results indicate that, in terms of morphological traits, common buckwheat germplasm is characterized by considerable diversity, which could be a consequence of outcrossing, given that common buckwheat is a self-incompatible species in which fertilization occurs via insect-mediated cross-pollination (Iwata et al. 2005; Grahic et al. 2016).

With respect to agronomic characterization, we found that the 50% flowering ranged from 38 to 46 days, averaging about 38 days. In terms of frequency distribution, 94% of accessions took 38–40 days to reach the 50% flowering stage, 3% took 41–43 days, and 3% took 44–46 days (Fig. 1a). In the comparison of collecting countries, we observed that germplasm accessions collected from Japan, Russia, China, Ukraine, Czech Republic, Belarus, Bhutan, Canada, and Nepal reached to 50% flowering in 38 days, whereas germplasm collected from India and Korea took 38–35 and 38–36 days for 50% flowering, respectively. The control varieties took 41 days for 50% flowering. Similarly, the time taken to reach 80% of maturity ranged from 73 to 95 days, with a mean of 89 days. In terms of frequency distribution, only 9.2% of accessions showed early maturation in the range of 73–80 days, whereas 50% took 81–90 days to reach maturity and 41% took 91–95 days (Fig. 1b). The range of days to maturity in different collecting countries was: Japan (73–94), China (73–88), Ukraine (73–95), Czech Republic (75–82), Russia (75–94), Korea (75–95), Canada (80), Bhutan (82), Belarus and Nepal (88), Pakistan and India (88–95). In control varieties, days to maturity ranged from 84 to 88 days.

Table 2 Country-wise distribution of 251 common buckwheat (*Fagopyrum esculentum*) accessions based on morphological traits

Country ^a	Stem color				Seed coat color							Seed shape				Winged-form	Total
	Stem color		Seed coat color					Seed shape									
	Pale green	Pale red	Red	Dark red	Total	White	Grey	Pale brown	Dark brown	Black	Total	Oval	Egg-shaped	Triangular			
KOR	39	–	169	–	208	–	–	155	53	–	208	–	200	5	3	208	
JPN	6	–	4	–	10	–	–	5	5	–	10	–	8	2	–	10	
RUS	4	–	5	–	9	–	–	7	2	–	9	–	9	–	–	9	
CHN	4	–	2	–	6	–	–	4	2	–	6	–	6	–	–	6	
PAK	–	–	4	–	4	–	–	3	1	–	4	–	2	–	2	4	
UKN	1	–	3	–	4	–	–	3	1	–	4	–	4	–	–	4	
CZE	2	–	1	–	3	–	–	1	2	–	3	–	3	–	–	3	
IND	–	–	3	–	3	–	–	3	–	–	3	–	3	–	–	3	
BLR	1	–	–	–	1	–	–	–	1	–	1	–	1	–	–	1	
BTN	–	–	1	–	1	–	–	1	–	–	1	–	1	–	–	1	
CAN	1	–	–	–	1	–	–	–	1	–	1	–	–	–	1	1	
NPL	–	–	1	–	1	–	–	–	1	–	1	–	1	–	–	1	
Total	58	–	193	–	251	–	–	182	69	–	251	–	238	7	6	251	
Control	1	–	1	–	2	–	–	1	1	–	2	–	2	–	–	2	

^aBLR, Belarus; BTN, Bhutan; CAN, Canada; CHN, China; CZE, Czech Republic; IND, India; JPN, Japan; KOR, Korea; NPL, Nepal; PAK, Pakistan; RUS, Russia; UKN, Ukraine

The 1000-seed weight (TSW) ranged from 21 to 42 g with a mean of 26.3 g. This range of TSW in our study is agreement with previous reported TSW in common buckwheat germplasm: 10.2–31.8 g (Baniya et al. 1995), 12–35 g (Grubben and Siemonsma 1996), 13.85–37.19 (Cepková et al. 2009), 24.50–33.08 g (Kapoor et al. 2018), 20.9–26.4 g (Jung et al. 2015). In term of frequency distribution 48% germplasm had TSW ranged from 20 to 25 g, 44% had 26–30 g, 7% had 31–35 and only 1% germplasm had above 35 g (Fig. 1c). The range of TSW according to collecting counties was Korea (21–41 g), Bhutan (22.3 g), Russia (22.7–35 g), India (23.7–25.7 g), China (23.7–34.3 g), Japan (24.7–42 g), India (25.3–25.7 g), Pakistan (25–30 g), Ukraine (25.3–30 g), Czech Republic (28.7–32.7 g), Belarus (32 g), Nepal (33.3 g), Canada (35.3 g). The germplasm accessions with the highest TSW were found in Korean and Japanese collections. In control varieties, TSW ranged from 28 to 32.7 g.

Evaluation for rutin and quercetin content

The HPLC peaks of standard and quantified rutin and quercetin content are shown in Supplementary Fig. S1. We found that the rutin content of seed was typically higher than that of quercetin, indicating that rutin is the major flavonoid in buckwheat seeds. These observations are consistent with the findings of Bai et al. (2015) and Raina and Gupta (2015), who reported rutin as the major flavonoid in buckwheat seeds. Overall, we found that the rutin content in common buckwheat seeds ranged from 7.22 to 47.86 mg/100 g DW, averaging 18.52 mg/100 g DW. In terms of frequency distribution, 69% of the germplasm had rutin content in the range of 11–20 mg/100 g DW, followed by 25% in the range of 21–30 mg/100 g DW and 4% in the range of 31–40 mg/100 g DW. In contrast, only 2% of germplasm had higher rutin content in the range of 41–50 mg/100 g DW (Fig. 2a). This range of rutin concentrations is in good accordance with earlier findings (Kitabayashi et al. 1995; Fabjan et al. 2003; Brunori and Vegvari 2007; Brunori et al. 2010; Raina and Gupta 2015; Kiproviski et al. 2015). In our results, the range of rutin according to collecting countries was: India (7.22–14.20 mg/100 g DW), Korea (8.64–47.86 mg/100 g DW), China (8.75–19.61 mg/

100 g DW), Pakistan (12.35–17.66 mg/100 g DW), Canada (14.47 mg/100 g DW), Russia (14.68–38.96 mg/100 g DW), Ukraine (17.35–22.36 mg/100 g DW), Nepal (17.45 mg/100 g DW), Japan (19.24–38.22 mg/100 g DW), Czech Republic (21.39–46.75 mg/100 g DW), Bhutan (25.80 mg/100 g DW), Belarus (45.54 mg/100 g DW). In control varieties, the rutin content ranged from 11.64 to 17.82 mg/100 g DW (Table 3). The mean content of rutin was in the order of Belarus > Czech Republic > Japan > Bhutan > Russia > Ukraine > Korea > Nepal > Pakistan > Canada > China > India. In contrast, in a study reported by Minami et al. (2001), a wide variation in rutin content was observed among common buckwheat accessions of Japanese, Chinese, Nepalese and European origin and some of the Nepalese accessions were considered to be useful breeding material. Recently, Yu et al. (2019) reported a variation of rutin content in diverse tartary buckwheat germplasm. In their study the content of rutin was in the order of Nepal > Bhutan > Japan > China > Pakistan > Slovenia > Indian. Similarly, Park et al. (2005) reported the content of rutin in tartary buckwheat germplasm in the order of Bhutan > Slovenia > China > Pakistan > Nepal > Japan > India. Similarly, higher rutin content was reported in accessions collected from Nepal than lines collected elsewhere (Kitabayashi et al. 1995).

Likewise, the content of quercetin ranged from 0 to 1.22 mg/100 g DW, averaging 0.35 mg/100 g DW. In terms of frequency distribution, we found that quercetin content was not detected in 20% germplasm, whereas 74% of germplasm had seed content in the range of 0.2–0.6 mg/100 g DW, followed by 5% with content in the range of 0.7–1.0 mg/100 g DW. Only 1% of germplasm had quercetin content greater than 1.0 mg/100 g DW (Fig. 2b). The range of quercetin content appears to be higher than that reported previously (Fabjan et al. 2003; Qin et al. 2010; Wiczowski et al. 2014; Bai et al. 2015; Kalinová et al. 2019). In contrast to the results obtained in the present study, Qin et al. (2010) detected quercetin in only one of the 18 common buckwheat accessions they evaluated for phenolic components. Similarly, Bai et al. (2015) and Fabjan et al. (2003) failed to detect quercetin in the seeds of the common buckwheat germplasm they examined. The range of quercetin according to collecting countries was: Belarus

Fig. 1 Frequency distribution of agronomic traits in 251 accessions of common buckwheat (*Fagopyrum esculentum*): **a** days to flowering, **b** days to maturity, **c** 1000-seed weight (g)

(1.22 mg/100 g DW), Korea (0–1.15 mg/100 g DW), Russia (0–1.05 mg/100 g DW), China (0–0.91 mg/100 g DW), Ukraine (0–0.52 mg/100 g DW), Japan (0.22–0.70 mg/100 g DW), India (0.24–0.33 mg/100 g DW), Pakistan (0.30–0.55 mg/100 g DW), Nepal (0.31 mg/100 g DW), Canada (0.35 mg/100 g DW), Czech Republic (0.38–0.69 mg/100 g DW), Bhutan (0.53 mg/100 g DW). In control varieties, the quercetin content ranged from 0.24 to 0.36 mg/100 g

DW (Table 3). These results indicate that buckwheat has a wide range of diversity in flavonoid content and genotype and area of the collection are important factors to be considered in evaluating buckwheat for high flavonoid content.

Agro-morphological characterization for rutin and quercetin content

With regards to morphological traits, although we found differences in the rutin and quercetin content of germplasm with pale green and red stem color, these were non-significant. The mean rutin content (20.35 mg/100 g DW) of germplasm with pale green

Fig. 2 Frequency distribution of flavonoid components in 251 accessions of common buckwheat (*Fagopyrum esculentum*): **a** rutin content, **b** quercetin content

Table 3 Ranges and means of agronomic traits and the content of rutin and quercetin in 251 common buckwheat (*Fagopyrum esculentum*) germplasm based on collecting countries

Country ^a	n		Days to 50% flowering	Days to 80% maturity	1000-seed weight (g)	Rutin (mg/100 g DW)	Quercetin (mg/100 g DW)
KOR	208	Range	38–46	75–95	21.0–41.0	8.64–47.86	0–1.15
		Mean	38	90	25.71 ± 0.05	17.76 ± 1.02	0.34 ± 0.04
JPN	10	Range	–	73–94	24.7–42.0	19.24–38.22	0.22–0.70
		Mean	38	84	31.00 ± 0.07	26.60 ± 0.96	0.44 ± 0.05
RUS	9	Range	–	75–94	22.7–35.0	14.68–38.96	0–1.05
		Mean	38	81	29.85 ± 0.07	23.17 ± 1.10	0.46 ± 0.04
CHN	6	Range	–	73–88	23.7–34.3	8.75–19.61	0–0.91
		Mean	38	83	27.70 ± 0.07	13.78 ± 0.93	0.44 ± 0.17
PAK	4	Range	–	88–95	25.0–30.0	12.35–17.66	0.30–0.55
		Mean	38	92	26.42 ± 0.03	14.91 ± 0.43	0.37 ± 0.02
UKN	4	Range	–	73–95	25.3–30.0	17.35–22.36	0–0.52
		Mean	38	83	27.17 ± 0.05	22.92 ± 1.12	0.25 ± 0.03
CZE	3	Range	–	75–82	28.7–32.7	21.39–46.75	0.38–0.69
		Mean	38	80	31.11 ± 0.04	36.60 ± 0.76	0.53 ± 0.02
IND	3	Range	38–45	88–95	23.7–25.7	7.22–14.20	0.24–0.33
		Mean	40	92	24.67 ± 0.06	10.42 ± 0.88	0.53 ± 0.02
BLR	1	Range	–	–	–	45.54 ± 1.45	1.22 ± 0.59
		Mean	38	88	32.0 ± 0.10		
BTN	1	Range	–	–	–	25.80 ± 0.58	0.53 ± 0.07
		Mean	38	82	22.3 ± 0.58		
CAN	1	Range	–	–	–	14.47 ± 1.85	0.35 ± 0.1
		Mean	38	80	35.3 ± 0.58		
NPL	1	Range	–	–	–	17.45 ± 0.56	0.31 ± 0.02
		Mean	38	88	33.3 ± 0.58		
Total	251	Range	38–46	73–95	21.0–42.0	7.22–47.86	0–1.22
		Mean	38	89	26.3 ± 0.05	18.52 ± 1.00	0.35 ± 0.04
Control	2	Range	–	84–88	28.0–32.7	11.64–17.82	0.29–0.36
		Mean	41	86	30.3 ± 0.03	14.73 ± 0.56	0.32 ± 0.08

^aBLR, Belarus; BTN, Bhutan; CAN, Canada; CHN, China; CZE, Czech Republic; IND, India; JPN, Japan; KOR, Korea; NPL, Nepal; PAK, Pakistan; RUS, Russia; UKN, Ukraine

stems was higher than that (17.96 mg/100 g DW) of germplasm with red-colored stems. Similarly, the mean quercetin content (0.36 mg/100 g DW) of germplasm with red stems was higher than that (0.30 mg/100 g DW) of germplasm with pale green stems. The rutin content of germplasm with red-colored stem ranged from 7.22 to 47.86 mg/100 g DW, whereas that of germplasm with pale green stem color ranged from 8.64 to 46.75 mg/100 g DW (Fig. 3a). Similarly, the quercetin content of germplasm with red stem color ranged from 0 to 1.15 mg/

100 g DW, whereas that of germplasm with pale green stem color ranged from 0 to 1.22 mg/100 g DW (Fig. 3b). Typically, the basal stems of buckwheat are red or pink, depending on the presence of anthocyanin. Previously, Sytar et al. (2014) demonstrated an association between the pink color of different parts of buckwheat plants and anthocyanin content, which was also found to be associated with rutin content. Consistently, Fang et al. (2019) reported that the selection of buckwheat germplasm for pink-colored seedlings is associated with high rutin content. Plants

with dark red stem color have been found to contain higher rutin content than plants with red–green or green–red stems (Klykov et al. 2016), thereby indicating that green stem color in buckwheat might be attributable to a lack of relevant enzymes in the

anthocyanin biosynthetic pathway (Matsuri et al. 2008).

Although we found no significant associations between mean rutin content and seed coat color in germplasm with dark brown and pale brown seed coat color, we did detect significant differences with

Fig. 3 Diversity in morphological traits of common buckwheat (*Fagopyrum esculentum*) germplasms associated with the content of rutin and quercetin

respect to quercetin content. Germplasm with dark brown seed coat color had a higher mean content of rutin (19.49 mg/100 g DW) than germplasm with pale brown seed coat color. The rutin and quercetin content in germplasm with pale brown seed coat color ranged from 7.22–47.86 to 0–1.15 mg/100 g DW, respectively. Similarly, the rutin and quercetin content in germplasm with dark brown seed coat color ranged from 9.01–46.75 to 0–1.22 mg/100 g DW, respectively (Fig. 3c, d). Recently, Yu et al. (2019) reported the highest content of rutin and quercetin in dark gray tartary buckwheat seed followed by dark brown, gray-brown and brown. Although it has previously been established that the flavonoid content in seeds is closely related to seed color and seed shape (Hou et al. 2017), in the present study, we failed to detect any significant differences in the mean rutin and quercetin content of germplasm possessing ovate, triangular, or winged-form seeds. The germplasm with winged-form seeds showed the highest mean rutin content (22.77 mg/100 g DW), followed by germplasm with triangular seeds (19.77 mg/100 g DW). Furthermore, the rutin and quercetin content in germplasm with ovate seeds ranged from 7.22–47.86 to 0–1.22 mg/100 g DW, which are higher than the content in germplasm with seeds of other shapes (Fig. 3e, f). Previously, a wide range of variation in the rutin content of tartary buckwheat germplasm has been observed, with gray-colored seeds having higher rutin content than black- and brown-colored seed, and slender seeds having a higher rutin content than notched and round seeds (Park et al. 2004; Sytar et al. 2016; Yu et al. 2019).

Additionally, several studies conducted on other major crop plants have also revealed an association between the seed color and phenolic components (Zhang et al. 2010; Shao et al. 2011; Wang et al. 2015). Although higher flavonoid content was reported in the seed coats than in the embryos of soybean and rice (Bordiga et al. 2014; Min et al. 2015), it has been emphasized that the type and quantity of flavonoids in seed coats and embryos may be related to plant genotype (Hou et al. 2017). Furthermore, Shen et al. (2009) and Shao et al. (2014) found that black- and red-colored rice grains contain higher flavonoid content than white-colored grains. Similarly, the blue-colored grains of barley germplasm have been shown to have higher mean levels of phenolic components than germplasm with purple- and black-colored grains

(Siebenhandl-Ehn et al. 2011), and the blue- and purple-colored grains of barley germplasm have been shown to higher mean phenolic content than black-grain barley (Kim et al. (2007). In rice, the pigmented bran contains larger amounts of soluble phenolics than non-pigmented rice bran (Ghasemzadeh et al. 2018). Likewise, a wide range for total phenolic content has also been detected among sorghum germplasm differing in grain color, with higher phenolic content being found in pigmented grains Lopez-Martinez et al. (2009) and Hu and Xu (2011). These results indicate that variation in seed color is closely associated with phenolic content.

In addition to seed coat color and seed shape, grain size also has a significant effect on the content of phenolic compounds, with small grains tending to contain higher levels of phenolic compounds than medium and large seeds. This is illustrated by the small seeds of tartary buckwheat that have with small seeds, contains higher flavonoid content than the large seeds of common buckwheat, and might be explained by the fact that phenolic compounds are more condensed in smaller grains (Kim et al. 2012).

With respect to agronomic traits, we failed to detect any significant differences in the rutin and quercetin content of germplasm taking 38–40, 41–43, and 44–46 days to reach 50% flowering (Fig. 4a, b). In contrast, we observed significant differences in the rutin content of germplasm taking 73–80, 81–90, and 91–95 days to reach maturity, although no similar significant differences were found with respect to quercetin content. The highest mean rutin content was found in early-maturing (73–80 days) followed by intermediate—(81–90 days) and late-maturing (> 91 days) accessions (Fig. 4c, d). We also detected significant differences in rutin and quercetin content with respect to TSW, with the highest mean rutin content (23.94 mg/100 g DW) being found in germplasm with a TSW > 35 g, although only 1% of the accessions evaluated had these high seed weights. Accessions with TSW of between 20 and 25 g accounted for the highest percentage of germplasm (48%), and these were characterized by rutin content ranging from 8.69 to 47.86 mg/100 g DW. These were followed by the 44% of germplasm with TSW of between 26 and 30 g, which had rutin content ranging from 7.22 to 46.75 mg/100 g DW (Fig. 4e, f).

Fig. 4 Diversity in agronomic traits of common buckwheat (*Fagopyrum esculentum*) germplasm associated with the content of rutin and quercetin

Correlation analysis

Correlation analysis (Fig. 5) revealed a significant positive correlation between the number of days to flowering and the number of days to reaching maturity and a significant negative correlation between the number of days to flowering and TSW. These results

are in line with the findings of Kapoor et al. (2018), who reported similar positive and negative correlations in common buckwheat germplasm. This could be due to the early transition from vegetative growth to reproductive development, which is characterized by less vegetative growth. In agreement, Li et al. (2012) found that late flowering was associated with

increased plant height and higher yields. We also detected negative and non-significant correlations between the number of days to flowering and rutin and quercetin content. Earlier, Oomah et al. (1996) reported less rutin content in early flowering genotypes than that of late cultivars with late flowering. Furthermore, the number of days taken to reach maturity was found to be significantly negatively correlated with TSW, rutin content, and quercetin content. The TSW was significantly positively correlated with rutin content but non-significantly and positively with quercetin content. However, the correlation between TSW and rutin content was not strong to any considerable degree, which is consistent with the findings of Brunori and Vegvari (2007) who also reported a weak correlation between grain yield and rutin content. Rutin content was found to be significantly positively correlated with quercetin content, which might be predicted given the fact that rutin

and quercetin are the major flavonoids in buckwheat seeds (Bai et al. 2015). A linear and positive correlation has likewise been detected in the seed rutin and quercetin content of tartary buckwheat germplasm (Yu et al. 2019).

Although buckwheat seeds generally contain high levels of rutin and low quercetin content, during the bread-making process the levels of these two flavonoids are reversed due to the conversion of rutin to quercetin, this process depends upon the rutin-degrading enzyme rutinase found in seeds, and imparts a bitter taste in buckwheat-derived food products (Yasuda and Nakagawa 1994). This bitter taste can, however, be eliminated by the hot water treatment of buckwheat seeds at temperatures in excess of 90 °C rutinase activity. However, there are also a number of non-bitter buckwheat varieties in which rutin remains under graded (Suzuki et al. 2014).

Fig. 5 Correlation between the agronomic traits and flavonoid components (rutin and quercetin) of common buckwheat (*Fagopyrum esculentum*) germplasm

Table 4 Eigenvalues, percentage of variance, and loadings of different principal components of common buckwheat (*Fagopyrum esculentum*) accessions

Traits	PC1	PC2	PC3	PC4	PC5
TSW ^a	0.383	− 0.469	0.493	0.621	0.072
DTF	− 0.294	0.485	0.785	− 0.048	− 0.244
DTM	− 0.552	0.249	− 0.175	0.612	0.479
Rutin	0.536	0.432	0.136	− 0.191	0.687
Quercetin	0.419	0.544	− 0.302	0.449	− 0.485
Eigenvalue	1.83	1.25	0.85	0.65	0.43
Variance (%)	36.50	25.00	16.91	12.97	8.63

^aTSW, 1000-seed weight; DTF, days to 50% flowering; DTM, days to 80% maturity

Fig. 6 Principal component analysis of 251 common buckwheat (*Fagopyrum esculentum*) germplasms accessions

Principal component analysis (PCA)

In an attempt to distinguish the common buckwheat germplasm based on their country of collection, PCA was performed based on the correlation matrix. PCA is used to analyze multidimensional data sets and to identify putative variables that provide maximum variability (Steiner et al. 2009). It also provides insights into different clusters and certain valuable traits, making it possible for breeders to identify distinct features of interest (Hussain 2015). The loadings, eigenvalues and the percentage of variance for PCA are presented in Table 4. In the present study,

we found that the first two principal components (PC1 and PC2), with an eigenvalue greater than 1, accounted for 36.5% and 25% of the total variability, respectively. Rutin and quercetin were the main contributors to the variability to PC1, whereas Quercetin, days to flowering and Rutin were the major contributor to PC2. The number of days to flowering and TSW were found to be the main contributors to PC3 (Table 4). According to the PCA biplot (Fig. 6), a high level of heterogeneity was observed among germplasm based on their agronomic traits and flavonoid content. The germplasm accessions with high rutin and quercetin contents were placed on the

Table 5 Promising common buckwheat (*Fagopyrum esculentum*) accessions with high rutin and quercetin content

Trait	Characteristics	Accession number
Early maturity	Below 80 days	225055 (UKN ^a), 250624 (CHN), 28844 (JPN), 179844 (RUS), 108885 (KOR), 250622 (CZE), 261921 (JPN), 104769 (KOR), 278145 (RUS), 104551 (KOR), 101282 (KOR), 278144 (RUS), 179843 (RUS), 261925 (CHN)
1000-seed weight	≥ 35 g	200686 (RUS), 302264 (CAN), 250614 (KOR), 235375 (KOR), 221968 (JPN)
Rutin	≥ 35 mg/100 g DW	264173 (KOR), 199286 (RUS), 250617 (JPN), 261923 (JPN), 179843 (RUS), 288929 (CZE), 185707 (KOR), 179846 (BLR), 250622 (CZE), 100801 (KOR)
Quercetin	≥ 1 mg/100 g DW	179843 (RUS), 103836 (KOR), 179846 (BLR)

^aBLR, Belarus; CAN, Canada; CHN, China; CZE, Czech Republic; JPN, Japan; KOR, Korea; RUS, Russia; UKN, Ukraine

upper right side plot, whereas the germplasm accessions with high TSW were placed on the lower right side plot. The control cultivars were placed at the center of PCA biplot. This information could be useful for plant breeders to select the most variable characters and promising accessions for buckwheat improvement.

Conclusion

The buckwheat accessions evaluated in this study were found to show considerable diversity with respect to morphological traits and flavonoid content as well as region from which accessions were collected. The detail of agro morphological traits and the content of rutin and quercetin for each accession are given as Supplemental Table S1. We believe that the findings of this study will provide a valuable basis for future buckwheat breeding programs and the development of high-yielding buckwheat cultivars for spring cultivation. We selected 32 promising accessions, which could be used as useful breeding material to improve the grain yield and content of rutin and quercetin in existing buckwheat cultivars (Table 5). The selected accession will be further evaluated for grain yield and flavonoid content under spring and fall cultivation. These accessions include 14 accessions with early maturity (below 80 days), 5 accessions with highest TSW (≥ 35 g), 10 with highest rutin content (≥ 35 mg/100 g DW) and 3 with highest quercetin content (≥ 1 mg/100 g DW). From the perspective of future studies, selected

germplasm with high rutin content could be characterized for low rutinase activity to minimize the degradation of rutin molecules during dough fermentation, which is responsible for the bitter taste that characterizes buckwheat-derived food products.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no financial or competing interests.

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