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Research paper

Differentiation and growth of a growing point until the stage of flower bud appearance in leading common buckwheat and Tartary buckwheat varieties in northern Japan

Shinya KASAJIMA*, Ikumi YOSHIMARU, and Hirotake ITOH

Faculty of Bioindustry, Tokyo University of Agriculture, Yasaka 196, Abashiri, Hokkaido 099-2493, Japan

* Corresponding author, *e-mail: s3kasaji@nodai.ac.jp*
Tel: +81-152-48-3823

Other e-mail addresses:
Ikumi Yoshimaru, yoshimaru193@yahoo.co.jp, Hirotake Itoh, h-ito@nodai.ac.jp

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ABSTRACT

Studies regarding the developmental stage of common buckwheat (*Fagopyrum esculentum* Moench) have not been adequately performed despite its importance in studying the yield-determining process. In addition, the difference between common buckwheat and Tartary buckwheat (*F. tataricum* (L.) Gaertn.) is still unclear. In the present study, the differentiation and growth of the growing point until the stage of flower bud appearance were evaluated in the common buckwheat variety 'Kitawasesoba' and the Tartary buckwheat variety 'Manten-Kirari', which are the leading buckwheat varieties in Hokkaido, Japan. With some exceptions, the developmental stages of 'Kitawasesoba' and 'Manten-Kirari' can be distinguished. Thus, leaf primordia, axillary flower bud, and terminal flower bud differentiations and growths were observed. Both the common and Tartary buckwheat varieties did not exhibit large differences in the morphology of the growing point. However, the two varieties showed differences in the rates of differentiation and growth.

INTRODUCTION

Buckwheat (family Polygonaceae) is a pseudocereal crop with beneficial effects on human health (Ikeda 2002; Ikeda et al. 2012). Common buckwheat (*Fagopyrum esculentum* Moench) and Tartary buckwheat (*F. tataricum* (L.) Gaertn.) are the well-known members of the *Fagopyrum* genus. Common buckwheat bears beautiful and abundant flowers. However, due to self-incompatibility, its seed set is generally low, implying poor seed yield (Woo et al. 2016). Currently, the seed yield of common buckwheat in Japan is less than 1,000 kg ha^{-1}. The yield of Tartary buckwheat is generally higher than that of common buckwheat as Tartary buckwheat is an autogamous plant with high fertilization efficiency. However, the seed yield of Tartary buckwheat is still much lower than that of major grain crops such as wheat and rice.

In buckwheat, the seed yield per unit area is determined by the yield component, i.e., the number of bloomed florets per unit area, fertilization rate, the percentage of ripened seeds, and seed weight (Ujihara and Matano 1975; Kasajima et al. 2011). The number of bloomed florets per unit area includes the number of flower clusters per unit area and the number of bloomed florets per flower cluster. In buckwheat, the number of flower clusters and the number of bloomed florets are considered to be important factors governing seed yield. In wheat, the grain number per spike is closely related to the formation and development of spikelets and florets (Miralles and Slafer 1999; Toyota et al. 2001). Similarly, in buckwheat, flower bud differentiation is thought to be related to the number of seeds based on the number of flower clusters and florets. Few studies have reported observations regarding flower bud differentiation in common buckwheat. Hagiwara et al. (1998) reported the criteria for determining the developmental stage of the growing point in common buckwheat. Although common buckwheat is important for examining the yield-determining process, detailed studies assessing its developmental stage have not yet been conducted. In addition, the difference between common buckwheat and Tartary buckwheat is still unknown.

In Japan, the largest buckwheat-producing region is Hokkaido, which lies in the northern part of the country. Common buckwheat production in Hokkaido accounts for approximately 40% of its total production in Japan (Morishita and Suzuki 2012). For approximately past three decades, the common buckwheat variety 'Kitawasesoba' has been the main cultivated variety of buckwheat in Hokkaido. In addition, there has been an increased cultivation of Tartary buckwheat in Hokkaido. Suzuki et al. (2014) developed a new Tartary buckwheat variety, 'Manten-Kirari', the flour of which contains only trace amounts of rutinosidase and thus, lacks the bitter taste associated with buckwheat. The cultivation and use of 'Manten-Kirari' have rapidly grown in Hokkaido. Thus, 'Kitawasesoba' and 'Manten-Kirari' are the leading varieties of common and Tartary buckwheat, respectively, grown in Hokkaido. There is a lack of basic research from the viewpoint of developmental stages to realize high-yield capabilities of these varieties.

The present study aimed to describe the differentiation and growth of a growing point until the stage of flower bud appearance in the leading common and Tartary buckwheat varieties 'Kitawasesoba' and 'Manten-Kirari', respectively, in Hokkaido, northern Japan.

MATERIALS AND METHODS

'Kitawasesoba' and 'Manten-Kirari' varieties developed by the NARO Hokkaido Agricultural Research Center were used in the present study (Inuyama et al. 1994; Suzuki et al. 2014). Under experimental conditions, the seeds of these varieties were sown in plastic planters measuring 56.5 × 17 × 16 cm (length × width × height) that were filled with commercial garden soil containing 0.374 g kg^{-1} N, 1.485 g kg^{-1} P_2O_5, and 0.242 g kg^{-1} K_2O. The experiment was conducted in a greenhouse with all sides open at the Faculty of Bioindustry, the Tokyo University of Agriculture in Hokkaido, Japan, in the summer of 2018. Ten planters of each variety were placed in the greenhouse, and approximately 120 plants of each variety were grown in a completely randomized design with two replications. A depression measuring approximately 2-cm in depth was made in the middle of the soil in the planters, and 24 seeds, equivalent to 250 seeds/square meter, were sown per planter on June 10, 2018. Plants were irrigated daily with tap water, and no fertilizer was applied.

Plants were sampled twice a week from the beginning of July to middle of August to observe the development of the growing point on the shoot. The growing points of three plant samples per plot were immediately dissected under a stereoscopic microscope to estimate the developmental stage of the growing points. The side views of the growing points were observed using a tablet in conjunction with the microscope. The vertical length of the growing point was recorded using the software in the tablet. The definitions of the developmental stages of both the varieties generally followed those reported by Hagiwara et al. (1998).

RESULTS AND DISCUSSION

In the present study, the developmental stages of the common buckwheat variety 'Kitawasesoba' and the Tartary buckwheat variety 'Manten-Kirari' were distinguished, with some exceptions, according to the criteria reported by in a study by Hagiwara et al. (1998) that used common buckwheat. Three primary developmental stages have been reported: (A) leaf primordia differentiation and growth period, (B) axillary flower bud differentiation and growth period, and (C) terminal flower bud differentiation and growth period. In the present study, the developmental stages of both common buckwheat and Tartary buckwheat were almost consistent with the aforementioned stages. The typical and easily-discernible pictures of each developmental stage are shown in Figs. 1–3.

Fig. 1 depicts leaf primordia differentiation and growth period in 'Manten-Kirari'. We did not observe the growing point with leaf primordia in 'Kitawasesoba'. The growing point in Fig. 1 A0 showed the formation of only leaf primordia. An axillary bud was observed on the growing point along with the growth of leaf primordial (Fig. 2 A1). In higher plants, vegetative growth can be divided into juvenile and adult phases (Bäurle and Dean 2006; Yoshikawa et al. 2013). In the present study, the difference between the juvenile and adult phases was difficult to discern, although the morphological characteristics of growing points were observed in the vegetative growth period.

Fig. 2 depicts axillary differentiation and the growth period in 'Kitawasesoba' and 'Manten-Kirari'. In Fig. 2 B0,

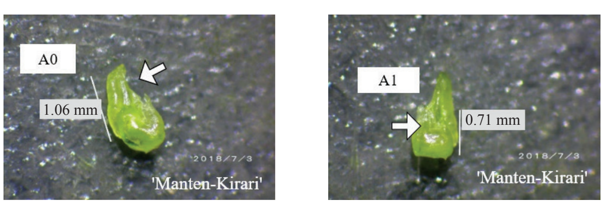

***Fig. 1.** Leaf primordia differentiation and growth period in 'Manten-Kirari'.*
(A0) Growing point initiates only leaf primordia. The arrow shows the leaf primordia. (A1) Leaf primordia and axillary buds differentiate on the growing point. The arrow shows the axillary buds.

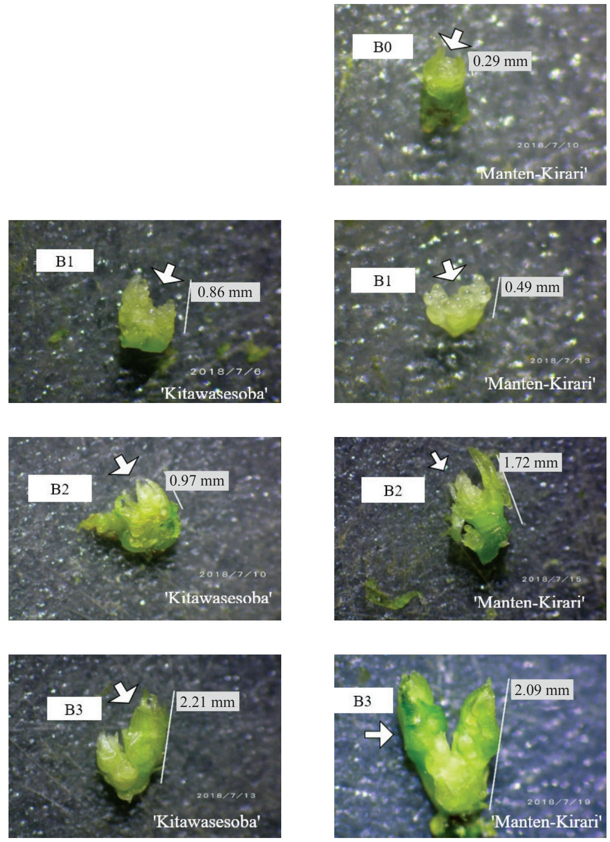

***Fig. 2.** Axillary bud differentiation and growth period in 'Kitawasesoba' (left) and 'Manten-Kirari' (right).*
(B0) Apical growing point and axillary bud primordia grew into a dome-shaped structure. The arrow shows the dome differentiated on the growing point. (B1) Numerous small projections formed on the surface of the dome differentiated at B0. The arrow shows the small projections. (B2) A flower bud grows and is covered with bract, which is indicated by the arrow. (B3) Flower bud grows and floret clusters covered with bract are visible. The arrow shows a floret cluster.

the apical growing point and axillary bud primordia grew into a dome-shaped structure. However, the differentiated dome on the growing point could not be observed in 'Kitawasesoba'. As seen in Fig. 2 B1, numerous small projections were formed on the surface of the dome differentiated in Fig. 2 B0. A flower bud grew and was covered with a bract (Fig. 2 B2). The flower bud grew, and floret clusters were found to be covered with a bract (Fig. 2 B3). In the growing point in B3, leaf primordia and flower buds simultaneously differentiated (Hagiwara et al. 1998). In general, buckwheat exhibits an indeterminate growth pattern that is based on indeterminate inflorescence.

Conversely, simultaneous ripening is a characteristic of determinate varieties of common buckwheat (Martinenco and Fesenko 1989). Therefore, the differentiation and growth of the growing point may vary between determinate and indeterminate common buckwheat varieties. Further studies that compare the growing point in determinate and indeterminate common buckwheat varieties are required to elucidate the effect of the overlapping period of vegetative and reproductive primordia on the agronomic characteristics of buckwheat.

Fig. 3 depicts terminal flower bud differentiation and growth period in 'Kitawasesoba' and 'Manten-Kirari'. As seen in Fig. 3 C0, the differentiation of the axillary flower bud ceased, and the apical growing point changed again into a dome-shaped structure. A dome-shaped growing point was found to be covered with small projections (Fig. 3 C1). Terminal flower bud growth and floret clusters formed from the small projections were visible (Fig. 3 C2). The floret clusters grew and were covered with bract (Fig. 3 C3). The results depicted in Figs. 1–3 show no large differences in the developmental morphology of the growing points of common buckwheat and Tartary buckwheat.

Fig. 4 depicts the mean vertical length of the growing points in 'Kitawasesoba' and 'Manten-Kirari'. The expansion of the size of the growing point in both 'Kitawasesoba' and 'Manten-Kirari' showed an S-shaped curve. However, the expansion was delayed in 'Manten-Kirari' compared with that in 'Kitawasesoba'. In addition, the onset of the expansion of the growing point was more rapid in 'Kitawasesoba' compared with that in 'Manten-Kirari', which may lead to a difficulty in identifying the developmental stage of the vegetative growth period in 'Kitawasesoba'. Thus, the sampling frequency should be increased to observe the growing point during the vegetative growth period of common buckwheat.

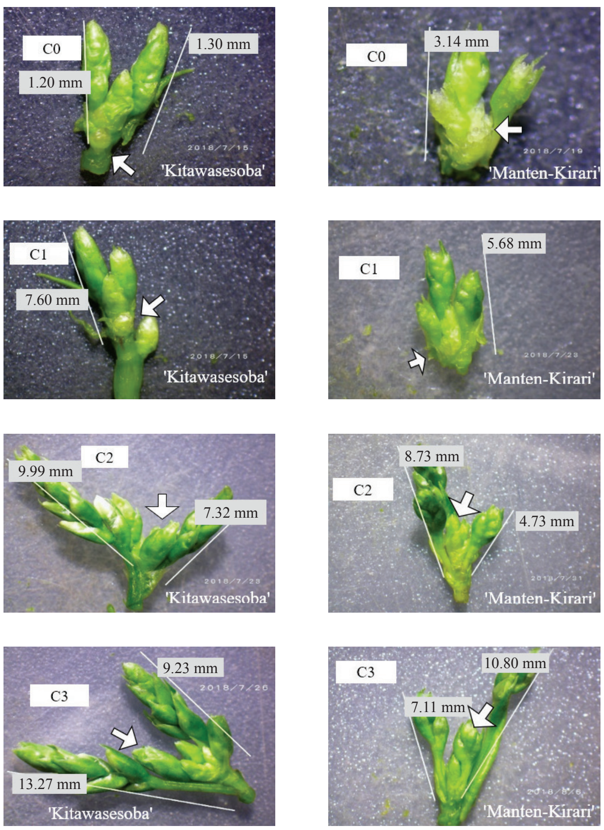

__Fig. 3.__ Terminal flower bud differentiation and growth period in 'Kitawasesoba' (left) and 'Manten-Kirari' (right).
(C0) Differentiation of axillary flower bud ceases, and the apical growing point changes again into the dome-shaped structure. The arrow shows the dome differentiated on the apical growing point. (C1) The dome-shaped growing point is covered with small projections (indicated by the arrow). (C2) Terminal flower bud growth and floret clusters formed from the small projections can be seen. The arrow shows visible floret clusters. (C3) Floret clusters grow and are covered with bract (indicated by the arrow).

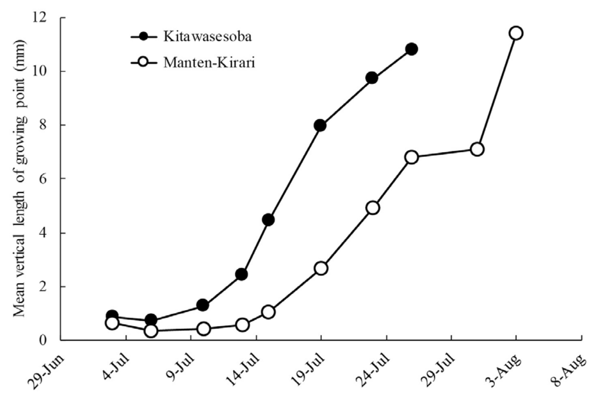

__Fig. 4.__ Mean vertical length of growing point in 'Kitawasesoba' and 'Manten-Kirari'.

In conclusion, there were no large differences in the morphology of the growing points of common buckwheat and Tartary buckwheat. However, differences were observed in the rates of differentiation and growth in the two varieties. In Japan, buckwheat is very sensitive to environmental stresses such as flooding stress during floral transition in the developmental stage. In further studies, we will conduct a more accurate evaluation of the growing point during floral transition and of its association with abiotic environments.

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REFERENCES

Bäurle, I. and C. Dean, 2006. The timing of developmental transitions in plants. CELL 125: 655-664. DOI: https://doi.org/10.1016/j.cell.2006.05.005

Hagiwara, M., N. Inoue and T. Matano, 1998. Variability in the length of flower bud differentiation period of common buckwheat. FAGOPYRUM 15: 55-64.

Ikeda K, 2002. Buckwheat composition, chemistry, and processing. ADV FOOD NUTR RES 44: 395-434. DOI: https://doi.org/10.1016/S1043-4526(02)44008-9

Ikeda, K., S. Ikeda, I. Kreft and R. Lin, 2012. Utilization of Tartary buckwheat. FAGOPYRUM 29: 27-30.

Inuyama, S., Y. Honda, S. Furuyama, M. Kimura and H. Kasano, 1994. The breeding and characteristics of a buckwheat cultivar, "Kitawasesoba." RES BULL HOKKAIDO NATL AGRIC EXP STN 159: 1-10. (In Japanese with English Summary)

Kasajima, S. and H. Itoh, 2011. Effect of shading during different growth phases on yield parameters of common buckwheat cv. Kitawasesoba in the northern region of Japan. FAGOPYRUM 28: 43-46.

Martinenko, G. E. and N. V. Fesenko, 1989. Selection of determinant buckwheat varieties. INPROC 4TH INT SYMP ON BUCKWHEAT OREL, USSR 342-243.

Morishita, T. and T. Suzuki, 2012. Buckwheat varieties and trend of cultivation in Hokkaido, Japan. RESEARCH JOURNAL OF FOOD AND AGRICULTURE 35: 9-12 (In Japanese).

Miralles, D. J. and G. A. Slafer, 1999. Wheat development. In Satorre E. H. and G. A. Slafer (Eds.), Wheat: ecology and physiology of yield determination. pp. 13-43, Food Products Press, New York.

Suzuki, T., T. Morishita, Y. Mukasa, S. Takigawa, S. Yokota, K. Ishiguro and T. Noda, 2014. Breeding of 'Manten-Kirari', a non-bitter and trace-rutinosidase variety of Tartary buckwheat (*Fagopyrum tataricum* Gaertn.). BREED SCI 64: 344-350. DOI: https://doi.org/10.1270/jsbbs.64.344

Toyota, M., I. Tsutsui, A. Kusutani and K. Asanuma, 2001. Initiation and development of spikelets and florets in wheat as influenced by shading and nitrogen supply at the spikelet phase. PLANT PROD SCI 4: 283-290. DOI: https://doi.org/10.1626/pps.4.283

Ujihara, A. and T. Matano, 1975. Characteristics of flowering, fertilization and fructification - approaches to yielding process. J AGRI SCI 30: 406-408. (In Japanese)

Woo, S. H., S. K. Roy, S. J. Kwon, S. W. Cho, K. Sarker, M. S. Lee, K. Y. Chung and H. H. Kim, 2016. Concepts, prospects, and potentiality in buckwheat (*Fagopyrum esculentum* Moench): a research perspective. In: Meiliang, Z., I. Kreft, S. H. Woo, N. Chrungoo and G. Wieslander (Eds.), Molecular Breeding and Nutritional Aspects of Buckwheat, pp. 21-49, Academic Press, London.

Yoshikawa, T., S. Ozawa, N. Sentoku, J. Itoh, Y. Nagato S. Yokoi, 2013. Change of shoot architecture during juvenile-to-adult phase transition in soybean. PLANTA 238: 229-237. DOI: https://doi.org/10.1007/s00425-013-1895-z

IZVLEČEK

Faze razvoja rastlin navadne ajde (*Fagopyrum esculentum* Moench) še niso bile ustrezno raziskane; so pa pomembne za formiranje pridelka ajde. Poleg tega še niso jasne morebitne razlike pri razvoju navadne in tatarske ajde (*F. tataricum* (L.) Gaertn.). V tej študiji sta bili proučeni rast in razvoj točke rasti vse do faze nastanka cvetnih brstov, pri navadni ajdi, sorta 'Kitawasesoba' in tatarski ajdi, sorta 'Manten-Kirari'. Ugotovljene so razlike v fazah razvoja med obema kultivarjema. Obe proučevani sorti sta vodilni pri pridelovanju ajde na otoku Hokkaido, Japonska. Raziskani so listni primordiji, stranski in glavni cvetni brsti in apikalna točka rasti. Glede morfologije točke rasti ni bistvenih razlik med kultivarjema, se pa rastline obeh raziskanih kultivarjev razlikujejo v stopnjah diferenciacije in rasti.

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Research paper

Changes of physicochemical properties and correlation analysis of common buckwheat starch during germination

Licheng GAO[1], Meijuan XIA[1], Zhonghao LI[1], Pengke WANG[1], Meng WANG[2], Jinfeng GAO[*1]

[1] *State Key Laboratory of Crop Stress Biology for Arid Areas, College of Agronomy, Northwest A&F University, Yangling, Shaanxi Province 712100, China*
[2] *Yulin Institute of Agricultural Sciences, Yulin, Shaanxi Province 719000, China*
* Corresponding author, *e-mail address: gaojf7604@126.com*

E-mail addresses:
1846756406@qq.com (Licheng Gao), 2631630429@qq.com (Meijuan Xia),
2817530110@qq.com (Zhonghao Li), (Pengke Wang), wangmeng3214@126.com (Meng Wang)

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Key words: common buckwheat, germination, physicochemical properties, starch

ABSTRACT

In order to clarify the physicochemical properties of starch during germination of common buckwheat, Xinong9976 was selected as the experimental material to study the main nutrients, particle structure, particle size distribution, transparency, aging value, pasting properties and the correlation between pasting properties and starch composition and main nutrients. The results showed that main nutrients were significantly different. The diameter of starch granules ranged from 2.36 to 8.89μm, and the shapes of starch granules were irregular with obvious holes and cracks on the surface. There were significant differences in starch transparency, aging value and pasting properties at different germination stages. Peak viscosity, through viscosity and final viscosity of germinated common buckwheat was significantly positive correlated with amylopectin content ($P < 0.05$) and breakdown, final viscosity and setback were significantly negatively correlated with amylose content ($P < 0.05$). The correlation analysis of starch pasting properties and main nutrients showed that breakdown, setback and crude fat content were significantly negatively correlated ($P < 0.01$), peak viscosity, through viscosity and final viscosity were significantly negatively correlated with crude fat content ($P < 0.05$), while the starch pasting properties had no significant correlation with other nutrients.

INTRODUCTION

Common buckwheat (*Fagopyrum esculentum*) belongs to Polygonaceae, it is an important minor grain crop in China (Nam et al. 2018). Common buckwheat is rich in nutrients, containing 10.6% -15.5% of protein, 1.2% - 2.8% of fat, 63% -71.2% of starch, as well as flavonoids, mineral elements and cysteine (Gimenez-Bastida and Zielinski 2015). Recently, with the improvement of people's living standard, it has become fashionable to pursue a balanced diet with scientific based nutrition. Therefore, common buckwheat products with the reputation of "the same origin of food and medicine" will be widely welcomed by people. Besides, the development and research of common buckwheat healthy food have broad market prospects, high economic value and social value (Yoshimoto et al. 2004). There have been many studies on the cultivation methods, yield and protein content of common buckwheat in China and abroad (Salehi et al. 2018, Fang et al. 2018, Khan et al. 2012) and studies on the starch properties have also been reported. However, changes of physicochemical properties of germinated common buckwheat starch is not reported. Starch is the main nutritional component of common buckwheat. Its physicochemical properties affect the nutritional properties of related products and are also related to the development of new uses of common buckwheat starch (Stibilj et al. 2004).

Germination is a dynamic process where plants come from resting state to the state with many physiological activities. Germination treatments can enhance the respiration of plants and significantly increase the species and number of enzymes (Mamilla and Mishra 2017). After germination, the starch content of mung bean decreased (Liu et al. 2014), while the amylase activity of kidney bean increased, leading to the changes in starch structure and composition (Yanli et al. 2018). Studies have showed that buckwheat has a high peak viscosity, hot paste stability and cold paste stability (Hara et al. 2007). Tartary buckwheat flavone content significantly increased after germination (Xiao-Peng et al. 2015). In addition, germination treatment can improve the edible and health value of buckwheat, enhance the resistance of starch paste and improve the stability of starch paste (Hara et al. 2007). Therefore, the study on the characteristics of germinated common buckwheat starch is of great significance to the development and utilization. In this experiment, the main nutrients, particle structure, physicochemical properties and the correlation between gelatinization characteristic value and main nutrients of germinated common buckwheat starch were studied by using cv. Xinong9976 as material to provide basis for the development and utilization of common buckwheat sprouts and bean flour and the deep processing of starch.

MATERIALS AND METHODS

1. Experimental materials

Cv. Xinong9976, a common buckwheat variety, provided by small grain laboratory of Northwest A&F University, was used for the experiment. Common buckwheat seeds with full grain and no disease were selected and were sterilized with 0.1% H_2O_2 for 10 min and soaked in the distilled water for 24 h. Then seeds were placed in a petri dish with two layers of filter paper, the cultivation of the sample under 25°C in dark for germination, respectively taking sample after 2, 4, 6 d, removed shell, dried at 40°C.

The dried sample was grinded and passed through a 0.100 mm mesh. Added 80% ethanol, 50°C, with the ultrasonic treatment (500 w) 30 min to remove flavonoids, then added the volume of distilled water, heated under the condition of 30°C for 24 h. Centrifuged (4000 rpm, 10 min) three times, scraped off the grayish-brown soft layer. Finally, dried at 40°C for 48 h and sieved with a 0.150 mm mesh.

2. Measurement of physicochemical properties

The morphology of starch granules was observed by scanning electron microscope (JSM-6390, Jeol Ltd, Tokyo Japan) at 2000 x magnification, the particle size distribution was determined by a laser diffraction particle size analyzer and the pasting properties were measured using a rapid visco analyzer (RVA) (Newport Scientific, Pty Ltd, Warriewood, Australia).

Starch transparency was determined using a modified version of (Zhou et al. 2014). 0.5g of the starch sample was blended with 50 mL distilled water and heated in a boiling water bath for 30 min. After the starch was completely gelatinized by stirring, the starch was removed and cooled to room temperature. Distilled water was used as a blank for zero adjustment and the transparency was measured at the wavelength of 620 nm with a visible-light spectrophotometer.

The starch aging value was determined as follows: 0.5g of the starch sample was blended with 50 mL distilled wa-

Table 1 *Changes of main nutrients of common buckwheat before and after germination*

Germination time/d	Water/%	Ash/%	Crude fat/%	Crude protein/%	Total starch/%	Amylose/%	Amylopectin/%
0	12.26 ± 0.09 a	1.12 ± 0.03 c	1.37 ± 0.01 a	9.22 ± 0.06 c	68.41 ± 0.11 a	24.11±0.28 a	44.30±0.39 a
2	11.21 ± 0.03 b	1.36 ± 0.02 b	1.04 ± 0.01 b	9.42 ± 0.10 c	58.77 ± 0.81 b	22.89±0.17 b	35.88±0.97 b
4	9.78 ± 0.01 c	1.44 ± 0.01 b	0.92 ± 0.01 c	10.83 ± 0.17 b	52.80 ± 0.09 c	21.85±0.25 c	30.95±0.32 c
6	7.73 ± 0.01 d	1.69 ± 0.03 a	0.84 ± 0.01 d	13.79 ± 0.20 a	47.66 ± 0.11 d	19.77±0.20 d	27.89±0.31 d

Note: *different letters in the same column mean significant difference of* $P<0.05$*, the same as below.*

ter and heated in a boiling water bath for 30 min and added distilled water to keep the total volume constant. Removed and cooled to room temperature, refrigerated for 24h, and defrosted at room temperature, and then centrifuged at 4000 rpm for 10 min, finally weighed the sediment quality. The starch aging value index was determined as follows: starch aging value = (weight of starch paste before centrifugation – weight of sediment quality) X 100.

3. Data analysis

Three parallel tests were conducted in the experiment. SPSS 17.0 was used for statistical analysis, Origin 9.0 was used for drawing, and LSD minimum significant difference test was used for the determination of significance of differences.

RESULTS AND DISCUSSION

Changes of the concentration of main nutrients

Main nutrients of common buckwheat were shown in table 1. The results showed that after germination, the contents of water, crude fat, total starch, amylose and amylopectin decreased significantly ($P < 0.05$), while the contents of ash and crude protein increased significantly ($P < 0.05$). Among them, the crude fat mass fraction decreased the most, from 1.37% to 0.84%, a decrease of 38.69% while the ash quality score increased the most, from 1.12% to 1.69%, increasing by 51%. In addition, the relative standard deviations of main nutrients in water, ash, crude fat, crude protein, total starch, amylose and amylopectin at different stages were 19.14%, 16.76%, 22.38%, 19.50%, 15.66%, 8.30% and 20.61%, respectively, indicating that the nutritional composition of the main nutrients in different stages of the germinated common buckwheat was significantly different.

After germination, the crude protein mass fraction increased exponentially, which may be caused by the decreased protease activity in the seeds of common buckwheat during the germination process, which effectively weakened the hydrolysis of related proteins and thus promoted the protein accumulation (Ikeda et al. 1984). The decrease of total starch mass fraction may be due to the activation of α-amylase and β-amylase in the sprouting of common buckwheat, which could promote the degradation of starch and provide part of sugars needed for the germination (Mohan et al. 2010). The decrease of fat mass fraction might be due to the action of lipase, which could decompose part of the fat into the energy required for the germination and growth of common buckwheat seeds.

Starch grain structure

As can be seen on Fig. 1a, the starch particles of mature common buckwheat seeds were complete with clear gaps, mostly spherical and oval in shape, with smooth surface and no holes or cracks. After the germination of 2 d, most starch granules were irregular in shape, while a few were spherical in shape. In addition, some of the crystalline structures of starch were destroyed, and a few starch granules showed cracks on the surface (Fig. 1b). In 4 d, the starch granules were disordered. A small number of starch granules were spherical in shape, while some starch granules were deformed and condensed together with the surrounding granules (Fig. 1c). And in 6 d, the starch granules were polygonal in shape with few of them being spherical. The crystalline structures of most starch granules were destroyed, and obvious cracks and holes appeared on the surface of most granules (Fig. 1d).

The table 2 showed that in the process of germination, starch granule size distribution was more dispersed, which indicated that the size of starch granules had ob-

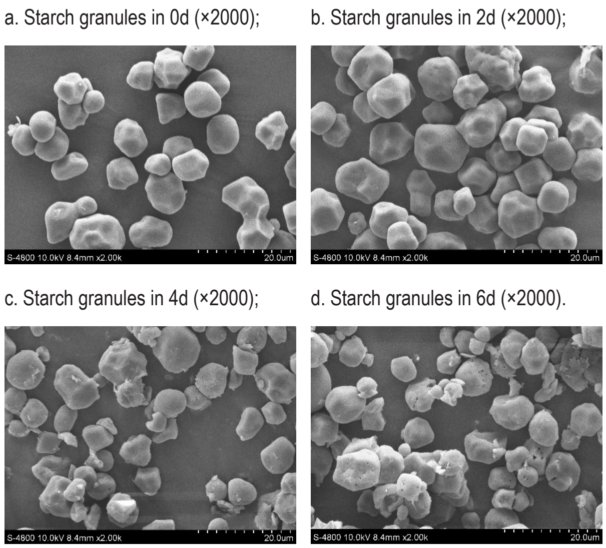

Fig.1. *Scanning electron microscopy of starch granules of common buckwheat at different germination stages*

vious differences. Chang found that the corn starch size ranged from 5.76 to 8.64 μm (Chang et al. 2004). Common buckwheat starch particles between 4.00 to 9.00 microns in diameter, which was smaller than the corn starch granules. After germination, the diameter, average sphericity and average aspect ratio of starch granules decreased significantly with the increase of germination time. In 6 d, the average diameter of starch granules decreased from 7.24 μm to 4.08 μm, a decrease of 43.65%. The average sphericity and the average aspect ratio decreased by 32.14% and 5.52%, respectively.

Starch transparency

Transparency is one of the important external characteristics of starch, which is directly related to the appearance and use of starch products (Wang et al. 2017).

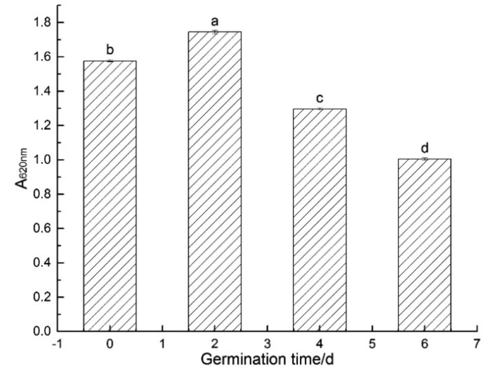

Fig.2. *Changes of the transparency of common buckwheat starch at different germination stages*

Transparency can reflect the mutual solubility of starch and water, and the transparency of buckwheat starch is positively proportional to the absorbance value, the higher the absorbance value is, the higher the transparency of the starch is (Li et al. 1997). As can be seen from Fig. 2, the transparency of common buckwheat starch first increased and then decreased with the extension of time in the germination process. And the transparency in different stages was significantly different ($P < 0.05$), the most transparent stage was 2 d and the absorbance value was 1.75, indicating that starch particles were completely expanded at this time, and there was no mutual association among the starch molecules after gelatinization. While the lowest transparency period was 6 d and the absorbance value was 1.00, which decreased by 36.30% compared with that of mature seeds. The average absorbance of starch in different germination stages was 1.41. After germination of 2d, starch transparency decreased significantly ($P < 0.05$), which may be due to the starch retrogradation, rearrangement of starch molecules and

Table 2 *Starch particle size distribution during the germination of common buckwheat*

Germination time/d	D10	D50	D90	Average sphericity	Average aspect ratio
0	4.97	7.86	8.89	0.84	1.45
2	4.68	6.64	7.98	0.76	1.43
4	3.29	5.17	6.97	0.68	1.40
6	2.36	4.05	5.84	0.57	1.37

Note: *D_{10}, D_{50} and D_{90} represented the critical particle size values when the minimum particle size was added up to 10%, 50% and 90% of the sample.*

scattering of light, thus reducing light transmission and starch transparency (Zhou et al. 2017).

Starch aging value

The essence of starch aging is that gelatinized starch molecules re-form hydrogen bonds during the cooling process(Jiamjariyatam et al. 2014). The aging process of starch can be regarded as the reverse process of gelatinization, but the degree of starch crystallization decreases after aging (Verma et al. 2018). It could be seen from Fig. 3 that there were significant differences in the aging value of common buckwheat starch in different germination stages (P < 0.05). The maximum aging value was 71.20% in mature grains. Subsequently, the aging value decreased gradually with the extension of germination time. Both 4d and 6d, the aging value decreased by 28.84%, 39.35%, respectively, which might be related to the weakened ability of buckwheat starch molecules to form hydrogen bonds again after germination (Liu et al. 2006). Starch aging not only makes food taste worse, but also reduces the digestibility (Verma et al. 2018). However, the aging value of common buckwheat gradually decreased during germination, indicating that common buckwheat sprouts were good in taste, easy to digest and had broad market development value.

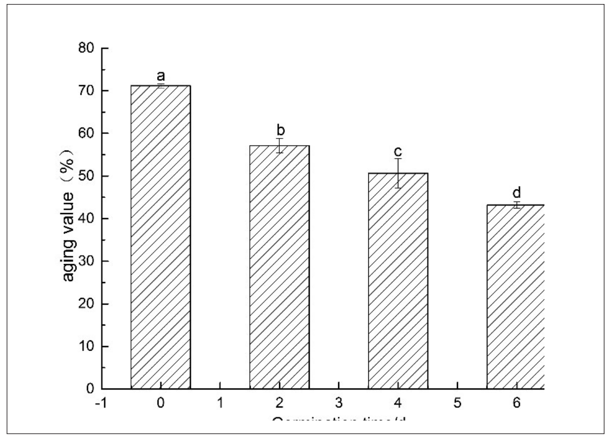

Fig.3. *Changes of the aging value of common buckwheat starch at different germination stages*

Pasting viscosity

Starch granules rapidly absorb water in aqueous solution due to thermal expansion, resulting in the fracture of intramolecular and intermolecular hydrogen bonds, and the process of gradual diffusion of starch granules is called starch paste (Jane et al. 1992). The pasting temperature was different in different germination stages due to the size of starch granules. The starch pasting properties of common buckwheat in the process of germination were shown in table 3, the results showed that starch pasting viscosities gradually decreased and significantly different (P < 0.05) in different period. After germination, peak viscosity, through viscosity, breakdown, final viscosity, setback, peak time and pasting temperature were lower than those of the mature grain starch.

Peak viscosity refers to the increase in the viscosity of starch paste caused by the friction between starch particles after full water absorption and expansion, which can reflect the expansion capacity of starch (Xiao-Li et al. 2008). As shown in table 3, the peak viscosity was 1394 - 2982 cp, the average peak viscosity was 2242 cp, and the difference was great. Through viscosity is caused by the sharp decrease in the viscosity of starch paste due to the fact that starch particles no longer friction with each other after they have expanded to the limit (Xiao-Li et al. 2008). The through viscosity was between 1335 and 2819 cp, and the average was 2131 cp. The breakdown is the difference between peak viscosity and through viscosity, which can reflect the thermal stability of starch paste. The smaller the breakdown is, the better the thermal stability is (Karim et al. 2000). The average breakdown was 111 cp, and the breakdown in mature grains was 2.76 times higher than that after germination, indicating that the starch

Table 3 *Characteristic values of gelatinization of common buckwheat starch during germination*

Germination time/d	peak viscosity /cp	Through viscosity /cp	breakdown/cp	final viscosity /cp	setback/cp	peak time /min	pasting temperature /°C
0	2982±37.64 a	2819±32.67 a	163±4.98 a	4765±34.43 a	1946±3.53 a	6.41±0.08 a	74.29±0.03 a
2	2587±18.50 b	2455±16.50 b	132±2.03 b	3982±41.51 b	1527±25.01 b	5.36±0.05 b	68.50±0.06 b
4	2005±7.84 c	1914±6.69 c	91±1.16 c	2933±17.53 c	1019±11.24 c	4.06±0.06 c	60.63±0.40 c
6	1394±11.29 d	1335±10.41 d	59±0.88 d	1988±18.02 d	654±7.62 d	2.24±0.04 d	50.73±0.34 d

had good thermal stability after germination and was suitable for the development of noodles and thickening agents. The final viscosity can reflect the retrogradation property of starch (Xiao-Li et al. 2008). After germination, the final viscosity decreased significantly, reaching 58.28% in 6d. The setback is the difference between final viscosity and through viscosity, which can reflect the stability of cold paste of starch. It can be seen from table 3 that the starch was not easy to age after germination and was suitable for making food such as common buckwheat instant noodles.

Correlation analysis of pasting properties and starch composition

Starch is the main component of common buckwheat grain, accounting for 60 -75% of the grain. The contents, composition and properties of buckwheat starch directly affect the processing technology of buckwheat food (Xin-Hua et al. 2009). The table 4 showed that peak viscosity, through viscosity and final viscosity of germinated common buckwheat was significantly positive correlated with amylopectin content ($P < 0.05$), which was the same as the relationship between the pasting viscosity and starch composition of the rice starch or germinated brown rice starch reported in previous studies, that was, the higher the content of amylopectin was, the higher the peak viscosity, through viscosity and final viscosity were. Breakdown, final viscosity and setback were significantly negatively correlated with amylose content ($P < 0.05$). Studies have shown that the short-term retrogradation of starch is mainly caused by the gelation order and dehydration crystallization of amylose molecules. However, setback was significantly negatively correlated with amylose content ($P < 0.05$), indicating that germinated common buckwheat with low amylose content was easy to retrogradate.

Correlation analysis of pasting properties and other nutrients

The correlation analysis of starch pasting properties and other nutrients was shown in table 5. In 6d, pasting properties was positively correlated and negatively correlated with the main nutrients. Breakdown and setback were significantly negatively correlated with crude fat content ($P < 0.01$), indicating that the thermal stability and cold paste stability gradually increased after germination with the decrease of crude fat content. Peak viscosity, through viscosity and final viscosity were negatively correlated with crude fat content ($P < 0.05$). There was no significant relationship between pasting viscosity and water content, which may be due to the fast growth rate and the need to consume more water for growth, resulting in low water content. Similarly, pasting viscosity had no significant relationship with ash content and crude protein content.

Table 4 *Correlation coefficient between pasting properties and starch composition (r/p)*

Varieties	peak viscosity /cp	Through viscosity /cp	breakdown/cp	final viscosity /cp	setback /cp	peak time /min	pasting temperature /°C
Total starch	0.973/0.149	0.974/0.147	0.960/0.180	0.967/0.164	0.956/0.189	0.910/0.273	0.872/0.326
Amylose	-0.996/0.059	-0.995/0.062	-0.999/0.028*	-0.998/0.044*	-1.000/0.019*	-0.995/0.064	-0.983/0.118
Amylopectin	1.000/0.017*	1.000/0.014*	0.997/0.048*	0.999/0.032*	0.996/0.057	0.976/0.141	0.954/0.194

Note: *Linear correlation coefficient between r. **: Significant correlation at the level of 0.01, *: Significant correlation at the level of 0.05, the same below.*

Varieties	peak viscosity /cp	Through viscosity /cp	breakdown/cp	final viscosity /cp	setback /cp	peak time /min	pasting temperature /°C
Water	0.769/0.442	0.771/0.439	0.737/0.472	0.753/0.457	0.727/0.482	0.631/0.565	0.564/0.618
Ash	-0.798/0.412	-0.795/0.415	-0.826/0.381	-0.812/0.397	-0.834/0.372	-0.899/0.288	-0.933/0.235
Crude fat	-0.998/0.039*	-0.998/0.042*	-1.000/0.009**	-0.999/0.024*	-1.000/0.000**	-0.991/0.084	-0.977/0.137
Crude protein	-0.772/0.438	-0.770/0.441	-0.802/0.407	-0.787/0.423	-0.811/0.398	-0.880/0.315	-0.917/0.261

Table 5 *Correlation coefficient between pasting properties and main nutrients (r/p)*

CONCLUSIONS

The results showed that in comparison to non-germinated grain, after germination, the concentration of main nutrients of common buckwheat were significantly different, where the content of crude fat, total starch, amylose and amylopectin decreased significantly while the content of ash and crude protein increased significantly. Starch granules were arranged in a disorderly manner, most of which were irregular in shape, few of which were spherical in shape. Moreover, the crystal structure of most starch granules was destroyed, and obvious cracks and voids appeared on the surface. In addition, starch size of mature common buckwheat was 4-9 μm. Pasting properties were closely related to the starch composition, and peak viscosity, through viscosity, final viscosity and setback were significantly positively correlated with amylopectin content, while breakdown, final viscosity and setback were significantly negatively correlated with amylose content. In addition, breakdown, setback and fat content were significantly negatively correlated, and peak viscosity, through viscosity and final viscosity were significantly negatively correlated with fat content, while pasting properties were not significantly correlated with other nutrients.

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REFERENCES

Chang Y H, Lin J H, Li C Y, 2004. Effect of ethanol concentration on the physicochemical properties of waxy corn starch treated by hydrochloric acid. Carbohydrate Polymers, 57(1):89-96.

Fang X, Li Y, Jiao N, et al, 2018. Effects of nitrogen fertilizer and planting density on the leaf photosynthetic characteristics, agronomic traits and grain yield in common buckwheat (Fagopyrum esculentum M.). Field Crops Research, 219:160-168.

Giménez-Bastida, J. A., & Zieliński, H, 2015. Buckwheat as a functional food and its effects on health. Journal of Agricultural and Food Chemistry, 63(36), 7896–7913.

Hara T, Matsui K, Noda T, et al, 2007. Effects of Preharvest Sprouting on Flour Pasting Viscosity in Common Buckwheat (Fagopyrum esculentum Moench). Plant Production Science, 10(3):361-366.

Ikeda K, Arioka K, Fujii S, et al, 1984. Effect on buckwheat protein quality of seed germination and changes in trypsin inhibitor content. Cereal Chemistry, 61(3), 236-238.

Jane, J.L, Chen J, 1992. Effect of amylose molecular size and amylopectin branch chain length on paste properties of starch. Cereal Chemistry, 69(1):60-65.

Jiamjariyatam R, Kongpensook V, Pradipasena P, 2014. Effects of amylose content, cooling rate and aging time on properties and characteristics of rice starch gels and puffed products. Journal of Cereal Science, 61:16-25.

Khan N, Takahashi Y, Katsubetanaka T, 2012. Tandem repeat inserts in 13S globulin subunits, the major allergenic storage protein of common buckwheat (Fagopyrum esculentum Moench) seeds. Food Chemistry, 133(1):29-37.

Karim A A, Norziah M H, Seow C C, 2000. Methods for the study of starch retrogradation. Food Chemistry, 71 (1) :9-36.

Liu R, Zhang D, He X, et al, 2014. The relationship between antioxidant enzymes activity and mungbean sprouts growth during the germination of mungbean seeds treated by electrolyzed water. Plant Growth Regulation, 74(1):83-91.

Li W, Lin R, Corke H, 1997. Physicochemical Properties of Common and Tartary Buckwheat Starch. Cereal Chemistry, 74(1):79-82.

Liu H, Nam E, Cui S W, 2006. Effects of yellow mustard mucilage on functional and rheological properties of buckwheat and pea starches. Food Chemistry, 95(1):83-93.

Mamilla R K, Mishra V K, 2017. Effect of germination on antioxidant and ACE inhibitory activities of legumes. LWT - Food Science and Technology, 75:51-58.

Mohan B H, Malleshi N G, Koseki T, 2010. Physico-chemical characteristics and non-starch polysaccharide contents of Indica and Japonica brown rice and their malts. LWT - Food Science and Technology, 43(5):0-791.

Nam T G, Kim D O, Eom S H, 2018. Effects of light sources on major flavonoids and antioxidant activity in common buckwheat sprouts. Food Science & Biotechnology, 27(1):1-8.

Salehi A, Mehdi B, Fallah S, et al, 2018. Productivity and nutrient use efficiency with integrated fertilization of buckwheat–fenugreek intercrops. Nutrient Cycling in Agroecosystems, 110(68–7):1-19.

Stibilj V, Kreft I, Smrkolj P, Osvald J, 2004. Enhanced selenium content in buckwheat (Fagopyrum esculentum Moench) and pumpkin (Cucurbita pepo L.) seeds by foliar fertilisation. Eur. Food Res. Technol. 219, 142–144.

Verma R, Jan S, Rani S, et al, 2018. Physicochemical and functional properties of gamma irradiated buckwheat and potato starch. Radiation Physics & Chemistry, 144:37-42.

Wang W, Zhou H, Yang H, et al, 2017. Effects of salts on the gelatinization and retrogradation properties of maize starch and waxy maize starch. Food Chemistry, 214:319-327.

Xiaopeng L, Xingxing B U, Haixia Z, et al, 2015. Effects of LED Lights on the Levels of Flavonoid during Germination of Tartary Buckwheat. Food Science, 36(3):86-89.

Xiao-Li Z, Wen-Yan X, Yi-Ming Z, 2008. Study on Relationship between Pasting Properties and Amylose Content of Different Buckwheat Varieties. Food Science, 10(1):47–55.

Xin-Hua L I, Han X F, Na Y U, 2009. Study on Properties of Buckwheat Starch. Food Science, 30(11):104-108.

Yoshimoto Y, Egashira T, Hanashiro I, Ohinata H, Takase Y, & Takeda Y, 2004. Molecular structure and some physicochemical properties of buckwheat starches. Cereal Chemistry, 81(4), 515–520.

Yanli M, Yifeng R, Ruijin Y, et al, 2018. Effect of white kidney bean extracts on estimated glycemic index of different kinds of porridge. LWT, 96:576-582.

Zhou H, Wang C, Shi L, et al, 2014. Effects of salts on physicochemical, microstructural and thermal properties of potato starch. Food Chemistry, 156(2):137-143.

Zhou Y, Wang H, Cui L, et al, 2017. Physicochemical Properties and Digestibility of Germination of Buckwheat Starch. Journal of the Chinese Cereals & Oils Association, 32(3):25-29 and 35.

IZVLEČEK

Za raziskavo fizikalno kemijskih lastnosti škroba tekom kalitve navadne ajde je bil izbran kultivar ‘Xinong9976’. Raziskava je vključevala glavna hranila, zgradbo delcev, razporeditev velikosti delcev, prosojnost, vrednost staranja, lastnosti gnetenja ter korelacijo med lastnostmi gnetenja, sestavo škroba in glavnimi nutrienti. Pri glavnih nutrientih so bile tekom kalitve značilne razlike. Premer škrobnih zrn je bil od 2.36 do 8.89μm, škrobna zrna so bila nepravilnih oblik in na površini z vidnimi odprtinami in razpokami. V različnih fazah kalitve so bile razlike glede prosojnosti, vrednosti staranja in končne viskoznosti. Najvišja vrednost viskoznosti, prava viskoznost in končna viskoznost so bile pozitivno povezane z vsebnostjo amilopektina ($P < 0.05$), več parametrov viskoznosti je bilo negativno povezanih z vsebnostjo amiloze ($P < 0.05$). Lastnost gnetljivosti škroba ni bila značilno povezana z vsebnostjo hranil.

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Research paper

Variation of rutin and quercetin contents in Tartary buckwheat germplasm

Je Hyeok YU[1], Soo Jeong KWON[1], Ju Young CHOI[1], Yong Hwan JU[1], Swapan Kumar ROY[1], Dong-Gyu LEE[1], Cheol Ho PARK[2], and Sun-Hee WOO[1*]

1 *Department of Crop Science, Chungbuk National University, Cheong-ju, Korea*
2 *College of Agricultural and Life Sciences, Kangwon National University, Chunchon, Korea*
* Corresponding author: *Sun-Hee Woo, Chungbuk National University, 1, Chungdae-ro, Seowon-gu, Cheongju-si, Chungbuk 28644, Korea, Tel: +82-43-261-2515, Fax: +82-43-273-2242, E-mail: shwoo@chungbuk.ac.kr*

E-mail addresses:
Je Hyeok YU- urbanhip@naver.com, Soo Jeong KWON- kwonsj1220@naver.com,
Ju Young CHOI- jychoi8519@gmail.com. Yong Hwan JU- joh7598@naver.com,
Swapan Kumar ROY- swapankhulna@gmail.com, Dong-Gyu LEE- dongguu00@naver.com,
Cheol Ho PARK- chpark@kangwon.ac.kr

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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 μmol.$m^{-2}.s^{-1}$ 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.

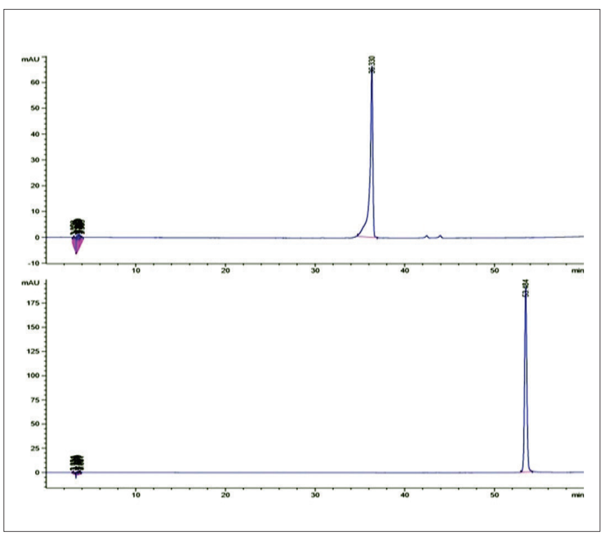

Figure 1. HPLC chromatogram patterns and retention time of rutin & quercetin

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-

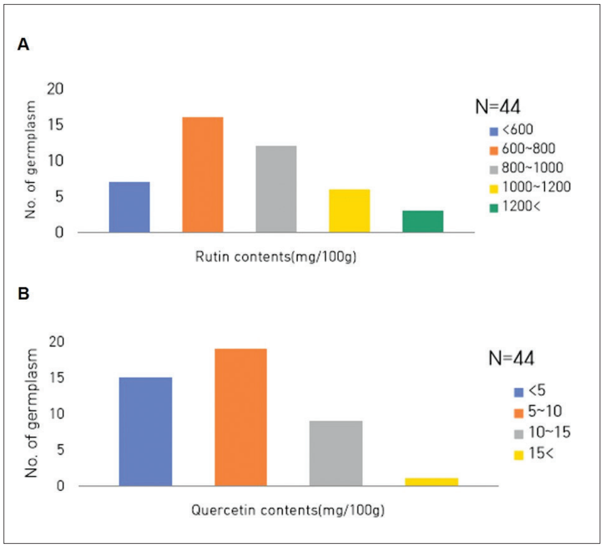

Figure 2. *Frequency distributions of rutin (A) and quercetin (B) contents in seed of Tartary buckwheat germplasm*

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-

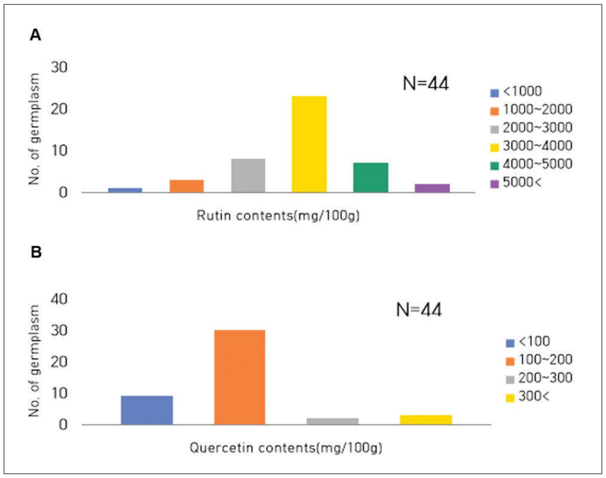

Figure 3. *Frequency distributions of rutin (A) and quercetin (B) contents in sprout of Tartary buckwheat germplasm*

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

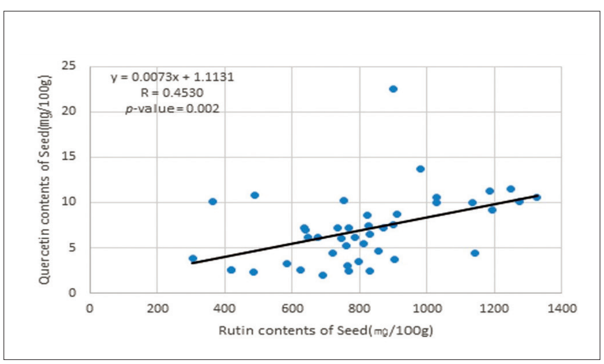

Figure 4. *Correlation between rutin and quercetin contents in seed of Tartary buckwheat germplasm*

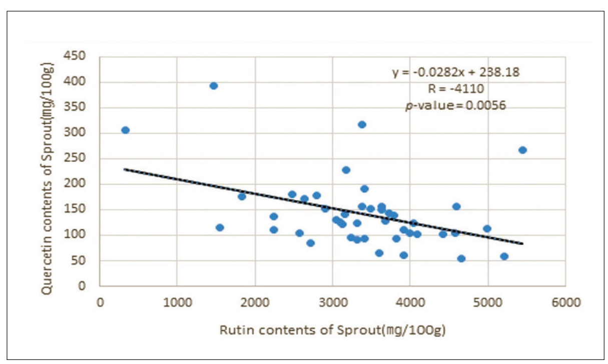

Figure 5. *Correlation between rutin and quercetin contents in sprout of Tartary buckwheat germplasm*

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.

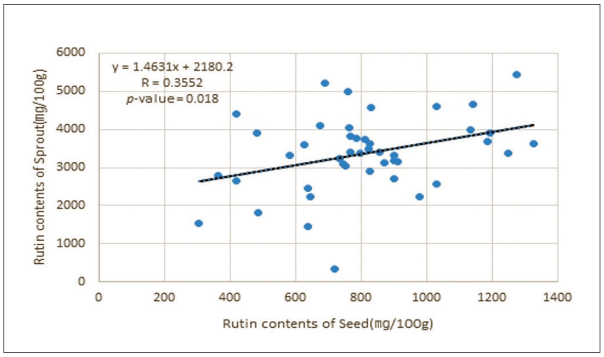

Figure 6. *Correlation between rutin contents in seed and sprout of Tartary buckwheat germplasm*

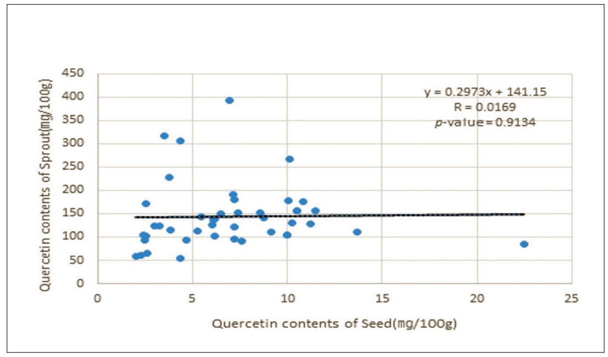

Figure 7. *Correlation between quercetin contents in seed and sprout of Tartary buckwheat germplasm*

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.

REFERENCES

Cavallini, L., A. Bindoli, and N. Siliprandi. 1978. Comparative evaluation of antiperoxidative action of silymarin and other flavonoids. Pharmacological Research Communications. 10: 133-136.

Daniel, R. S., K. S. Devi, K. T. Augusti, and C. R. S. Nair. 2003. Mechanism of action of antiatherogenic and related effects of *Ficus bengalensis* Linn. flavonoids in experimental animals. Indian Journal of Experimental Biology. 41(4): 296-303.

Formica, J. V., and W. Regelson. 1995. Review of the biology of quercetin and related bioflavonoids. Food and Chemical Toxicology. 33: 1061-1080.

Golob, A., V. Stibilj, I. Kreft, and M. Germ. 2015. The feasibility of using Tartary buckwheat as a Se-containing food material. Journal of Chemistry, Article ID 246042, 1-4.

Graf, B. A., P. E. Milbury, and J. B. Blumberg. 2005. Flavonols, flavones, flavanones, and human health: epidemiological evidence. Journal of Medicinal Food. 8: 281-290.

Halliwell, B., J. M. C. Gutteridge, and C. E. Cross. 1992. Free radicals, antioxidants and human disease: where are we now. Journal of Laboratory and Clinical Medicine. 119(6): 598-620.

He, J., M. J. Klag, P. K. Whelton, J. P. Mo, J. Y. Chen, M. C. Qian, P. S. Mo, and G. Q. He. 1995. Oats and buckwheat intakes and cardiovascular disease risk factors in an ethnic minority of China. The American Journal of Clinical Nutrition. 61: 366-372.

Jeon, A. Y. 2012. Effects of LED light conditions on growth and analysis of functional components and metabolites in buckwheat. MS Thesis, Chungbuk National University.

Kim, S. J., I. S. M. Zaidul, T. Maeda, T. Suzuki, N. Hashimoto, S. Takigawa, T. Noda, C. Matsuura-Endo, and H. Yamauchi. 2007. A time-course study of flavonoids in the sprouts of tartary (*Fagopyrum tataricum* Gaertn.) buckwheats. Scientia Horticulturae. 115: 13-18.

Kim, S. L., S. K. Kim, and C. H. Park. 2004. Introduction and nutritional evaluation of buckwheat sprouts as a new vegetable. Food Research International. 37: 319-327.

Kim, Y. S., J. G. Kim, Y. S. Lee, and I. J. Kang. 2005. Comparison of the chemical components of buckwheat seed and sprout. Journal of the Korean Society of Food Science and Nutrition. 34(1): 81-86.

Kimura, M., and H. Yamada. 1984. Interaction in the antibacterial activity of flavonoids from *Sophora japonica* L. to propionibacterium. Journal of the Pharmaceutical Society of Japan. 104: 340-346.

Kreft, I., K. J. Chang, Y. S. Choi and C. H. Park. 2003. Ethnobotany of buckwheat. Jinsol Publishing Co. Seoul. 154.

Lamson, D. W., and M. S. Brignall. 2000. Antioxidants and cancer, part 3: quercetin. Alternative Medicine Review. 5(3): 196-208.

Lee, J. H. 2007. Studies of antioxidant and anti-inflammatory activity for *Elsholtzia splendens*. Annals of Plant Resources Research. 6: 147-161.

Lee, J. M., J. H. Park, W. M. Chu, Y. M. Yoon, E. Park, and H. R. Park. 2011. Antioxidant activity and alpha-glucosidase inhibitory activity of stings of *Gleditsia sinensis* extract. Journal of Life Sciences. 21(1): 62-67.

Lee, J. S., S. S. Song, Y. S. Maeng, Y. K. Chang, and J. S. Ju. 1994. Effects of buckwheat on organ weight, glucose and lipid metabolism in streptozocin-induced diabetic rats. Korean Journal of Nutrition. 27: 819-827.

Lee, M. H., S. J. Kim, and S. N. Park. 2013. Development of porous cellulose-hydrogel system for enhanced transdermal delivery of quercetin and rutin. Polymer (Korea). 37(3): 347-355

Lee, M. K., S. H. Park, and S. J. Kim. 2011. A time-course study of flavonoids in buckwheats (*Fagopyrum species*). Jounal of Agricultural Science. 38(1): 87-94.

Lim, Y. S., B. J. Park, C. H. Park, J. I. Park, Y. S. Kim, K. H. Park, Y. K. Kang, and K. J. Chang. 2009. Labor-saving practices in tartary buckwheat (*Fagopyrum tataricum*) production. Korean Journal of Plant Resources. 22(4): 359-363.

Margna, U., and E. Margna. 1978. Differential biosynthesis of buckwheat flavonoids from endogenous substrates. Biochemie und Physiologie der Pflanzen. 173: 347-354.

Park, B. J., J. I. Park, K. J. Chang, and C. H. Park. 2005a. Comparison in rutin content of tartary buckwheat (*Fagopyrum tataricum*). Korean Journal of Plant Resources. 18(2): 246.

Park, B. J., K. J. Chang, J. I. Park, and C. H. Park. 2004. Effects of temperature and photoperiod on the growth of tatary buckwheat (*Fagopyrum tataricum*). Korean Journal of Plant Resources. 17(3): 352-357.

Park, B. J., S. M. Kwon, J. I. Park, K. J. Chang, and C. H Park. 2005b. Phenolic compound in common and tartary buckwheat. Korean Journal of Crop Science. 50(1): 175-180.

Park, H. S. 2011. Comparison of antioxidant activities of wild grape seed (*Vitis coignetiae* seed) extracts by solvents. The Korean Journal of Culinary Research. 17(1): 270-279.

Park, M. H., C. Choi, and M. J. Bae. 2000. Effect of polyphenol com- pounds from persimmon leaves (*Diospyros kaki* folium) on allergic contact dermatitis. Journal of the Korean Society of Food Science and Nutrition. 29(1): 111-115.

Park, Y. M., J. B. Jeong, J. H. Seo, J. H. Lim, H. J. Jeong, and E. W. Seo. 2011. Inhibitory effect of red bean (*Phaseolus angularis*) hot water extracts on oxidative DNA and cell damage. Korean Journal of Plant Resources. 24(2): 130-138.

Shin, J. G., J. W. Park, J. K. Pyo, M. S. Kim, and M. H. Chung. 1990. Protective effects of a ginseng component, maltol (2-methyl- 3-hydroxy-4-pyrone) against tissue damages induced by oxygen radicals. International Symposium on Ginseng. 45-48.

Shin, D.H., A.H. Mostafa Kamal, T. Suzuki, Y.H. Yun, M.S. Lee, K.Y. Chung, H.S. Jeong, C.H. Park, J.S. Choi, and S.H. Woo. 2010. Reference proteome map of buckwheat (*Fagopyrum esculentum* and *Fagopyrum tataricum*) leaf and stem cultured under light or dark. Australian Journal of Crop Science, 4(8): 633-641.

Suzuki, T., M. Kimura, M. Kawakatsu, K. Nakatsuka, S. J. Kim, Y. Mukasa, S. Yokota, H. Yamauchi, S. Takigawa, T. Noda, N. Hashimoto, and C. Matsuura-Endo. 2008. "Hokkai T 9" and "Hokkai T 10": new tartary buckwheat varieties for sprouts and dried powders. Hokkaido Agricultural Research. 188: 45-53.

Wang, Q., T. Ogura and L. Wang. 1995. Research and development of new products from bitter-buckwheat. Current Advances in Buckwheat Research. 1: 873-879.

Watanabe, M., and M. Ito. 2002. Changes in antioxidative activity and flavonoids composition of the extracts from aerial parts of buckwheat during growth period. Nippon Shokuhin Kagaku Kogaku Kaishi. 49: 119-125.

Wojcicki, J., B. Barcew-Wiszniewska, L. Samochowiec, and L. Rozwsicka. 1995. *Extractum fagopyri* reduces atherosclerosis in high fat diet fed rabbits. Die Pharmazie. 50: 560-562.

Zhu, M., Y. Gong, Z. Yang, G. Ge, C. Han, J. Chen. 1999. Green tea and its major components ameliorate immune dysfunction in mice bearing lewis lung carcinoma and treated with the carcinogen NNK. Nutrition and cancer. 35(1): 64-72.

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.

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