Postprint Study on Total Flavonoids, Crude Protein and Protein Component Contents in Different Strains of Self-fertile Sweet Buckwheat, Golden Tartary Buckwheat and Rice Tartary Buckwheat

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Abstract

To investigate the nutritional and health value of new buckwheat varieties, this study examined the variation in crude protein, total flavonoids, protein component contents, and fruit traits of seeds from 56 different lines of self-fertile common buckwheat, golden tartary buckwheat, and rice tartary buckwheat. The results showed that the average crude protein contents in seeds of common buckwheat, golden tartary buckwheat, and rice tartary buckwheat were 13.19%, 15.44%, and 11.75%, respectively; the average total flavonoid contents were 0.14%, 2.50%, and 2.09%, respectively; the average albumin contents were 5.22%, 6.13%, and 4.56%, respectively; the average globulin contents were 1.29%, 1.15%, and 0.91%, respectively; the average prolamin contents were 0.42%, 0.58%, and 0.55%, respectively; the average glutelin contents were 2.66%, 3.36%, and 2.80%, respectively. The protein components of all three buckwheat types followed the pattern albumin > glutelin > globulin > prolamin. Regarding fruit traits, common buckwheat showed the highest coefficients of variation for thousand-grain weight, fruit area, and fruit diameter, while rice tartary buckwheat exhibited the highest coefficients of variation for fruit perimeter, fruit length-to-width ratio, fruit length, fruit width, and 50 mL volume weight. Correlation analysis revealed that in common buckwheat, the correlations of crude protein content with fruit length-to-width ratio and fruit length; in golden tartary buckwheat, the correlations of crude protein content with fruit perimeter and fruit length; and in rice tartary buckwheat, the correlations of crude protein content with fruit width as well as total flavonoid content with fruit area, fruit width, fruit diameter, and 50 mL volume weight all reached significant or highly significant levels. This study identified buckwheat lines with high protein content or high flavonoid content, including common buckwheat (1808-166, superior line of Guitian No. 2), golden tartary buckwheat (Duoku 74, Duoku 78), and rice tartary buckwheat (1906-136, black-grain buckwheat, 43-2). These results provide valuable guidance for the breeding of superior buckwheat varieties and the development of new buckwheat products.

Full Text

Analysis of Total Flavonoids, Crude Protein, and Protein Components in Different Lines of Self-Fertile Common Buckwheat, Golden Tartary Buckwheat, and Rice Tartary Buckwheat

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Abstract: To investigate the nutritional and health value of new buckwheat varieties, this study examined variations in crude protein, total flavonoids, protein components, and fruit traits across 56 different lines of self-fertile common buckwheat, golden tartary buckwheat, and rice tartary buckwheat. The results showed that average crude protein contents in common buckwheat, golden tartary buckwheat, and rice tartary buckwheat seeds were 13.19%, 15.44%, and 11.75%, respectively, while average total flavonoid contents were 0.14%, 2.50%, and 2.09%. Average albumin contents were 5.22%, 6.13%, and 4.56%; globulin contents were 1.29%, 1.15%, and 0.91%; gliadin contents were 0.42%, 0.58%, and 0.55%; and glutenin contents were 2.66%, 3.36%, and 2.80%, respectively. All three buckwheat types followed the protein component pattern of albumin > glutenin > globulin > gliadin. Among fruit traits, common buckwheat showed the highest coefficients of variation for 1,000-fruit weight, fruit area, and fruit diameter, while rice tartary buckwheat exhibited the highest coefficients of variation for fruit perimeter, length-width ratio, fruit length, fruit width, and 50 mL volumetric weight. Correlation analysis revealed significant or highly significant relationships between crude protein content and fruit length-width ratio and fruit length in common buckwheat; between crude protein content and fruit perimeter and fruit length in golden tartary buckwheat; and between crude protein content and fruit width, as well as between total flavonoid content and fruit area, fruit width, fruit diameter, and 50 mL volumetric weight in rice tartary buckwheat. This study identified high-protein or high-flavonoid buckwheat lines including common buckwheat (1808-166, Guitian No. 2 elite line), golden tartary buckwheat (Duoku 74, Duoku 78), and rice tartary buckwheat (1906-

136 black rice buckwheat, 43-2). These findings provide valuable guidance for breeding superior buckwheat varieties and developing new buckwheat products.

Keywords: self-fertile common buckwheat; golden tartary buckwheat; rice tartary buckwheat; crude protein; total flavonoids; fruit traits

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Buckwheat belongs to the Polygonaceae family, genus *Fagopyrum*, comprising approximately 23 species of annual or perennial herbs. The two primary cultivated grain species are common buckwheat (*Fagopyrum esculentum*) and tartary buckwheat (*Fagopyrum tataricum*) (Li, 1998). Buckwheat contains not only the five essential nutritional elements required by humans but also abundant flavonoid compounds such as rutin and quercetin (Yin et al., 2002), endowing buckwheat products with excellent nutritional and health-promoting functions.

Protein represents a major nutritional active component in buckwheat (Du et al., 2004), with average seed protein content higher than that of major cereal crops like rice and maize (Yang, 2008). Research has demonstrated that buckwheat protein contains well-balanced essential amino acids (Shu et al., 2005; Ruan and Chen, 2008) and exhibits various health benefits including lowering blood cholesterol (Kayashita et al., 1997), anti-aging effects (Zhang et al., 1999), anti-cancer properties (Kayashita et al., 1999; Liu et al., 2001), gallstone inhibition (Tomotake et al., 2002), fat accumulation suppression (Kayashita et al., 1995), blood pressure reduction (Yin et al., 2002), and anti-leukemia activity (Wang et al., 2002). Consequently, buckwheat represents a highly valuable protein resource. Based on solubility characteristics, buckwheat proteins can be classified into albumin, globulin, gliadin, and glutenin, with glutenin being the primary protein responsible for dough properties (Chen, 2012) and thus holding development potential for gluten-based food products. Additionally, buckwheat contains flavonoid compounds not found in other cereals, which constitute the main functional components responsible for its health benefits (Zhao and Wang, 2008). Long-term consumption has been associated with hypertension reduction, cardiovascular disease prevention, antioxidant activity, and anti-cancer effects (Pu et al., 2019).

Common buckwheat suffers from self-incompatibility, resulting in low natural seed set rates and unstable yields. Tartary buckwheat typically has thick hulls that are difficult to remove, requiring soaking, steaming, drying, and subsequent dehulling processes that compromise quality (Chen, 2018). To address these issues, we have developed new buckwheat varieties through hybridization between self-incompatible and wild-type self-fertile common buckwheat, crosses between thick-hulled and thin-hulled tartary buckwheat, and interspecific hybridization between golden buckwheat and tartary buckwheat. These efforts have yielded new lines of self-fertile common buckwheat, rice tartary buckwheat (thin-hulled tartary buckwheat), and golden tartary buckwheat (multiple tartary buckwheat) (Chen, 1999; Chen et al., 2015; Chen et al., 2018). These varieties differ from conventional self-incompatible common buckwheat and thick-hulled tartary buckwheat, representing novel buckwheat types. Previous studies have focused primarily on genetic analysis of agronomic traits in these new buckwheat types (Guo, 2016; Hou, 2018), analysis of hull rate and related traits in rice tartary buckwheat (Cui et al., 2019), and effects of sowing season and planting methods on main agronomic traits (Yang et al., 2020). However, research on quality analysis and the relationship between quality indices and fruit traits in these new buckwheat types remains limited. Therefore, investigating the quality and fruit traits of new buckwheat types is crucial for technology transfer and advancing buckwheat industry development.

This study examined 56 lines across three cultivated buckwheat species to investigate variation in quality and fruit traits, explore correlations between quality indices and fruit characteristics, and identify high-protein and high-flavonoid buckwheat lines, thereby providing a foundation for breeding superior varieties and evaluating new buckwheat types.

1.1 Materials

The 56 experimental materials selected for this study were obtained from the Research Center of Buckwheat Industry Technology (RCBIT), Guizhou Normal University, comprising 9 common buckwheat lines, 9 golden tartary buckwheat lines, and 38 rice tartary buckwheat lines (Table 1).

1.2 Methods

1.2.1 Sample Preparation Mature, uniformly sized buckwheat fruits from each line were selected, dried, and weighed (10–15 g). The hulls were carefully removed with tweezers, and the seeds were ground into powder for subsequent analysis.

1.2.2 Determination of Total Flavonoid Content Total flavonoids were extracted using ethanol immersion and quantified by aluminum chloride colorimetry (Wang and Duan, 2012; Wang et al., 2019). For extraction, 0.05 g of buckwheat powder was placed in a 2 mL centrifuge tube with 1.5 mL of 80% ethanol, incubated at 70°C for 5 hours with shaking every 30 minutes, sonicated for 10 minutes, and centrifuged at 8,000 rpm for 10 minutes. The supernatant was collected and diluted to 5 mL with 80% ethanol. For quantification, a standard curve was prepared using rutin standards (0–0.35 mg/mL). To 0.10 mL of standard or sample solution, 1.0 mL of 1% $AlCl_3$ solution was added, vortexed, and absorbance measured at 420 nm using a microplate reader. Flavonoid content was calculated from the standard curve.

1.2.3 Determination of Protein Components Protein components were sequentially extracted using the method of Zhang et al. (2017). Albumin was extracted with 0.01 $mol \cdot L^{-1}$ Tris-HCl buffer (pH 7.5), globulin with 0.01 $mol \cdot L^{-1}$ Tris-HCl (pH 7.5) containing 0.5 $mol \cdot L^{-1}$ NaCl, gliadin with 60% n-propanol, and glutenin with a mixture of 0.5% sodium tartrate, 0.24% copper sulfate, 1.68% potassium hydroxide, and 50% n-propanol. Protein content was determined by Coomassie brilliant blue colorimetry (Li, 2011). Standard curves were prepared using bovine serum albumin (0–0.10 mg/mL), with absorbance measured at 595 nm after adding 3.0 mL of Coomassie brilliant blue solution and incubating for 2 minutes.

1.2.4 Determination of Crude Protein Crude protein content was determined according to the national food safety standard GB5009.5-2016 using the automatic Kjeldahl method with an ATN-300 automatic Kjeldahl analyzer.

1.2.5 Evaluation of Fruit Traits Two hundred buckwheat fruits were randomly selected from each line to measure 1,000-fruit weight, fruit length, fruit width, and fruit diameter using a Wanshen SC-A seed counter (Hangzhou Wanshen Detection Technology Co., Ltd.). The 50 mL volumetric weight was determined by filling a 50 mL container with buckwheat fruits and recording the average weight of three replicates.

1.2.6 Data Processing and Analysis Data were processed and analyzed using Microsoft Excel and SPSS 22.0 software.

2.1 Variation in Quality Traits of Common Buckwheat

As shown in Table 2 , crude protein content in common buckwheat lines ranged from 12.48% to 14.05%, total flavonoids from 0.07% to 0.21%, albumin from 3.93% to 6.64%, globulin from 0.94% to 1.72%, gliadin from 0.28% to 0.52%, and glutenin from 1.13% to 4.24%. The highest crude protein content (14.05%) was observed in line T1, the highest total flavonoid content (0.21%) in T3, the highest albumin content (6.64%) in T4, the highest globulin content (1.72%) in T6, the highest gliadin content (0.52%) in T3, and the highest glutenin content (4.24%) in T6.

2.2 Variation in Quality Traits of Golden Tartary Buckwheat

As shown in Table 3 , crude protein content in golden tartary buckwheat lines ranged from 13.44% to 18.20%, total flavonoids from 2.32% to 2.81%, albumin from 4.42% to 7.30%, globulin from 0.90% to 1.40%, gliadin from 0.27% to 0.79%, and glutenin from 2.19% to 4.33%. The highest crude protein content (18.20%) was observed in line D2, the highest total flavonoid content (2.81%) in D1, the highest albumin content (7.30%) in D3, the highest globulin content (1.40%) in D5, the highest gliadin content (0.79%), and the highest glutenin content (4.33%) in D4.

2.3 Variation in Quality Traits of Rice Tartary Buckwheat

As shown in Table 4 , crude protein content in rice tartary buckwheat lines ranged from 9.48% to 14.19%, total flavonoids from 1.63% to 2.56%, albumin from 2.68% to 7.01%, globulin from 0.42% to 1.29%, gliadin from 0.17% to 0.75%, and glutenin from 1.74% to 3.99%. The highest crude protein content (14.19%) was observed in line M6, the highest total flavonoid content (2.56%) in M38, the highest albumin content (7.01%) in M6, the highest globulin content (1.29%) in M26, the highest gliadin content (0.75%) in M29, and the highest glutenin content (3.99%) in M12.

2.4 Comparison of Quality Traits Among Buckwheat Types

As shown in Table 5 , golden tartary buckwheat exhibited significantly higher crude protein, total flavonoid, and glutenin contents compared to common buckwheat and rice tartary buckwheat. Rice tartary buckwheat had the lowest crude protein content, with both crude protein and globulin significantly lower than in common buckwheat and golden tartary buckwheat. Common buckwheat showed the lowest total flavonoid and glutenin contents, significantly lower than both golden and rice tartary buckwheat.

2.5 Variation in Fruit Traits Among Buckwheat Types

Evaluation of fruit traits revealed considerable variation among buckwheat types (Table 6). In common buckwheat, 1,000-fruit weight ranged from 28.21 to 47.23 g (mean 33.50 g), fruit area from 18.64 to 27.08 mm^2 (mean 20.60 mm^2), fruit perimeter from 17.12 to 20.90 mm (mean 18.02 mm), length-width ratio from 1.36 to 1.59 (mean 1.43), fruit length from 6.06 to 7.61 mm (mean 6.47 mm), fruit width from 4.26 to 5.22 mm (mean 4.56 mm), fruit diameter from 4.85 to 5.84 mm (mean 5.08 mm), and 50 mL volumetric weight from 22.06 to 25.74 g (mean 23.81 g).

In golden tartary buckwheat, 1,000-fruit weight ranged from 29.33 to 42.75 g (mean 34.81 g), fruit area from 16.03 to 20.91 mm^2 (mean 17.71 mm^2), fruit perimeter from 16.19 to 18.91 mm (mean 16.99 mm), length-width ratio from 1.27 to 1.50 (mean 1.39), fruit length from 5.44 to 6.78 mm (mean 5.89 mm), fruit width from 3.96 to 4.56 mm (mean 4.29 mm), fruit diameter from 4.50 to 5.14 mm (mean 4.72 mm), and 50 mL volumetric weight from 22.37 to 24.59 g (mean 23.49 g).

In rice tartary buckwheat, 1,000-fruit weight ranged from 11.70 to 20.16 g (mean 13.89 g), fruit area from 7.45 to 11.88 mm^2 (mean 8.65 mm^2), fruit perimeter from 10.59 to 13.76 mm (mean 11.55 mm), length-width ratio from 1.25 to 1.75 (mean 1.44), fruit length from 3.71 to 5.25 mm (mean 4.13 mm), fruit width from 2.58 to 3.62 mm (mean 2.90 mm), fruit diameter from 3.07 to 3.86 mm (mean 3.29 mm), and 50 mL volumetric weight from 22.00 to 28.66 g (mean 25.92 g).

2.6 Correlation Analysis Between Quality and Fruit Traits

Correlation analysis revealed distinct relationships among traits in different buckwheat types (Table 7). In common buckwheat, crude protein content showed highly significant positive correlations with fruit length-width ratio and significant positive correlations with fruit length. Total flavonoid content exhibited significant negative correlations with globulin and glutenin contents but no significant correlations with fruit traits. Albumin content showed highly significant negative correlations with fruit length-width ratio, while globulin and glutenin contents were significantly positively correlated. Among fruit traits, all characteristics except length-width ratio and 50 mL volumetric weight showed significant or highly significant positive correlations with each other.

In golden tartary buckwheat, crude protein content was significantly negatively correlated with gliadin content, fruit perimeter, and fruit length. Total flavonoid content showed no significant correlations with any traits. Albumin content was highly significantly negatively correlated with 1,000-fruit weight, fruit area, and fruit perimeter, and significantly negatively correlated with fruit length, fruit width, and fruit diameter. Gliadin content was highly significantly positively correlated with glutenin content. Among fruit traits, 1,000-fruit weight showed significant or highly significant positive correlations with fruit area, perimeter, length, width, and diameter. Fruit area was significantly or highly significantly positively correlated with fruit perimeter, length, width, and diameter. Fruit perimeter was significantly or highly significantly positively correlated with fruit width and diameter. Fruit length-width ratio was significantly positively correlated with fruit length, while both fruit length and width were highly significantly positively correlated with fruit diameter.

In rice tartary buckwheat, crude protein content was significantly positively correlated with total flavonoid content and highly significantly positively correlated with albumin and globulin contents, but highly significantly negatively correlated with fruit width. Total flavonoid content was significantly negatively correlated with 1,000-fruit weight, fruit area, and fruit diameter, highly significantly negatively correlated with fruit width, and significantly positively correlated with 50 mL volumetric weight. Albumin content was highly significantly positively correlated with globulin content, while gliadin content was significantly positively correlated with glutenin content. Among fruit traits, 1,000-fruit weight showed no significant correlation with length-width ratio, and fruit area and perimeter showed no significant correlation with length-width ratio, which in turn showed no significant correlation with fruit diameter or 50 mL volumetric weight.

Discussion

3.1 Buckwheat Seed Quality

This study compared crude protein and total flavonoid contents among self-fertile common buckwheat, rice tartary buckwheat, and golden tartary buckwheat, revealing significant differences among varieties. Golden tartary buckwheat exhibited the highest crude protein, glutenin, and flavonoid contents, while rice tartary buckwheat showed higher flavonoid and glutenin contents than common buckwheat. Given that golden tartary buckwheat possesses excellent characteristics such as strong adaptability, stress resistance, and regeneration capacity, enabling multiple harvests from a single sowing (Chen, 2018), it should be prioritized as a key breeding objective in future buckwheat improvement programs. The difficulty of dehulling has long been a major challenge in buckwheat industrialization, but rice tartary buckwheat features thin hulls and easy dehulling, making high-protein, high-flavonoid rice tartary buckwheat lines valuable breeding materials. Although common buckwheat contains relatively high crude protein, its flavonoid content is generally low, suggesting that breeding efforts should focus on enhancing protein content as a protein source. Glutenin is the main component of dough gluten proteins, and the glutenin contents of 2.66%, 3.36%, and 2.80% found in common buckwheat, golden tartary buckwheat, and rice tartary buckwheat, respectively, are significant for buckwheat flour dough properties.

Previous research on buckwheat protein and flavonoid content has primarily focused on common buckwheat and conventional tartary buckwheat, with limited studies on new buckwheat types. Liu et al. (2007) reported average total flavonoid and protein contents of 2.46% and 14.30% in 76 tartary buckwheat accessions. Rao et al. (2016) documented a mean flavonoid content of 2.078% in 100 thin-hulled tartary buckwheat lines. Shi et al. (2011) investigated buckwheat performance across different ecological regions, finding protein content ranging from 13.79% to 20.96% and flavonoid content from 0.04% to 0.25% in seven common buckwheat varieties. Our results are generally consistent with these previous studies, though some differences with Wang et al. (2015) may be attributed to variations in experimental materials and cultivation environments, as crop quality is influenced by both genetic and environmental factors (Shi et al., 2011). This study demonstrates that golden tartary buckwheat has significantly higher crude protein and total flavonoid contents than rice tartary buckwheat and common buckwheat, indicating its advantages in breeding superior varieties and its increasing importance in buckwheat industrialization research. New types of tartary buckwheat represent a promising direction for buckwheat genetic improvement. The high-protein and high-flavonoid lines identified in this study can be applied in breeding programs and production to enhance the nutritional and health value of new buckwheat varieties and products.

3.2 Relationship Between Fruit Traits and Seed Quality

Few studies have reported on correlations between buckwheat fruit traits and seed quality. Lü et al. (2020) found significant positive correlations between tartary buckwheat flavonoid content and seed area and perimeter, which differs from our findings, likely due to differences in experimental materials. This study revealed that common buckwheat crude protein content was highly significantly or significantly positively correlated with fruit length-width ratio and fruit length. Golden tartary buckwheat crude protein content was significantly negatively correlated with fruit length. Rice tartary buckwheat crude protein content was highly significantly negatively correlated with fruit width, while total flavonoid content showed significant or highly significant correlations with fruit area, fruit width, fruit diameter, and 50 mL volumetric weight. These findings suggest that selection based on these correlated traits could improve breeding efficiency for developing new buckwheat types with high protein and flavonoid contents.

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Note: Figure translations are in progress. See original paper for figures.