Correlation and genetic analysis of seed shell thickness and yield factors in Tartary buckwheat (*Fagopyrum tataricum* (L.) Gaertn.)

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In order to solve the difficult problem of the outer shell covering in the processing of Tartary buckwheat, we conducted a genetic analysis in segregating F₂ and F₃ populations derived from a hybrid between ‘Yunqiao No. 1’ and ‘Rice buckwheat’, and the F₃ population was used to analyze the phenotypic and genetic correlation among the traits. The results showed that the variety with a value of trait for seed shell thickness over 0.20 mm is a hard-shelled type (The thick shell type = seed shell rate > 20%), and that with a value of trait for seed shell thickness below 0.15 mm is a easily-shelled type (The thin shell type = seed shell rate < 20%), while that with a value of trait for seed shell thickness ranging from 0.15 mm to 0.20 mm is a hard-shelled type or easily-shelled type. In addition, alleles for traits of number of seed per plant and total seed weight per plant have larger dominance variance relative to their additive variance, indicating that genes controlling these traits have larger dominant effects, it is not suitable for the selection of single plant in offspring plants at the early stage of development, because these traits do not show up then. The alleles for traits of 1000 kernel weight and seed shell thickness have larger additive variance relative to their dominant variance, indicating that genes governing these traits have greater additive effects, with which the single plant can be selected in the progeny at the early stage of development. Although, the value of seed shell thickness has been shown to correlated positively with that of 1000 kernel weight, almost all the seeds of easily-shelled type are those with thin shell. However, ideal single plants with easily-shelled trait are those with intermediate phenotypes of seed shell thickness and 1000 kernel weight, by which the traits of large number of seeds per plant and total seed weight per plant could be selected. In the progeny population of this study, there were excellent single plants with high-yield and easily-shelled traits, of which the value of seed shell thickness was 0.17 mm (0.15 mm to 0.20 mm), the value of 1000 kernel weight was 14 g, the value of number of seeds per plant was 1137 and value of total seed weight per plant was 15.9 g. The results showed that taking the hybrid combinations of easily-shelled trait with the trait of seed shell thickness was the most effective selection indexes to breed the high-yield buckwheat varieties with the trait of easy shelling.

**Key Words:** Tartary buckwheat, seed shell thickness, genetic parameter, genetic correlation, phenotypic correlation, hybridization breeding.
Buckwheat grains, tea and flours can be made of Tartary buckwheat removed the hulls; removing buckwheat hulls is one of the most important procedures to get high quality buckwheat food (Chen 1999, Yin et al. 2002). It is highly inefficient to remove the Tartary buckwheat hulls with traditional processing, by which it is not only difficult to remove hulls thoroughly, but also easily to crash the seeds; so de-hulling is one of the important techniques for Tartary buckwheat processing to ensure the quality of buckwheat production (Chen 1999). Certain techniques have been created for buckwheat de-hulling, which include the procedures of soaking, cooking, drying and the last step, hulling, by which the conversion cost of Tartary buckwheat increases greatly, the nutritional values and health-promoting components decline significantly and there is obvious changes in color under the hull of the grain, by which the product quality of buckwheat reduces remarkably. According to value of seed shell thickness, which can be evaluated by the parameters of seed shell rate, Tartary buckwheat can be divided into two types: thick shell type (seed shell rate > 20%–hard-shelled type) and thin shell type (seed shell rate < 20%–easily-shelled type) (Chen 2012). All in all, the phenotype of seed shell thickness is one of the most important target traits for studying the easily-shelled Tartary buckwheat.

Crossbreeding plays an important role in the innovative use of germplasm resources and breeding new varieties for varieties of Tartary buckwheat, which has been widely applied in breeding cultivars of rice, maize, wheat and other crops, but not yet been used in buckwheat breeding, because the flowers of buckwheat are cleistogamous in nature, whose floral organs are very small during anthesis, the sizes of flower and bud are about 2–3 mm and 1 mm in diameter, respectively, and it is nearly impossible to pollinate after emasculation. Mukasa et al. (2007) successfully crossbred Tartary buckwheat using warm decoction to remove stamens, and Suzuki et al. (2014a, 2014b) used the same crossbreeding method to breed Tartary buckwheat hybrid ‘Manten-Kirari’. In recent years, researches have been conducted on genetic analysis of agronomic traits and molecular marker for hybridization of Tartary buckwheat (Chen et al. 2015, Li et al. 2007, 2011, 2012, 2015a, Wang and Campbell 2007, Wang 2011, 2012).

In order to solve the tough problem of Tartary buckwheat shelling, varieties with the phenotype of easily-shelled trait should be bred. It is very important to study the genetics of germplasm resources and select the molecular markers for thin shell genes and other related genes, with which the plant breeding efficiency of easily-shelled buckwheat variety can be increased by purifying selection. In this study, research focuses on the genetic rules of thin shell characters, the phenotypic correlations and genetic correlations between the thin shell characters and other relative characters, which lay the foundations for the breeding of high-yielding Tartary buckwheat varieties with thin shell, and solve the problem of buckwheat de-hulling, by which the additional value of Tartary buckwheat can be increased, the seed yield of Tartary buckwheat can be improved. Therefore, it is significant to do this research for breeding buckwheat varieties with high quality.

**Materials and Methods**

**Plant materials**

Experimental material of female material ‘Yunqiao No. 1’ is a variety created with 60Co γ-radiation, selected and bred using systematic breeding program from Qujing local Tartary buckwheat by Biotechnology and Germplasm Resources Research Institute, Yunnan Provincial Academy of Agricultural Sciences. The characteristics of the variety, ‘Yunqiao No. 1’, are as follows: its growth period is short, it is a widely adaptable variety, it is a variety with hard-shelled trait, and 1000 kernel weight is about 22.4 g (Li et al. 2015b). The male material ‘Rice buckwheat’ is one of local varieties cultivated in certain parts of Yunnan and Sichuan (Chen 2012), whose characteristics are that: its growth period is long, it is a narrowly adaptable variety, it is a variety with easily-shelled trait, and 1000 kernel weight is about 16.4 g (Li et al. 2016) (Fig. 1).

**Fig. 1.** Female (Yunqiao No. 1) and male (Rice buckwheat) seeds.
Experimental method

Field trails were carried out in the buckwheat test base, Dianxin village, the town of Xianjie, Kunming city, Yunnan Province, P.R. of China (102°25′E Longitude, 24°45′N Latitude, 1887 m Altitude). The F₁ generation was obtained by means of hybridizing ‘Yunqiao No. 1’ (hard-shelled variety) with ‘Rice buckwheat’ (easily-shelled variety) in September, 2013. Before flowering hybridization was conducted by removing the male flower buds from the female parent using tweezers with portable magnifying glass, pollinating female flowers with the stamen of male parent, and then bagging the pollinated flower buds. The success of hybridization can be judged at this point by using SSR marker (BM27) in F₁ plants, which has a band, while the female parent ‘Yunqiao No. 1’ has no band, and the male parent ‘Rice buckwheat’ has a band. There were 232 seeds obtained from the self-intersection of F₁ in March, 2014. Fifty seeds from each parent as well as 232 seeds from F₂ populations were sown in rows with 10 cm distance between them in July, 2014. Fifty seeds from each parent and the F₃ generation of 232 strains, respectively, were planted in rows with 10 cm distance between plants in July, 2015. Planting was done in rows, which were with a row distance of 0.4 m; there were two rows in each of the plot, of which the area was 2 m².

Phenotypic measurements

Ten individuals of each parent and 232 individuals of F₂ population were investigated in 2014. Ten individuals of both male and female parent as well as 232 strains of F₃ population were studied in 2015. The parameters of the 1000 kernel weight, number of seeds per plant, total seed weight per plant and seed shell thickness were measured when 70–80% individuals of the population were mature in the years of 2014 and 2015. The value of seed shell thickness was measured by vernier caliper (111-101V-10G; Guilin Guanglu Digital Measurement and Control Co., Ltd.) after shelling process. The trait of cleavage of seed coat was used to discriminate between F₃ and F₄ progeny groups, no cleavage is particularly noticeable in the case of hard-shelled seeds, and cleavage is obvious in easily-shelled seeds.

Data analysis

Analysis of the expression values of the agronomic characters of the parents as well as F₂ and F₃ populations

Data were analyzed using JMP version 9.0.2 (SAS Institute, Cary, NC, USA); the f-test was used to compare the population variances of the four traits.

Estimation of genetic parameters

The genetic parameters and heritability were estimated from the within-generation variances of each trait (Mather and Jinks 1971). Assuming that within-generation variances are composed of an additive variance component (A), a dominance variance component (D) and an environmental variance component (E), the phenotypic variance (Vₚ) of the F₂ generation was calculated as follows:

\[ Vₚ(F₂) = 1/2A + 1/4D + E₁, \]

and that of the F₃ as follows:

\[ Vₚ(F₃) = 3/4A + 3/16D + E₁. \]

The number of individuals of each parent was different. Hence, we defined E₁ as follows (National Agricultural Research Center 1995):

\[ E₁ = (S₁ + S₂)(n₁ + n₂ - 2), \]

where S₁ and S₂ are the sums of squares, and n₁ and n₂ are the numbers of plants, of each respective parent. The broad-sense (h²ₑ[F₁]) and narrow-sense (h²ₑ[F₃]) heritability estimates were defined as follows:

\[ h²ₑ[F₁] = \frac{(1 - (1/2)^{-t})A + (1/2)^{-t}(1 - (1/2)^{-t})D}{(1 - (1/2)^{-t})(A + (1/2)^{-t}(1 - (1/2)^{-t})D) + E₁} \]

and

\[ h²ₑ[F₃] = \frac{(1 - (1/2)^{-t})A}{(1 - (1/2)^{-t})(A + (1/2)^{-t}(1 - (1/2)^{-t})D) + E₁} \]

where \( t \) is the generation. Furthermore, using the additive variances, we determined true-sense heritability—which is independent of generation—defined as follows (Ukai 2002):

\[ h²_{true} = \frac{A}{A + E}. \]

Estimation of phenotypic and genetic correlation coefficients

In the F₃ population, phenotypic correlations (\( r_p \)) and genetic correlations (\( r_G \)) among traits were estimated as follows (Ukai 2002):

\[ r_p = \frac{\text{Cov}(Yₐ,Yₜ)}{\sqrt{\text{Var}(Yₐ) \times \text{Var}(Yₜ)}}, \]

and

\[ r_G = \frac{\text{Cov}(Yₐ,Yₜ) - \text{Cov}(eₐ,eₜ)}{\sqrt{(\text{Var}(Yₐ) - \text{Var}(eₐ)) \times (\text{Var}(Yₜ) - \text{Var}(eₜ))}}, \]

where \( Yₐ \) and \( Yₜ \) are the phenotypic variances of traits A and B, respectively and \( eₐ \) and \( eₜ \) are the environmental variances of traits A and B, respectively.

Results

Distributions and expression value of parents, F₂ and F₃ generations each agronomic trait

In this study, genetic analysis was carried out in F₂ and F₃ population of ‘Yunqiao No. 1’ and ‘Rice buckwheat’, the distribution of the agronomic traits are shown in Fig. 2. Among the four agronomic traits, there were significant differences (P < 0.01) in the average values among ‘Yunqiao No. 1’, ‘Rice buckwheat’ and the F₂ and F₃ generations. It indicates that the genetic background of these two varieties is different, and the results confirmed that the accuracy of
was 3:1, showing the genetic model for single genes. And alleles of the non-cracking (hard-shelled) trait and cracking (easily-shelled) one for the fruit shell were not separated with the value of seed shell thickness, suggesting that the two alleles may be linked closely.

Genetic parameters of each agronomic trait

The genetic parameters of four agronomic traits are shown in Table 2. The additive variance for the traits of 1000 kernel weight and seed shell thickness exceeded dominant variance, and the average degrees of dominance were less than 1.0. The dominant variance for the traits of number of seed per plant and total seed weight per plant exceeded additive variance, and the average degrees of dominance greater than 1.0. The values of broad-sense heritability for the traits of number of seed per plant, 1000 kernel weight, total seed weight per plant and seed shell thickness were 0.78, 0.77, 0.74 and 0.78 in the F2 generation, those were 0.78, 0.82, 0.75 and 0.81 in the F3 generation, respectively. The values of narrow-sense heritability for the four traits in the F3 generation was higher than those in the F2 generation. The value of narrow-sense heritability for the trait of 1000 kernel weight (0.77) was the highest one, followed by that for seed shell thickness (0.65) and that for number of seeds per plant (0.45), and the value of heritability for total seed weight per plant (0.31) was the lowest one in F3 population. The value of true-sense heritability ranged from 0.62 (total seed weight per plant) to 0.84 (1000 kernel weight).
Phenotypic and genetic correlation among agronomic traits

The phenotypic correlation and genetic correlation coefficient among agronomic traits are shown in Table 3. The values for number of seed per plant and 1000 kernel weight were phenotypically and genetically correlated with those for total seed weight per plant (r = 0.925, 0.940; r = 0.966, 0.942), but there were no statistically significant differences in the parameters between that for number of seed per plant and that for 1000 kernel weight. The values for seed shell thickness were phenotypically correlated with those for 1000 kernel weight (r = 0.537), and were genetically correlated with those for 1000 kernel weight (r = 0.806) and those for total seed weight per plant (r = 0.959), but there was no significant correlation with those for number of seed per plant.

Discussion

In the present study, the separation ratio of thick shell type (hard-shelled type) and thin shell type (easily-shelled type) was about 3:1 (Fig. 2D), indicating that thin shell type was controlled by a single recessive gene, which was consistent with the results of Mukasa et al. (2009) and Chen et al. (2015). In addition, it can be determined from Table 1 that the varieties, of which the values of seed shell thickness are greater than 0.20 mm, are hard-shelled types (The thick shell type = seed shell rate > 20%), and those with the values less than 0.15 mm, are easily-shelled types (The thin shell type = seed shell rate < 20%); while the varieties with the values of seed shell thickness from 0.15 mm to 0.20 mm are hard-shelled types or easily-shelled type. In F2 progeny, single plants with the trait of small seed shell thickness existing in groups are hard-shelled types, while single plants with the trait of large seed shell thickness are easily-shelled type. It shows that the hard-shelled trait is not necessarily in a thick shell type, and the easily-shelled trait is not necessarily in a thin shell type. Therefore, we should not blindly select the single plant with the trait of small seed shell thickness when selecting the easily-shelled varieties. Because the trait of husking is one of qualitative traits and the trait of seed shell thickness is one of a quantitative traits controlled by polygene, the selection of high-yield Tartary buckwheat varieties with easily-shelled and high-yield traits can be done by using qualitative genes for the varieties with the easily-shelled trait and quantitative genes for selecting varieties with high-yield trait.

The broad-sense heritability of the 4 traits ranges from 0.74 to 0.82, of which there is no difference (Table 2). The results indicate that all traits examined were affected by environmental factors to some extent. Previously, Li et al. (2011) reported that by means of a 6 parent diallel cross of Tartary buckwheat, the values of traits of number of seeds per plant, 1000 kernel weight and total seed weight per plant, were examined and the values of broad-sense heritability ranged from 0.87 to 0.98. These differences in heritability estimates may come from the estimation methods adopted for environmental variances. They estimated the environmental variance E1 based on the means of six parental lines with two replications while, in the present study, we estimated E1 from the distributions of two parental line.
populations according to the procedure described by the National Agricultural Research Center (1995). Li et al. (2012) used the same method to determine total seed weight per plant. The value of broad-sense heritability of F2 and F3 generations was 0.73 and 0.75, respectively, which was almost the same as that of 0.74 and 0.75 in this experiment. It showed that the results of the two experiments were consistent and very close to those of Kitabayasi et al. (1995b), indicating the stability of the broad-sense heritability of total seed weight per plant.

Unlike the values of broad-sense heritability, those of narrow-sense heritability differed among the traits. There was a highest value of narrow-sense heritability for 1000 kernel weight, which was followed by that for seed shell thickness, the values for number of seed per plant and total seed weight per plant were the lowest in both F2 and F3. The lowest value of narrow-sense heritability for trait of total seed weight per plant was attributed to the large dominance variance associated with this trait. True-sense heritability defined by Ukai (2002), which is independent of the generation in which the heritability is measured, was also the lowest value for trait of total seed weight per plant among the four traits.

In rice, broad-sense and narrow-sense heritability estimates of the traits of 1000 kernel weight and total seed weight per plant were 0.74 and 0.55, 0.79 and 0.37, respectively, in F2 generation (Ahmadikhan 2010). The broad-sense heritability of trait for 1000 kernel weight in Tartary buckwheat was nearly equal to that in rice, but its narrow-sense heritability was larger than that in rice. This suggested that the additive effect for trait of 1000 kernel weight was large in Tartary buckwheat, and it indicated that more rapid fixation of alleles for this trait was possible. Compared with that of rice, the broad-sense heritability of total seed weight per plant of Tartary buckwheat is similar, but the narrow-sense heritability is small, indicating that the stability of alleles of total seed weight per plant of Tartary buckwheat is slower than that of rice.

Broad-sense heritability of the four traits was nearly equal to each other between F2 and F3; however, as the generations advanced, narrow-sense heritability increased. And the true-sense heritability is higher than that narrow-sense heritability of F2 generation. These results are natural, since the contribution of dominance variance in narrow-sense heritability decreases with the generation.

Our data also indicated significant positive phenotypic and genetic correlations between traits for number of seed per plant and 1000 kernel weight with total seed weight per plant. This is consistent with previous studies by Li et al. (2013), Chen et al. (2015), Morishita et al. (2006) and Joshi (2005). In addition, the values of number of seed per plant and 1000 kernel weight of sorghum (Padi 2007), Rice (Iftekharuddaula et al. 2001, Xu et al. 2008) chickpea (Arshad et al. 2004) and other crops also showed a high positive correlation with those of total seed weight per plant. The relationship between these traits of Tartary buckwheat is similar to that of other crops. In addition, there are significant positive phenotypic and genetic correlations between the traits of seed shell thickness and 1000 kernel weight. This is consistent with the results that there was significant negative correlation between 1000 kernel weight and thin shell (Chen 2015). Similarly, according to the data presented here, it suggests that it may be difficult to breed Tartary buckwheat varieties with the trait of thin shell as well as the trait of large grain.

Therefore, in order to breed high-yielding varieties with easily-shelled trait, it is necessary to select individual plants for the traits with high narrow-sense heritability such as 1000 kernel weight and seed shell thickness, which are not susceptible to environmental factors in the early progenies of hybrid combinations. However, the traits of 1000 kernel weight and seed shell thickness are quantitative traits, controlled by multiple genes. Therefore, the strains with the phenotype of easily-shelled trait, which was controlled by a single recessive gene, were selected, after the progeny were continuously reproduced and the genotypes were fixed; it was effective to select optimal strains by means of bulk selection, according to the traits of number of seeds per plant and total seed weight per plant, which were easily affected by environmental factors and with a much lower narrow-sense heritability. Although, the trait of seed shell thickness is positively correlated with the trait of 1000 kernel weight, and almost all the varieties with the easily-shelled are the varieties with small value of seed shell thickness. However, ideal single plants with easy-shelled trait, moderate values in traits of seed shell thickness and 1000 kernel weight, and large values in number of seeds per plant and total seed weight per plant, could be selected. In the progeny population of this study, there were excellent single plants with high-yield and easily-shelled traits, of which the values of seed shell thickness, 1000 kernel weight, number of seeds per plant and total seed weight per plant were 0.17 mm (0.15 mm to 0.20 mm), 14 g, 1137 and 15.9 g, respectively. Therefore, when selecting varieties with high-yield and easily-shelled traits, we should selected varieties through the hybridization of easily-shelled varieties with small seed shell thickness as well as hard-shelled varieties with large seed shell thickness, instead of blindly choosing the performance values in the seed shell thickness or easily-shelled trait; the combinations of easily-shelled trait with the trait of seed shell thickness are the most effective selection indexes from the descendant segregation population.

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