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Diversity of Tartary Buckwheat (*Fagopyrum tataricum*) Landraces from Liangshan, Southwest China: Evidence from Morphology and SSR Markers

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Abstract: Tartary buckwheat (*Fagopyrum tataricum*) has been cultivated for over one thousand years in the Liangshan Prefecture of Sichuan, China. Growing population pressures, economic modernization pressures, and the erosion of traditional culture have led to the rapid loss of area covered by Tartary buckwheat landraces. Morphological and molecular characterization of 112 Tartary buckwheat accessions from 29 populations were assessed based on 10 morphological traits of seeds and 10 SSR markers, respectively. The coefficient of variation and Shannon index showed diversity within the morphological characteristics of the seeds. All accessions were divided into three categories according to phylogenetic dendrogram analysis, which was consistent with folk nomenclature and taxonomy. Genetic analysis using SSR markers identified 45 alleles with a mean value of 4.5 alleles per locus. The high average *PIC* value (0.459) indicated polymorphism of the SSR markers. The genetic similarity coefficient of the 112 Tartary buckwheat accessions showed a high level of genetic diversity ranging from 0.130 to 0.978. The genetic structure analysis revealed high genetic differentiation (*Nei* = 0.255). The folk nomenclature, folk taxonomy, and sociocultural norms may also contribute to a significant influence on the diversity of folk nomenclature and taxonomy. The assessment of the genetic diversity of Tartary buckwheat landraces and detection of SSR loci associated with traits could be used as scientific guidance for selecting Tartary buckwheat seed for improved production relative to local farmers and consumer preferences. Local traditional knowledge (seed exchange network) and culture also contribute to breeding and the maintenance of the genetic diversity of Tartary buckwheat.

Keywords: Tartary buckwheat; genetic diversity; genetic structure; morphological traits; traditional knowledge

1. Introduction

Tartary buckwheat (*Fagopyrum tataricum*) is an annual crop belonging to the family Polygonaceae, comprising twenty different species [1]. As two major cultivated species,

Tartary buckwheat and common buckwheat (*Fagopyrum esculentum* Moench) are widely cultivated in Asia, Europe, and North America [2]. The global production of Tartary buckwheat and common buckwheat has exceeded 2.9 million tons in 2018, and China was one of the major producing countries [2]. It is believed to have an origin in southwest China, encompassing Sichuan, Yunnan, and Tibet, and continues to be cultivated and domesticated in mountainous areas of western China and the Himalayas [3,4]. Recent research showed that two independent domestication events occurred in southwestern and northern China, resulting in diverse characteristics of modern Tartary buckwheat varieties [5]. Tartary buckwheat has become a popular food crop due to its high rutin content, abundant nutrients, and high levels of anti-oxidants, anti-inflammatories, and anti-hypertensives [6–8]. It has been considered to be an alternative food subsistent and market crop for mountain environments and as a minor cereal for specialty foods in Asia, Europe, North America, and South Africa [9]. Tartary buckwheat has been used as an important raw material for tea, noodles, and cakes, as a green vegetable, as a flower source for honey, and as a green manure cover crop and smother crop to crowd-out weeds [10]. Tartary buckwheat seeds are rich in proteins, vitamin B, dietary fibers, resistant starch, polyunsaturated fatty acids, and a variety of minerals that are an essential part of human diets [11]. In addition, various flavonoids have been identified in Tartary buckwheat [12].

The growing population pressure on agricultural lands, urbanization, and rapid modernization is eroding the vast diversity of crop biodiversity globally. The conservation of crop genetic diversity has been a worldwide concern [13]. Crop agricultural biodiversity in the form of crop landraces is also referred to as traditional varieties, and it plays a key role both in crop breeding and the current food security of local peoples in mountainous regions [14]. The landraces are being lost rapidly due to economic development and other human activities, including climate change. In China, monoculture and high-yielded varieties have been extended throughout the country, causing significant loss of local landraces. In the remote areas of southwest China, a limited number of landraces are still cultivated and maintained by local people, who depend on these varieties for their livelihoods [15,16].

Liangshan Yi Autonomous Prefecture, in southwest China, is the largest Yi-dominated community in China. It is located in the center of what is considered to be the origin of cultivated Tartary buckwheat [1]. Yi people value Tartary buckwheat as a dietary staple, for feeding livestock, as well as being an integral part of their cultural story of creation and for festivals. Yi people associate Tartary buckwheat with safety, health, fertility, and high yield of crops [17]. They maintain a traditional seed system to conserve the diversity of Tartary buckwheat landraces, where they identify different varieties based on seed shape, size, color, and time of maturity [18].

Genetic diversity plays a crucial role in the survival of any species, as it influences the adaptability of a population under changing environmental conditions [19,20]. Morphological descriptors together with biochemical and molecular markers are available for studying the genetic diversity of Tartary buckwheat [21]. Diversity in morphological characteristics of buckwheat seeds is important in buckwheat breeding [22]. Kernel shape is a key morphological descriptor evaluating the genetic diversity of buckwheat genetic resources. Tartary buckwheat fruit is a trihedral or tetrahedron achene, usually with rounded edges and lateral longitudinal grooves, which significantly hinders shelling. The shape is most often classified as either triangle type or ovate type. The grain color of Tartary buckwheat is diverse, ranging from black, gray, purple, to brown. Some seeds have unique traits with thorns and wings. Few studies on the genetic diversity of Tartary buckwheat, combining morphological diversity of seed characteristics with molecular marker techniques, have been conducted [23].

Several molecular marker studies have been carried out on Tartary buckwheat genetic diversity [24–26]. Simple sequence repeat (SSR) markers are used widely for the analysis of genotype and map construction because of their abundance, random distribution within the genome, high polymorphism information content (*PIC*), and stable co-dominance [27].

However, few studies have detected and identified key loci and genes associated with Tartary buckwheat's important agronomic traits, such as 1000-grain weight, kernel shape, and rutin content.

Hou et al. analyzed the population genetic structure of Tartary buckwheat using SSR data to determine that the crop could be clustered into two separate subgroups (Loess plateau and Yunnan–Guizhou plateau) [28]. Ma et al. found that using the SSR markets gave consistent results to the phylogenetic relationships of *Fagopyrum* species revealed using other marker systems [29]. Li et al. constructed an EST-SSR fingerprint and analyzed the genetic diversity of 35 registered Tartary buckwheat varieties, finding a close genetic relationship among the 35 varieties and a narrow genetic basis; the genetic relationship was in accordance with their breeding history [30]. Few studies have compared the role of traditional seed systems and their correlation with molecular markers.

The purpose of this study was to investigate the genetic diversity of Tartary buckwheat landraces in Liangshan Yi Prefecture using morphological traits of seed and SSR markers and to identify the correlation between morphological traits of seeds and molecular markers. The results may provide an effective guide for the protection, breeding, and future development of Tartary buckwheat.

2. Materials and Methods

2.1. Plant Materials

One hundred and twelve accessions of Tartary buckwheat landraces were selected for analysis as a representative sample based on different agronomic traits and local names from 29 Yi ethnic villages (Figure S1) in Liangshan Prefecture, Sichuan Province in 2017–2018. The samples were maintained as an active collection at the Minzu University of China and were used for morphological characteristics of seed analysis, SSR polymorphism detection, and genetic diversity analysis. The seed morphological characteristics of all accessions were recorded and then cultivated in an incubator for later DNA extraction. The accessions from 29 Yi people villages were considered as 29 different Tartary buckwheat populations for genetic structure analysis.

During the collection of the Tartary buckwheat landraces seeds, an ethnobotanical survey was also conducted based on key informant interviews and semi-structured interviews [31,32], to obtain information on traditional culture and knowledge affecting the management of the diversity of Tartary buckwheat landraces and the dynamics of the seed exchange network.

2.2. Phenotypic Data Analysis

The morphological characteristics of seeds were analyzed using observed traits based on the Description Standard and Data Standard of Buckwheat Germplasm Resources [33], which mainly included kernel shape, husk color, longitudinal groove, thorn, wing, a bulge in the ridge, apex tip, slender achene, seed length, and 1000-grain weight. Thirty-five biological replicates were used for statistical analysis of each trait. All qualitative traits were assigned values according to different phenotypes (Table 1). The quantitative traits data were classified into 10 grades, 1 grade < $X - 2\delta$, 10 grades > $X + 2\delta$, where each grade interval is 0.5δ between 1 and 10 grades, and δ is the standard deviation [34]. After standard quantification of ten traits, the average, minimum value, maximum value, standard deviation, and coefficient of variance were analyzed. Shannon index was calculated by using the following formula: $Hs = -\sum pi * lnpi$. When the germplasm was clustered according to the diversity index of morphological traits as well as quantitative and quality traits, the hierarchical model of quantitative traits was conducted. Then, phylogenetic dendrogram (UPGMA) and principal component analysis (PCA) were conducted by OriginPro software based on the qualitative and quantitative traits of seeds. To present the diversity of seed agronomic traits visually, a heat map was also produced using GraphPad Prism based on data standardization through SPSS 20.0 (Z-score method).

Table 1. The main morphological traits of Tartary buckwheat seeds and their given valuations.

Agronomic Traits	Given Valuations
Kernel shape	Triangular (1), ovate triangular (2), triple cone (3), quadrangular (4), polygonal (5), ovate quadrangular (6), ovate triple cone (7)
Husk color	Gray (1), dark gray (2), light gray (3), purple black (4), brown (5),light brown (6), purple (7), light purple (8)
Longitudinal groove	Yes (1), no (2), indistinct (3)
Thorn	Yes (1), no (2)
Wing	Yes (1), no (2)
Bulge in ridge	Yes (1), no (2)
Apex tip	Yes (1), no (2)
Slender achene	Yes (1), no (2)
Kernel length	The length of kernel (mm)
1000-grain weight	The weight of 1000 pure seeds in the dry air (g)

Note: The values represent different phenotypes of different agronomic traits, which were used to distinguish between phenotypes of the same agronomic trait.

2.3. DNA Isolation and PCR Amplification

DNA was extracted from leaf samples from each accession by a plant genomic DNA isolation kit (Tiangen, China). Eighty SSR primers were designed as previously reported [28,30,35,36] (Supplementary Table S1). All primers were constructed by the General Biosystems Company (Changzhou) and tested on all the isolated DNA samples, out of which ten pairs of SSR primers were selected for further study, as they produced clear bands across all the samples (Table 2).

Table 2. Variations in 10 morphological traits of Tartary buckwheat landrace seeds.

No.	Trait	Minimum	Maximum	Range	Average	SD	CV (%)	Shannon-Index
1	Kernel shape	1	7	6	2.35	1.35	57.45	0.32
2	Husk color	1	8	7	3.08	1.85	60.06	0.31
3	Longitudinal groove	1	3	2	1.27	0.48	37.80	0.27
4	Thorn	1	2	1	1.92	0.27	14.06	0.20
5	Wing	1	2	1	1.96	0.21	10.71	0.14
6	Bulge in ridge	1	2	1	1.93	0.26	13.47	0.19
7	Apex tip	1	2	1	1.93	0.26	13.47	0.19
8	Slender achene	1	2	1	1.96	0.19	9.69	0.12
9	Kernel length	3.00	7.00	4.00	5.02	0.86	17.13	0.17
10	1000-grain weight	16.58	29.57	12.99	22.45	2.57	11.45	0.24

The PCR mixture contained 1 × Buffer, 0.2 mM dNTP (each), 0.3 µM of each primer, 0.1 units Taq DNA Polymerase, and 20 ng of template DNA for a final volume of 20 µL. The PCR process consisted of denaturation for 5 min at 94 °C, then 35 cycles of 30 s at 94 °C, and annealing at 54 °C for 40 s of extension at 72 °C. The samples were stored at 4 °C until analysis was carried out. The detection of amplification products was carried out by capillary electrophoresis (CE) with a 3730XL DNA Analyzer (Applied Biosystems, Waltham, MA, USA).

2.4. Genetic Diversity and Structure Analysis

The number of different alleles (Na), number of effective alleles (Ne), Shannon diversity index (*I*), expected (*He*), and observed (*Ho*) heterozygosity were used to determine genetic parameters [37]. The polymorphic index content (*PIC*) locus was determined by Cervus vison 3.0.7 software (Copyright Tristan Marshal, Field Genetic, Ltd., London, UK), which reveals the potential of markers in genetic variability. Similarity coefficients were calculated using the *similarity* program in NTSYS-pc 2.1 software. Genetic distance was

calculated according to the *genetic distance* = 1 − *similarity coefficient*. A phylogenetic dendrogram was constructed using the algorithm UPGMA (unweighted pair group method of arithmetic clustering) by MEGA v.7 [38]. Then, the genetic diversity of Tartary buckwheat's 29 populations (villages) was also analyzed based on the above methods. Moreover, the Mantel test was conducted to analyze Euclidean geographic distances and genetic distances through adegenet analysis package [39]. In addition, Pearson's correlation analysis ($p < 0.05$, $p < 0.01$) was used to calculate the correlation coefficient between each agronomic trait and the degree of association between the trait and the SSR loci.

3. Results

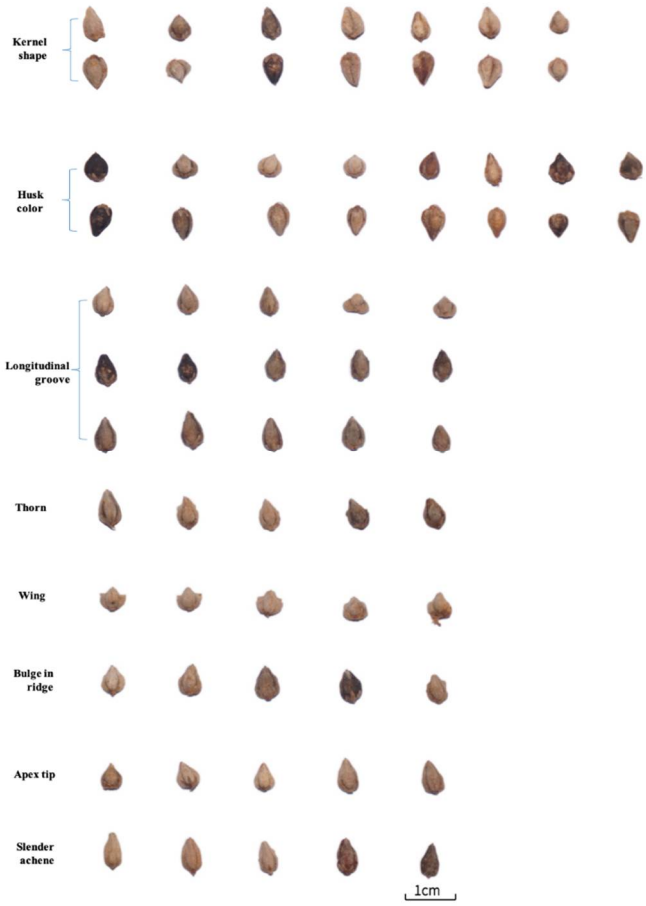
3.1. Morphological Traits Diversity of Seeds

The morphological traits of Tartary buckwheat seeds were diverse, and ten different traits, including eight quality traits (Figure 1A) and two quantitative traits, were recorded. Husk color was the most highly variable, with gray, dark gray, light gray, purple-black, brown, light brown, purple, and light purple, as shown in Figure 1B. The second most highly variable trait was kernel shape. There were seven different kernel shapes recorded, which include triangular, ovate triangular, triple cone, quadrangular, polygonal, ovate quadrangular, and ovate triple cone (Figure 1A). Longitudinal groove was the third most diverse trait, including longitudinal groove, no longitudinal groove, and indistinct, which was analyzed for the first time. Tis trait could be used in the classification of different Tartary buckwheat landraces. In addition, thorn, wing, bulge in ridge, apex tip, and slender achene were also variable in Tartary buckwheat landraces.

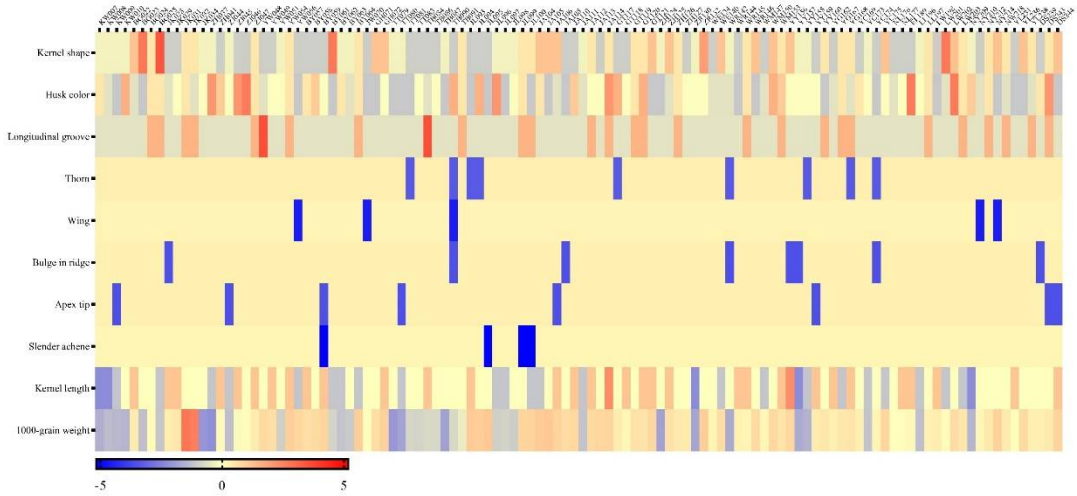
The statistical parameters (minimum, maximum, range, average, standard deviation (SD), and coefficient of variation (CV)) related to morphological traits of Tartary buckwheat landraces are presented in Table 2. There were extensive variations in Tartary buckwheat landraces with the CV of 10 morphological traits ranging between 9.69% and 60.06%. Husk color showed the highest CV (60.06%). The second highest was kernel shape with a CV value of 57.45%. The third highest was longitudinal groove with a 37.80% CV value. Other traits with lower CV values included kernel length (17.13%), thorn (14.06%), bulge in ridge (13.47%), apex tip (13.47%), 1000-grain weight (11.45%), wing (10.07%) and slender achene (9.69%).

The Shannon index of morphological traits in Tartary buckwheat landraces seeds was calculated using $Hs = -\sum pi \cdot lnpi$ after the results of traits evaluation were normalized (Table 2). The average diversity index of qualitative and quantitative traits was 0.22 and 0.21, respectively, which indicated that there was more extensive genetic diversity in qualitative traits. The highest Shannon diversity index value was that of kernel shape (0.32), followed by husk color (0.31), and the third-highest was longitudinal groove with 0.27, whereas the lowest was slender achene (0.12). The diversity index of the two quantitative traits, kernel length and 1000-grain weight, was 0.17 and 0.24, respectively.

The cluster analysis, which used genetic distance, divided the 112 Tartary buckwheat accessions into three categories at the 0.72 genetic distance value (Figure 1C). The first category consisted of 26 accessions, which were characterized by kernel shape (triple cone and ovate triple cone), husk color (purple), no longitudinal groove, relatively higher husk length, and 1000-weight. This category was labeled as a large seed group. The second category included 20 accessions, which have polygonal kernel shape with longitudinal groove and gray husk color, also called the large particle. The third category was the largest one, including a total of 66 accessions, which have triangular and ovate triangular kernel shapes, with longitudinal groove, wing, thorn, and relatively lower kernel length and 1000-weight.

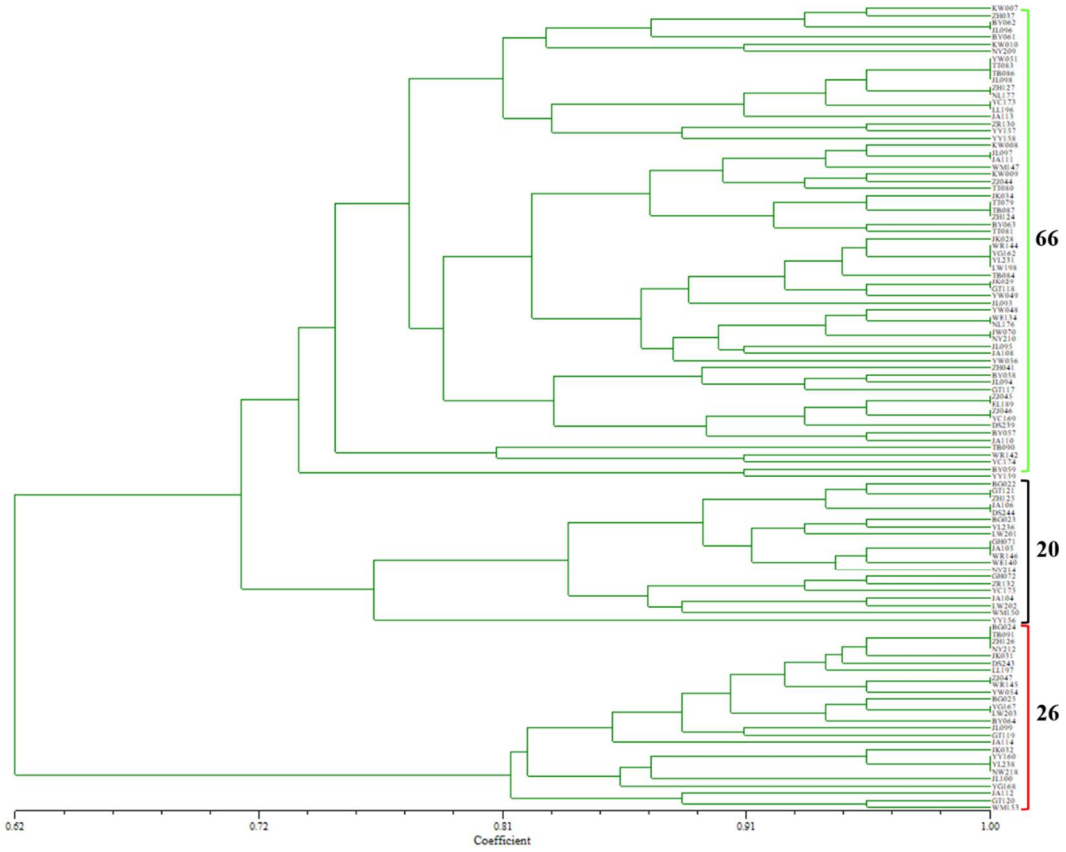


(**A**)



(**B**)

Figure 1. *Cont*.



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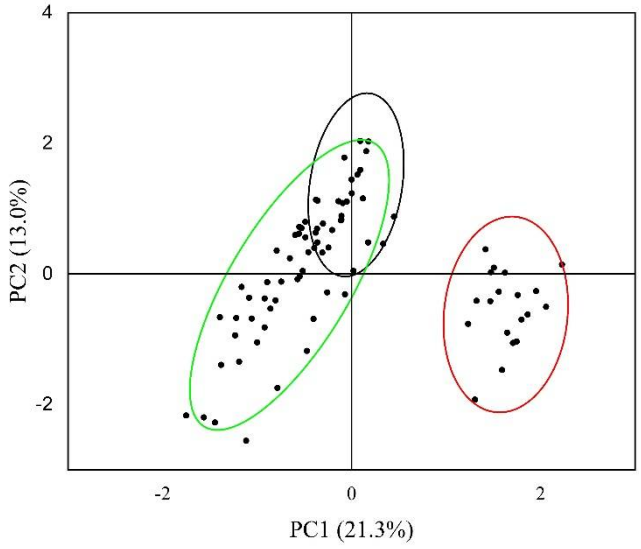

(**D**)

Figure 1. (**A**) Morphological trait diversity of Tartary buckwheat seeds. (**B**) The heat map of ten morphological traits of seeds. (**C**) UPGMA dendrogram of morphological traits in Tartary buckwheat landraces seeds. (**D**) Principal component analysis (PCA) of 112 Tartary buckwheat accessions based on morphological traits.

To further evaluate the morphological traits variations of 112 accessions, we performed principal component analysis (PCA). Based on the first two principal components, the genotype-trait biplots were generated (Figure 1D). The variance contribution of PC1 was 21.28%, which was composed of 55 accessions (the absolute value of eigenvalue > 0.7),

including BG022, BG023, JK029, ZJ045, YW48 with triangular kernel shape, longitudinal groove, and kernel length between 4.4 and 5.0 mm. The variance contribution of the second PC axis was 12.96%. In this group, triple cone kernel shape, dark purple husk color, and more than 22.59 g 1000-weight were the main characteristics, which included 15 accessions, including BG024, JK031, JK032, YW054, and JL099. Due to its dark color and high 1000-weight, this group was classified as a special large group.

3.2. Polymorphism and Allelic Diversity

From the eighty primers tested using 6 of 112 accessions from different villages for PCR amplification, a total of 10 primers (Table S2), with good repeatability and abundant polymorphism, were used to investigate the polymorphisms among the 112 Tartary buckwheat accessions from 29 villages. The genetic diversity parameters of 10 SSR primers in Tartary buckwheat were evaluated (Table 3). These loci generated a total of 45 alleles with an average of 4.5 alleles per locus. SSR primer P59 showed the highest number of alleles (24), while primer P22 had the lowest number (2). The fragment size of all amplification products ranged from 186 to 224 bp. The average numbers of *Na* and *Ne* per primers were 4.500 (ranging from 2.000 to 5.000 for P22 and P1) and 2.229 (ranging from 1.368 to 2.912 for P60 and P6), respectively. The Shannon's information index (*I*) was between 0.488 and 1.366, with an average of 0.934. P12 exhibited the highest *PIC* (0.596), whereas P3 exhibited the lowest 0.248 (P3), and the mean of all ten primers was 0.459. The overall observed heterozygosity (*Ho*) was between 0.340 and 0.729, and the mean was 0.480 (Table 3).

Table 3. Polymorphism analyses of 10 SSR primer-pairs.

Primers	*Na*	*Ne*	*I*	*Ho*	*He*	*PIC*
P1	5.000	2.873	1.116	0.345	0.655	0.581
P3	3.000	1.394	0.488	0.716	0.284	0.248
P6	3.000	2.912	1.083	0.340	0.660	0.582
P12	4.000	2.784	1.200	0.356	0.644	0.596
P22	2.000	1.987	0.690	0.501	0.500	0.373
P58	5.000	2.686	1.117	0.370	0.631	0.565
P59	9.000	2.357	1.366	0.422	0.578	0.559
P60	5.000	1.368	0.577	0.730	0.271	0.256
P64	6.000	1.826	0.902	0.546	0.454	0.414
P70	3.000	2.106	0.797	0.473	0.527	0.412
Total	45.000					
Average	4.500	2.229	0.934	0.480	0.520	0.459

The unweighted pair group with arithmetic mean (UPGMA) dendrogram of 112 Tartary buckwheat accessions was constructed based on the genetic similarity coefficient (Figure 2). The genetic similarity coefficient ranged from 0.130 (between JL100 and NL 176) to 0.978 (BY057 and JA108, JL096 and JA113, NY209 and WR145, WM153 and YY156, YY158, YY159, YY160). The cluster analysis indicated that the 112 Tartary buckwheat accessions could be divided into five categories (I, II, III, IV, V) at the 0.520 similarity coefficient (Figure 2). In cluster V, there were 10 accessions, which included two sub-clusters (Va and Vb). Sub-cluster Va included six accessions (YY156, YY157, YY158, YY159, YY160, and WM 153) from two adjacent villages. Vb included JA104, GT118, GT120, and GT121 from two adjacent villages. Clusters III and IV included only one accession, BG022 and NL176, respectively. Cluster II included five accessions from different two sub-clusters IIa (TB084, YL231, and DS239) and IIb (JL099 and JA110). Cluster I was also divided into two sub-clusters (Ia and Ib), and 96 and 1 (JL094) accessions were clustered into Cluster Ia and Cluster Ib, respectively.

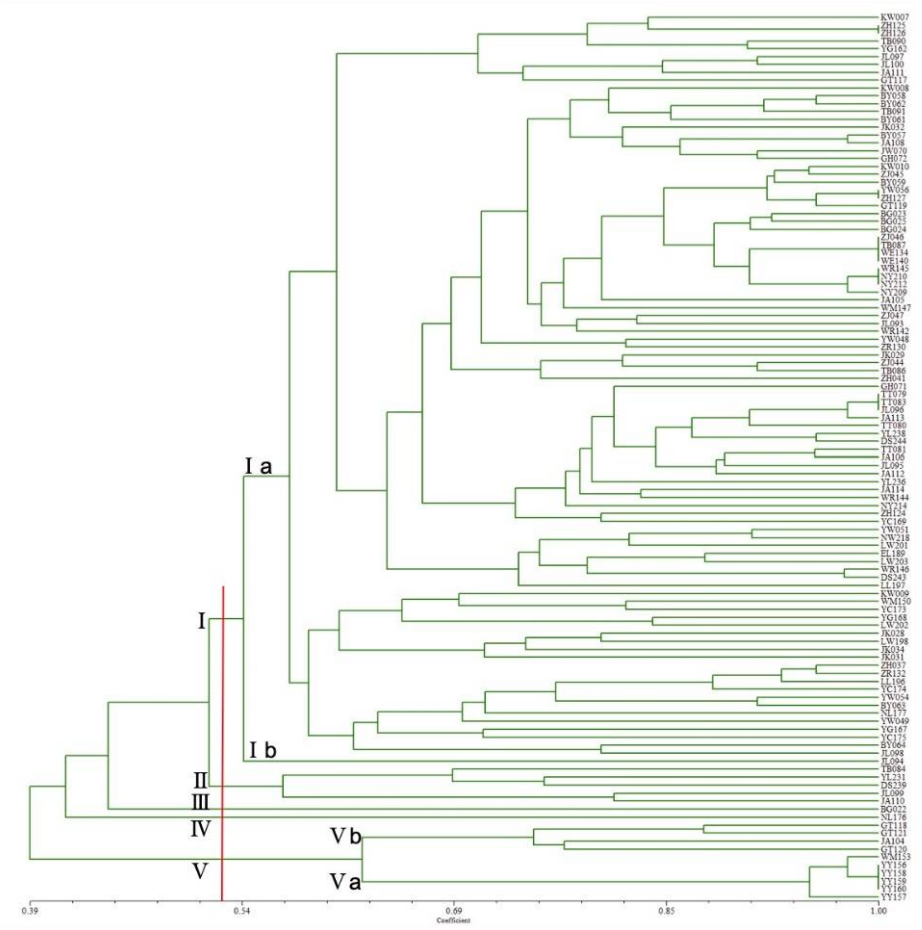

Figure 2. UPGMA dendrogram of 112 Tartary buckwheat landraces accessions based on genetic similarity. (I, II, III, IV, and V represents five different clusters, and a and b represent a subclass.)

3.3. Genetic Diversity of Tartary Buckwheat's 29 Populations (Villages) Based on SSR Analysis

The results of the genetic diversity of Tartary buckwheat populations are shown in Table S3. The means of observed alleles (*Na*) and effective alleles (*Ne*) were 1.738 (ranging from 1.083 to 2.500 for JW/WE and JA) and 1.535 (ranging from 1.083 to 2.082 for JW/WE and WM), respectively. The average Shannon index (*I*) was 0.393, WM showed the highest *I* (0.712), whereas the lowest was 0.058 (JW). The observed heterozygosity (*Ho*), expected heterozygosity (*He*) and *Nei* at the population level were 0.170, 0.306, and 0.255, respectively. WM also exhibited the highest *He* (0.556) and *Nei* (0.463). The levels (lowest to highest) of *I*, *He*, and *Nei* were consistent. WM had the highest genetic diversity based on all three measures, i.e., *I*, *He*, and *Nei*, followed by JL and YG, whereas the lowest values were found in JW and WE (Table S3).

A dendrogram based on genetic distance was conducted using the UPGMA algorithm (Figure 3). The results indicated three large clusters covering the 29 Tartary buckwheat populations. In cluster I, there were 25 populations, which included two sub-clusters (Ia and Ib). Sub-cluster Ib included 24 populations, whereas Ia included one population (WM). Clusters II and III included three (EL, LL and NL) and one (YY) populations. Meanwhile, the Mantel test results showed that there is a significant correlation between population geographic distance and genetic distance ($p < 0.05$), which indicated the genetic structure associated with geographic patterns, except for BG and WR ($p > 0.5$).

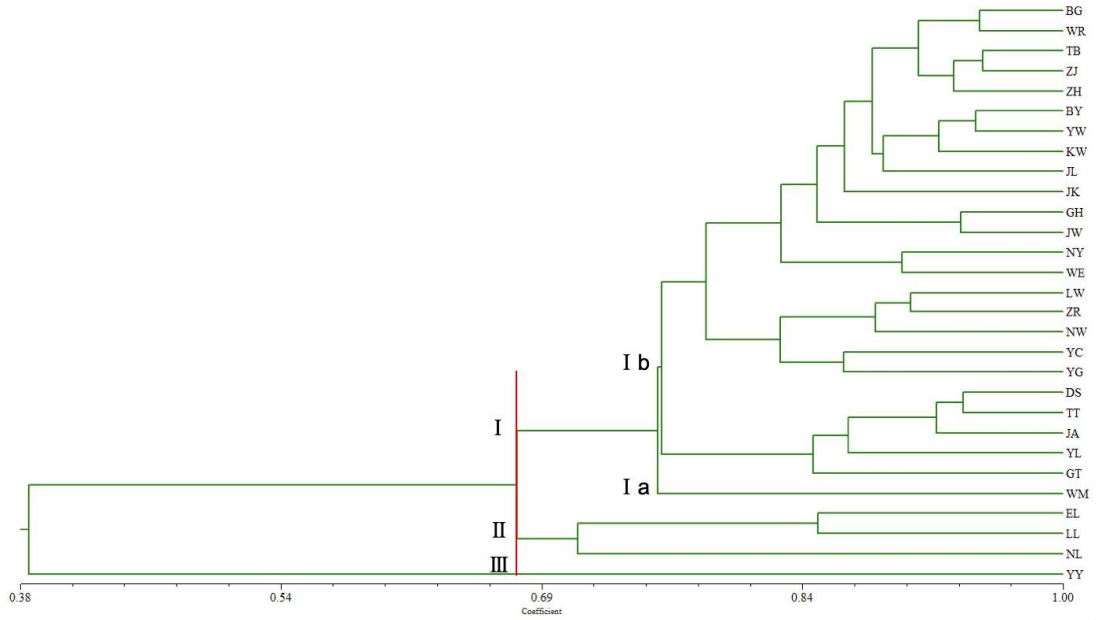

Figure 3. UPGMA dendrogram generated from 10 SSR data showing relationships of twenty-nine Tartary buckwheat populations. (I, II, and III represents three different clusters, and a and b represent a subclass.)

3.4. The Relationship between Morphological Traits of Seeds and SSR Markers

The correlation coefficients between 10 SSR loci and 10 morphological traits of seeds were detected (Table 4). Some loci were significantly correlated with multiple morphological traits. For instance, P58 was associated with polygonal, gray, light gray, thorn, bulge in ridge, kernel length, and 1000-grain weight. P60 was associated with seven morphological traits. Meanwhile, some loci were associated with only a single trait (P6, P70). Five loci (P12, P22, P58, P60, P70) were associated with 1000-grain weight, while only one locus (P58) was associated with a polygonal shape. The discovery of these loci associated with morphological traits will greatly promote the improvement of Tartary buckwheat varieties and speed up the genetic breeding process of buckwheat.

Table 4. SSR marker loci associated with morphological traits ($p < 0.05$).

Locus	Polygonal	Gray	Light Gray	Brown	Thorn	Bulge in Ridge	Apex Tip	Slender Achene	Kernel Length	1000-Grain Weight
P1			0.190			0.232				
P3							0.342	−0.344		
P6							0.209			
P12			0.219							0.192
P22										−0.200
P58	−0.204	−0.218	−0.333		0.192	0.224			−0.210	−0.237
P59			0.225	0.194	0.253	0.242			0.244	
P60		−0.193		0.278	0.321	0.192		0.222	0.244	0.263
P64								0.337	−0.500	
P70										0.189

4. Discussion

The present study analyzed the diversity of Tartary buckwheat landraces' seed morphological traits and molecular characterization. Morphological analysis has provided helpful information for characterizing genetic resources [40,41]. However, evaluating genetic diversity by morphological analysis alone has limitations. Molecular markers developed from genome sequences have been used successfully for the analysis of genetic diversity and population structures of Tartary buckwheat [28,40]. By combining these

agronomic and molecular assessment tools, Tartary buckwheat landraces were found to be highly genetically diverse with some seed morphological traits correlated with the SSR molecular markers.

4.1. Genetic Diversity of Morphological Traits of Seed in Tartary Buckwheat

The analysis of variance and the Shannon index revealed the diversity of the seeds' morphological traits. Kernel shape and husk color were the first two highly variable traits. They were also frequently used in folk nomenclature and taxonomy based on diversity traits. The Tartary buckwheat landraces were divided into three types, including small seed, large seed, and lanky landraces based on kernel shape by local Yi people.

The husk color and kernel shape are an important basis for traditional classification and folk nomenclature by local Yi people. According to husk color, black, brown (yellow), and gray Tartary buckwheat landraces were classified and named. Folk nomenclature and taxonomy were based on size, shape, color, and other characteristics of biology [42,43].

Folk nomenclature and taxonomy and sociocultural norms have contributed to the crop genetic diversity for numerous crops [43–45]. In Liangshan Yi Prefecture, folk taxonomies of Tartary buckwheat are based on the seeds' morphological traits. Yi people select seeds according to seeds' morphological traits, providing selection pressure on the next generation of seeds of each variety to be planted, thus influencing the diversity of their Tartary buckwheat germplasm resources [46]. Local culture practices are not uniform across the Liangshan Yi Prefecture. Different villages may require different colors and shapes of Tartary buckwheat in their rituals.

Through cluster analysis, all accessions were divided into three categories. Cluster I (large seed size) and III (small seed size) were the two main categories cultivated by local Yi people who live halfway up the mountain (2000–3500 m). These two categories of landraces are also grown in different agroecological zones. Cluster I is cultivated in low altitudes (2000 m), has a short maturation period, and has high flood tolerance. Cluster III comprises cold-tolerant varieties, cultivated in high-altitude mountains (2500–3000 m). According to the local Yi people, small-seeded landraces are their favorite because of their good flavor and high yield of flour. They cultivate some large seed landraces at the foot of the mountains because their maturation period is longer than small seed varieties, allowing the people to stagger the harvest. Above all, farmers' knowledge and any activities of farmers to maintain landraces were conducive to the maintenance of landraces and should be referenced by modern breeding techniques.

4.2. Molecular Characterization and Genetic Diversity of Tartary Buckwheat Landraces

In total, 45 alleles were detected among 112 accessions, with an average of 4.5 alleles per locus. This is similar to a study by Senthilkumaran [41], which revealed average values of 6.5 alleles per SSR locus, after analyzing diversity in buckwheat from the northwestern Indian Himalayas. In contrast, only 1.1 alleles per SSR were reported by Kishore et al. [47] in a diversity assessment of the population genetic diversity of Tartary buckwheat in western Himalaya. The difference in the number of alleles between studies could be explained by the large regional difference in the varieties of Tartary buckwheat tested. Among the SSR markers, the *PIC* value was between 0.248 and 0.596, and the average was 0.459, which means that polymorphism information could be presented by locus. Our results were similar to those of Li et al. [30], who showed an average 0.440 *PIC* value, which was higher than Kishore's result [47]. The genetic similarity coefficient of 112 Tartary buckwheat accessions, ranging from 0.130 to 0.978, and the Shannon-index of 0.934 both indicated that the germplasm resources of Tartary buckwheat landraces in Liangshan Yi Prefecture had higher diversity than the populations tested by Kishore et al. [47].

The cluster analysis using the UPGMA algorithm, which grouped the 112 accessions into five clusters, was similar to the results of the morphological traits cluster analysis. The results of association analysis between morphological traits of seeds and molecular

markers showed that selected loci were associated with morphological traits, with some loci being found to be associated with one or more traits.

4.3. Genetic Structure of Tartary Buckwheat Populations

Understanding the genetic structure of Tartary buckwheat populations is an important method for evaluating the diversity and on-farm management of Tartary buckwheat landraces [41]. Higher genetic diversity has been related to greater adaptation potential to the environment [48]. The average *I* and *Nei* values of the 29 populations were 0.393 (ranging from 0.058 to 0.712) and 0.255 (ranging from 0.042 to 0.463), respectively, indicating high genetic diversity of Tartary buckwheat landraces in Liangshan Yi Prefecture with larger differences in genetic background. In particular, the genetic diversity of WM, JL, and YG was relatively high, which may be related to the complex and diverse mountain environment, traditional farming culture, and customs of these villages. The genetic similarity coefficient of 29 populations was between 0.221 (between YY and WE) and 0.950 (between BG and WR). The genetic distance and geographical distance between BG and WR were 0.051 and 130 km, respectively, which showed no positive correlation ($p > 0.05$). Maybe due to the kinship between the two villages, there is a high frequent seed exchange of Tartary buckwheat landraces, potentially leading to the greater genetic similarity found in these villages. Bajracharya et al. found similar results when they examined seed systems and molecular diversity for Himalayan barley varieties [14]. Kinship and frequent seed exchange (Figure 3) may explain why genetic distance and geographical distance were not related. In contrast, in YY village, genetic similarity with other villages was low between 0.221 and 0.518, indicating less exchange of genetic materials with other villages. This village is part of the China Important Agricultural Heritage Systems sites, where to be a member the village, it is required to continue traditional Tartary buckwheat cultivated practices.

The study area encompassed a large difference in altitude, providing a wide diversity of microclimates and affecting the amount and distribution of genetic diversity across sites [49]. Yi people's culture and traditional knowledge are the key factors in shaping the diversity of Tartary buckwheat landraces, through festivals that use specific Tartary buckwheat foods, such as weddings and celebrating the Yi New Year. Unpredictable changes in temperature and precipitation, altitudinal differences, staggering harvest periods because of a variation in maturation periods, and different taste preferences influence which Tartary buckwheat varieties are grown in different communities. Farmer seed exchange networks allow for the transfer of seed varieties via farmer-to-farmer gifting, swapping, bartering, or purchase and trade or sale, which occurs outside of the commercial seed sector and formal seed regulation. Seed networks can support seed flow and shape gene flow in traditional agricultural systems [50,51]. The local Yi people often exchanged Tartary buckwheat landraces seed with neighbors and relatives both inside and outside villages. Social and cultural knowledge affects the selection and structure of crop varietal diversity [52].

5. Conclusions

Our results revealed the high genetic diversity of Tartary buckwheat landraces for the Yi people in Liangshan County, southwestern China, and the consistency of folk nomenclature, taxonomy, morphological traits, and genetic diversity measured through SSR markers. Additionally, some SSR loci-associated morphological traits were also found. Local traditional knowledge and its related seed exchange network were an integral part in maintaining high levels of genetic diversity of Tartary buckwheat landraces in the study area. These results can provide important references for the breeding of Tartary buckwheat.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12051022/s1, Figure S1: Sampling location of 29 populations (villages) of Tartary buckwheat (*Fagopyrum tataricum*) landraces, in Meigu and Zhaojue county of Sichuan Province, China; Table S1: Information of 80 primers; Table S2: Information of 10 SSR primers; Table S3 Genetic diversity of Tartary buckwheat population.

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