HortScience 60(4):467–475. 2025. https://doi.org/10.21273/HORTSCI17907-24

Study on Nut Phenotypic Diversity of Ancient Walnut Germplasm Resources in Xinjiang

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Keywords. germplasm resources, phenotypic diversity, Xinjiang aged walnut

Abstract. **Using morphological difference analysis and other methods, this study investigated the phenotypic trait diversity of ancient walnut germplasm nuts in Xinjiang, providing theoretical references for the study of walnut germplasm origin and resource protection. We measured 16 qualitative traits and 12 quantitative traits of 304 Xinjiang wild walnut germplasm and evaluated the phenotypic traits of ancient Xinjiang walnuts using normal distribution, cluster analysis, and correlation analysis methods. The results showed that among the 304 resources, the coefficients of variation for traits such as single fruit weight, single kernel weight, kernel percentage, and shell thickness were relatively high. Correlation analysis revealed that there were positive correlations between single fruit weight and nut length, shell carcass and single kernel weight, among other 45 pairs of data. There were significant negative correlations between transverse diameter and fruit shape index, shell thickness and suture width, and extremely significant negative correlations between shell thickness and single kernel weight, fruit shape index and shell carcass. The resource materials could be divided into six categories, with the results mainly related to nut color, nut shape, kernel odor, fruit surface smoothness, single fruit weight, kernel percentage, shell thickness, and nut dimensions. Ancient Xinjiang walnuts exhibit rich genetic diversity in phenotypic traits. Single kernel weight, single fruit weight, kernel percentage, and shell thickness can serve as reference standards for evaluating the quality and breeding of ancient Xinjiang walnuts in China.**

Walnut (*Juglans regia* L.) is a perennial deciduous tree (Xi 1996). The lifespan of walnut plants can exceed a century, and there are trees more than 500 years old in Xinjiang, Tibet, and other regions (Pei 2011). Chinese walnuts are one of the centers of walnut origin in the world (Xi 1981, 1990). Regarding Chinese walnuts, Zhang (1987), Xi (1987), and Wu et al. (2000) have all listed Xinjiang as one of the important distribution areas of walnuts (Li et al. 2014, 2019; Xi 2015). Archaeological excavations in the Junggar Basin of Xinjiang have found the presence of walnut pollen in the lower rock formation of the Oligocene, providing evidence for the origin of Chinese walnuts (Beijing Institute of Botany, Chinese Academy of Sciences, Nanjing Institute of Geology and Palaeontology, "Chinese Cenozoic Plants" compilation group 1978). As early as 1300 to 1500 years ago, walnuts were cultivated in Xinjiang. For example, walnuts were excavated from the North and South Dynasty ruins (Tukuzasail, Bachu County) and the Tang Dynasty No. 195 ancient tomb (Astana, Turpan City) (Yan 1994). As one of the main walnut-producing regions, Xinjiang has a long history of walnut cultivation and abundant resources of seed-grown walnuts (Zhao et al. 2011). Moreover, Xinjiang walnuts differ from those in other regions, forming high-quality characteristic walnut germplasm resources that are adapted to the region's arid and rain-scarce conditions, large temperature differences between day and night, and frequent sandstorms (Wang 2015). The local seed-grown farm type is the main germplasm resource pool. Due to cross-pollination and reproduction through seeds, the offspring variation types are abundant (Wang 2010). However, due to climate change, the spread of pests and diseases, and human disorderly economic development activities, the ancient seed-grown germplasm resources are ecologically fragile. In addition, with the increased promotion of intensive walnut cultivation and improved variety grafting measures, some locally adapted seed-grown farm types have been transformed into excellent cultivars, resulting in a large waste of walnut germplasm resources that have not been explored and have potential utilization value (He et al. 2013). Therefore, it is necessary to take measures such as controlling human economic activities and reducing grazing as soon as possible, deeply excavate high-quality wild or ancient germplasm resources, and develop and protect them simultaneously to achieve sustainable development (Guorong and Zheng 2010; Kang 2013).

Globally, walnut cultivation is primarily concentrated in countries such as China, the United States, Iran, and Türkiye. These countries not only possess abundant walnut germplasm resources but also have advanced technologies and rich experiences in walnut cultivation techniques, variety improvement, pest and disease control, and so forth. Currently, with the in-depth study of walnut germplasm resources, our understanding of their genetic diversity, phenotypic characteristics, growth habits, disease resistance, and so on, is continuously deepening, providing a scientific basis for the efficient cultivation of walnuts, the breeding of new varieties, and the sustainable development of the industry in the future. In terms of phenotypic evaluation and diversity analysis of walnuts, researchers have adopted various methods including physical measurements, growth records, biochemical analysis, and molecular marker techniques. The application of these methods helps to deeply understand the genetic and phenotypic characteristics of walnuts, laying the foundation for breeding new varieties with good performance. Future research directions include further analyzing walnut genetic information through modern molecular biology techniques such as multiparent crossing groups and QTL mapping, as well as evaluating the adaptability and productivity of different germplasms through multienvironmental field experiments. These studies not only promote the sustainable development of the walnut industry but also provide consumers with more high-quality walnut products. Through in-depth research and rational utilization of walnut germplasm resources, combined with the development of modern agricultural technologies, it is expected to further enhance the overall competitiveness and sustainable development capacity of the walnut industry. Phenotypic diversity is a comprehensive reflection of environmental and genetic diversity, and it is also an important method for studying biodiversity (Akça et al. 2020; Arab et al. 2020, 2023; Hassani et al. 2020; Ivan Ivanovich et al. 2024; Jafari Sayadi et al. 2011; Sarikhani et al. 2023; Vahdati 2013; Vahdati et al. 2019). The phenotypic characteristics of plant populations are influenced by geographical environmental conditions. Therefore, by analyzing species characteristics and geographical distribution information, we can deeply understand the diversity of germplasm resources, genetic variation, and the genetic relationships between different regions in the area. In addition, this article reveals the performance of these germplasm resources under different environmental pressures such as drought, salinity, low temperature, and other adverse conditions, as well as their diversity in growth habits, growth rate, yield, and fruit quality. This information can also help us effectively divide germplasm resources, thereby formulating reasonable development and utilization plans and breeding strategies.

This experiment primarily focuses on the ancient walnut germplasm resources from four regions in southern Xinjiang, namely Aksu, Kashgar, Hotan, and Korla. We collected and organized relevant walnut resources and used morphological characteristics to identify the phenotypic traits of nuts. In addition, we conducted a comprehensive analysis of the origin, feasibility, and effectiveness of these ancient walnut resources in Xinjiang. This study expands the scope of research. We not only focused on the ancient walnut germplasm resources in the four regions of southern Xinjiang but also conducted extensive surveys and collections in surrounding areas, broadening the geographical scope of the study. This aids us in gaining a more comprehensive understanding of the distribution characteristics and genetic variation of walnut germplasm resources in Xinjiang, providing richer reference information for formulating regional walnut industry development plans.

Materials and Methods

Material

The distribution of walnut resources in Xinjiang is primarily determined by human factors, exhibiting distinct regional characteristics and a discontinuous distribution pattern. Due to minimal influence from exotic species, its distribution is closely linked to local production activities, primarily shaped by human migration and settlement. By reviewing relevant literature, we can roughly determine the distribution range of Xinjiang's aged walnut germplasm resources. When selecting survey subjects, priority should be given to walnut trees that are early-bearing, have distinct phenotypic characteristics, and exhibit rich variation in flowers and buds. Using a line survey method, county-based field surveys were conducted on Xinjiang's aged walnut germplasm resources. Sampling was carried out from Sep to Oct 2022 in 17 counties (cities) across four regions: Kashgar, Hotan, Aksu, and Bayingolin Mongol Autonomous Prefecture, with a total of 17 sampling points and 304 samples. All samples were collected from local walnut germplasm resources of the seedling type, grown in farmyards, around villages, and in farmlands, with a trunk diameter at breast height greater than 80 cm.

Method

Mature walnut fruits were randomly selected from the periphery of the tree crown, and 10 mature walnut nuts that had been naturally sun-dried were taken. An electronic analytical balance (accuracy of 0.001 g) was used to weigh the single nut mass and single nut kernel mass. A digital vernier caliper (accuracy of 0.01 mm) was used to measure the longitudinal, transverse, and edge diameters of the walnut nut; the average of the three diameters; the length without the suture line; the width without the suture line; the shell; and the thickness of the kernel shell. For 16 walnut nut traits, refer to the nut grade standards for description, observation, and assignment of values (Deng et al. 2018; Liu 2007; Xi 2015). For 12 quantitative walnut nut traits measurement methods were used.

Data processing

Data processing was conducted using WPS software; data analysis was performed using SPSS25.0 and DPS9.01 software, and the remaining tasks were handled by Origin2021 software for plotting.

Quality and shape analysis. Based on the nut grade standards, 16 quality traits of walnut nuts were observed and assigned values according to the observation method (Table 1), and the Shannon-Wiener index (H') was calculated.

Normal analysis of quantitative traits. Twelve quantitative traits of walnut nuts were measured using the measurement method, and the frequency histograms were plotted based on Table 1. The average value ($\bar{X}$), standard deviation (*SD*), coefficient of variation (*CV*), variance (Var), maximum value (Max), minimum value (Min), range

Received for publication 14 Nov 2024. Accepted for publication 13 Jan 2025.

Published online 3 Mar 2025.

S.S.C. and Z.Z.G. contributed equally to this work.

This research work was supported by the National Natural Science Foundation of China (32160698); the South Xinjiang Key Industry Innovation Development Support Program (2022DB022); the Tarim University Scientific Research Innovation Project (TDGR202331); "Tianshan Talents" Training Program for Young Top-notch Scientific Talents focused on Walnut Germplasm Resources Collection (2022TSYCCX0120); Evaluation, Creation, and Characteristic Hard Shell Development Research; the South Xinjiang Horticultural Research Center Scientific Research Condition Construction Project (TDZKKY202204); and the *Populus euphratica* talent project (TDZKBS202419), laying a solid foundation for the research conducted.

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Table 1. Xinjiang Walnut germplasm resources identification characters and standards.

No.	Characters	Evaluation
1	Nut shape	1 = Roundness, 2 = Approximate roundness, 3 = Ellipse, 4 = Short ellipse, 5 = Length ellipse, 6 = Ovum, 7 = Converse oval, 8 = Cylinder, 9 = Circumference, 10 = Triangle
2	Nut smoothness	1 = Smooth beauty, 2 = Smooth, 3 = Coarseness
3	Nutshell color	1 = Light yellow, 2 = Brown yellow, 3 = Light brown, 4 = Brown, 5 = Black brown, 6 = Black
4	Apex and shoulder of nut	1 = Round, 2 = Tine round, 3 = Obtuse tine, 4 = Tine, 5 = Flat, 6 = Concave
5	Nut base	1 = Tine round, 2 = Round, 3 = Flat
6	Suture line character	1 = Swelling, 2 = Flat, 3 = Concave
7	Suture line tightness	1 = Loose, 2 = More loose, 3 = Tight
8	Rill	1 = Sparce, 2 = Dense
9	Bare kernel	1 = Unbare, 2 = Tiny bare, 3 = Bare
10	Minor septum	1 = Degenerate or membrane, 2 = Leathery, 3 = Bony
11	Major septum	1 = Membrane, 2 = Leathery, 3 = Bony
12	Difficulty of taking kernel	1 = Easy, 2 = Intermediate, 3 = Difficult
13	Fullness of kernel	1 = Full, 2 = Almost full, 3 = Shriveled
14	Kernel skin color	1 = Light yellow, 2 = Yellowish brown, 3 = Brown, 4 = Black brown, 5 = Mauve, 6 = Black
15	Nut meat	1 = Whole kernel, 2 = Halves, 3 = 1/4 grain, 4 = 1/8 grain, 5 = Small pieces
16	Nut odor	1 = Normal, 2 = Other
17	Nut mass/g	Weigh 10 naturally dried mature nuts on a balance and take the average value
18	Nut length/mm	Measure the length of walnut nut tip to bottom with a vernier caliper
19	Nut width/mm	Measure the width between the middle of two suture lines of walnut nuts with a vernier caliper
20	Nut height/mm	Measure the width of half of the abdomen on both sides of walnut nuts with a vernier caliper
21	Nut three diameter mean/mm	The average longitudinal, transverse, and angular diameters of nuts
22	Remove the suture length/mm	Measure the longitudinal diameter of walnut nuts with a vernier caliper, removing the length from the tip to the bottom of the walnut
23	Remove suture width/mm	Measure by vernier caliper The width of walnut nuts, excluding the width of the protruding stitching lines on both sides, measured in units:mm
24	Nutshell carcass/mm	Measure by vernier caliper "Along the vertical direction of the walnut suture line, on both sides of the walnut abdomen near the bottom 1/3" Width, unit:mm
25	Husk thickness/mm	Measure by vernier caliper The thickness of the shell of walnut nuts, unit:mm
26	Kernel mass/g	The weighing scales The quality of walnut kernel after removing the shell, unit:g
27	Kernel ratio/%	(Nut mass/Kernel mass) × 100
28	Indexes of nut shape	Nut length/Nut width

(R), and Shannon-Wiener index (H′) of each trait were calculated;

$$\text{Coefficient of variation } (CV) = [\text{Standard deviation } (SD)/\text{Mean } (\bar{X}) \times 100\%].$$

Shannon-Weiner index:

$$H = -\Sigma_{i=1}^{k} P_i \ln P_i$$

In the formula, P_i represents the percentage of the i-th level trait in a certain trait relative to the total number of traits.

Cluster analysis of phenotypic traits. Before conducting cluster analysis of phenotypic traits, the data should be standardized and organized. The systematic clustering method was employed, with Euclidean distance as the genetic distance, and the unweighted pair group method with arithmetic mean used for cluster analysis to generate a cluster diagram.

Correlation analysis. Correlation analysis was conducted using the Pearson correlation coefficient method. * denotes correlation at the 0.05 level and ** denotes correlation at the 0.01 level.

Diversity of Quality Traits of Ancient Walnut Germplasm Resources in Xinjiang

Diversity analysis of overall quality traits. The frequency and Shannon-Wiener diversity index were calculated for 16 quality traits (numbered 1 to 16) of 304 ancient walnut germplasm resources from Xinjiang. The study found (Table 2) that the nut shapes of the ancient walnut germplasm resources from Xinjiang are mainly oval and obovate, accounting for 34.21% and 22.01%, respectively; the nut smoothness is mainly relatively smooth, accounting for 53.95%; the nut color is mainly light brown and brownish yellow, accounting for 47.04% and 32.57%, respectively; the shape of the nut top and shoulder is mainly flat and blunt, accounting for 42.43% and 27.96%, respectively; the shape of the nut bottom is mainly pointed and round, accounting for 44.40% and 36.51%, respectively; the suture line feature is mainly raised, accounting for 94.41%; the suture line is mainly tight, accounting for 80.59%; the kernel shell groove is evenly distributed, with half being sparse and half being dense; the kernel exposure is mainly not exposed, accounting for 99.34%; the internal fold wall degeneration or membrane and bone quality is mainly 56.91% and 26.65%, respectively; the septum is mainly leathery and membranous, accounting for 53.29% and 34.87%, respectively; the kernel extraction is easy; the kernel plumpness is mainly relatively plump and plump, accounting for 57.90% and 30.26%, respectively; the kernel skin color is mainly yellowish brown and brown, accounting for 33.55% and 33.22%, respectively; the difficulty of nut kernel extraction is mainly 1/8 kernel and 1/4 kernel, accounting for 68.49% and 29.29%, respectively; and the kernel smell is mainly normal, accounting for 99.34%.

The Shannon-Wiener index of the ancient walnut germplasm resources in Xinjiang ranges from 0.06 to 2.69, with an average of 1.06. Among them, the Shannon-Wiener index for nut shape is the highest, reaching 2.69. The Shannon-Wiener index for nut smoothness, nut color, shape of the top and tip of the fruit, shape of the bottom of the fruit, and the membrane of the kernel is all above 1.00, indicating rich diversity. However, the Shannon-Wiener index for the presence of exposed kernels is the lowest, at 0.06, indicating relatively poor diversity compared with other traits.

The Diversity of Quantitative Traits of Ancient Walnut Germplasm Resources in Xinjiang

Distribution of overall quantitative traits. The frequency distribution of 12 quantitative traits of ancient walnut germplasm resources in Xinjiang was analyzed, and the results are shown in Figures 1 to 12. In addition, a Kolmogorov-Smirnov (K-S) test was conducted on these quantitative traits (Table 3). Based on the significance level of > 0.05, the frequency distribution

Table 2. Analysis of diversity for 16 qualitative characters.

Characters	Evaluation	Germplasm copies	Frequency	H′
Nut shape	Roundness	15	4.93	2.69
	Approximate roundness	14	4.6	
	Ellipse	104	34.21	
	Short ellipse	8	2.63	
	Length ellipse	28	9.21	
	Ovum	12	3.94	
	Converse oval	67	22.01	
	Cylinder	20	6.58	
	Circumference	36	11.84	
Nut smoothness	Smooth beauty	57	18.75	1.44
	Smooth	164	53.95	
	Coarseness	83	27.3	
Nutshell color	Light yellow	1	0.33	1.77
	Brown yellow	99	32.57	
	Light brown	143	47.04	
	Brown	40	13.91	
	Black brown	16	5.26	
	Black	5	1.65	
Apex and shoulder of nut	Round	25	8.22	2.14
	Tine round	24	7.9	
	Obtuse tine	85	27.96	
	Tine	14	4.61	
	Flat	129	42.43	
	Concave	27	8.88	
Nut base	Tine round	135	44.4	1.51
	Round	111	36.51	
	Flat	58	19.08	
Suture line character	Swelling	287	94.41	0.31
	Flat	17	5.59	
Suture line tightness	Loose	7	2.3	0.56
	More loose	52	17.11	
	Tight	245	80.59	
Rill	Sparce	159	52.3	1
	Dense	149	47.7	
Bare kernel	Unbare	302	99.34	0.06
	Bare	2	0.65	
Minor septum	Degenerate or membrane	173	56.91	0.89
	Leathery	50	16.447	
	Bony	81	26.65	
Major septum	Membrane	106	34.87	1.01
	Leathery	162	53.29	
	Bony	36	11.84	
Difficulty of taking kernel	Easy	249	81.91	0.37
	Intermediate	8	2.632	
	Difficult	47	15.46	
Fullness of kernel	Full	92	30.26	0.98
	Almost full	176	57.9	
	Shriveled	36	11.84	
Kernel skin color	Light yellow	48	15.79	0.95
	Yellowish brown	102	33.55	
	Brown	101	33.22	
	Black brown	44	14.47	
	Mauve	8	2.63	
	Black	1	0.33	
Nut meat	Whole kernel	33	10.86	0.7
	Halves	33	10.86	
	1/4 Pieces	88	29.2928.95	
	1/8 Medium pieces	117	68.49	
	Small pieces	33	11.2410.86	
Nut odor	Normal	302	99.34	0.57
	Other	2	0.66	

diagram follows a normal distribution. Among the quantitative traits of ancient walnut germplasm resources in Xinjiang, except for the kernel yield, which does not follow a normal distribution, the other traits conform to a normal distribution.

Skewness refers to the symmetry of the distribution, with a value less than 0 indicating left or negative skew, and a value greater than 0 indicating right or positive skew. Among the 12 main quantitative traits of ancient walnut germplasm in Xinjiang, kernel yield and single fruit weight exhibit left skew and the rest exhibit right skew. In terms of absolute values, kernel yield and suture width have the largest values, indicating a greater degree of skew.

Kurtosis refers to the steepness of a distribution, with a sharp peak indicating steepness (kurtosis value greater than 0) and a flat peak indicating flatness (kurtosis value less than 0). Among the 12 main quantitative traits of ancient walnut germplasm resources in Xinjiang, the distribution pattern of the length of the suture line is a flat peak and the rest are sharp peaks. Among them, the absolute values are the largest for the width of the suture line and the kernel yield, which means that the difference in steepness of the distribution pattern is also more obvious.

Variation and genetic stability of overall quantitative traits

The variation of quantitative traits among different individual plants of ancient walnuts in Xinjiang is shown in Table 4. There are significant differences in the quantitative traits of nut among different ancient walnut germplasm individual plants. The *CV* of quantitative traits among 304 ancient walnut germplasm resources ranges from 7.42% to 34.00%, with an average of 14.05%. Among them, the *CV* for single nut weight is the highest, at 34.00%. The variation coefficients for single nut weight, kernel yield, shell thickness, and nut transverse diameter are the next highest, with nut transverse diameter having the smallest variation coefficient. The single nut weight of HHT12 ancient walnut germplasm from Hotan County is the highest, significantly higher than other germplasm, at 25.978 g. The second highest is KYC17 from Yecheng County and the lowest is HMY20 from Moyu County. The single nut weight of KELT06 ancient walnut germplasm from Luntai County is the highest, at 14.381 g, and the lowest is KYC12 from Yecheng County. The smallest nut diameter is found in ASM15 from Tianshan Shenmuyuan in Wensu County and the largest is found in HHT123 from Hotan County. The maximum kernel yield is found in KELT07 from Luntai County, which is 6.88 times higher than the minimum found in AKC23 from Kuche County. The maximum shell thickness is found in HYT03 from Hotan County, while the minimum is found in ASM07. The maximum shell thickness is 3.18 times the minimum.

The Shannon-Wiener diversity index for 12 quantitative traits ranged from 8.159 to 8.2430, with an average of 8.227. Among them, the highest index was for nut transverse diameter, reaching 8.243, whereas the lowest was for single nut weight, at 8.159. All quantitative traits had Shannon-Wiener indices above 8.000, indicating rich genetic diversity in Xinjiang's ancient walnut germplasm resources.

Correlation Analysis of Quantitative Traits of Ancient Walnut Germplasm Resources in Xinjiang

The Pearson product moment correlation coefficient was used to analyze the correlation among different quantitative traits of 304 ancient walnut germplasm resources from Xinjiang (Table 5). There were 49 pairs of correlation coefficients that reached a significant level or above, indicating a high correlation among the quantitative traits of ancient walnut nuts from Xinjiang. Among them, the main

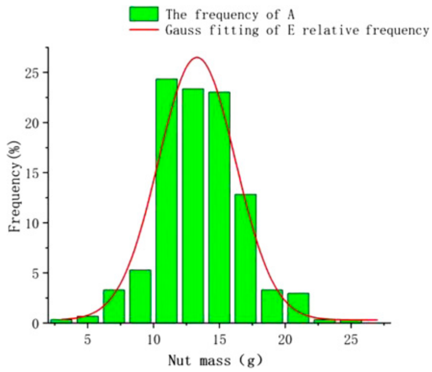

Fig. 1. Frequency distribution of nut mass. In Figs. 1–12, A represents an acceptable quality level, which is the upper limit of the allowable defective rate, and E indicates the defective rate level beyond the acceptable range.

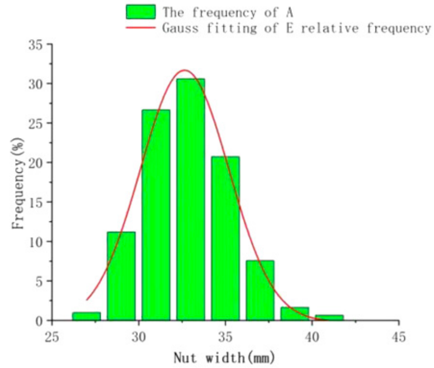

Fig. 3. Frequency distribution of nut width.

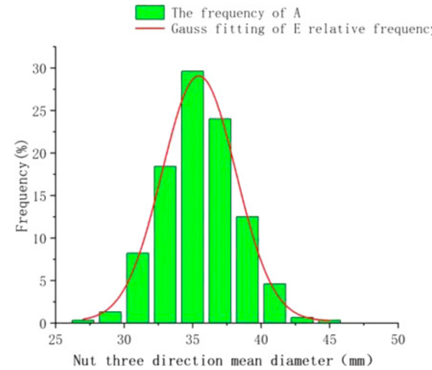

Fig. 5. Frequency distribution of nut three direction mean diameter.

economic traits related to actual production, such as single fruit weight, kernel yield, and kernel weight, showed significant positive correlations with most other traits; nut transverse diameter and fruit shape index, shell thickness, and suture width showed significant negative correlations; shell and fruit shape index, kernel yield, and shell thickness showed extremely significant negative correlations.

Cluster Analysis of Ancient Walnut Germplasm Resources in Xinjiang

Cluster analysis was conducted on 28 phenotypic traits of 304 ancient walnut germplasm from Xinjiang (Fig. 13). The overall phenotypic trait variation of these ancient walnut germplasm from Xinjiang is quite rich. When using a squared Euclidean distance of 20, they can be divided into six major clusters.

Category 1 includes 10 ancient walnut germplasm, mainly sourced from Xinhe and Kuche County in Aksu. The most prominent characteristics of its nut traits are black nut color, cylindrical nut shape, and relatively dry and shriveled kernel.

Category 2 includes 10 ancient walnut germplasm samples, primarily sourced from Aksu Kuche and Xinhe County. The most prominent characteristics of their nut traits are the raised stitch line, tight stitch line, sparse shell groove, thin shell thickness, no kernel exposure, and a higher proportion of bone in the inner fold wall and septum.

Category 3 includes one ancient walnut germplasm, sourced from Wensu County, Aksu (AWS06). The most prominent trait of its nut characteristics is the unpleasant smell of the kernel, whereas the quality and yield of the kernel are relatively high.

Category 4 includes four ancient walnut germplasm, mainly produced in Hotan County and Qiemo County (HHT12, KEQM05, KEQM06, KELT07). The most prominent characteristics of its nut traits are higher single fruit weight, nut diameter, and kernel yield compared with other germplasm.

Category 5 includes two ancient walnut germplasm, with their main production areas being Aksu Shenmuyuan (HMY20) and Hotan Moyu County (ASM15). The most prominent feature of their nut traits is that the nut shape is oval, with relatively small nut rib diameter and shell.

Category 6 includes 277 ancient walnut germplasm, mainly produced in various counties and cities of Aksu, Kashgar, Hotan, and Korla. The germplasm resources exhibit a rich diversity of traits.

Discussion

Phenotypic trait variation is the most direct manifestation of genetic variation, resulting from the interaction between genetic factors and external environment. Studies have found that qualitative traits are controlled by a single gene, with a simple correspondence between genotype and phenotype; however, because of the inability to accurately measure them, only simple descriptions of qualitative traits can be made (Xu 2009). Quantifying the occurrence of a trait and plotting a histogram based on its frequency can reflect the degree of variation in quantitative traits among species or individuals (Liu 2015). Among the 12 quantitative traits, except for the kernel percentage, which does not follow a normal distribution, the other traits follow a normal distribution. In the distribution pattern of quantitative traits, the kernel percentage and single fruit weight exhibit left skewness, while the rest exhibit right skewness; the length of the suture line is a flat-top peak, while the rest are pointed peaks; among them, the absolute values are largest for the kernel percentage and the width of the suture line. Combining the variation range, frequency distribution, and K-S test results, it can be seen that as the variation range increases, the distribution of traits gradually deviates from normality (Dong 2008; Sun 2016). This also indicates that the variation types of nut traits in Xinjiang old walnut germplasm are diverse, with high genetic diversity and great breeding potential. Therefore, based on the differences in phenotypic traits of Xinjiang old walnuts, breeding objectives can be selected separately, providing theoretical support for better utilization of walnut resources.

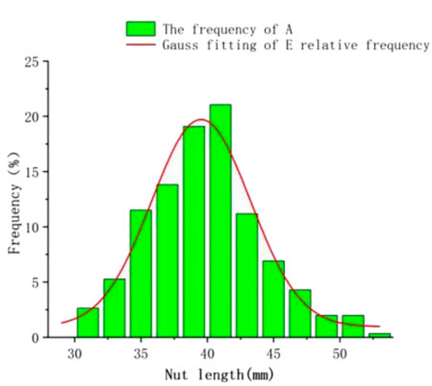

Fig. 2. Frequency distribution of nut length.

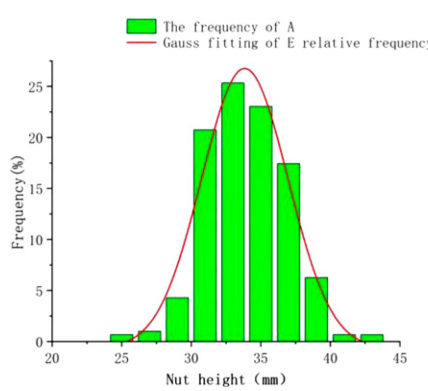

Fig. 4. Frequency distribution of nut height.

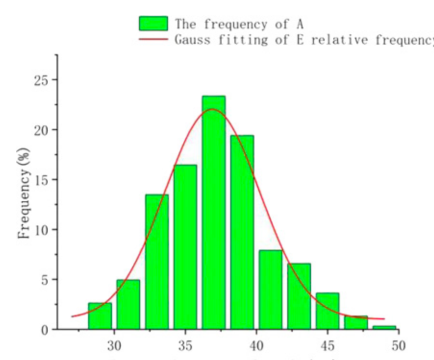

Fig. 6. Frequency distribution of remove the suture length.

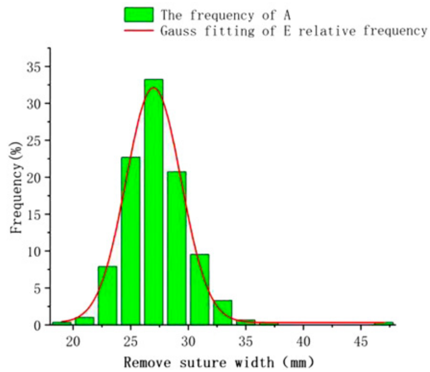

Fig. 7. Frequency distribution of remove carcass.

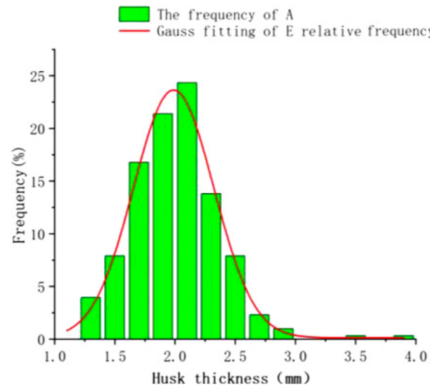

Fig. 9. Frequency distribution of husk thickness.

The author's research has found that the ancient walnuts in Xinjiang exhibit a rich variety of morphological variations, indicating high genetic diversity, which is related to their geographical distribution. Due to the widespread distribution of ancient walnut germplasm, it has developed relatively unique characteristics that adapt to the local climate, thus increasing the likelihood of phenotypic trait variation (Xu et al. 2016). A higher *CV* indicates a greater environmental impact, whereas a lower *CV* indicates better stability and less susceptibility to environmental factors such as climate and soil (Chuanyou et al. 2007). Juan et al. (2014) believe that when the *CV* is above 10%, the variation and differentiation of phenotypic traits are more obvious. In this study, the *CV* for 12 quantitative traits of ancient walnuts in Xinjiang ranged from 7.42% to 34.00%, with an average of 14.05%, indicating that ancient walnuts in Xinjiang exhibit rich genetic diversity in phenotypic traits. Among them, the *CV* for single nut weight is the highest, at 34.00%, followed by variations in single nut weight, kernel yield, and shell thickness, with the smallest *CV* for nut transverse diameter at 7.42%. This is consistent with the conclusion of Xuelin et al. (2012) that the single nut weight of walnuts in Nyingchi, Tibet, is susceptible to environmental influences.

There is a widespread correlation among different fruit traits (Lou et al. 2017; Yuankai et al. 2011), and the selection of these traits based on their correlation provides a theoretical foundation for future gene selection breeding. Correlation analysis indicates a high degree of correlation among the quantitative traits of Xinjiang ancient walnut nuts. Generally, single fruit weight, single nut kernel weight, nut length, nut width, nut rib diameter, and shell weight show a positive correlation with most traits. Among them, 11 indicators, including kernel yield, single nut kernel weight, nut width, nut rib diameter, and nut three diameter mean, are positively correlated with single fruit weight. The thickness of the nutshell exhibits a highly significant negative correlation with kernel yield and the width of the suture line, indicating that the higher the kernel yield and the width of the suture line, the thinner the nutshell thickness. This finding aligns closely with the conclusions of Zhao Mengjun (2016) regarding the phenotypic diversity of local walnuts in Qinghai. The correlation among traits is of significant guidance for early selection of traits. Therefore, traits such as single fruit weight, single nut kernel weight, kernel yield, and nutshell thickness can serve as primary research and reference objects in the study of genetic diversity of Xinjiang walnut germplasm resources.

Through cluster analysis of individual traits of different germplasm, the walnut germplasm resources in Xinjiang can be divided into seven major categories. The overall phenotypic trait variation types of ancient walnut germplasm in Xinjiang are very rich, but the first five categories all have relatively special germplasm classifications. Moreover, the tree age is also relatively large among the 304 germplasm (circumference of more than 4.0 m), reflecting the complex genetic background of ancient walnuts in Xinjiang. Among them, it is particularly noteworthy that in the first and second sister groups, Kuche and Xinhe are clustered as the main germplasm. The ancient tree species in the Kuche-Xinhe region are highly likely to have been brought to Kuche and surrounding areas from the Yili region by farmers and herdsmen crossing the Tianshan Mountains via the "Wusun Ancient Trail" during the "Thirty-Six Kingdoms of the Western Regions" period (Lin 2013; Ren 2009) or even earlier. The central and western regions of the Tarim Basin in Xinjiang have a relatively concentrated distribution of rivers and lakes, and the oases are relatively close to each other, thus forming a very special nomadic migration and oasis culture (Mamuti 2009). In the first cluster, there is a clustering of germplasm from Yarkand (KSC02 KSC07) and the fifth cluster, which includes Shenmuyuan (ASM15) and Moyu (HMY20) germplasm with thin-skinned traits. This is most likely due to exchanges between nomadic peoples living in the Tarim River, Hotan River, and Yerqiang River areas. At the same time, the Tianshan Shenmuyuan and Yili Wild Walnut Valley are located on both sides of the Tianshan Mountains (with a straight-line distance of only more than 100 km). As a refuge for ancient temperate vegetation in the Tertiary period, the Tianshan Shenmuyuan with a "sacrifice effect" and retaining thin-skinned germplasm has been preliminarily identified by Zhang (2010) as a branch of Yili Wild Walnut. Therefore, combining the second and fifth clusters, it can be found that there is a convergence of ancient resources with thin-skinned traits in Moyu County, strongly indicating the spread, exchange, and infiltration of walnut genes among Kuche, Xinhe, Moyu, and Shenmuyuan, and once again proving the close relationship between Wensu

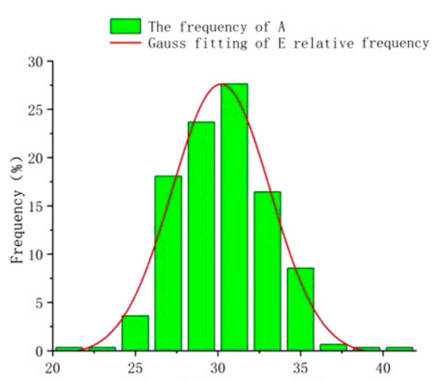

Fig. 8. Frequency distribution of nutshell suture width.

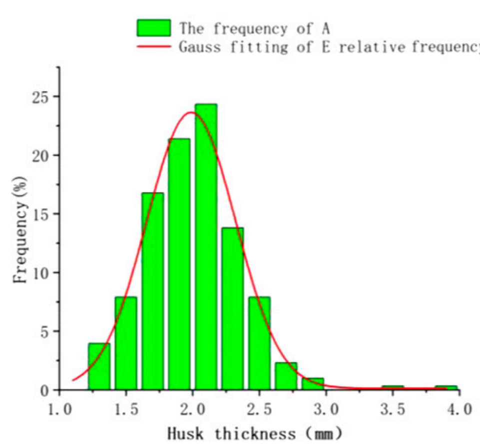

Fig. 10. Frequency distribution of kernel mass.

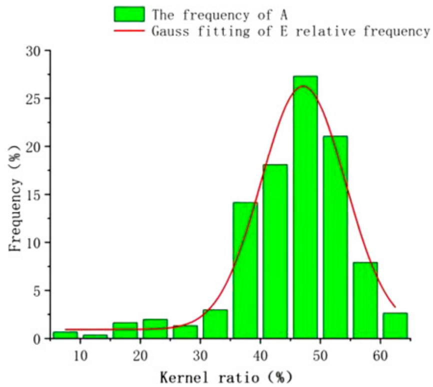

Fig. 11. Frequency distribution of kernel ratio.

Table 3. Kolmogorov-Smirnov test for 12 quantitative characters.

Characters	Skewness	Kurtosis	Test statistic	Asymptotic significance (two-tailed)
Nut mass/g	−0.219	0.931	0.860	0.451[ii]
Nut length/mm	0.360	0.130	0.926	0.358
Nut width/mm	0.347	0.351	0.558	0.915[ii]
Nut height/mm	0.141	0.553	0.684	0.738
Nut three diameter mean/mm	0.170	0.810	0.466	0.982
Remove the suture length/mm	0.225	−0.022	0.946	0.332
Remove suture width/mm	1.250	7.082	1.034	0.235
Nutshell carcass/mm	0.241	0.647	0.544	0.929[ii]
Husk thickness/mm	0.697	2.508	0.852	0.462
Kernel mass/g	0.253	1.203	1.076	0.197
Kernel ratio/%	−1.107	2.284	1.358	0.050
Indexes of nut shape	0.425	0.146	1.103	0.175

[i] Lilliefors Significance Correction.
[ii] This is a lower bound of the true significance.

Table 4. Analysis of diversity for 12 quantitative characters.

Characters	Mean	Max.	Min.	R	Var	*SD*	*CV*/%	H′
17	13.534	25.978	2.596	23.382	10.345	3.216	23.765	8.206
18	39.818	52.293	30.193	22.1	18.493	4.3	10.8	8.2395
19	32.867	41.331	26.46	14.871	5.947	2.439	7.42	8.243
20	33.961	43.465	25.07	18.395	8.084	2.843	8.372	8.2428
21	35.548	44.461	27.818	16.643	7.689	2.773	7.8	8.235
22	37.177	48.095	28.107	19.988	14.298	3.781	10.171	8.24
23	27.328	46.593	18.95	27.643	7.746	2.783	10.184	8.24
24	30.294	41.49	20.463	21.027	7.916	2.814	9.288	8.241
25	1.996	3.829	1.203	2.626	0.125	0.354	17.725	8.225
26	6.371	14.381	0.512	13.869	4.694	2.167	34.007	8.159
27	45.321	63.516	9.226	54.289	83.92	9.161	20.213	8.214
28	1.213	1.552	0.928	0.624	0.012	0.108	8.881	8.242

Note: Characters in the table, 17 to 28 were the 12 quantitative characters. Same as Table 3.

Shenmuyuan and Yili Wild Walnut. Xinjiang, located in the inland hinterland of northwest China, serves as a vital passage for east-west exchanges. It developed into an important node, trade route, and transit point on the ancient Silk Road. Therefore, I speculate that in the fourth cluster, the characteristic germplasm of Qomul and Hotan counties with the "big fruit" trait gene was propagated and multiplied due to the ancient Silk Road – "Xinjiang Southern Route" (Zhang and Western Jin date unknown). By studying the historical backgrounds of "nomadic oasis civilization," "Wusun Ancient Trail," "Silk Road," and Xinjiang, it can be determined that the ancient walnut germplasm in Xinjiang was mainly spread by human activities in a dispersed, convergent, and radiating pattern along rivers. However, for the sixth cluster, the ancient walnut germplasm was not completely clustered according to sampling points, but rather clustered in small groups in scattered sampling locations and then crossed and mixed with each other. Although the germplasm from different regions has different sources, their trait characteristics are relatively consistent. The reason for this phenomenon may be that modern human introduction and seed selection have promoted gene exchange among Xinjiang walnut germplasm. During this large-scale seed dissemination process, while maintaining their own species genetics, Bin et al. (2012) and others also believe that Xinjiang walnut germplasm resources may have undergone moderate gene exchange due to seed dissemination or human seed selection, and the infiltration of certain genes from other regions has also caused variations. At the same time, errors in the judgment of qualitative traits can also cause interference. Through phenotypic trait genetic diversity surveys and research, we can clarify the overall richness of phenotypic genetic diversity, providing reliable phenotypic data for in-depth study of the origin of Xinjiang walnuts and whole-genome association analysis through genome sequencing technology, elucidating the molecular mechanism of trait formation. This is of great significance for mining key genes controlling traits and breeding high-quality varieties.

Conclusion

The ancient walnuts from Xinjiang exhibit significant phenotypic trait variations among different germplasms, boasting rich genetic diversity. Their nuts are oval or obovoid in shape, relatively smooth, and come in shades of brownish yellow or light brown. The shape of the nut top and shoulder is flat or bluntly pointed, while the nut bottom is pointedly round or round. The nutshell has sparse or dense grooves, with the inner fold wall being membranous or bony, the septum being

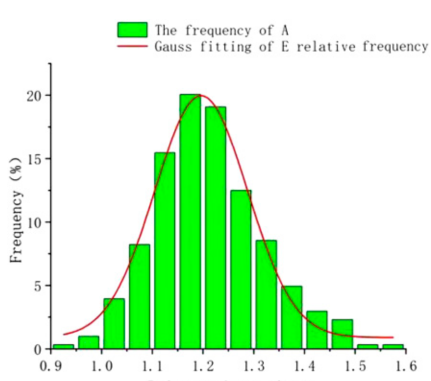

Fig. 12. Frequency distribution of indexes of nut shape.

Table 5. Correlation coefficients of different quantitative traits.

Correlation coefficients	17	18	19	20	21	22	23	24	25	26	27	28
17	1											
18	0.51**	1										
19	0.68**	0.57**	1									
20	0.65**	0.55**	0.81**	1								
21	0.68**	0.87**	0.86**	0.86**	1							
22	0.49**	0.94**	0.55**	0.54**	0.83**	1						
23	0.49**	0.45**	0.70**	0.71**	0.68**	0.47**	1					
24	0.49**	0.30**	0.69**	0.80**	0.63**	0.31**	0.56**	1				
25	0.27**	0.06	0.09	0.13*	0.1	0.04	−0.11*	0.11*	1			
26	0.87**	0.39**	0.55**	0.49**	0.53**	0.36**	0.45**	0.37**	−0.02	1		
27	0.39**	0.06	0.16**	0.07	0.1	0.04	0.24**	0.06	−0.36**	0.75**	1	
28	0.05	0.74**	−0.13*	0	0.35**	0.69**	−0.03	−0.19**	0	0.01	−0.06	1

Note: Characters in the table, 17 to 28 were the 12 quantitative characters. Same as Table 3. ** and * denote significant difference at the 0.01 and 0.05 levels (double tail), respectively.

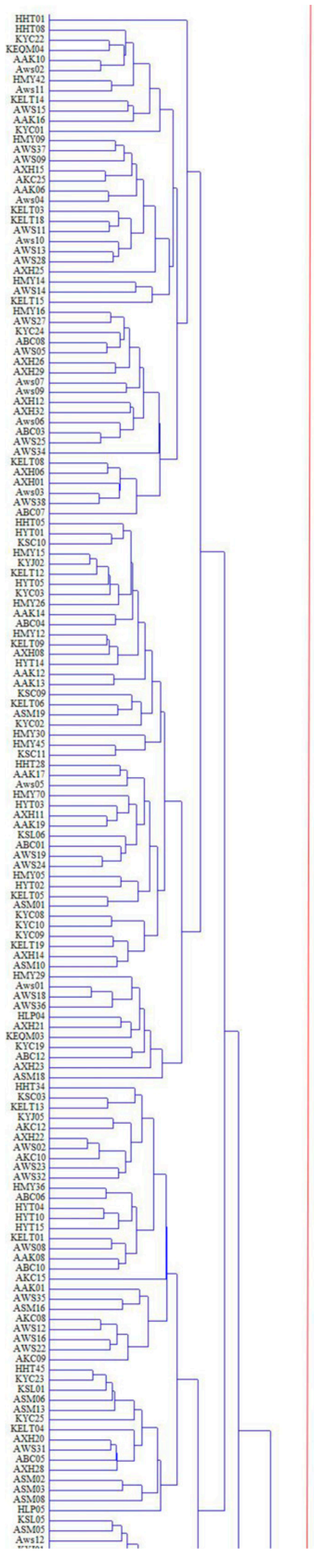

Fig. 13. Dendrogram of between groups linkage method cluster analysis based on squared Euclidean distance of 304 Xinjiang landrace Walnut.

leathery or membranous, and the seed kernel being relatively plump or plump. The skin color of the seed kernel is yellowish brown or brown, with the nut yielding one-eighth or one-fourth of the kernel. The suture line is

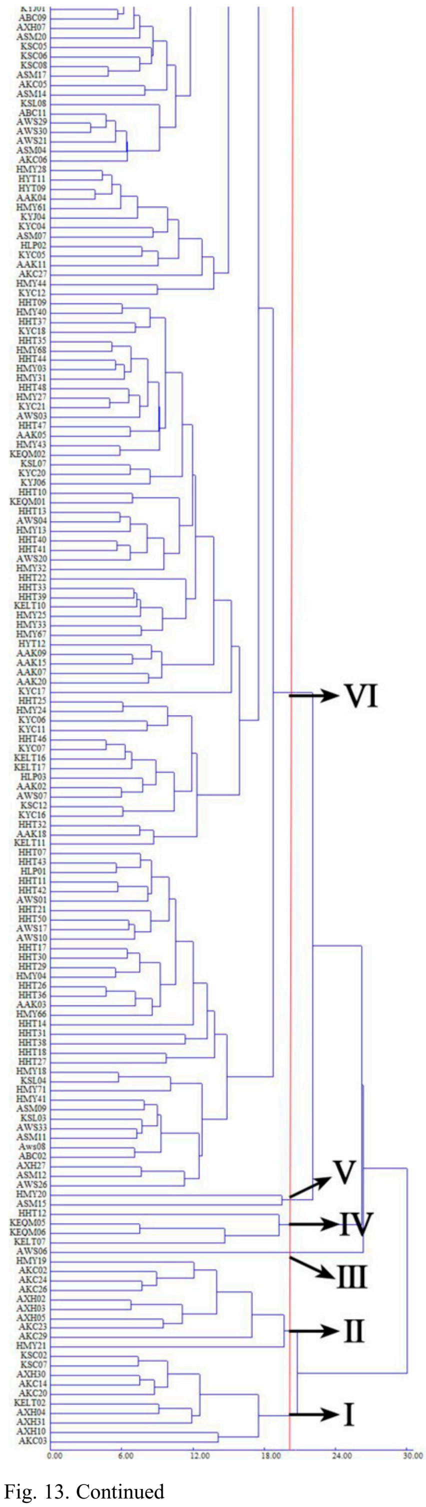

Fig. 13. Continued

tightly raised, without exposing the kernel, making kernel extraction easy, and the seed kernel smells normal. The diversity of nut shape, nut color, shape of the nut top and tip, nut kernel yield, seed kernel skin color, and the three diameters of the nut is relatively rich; however, the diversity of kernel exposure and single kernel quality is relatively poor compared with other traits. The genetic relationship and geographical distribution of Xinjiang walnut germplasm are somewhat related, but they are not completely clustered according to the sampling locations. The clustering method mainly involves cross-mixing clustering of sampling locations. However, a small number of samples from the same sampling location are clustered together, albeit scattered. This is particularly evident in the walnuts from Wensu County and Hotan County, as well as Xinjiang wild walnuts. This study is the first to investigate the traits of 304 Xinjiang wild walnut germplasm covering four prefectures in southern Xinjiang. Its growth environment encompasses most of the ecological conditions in southern Xinjiang. Identifying and evaluating the genetic diversity of 28 phenotypic traits can provide scientific support for the exploration, innovation, and protection of existing walnut germplasm resources, improve breeding efficiency, provide a basis for the selection of core germplasm in the future, and lay a preliminary foundation for gene mapping of walnut phenotypic-related traits.

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