

Article

Phenotypic Variation in Seed Morphochemical and Seedling Traits in Four Chinese Provenances of *Xanthoceras sorbifolium*

Yuxin Chen ¹, Kexin Wang ¹, Zishuo Zhang ¹, Lijin Ou ¹, Xiaofei Luo ¹, Fei Zhu ¹, Peter M. Hirst ² and Yan Ao ^{1,*}

¹ Key Laboratory for Silviculture and Conservation, Ministry of Education, Beijing Forestry University, 35 East Qinghua Road, Haidian District, Beijing 100083, China; cyx0212@bjfu.edu.cn (Y.C.); puppydance@bjfu.edu.cn (K.W.); zhangzishuo@bjfu.edu.cn (Z.Z.); oulijin@bjfu.edu.cn (L.O.); luofei1021@bjfu.edu.cn (X.L.); zhufei@bjfu.edu.cn (F.Z.)

² Department of Horticulture and Landscape Architecture, Purdue University, West Lafayette, IN 47907, USA; hirst@purdue.edu

* Correspondence: aoyan316@163.com; Tel.: +86-10-62337938

Abstract: Variability in seed and seedling traits of *Xanthoceras sorbifolium* Bunge was evaluated at the population level. Seed samples were collected from four provenances in China and examined for variations in morphometric traits, chemical components, and seedling growth in the nursery stage. There were significant differences in the seed length, width, dry weight, 1000-seed weight, oil concentration, Mg and Cu concentrations, root biomass, and root–stem biomass ratio. The largest seed in terms of size and weight was from Ongniud Banner, Inner Mongolia (OB), but these seeds also had the lowest seed oil concentration. At the end of the first growing season in the nursery, seedlings stopped growing one month earlier in height than in diameter. The provenance difference in height was significant at the first 2 months after sowing but disappeared later. Genotypic variance (V_g) was found to be higher than corresponding environmental (V_e) variance for seed length, seed width, seed dry weight, 1000-seed weight, diameter, and root biomass, indicating that these parameters were strongly inherited and there was ample scope for improvement. Moreover, correlations between seed and seedling traits and climatic and geographical factors were assessed. Some significant intercharacter correlations were found, such as between seed length, width and seed weight, between oil concentration and seed size, and between seedling height, diameter, and root biomass. Combining the seed- and seedling-related parameters, our results indicated that Mulei, Xinjiang (ML) may be used as an ideal material for a further improvement program.

Keywords: *Xanthoceras sorbifolium* Bunge; biofuel; seed and seedling characters; broad-sense heritability (H^2); geographical factors



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1. Introduction

Xanthoceras sorbifolium Bunge, commonly known as yellow horn, is an oil-rich seed species that belongs to the Sapindaceae family. The oil concentration is about 55–66% in the seed kernel. Historically, *X. sorbifolium* seed oil was used for illuminating oil and cooking oil [1]. Recently, it has been used to produce renewable and environmentally friendly biodiesel and was considered as one of the eight species listed by the national authorities for the production of biodiesel. Moreover, so far, the planted area of yellow horn has increased to 5×10^5 ha. [2]. As an alternative to petrodiesel, the production of *X. sorbifolium* has received much attention [3–7]. It was reported that yellow horn has experienced extensive management and a ratio of female to male flowers over the past several decades, which has led to low seed yields, i.e., the average yield of mature trees was 670 kg/ha, while 2000 kg/ha was expected under the elite germplasm choice and optimal cultivation and management [8,9]. The performance can be improved if the seed from a broad genetic base or from provenance selection is used to match specific site conditions.

Genetic variation within and between populations and seed sources are essential to exploit the improvement potential. Provenance variation, with respect to seed and seedling morphological, anatomical, physiological, and biochemical traits, has been well studied for a number of tree species [10–12]. Therefore, a screening test is necessary to take advantage of the naturally available genetic variation so to select the ideal planting material for better productivity.

X. sorbifolium has large range of distribution in North China, extending from 28°34′–47°20′ N, 73°20′–120°25′ E, and at a mean annual rainfall of between 43 and 969 mm [8]. Since the environmental conditions vary extensively within the natural range of the species, genetic variation among *X. sorbifolium* provenances would be expected in some traits. However, to date, little work has been done on the relationship between seed traits and seedling growth performance. In this study, seeds of four provenances from across the natural range of *X. sorbifolium* were examined for differences in both seed morphochemical traits and seedling growth and architectural traits in the nursery. The specific aims of this study were to: (1) determine the variation in seed and seedling traits among four different *X. sorbifolium* provenances; (2) evaluate the broad-sense heritability and correlations among traits in *X. sorbifolium*; (3) assess the correlations among seed and seedling traits and correlations between these traits and environmental conditions; (4) evaluate the four provenances based on these traits. The results will illustrate the relationship between the morphological and chemical traits of seeds from four provenances and help to choose seeds with desired chemical components based on that relationship. Furthermore, this study will offer guidance on how to predict the seedling growth performance according to the seed morphochemical traits.

2. Materials and Methods

2.1. Plant Materials

X. sorbifolium seeds were harvested from four provenances in China, arrayed from east to west: Ar Horqin Banner, Inner Mongolia Autonomous Region (AB); Ongniud Banner, Inner Mongolia Autonomous Region (OB); Shanxian, Henan Province (SX); Mulei, Xinjiang Uygur Autonomous Region (ML). The four provenances span the natural distribution range of the species in China, with the AB, ML, and SX provenances representing the northeastern, northwestern, and southern ranges of distribution of the species, respectively [13]. The geographic locations and climatic conditions of the different provenances are presented in Table 1 and Figure 1. These trees were established by farmers from locally collected seeds from wild sources. In each provenance, to ensure the maximum genetic variation within the population, 2 kg of seeds were collected from all directions of the outer crown of 30 dominant candidate trees. These trees were spaced 30–40 m apart and the seeds were extracted from fruits collected at the peak of the fruit season (July–August) in 2014. All seeds from the same provenance were pooled. Seeds were placed in partially sealed polyethylene bags (100 µm thick, permeable to carbon dioxide and oxygen, yet largely impermeable to moisture), and stored at 2 °C.

Table 1. Geographical location and climatic factors of four *X. sorbifolium* provenances.

Provenance	Longitude	Latitude	Altitude (m)	Mean Annual Temperature (°C)	Mean Temperature in Jan. (°C)	Mean Temperature in Jul. (°C)	Mean Annual Rainfall (mm)	Frost-Free Period (d)
AB	120° 01′ E	43°21′ N	550	5.5	−9.5	24.7	340	125
OB	119°02′ E	42°56′ N	639	8.8	−7.4	26.7	320	130
SX	111°37′ E	34°24′ N	760	15.9	3.5	28.0	650	219
ML	92°16′ E	43°14′ N	1084	6.7	−15.6	24.5	294	139

AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei. Climatic resource: <https://www.ventusky.com> (accessed on 9 October 2020).

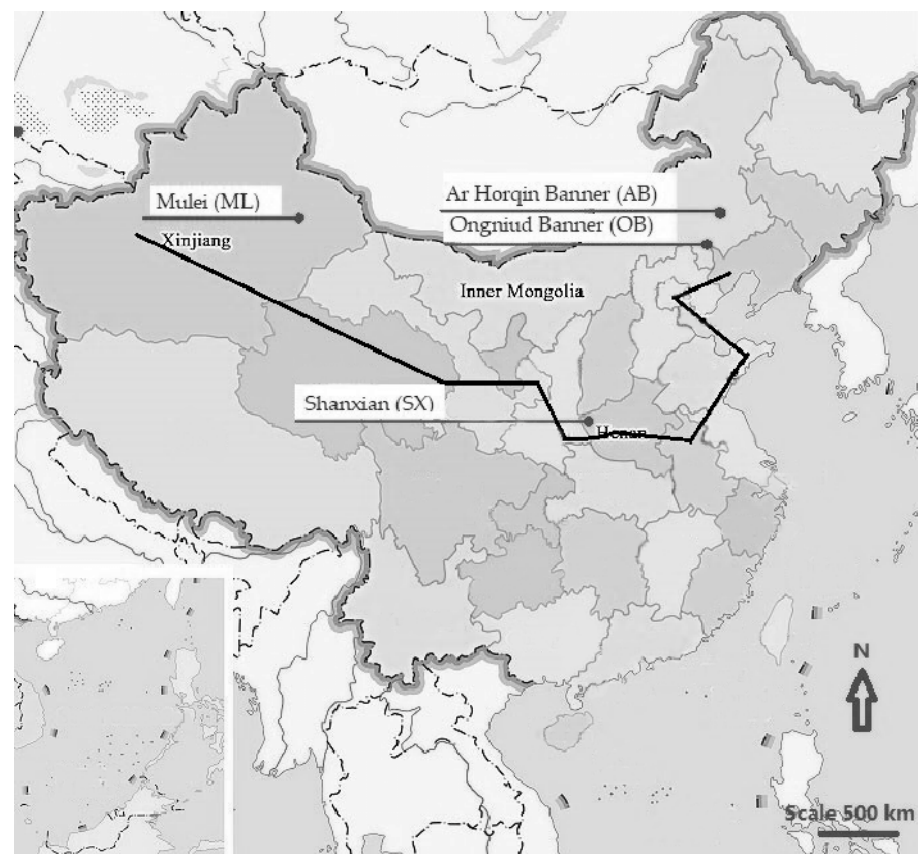


Figure 1. Map of China showing locations of four sampled *X. sorbifolium* populations. The line represents the limit of *X. sorbifolium* distribution.

2.2. Determination of Seed Characteristics

Determination of seed characteristics was made on each of the 40 seed replicates, drawn at random from the pooled samples from each provenance. Measurement of each trait was performed on the specified number of samples. Five samples of 40 seeds from each provenance were used to measure seed size. Seed length (SL) and width (SW) were measured using a digital vernier caliper (Deli LLC, Ningbo, China). The 1000-seed weight (1000 SW) was determined by measuring the fresh weight (digital balance, UX820H, Shanghai Xiangfan Instruments Ltd., Shanghai, China) of eight samples of 100 seeds, and the 1000-seed weight was calculated. After measuring the morphological parameters, the seeds were oven dried at 80 °C for 4–5 h to determine individual seed dry weight (SDW).

Dried seeds were then crushed into powder, and about 0.2 g of each sample was sieved through a 0.25 mm screen. Determination was carried out after four technical replications of sieving. The samples were wet-digested using the H_2SO_4 – H_2O_2 method [14]. Total nitrogen concentration was determined by the micro-Kjeldahl method (UDK-152, Velp Scientifica, Deer Park, NY, USA) [15]. Seed crude protein concentration (SCPC) was then estimated by the multiplication of factor 6.25 with N concentration [16]. Seed soluble sugar concentration (SSSC) was determined on five replicates using an anthrone colorimetry method [17].

To determine seed oil concentration (SOC), each 100-seed sample was dried to a constant weight at 80 °C, and then pulverized with ball mills. The organic solvent was petroleum ether (boiling point, 60 °C). The kernel oil components were extracted using Soxhlet extraction (Soxtec 8000, FOSS, Hillerød, Denmark) through the following process: boil pulverized seeds at 120 °C for 5 min, leach oils for 1 h, and recover oils for 25 min. The kernel oil weight of five replicates was calculated according to the weight difference between the extraction products and the samples [3].

Seed mineral concentrations (N, P, K, Ca, Mg, Cu, Zn, Fe, and Mn) of five replicates were determined using atomic-absorption spectrophotometry (Varian SpectrAA 220FS, VARIAN Technology Co., Ltd., Palo Alto, CA, USA). Samples were prepared according to the standard method as described in AOAC [18].

2.3. Assessment of Seedling Traits

For assessment of the variability in seedling growth parameters, a nursery experiment was performed in a greenhouse at the Beijing Forestry University Forest Science Co., Ltd. (Beijing, China, 40°00' N, 116°34' E). Four replicates of 60 seeds per provenance were randomly sampled and arranged in a completely randomized design. Seeds were sown on 2 April 2014 (one seed per container) at a depth of 1–2 cm in a 1050 mL plastic cuboid-shaped container (8 cm diameter × 20 cm deep) filled with a peat: vermiculite 3:1 ratio by volume. Trays were placed on rolling benches under natural light in the greenhouse and rotated weekly to minimize microsite effects. The seedlings were irrigated as needed and grown in temperatures of 24:21 °C (day:night).

Eight seedlings were selected randomly from each replicate, resulting in a total of 32 seedlings per provenance. Thirty days after sowing, seedling height (H) and diameter (D) at ground level were recorded on selected seedlings every 30 days until growth cessation (150 days after sowing) [19,20]. In late October, a final destructive sampling was performed. The eight seedlings (per replicate) were excavated and washed gently until free of growing medium and separated into stems and roots. Each plant tissue type was oven-dried at 70 °C for 48 h and then weighed using precision weighing balance to determine dry mass. Total biomass (TB) was calculated as the sum of root and stem dry mass (SB).

Prior to measuring the root dry mass (RB), the roots were scanned on a professional scanner (Epson Expression 1680 Pro, Seiko Epson Corporation, Nagano, Japan) and then analyzed by an image analysis system (WinRHIZO, Regent Instruments Inc., Quebec City, QC, Canada) to determine root length, surface area, and volume (RL, RA, and RB).

2.4. Statistical Analysis

All results were reported as the mean ± standard error (SE). Statistical analysis was performed using SPSS 24.0 (Chicago, IL, USA). The explore function was used to examine data for normality and homogeneity prior to analysis. Analysis of variance (ANOVA) was conducted to test the effects of provenance on the measured parameters. When the ANOVA analysis found significant differences among provenances, Duncan's test was conducted to detect differences among provenance means ($\alpha = 0.05$). The Pearson's correlation coefficient was applied to assess correlations among the different seed and seedling traits. The V_p , representing total phenotypic variance in the provenances, consists of the V_g (genotypic variance) and V_e (environmental variance). The broad sense heritability (H^2) and coefficient of variance (CV) were calculated by the following equations [21–23]:

$$H^2 = \frac{V_g}{V_p} \quad (1)$$

$$\sigma_g^2 = \frac{V_P - \sigma_e^2}{B} \quad (2)$$

$$CV = \frac{S}{\bar{X}} \quad (3)$$

where V_g and V_p are the estimates of the genetic (σ_g^2) and residual (σ_e^2) variances derived from the expected mean squares of the variance analysis, B is the number of replicates, S is the standard deviation, and $\bar{X}$ is the mean of the sample data.

The logistic curve was used to fit the annual growth of the seedling height and ground diameter [24]. The result was calculated by the following equation:

$$y = \frac{k}{1 + ae^{-bt}}, \quad (4)$$

where y is the growth of the seedlings, t is the growth time (calculated from the sowing day, with the sowing day considered as 0), a and b are the undetermined coefficients, and k is the upper limit value of growth theoretical limitation.

3. Results

3.1. Seed Morphology and Chemical Component Concentrations

Significant differences among the provenances were detected for all the seed morphometric characters, indicating a genetic variability among the provenances (Table 2) and the specific results of variance analysis are given in Tables S1–S8. Overall, the seeds from the OB provenance were the largest, measured in terms of seed length, seed width, seed dry weight, and 1000-seed weight. All the provenances differed significantly ($p < 0.001$) from each other for the 1000SW. SW varied more across provenances than the seed length and width. The H^2 values varied from 0.608 (SDW) to 0.939 (1000SW). The coefficient of variation ranged between 3.78% and 9.16% (Table 3).

Table 2. Variation in seed morphological parameters among four *X. sorbifolium* provenances.

Provenance	SL (mm)	SW (mm)	SDW (g)	1000SW (g)
AB	13.43 ± 0.10 a	12.24 ± 0.12 b	0.71 ± 0.02 a	803.87 ± 5.33 b
OB	14.21 ± 0.11 b	12.92 ± 0.03 c	0.80 ± 0.01 b	952.92 ± 8.13 d
SX	13.16 ± 0.13 a	11.87 ± 0.11 a	0.70 ± 0.02 a	755.26 ± 9.76 a
ML	13.10 ± 0.12 a	12.43 ± 0.08 b	0.79 ± 0.01 b	850.81 ± 10.15 c
CV (%)	3.79	3.48	7.11	9.16
p value	<0.001	<0.001	<0.001	<0.001

AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei; CV—coefficient of variation; SL—seed length; SW—seed width; SDW—seed dry weight; 1000SW—1000-seed weight. Mean ± SE marked with different letters in a column differ significantly according to Duncan's test $\alpha = 0.05$.

Table 3. Variances and broad-sense heritability of seed parameters in *X. sorbifolium*.

Parameter	Ve	Vg	Vp	CV (%)	H^2
SL	0.057	0.247	0.304	3.78	0.813
SW	0.045	0.181	0.226	3.48	0.801
SDW	0.001	0.002	0.003	7.15	0.608
1000SW	454.942	7026.127	7481.069	9.16	0.939
SCPC	21.663	5.024	26.687	15.02	0.188
SSSC	69.452	20.502	89.954	70.38	0.228
SOC	2.462	1.297	3.759	2.84	0.345

SL—seed length; SW—seed width; SDW—seed dry weight; 1000 SW—1000-seed weight; SCPC—seed crude protein concentration; SSSC—seed soluble sugar concentration; SOC—seed oil concentration; Ve—environmental variance; Vg—genotypic variance; Vp—phenotypic variance; CV—coefficient of variation; H^2 —broad-sense heritability.

The results in Table 4 show that there was no significant difference among the provenances in seed crude protein concentrations and seed soluble sugar concentrations. These traits also had lower H^2 value estimates, ranging between 0.188 and 0.228. SSSC, displayed the maximum environmental, genotypic, and phenotypic variances, and the highest variability (Table 3). The environmental effects on the SCPC and SSSC were remarkably higher than the genotypic effects, as shown by the low H^2 values. The provenance was found to significantly affect the seed oil concentration (SOC) (Table 4). Variation in the SOC was low in terms of phenotypic, genotypic and environmental variances (Table 3).

Table 4. Variation in concentrations (%) of seed crude protein, soluble sugar, and oil among four *X. sorbifolium* provenances.

Provenance	SCPC (%)	SSSC (%)	SOC (%)
AB	34.22 ± 1.17	8.85 ± 4.99	62.14 ± 0.65 b
OB	36.82 ± 2.09	8.36 ± 3.28	59.92 ± 0.26 a
SX	35.79 ± 0.74	11.02 ± 2.44	62.76 ± 0.19 b
ML	34.36 ± 1.11	21.29 ± 2.60	63.18 ± 0.44 b
CV (%)	7.80	70.39	2.84
p value	0.469	0.063	<0.001

AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei; CV—coefficient of variation; SCPC—seed crude protein concentration; SSSC—seed soluble sugar concentration; SOC—seed oil concentration. Mean ± SE marked with different letters in a column differ significantly according to Duncan's test $\alpha = 0.05$.

The most dominant minerals in the seeds were N, K, Mg, P, and Ca, while other minerals (Fe, Zn, Cu, and Mn) were present at much lower levels (Table 5). The provenance only significantly affected Mg and Cu concentrations. Seeds from OB had the lowest Mg concentration, while seeds from the ML provenance showed the highest Mg concentration. The Cu concentration in seeds from the AB and OB provenances was significantly higher than those from SX and ML provenances (Table 5).

Table 5. Variation in seed mineral concentrations among four *X. sorbifolium* provenances (mg/kg; dry weight).

Provenance	N	P	K	Ca	Mg	Cu	Zn	Fe	Mn
AB	22,326 ± 0.076	1992 ± 0.020	8448 ± 0.007	1792 ± 0.005	2202 ± 0.003 b	8.339 ± 0.367 b	20.906 ± 0.504	98.890 ± 7.544	3.295 ± 0.309
OB	23,640 ± 0.107	1760 ± 0.017	8452 ± 0.002	1816 ± 0.004	2117 ± 0.001 a	8.412 ± 0.735 b	22.480 ± 0.1234	100.050 ± 6.049	3.386 ± 0.472
SX	24,296 ± 0.126	1562 ± 0.010	8422 ± 0.005	1841 ± 0.003	2194 ± 0.002 b	7.265 ± 0.128 ab	21.201 ± 0.708	89.712 ± 3.900	3.957 ± 0.471
ML	21,671 ± 0.126	2234 ± 0.039	8502 ± 0.006	1627 ± 0.011	2205 ± 0.002 b	6.410 ± 0.523 a	18.553 ± 1.142	85.456 ± 1.689	3.568 ± 0.106
CV (%)	9.79	26.78	1.23	8.33	2.35	16.10	10.77	12.17	20.11
p value	0.360	0.281	0.776	0.153	0.025	0.040	0.073	0.200	0.613

AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei; CV—coefficient of variation. Mean ± SE marked with different letters in a column differ significantly according to Duncan's test $\alpha = 0.05$.

3.2. Seedling Morphology and Biomass

The broad-sense heritability was relatively higher in the seedling diameter (0.712) and root biomass (0.845), while the minimum H^2 value was recorded for the seedling height (0.017). According to the coefficient of variation values, the root length, stem biomass, and root area had strong variability (Table 6).

Table 6. Variances and broad-sense heritability of seedling parameters in *X. sorbifolium*.

Parameters	Ve	Vg	Vp	CV (%)	H^2
H	5.500	0.094	5.594	11.07	0.017
D	0.059	0.146	0.205	15.78	0.712
RB	0.053	0.289	0.342	23.64	0.845
SB	0.038	0.013	0.051	41.13	0.248
TB	0.098	0.360	0.458	22.75	0.787
RL	17,894.730	7912.087	25,806.810	61.82	0.307
RA	581.626	373.758	955.385	54.93	0.391
RV	2.709	0.349	3.058	50.35	0.114

H—height; D—diameter; RB—root biomass; SB—stem biomass; TB—total biomass; RL—root length; RA—root area; RV—root volume. Ve—environmental variance; Vg—genotypic variance; Vp—phenotypic variance; CV—coefficient of variation; H^2 —broad-sense heritability.

Seedlings grew rapidly during the first two months after sowing (Figure 2). Differences in seedling height among the provenances could be seen one month after sowing, and these differences remained fairly stable throughout the growing season. Seeds from provenances AB and ML produced the tallest seedlings, and the seedlings produced by those from OB

and SX were the shortest (Figure 2). Seedling ground diameter increased rapidly during the first three months and increased at a lower rate during 3–5 MAS. Five months after sowing, the height–diameter ratio (H/D) was higher in the seedlings from the ML than in the SX provenance. However, the seedlings from the ML provenance were significantly lower than those from the SX and AB provenances in terms of the root–stem biomass ratio (R/S) (Figure 3). Seedling height was determined primarily by the environment and was virtually unaffected by the genotype or provenance (Table 6). Conversely, 71% of the variation in the seedling diameter could be caused by the provenance.

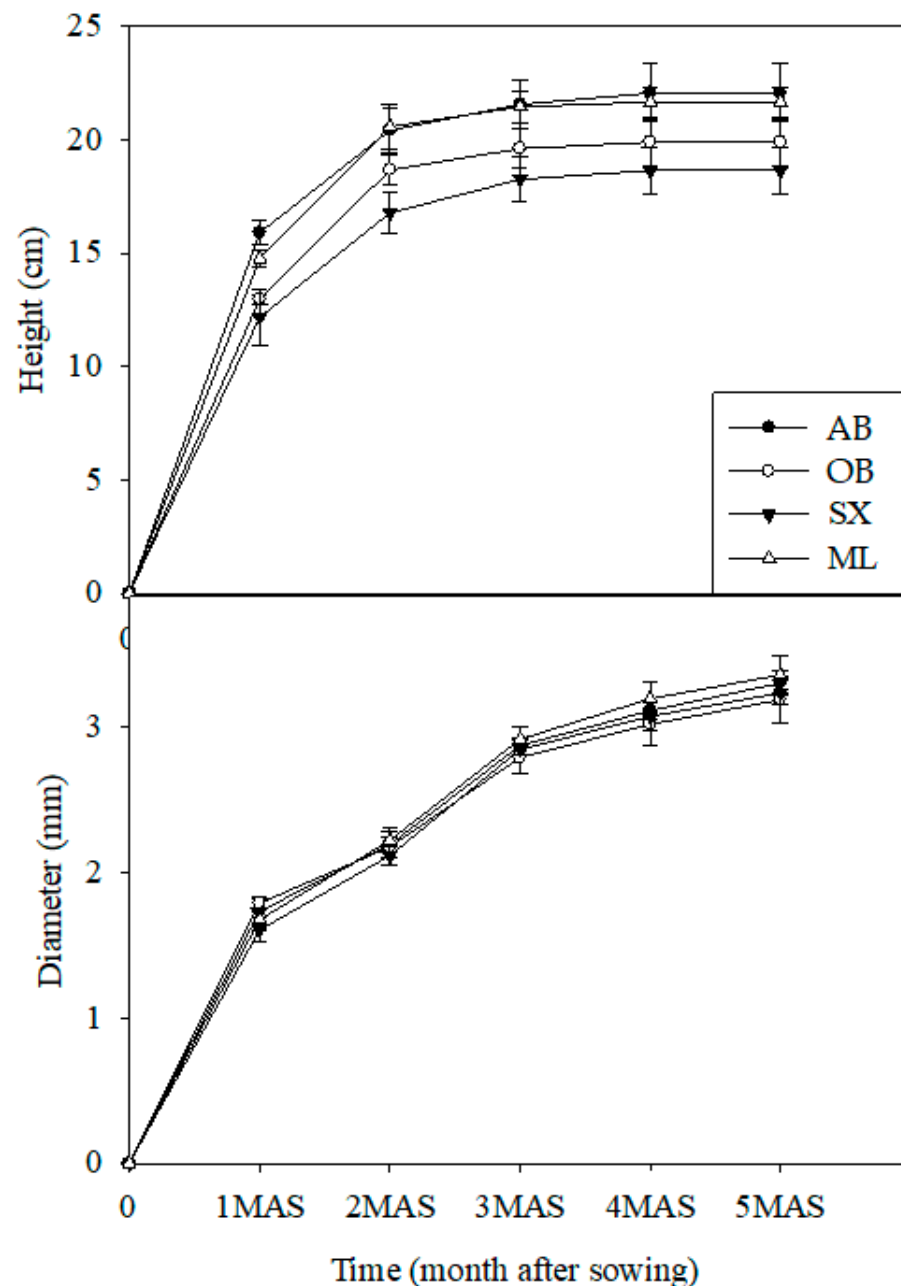


Figure 2. Monthly seedling growth in height and diameter of four provenances of *X. sorbifolium*. AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei. Bars marked with different letters differ significantly according to Duncan’s test $\alpha = 0.05$.

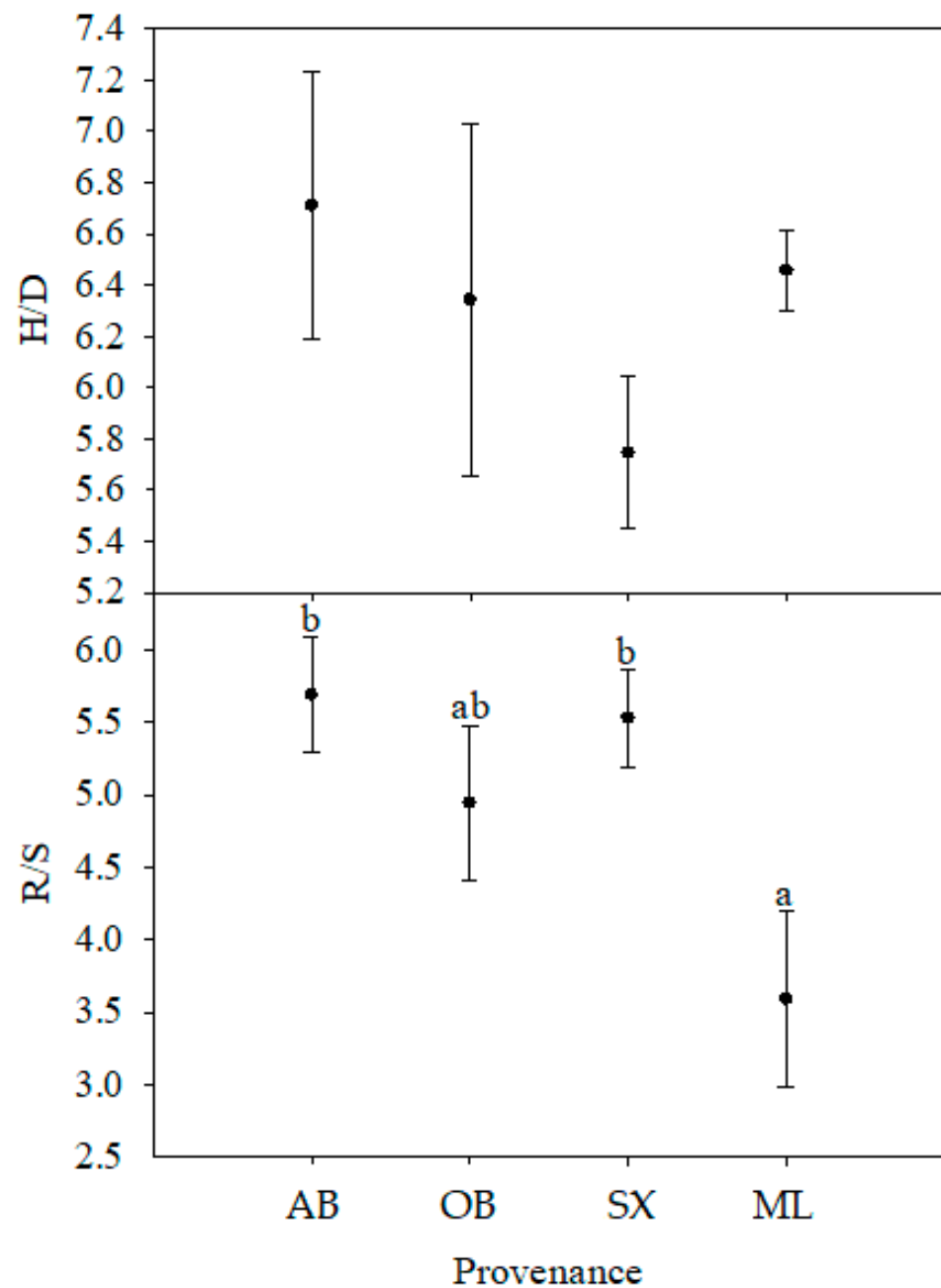


Figure 3. Effects of provenance on height–diameter ratio (H/D) and root–stem biomass ratio (R/S) in *X. sorbifolium* (mean $\pm$ SE). AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei. Bars marked with different letters differ significantly according to Duncan’s test $\alpha = 0.05$.

The logistic growth curves of the seedlings from the four provenances are fitted in Figure 4 (diameter) and Figure 5 (height). All of the diameter growth curves indicated the ‘fast–slow’ trend, while height growth curves of OB and ML showed a ‘slightly slow–fast–slow’ trend in the four provenances. The estimated parameters are given in Tables 7 and 8. The average determination coefficient of the ground diameter (0.923) and height (0.986) indicated the feasibility of the fitting equations. Variance component analysis of the estimated parameters are given in Tables S5 and S6.

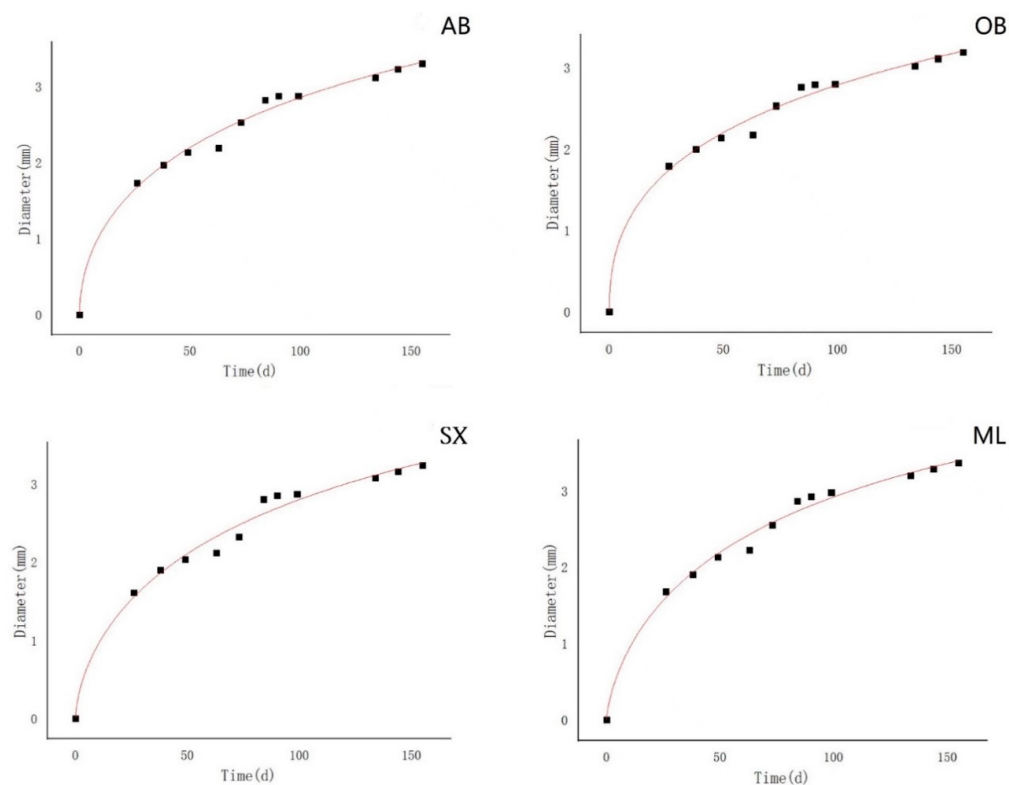


Figure 4. Logistic fitting curves of seedling diameter of *X. sorbifolium* from different provenances. AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei.

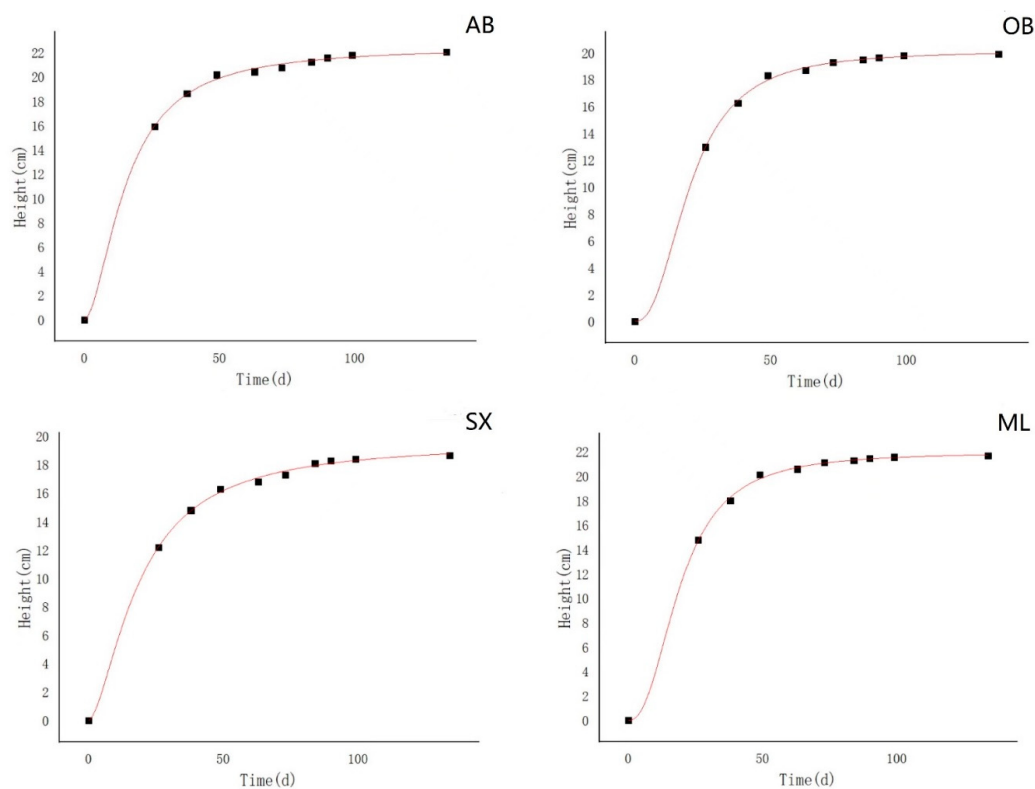


Figure 5. Logistic fitting curves of seedling height of *X. sorbifolium* from different provenances. AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei.

Table 7. Estimated parameters of logistic curve of seedling ground diameter of *X. sorbifolium* from different provenances.

Provenance	k	a	b	R ²	F
AB	3.137	4.393	0.045	0.920	318.190 **
OB	2.986	4.361	0.050	0.911	298.540 **
SX	3.123	4.536	0.042	0.925	323.370 **
ML	3.231	4.689	0.044	0.935	372.080 **

AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei; k—growth limitation; a, b—undetermined coefficients; R²—determination coefficient. ** Correlation is significant at the 0.01 level.

Table 8. Estimated parameters of logistic curve of seedling height of *X. sorbifolium* from different provenances.

Provenance	k	a	b	R ²	F
AB	21.061	22.182	0.157	0.986	1552.000 **
OB	19.328	16.580	0.129	0.989	1830.480 **
SX	17.740	13.839	0.123	0.978	936.940 **
ML	21.101	17.641	0.137	0.989	1883.750 **

AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei; k—growth limitation; a, b—undetermined coefficients; R²—determination coefficient. ** Correlation is significant at the 0.01 level.

At the end of the first growing season, the biomass of the SX seedlings was 51% larger than the mean from the other provenances for the root biomass, and 45% larger for the total biomass (Table 9 and Figure 3). The highest value of CV was recorded in the stem biomass (41.13%), followed by the root biomass (23.64%).

Table 9. Seedling root biomass, stem biomass, and total biomass of four provenances of *X. sorbifolium*.

Provenance	Root Biomass (g)	Stem Biomass (g)	Total Biomass (g)
AB	2.080 ± 0.124 a	0.367 ± 0.009	2.447 ± 0.121 a
OB	2.128 ± 0.201 a	0.439 ± 0.048	2.567 ± 0.232 a
SX	3.231 ± 0.161 b	0.586 ± 0.019	3.817 ± 0.161 b
ML	2.199 ± 0.146 a	0.698 ± 0.182	2.897 ± 0.278 a
CV (%)	23.64	41.13	22.75
p value	0.001	0.113	0.002

AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei; CV—coefficient of variation. Mean ± SE marked with different letters in a column differ significantly according to Duncan's test $\alpha = 0.05$.

The phenotypic, genotypic, and environmental variances of the total biomass (0.458, 0.360, and 0.098, respectively) were higher than those observed for either the root or stem biomass. Both root biomass and total biomass exhibited high H² values (Table 6).

3.3. Seedling Root Architecture

There was a general trend for the seedlings from provenance ML to have the highest total root length, area, and volume, and those from OB exhibited the lowest levels for these parameters (Table 10). However, the provenances had no significant effects on the total root length, area, or volume. The root composition of the seedlings from AB differed from that of the seedlings from the other provenances in a significantly lower ratio of smaller roots and a significantly higher ratio of intermediate-sized roots. For all provenances, roots with a diameter < 2 mm averagely contributed to a greater proportion (89.23%) of the total root length, and roots with a diameter between 2 and 5 mm ranked the second (8.37%). As might be expected, the root area had a similar trend.

Table 10. Root length, area, and volume ratio of different root diameter class (<2, 2–5, >5 mm) to total root of four provenances of *X. sorbifolium* seedlings.

Provenance	Ratio of Different Root Diameter Class to Total Root Length (%)			Total Root Length (cm)
	Diameter < 2 mm	2 mm ≤ Diameter < 5 mm	Diameter ≥ 5 mm	
AB	82.78 ± 3.44 a	15.19 ± 3.24 b	2.03 ± 0.83	233.995 ± 63.535
OB	90.36 ± 1.92 b	6.54 ± 2.20 a	3.11 ± 1.68	150.917 ± 36.129
SX	91.53 ± 0.89 b	6.09 ± 1.05 a	2.38 ± 0.99	258.698 ± 22.300
ML	92.26 ± 2.56 b	5.64 ± 2.78 a	2.10 ± 1.48	422.449 ± 114.584
CV (%)	7.01	77.37	111.93	13.56
p value	0.046	0.042	0.931	0.114

Provenance	Ratio of Different Root Diameter Class to Total Root Area (%)			Total Root Area (cm ²)
	Diameter < 2 mm	2 mm ≤ Diameter < 5 mm	Diameter ≥ 5 mm	
AB	46.87 ± 6.89	41.50 ± 7.50	11.64 ± 4.97	74.920 ± 21.196
OB	57.62 ± 8.89	22.63 ± 8.73	19.76 ± 10.81	43.437 ± 13.619
SX	52.41 ± 5.94	28.53 ± 6.64	19.06 ± 7.92	58.895 ± 7.090
ML	59.04 ± 10.77	23.87 ± 10.28	17.09 ± 11.78	93.464 ± 24.687
CV (%)	32.97	64.76	114.21	61.39
p value	0.727	0.396	0.923	0.273

Provenance	Ratio of Different Root Diameter Class to Total Root Volume (%)			Total Root Volume (cm ³)
	Diameter < 2 mm	2 mm ≤ Diameter < 5 mm	Diameter ≥ 5 mm	
AB	21.22 ± 6.17	52.22 ± 9.94	26.56 ± 11.86	4.964 ± 1.628
OB	31.15 ± 13.50	33.01 ± 13.75	35.84 ± 19.41	3.556 ± 1.537
SX	17.77 ± 7.60	42.40 ± 12.92	39.83 ± 14.70	4.007 ± 1.045
ML	29.06 ± 11.90	38.09 ± 15.45	32.85 ± 17.80	5.172 ± 1.297
CV (%)	87.76	67.51	99.56	66.53
p value	0.766	0.766	0.948	0.822

AB—Ar Horqin Banner; OB—Ongniud Banner; SX—Shanxian; ML—Mulei; CV—coefficient of variation. Mean ± SE marked with different letters in a column differ significantly according to Duncan's test $\alpha = 0.05$.

A considerable amount of variability was observed between the provenances for the root architecture, as evidenced by the CV value. The CV values for the root length, area, and volume were found to be 13.56%, 61.39%, and 66.53%, respectively. Moreover, fairly low H^2 values of the root length, area, and volume indicated that the root architecture was mainly affected by the environment.

3.4. Correlation Analysis for Seed and Seedling Traits

Correlation analysis demonstrated relationships among seed traits (Table 11), and relevant observation numbers are given in Tables S10–S12. Seed dry weight, 1000SW, SL, and SW showed significant positive correlations with each other. SOC was negatively correlated with seed length and width, and a similar result was found in the seed Mg concentration. SSSC was negatively correlated with Cu and Zn concentrations. Among seed mineral concentrations, significant positive correlations were found between K and P, Ca and N, Zn and Fe, Fe and Cu, and Zn and Cu. Negative correlations were found between N and P, P and Ca, K and Ca, and Zn and Mg.

At the provenance level, seedling morphological traits (height and diameter) and biomass (RB, SB, and TB), RL, RA, and RV were found to have strong positive relationships (Table 12). RL was positively associated with TB and H/D. Seedling height exhibited significant correlations with all other parameters. As might be expected, diameter was positively correlated with biomass, but negatively with H/D. The result at the individual level corresponded with the provenance level, and related correlation coefficients are given in Table S9.

Table 11. Correlation coefficients among seed traits of four provenances of *X. sorbifolium*.

	1000SW	SL	SW	SSSC	SCPC	SOC	N	P	K	Ca	Mg	Cu	Zn	Fe	Mn
SDW	0.789 **	0.554 *	0.844 **	0.065	0.257	−0.461 *	−0.251	0.256	0.170	−0.338	−0.304	−0.079	0.097	0.024	0.0130
1000SW		0.785 **	0.929 **	−0.015	0.308	−0.664 **	−0.025	0.086	0.063	−0.062	−0.542 *	0.207	0.219	0.245	−0.190
SL			0.747 **	−0.360	0.392	−0.852 **	0.108	−0.143	−0.136	0.242	−0.526 *	0.401	0.473*	0.388	−0.148
SW				−0.148	0.298	−0.645 **	−0.199	0.262	0.187	−0.219	−0.458 *	0.283	0.257	0.238	−0.126
SSSC					−0.110	0.398	0.001	0.081	0.197	−0.266	0.322	−0.516 *	−0.541 *	−0.452	−0.049
SCPC						−0.415	0.077	−0.398	−0.008	0.313	−0.459 *	0.065	0.265	0.079	−0.010
SOC							−0.251	0.265	0.184	−0.281	0.528 *	−0.296	−0.465 *	−0.206	0.306
N								−0.533 *	−0.127	0.602 **	−0.012	−0.179	0.031	−0.143	−0.212
P									0.573 **	−0.744 **	0.245	−0.006	−0.242	−0.075	−0.073
K										−0.629 **	0.141	0.071	−0.035	−0.293	−0.012
Ca											−0.098	0.128	0.207	0.385	0.095
Mg												−0.431	−0.571 **	−0.377	0.223
Cu													0.781 **	0.772 **	0.271
Zn														0.587 **	0.351
Fe															0.377

SDW—seed dry weight; 1000SW—1000-seed weight; SL—seed length; SW—seed width; SSSC—seed soluble sugar concentration; SCPC—seed crude protein concentration; SOC—seed oil concentration. * Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

Table 12. Correlation coefficients among seedling traits of *X. sorbifolium* from four provenances.

	RA	RV	H	D	RB	SB	TB	R/S	H/D
RL	0.896 **	0.543 *	0.367	−0.359	0.217	0.070	0.570 **	0.211	0.521 *
RA		0.813 **	0.290	−0.277	0.227	0.080	0.394	0.250	0.407
RV			0.156	−0.079	0.274	0.051	0.236	0.322	0.176
H				0.379 **	0.292 **	0.539 **	0.389 **	−0.274 **	0.746 **
D					0.391 **	0.397 **	0.442 **	−0.042	−0.274 **
RB						0.364 **	0.974 **	0.360 **	0.030
SB							0.564 **	−0.305 **	0.265 **
TB								0.246 **	0.090 *
R/S									−0.250 **

RL—root length; RA—root area; RV—root volume; H—height; D—diameter; RB—root biomass; SB—stem biomass; TB—total biomass; R/S—root–stem biomass ratio; H/D—height–diameter ratio. * Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

The correlations between seed and seedling traits (Table 13) revealed that 1000SW and SW showed significantly positive relationships with the diameter, but negative relationships with the RB, TB, and H/D. The SSSC exhibited positive correlations with the RL, RA, and SB, but negative correlation with the R/S. Seed Mg, Cu, Zn, and Fe concentrations were significantly correlated with the RL, SB, and H/D.

Table 13. Correlation coefficients between seed and seedling traits of four provenances of *X. sorbifolium*.

	RL	RA	RV	H	D	RB	SB	TB	R/S	H/D
SDW	0.213	0.139	0.010	−0.094	0.420	−0.321	0.222	−0.212	−0.430	−0.298
1000SW	−0.142	−0.135	−0.121	−0.415	0.659 **	−0.559 *	−0.069	−0.504 *	−0.273	−0.687 **
SL	−0.231	−0.105	−0.008	−0.326	0.598 *	−0.306	−0.170	−0.316	0.048	−0.564 *
SW	−0.160	−0.117	−0.104	−0.396	0.647 **	−0.554 *	−0.265	−0.560 *	−0.107	−0.672 **
SSSC	0.620 **	0.515 *	0.420	−0.011	−0.277	0.022	0.617 *	0.209	−0.528 *	0.228
SCPC	−0.031	0.126	0.360	−0.012	0.598 *	0.017	−0.052	−0.002	−0.018	−0.386
SOC	0.167	0.018	0.023	0.317	−0.550 *	0.281	0.272	0.326	−0.176	0.565 *
N	−0.071	−0.203	−0.376	−0.050	0.055	0.313	0.169	0.322	0.247	−0.103
P	0.044	−0.053	−0.131	0.073	−0.104	−0.327	−0.200	−0.344	−0.190	0.129
K	−0.033	−0.108	−0.144	0.018	−0.120	−0.166	−0.193	−0.203	−0.098	0.094
Ca	−0.206	−0.111	−0.027	0.060	0.272	0.205	0.055	0.194	0.209	−0.162
Mg	0.459 *	0.386	0.136	0.250	−0.687 **	0.112	0.409	0.223	−0.097	0.707 **
Cu	−0.633 **	−0.470 *	−0.233	−0.322	0.354	−0.254	−0.740 **	−0.446	0.456	−0.518 *
Zn	−0.497 *	−0.425	−0.298	−0.189	0.369	0.061	−0.567 *	−0.122	0.398	−0.481 *
Fe	−0.434	−0.359	−0.219	−0.210	0.367	−0.255	−0.378	−0.337	0.247	−0.455 *
Mn	0.008	−0.061	−0.062	0.084	−0.080	0.237	0.094	0.234	0.006	0.095

SDW—seed dry weight; 1000SW—1000-seed weight; SL—seed length; SW—seed width; SSSC—seed soluble sugar concentration; SCPC—seed crude protein concentration; SOC—seed oil concentration; RL—root length; RA—root area; RV—root volume; H—height; D—diameter; RB—root biomass; SB—stem biomass; TB—total biomass; R/S—root–stem biomass ratio; H/D—height–diameter ratio. * Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

3.5. Correlations between Geographical Factors, Climatic Factors, and Seed/Seedling Traits

With regard to the relationship between the geographical and climatic factors of the provenances and the parameters studied, the SW, SDW, and 1000SW showed significant negative correlations with the mean temperature in January and the mean annual rainfall (Table 14). Of the seed chemical component concentrations, the SSSC was significantly negatively correlated with the longitude and positively correlated with the altitude, while opposite correlations with longitude and altitude were found in the Cu, Zn, and Fe concentrations. Both the RB and TB showed significantly positive correlations with the

temperature, rainfall, and frost-free period, while the SB showed a significantly positive correlation with the altitude.

Table 14. Correlation coefficients between geographical factors, climatic factors, and seed/seedling parameters of four provenances of *X. sorbifolium*.

	Longitude	Latitude	Altitude	Mean Annual Temperature	Mean Temperature in Jan.	Mean Temperature in Jul.	Mean Annual Rainfall	Frost-Free Period
SL	0.534 *	0.280	−0.494 *	−0.162	−0.021	0.174	−0.331	−0.408
SW	0.071	0.619 **	−0.053	−0.480 *	−0.493 *	−0.224	−0.691 **	−0.667 **
SDW	−0.307	0.463 *	0.332	−0.327	−0.496 *	−0.212	−0.551 *	−0.441
1000SW	0.075	0.583 **	−0.042	−0.419	−0.452 *	−0.137	−0.672 **	−0.638 **
SSSC	−0.619 **	0.124	0.604 **	−0.121	−0.342	−0.296	−0.145	−0.010
SCPC	0.213	0.000	−0.170	0.086	0.101	0.250	−0.049	−0.062
SOC	−0.608 **	0.142	0.595 **	−0.126	−0.348	−0.283	−0.170	−0.034
N	0.253	−0.379	−0.208	0.406	0.440	0.467	0.360	0.325
P	−0.310	0.414	0.267	−0.429	−0.489	−0.491	−0.403	−0.351
K	−0.224	0.209	0.211	−0.196	−0.267	−0.225	−0.220	−0.170
Ca	0.518 *	−0.323	−0.490	0.318	0.482	0.434	0.337	0.228
Mg	−0.331	−0.091	0.277	−0.032	−0.073	−0.285	0.157	0.183
Cu	0.664 **	0.124	−0.666 **	−0.122	0.139	0.105	−0.097	−0.247
Zn	0.582 *	−0.167	−0.544 *	0.207	0.378	0.414	0.158	0.049
Fe	0.504 *	0.165	−0.508 *	−0.157	0.044	0.032	−0.148	−0.260
Mn	−0.118	−0.333	0.142	0.331	0.253	0.250	0.321	0.354
RL	−0.543 *	0.089	0.521 *	−0.108	−0.288	−0.288	−0.094	0.016
RA	−0.371	0.223	0.327	−0.270	−0.350	−0.411	−0.199	−0.142
RV	−0.208	0.248	0.177	−0.266	−0.304	−0.321	−0.236	−0.204
H	−0.239	−0.305	0.242	0.266	0.174	0.109	0.314	0.359
D	0.346	0.183	−0.289	−0.042	0.001	0.249	−0.259	−0.283
RB	−0.023	−0.854 **	0.079	0.828 **	0.747 **	0.684 **	0.846 **	0.863 **
SB	−0.573 *	−0.153	0.592 *	0.178	−0.072	0.007	0.111	0.252
TB	−0.205	−0.778 **	0.258	0.764 **	0.615 *	0.586 *	0.758 **	0.818 **
R/S	0.628 **	−0.305	−0.627 **	0.241	0.492	0.327	0.362	0.205
H/D	−0.414	−0.308	0.377	0.187	0.090	−0.116	0.363	0.414

SL—seed length; SW—seed width; SDW—seed dry weight; 1000SW—1000-seed weight; SSSC—seed soluble sugar concentration; SCPC—seed crude protein concentration; SOC—seed oil concentration; RL—root length; RA—root area; RV—root volume; H—height; D—diameter; RB—root biomass; SB—stem biomass; TB—total biomass; R/S—root–stem biomass ratio; H/D—height–diameter ratio. * Correlation is significant at the 0.05 level.

** Correlation is significant at the 0.01 level.

4. Discussion

The distribution of *X. sorbifolium* over a wide geographical and climatic range is expected to be reflected in the genetic constitution of its populations. The source of the variation in seed traits has been previously documented in *X. sorbifolium* in different provenances [25–27]. However, most of the reports neglected the relationship between the seed and the seedling traits. In the present study, a considerable variation in the seed and seedling traits was observed among the provenances. The variation suggested the combined effects of the genotype and the environment, such as geographical and climatic factors.

Seed morphological traits showed significant correlations among themselves (Table 11), revealing that these traits are independent and genetically controlled. Similar findings were also reported for *Pinus wallichiana* [12], *Acacia catechu* Willd. [28], and *Tectona grandis* L.f. [29]. In addition, this was also supported by the result of the variability analysis. Most of the seed morphological traits were controlled strongly by genotypic factors, while environmental factors played a minor role in shaping these characters (Table 3), which was in accordance with the trend of the H^2 values. The SW, SDW, and 1000SW were significantly negatively correlated with the mean annual rainfall (Table 14), indicating that *X. sorbifolium* is sensitive to waterlogging. Similar results were also found in *Haloxylon ammodendron* [30]. Both the

RB and TB had stronger positive relationships with the mean temperature in January rather than in July. Wulff (1995) also suggested that low temperature played a more important role in increasing the seed mass during seed development and maturation [31,32]. Among the provenances, seeds from OB showed the maximum seed length and width (Table 2). According to the previous reports [33,34], this could be attributed to the extremely harsh climate and the long periods of moisture stress that OB experienced. Thus, its seeds needed to store more nutrition for the seedlings to survive after germination.

Among seed chemical components (SCPC, SSSC, and SOC), the H^2 values ranged from 0.188 to 0.345, indicating that these parameters were prone to be influenced by the environment (Table 3). The SOC was negatively correlated with the seed length, width, and weight (Table 11), which implied small seeds contain more oil than big ones. A similar result was found in *Jatropha curcas* [35]. The positive relationship of the SSSC and the altitude could be attributed to the fact that important environmental conditions and rainfall change rapidly with elevation [36], and sunlight intensity increases with altitude, which is helpful for the accumulation of soluble sugar. It is hypothesized that *X. sorbifolium* growing at higher altitude level contains more soluble sugar in the seeds. However, further research is needed to elucidate the effect of altitude on this species. The SSSC was significantly negatively correlated with the longitude. This may be because rainfall increases with longitude, and more rainfall and less sunlight lead to low seed soluble sugar concentrations. According to the mineral concentrations, N, P, K, Ca, and Mg were the predominant elements in the seeds, and concentrations of Zn, Fe, and Cu were highly and positively correlated (Table 11), which were presumably due in part to the charge neutralization produced by the phytate polyanion. This anion is responsible for the bulk of P content in seeds. The positive relationship between Zn and Fe concentrations may result from the fact that Zn and Fe mobilization, uptake, distribution, and accumulation in the plant are controlled by the same genetic and molecular mechanisms [37].

Seedlings of different provenances, when grown under identical environment conditions, often display different patterns of growth [38–40]. Provenance variation in the seedling diameter at the nursery stage has been observed for *Dalbergia sissoo* Roxb. after 6 months [41] and for *Tectona grandis* after 8 months [29]. In our study, there was no variation in the seedling diameter throughout the first growing season. Though the seedling height varied significantly at the early stage of growth (1 month and 2 months), the difference disappeared after 3 months. These findings suggest that the expression of genetic potential for seedling growth is species-specific. Meanwhile, the logistic growth model is usually S-shaped, and it includes the emergence stage, seedling stage, rapid growth stage, and late growth stage. In the present study, the seedling diameter and height entered the rapid growth stage quickly probably because the seeds were germinated in advance and the seedlings grew in a greenhouse with ideal temperature and moisture conditions.

Identifying the association between the seed traits and seedling quality parameters would allow us to manipulate the breeding process by selecting seeds. Positive correlations of diameter with the SL, SW, and 1000SW depicted that the provenance which yielded big seeds tended to show greater seedling diameter as compared to the provenance with small seeds (Table 13). A similar relationship has been obtained in many species [12,42]. To a certain extent, the larger seeds are known to have greater cotyledons with more mobilizable carbohydrate reserves and nutrients [43]; therefore, large seeds could provide an advantage for early seedling establishment. The SSSC showed significantly positive correlations with the RL and RA, and SB, indicating that soluble sugar could provide a nutrition supply for seedling root and stem growth. The advantages provided by the large seeds were also reflected in the seedling traits. In this study, the seedlings developed from large seeds showed a greater survival ratio because of the more water and nutrients absorbed by the larger root diameter and volume. Therefore, among all the seedling traits, the root diameter is considered to be the most effective factor for predicting the future growth state [19].

Heritability provides an index of the relative roles of heredity and environment in the expression of various traits [44,45]. In our study, the SL, SW, SDW, 1000SW, D, and RB had

relatively strong heritability (Tables 3 and 6). Therefore, these traits could be improved through clonal selection. On the other hand, these traits are still weakly correlated with environmental and geographic factors. Therefore, future research directions should be: first, carry out further molecular studies to verify genotypic and environmental effects; second, test field growth performance of all provenances more fully in subsequent years across a much broader range of environmental conditions; third, select and analyze additional provenances to get more precise correlation with geographic data.

5. Conclusions

The present study indicated that seed- and seedling-related traits varied considerably among the provenances of *X. sorbifolium*. Seeds from the OB provenance had the largest size and the lowest SOC and Mg concentrations. The ML provenance demonstrated the maximum values of oil, soluble sugar, P, K, and Mg concentrations, diameter, root length, area, and volume, and comparatively higher values of seed size parameters and biomass. The H^2 was high in seed and seedling morphological parameters (seed length, width, weight, seedling diameter, and root biomass). These parameters were also weakly correlated with environmental factors. Seed length, width, and weight were positively correlated with each other. Seed length and width showed negative correlation with the SOC, which meant smaller seeds contain more oil. Additionally, the SSSC was negatively correlated with the longitude and positively correlated with the altitude, indicating that seeds collected from a higher altitude may contain more soluble sugar. Among the four provenances, the ML provenance was recommended as an ideal material for the further improvement program according to the assessment of seed and seedling traits.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/f13060959/s1>, Table S1: Variance analysis of seed morphological parameters among four *X. sorbifolium* provenances; Table S2: Variance analysis in concentrations (%) of seed crude protein, soluble sugar and oil among four *X. sorbifolium* provenances; Table S3: Variance analysis of seed mineral contents among four *X. sorbifolium* provenances (mg/kg; dry weight); Table S4: Variance analysis of height–diameter ratio (H/D) and root–stem biomass ratio (R/S); Table S5: Variance analysis of logistic curve fitting of seedling diameter of four provenances of *X. sorbifolium*; Table S6: Variance analysis of logistic curve fitting of seedling height of four provenances of *X. sorbifolium*; Table S7: Variance analysis of seedlings root biomass, stem biomass, and total biomass of four provenances of *X. sorbifolium*; Table S8: Variance analysis of root length, area and volume ratio of different root diameter class (<2, 2–5, >5 mm) to total root of four provenances of *X. sorbifolium* seedlings; Table S9: Correlation coefficients among seedling traits of *X. sorbifolium* individuals; Table S10: The observation numbers of seed and seedling traits of *X. sorbifolium* (5 replicates); Table S11: The observation numbers of seed and seedling traits of *X. sorbifolium* (4 replicates); Table S12: The observation numbers of seed oil concentration of *X. sorbifolium* (9 replicates);

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