# PHENOTYPIC VARIATION OF NATURAL POPULUS PRUINOSA SCHRENK POPULATIONS SHAPED BY GEOGRAPHICAL AND CLIMATIC FACTORS

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> > (Received 4th Sep 2021; accepted 6th Dec 2021)

**Abstract.** *Populus pruinosa* Schrenk (*P. pruinosa*) is an important domestic forest species, only distributed in Xinjiang in China. To better protect the genetic germplasm resources of *P. pruinosa populations*, we study the phenotypic variations of leaf, seed, and fruit traits affected by geographical and climatic factors through investigation of natural *P. pruinosa* populations. Here, twenty-eight traits associated with leaf, seed and fruit characteristics were measured in the seventeen populations. The results of phenotypic variation show that leaf phenotypic traits directly exposed to the air undergo the greater morphological changes in order to adapt to changes in the external environment. The changes of phenotypic traits of different natural *P. pruinosa* among populations (44.45%) was significant more than that within populations (18.14%), suggesting that the former was the main source of the total variation in phenotypic traits. The correlation analysis of phenotypic traits of natural *P. pruinosa* populations and environmental factors show that changes in annual average precipitation and evaporation have the most significant effects on the phenotypic traits. These results indicate that the phenotypic traits of natural *P. pruinosa* populations in the arid area are more sensitive to the damp environment over a long period of evolution.

Keywords: natural forest, quantity trait, morphological change, population variation

**Abbreviations:** CA, capsule area (cm<sup>2</sup>); CI, capsule index; CL, capsule length (cm); CP, capsule perimeter (cm); CW, capsule width (cm); GI, germination index (%); GP, germination potential (%); GR, germination rate (%); LA, leaf area (cm<sup>2);</sup> LBA, leaf base angle; LL, leaf length (cm); LLW, left leaf width (cm); LP, leaf perimeter (cm); LSI, leaf shape index; LTA, leaf tip angle; LW, leaf width (cm); PEL, petiole length (cm); PL, podetium length (cm); SA, seed area (mm<sup>2</sup>); SL, seed length (mm); SP, seed perimeter (mm); SSI, seed shape index; SW, seed width (mm); TSW, thousand seeds weight (g); VA, vein angle; VD, vein density (n); VN, vein number (n); WBD, wide base distance (cm)

## Introduction

Global climate change have seriously affected the distribution and behaviour of most species, leading to a gradual increase in the number of endangered species, and it has also increased people's attention to biodiversity, ecosystem integration, and ecological health. As a result, conservation biology has naturally become a popular topic in academic research (Hoban and Schlarbaum, 2014; Trombulak et al., 2004; Flanagan et al., 2018). The genetic diversity of a species is not only the responded to environmental changes but also an important component of biodiversity (Flower et al., 2018). Plant phenotypic diversity is the result of interactions between genetic and environmental factors, and plays an important role in adaptive evolution (Wagner and Mitchell-Olds, 2018; Ghalambor et al., 2015; Hendry, 2015). Environmental changes can cause a plasticity effect on phenotypic traits of species, which can be produced by genetic differentiation at the population level or by individuals changing their phenotypes or choosing an environment to adapt to the new habitat (Sun et al., 2016; Edelaar et al., 2017; Hallsson and Björklund, 2012). Due to the plasticity of phenotypic traits, phenotypic diversity of species is ubiquitous in organisms (Holloway, 2002). Hence, Species can be genetically marked at the morphological, physiological and molecular levels, all of which give us a further knowledge of the genetic diversity of a particular species. Among them, phenotype determination at the morphological level is the most realistic and direct methods (Sreekanth et al., 2014; Xu et al., 2016). Studies on phenotypic traits could not only reveal phenotypic differences among and within populations, but also reflect the characterization of genetic variation (Brochmann et al., 1992). Up to now, stable plant phenotypic traits have been widely used by many scholars in research of cash crops (Rabara et al., 2014; Khadivi, 2018; Mashilo et al., 2017; Ngwepe et al., 2021), forest trees (Singh et al., 2015; Li et al., 2018), horticultural varieties (Zhang et al., 2017) and invasive species (Wang et al., 2018; Dong et al., 2015; Liu et al., 2014).

Leaf morphology is closely related to plant nutrition, physiology and ecological factors, so leaf phenotypic variation have a high research value (Chechowitz et al., 1990). For example, with the increase of altitudinal gradients, the plant populations show that leaves become smaller and thicker, along with the increase of nutrient content (Bresson et al., 2011). Under drought stress, the morphological and anatomical structure of leaves show the obvious characteristics of drought resistance, improve the photosynthetic capacity and the content of osmotic regulation substances (Zhai, 2020). Secondly, the phenotypic traits of fruit and seed can not only determine the mode and ability of species dispersal, but also affect seed germination and seedling settlement, and then affect the distribution pattern of populations (Li et al., 2013). For example, the variation pattern of seed characteristics on a large scale is usually related to climate and latitude gradient (García et al., 2010; Zhao et al., 2015). The zonal changes of seed morphological characters have become one of the contents of seed geography research (Fang and Yu, 2012). Therefore, it is imperative to measure the phenotypic traits of the *P. pruinosa* leaf, seed and fruit to understand the effects of environmental filtering on plant functional traits and the resulting biogeographic distribution pattern of species (Wright et al., 2007).

*Populus pruinosa* Schrenk and *Populus euphratica* Oliv. belong to the sect. Turanga in Populus of Salicaceae. *P. pruinosa* is only distributed in Xinjiang in China and mainly exists near the Hetian River and Ye Er-Qiang River and along the upstream coast of the Tarim Basin, where it often forms a large-scale mixed forest with *P. euphratica* (Yuan et al., 2020). Because of its excellent resistance to saline and alkali conditions, drought, heat and sand, *P. pruinosa* forests have become the main species used to maintain the balance of the ecosystem and the survival of the desert "green corridor" in the extremely arid area of the Tarim Basin. it played an irreplaceable role in maintaining the regional ecological balance and the safe development of the local society and economy. Although recent researches involved the anatomical structural indexes of heteromorphic leaves (Zhai et al., 2019) and the genetic structure investigated by SSR molecular markers (Zhang et al.,

2012), studies on the genetic structure and environmental adaptability of natural *P. pruinosa* by analyzing the phenotypic variation of leaf, fruit and seed have not been reported. Changes in phenotypic traits were indispensable for describing species variation. Here, the phenotypic traits were measured and analysed in the *P. pruinosa* populations, and the genetic diversity of different natural *P. pruinosa* populations was studied as follows: (1) Analyse the phenotypic traits of different natural populations areas to study the geographical variation characteristics of morphological changes. (2) Explore the source of population variation and evaluate the impact of different environmental factors in phenotypic traits. The aim of our study was to advance our knowledge of the biological characteristics of different *P. pruinosa* populations, promote the collection of genetic germplasm resources and breeding of high-quality germplasm resources of the species.

## Materials and methods

## Study site

When investigating the distribution of *P. pruinosa* in the northwestern China, experimental materials were ultimately collected from 17 natural populations (*Fig. 1*). The date of sampling was collected from May to September in 2020. For each natural population, the tree age was calculated through the Logistic equation (Gu et al., 2013), 15 50-year-old trees displaying good growth that are free from pests are randomly selected as the samples, with the distance between the samples being greater than 100 m. For each tree, 3 replicates of matured broad ovate leaves, 15 replicates of fruit and 10 replicates of seeds are used. The longitude, latitude and altitude values are measured in the field by a GPS device, and other environmental factors were obtained by Wheat A software (http://www.wheata.cn) (*Table A1*). The general geographic distribution map is shown in *Figure 2*.



*Figure 1.* Sampling sites of natural Populus pruinosa populations. (A) natural zpx population, (B) natural clx population, (C) natural ale population, (D) natural scx population



Figure 2. Geographical distribution map of 17 natural Populus pruinosa populations

## Measurement of phenotypic traits

The leaves, fruits of natural P. pruinosa populations were photographed by using a digital camera, and its length, area, perimeter and angle of phenotypic traits were measured by *pruinosa* software (https://imagej.net/software/fiji/downloads). The sampling leaves were collected at the third nodes from the base of current branch. The leaf traits included leaf length/cm (LL), leaf width/cm (LW), wide base distance/cm (WBD), left leaf width/cm (LLW), petiole length/cm (PEL), leaf tip angle (LTA), leaf base angle (LBA), vein angle (VA), leaf area/cm<sup>2</sup> (LA), leaf perimeter/cm (LP), vein numbers/n (VN), leaf shape index (LSI) and vein density/n (VD). The fruit traits included capsule length/cm (CL), capsule width/cm (CW), capsule index (CI), capsule area/cm<sup>2</sup> (CA), capsule perimeter/cm (CP) and podetium length/cm (PL). The seed traits included seed length/mm (SL), seed width/mm (SW) seed area/mm<sup>2</sup> (SA), seed perimeter/mm (SP), seed shape index (SSI), germination rate (GR), germination potential (GP) and the germination index (GI), thousand seeds weight (TSW). The WBD is the distance from the maximum leaf width to the leaf base. The LLW refers to the distance from the left edge of the widest leaf to the centre. The LTA refers to the angle between the blade edge and the main vein at the blade tip. The VN refers to the number of primary veins on the left and right sides.

The computational formulas are as follows:

Vein density = 
$$\frac{\text{vein numbers}}{2 \times \text{ength}} \times 100\%$$
 (Eq.1)

Leaf shape index = 
$$\frac{\text{leaf length}}{\text{leaf width}} \times 100\%$$
 (Eq.2)

The seed traits were measured by stereoscopic microscopy. The thousand seeds that was uniformly and randomly selected from 15 trees for each population was measured 4

times by an electronic balance with an accuracy of 0.0001 g. Finally, the seeds from 5 randomly selected trees for each population were subjected to germination experiments with 3 replicates per tree and 100 seeds per replicate.

A solution of 1% NaClO was used to saturate seeds for an hour, after which the seeds were immediately washed with distilled water and blotted with absorbent paper. We placed the seeds evenly into Petri dishes lined with 2 layers of sterilized filter paper, added 8 mL distilled water to each Petri dish, weighed each dish, and then moved the Petri dishes into a light incubator for culturing. The experimental conditions were as follows: 08:00-24:00, light, temperature 30 °C; 0:00-08:00, dark, temperature 25 °C. Water was added to the dish at 14:00 every day to maintain moisture. The germination of seeds was recorded every day. all the germination experiments were ended at 7 days. The relevant formulas of seed germination are as follows:

Seed germination rate (GR) = 
$$\frac{\text{numbers of seed germination}}{\text{numbers of experimental seeds}} \times 100\%$$
 (Eq.3)  
Germination potential (Gp) =  $\frac{\text{max numbers of seed germination}}{\text{number of experimental seeds}} \times 100\%$  (Eq.4)

Germination index (GI) =  $\sum \frac{\text{Numbers of seed germination per day (Gt)}}{\text{seed germination days (Dt)}} \times 100\%$  (Eq.5)

## Statistical analysis

The observed and recorded data were sorted and summarized in the Excel 2016 software. The coefficient of variation (CV) was calculated according to the average (X) and standard deviation (SD) of phenotypic traits of *P. pruinosa*. The significance test using Duncan multiple comparison method, Nested design of variance and Person related analyses of 28 phenotypic traits in the seventeen populations were performed with SPSS 19.0 software (IBM Corp., Armonk, NY, USA). the linear model of nested design of variance:  $Y_{ijk} = u + S_i + T_{(i)j} + e_{(ij)k}$ , where  $Y_{ijk}$  was the K<sup>th</sup> observation of the J<sup>th</sup> individual of the i<sup>th</sup> population;  $\mu$  was the overall value; S<sub>i</sub> was the effected value in the i<sup>th</sup> population;  $T_{(i)j}$  was the effected value of the J<sup>th</sup> individual in the i<sup>th</sup> population; and  $e_{(ij)k}$  was the random error. The formula of differentiation coefficients of phenotypic traits (V<sub>st</sub>) was as follows (Ge et al., 1988).

Seed germination rate (GR) =  $\frac{\text{variance among populations } (\delta_{2t/s})}{\text{variance among and within population } (\delta_{2t/s+\delta_{2s}})} \times 100\%$  (Eq.5)

### Results

### Phenotypic variation

The extreme value ratio of phenotypic traits could reflect the evolutionary and adaptive potential of traits in response to environmental changes (Benstock and Cegla, 2017). In terms of evolutionary potential (*Table 1*), these phenotypic traits ranged from high to low as follows: CA > CP > SA > CW > CI > LA > PL > CL > VD > PEL > TSW > WBD > VA > SL > LL > LTA > LP > SP > LBA > LW > LSI > LLW > SSI > SW > VN. The results showed that the capsule area, capsule perimeter and seed area had a relatively high evolutionary potential, but the seed shape index, seed width and vein numbers were low. The standard deviation can reflects the degree of dispersion of

a dataset. The orders of standard deviations of different phenotypic traits were comprehensively analysed as follows: VD > LTA > LBA > VA > LA > LP > CP > VN > PEL > LW > LL > CI > LLW > CA > WBD > SP > SSI > CL > CW > SL > PL > SA > LSI > SW > TSW. The average levels of leaf traits (SD = 4.499) was more larger than fruit trait (SD = 0.379) and seed trait (SD = 0.119). The results show that the leaf phenotypic traits have undergone greater morphological changes than fruit and seed changes.

Traits	Min	Max	Median	Extreme difference value	Average	Standard deviation	Coefficient of variation (%)
LL	3.085	4.938	4.234	1.852	4.218	0.536	12.709
LW	4.956	7.116	5.941	2.16	5.834	0.673	11.545
WBD	1.199	2.072	1.598	0.873	1.643	0.258	15.726
LLW	2.504	3.485	2.945	0.981	2.91	0.325	11.158
PEL	2.773	5.064	3.098	2.291	3.35	0.687	20.499
LTA	90.686	142.946	115.617	52.26	117.606	14.235	12.104
LBA	84.479	129.583	111.566	45.104	111.483	10.683	9.583
VA	23.31	40.115	34.965	16.805	34.47	4.303	12.483
LA	11.668	27.354	20.372	15.686	19.668	4.269	21.707
LP	13.423	20.784	17.827	7.36	17.177	2.202	12.82
VN	8.201	11	9.25	2.799	9.45	0.766	8.106
LSI	0.59	0.833	0.734	0.243	0.731	0.068	9.256
VD	91.215	166.754	118.201	75.538	117.494	19.479	16.579
Leaf average	26.007	43.234	34.334	17.227	34.31	4.499	13.406
CA	0.141	1.269	0.347	1.128	0.442	0.293	66.388
CP	1.602	5.66	3.619	4.058	3.638	0.988	27.154
PL	0.317	0.736	0.358	0.418	0.416	0.119	28.63
CL	0.583	1.348	0.942	0.765	0.964	0.186	19.289
CW	0.27	0.845	0.372	0.575	0.423	0.157	37.153
CI	1.313	3.232	2.598	1.919	2.432	0.533	21.912
Fruit average	0.704	2.182	1.373	1.477	1.386	0.379	33.421
SL	0.658	1.115	0.944	0.457	0.94	0.127	13.529
SW	0.313	0.424	0.384	0.111	0.377	0.027	7.075
SA	0.157	0.549	0.279	0.391	0.29	0.084	28.811
SP	1.708	2.642	2.337	0.934	2.288	0.255	11.137
SSI	2.12	2.897	2.495	0.778	2.492	0.21	8.424
TSW	0.045	0.082	0.057	0.037	0.058	0.01	17.606
Seed average	0.834	1.285	1.083	0.451	1.074	0.119	14.43
GR	0.075	1.049	0.258	0.973	0.343	0.259	75.503
GP	0.005	0.381	0.108	0.376	0.144	0.114	79.242
GI	1.076	14.982	3.686	13.906	4.898	3.698	75.505
Total average	9.182	15.567	12.263	6.385	12.257	1.666	24.701

Table 1. Phenotypic variation of natural Populus pruinosa populations

## Differentiation among populations

The analysis of variance (ANOVA) results for the 24 phenotypic traits of the leaves, fruits and seeds among and within *P. pruinosa* populations was showed (*Table 2*). The Left leaf width was highly significantly different among populations and not significantly different within populations. The capsule area, podetium length and

capsule width showed no significant differences within and among populations. The capsule length was no significant differences within populations but highly significantly different among populations. The other traits were all highly significantly different within and among populations, which showed the phenotypic variation existed in the morphological characters.

Traits		Mean squared		F						
	Among population	Within population	Random errors	Among population	Within population					
LL	13.37	1.73	0.37	7.74**	4.73**					
LW	20.81	2.06	0.32	10.09**	6.49**					
WBD	2.94	0.46	0.19	6.33**	2.46**					
LLW	251.45	219.04	3.79	1.15	57.74**					
PEL	20.07	1.56	0.36	12.89**	4.30**					
LTA	54037.36	1554.85	905.05	34.75**	1.72**					
LBA	10458.7	973.28	304.58	10.75**	3.20**					
VA	1171.58	87.56	37.81	13.38**	2.32**					
LA	903.92	94.5	14.67	9.57**	6.44**					
LP	230.42	18.3	2.79	12.59**	6.56**					
VN	41.77	5.87	2.79	7.11**	2.10**					
LSI	0.22	0.04	0.01	5.94**	4.07**					
VD	13850.51	1825.34	725.93	7.59**	2.51**					
CA	1.79	1.56	4.84	1.15	0.32					
CP	97.82	6.55	2.75	14.93**	2.38**					
PL	0.52	1.55	4.82	0.33	0.32					
CL	4.74	1.91	4.29	2.48**	0.45					
CW	0.69	1.35	4.81	0.51	0.28					
CI	25.39	3.16	3.45	8.05**	0.92					
SL	1.27	0.15	0.01	8.28**	19.38**					
SW	0.06	0.01	0.003	4.29**	4.97**					
SA	0.32	0.04	0.003	7.44**	16.04**					
SP	5.98	0.75	0.04	7.96**	18.85**					
SSI	3.77	0.52	0.13	7.31**	4.05**					

Table 2. Variance analysis of phenotypic traits of natural populus pruinosa populations

\*P < 0.05; \*\*P < 0.01, the same applies below

The variance component accounted for 18.14% and 44.45% among the intrapopulation and interpopulation of total variation respectively, which indicated that there existed some extent of variation. The differentiation coefficients of the phenotypic traits ranged from 2.92% to 98.71%, with an average of 71.02%. At the population level, the capsule index of different populations existed in the largest differentiated coefficients (98.71%), followed by capsule perimeter (96%) and leaf tip angle (94.17%). The trait of the least differentiated coefficients was left leaf width (2.92%) (*Table 3*). In In general, the variance of most phenotypic traits among population was larger than that within population, so the former was the main source of phenotypic variation.

Traits	Va	riance compone	nt	Percentage of	of variance con	Differentiation			
	Among	Within	Random	Among	Within	Random	coefficients of phenotypic		
	population	population	errors	population	population	errors	traits (Vst) (%)		
LL	0.78	0.45	0.37	48.65	28.46	22.88	63.09		
LW	1.25	0.58	0.32	58.16	27.05	14.79	68.25		
WBD	0.16	0.09	0.19	37.01	20.59	42.39	64.25		
LLW	2.16	71.75	3.79	2.78	92.34	4.88	2.92		
PEL	1.23	0.40	0.36	61.89	19.97	18.14	75.60		
LTA	3498.83	216.60	905.05	75.72	4.69	19.59	94.17		
LBA	632.36	222.90	304.58	54.52	19.22	26.26	73.94		
VA	72.27	16.58	37.81	57.05	13.09	29.85	81.34		
LA	53.96	26.61	14.67	56.66	27.94	15.41	66.97		
LP	14.14	5.17	2.79	63.99	23.39	12.62	73.23		
VN	2.39	1.03	2.79	38.53	16.53	44.94	69.98		
LSI	0.01	0.01	0.01	39.88	30.45	29.68	56.71		
VD	801.68	366.47	725.93	42.33	19.35	38.33	68.63		
CA	0.02	0.22	4.84	0.30	4.31	95.39	6.57		
CP	6.08	0.25	2.75	66.93	2.79	30.28	96.00		
PL	0.07	0.22	4.82	1.35	4.27	94.38	23.95		
CL	0.19	0.16	4.29	4.07	3.42	92.51	54.33		
CW	0.04	0.23	4.81	0.86	4.54	94.60	15.93		
CI	1.48	0.02	3.45	29.96	0.39	69.65	98.71		
SL	0.07	0.01	0.01	76.84	15.02	8.15	83.65		
SW	0.002	0.001	0.002	43.73	15.85	40.41	73.39		
SA	0.02	0.004	0.003	73.24	15.98	10.79	82.09		
SP	0.35	0.07	0.04	75.85	15.48	8.67	83.05		
SSI	0.22	0.04	0.13	56.61	10.13	33.26	84.82		
Mean	_			44.45	18.14	37.41	71.02		

*Table 3.* Variance components and differentiation coefficients of phenotypic traits of natural populus pruinosa populations

## Variation in phenotypic traits in different populations

The 24 phenotypic traits of *P. pruinosa* leaf, fruit and seed traits among populations can be showed (*Table 4*). The gmx population had the largest leaf base angle, while wide base distance, vein numbers and leaf shape index were the smallest. The lpx population had the largest vein numbers and seed width. The mfx population had the smallest leaf tip angle, leaf base angle and vein angle. The psx population had the largest leaf tip angle, seed area and seed shape index, while its capsule area, capsule perimeter, podetium length, capsule length and capsule width were the smallest. The ytx population had the largest wide base distance, leaf shape index, seed length and seed perimeter. The vein density of the scx population was the smallest. The slx population had the largest leaf length, leaf width, left leaf width, petiole length, leaf area, leaf perimeter and capsule index. The vein angle of the ale population was the largest. The capsule length of the nlkx population was the largest. The vein density, capsule area, capsule perimeter, podetium length, and capsule width of the cbce population were the largest, while its leaf length, leaf area, capsule index, seed length, seed width, seed area, seed perimeter and seed shape index were the smallest. The leaf width, left leaf width, petiole length and leaf perimeter of the kkdl population were the smallest. The phenotypic traits of the ylx, clx, myx, bcx, mgtx and zpx populations were all moderate, revealing no special phenotypic characteristics. The phenotypic traits of P. pruinosa leaf, fruit and seed had the significant differences among populations, but the overall performance was continuous variation.

Populations	LL	LW	WBD	LLW	PEL	LTA	LBA	VA	LA	LP	VN	LSI
qmx	4.09±0.28cde	6.94±0.41a	1.20±0.24f	3.48±0.23a	4.07±0.34b	101.61±21.42fg	129.58±15.38a	35.90±2.38abcd	23.52±2.36ab	20.73±1.30a	8.20±0.91g	0.59±0.04f
ylx	3.76±0.25ef	5.53±0.42cdef	1.34±0.14ef	2.76±0.24cdef	3.14±0.31cde	113.68±12.30def	108.85±11.62de	34.47±3.46cd	17.00±2.23cde	16.06±1.11de	8.60±0.90fg	0.68±0.06de
clx	$4.42 \pm 0.48$ abcd	6.26±0.90bc	1.57±0.19cde	3.14±0.47abc	3.68±0.40bc	121.59±17.82bcde	101.67±16.28e	29.99±4.70e	21.80±5.88b	17.94±2.45bcd	10.33±1.59abc	0.71±0.06cde
lpx	4.66±1.05abc	6.14±0.49bc	2.04±0.43a	3.07±0.21bc	3.06±0.49cde	138.26±17.07ab	116.84±14.28bcd	34.97±4.75cd	21.89±3.97b	18.08±1.63bcd	11.00±1.31a	0.76±0.14abcd
mfx	4.23±0.54bcde	5.24±0.77efg	1.95±0.26ab	2.61±0.41efg	3.49±0.66bcd	90.69±14.63g	84.48±11.79f	23.31±3.29f	16.40±4.72cdef	15.69±2.24ef	9.18±1.29defg	0.82±0.09ab
psx	4.11±0.63cde	$5.72 \pm 0.83$ bcdef	1.63±0.46bcde	$2.78\pm0.40$ cdef	3.00±0.34de	142.95±34.40a	116.62±22.75bcd	37.20±5.12abcd	18.88±3.98bcd	16.72±2.05cde	9.95±1.68bcd	0.73±0.14bcd
ytx	4.60±0.77abc	5.62±0.78cdef	2.07±0.44a	2.78±0.42cdef	2.79±0.81ef	133.22±33.01abc	106.95±16.38de	35.48±6.85bcd	19.59±4.07bcd	16.85±1.67cde	8.80±0.99efg	0.83±0.18a
myx	4.59±0.87abc	6.01±1.24bcd	1.88±0.45abc	$2.94{\pm}0.65$ bcde	3.14±1.06cde	117.82±14.02cdef	111.57±12.48cde	34.72±4.31cd	22.41±8.43ab	18.05±4.00bcd	10.62±1.88ab	0.78±0.10abcd
bcx	4.17±0.76cde	5.94±0.98bcde	1.55±0.35cde	$2.98{\pm}0.46$ bcde	3.58±0.71bcd	115.62±15.26cdef	123.78±15.57ab	30.09±6.25e	20.37±6.49bc	17.83±2.94bcd	9.49±1.32cdef	0.71±0.13cde
mgtx	4.41±0.79abcd	6.46±1.04ab	1.54±0.50cde	3.24±0.56ab	3.93±1.13b	113.22±16.04def	122.13±12.17abc	33.14±4.27de	23.32±7.87ab	19.28±3.11ab	9.04±0.95defg	0.69±0.08cde
SCX	4.87±0.93a	5.95±0.88bcde	1.75±0.553abcd	3.00±0.42bcd	4.04±0.74b	129.44±18.45abcd	111.38±9.91cde	39.13±7.35ab	22.86±7.14ab	18.27±2.67bc	8.56±0.59fg	0.82±0.15ab
slx	4.94±0.67a	7.12±0.63a	1.83±0.41abc	3.48±0.32a	5.06±1.06a	108.69±21.68efg	121.49±11.33abc	38.12±3.90abc	27.35±4.88a	20.78±1.90a	9.07±0.92defg	0.70±0.08cde
zpx	3.71±0.84efg	5.42±0.93def	1.55±0.23cde	2.69±0.48defg	2.87±0.58ef	103.56±10.40fg	103.86±13.30e	30.61±4.45e	15.61±5.11def	15.24±2.53efg	9.71±1.26bcdef	0.69±0.11cde
ale	4.83±0.71ab	6.17±0.29bc	1.60±0.27cde	3.10±0.16bc	2.99±0.31de	132.32±22.57abcd	117.44±12.40bcd	40.12±4.69a	23.22±3.15ab	18.71±1.47abc	9.86±0.69bcde	0.78±0.10abc
nlkx	3.94±0.51de	5.16±0.40fg	1.76±0.39abcd	2.60±0.18efg	2.77±0.47ef	122.95±33.56bcde	102.99±8.60e	40.02±4.87a	15.98±2.63cdef	15.14±1.17efg	9.05±0.99defg	0.77±0.10abcd
cbce	3.16±0.45g	5.02±0.62fg	1.43±0.25def	2.51±0.33fg	2.80±0.56ef	110.36±19.33ef	103.59±10.44e	33.23±4.88de	12.18±3.02f	13.65±1.77fg	9.40±1.02cdef	0.63±0.04ef
kkdl	3.29±0.61fg	4.55±1.11g	1.32±0.31ef	2.32±0.57g	2.30±0.67f	106.04±20.06efg	111.87±10.75cde	36.26±3.20abcd	12.48±4.76ef	13.22±2.98g	9.25±1.09defg	0.75±0.13abcd

Table 4. Variation of phenotypic traits in different populations

### Table 4. Continued

Populations	VD	CA	СР	PL.	CL	CW	CI	SL	SW	SA	SP	SSI
amx	100 92±9 05efg	0.47±0.15cde	4 16±0 77b	0.43±0.05bcde	1 23±0 22ab	0.44±0.07cde	2.81±0.28bc	1 05±0 04ab	$0.39\pm0.02$ bc	$0.31\pm0.02$ hc	2.49±0.10ab	2.70±0.12bc
vlx	116.08±17.11cdef	$0.52\pm0.12$ cd	4.09±0.65b	$0.48\pm0.10$ bc	$0.99\pm0.18$ cdef	$0.48\pm0.09$ cd	2.09±0.22f	0.88±0.05def	$0.37\pm0.03c$	$0.26\pm0.03$ bc	$2.20\pm0.13$ de	2.38±0.14ef
clx	118.77±21.82bcde	0.31±0.09efg	3.28±0.48def	0.35±0.07de	0.99±0.19cdef	0.35±0.05efgh	2.85±0.34bc	0.91±0.11cde	0.37±0.04c	0.26±0.06bc	2.23±0.24cde	2.50±0.21de
lpx	123.25±26.91bcd	0.26±0.13fgh	3.13±0.61ef	0.33±0.06de	0.85±0.15efg	0.29±0.05gh	2.98±0.22b	1.08±0.11a	0.42±0.04a	0.35±0.06b	2.58±0.23a	2.56±0.19cd
mfx	109.84±13.71cdefg	0.24±0.04gh	3.03±0.28f	0.33±0.03de	0.80±0.11fg	0.31±0.04fgh	2.62±0.22cd	0.92±0.10cde	0.37±0.03c	0.27±0.05bc	2.23±0.21cde	2.48±0.18de
psx	125.86±33.28bc	0.14±0.04h	1.60±0.20g	0.32±0.10e	0.58±0.09h	0.27±0.04h	2.22±0.27ef	1.10±0.18a	0.38±0.05c	0.55±0.90a	2.60±0.38a	2.90±0.31a
ytx	98.13±11.92fg	0.25±0.07gh	2.21±0.33g	0.32±0.12e	0.85±0.14fg	0.34±0.07efgh	2.60±0.41cd	1.11±0.11a	0.39±0.03bc	0.34±0.05b	2.64±0.24a	2.84±0.22ab
myx	118.91±21.57bcde	0.35±0.14efg	3.72±0.73bcdef	0.36±0.05de	1.00±0.26cdef	0.35±0.06efgh	2.83±0.37bc	0.96±0.09cd	0.39±0.03bc	0.28±0.07bc	2.34±0.19bcde	2.48±0.23de
bcx	118.20±26.04bcde	0.32±0.05efg	3.31±0.27def	0.34±0.10de	0.87±0.07efg	0.37±0.04efgh	2.36±0.26def	0.99±0.11bc	0.39±0.03bc	0.30±0.05bc	2.40±0.22bc	2.58±0.24cd
mgtx	106.72±24.39cdefg	0.36±0.08efg	3.62±0.52bcdef	0.38±0.11cde	0.94±0.18defg	0.39±0.06def	2.41±0.39de	0.98±0.09bc	0.38±0.03bc	0.30±0.05bc	2.36±0.20bcd	2.56±0.22cd
scx	91.22±18.45g	0.30±0.07efgh	3.35±0.50cdef	0.34±0.06de	0.95±0.18defg	0.33±0.03fgh	2.85±0.44bc	1.04±0.05ab	0.41±0.01ab	0.33±0.03bc	2.47±0.11ab	2.55±0.11cde
slx	93.35±12.37g	0.31±0.05efg	3.82±0.50bcde	0.45±0.11bcd	1.06±0.09bcde	0.33±0.03fgh	3.23±0.21a	0.94±0.07cde	0.37±0.03c	0.27±0.04bc	2.27±0.15cde	2.59±0.16cd
zpx	136.11±28.03ab	0.35±0.11efg	3.34±0.56def	0.34±0.05de	0.89±0.12efg	0.39±0.07defg	2.24±0.11ef	0.87±0.16ef	0.38±0.05bc	0.27±0.08bc	2.16±0.36ef	2.26±0.22fg
ale	105.17±19.67defg	0.43±0.07def	3.96±0.38bcd	0.40±0.08cde	1.17±0.12abc	0.43±0.04cde	2.71±0.09bc	0.94±0.17cde	0.39±0.03bc	0.30±0.08bc	2.34±0.37bcde	2.41±0.31def
nlkx	120.30±23.43bcde	1.05±0.41b	5.51±1.45a	0.64±0.31a	1.35±0.42a	0.75±0.22b	1.78±0.14g	0.72±0.16g	0.33±0.06d	0.19±0.07bc	1.85±0.37gh	2.21±0.37g
yl-cbce	152.81±21.34a	1.27±0.68a	5.66±2.25a	0.74±0.40a	1.11±0.57bcd	0.85±0.42a	1.31±0.24h	0.66±0.08g	0.31±0.04d	0.16±0.04c	1.71±0.20h	2.12±0.23g
yl-kkdl	147.92±31.60a	0.59±0.25c	4.06±1.30bc	0.52±0.19b	0.76±0.33gh	0.52±0.21c	1.46±0.29h	0.81±0.09f	0.36±0.03c	0.21±0.05bc	2.02±0.22fg	2.26±0.34fg

Different lowercase letters in the same column indicated the significant differences (p<0.05)

### Correlations between phenotypic traits

As it was showed (*Table 5*), there were significant correlations among 28 phenotypic traits related to leaf, fruit and seed. For example, in the significant correlation associated with leaf traits, leaf length among 28 phenotypic traits (n = 6) had more positive correlation numbers than other traits, followed by leaf width (n = 5), left leaf width (n = 5), leaf base angle (n = 3). There were few negative correlations with leaf traits. The larger leaf length, leaf width and left leaf width had great influence on leaf morphology. In the significant correlation associated with fruit traits, capsule area (n = 4) had more positive correlation numbers than other traits, followed by capsule perimeter (n = 3), vein density (n = 3). The leaf length (n = 4) had more negative correlation numbers than other trait, followed by leaf area, leaf perimeter. The fruit correlation traits was more greatly affected by leaf length, capsule area, capsule perimeter and vein density. In the significant correlation associated with seed traits, seed length (n = 5) had more positive correlation numbers than other traits, followed by leaf length, leaf area, leaf perimeter, capsule index and seed width. The capsule area (n = 5), capsule perimeter (n = 5), podetium length (n = 5) and capsule width (n = 5)had more negative correlation numbers than other trait, followed by vein density. The results indicated seed morphological changes was more greatly affected by leaf traits, followed by fruit traits and seed traits.

### Correlations of the phenotypic traits with environmental factors

The 28 phenotypic traits of *P. pruinosa* were not significantly correlated with longitude, annual average temperature, annual max temperature, soli temperature and soil moisture, which indicated that these phenotypic traits were relatively stable and not easily changed by these influences. The latitude was significantly positively correlated with vein density, capsule area, capsule perimeter, podetium length and capsule width. The altitude was significantly positively correlated with leaf length, leaf width, left leaf width, leaf area, leaf perimeter, capsule index, seed length, seed width, seed perimeter and seed shape index. The sunlight intensity was significantly positively correlated with leaf length, leaf width, left leaf width, leaf area, leaf perimeter, leaf shape index, seed length, seed width, seed perimeter and seed shape index. The relative humidity was significantly positively correlated with capsule area, podetium length and capsule width and so on (Table 6). In terms of the number of significant correlations with the phenotypic traits of *P. pruinosa*, the environmental factors were ranked as follows: count = 16 (annual average precipitation, evaporation) > count = 15 (altitude, latitude, sunlight intensity) > count = 9 (relative humidity) > count = 4 (annual min temperature) > count = 3 (topsoil organic carbon > count = 1(topsoil texture classification) > count = 0 (longitude, annual average temperature, annual max temperature, soil temperature, soil moisture).

### Discussion

Variation in plant phenotypic traits result from genetic variation in "composition" and gene expression influenced by environmental factors (Burns and Strauss, 2012). The richer the phenotypic variation, the better the adaptability of plants to different environments (Verbeeck et al., 2014; Saenger and West, 2018). It has also been showed that phenotypic plasticity could respond to environmental changes, indicating that the

same genotype expressed different phenotypes in different environments, or occurred through genetic variation in average phenotypes, but the evolutionary potential is not significant (Brunet and Larson-Rabin, 2012). Studies of Styrax tonkinensis populations show that the phenotypic variation within populations (69.60%) is greater than that among populations by using the molecular markers (Li et al., 2012), but the phenotypic variation among populations (59.08%) is greater than that within populations by using phenotypic traits (Liu et al., 2011). Similar to the results of P. pruinosa populations, the variation within population (88%) is the main source of phenotypic variation by using the molecular markers (Zhang et al., 2012). This research of P. pruinosa populations shows that the variation among population (71.02%) is greater than that within populations. Accordingly, the variation among population is the main source of the population variation by using phenotypic traits (Table 3). The possible reasons are as follows. Although the P. pruinosa has the biological characteristics of wind pollination, in fact, the natural populations are distributed in the extreme desert region and the effective distance of seed dispersal may be less than the actual distance, which increases the difference between different population. In addition, Xinjiang is located in arid and semi-arid regions. There are large conflicts between the supply and demand of water resources in different distribution areas, and the frequent climate changes (Zhang et al., 2020), which has caused obvious environmental heterogeneity among different populations. The uplift of the Tianshan Mountains directly blocks the gene flow between the north and south of Xinjiang (Zeng et al., 2018; Jia et al., 2020), and blocks the transport of water vapor from north to south of Xinjiang. The huge geographical and environmental differences are an important reason for the differences of natural P. pruinosa populations. Finally, P. euphratica has the multiple pedigrees and forms a parallel group with P. pruinosa. There is also a strong gene introgression between P. pruinosa and P. euphratica populations (Ma et al., 2018), which increases the genetic diversity of P. pruinosa population and promotes the phenotypic diversity of different populations. Therefore, there are significant differences in the phenotypic traits of different natural P. pruinosa populations (Table 4).

Plant trait correlation can indirectly reflect the phenotype of another trait through the phenotype of one trait, improve the selection efficiency and accelerate the breeding process (Rui et al., 2018). For example, leaves are vegetative organs that provide energy to plants. Changes in leaf shape result from responses to the ecological environment and evolutionary history (Nicotra et al., 2011). Leaf shape is correlated with other phenotypic traits. For example, Mclellan finds significant correlations between leaf shape and the presence, number and size of trichomes in plants (Mclellan, 2005). As is showed (*Table 5*), the correlations between most phenotypic traits of natural *P. pruinosa* populations have a significant level, indicating that these phenotypic traits show a strong linkage effects to adapt the environmental changes.

An important content of plant ecology research is to determine and quantify the dominant dimensions of the ecological variables of each species and try to explain its mechanism (Wright et al., 2007). By analyzing the phenotypic variation based at the population level for specific plant groups on a larger scale, studying the relationship between phenotypic traits and geography-climate factors could often reveal the pattern of plant variation and its ecological response strategies more accurately (Yang et al., 2016; Meng et al., 2017). For example, different environmental factors may affect the distribution range of plants in response to climate change (Mccauley et al., 2014; Galván-Hernández et al., 2015).

Table 5. Correlations between phenotypic traits

Traits	LL	LW	WBD	LLW	PEL	LTA	LBA	VA	LA	LP	VN	LSI	VD	CA	СР	PL	CL	CW	CI	SL	SW	SA	SP	SSI	TSW	GR	GP
LL																											
LW	0.71**																										
WBD	0.61**	0.06																									
LLW	0.70**	0.99**	0.02																								
PEL	0.57*	0.82**	0.04	0.82**																							
LTA	0.42	0.11	0.37	0.09**	-0.22																						
LBA	0.26	0.63**	-0.33	0.64**	0.37	0.26																					
VA	0.23	0.184	-0.06	0.19	-0.01	0.57*	0.52*																				
LA	0.88**	0.94**	0.26	0.94**	0.77**	0.26	0.58*	0.27																			
LP	0.79**	0.98**	0.12	0.98**	0.80**	0.15	0.64**	0.21	0.97**																		
VN	0.17	-0.01	0.37	-0.04	-0.28	0.39	-0.09	-0.14	0.06	-0.05																	
LSI	0.48	-0.28	0.75**	-0.29	-0.24	0.41	-0.42	0.08	0.03	-0.13	0.19																
VD	-0.83**	-0.70**	-0.35	-0.70**	-0.7**	-0.145	-0.27	-0.21	-0.80**	-0.79**	0.38	-0.30															
CA	-0.63**	-0.46	-0.36	-0.43	-0.35	-0.21	-0.19	0.21	-0.57*	-0.52*	-0.20	-0.35	0.52*														
СР	-0.43	-0.19	-0.40	-0.15	-0.08	-0.38	-0.05	0.21	-0.30	-0.25	-0.23	-0.40	0.31	0.89**													
PL	-0.63**	-0.40	-0.40	-0.38	-0.25	-0.25	-0.12	0.27	-0.52*	-0.48	-0.27	-0.40	0.48*	0.97**	0.88**												
CL	0.05	0.27	-0.21	0.31	0.20	-0.17	0.12	0.36	0.18	0.24	-0.27	-0.30	-0.201	0.60*	0.79**	0.55*											
CW	-0.67**	-0.48*	-0.41	-0.45	-0.39	-0.22	-0.18	0.20	-0.60*	-0.55*	-0.25	-0.36	0.53*	0.993**	0.88**	0.95**	0.58*										
CI	0.90**	0.81**	0.48	0.80**	0.68**	0.18	0.26	0.002	0.89**	0.85**	0.18	0.23	-0.80**	-0.73**	-0.47	-0.71**	0.02	-0.77**									
SL	0.67**	0.55*	0.36	0.53*	0.32	0.45	0.43	0.06	0.64**	0.63**	0.07	0.28	-0.61**	-0.86**	-0.83**	-0.85**	-0.45	-0.86**	0.70**								
SW	0.63**	0.45	0.31	0.45	0.21	0.35	0.38	0.02	0.57*	0.52*	0.21	0.33	-0.49*	-0.83**	-0.68**	-0.854**	-0.39	-0.83**	0.69**	0.87**							
SA	0.41	0.31	0.23	0.26	0.10	0.60*	0.33	0.15	0.37	0.355	0.19	0.21	-0.30	-0.70**	-0.84**	-0.68**	-0.59*	-0.69**	0.39	0.82**	0.60*						
SP	0.68**	0.55*	0.36	0.53*	0.29	0.47	0.44	0.08	0.64**	0.63**	0.09	0.288	-0.61**	-0.85**	-0.80**	-0.85**	-0.43	-0.85**	0.71**	0.99**	0.88**	0.82**					
SSI	0.57*	0.54*	0.31	0.50*	0.37	0.42	0.40	0.07	0.59*	0.60*	-0.05	0.18	-0.60*	-0.74**	-0.81**	-0.70**	-0.43	-0.74**	0.59*	0.92**	0.60*	0.84**	0.91**				
TSW	0.26	0.30	-0.04	0.33	0.10	0.12	0.40	0.24	0.31	0.37	-0.14	0.01	-0.33	-0.26	-0.12	-0.33	0.14	-0.27	0.40	0.49*	0.64**	0.20	0.50*	0.27			
GR	0.11	-0.16	0.26	-0.149	-0.17	0.18	-0.32	0.03	-0.09	-0.11	0.13	0.33	-0.06	0.16	0.10	0.05	0.28	0.17	-0.06	-0.19	-0.23	0.03	-0.17	-0.15	-0.25		
GP	0.20	0.07	0.26	0.03	-0.09	0.03	-0.01	0.03	0.17	0.11	0.43	0.2	0.02	-0.09	0.02	-0.13	0.07	-0.12	0.20	0.04	0.10	-0.01	0.05*	-0.01	0.11	-0.10	
GI	0.18	-0.07	0.30	-0.07	-0.06	0.16	-0.29	0.07	-0.01	-0.04	0.11	0.31	-0.12	0.14	0.11	0.06	0.31	0.15	0.01	-0.20	-0.25	0.02	-0.18	-0.13	-0.29	0.98**	-0.11

\* p < 0.05, \*\* p < 0.01, the same below

Traits	Longitude	Latitude	Altitude	AAVT	AMAT	AMIT	AAP	SI	RH	EV	ST	SM	TTC	TOC
LL	-0.43	-0.67**	0.74**	0.05	-0.07	0.38	-0.58*	0.62**	-0.43	-0.61**	-0.06	0.17	0.36	-0.32
LW	-0.12	-0.58*	0.62**	0.19	0.14	0.42	-0.65**	0.56*	-0.51*	-0.64**	0.12	0.30	0.14	-0.12
WBD	-0.33	-0.38	0.37	0.09	-0.01	0.24	-0.22	0.35	-0.28	-0.24	0.06	-0.23	0.20	-0.25
LLW	-0.11	-0.55*	0.61**	0.15	0.12	0.37	-0.62*	0.53*	-0.47	-0.61**	0.08	0.32	0.16	-0.13
PEL	-0.15	-0.45	0.45	0.03	0.01	0.20	-0.46	0.47	-0.28	-0.47	-0.10	0.17	0.01	0.13
LTA	-0.31	0.01	0.11	0.01	-0.10	0.08	-0.01	-0.13	0.06	-0.06	0.12	0.23	0.34	-0.33
LBA	-0.07	-0.17	0.08	0.18	0.14	0.28	-0.23	0.15	-0.18	-0.21	0.16	0.44	-0.09	0.10
VA	-0.29	0.29	-0.27	-0.25	-0.27	-0.18	0.35	-0.34	0.41	0.31	-0.32	0.30	-0.21	0.23
LA	-0.28	-0.63**	0.68**	0.15	0.06	0.44	-0.64**	0.60*	-0.47	-0.64**	0.03	0.29	0.22	-0.18
LP	-0.14	-0.64**	0.66**	0.17	0.12	0.42	-0.67**	0.63**	-0.52*	-0.66**	0.08	0.31	0.22	-0.20
VN	-0.15	-0.10	0.22	0.22	0.12	0.24	-0.14	-0.01	-0.23	-0.15	0.37	-0.09	0.15	-0.21
LSI	-0.39	-0.22	0.24	-0.13	-0.22	0.02	-0.01	0.18	0.02	-0.05	-0.23	-0.17	0.31	-0.27
VD	0.19	0.63**	-0.64**	0.01	0.05	-0.28	0.56*	-0.65**	0.38	0.57*	0.15	-0.18	-0.34	0.30
CA	0.24	0.76**	-0.75**	-0.18	-0.05	-0.51*	0.80**	-0.70**	0.58*	0.81**	-0.22	-0.25	-0.44	0.43
CP	0.24	0.59*	-0.58*	-0.22	-0.09	-0.46	0.63**	-0.52*	0.47	0.64**	-0.30	-0.18	-0.43	0.44
PL	0.27	0.81**	-0.82**	-0.10	0.02	-0.45	0.80**	-0.73**	0.57*	0.82**	-0.19	-0.23	-0.60*	0.58*
CL	0.17	0.16	-0.07	-0.21	-0.11	-0.28	0.25	-0.11	0.16	0.23	-0.29	-0.12	-0.06	0.08
CW	0.25	0.76**	-0.75**	-0.19	-0.07	-0.51*	0.81**	-0.71**	0.59*	0.82**	-0.23	-0.22	-0.42	0.40
CI	-0.24	-0.79**	0.83**	0.10	0.02	0.41	-0.78**	0.76**	-0.59*	-0.81**	0.06	0.12	0.39	-0.30
SL	-0.17	-0.69**	0.64**	0.21	0.13	0.47	-0.74**	0.66**	-0.55*	-0.74**	0.24	0.30	0.45	-0.44
SW	-0.27	-0.68**	0.62**	-0.01	-0.11	0.32	-0.69**	0.60*	-0.42	-0.73**	0.07	0.41	0.46	-0.44
SA	-0.17	-0.40	0.39	0.16	0.07	0.34	-0.49*	0.34	-0.32	-0.50*	0.31	0.38	0.37	-0.43
SP	-0.16	-0.70**	0.65**	0.22	0.13	0.48*	-0.75**	0.66**	-0.57*	-0.76**	0.26	0.31	0.48	-0.48
SSI	-0.05	-0.57*	0.54*	0.37	0.31	0.52*	-0.65**	0.59*	-0.57*	-0.63**	0.34	0.16	0.36	-0.36
TSW	-0.01	-0.41	0.27	-0.30	-0.26	-0.14	-0.33	0.40	-0.11	-0.42	-0.19	0.10	0.39	-0.14
GR	-0.05	0.01	0.16	-0.27	-0.24	-0.29	0.22	-0.06	0.10	0.19	-0.10	-0.10	0.39	-0.50*
GP	-0.01	-0.23	0.15	0.21	0.15	0.31	-0.17	0.29	-0.23	-0.15	0.08	-0.24	0.37	-0.50*
GI	-0.09	-0.02	0.17	-0.25	-0.23	-0.24	0.20	-0.03	0.07	0.17	-0.10	-0.12	0.31	-0.41
Count	0	15	15	0	0	4	16	15	9	16	0	0	1	3

Table 6. Correlations of the phenotypic traits with environmental factors

AAVT = annual average temperature; AMAT = annual max temperature; AMIT = annual min temperature; AAP = annual average precipitation; SI = sunlight intensity; HR = relative humidity; EV = evaporation; ST = soil temperature; SM = soil moisture; TTC = topsoil texture classification; TOC = topsoil organic carbon

The different geographical factors could affect plant phenotypic traits and seed germination rate and quality (Yan et al., 2014; Butnor, 2019). Plants could adapt to the warmth of their surroundings by adjusting their phenotypic morphology (Supratya et al., 2020; Bahuguna and Jagadish, 2015). As is showed (*Table 6*), most phenotypic traits and environmental factors in the study are significantly correlated, indicating that changes in habitat conditions have a great influence on the phenotypic traits of *P. pruinosa*. For example, annual average precipitation and evaporation are significantly correlated with most of the phenotypic traits, while longitude, annual average temperature and annual maximum temperature have no significant correlations with the phenotypic traits. The results indicate that the annual average precipitation and evaporation are the main factors driving the variation of phenotypic traits of *P. pruinosa*, and the tropism evolution of phenotypic traits is to meet the growth needs of extreme drought environments in different desert regions.

The evaluation of phenotypic traits is an important step in the identification and protection of germplasm resources and plays a vital role in the comprehensive potential of agronomic traits and breeding (Scarano et al., 2014). *P. pruinosa* is one of the few

species that only exists in extreme desert environments, which requires that the plant has a regulatory mechanisms allowing them to adapt to changes in harsh conditions. it means that the germplasm resources of natural P. pruinosa populations have high economic value and social benefits. Currently, the survival rate of asexual reproduction from natural P. pruinosa populations have been very low. So seed reproduction is an extremely important method for population reproduction of *P. pruinosa*. Moreover, the population habitat fragmentation caused by human factors and the increasingly poor ecological environment lead to a dramatically sharp increase in the decline rate of P. pruinosa populations; thus, in order to better protect the existing genetic resources and achieve sustainable growth of natural P. pruinosa populations, we suggest the following: (1) Implement in situ and off-site protection mechanisms to maximize the ecological value and economic benefits of genetic resources. (2) In the future, explore excellently genetic stress-resistance resources in different populations that are adapted to environmental factors such as salt and alkali, drought and heat at the molecular level to provide a basis for breeding protection. (3) Variation among populations is the result of adaptation to the environment and evolution, and conservation and utilization should be strengthened. Screening excellent germplasm resources will be beneficial to the conservation of existing P. pruinosa. (4) Superior natural populations selected from different provenances will be pollinated by seed reproduction to build a parent forest or seed orchard and obtain capsule tree species with high ecological value.

## Conclusions

The phenotypic diversity associated the leaf, fruit and seed traits of seventeen natural P. pruinosa populations show that there are abundant variations in phenotypic traits. In addition, there are significant differences in phenotypic traits between populations and within populations, and the variation among populations is greater than that within populations. The different geographical and climatic factors have significant influence on the variation of phenotypic traits, The results indicate that the differences of adaptation mechanisms and the sensitivity in response to environmental factors of plant phenotypic traits may lead to the different patterns of geographic variation in morphological changes during the growth and development P. pruinosa populations. It is imperative that all of natural P. pruinosa populations in China is studied by molecular markers in the future to fully reveal the variation law and genetic diversity level of different groups. Finally, using resistance genes associated with molecular markers, such as salt-tolerance gene and water-stressed gene, is to distinguish the differences among populations, construct core collections of populations from the level of genetic diversity, and consider the populations of phenotypic diversity to achieve the vital protection of natural P. pruinosa populations.

Acknowledgements. We were greatful to the Xinjiang Production & Construction Corps Key Laboratory of Protection and Utilization of Biological Resources in Tarim Basin (grant number BRZD2003) and Xinjiang Production and Construction Corps Key Field Innovative team Building Project "*Populus euphratica* Germplasm Resources Protection and Mining Utilization" (grant number 2018CB003). Thanks to Shanhe Zhang, Jianhao Sun, Xiaoli Han, Xiu Li, Jianing Wang, Xiaoshan Dong, and Zijian Wang who participated in this sample collection.

Conflict of interests. The authors declare no conflict of interests.

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DOI: http://dx.doi.org/10.15666/aeer/2002\_10651083

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### APPENDIX

Table A1. General information of seventeen natural Populus pruinosa populations in Xinjiang

Dut	<b>G</b> *4	C. I.	Longitude	Latitude	Altitude	AAVT	AMAT	AMIT	AAP	SI	RH	EV	ST	SM	TTC	тос
Kegion	Site	Code	(°E)	(°N)	(m)	(°C)	(°C)	(°C)	(mm)	(J/m²/d)	(%)	(mm)	(°C)	(m <sup>3</sup> /m <sup>3</sup> )	nc	(%)
Bayingol Mongolian Autonomous Prefecture, Southern Xinjiang	Qiemo County	qmx	85°23′32″	38°18′13.00″	1212.5	11.21	39.72	-20.36	35.55	253.71	28.43	39.65	7.78	0.17	9	0.99
Bayingol Mongolian Autonomous Prefecture, Southern Xinjiang	Yuli County	ylx	85°48′27.9″	41°08′24.5″	899	13.16	39.34	-17.3	64.28	234.46	33.53	65.68	15.83	0.18	9	0.42
Hotan, Southern Xinjiang	Qira County	clx	81°06′43.48″	36°49′35.29″	1454	12.06	38.4	-19.74	62.21	237.47	31.27	57.61	9.54	0.09	13	0.43
Hotan, Southern Xinjiang	Lop County	lpx	80°56′46.32″	39°33'32.69"	1123.2	12.24	38.38	-19.01	64.9	236.92	30.08	57.69	15.34	0.2	9	0.42
Hotan, Southern Xinjiang	Minfeng County	mfx	82°47′38.27″	37°38'37.05"	1308	12.46	39.59	-18.51	46.78	257.63	27.02	54.67	9.31	0.08	9	0.42
Hotan, Southern Xinjiang	Karakax County	myx	80°11′15.33″	37°46′14.09″	1248.2	13.91	39.48	-14.43	51.26	254.82	28.25	59.38	10.22	0.09		
Hotan, Southern Xinjiang	Pishan County	psx	79°0′28.37″	37°36′44.54″	1299.7	13.81	39.31	-14.48	56.32	253.23	27.88	69.25	10.3	0.1	10	0.43
Hotan, Southern Xinjiang	Yutian County	ytx	81°23′33.44″	37°16′37.04″	1288.8	13.47	39.26	-16.18	52.25	255.25	26.97	54.7	16.53	0.08	13	0.43
Kashgar, Southern Xinjiang	Marabishi County	bcx	78°18′29.00″	39°46′22.78″	1126.7	12.68	38.43	-16.13	84.93	237.6	34.31	125.35	6.93	0.32	5	0.49
Kashgar, Southern Xinjiang	Makit County	mgtx	74°17'30.13"	39°19′31.14″	1143.2	2.22	26.96	-26.43	126.08	238.79	48	104.05	-9.8	0.23	11	1.41
Kashgar, Southern Xinjiang	Yarkant County	scx	77°21′28.48″	38°24'43.48"	1207.5	12.79	37.87	-15.14	66.47	247.8	30.83	71.11	8.25	0.14	2	2.94
Kashgar, Southern Xinjiang	Shule County	slx	76°10′25.00″	38°0'17.37"	1270.8	4.21	28.38	-22.11	87.08	241.82	39.14	81.14	2.61	0.22	9	0.58
Kashgar, Southern Xinjiang	Zepu County	zpx	76°58'04.51"	38°02'06.45"	1407	10.6	35.23	-16.56	71.4	245.29	32.38	74.96	5.96	0.21	9	0.41
Aksu, Southern Xinjiang	Alaer County	ale	79°47′40.46″	40°21′31.06″	1049.3	12.84	38.57	-17.28	88.28	234.01	34.36	95.99	15.46	0.22	9	0.42
Ili Kazak Autonomous Prefecture, Southern Xinjiang	Nilka County	nlk	82°12′32.30	43°35′53.58″	754.8	7.16	34.29	-26.43	255.77	217.19	44.95	273.71	2.48	0.1	9	0.46
Ili Kazak Autonomous Prefecture, Southern Xinjiang	Qapqal Xibe Autonomous County	cbce	80°40′48.36″	43°50′34.87″	538.5	11.14	37.82	-22.11	219.21	215.61	43.69	244.66	7.11	0.11	2	2.94
Ili Kazak Autonomous Prefecture, Southern Xinjiang	Cocodala city	kkdl	80°47′20.93″	43°55′47.64″	553.3	11.14	37.82	-22.11	219.21	215.61	43.69	244.66	3.64	0.13	2	2.94

AAVT = annual average temperature; AMAT = annual max temperature; AMIT = annual min temperature; AAP = annual average precipitation; SI = sunlight intensity; HR = relative humidity; EV = evaporation; ST = soil temperature; SM = soil moisture; TTC = topsoil texture classification; TOC = topsoil organic carbon

APPLIED ECOLOGY AND ENVIRONMENTAL RESEARCH 20(2):1065-1083. http://www.aloki.hu • ISSN 1589 1623 (Print) • ISSN 1785 0037 (Online) DOI: http://dx.doi.org/10.15666/aeer/2002\_10651083 © 2022, ALÖKI Kft., Budapest, Hungary