Morphology and SSR markers reveal the genetic diversity of Elymus species germplasm in northwestern China

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Abstract

The relationship between the genetic diversity and genetic characteristics of wild plant germplasm can provide insights for better utilization and conservation of genetic resources. Bunchgrass species in the genus *Elymus* are important for forage and grassland restoration in Northwest China. In this study, eight phenotypic traits were evaluated in 81 accessions of four *Elymus* species in the northwest region of China, and genetic diversity analysis was performed using 16 simple sequence repeat (SSR) markers. In the phenotypic trait analysis, *Elymus sibiricus* had the highest coefficient of variation for single-plant weight(58.24%) and the lowest coefficient of variation for the number of spikelets(0.005%). Clustering based on phenotypic traits placed all varieties into four groups, which were also supported by principal component analysis (PCA). 16 pairs of SSR markers were screened with high polymorphism, with a polymorphism information content (PIC) range of 0.3648–0.7794 and an interspecific PIC range of 0.465–0.611 in *Elymus* species. The unweighted pair-group method with arithmetic mean approach applied to SSR marker data also divided the 81 accessions into four groups, similar to the results based on phenotypic traits. The results of PCA and population structure analysis based on SSR data were similar. The findings presented here will facilitate the collection and conservation of *Elymus* germplasm resources and provide theoretical references for the future classification, breeding, innovation, utilization, and conservation of germplasm.

1. Introduction

Elymus spp., important members of the wheat family (Gramineae), are high-quality perennial forage grains found in grasslands and meadows with medium- to fast-drying meristems and represent an important component of forages with extremely high nutritional value (Dewey., 1984, Yang et al., 2017). Because these species have excellent traits such as disease resistance, insect resistance, drought tolerance, and salt tolerance, which are lacking in wheat crops, they are important germplasm resources in wheat breeding (Li et al., 2021). This group of forage grasses is widely distributed, mainly in Central Asia, Russia, Mongolia, China, Korea, Japan, and northern India. In China, it is widely distributed in grasslands below 3000 m on the Qinghai-Tibetan Plateau (Yang et al., 2017, Cui et al., 2019). As a result of their wide distribution, these forage grasses harbour large numbers of genes and contain rich genetic resources.

The genetic variation within an organism is known as genetic diversity, and it is the basis of species diversity (Salgotra and Chauhan, 2023). To some extent, species diversity is a measure of the biodiversity of a region and is therefore the key to biodiversity, as it reflects both the complex relationship between organisms and their environment and the abundance of biological resources (Hamilton et al., 2005). Genetic markers are an essential resource in research on species diversity, and they play an important role in the establishment and development of genetic technology (Wambugu and Henry, 2022). With the development of genetic markers, the methods for quantifying genetic diversity have evolved from the macro- to microscopic level (Yasui et al., 2020, Raza et al., 2016). There are four main types of genetic markers: morphological markers, cytological markers, biochemical markers, and molecular markers (Yang

et al., 2013). Among them, morphological and molecular markers play complementary roles in the study of genetic diversity.

Morphological markers are based on visually observable traits such as flower colour, seed shape, growth habit, and pigmentation (Aworunse et al., 2023). These markers are tagged by visualizing the external plant phenotype, which does not require expensive technology, but some marker traits are often susceptible to environmental variation that cannot be separated from genotypic variation (Govindarai et al., 2015). Morphological variables have also been used to describe phenotypic variability among grass ecotypes, such as in a study of the northern U.S. grassland plant switchgrass, where morphological markers were used to distinguish between upland and lowland ecotypes and to determine high phenotypic variability among populations collected from nearby areas (Cortese et al., 2010).

Genetic diversity can be assessed based on morphological markers, but it is difficult to classify some varieties precisely based on their morphological characters alone, hence the use of molecular markers (kumar Ganesan et al., 2014). Molecular markers are DNA sequences located at known locations on chromosomes, genes whose phenotypic expression is often easily discernible and used to detect individuals, or probes used to mark chromosomes, nuclei, or loci (Semagn et al., 2006, Grover et al., 2016). Molecular markers display polymorphism, which may arise due to nucleotide alterations or mutations at genomic loci, making it possible to identify genetic differences between individual organisms or species (Garrido-Cardenas et al., 2018, Coates et al., 2018). Molecular markers can be used in many different fields, such as genetic mapping, paternity testing, detection of mutated genes associated with hereditary diseases, variety identification, marker-assisted breeding, population history reconstruction, epidemiology, food safety, and population studies (MirMohammadi et al., 2019). Various molecular markers (restriction fragment length polymorphisms (RFLPs), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), and simple sequence repeats (SSRs)) have been successfully applied in studies of germplasm genetic diversity (Semagn et al., 2006). Sixty different Bermuda grass varieties from five geographical regions of China were analysed using SSR markers to determine the genetic diversity of quality-related traits (Gitau et al., 2017).

A good understanding of the genetic diversity of wild germplasm resources is central to the effective conservation of their gene pool, the comprehension of their evolutionary processes, and their effective use in breeding strategies (Ma et al., 2012). In recent years, interest in the genetic structure of natural populations of grass species has increased due to the need to expand the knowledge of genetic variation in cultivated species.

The current study aimed to further clarify the genetic diversity of common *Elymus* species and the genetic relationships among the major distributed *Elymus* species in China. To this end, 81 accessions were collected from widely grown wild resources in four provinces of northwestern China, and their genetic relationships, population structure, and interspecific genetic relationships were comprehensively evaluated. Phenotypic characters were combined with SSR molecular markers for comprehensive identification and evaluation. This study provides useful information for the identification, classification,

and breeding of Chinese *Elymus* species as well as a theoretical reference for exploring the origin and domestication of *Elymus*.

2. Materials and Methods

2.1. Plant Materials

Eighty-one accessions of wild *Elymus* spp. were collected from China, including *Elymus cylindricus* (26), *Elymus breviaristatus* (25), *Elymus nutans* (19), and *Elymus sibiricus* (11), which are currently concentrated in the greater northwestern region of China (Fig. 1A, B and Table S1). All of the collected accessions were grown in the germplasm resource nursery of China Agricultural University (Beijing). The materials were grown in a greenhouse with a distance of 10 cm between plants, and standard weeding and agronomic practices were implemented regularly to provide adequate plant growth conditions. There were three replicates of each variety, and 20 plants were grown in each of the replicates.

2.2. Phenotype Assessment

The collected accessions were evaluated for a total of eight phenotypic traits of leaves, stems, spikes, and seeds (Table 1). The aboveground stem- and leaf-related traits were investigated 90–110 days after planting, and the spike and other traits were investigated by the late tassel stage. The traits related to the single-plant weights and seed yield per plant were investigated after maturity. Phenotypic trait data were recorded as described above. Twenty individuals were randomly selected for each accession, and their traits were measured.

2.3. DNA Extraction

Fresh young leaf tissue was collected from greenhouse plants after 8 weeks (approximately 200 mg in 2.0 ml centrifuge tubes) and frozen in liquid nitrogen. DNA was extracted using a cetyltrimethylammonium bromide (CTAB) protocol (Doyle, 1991). DNA quality and concentration were assessed by comparing samples to known λ DNA standards using a 1% (w/v) agarose gel and a Nanodrop ND1000 spectrophotometer (Nanodrop Technologies, DE, USA). Isolated genomic DNA was diluted to 10 ng/μl and stored at -20°C for SSR analysis.

2.4. SSR Genotyping

Sixteen SSR markers with clear bands and polymorphisms were selected from the 53 previously developed SSR markers (Table 2) for further analysis (Lei et al., 2014). The primers were synthesized by Shanghai Biotechnology Bioengineering Co. The PCR amplification reaction system was 10 μl, containing 4 ml of 2 Taq PCR mix (each dNTP at 500 mM, 20 mM Tris-HCl, 100 mM KCl, 3 mM $MgCl_2$) (Tiangen Biotechnology Co., Ltd., Beijing, China), 2 μl of each primer pair (10 mM) and 1 μl of template DNA (20 ng/μl), and enough ddH_2O to reach the total volume. The following cycling parameters were used for the amplification reactions: predenaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s,

annealing at 55°C for 30 s, and 72°C for 30 s and final extension at 72°C for 10 min. The amplified PCR products were detected on an 8% nondenaturing polyacrylamide gel. Silver nitrate staining was performed, and images were collected for recording and analysis.

2.5. Data Analysis

Statistical analyses of maximum, minimum, mean, standard deviation (SD), and coefficient of variation (CV) values were performed for the trait survey results in Table 1 using SPSS 25.0 software. Systematic clustering and principal component analysis (PCA) were performed based on Euclidean distances using Origin 2022 software. The SSR marker polymorphic bands were labelled for the presence (1) and absence (0) of primers at each locus, and a binary matrix was constructed and statistically analysed. The number of alleles (*Na*), effective allele number (*Ne*), allele frequency, Shannon's diversity index (*I*), observed heterozygosity (*Ho*), expected heterozygosity (*He*), Nei's (1973) genetic diversity index (*H*) and polymorphism information content (*PIC*) were analysed using PowerMarker software version 3.5.1 (Liu et al., 2005). Analysis of population structure was performed by Bayesian modelling in STRUCTURE software version 2.3.4 (Pritchard et al., 2000). K (the number of clusters) was estimated in the range of 2–10, and the software was run 15 times to determine this value. Estimates were obtained using a Markov chain Monte Carlo (MCMC) method for 100,000 iterations followed by a burn-in period of 500,000 iterations. STRUCTURE HARVESTER (Earl et al., 2012), which determines the best K based on the probability of data given K and ΔK (Evanno et al., 2005), was used to estimate the most likely number of population subgroups (K).

3. Results

3.1. Phenotypic Diversity Analysis

For all *Elymus* accessions (Table 3), the CV values for the eight quantitative traits ranged from 0.005–58.24%, and the CV values for single-plant weight exceeded 30%, with the highest values observed for single-plant weight and the lowest values for the number of spikelets. Plant height showed the most variation (15.73 cm), followed by main stem length (7.49 cm), the number of spikelets (6.30), single-plant weight (5.47 g), leaf length (4.28 cm), spikelet length (0.36 cm), seed yield per plant (0.22 g) and leaf width (0.15 cm). Of the four species, *E. sibiricus* had the highest CV for plant weight (58.24%), and *E. breviaristatus* had the lowest CV (0.39%). *E. cylindricus* had the highest CV for spikelet number (6.50%), and *E. sibiricus* had the lowest CV (0.005%). Both the maximum and minimum CV values were observed in *E. sibiricus*, *E. cylindricus* had the highest CV for plant height, and *E. sibiricus* had the lowest CV for this trait. *E. cylindricus* had the highest CV for main stem length, and *E. nutans* had the lowest CV. *E. cylindricus* had the highest CV for leaf length, and *E. sibiricus* had the lowest CV. *E. sibiricus* had the highest CV for leaf width, and *E. nutans* had the lowest CV. The CV of spike length was highest in *E. sibiricus* and lowest in *E. cylindricus*, and the CV of seed yield per plant was highest in *E. sibiricus* and lowest in *E. breviaristatus*. Phenotypic analysis showed that wild species of Chinese *Elymus* have rich

diversity. However, some species have low diversity, and the number of accessions may be an important reason; further analysis will require a large number of accessions.

A cluster dendrogram was created for 81 *Elymus* accessions based on eight phenotypic characters (Fig. 2A). These 81 accessions were divided into four main groups, which corresponded to the four species. *E. cylindricus* was placed in Group G1, which had the highest mean values for main stem length, plant height, seed yield per plant, and single-plant weight and included all collection areas, and *E. breviaristatus* was placed in Group G2, which had the highest mean values for spike length and number of spikelets and included all collection areas. *E. sibiricus* belonged to G3, which had the lowest mean values for all phenotypic characters except leaf length and spike length, and *E. nutans* was assigned to group G4, which had intermediate mean values for phenotypic characters and included all collection areas.

PCA was used to examine the phenotypic traits (Fig. 2B). As shown, two principal components (PCs) accounted for 56.6% (PC1) and 24.4% (PC2) of the total variation. PC1 was dominated by main stem length, plant height, leaf length, single-plant weight, and seed yield per plant. PC2 combined leaf width and spike length (Table S2). As in the clustering dendrogram, the 81 accessions were divided into four groups. *E. breviaristatus* accessions were distant from the other three groups, while the other three groups were very close to each other. This suggests that *E. breviaristatus* is distantly genetically related to the other accessions, while *E. cylindricus*, *E. nutans*, and *E. sibiricus* are closely genetically related.

3.2. Polymorphism Analysis of Molecular Markers

Altogether, 89 bands were amplified by 16 SSR primers, and 77 of these bands were polymorphic, yielding a proportion of polymorphic loci equal to 86.52%. A total of 74 pairs of alleles were amplified, and the largest *Na* values (7) were found when using primers ESGS125, ESGS155, and ESGS218, with an average of 4.63 alleles per primer. The mean values of *Ne*, *I*, *Ho*, and *He* were 1.748, 0.700, 0.222, and 0.413, respectively. The *PIC* values ranged from 0.3648 to 0.7794, with an average of 0.6182 (Table 4). The range of the observed number of alleles (*Nr*) was 2–4 for *E. cylindricus* and *E. nutans*, 2–5 for *E. breviaristatus*, and 2–3 for *E. sibiricus*. The highest values for *Na*, *H*, and *PIC* were observed for *E. nutans*, and the lowest values were observed for *E. sibiricus*. The lowest value for both *Ne* and *He* was in *E. cylindricus*, the highest value for both *Ne* and *Ho* was in *E. sibiricus*, and the lowest value for both *Ho* was in *E. nutans* (Table 5).

Cluster dendrogram analysis was performed based on the polymorphic band data of SSR markers (Fig. 3a). Based on the cluster analysis of the SSR data, the 81 accessions could be divided into four groups. In general, the groups corresponding to the species could be distinguished, but there were very few cases where the same accessions were not clustered together. Specifically, group G1 contained an accession of *E. breviaristatus* (Espp-6), and group G2 contained an accession of *E. sibiricus* (Espp-27). The results of PCA were in general agreement with those of the molecular marker cluster analysis (Figure. 3b). The 81 accessions could be divided into four groups. The accessions of *E. cylindricus* and *E. breviaristatus* could be separated, while those of *E. nutans* and *E. sibiricus* were clustered together.

Based on the output of STRUCTURE HARVESTER, the optimal K value was 4 (i.e., where ΔK reached its maximum value) (Figure S1). At K = 2, the 81 accessions were categorized into two subgroups, with green representing the first subgroup (45 accessions) and red representing the second subgroup (36 accessions). The first subgroup included all accessions of *E. cylindricus* and *E. nutans*, and the second subgroup included accessions of *E. breviaristatus* and *E. sibiricus.* When K = 3, the accessions of *E. cylindricus* and *E. nutans* were clustered into a group independently, while the other accessions were clustered into another group. At K = 4, red represents the first subgroup (26 accessions), yellow represents the second subgroup (25 accessions), green represents the third subgroup (11 accessions), and blue represents the fourth subgroup (19 accessions). These four subgroups correspond to the accessions of *E. cylindricus*, *E. breviaristatus*, *E. nutans*, and *E. sibiricus* (Fig. 4).

4. Discussion

4.1. Analysis of Genetic Diversity by Combining Phenotypic Traits and Molecular Marker Identification

Phenotypic diversity is the outward expression of genetic diversity and is the most fundamental metric for germplasm resource selection and genetic background studies (Sartie et al., 2012, Zhang et al., 2019). The magnitude of the CV indirectly reflects the abundance of phenotypic diversity in a population, and when the value is large, it indicates a high level of phenotypic diversity in the population (Sun et al., 2019). Maintaining a large pool of variation in a plant population is beneficial and allows the population to be well adapted to most environmental conditions (Lande et al., 1987). Genetic diversity is commonly used for morphological characterization, variety identification, and selection of breeding material because it is simple and easy to document phenotypic differences (Bunlungsup et al., 2016). In this study, eight phenotypic traits of 81 *Elymus* accessions belonging to four species were analysed, and the results showed high diversity among the species but low diversity within them, which may be due to insufficient sampling, necessitating further expansion of sample collection.

Molecular markers have been used extensively for genetic diversity analysis of different species (Denwar et al., 2019, Yin et al., 2023). In this paper, sixteen SSR primers were selected to analyse the genetic diversity of 81 accessions of four species of *Elymus*. The SSR markers all showed good amplification and could effectively and accurately capture genetic variation in the *Elymus* species. Seventy-four alleles were amplified by SSR markers, and the average number of alleles observed per marker locus (*Na*) was 4.63, with a range of 3–7, indicating polyploidy in the population. Yan et al. (Yan et al., 2010) obtained similar results regarding the genetic diversity of germplasm resources of *E. sibiricus* by using SRAP markers. Analysis of genetic diversity based on expected heterozygosity (*He*) and Shannon's index (*I*) can shed light on the relationship between the magnitude and spatiotemporal distribution of genetic variation and the environmental conditions experienced by the species, thus providing a powerful tool for developing conservation strategies for endemic species (van et al., 2015). Ma et al. (2008) reported a Shannon's diversity value (*I* = 0.41) similar to that observed for the species in this study. The Shannon

diversity value for E. nutans (I = 0.45) reported (Ma et al., 2014), is lower than the value for this species in this study. In the present study, SSR markers revealed moderately high values of genetic diversity for *E. breviaristatus*. When the mean expected heterozygosity (*He* = 0.421) and Shannon index (*I* = 0.737) calculated at the population level were compared with those of other *Elymus* species analysed with dominant markers (e.g., RAPD, ISSR, and AFLP), *E. breviaristatus* exhibited relatively high levels of diversity (Yu et al., 2019), similar to those in *E. nutans* (Chen et al., 2009)d *trachycaulus* (Gaudett et al., 2005). In contrast, some *Elymus* species, such as *E. alaskanus* (Zhang et al., 2002)d *fibrosus* (Díaz et al., 2000), showed very low *He* estimates compared to those of *E. breviaristatus*.

In conclusion, the clustering analysis of 81 Chinese *Elymus* accessions based on polymorphic band data for molecular markers yielded results in good congruence with those from the clustering analysis of phenotypic traits. The difference between the two sets of results was that phenotypic clustering placed different materials of the same species together, while the molecular markers showed a few materials of other species in the same category. This may be due to the limited number of phenotypic traits and some traits being ignored, resulting in inadequate analysis. This may also be due to incomplete genomic information for *Elymus*, the high difficulty of polymorphic marker development, the lack of molecular markers closely related to phenotypic traits, and the application of molecular markers in different populations of *Elymus*, where genetic maps cannot be integrated, resulting in low density and poor generalizability. These factors may explain the differences between the results based on molecular markers and phenotypic traits. Therefore, combined analysis using both methods could be a good approach for identifying *Elymus* species (Denwar et al., 2019).

4.2. The Value of Genetic Diversity

The genetic diversity of germplasm resources is a potentially valuable resource for improving a wide range of agronomic traits. Expanding the genetic base is also an important strategy for screening and developing new germplasm or breeding lines with desirable traits. Abundant wild germplasm can provide favourable genes or alleles and improve the quality, yield, and resistance of resources through crosses and infiltration lines (Angessa et al., 2016). Two wild *E. sibiricus* materials with genetic and phenotypic differences were crossed to obtain F_1 hybrids, and molecular marker and phenotypic diversity analyses of the hybrids revealed abundant genetic and phenotypic variation (Zhang et al., 2016). Five hybrid populations were established by crossing seven wild Siberians with large genetic variation, and high levels of genetic diversity were found in the hybrid populations using SSR markers. The results also indicated that more genetic variation could be captured in the hybrid populations by crossing (Zhao et al., 2017). In this study, we documented the genetic variation and population structure of *E. sibiricus* in the wild in Northwest China. These wild species with rich genetic diversity and desirable traits could serve as important genetic resources for future *E. sibiricus* breeding programs.

4.3. Proposal for Conservation

The results of this study suggest low diversity in *Elymus* species (*Elymus sibiricus*), which may be related to local human and animal activities and environmental degradation. We suggest in situ and ex situ

conservation strategies to limit the decline of the species. First, an in situ conservation strategy should be adopted to protect and restore all existing populations to ensure the genetic and evolutionary continuation of the resource in its natural environment. Population size is an important consideration for the recovery of endangered and rare species (Brzosko et al., 2015). Maintaining moderate grazing in the focal area promotes the maintenance of genetic variation in small and dispersed populations through frequent gene flow between populations and prevents the loss of genetic diversity due to random genetic drift. When grazing animals move around pastures, they can facilitate seed dispersal and increase gene flow (Brzosko et al., 2015, Gitzendanner et al., 2000). Second, for ex situ conservation, a germplasm repository for the species needs to be established. A reasonable approach to ex situ conservation is to collect and preserve germplasm material from a relatively small number of individuals to avoid disturbing the population (Endris et al., 2023).

5. Conclusions

In China, the germplasm of wild *Elymus* species shows a high degree of intra- and interspecific diversity. Phenotypic and molecular markers are very effective tools for detecting *Elymus* diversity. The best way to identify genetic differences is to combine molecular and phenotypic data to obtain more information and clarify genetic relationships. The results of the cluster analysis showed that *E. breviaristatus* was the most distantly related to the other species, while *E. cylindricus*, *E. nutans*, and *E. sibiricus* were the most closely related, a result further confirmed by PCA and population structure analysis. In conclusion, the results of this study provide a theoretical basis for determining the genetic differences and resource types of wild *Elymus* species in China.

Declarations

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

Author Contributions

Q.Z. and S.L. designed the experiments; S.G. carried out the experiments and wrote the manuscript; H.T. revised the manuscript; S.G. and Q.Z. analyzed the data. All authors have read and agreed to the published version of the manuscript.

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Tables

Table.1 The descriptions used in this study for phenotypic evaluation of *Elymus* spp.

Determine the traits	Determination method
Main stem length	Average main stem height of 20 plants
Plant height	Average height of 20 plants
Leaf length	Average leaf length of 20 plants
Leaf width	Average leaf width of 20 plants
No.of spikelets	Average number of spikelets for 20 plants
Spikelet length	Average spikelet length of 20 plants
Weight of individual plant	Average weight of 20 plants
Seed yield per plant	Average seed yield of 20 plants

Table.2 Information on the SSR markers used in this study

Marker	Repeat motif	Primer sequence (5′–3′)	Tm (°C)	Size (bp)
ESGS4	(GA)14	F: GTGCCCTATCAAGATTACG	53.2	195–205
		R: TTCATCGGGACACCTTTT	56.4	
ESGS25	(GA)13	F: AAGTGCCAACTAGGAGTT	49.5	80–90
		R: CATCACCATTTTACAGGG	51.9	
ESGS41	(CT)14	F: TACATCCACAACTTGAGCACC	58.6	213–217
		R: CACAACTCACAAGCAGGACAC	59.4	
ESGS45	(CT)29	F: GCAAGAGCAAGAGGAAGGAG	59.3	167–175
		R: AGGCAAGCTGTTCAACAAAA	58.6	
ESGS46	(AC)21	F: CAAGTATCACATTTATCACCA	51.1	132–136
		R: GCAGAGTGTAAAACTATTTGA	50.0	
ESGS55	(CT)12	F: CTGAATTATTTTGACATT	42.5	160–168
		R: TGTAACATTTGAGAGAGA	43.2	
ESGS61	(CT)7	F: TATTGGTTTCCTACACCCACTCA	60.6	180–184
		R: GGTGACCTAGATAGACAACGTGC	60.1	
ESGS87	(GA)13	F: AAGTGCCAACTAGGAGTTTG	54.6	120–124
		R: ACATCACCATTTTACAGGGA	55.3	
ESGS98	(CT)7	F: AAAGAAAAGGAAATAAACG	49.1	160–166
		R: GGAGGTGGGAGACGAACTG	60.7	
ESGS117	(CT)9	F: CGAGGCGAGGTAAAAAGTATA	56.4	218–230
		R: GTTGTCACGTTTGAAGCAAGT	57.9	
ESGS124	(CT)7	F: GCGAGGTAAAAAGTATAG	45.0	218–230
		R: TCACGTTTGAAGCAAGTA	50.8	
ESGS125	(CT)9	F: AAAAAGAAAGAAAAAACGG	51.6	113–137
		R: CGAAGCTGACATGCGGGGC	69.7	
ESGS155	(CT)21	F: GCCACTAATAGGGTTTTTTC	53.3	134–144
		R: CCCACTAACTCACTCACACA	54.4	
ESGS169	(TG)9	F: ATGGAATTTATATTAGTTTG	44.1	280–290
		R: TTATTTTTTCTACTCCTATG	44.3	

ESGS218	(CA)9	F: ACAACACCAACTCTCCCGC	61.1	150–158
		R: CTTATTTCAGACGACCCAC	53.0	
ESGS243	(AG)12	F: TGTCACCCTGGTCCTGCC	63.3	100–104
		R: ATAACACACAAACTCTCG	45.9	

Table.3 Statistics analyses of intra- and interspecific quantitative traits of four *Elymus* species

Species	Trait	Mean	Maximum	Minimum	Range	*SD*	CV (%)
E. cylindricus	Main stem length (cm)	78.26	83.91	75.24	8.67	5.32	6.79
	Plant height(cm)	92.09	97.85	86.55	11.31	6.50	7.06
	Leaf length(cm)	14.41	16.79	13.07	3.72	0.97	6.74
	Leaf width(cm)	0.50	0.56	0.44	0.11	0.007	1.50
	No.of spikelets	47.47	50.40	44.20	6.20	3.09	6.50
	Spikelet length(cm)	0.82	0.88	0.76	0.11	0.007	0.90
	Weight of individual plant(g)	24.65	28.49	21.26	7.24	1.96	7.94
	Seed yield per plant (g)	0.55	0.68	0.38	0.30	0.004	0.71
E. breviaristatus	Main stem length (cm)	70.90	74.66	67.97	6.69	0.09	0.13
	Plant height(cm)	86.73	92.12	77.84	14.28	4.82	5.55
	Leaf length(cm)	12.76	16.20	11.32	4.88	0.76	5.97
	Leaf width(cm)	0.65	0.75	0.58	0.17	0.002	0.27
	No.of spikelets	50.26	54.45	45.88	8.58	0.005	0.01
	Spikelet length(cm)	1.150	1.90	0.94	0.96	0.009	0.78
	Weight of individual plant(g)	16.57	18.73	14.59	4.14	0.06	0.39
	Seed yield per plant (g)	0.41	0.52	0.31	0.21	0.001	0.27
E. nutans	Main stem length (cm)	68.94	70.76	67.47	3.30	0.04	0.06
	Plant height(cm)	82.49	101.09	75.13	25.97	2.74	3.32
	Leaf length(cm)	11.31	15.02	10.03	4.99	0.76	6.73
	Leaf width(cm)	0.40	0.51	0.36	0.15	0.0002	0.061
	No.of spikelets	39.77	42.00	35.35	6.65	0.26	0.66
	Spikelet	0.89	0.99	0.81	0.19	0.003	0.31

	length(cm)						
	Weight of individual plant(g)	21.03	24.38	18.77	5.61	8.54	40.59
	Seed yield per plant (g)	0.31	0.40	0.20	0.20	0.004	1.37
E. sibiricus	Main stem length(cm)	62.22	68.48	57.18	11.30	2.86	4.60
	Plant height(cm)	70.76	78.81	67.48	11.34	0.76	1.07
	Leaf length(cm)	11.84	14.36	10.82	3.54	0.65	5.52
	Leaf width(cm)	0.39	0.47	0.31	0.17	0.01	1.78
	No.of spikelets	27.43	29.78	26.00	3.78	0.001	0.005
	Spikelet length(cm)	0.97	1.07	0.89	0.18	0.01	1.17
	Weight of individual plant(g)	14.19	17.38	12.50	4.88	8.26	58.24
	Seed yield per plant (g)	0.21	0.28	0.12	0.17	0.01	3.45

Table.4 Hereditary differentiation parameters of 81 *Elymus* accessions revealed by 16 SSR markers with polymorphism

Marker	*N*a	*N*e	*I*	*H*o	*H*e	*H*	*PIC*
ESGS4	4	1.424	0.537	0.179	0.288	0.5980	0.5188
ESGS25	4	1.843	0.701	0.360	0.437	0.6344	0.5705
ESGS41	3	1.734	0.699	0.276	0.412	0.6184	0.5489
ESGS45	4	1.731	0.676	0.386	0.417	0.6538	0.5944
ESGS46	3	1.697	0.659	0.212	0.410	0.5816	0.5100
ESGS55	4	1.725	0.705	0.239	0.411	0.6294	0.5722
ESGS61	3	1.438	0.449	0.137	0.284	0.4012	0.3648
ESGS87	3	1.841	0.749	0.176	0.454	0.6593	0.5849
ESGS98	4	1.862	0.704	0.173	0.461	0.6605	0.6087
ESGS117	6	2.038	0.914	0.229	0.509	0.7952	0.7638
ESGS124	6	1.823	0.748	0.183	0.432	0.7431	0.7006
ESGS125	7	2.066	0.952	0.183	0.510	0.7950	0.7650
ESGS155	7	1.835	0.800	0.203	0.454	0.7880	0.7560
ESGS169	6	1.757	0.745	0.189	0.418	0.7639	0.7250
ESGS218	7	1.643	0.636	0.242	0.388	0.8073	0.7794
ESGS243	3	1.504	0.529	0.189	0.328	0.6017	0.5286
Mean	4.63	1.748	0.700	0.222	0.413	0.6707	0.6182
Total	74	27.961	11.203	3.556	6.613	10.7308	9.8916

*Note: N*a, observed number of alleles; *N*e, effective number of alleles; *I*, Shannon's diversity index; *H*o, observed heterozygosity; *H*e, expected heterozygosity; *H*, Nei's gene diversity; PIC, Polymorphism information content.

Table 5 Parameters of genetic differentiation among *Elymus* species revealed by 16 polymorphic SSR markers

Species	*N*a	*N*r	*N*e	*I*	*H*o	*H*e	*H*	*PIC*
E. cylindricus	2.688	2-4	1.688	0.669	0.219	0.398	0.523	0.501
E. breviaristatus	3.125	2-5	1.772	0.737	0.208	0.421	0.631	0.579
E. nutans	2.875	2-4	1.734	0.715	0.207	0.417	0.655	0.611
E. sibiricus	2.500	2-3	1.797	0.680	0.256	0.418	0.489	0.465

Note: *N*a, observed number of alleles; *N*r, range of observed number of alleles; *N*e, effective number of alleles; *I*, Shannon's diversity index; *H*o, observed heterozygosity; *H*e, expected heterozygosity; *H*, Nei's gene diversity; PIC, Polymorphism information content.

Figures

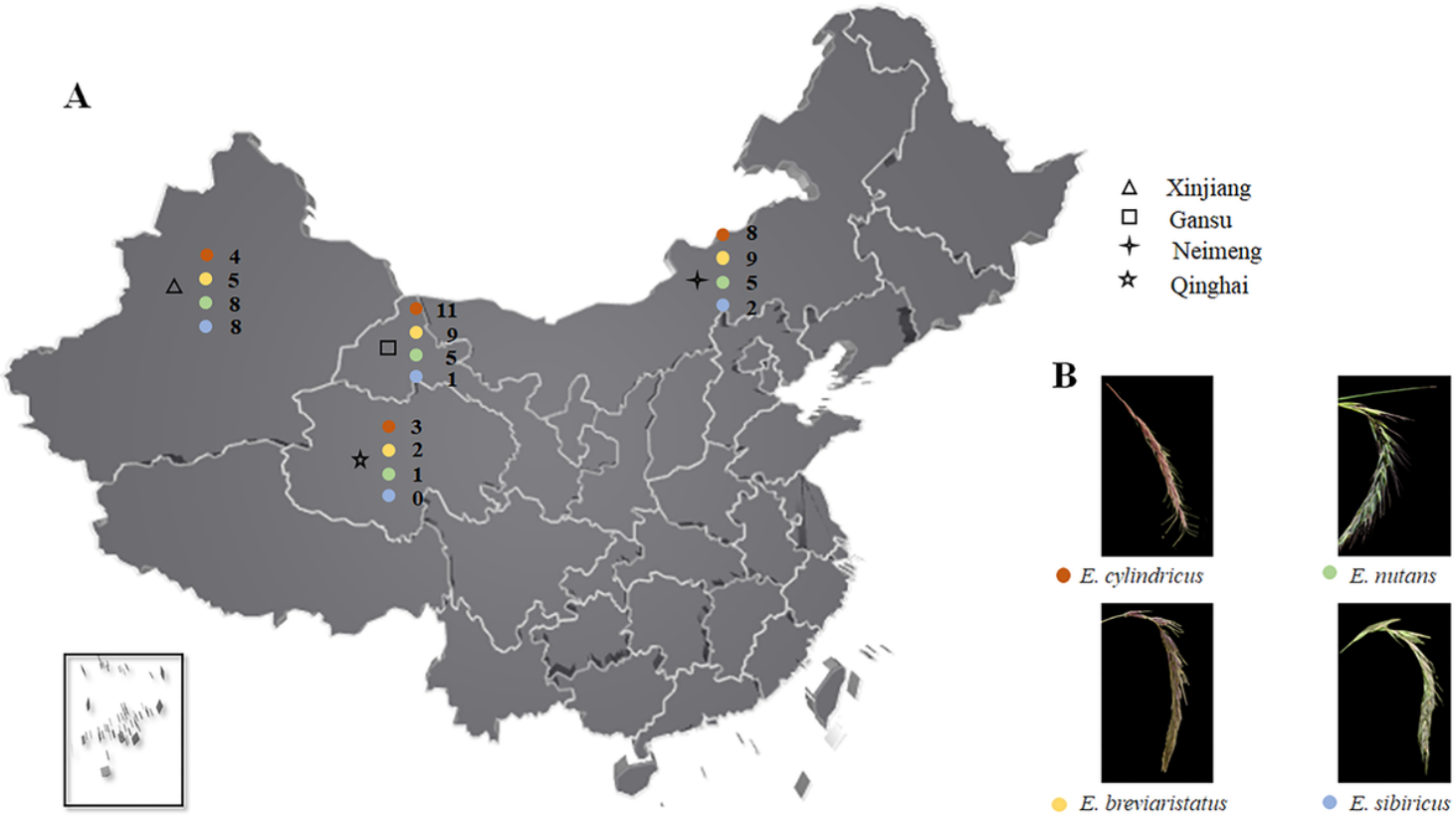

Figure 1

Geographical distribution of different *Elymus* accessions and the number of accessions in different provinces (A), and local phenotypic images of four *E.* species in China (B). Numbers indicate the number of all resources collected in each province, solid circles of different colors indicate different *Elymus* species, and different shapes represent different provinces.

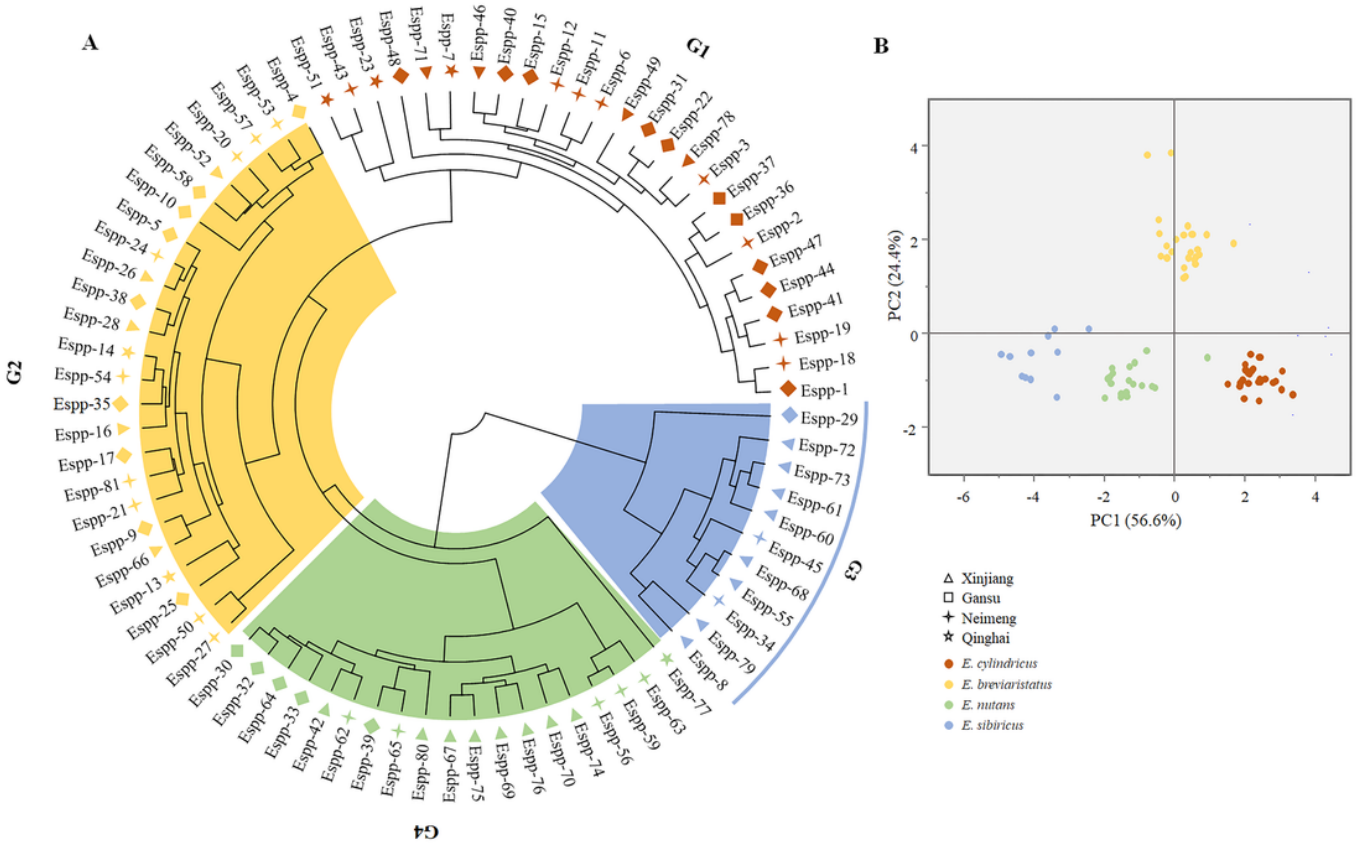

Figure 2

Cluster dendrogram (A) and PCA (B) of 81 *Elymus* accessions on the basis of phenotypic traits. G" is an abbreviation for group, and G1-G6 represent different groups.

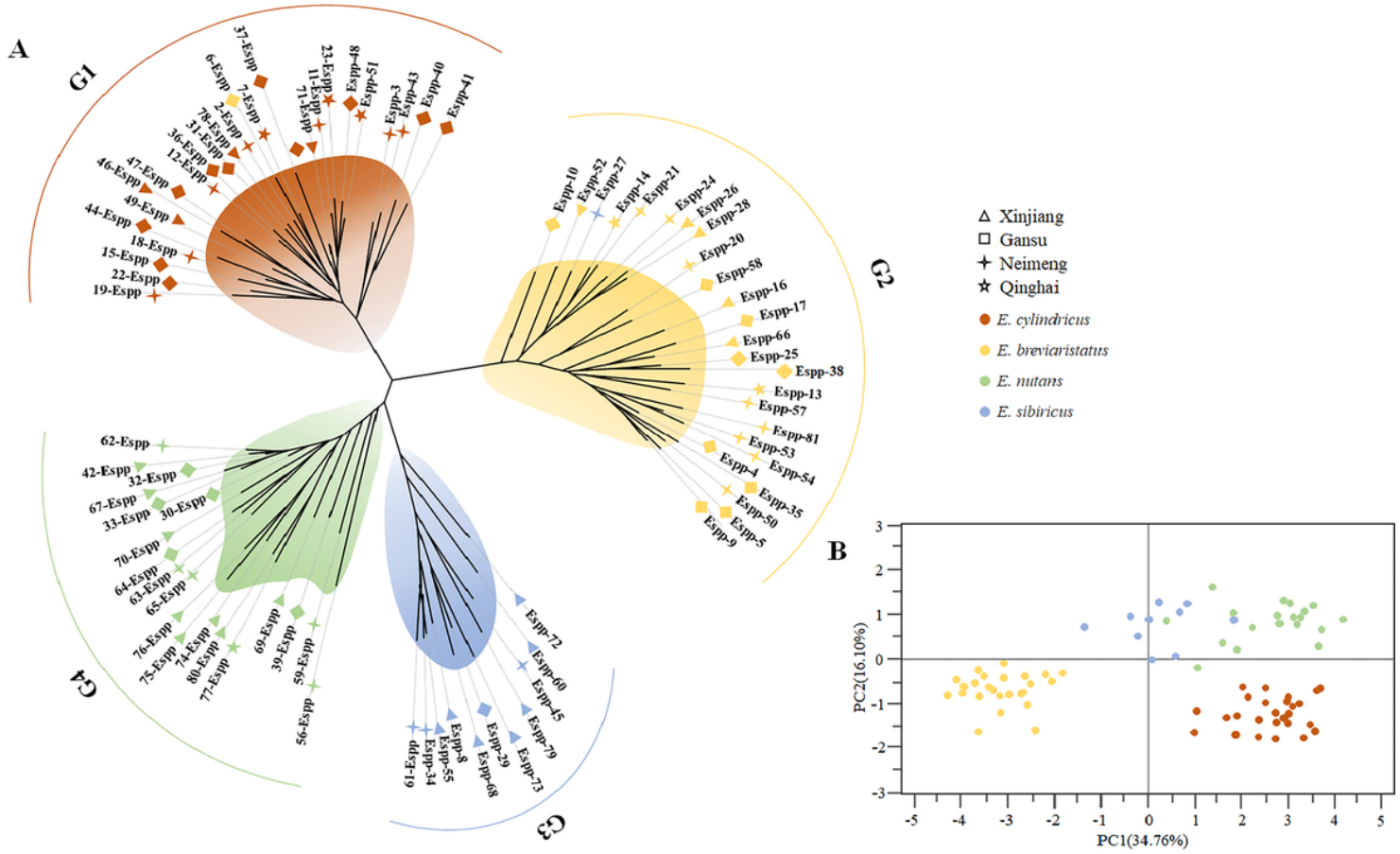

Figure 3

UPGMA clustering dendrogram (A) and PCA (B) for 81 *Elymus* accessions based on SSR polymorphism data." G" is an abbreviation for group, and G1-G6 represent different groups.

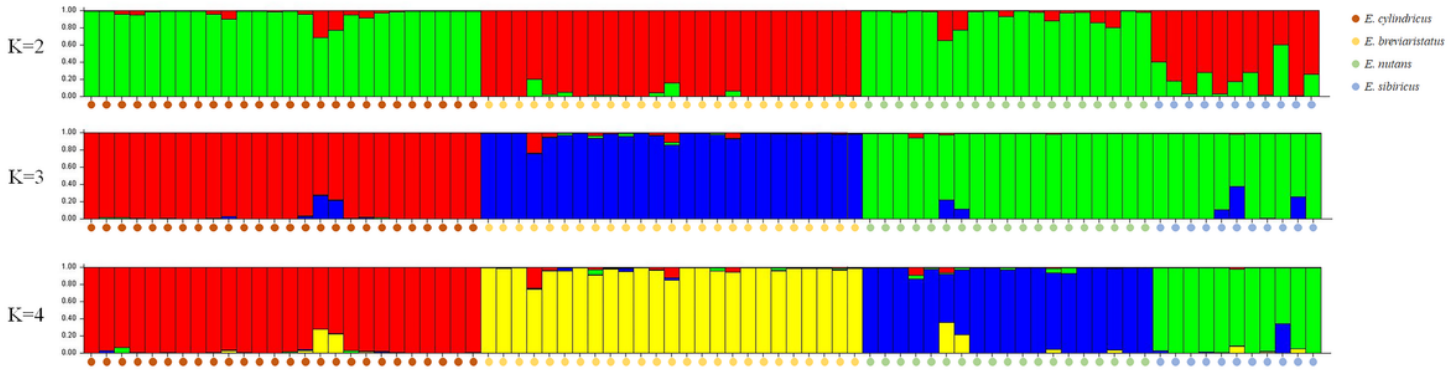

Figure 4

Population structure analysis of 81 *Elymus* accessions: population genetic structure of all accessions at K = 2, 3 and 4.

Supplementary Files

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