Germplasm evaluation of an Eurasia steppe native specie--sheepgrass (*Leymus chinensis)*

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Sheepgrass (*Leymus chinensis* (Trin.) Tzvel) is an advantageous perennial native grass in China and other northern Eurasian countries having steppe. As an important forage grass of great value in animal husbandry, sheepgrass is well known for its abundant foliage, high palatability and high nutritive content. Sheepgrass is also valuable in grassland restoration and conservation since it is a perennial grass with a rhizome network to fix the soil and can survive well in stressful environments. Terefore, the collection, evaluation and utilization of sheepgrass are necessary for protecting grassland biodiversity, for establishing artificial pasture, restoring degraded grassland, and the development of forage industry and animal husbandry in Eurasia's native steppe. Here, we reviewed our previous studies on the collection, evaluation of phenotypic diversity for germplasm resources, distribution and domestication of wild sheepgrass, and application of sheepgrass new varieties.

Introduction

Sheepgrass (*Leymus chinensis* (Trin.) Tzvel) is a vital species in Eurasian steppe which is widely distributed and utilized for grazing. It is a perennial gramineous plant belonging to *Leymus*, Triticeae, and Poaceae. As a most important forage grass of nutritional value in ruminant husbandry, sheepgrass is well known for its abundant foliage, high palatability and high crude protein content. It plays an important role in the maintenance of biodiversity, protection of ecological environment and grassland ecological environment and saline land in northern China (Wu et al. 2018) and Mongolia. Based on the morphological and developmental knowledge, the cultivation sheepgrass has become the focus of many grass breeders (Liu et al. 2022a). In recent decades, a variety of new cultivars of sheepgrass, such as "Zhongke," were domesticated from wild types and verified for grass and seed production (Liu et al. 2016). Recently, we demonstrated different utilization models, such as restoration and improvement of saline alkali land, desertification land, and degraded grassland, ecological restoration of soil erosion areas, and control of exotic harmful species of *Cenchrus spinifex* Cav, etc. (Liu et al. 2022b).

1. Distribution of wild sheepgrass

Sheepgrass is widely distributed at around 36° to 62° North latitude and 120° to 132° East longitude. It is mainly distributed and probably originates at the east end of the Eurasian steppe at altitudes 600 to 2 400 m. In China, sheepgrass is mainly distributed in the northeastern provinces, including Heilongjiang, Jilin, Liaoning, Inner Mongolia, Hebei, Shanxi, Beijing, Tianjin, Shaanxi, Ningxia, Gansu, Qinghai and Xinjiang. Sheepgrass also grows in Russia, Mongolia, North Korea, and Japan. Sheepgrass is distributed worldwide in an estimated area of 280 million ha, half of which is in China. It is mainly distributed in eastern pastures of Inner Mongolia and in the Songnen Plain in northeastern China (Figure 1).

Figure 1 Prairie of wild sheepgrass

2. Genomic composition and Phenotypic diversity of sheepgrass

On the basis of the morphological observation, meiotic chromosome pairing of inter-generic hybrids, DNA hybridization patterns, and variation in repeated nucleotide sequences, previous studies have concluded that one genome of sheepgrass (NsNsXmXm) originated from the genus *Psathyrostachys* (NsNs). Based on the sequences ITS and trnL-F, we concluded that the maternity of sheepgrass is the Ns genome, and the paternity is Xm genome (*Psathyrostachys*) (Liu et al. 2008). This species consists of yellow-green and gray-green leaf color types (Figure 2). The two ecotypes show mosaic distribution and share the same geographic, climate, and soil conditions at the wild site. According to taxonomic revisions, the genus *Leymus* Hochst comprises approximately 65 polyploid taxa that range from tetraploids (2n = 4x = 28) to octoploids (2n = 8x = 56) and dodecaploids (2n = 12x = 84) (Zhang et al. 2006; Sha et al. 2010; Tang et al. 2012). Additionally, genetic diversity in 37 morphological characters of 293 accessions was assessed in three successive years. Compared with the yellow-green type of sheepgrass, the grey-green type had significantly ($P < 0.05$) more genetic diversity. The highest genetic diversity index of accessions was observed from the region of longitude of 124-128°E, suggesting the most abundant germplasm of sheepgrass in this region (Liu et al., 2008). These findings could provide a solid framework to assess genetic diversity, breeding, evolution of sheepgrass in China.

Figure 2 A total of 293 accessions of sheepgrass. A: Gray-green type sheepgrass; B: Yellow-green type sheepgrass; C: Germplasm nursery of sheepgrass

3. Domesticated varieties and ecological restoration models of sheepgrass

Traditional breeding techniques (distant hybridization and recurrent selection) are adopted to domesticate and breed new varieties. The Institute of Grassland Research of CAAS worked with Heilongjiang Animal Science Institute to create the Northeastern sheepgrass variety with relative higher seed production. Inner Mongolia Agricultural University developed the "Nongmu" No. 1 sheepgrass variety through mass selection of individual plants for several generations, and it has higher seed setting rate. Keping Wang, a researcher in Jilin Biology Research Institute, selected the "Jisheng" No. 1-4 sheepgrass varieties with higher saline dolerance. From 2014 to 2021, we selected 7 new varieties (Zhongke) with higher seed production,higher germination rate and higher hay yield, this provided a foundation for grassland ecological restoration. The varieties could be used for riching the seed bank of grassland, improving depleted natural pasture, and building water-saving ecological pasture on saline-alkali soil. The varieties have large potentials in the northern farming–pastoral mix zone, typical grassland, and saline region, and they can significantly improve the grassland ecological environment and governance of the saline land in northern China (Liu et al. 2019).

In recent years, sheepgrass cultivares have been planted in the pastures of Xinjiang, Gansu, Ningxia, Inner Mongolia, Hebei, Henan, and Heilongjiang in China, and have formed the basis for an ecological restoration technology model that can be promoted, replicated and applied. Based on our previous studies, different utilization models have been formed, including saline land ecological restoration, desertification land management, saline alkali land improvement, degraded grassland improvement, ecological restoration of soil erosion areas, and control of exotic harmful species *Cenchrus spinifex* Cav (Figure 3). We also summarized the mature technical points of different utilization models, providing scientific guidance for ecological restoration projects in the future (Dong et al. 2022).

Figure 3 Ecological restauration model of saline land. The first year A, second year B and third year growth of "Zhongke" sheepgrass.

4. Future sheepgrass breeding directions

Sheepgrass has a wide distribution, adaptability, high livestock value and ecological environment value, and the demand of the new variety and seeds is very strong in China. Many interesting and important works prospected in near future as followings:

4.1. One of The priorities is the seed production of actual cultivar in large quantities.Because since a long long time,this species produced small number of seed due to its low seed setting number.

4.2. Applying the seeds produced by new varieties to degraded grassland.In this way,we wish,not only restoring the degenerated grassland, but also reading the forage yield for local herdsmen.

4.3. Expand the area of ecological restoration in the native grassland,such as West region of Heilongjiang and Jilin provinces,East region of Inner-Mongolia,and the similar ecological areas.This is a very urgent issue because the ecological system in those areas are very critical in face of increasing uncertainty in future climate change.

4.4. The traditional breeding method is practice and still important, but Sheepgrass sexual life cycle is very long and the actual breeding efficiency is very low. Future researchers need new molecular tools such as marker-assisted breeding technology, gene editing technology etc.to enhance their breeding efficiency.

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