

Review



The Current Status, Problems, and Prospects of Alfalfa (*Medicago sativa* L.) Breeding in China

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Abstract: This paper reviews the current status, methodology, achievements, and prospects of alfalfa (*Medicago sativa* L.) breeding in China. There are 77 cultivars that have been registered in the country, these include 36 cultivars bred through breeding programs, 17 introduced from overseas, 5 domesticated from wild ecotypes, and 19 through regional collection/breeding programs. Cultivars have been selected for cold resistance, disease resistance, salt tolerance, grazing tolerance, high yield, and early maturity. Most of these cultivars have been developed through conventional breeding techniques, such as selective and cross breeding, and some are now being evaluated that have been developed through the application of transgenic technology. The major problems for alfalfa breeding in China include low success rate, slow progress to breed resistant varieties, lack of breeding materials and their systematic collection, storage and evaluation, lack of advanced breeding techniques, and low adoption rate of new cultivars. There are gaps in alfalfa breeding between China and the developed world. Improvement of alfalfa breeding techniques, development of cultivars with adaptations to different regions within China, and the protection and utilization of alfalfa gremplasm resources have been identified as major strategies to improve the efficiency of alfalfa breeding in China.

Keywords: China; alfalfa; varieties; breeding; selection; Medicago sativa L.

1. Introduction

China has abundant genetic resources of *Medicago* spp. with 12 species *M. sativa* subsp. *sativa* L., *M. ruthenica* L. Ledeb., *M. pubescens* Sirj., *M. archiducis-nicolai* Sirjaev., *M. platycarpos* L. Trautv., *M. sativa* subsp. *falcata*, *M. sativa* subsp. *x varia*, *M. polymorpha* L., *M. minima* L. Grnfb, *M. lupulina* L., and *M. orbicularis* L. Bart., which are mainly distributed in northern and southwest China [1].

M. sativa L. is a perennial species of the genus, originating in Asia Minor, Transcaucasia, Iran and Turkmenistan highlands. The common name "alfalfa" refers to the main species *M. sativa* subsp. *sativa* but may also describe *M. sativa* subsp. *falcata* and *M. sativa* subsp. *x varia* (a hybrid between subsp. *sativa* and subsp. *falcata*) which is closely related to *M. sativa* in morphology. Among the *Medicago* species, *M. sativa* has the largest cultivation area in the world at present because of its numerous superior traits such as cold resistance, salt tolerance, wide adaptability, high yield, good herbage quality, resistance to frequent cutting, good persistence, soil amelioration, and economic benefit. It is therefore regarded as "the queen of forages". As the concept of "Pasture-based livestock industries" gained prominence in China in recent years, and with the application of new policies such as subsidy for grassland ecological protection, revitalization of alfalfa for dairying, and conversion of crops to forest and grassland, *M. sativa* has become the most widely used species in integrated farming systems, grazing, and ecological conservation in China. It is therefore imperative to develop new alfalfa varieties

to meet production targets under local conditions, which presents both opportunities and challenges for alfalfa breeding. Although there have been previous reviews of alfalfa breeding in China [2–4] these have not been widely available to an international audience, this review seeks to provide an updated review of the status of alfalfa breeding in China and a comment on the future needs for research and development.

2. Alfalfa Breeding in China

Forage breeding in China commenced about half a century later than the developed world. Until 1949, only a few scientists collected and evaluated wild forage germplasm for agronomic adaptation. Modern herbage breeding programs commenced in the 1950s and have been developed extensively since the 1980s. The National Committee for the Examination and Approval of Pasture Variety (NCEAPV) was established officially in 1986, and has promoted the selection and development of new pasture varieties, the collection and domestication of local ecotypes, and the introduction of foreign forage germplasm.

As of 2015, a total of 77 Alfalfa varieties were registered by NCEAPV, including 36 domestic varieties, 17 introduced varieties, 5 domesticated varieties, and 19 local varieties [5,6]. These do not include varieties registered by regional authorities (provinces and autonomous regions).

Varieties developed in China can be divided into eight categories based on their main characteristics (Figure 1), which have been described briefly below:

- 1. *High yield*: such as cv. Gannong 3 and 4;
- 2. *Disease resistance*: such as cv. Zhonglan 1, which has high resistance to downy mildew (*Peronospera trifoliorum*), and moderate resistance to brown leaf spot and rust disease, with resulting increases of herbage production of ca. 30% compared with cv. Longdong in the presence of these diseases. The resistance to downy mildew and brown leaf spot of cv. Xinmu 4 is better than cv. Xinjiang Daye;
- 3. *Cold resistance*: such as cvv. Longmu 801, Longmu 803, Longmu 806, Longmu 808, Caoyuan 1, Caoyuan 2, Caoyuan 3, Tumu 1, Xinmu 1, Xinmu 3, and Chicao 1. Most of these have been developed for planting in high latitude and high altitude areas in North China;
- 4. *Salt tolerant*: such as cvv. Zhongmu 1, Zhongmu 3, and Zhongmu 5; these have more than 10% higher yields compared with control cultivars when planted in saline-alkali soil;
- 5. *Grazing tolerance*: creeping-rooted types alfalfa such as cvv. Gannong 2, Zhongmu 2, and Gongnong 3. This suitability to grazing is conferred through strong root systems and greater ground cover. These cultivars are also highly suitable for conservation of water and soil, wind-breaks and sand/dune stability, soil reinforcement, and slope protection which represent other important areas of use for alfalfa in the pastoral regions of China;
- 6. *Early maturity*: such as cv. Xinmu 2, which matures earlier (about three to five days) than cv. Xinjiang Daye, and has high rates of regrowth following grazing;
- 7. *Lower fiber concentrations*: such as cv. Gannong 7 which has Neutral Detergent Fiber(NDF) and Acid Detergent Fiber (ADF) concentrations 2 percentage points lower than that in other alfalfa, and crude protein 1 percentage point higher than other cultivars, and hence improved palatability;
- 8. *Insect resistance*: such as cv. Gannong 5, with high levels of resistance to aphids with yields increased 14.22% [7] compared with the control cv. Golden Empress, cv. Caoyuan 4 is a variety developed for regions with serious thrip infestations.



Figure 1. The number and reported general attributes of alfalfa varieties bred in China.

The introduction of new alfalfa cultivars from overseas has been increasing with the rapid development of the alfalfa industry in China. The number of alfalfa varieties imported to China has increased dramatically to a current level of approximately 400. There are presently 17 varieties registered by the NCEAPV, including cvv. AmeriGraze 401+Z, Derby, Sanditi, and WL525HQ. (Table 1). This has been attributed to the improved production and forage quality that can be directly utilized for animal production. These importations also contribute to the pool of the alfalfa germplasm resources for alfalfa breeding in China.

Num.	Cultivar	Country	Main Characteristics
1	AmeriGraze 401+Z	USA	Cold tolerance, regeneration
2	Derby	France	Lodging resistance, cold tolerance, high yield
3	Eureka	Australia	Heat tolerance
4	Golden Empress	USA	Cold tolerance, drought tolerance, regeneration
5	Crown	USA	Cold tolerance, drought tolerance, regeneration
6	Sanditi	France	Lodging resistance, cold resistance, yield
7	Sitel	France	Lodging resistance, yield
8	Victor	Canada	Drought tolerance, insect resistance, heat tolerance
9	Victoria	Canada	Moderate cold tolerance, heat tolerance
10	WL232HQ	USA	Cold tolerance, regeneration
11	WL323ML	USA	Cold tolerance, yield
12	Rambler	Canada	Cold tolerance, drought tolerance, grazing tolerance
13	Caribou	Canada	Cold tolerance
14	WL525HQ	USA	Heat tolerance, yield
15	Wisdom	USA	Heat tolerance
16	WL343HQ	USA	Resistance to diseases and insect pests, cold tolerance, regeneration, frequent cutting tolerance
17	Qiuliu	Russia	Cold tolerance, drought tolerance, saline-alkali soil tolerance

Table 1. Introduced *Medicago sativa* varieties registered by the National Committee for the Examination and Approval of Pasture Varieties.

Local varieties are mainly distributed in Xinjiang, Inner Mongolia, Gansu, Shaanxi, Shanxi, Hebei, Shandong, Heilongjiang, Jiangsu, and Yunnan provinces. They have resistance to environmental stresses, for example, cv. Zhaodong and cv. Aohan are resistant to cold.

Alfalfa has been cultivated for more than 2000 years in China; a nation with vastly diverse climate, soil, and other ecological conditions across different regions. Under the long-term influence of natural conditions and farming conditions in different parts of the country, the formation of the local varieties has primarily been achieved by natural selection (Local varieties, Table 2). These local varieties play a significant role in production, are extremely valuable germplasm resources, and have strong resistance, wide adaptability, and yield stability.

Num.	Cultivar	Region	Main Characteristics
1	Beijiang	Xinjiang	Drought tolerance, cold tolerance, poor regeneration
2	Xinjiang Daye	Xinjiang	Large leaves, good regeneration
3	Hexi	Gansu	Late maturing, poor regeneration
4	Longdong	Gansu	Drought tolerance, poor regeneration
5	Longzhong		Drought tolerance
6	Tianshui	Gansu	
7	Guanzhong	Shanxi	Early regrowth, early maturing
8	Shanbei	Shanxi	Late maturing, drought tolerance
9	Jinnan	Shanxi	Good regeneration, early maturing
10	Pianguan	Shanxi	Late maturing
11	Aohan	Inner Mongolia	Drought tolerance, cold tolerance, suitable for dryland cultivation
12	Junggar	Inner Mongolia	Drought tolerance, suitable for dryland cultivation
13	Cangzhou	Hebei	Comparative tolerance to saline-alkali soils
14	Weixi	Hebei	Drought tolerance
15	Baoding	Hebei	Good regeneration
16	Zhaodong	Heilongjiang	Cold tolerance
17	Huiyin	Jiangsu	Heat tolerance, early maturing
18	Chuxiong	Yunnan	Annual or biennial, high nitrogen-fixing ability, cold tolerance, drought tolerance
19	Wudi	Shandong	Highly salt tolerant

Table 2. Local alfalfa varieties registered by the National Committee for the Examination and Approval of Herbage Varieties.

The five wild varieties registered for cultivation are cvv. Aletai, Longdong, Hulunbeier, Deqin, and Qingshui. Among them, cv. Qingshui is the first rhizomatous alfalfa registered in China, and plays an important role in production for some regions. The width of the crown is 25–30 cm with well-developed underground horizontal roots, which enables the variety to produce abundant shoots (25–46 shoots/plant) and to have a strong regenerative ability following grazing.

3. Breeding Methods

Alfalfa is a perennial predominantly cross-pollinated plant; self-fertility is affected by many factors. Morphological features and physiological characteristics prevent alfalfa from self-pollination which results in low selfing rates. Hence, alfalfa is classified as a cross-pollinated species, and its natural cross-pollination rate is between 25% and 75%. As an autotetraploid, it has its specific requirements in breeding methods and selection of offspring that are different to diploid plant species; these factors have also contributed to the delay in utilizing some modern breeding technologies in alfalfa compared to major food crops. Currently selective breeding, cross breeding, male sterile line breeding, space breeding, and biotechnology-assisted breeding have been adopted for alfalfa breeding in China, among which selective breeding and cross breeding are the most widely applied.

3.1. Selective Breeding

Selective breeding is the selection of improved varieties and genotypes with advantageous characteristics to develop new varieties with specific breeding objectives. It is an important method to improve both existing cultivars and breed new varieties from germplasm collections or other sources. The methods of selective breeding include individual selection, mass selection, improved mass selection, group selection, and recurrent selection etc. [8]. There are a large number of new varieties developed and registered in China using this method, including cvv. Zhongmu 1, Zhongmu 2, Zhongmu 3, Gongnong 1, Gongnong 2, Gongnong 3, Xinmu 1, Xinmu 2, Xinmu 3, and Gannong 2. Selective breeding is practical, effective, manageable, and is one of the most commonly used methods in alfalfa breeding in China.

3.2. Cross Breeding

Cross breeding uses cross-pollination between different species or sub-species to breed varieties by selection from the hybrid progeny. It can recombine the parents' genes to form a variety of different types and provide abundant material for selection. Genetic recombination allows the accumulation of desirable alleles from parents that differ for major traits. Choosing the correct parents and making suitable combinations are critical for cross breeding. Among alfalfa varieties that have been bred using cross breeding and registered in China are cv. Gannong 3 and Tumu 2. *M. sativa* subsp *x. varia* cvv. Caoyuan 1, Caoyuan 2, Tumu 1, and Gannong 1 were developed through crossing subsp. *sativa* and subsp. *falcata.* Intergeneric reciprocal hybridization combined with radiation breeding techniques were used to develop *Melilotoides ruthenicus* Sojak hybrids cvv. Longmu 801 and Longmu 803.

3.3. Male Sterile Line Breeding

Male sterile line breeding is where the male organs in flowers do not function to produce pollen whereas the female organs function normally. No seeds of male sterile lines are produced by self-pollination but seeds can be produced when pollinated with other non-male sterile lines.

The first alfalfa male sterile line was found in Canada in 1958. Since then researchers in the USA, Russia, Hungary, Bulgaria, France, and Japan used male sterile lines in their breeding programs. Petkov discovered that the maintainer line (line to help maintain the male sterile lines) for sterile line breeding in 1979. Other researchers conducted hybridization experiments with the sterile line and obtained excellent hybrids, which showed obvious heterosis. Herbage yield increased by 30% compared with control species, with improved resistance to stress. From the 1980s, researchers have studied a range of aspects related to the basic biology and agronomic suitability of male sterility in alfalfa breeding including pollen sterility and fertility, regarding the characterization of male sterile lines, and investigated the sterility mechanism of alfalfa pollen. More recently, Hybrid force-620 that was bred with male sterile line technique by Dairyland company in the US has been widely sown by dairy farmers due to its high yield and improved quality [9].

In China, Yongfu Wu [10] from Inner Mongolia University selected and bred six male sterile lines from *M. varia* Martin. cv. Caoyuan 1; scientists from the Institute of Animal Science, Chinese Academy of Agricultural Sciences developed three male sterile lines from *M. sativa* cv. Atlantic Ocean; and scientists from the Institute of Grassland Science, Jilin Academy of Agricultural Sciences pioneered the male sterile variety test in alfalfa and obtained F1 seeds under open pollination [2]. However, there has been no practical use of male sterile line technology in the development of new cultivars of *M. sativa* in China.

3.4. Biotechnology Assisted Breeding

Biotechnology assisted breeding is the use of genomic techniques such as molecular markers and transgenesis to incorporate new traits or increase the rate of genetic gain [11–14]. Research on biotechnology assisted breeding for alfalfa in China began in late 1970s. We used tissue culture and somatic cell hybridization initially, and then moved to the development and application of transgenesis and molecular markers. At present, studies are focused on the transformation and expression of genes related to stress resistance and tolerance. Although there are few varieties developed using this technology to date, the methods adopted in China are briefly summarized below.

3.5. Transgenic Technology

Different genetic transformation methods significantly affect the efficiency of exogenous genes transfer into *M. sativa*, which is the basis for the acquisition and development of transgenic plants. There are three main methods in genetic transformation of *M. sativa*, germplasm line transformation, direct transformation, and indirect transformation. Germplasm line transformation method uses a plant's own pollen, ovary, and other germ cells to introduce exogenous genes, such as via the

ovary injection and pollen tube method. Direct transformation methods use chemical or physical methods to introduce exogenous DNA into plant cells and obtain transgenic plants. Biolistic, ultrasonic, polyethelene glycol (PEG)-mediated, and microinjection are all examples of direct transformation methods. Indirect transformation methods use carriers to introduce DNA into the plant cell, and Agrobacterium-mediated transformation is the major example of this method.

In genetically modified breeding for alfalfa salt tolerance [15], Li et al. overexpressed the Alfinl transcription factor in alfalfa via Agrobacterium-mediated transformation, which enhanced the expression of endogenous gene MsPRP2 and improved the salt tolerance of transgenic alfalfa plants. Liang et al. [14] studied several factors that influence the efficiency of Agrobacterium-mediated genetic transformation in alfalfa and obtained transgenic plants expressing the betaine aldehyde dehydrogenase (BADH) gene. Yan et al. [16] showed that the BADH gene can enhance the salt tolerance of alfalfa. Wang et al. [17] used the vacuum infiltration-aided Agrobacterium-mediated method to transfer salt tolerance gene AtNHXl into Alfalfa and obtained salt tolerant somatic embryos. Pan et al. [18] used Agrobacterium EHA105-mediated cotyledon dissemination method to transfer Cg-NHXl gene into cv. Xinjiang Daye. Zhao et al. [19], transferred the Atriplex dimorphostegia NHX salt tolerance gene into aseptic seedling leaves and cotyledons of cv. Xinmu No.1 and cv. Xinjiang Daye, and obtained salt tolerant plants. Li et al. [20] studied genetic transformation of M. sativa and transferred DsNRT2 (a Nitrate Transporter) that was cloned from Dunaliella salina into cv. Zhongmu No.1 and 7 with positive seedlings obtained. Liu et al. [21] transferred the HAL1 gene that was cloned from yeast into the embryogenic callus of cv. Longmu 803 with the Agrobacterium-mediated method and obtained 11 transgenic plants. Expression of HAL1 gene improved the salt tolerance characteristics of alfalfa. Sui [22] studied a vacuolar Na⁺/H⁺antiporter gene (ScNHXI) from Suaeda corniculata, which was introduced into *M. sativa* and improved salt tolerance of transgenic alfalfa. Sheng [23] successfully introduced the DREB2A gene into a range of alfalfa genotypes cvv. Zhaodong, Aoha, Gongnong 1, and Gongnong 2, primarily from Heilongjiang province with the aim of improving drought tolerance characteristics. Wang et al. [24] transferred the LEA3 gene from barley into cv. Zhongmu 1 through biolistic transformation and obtained an increased survival rate of transgenic plants under high salt stress. The salt tolerance of the transgenic plants was greater than that of the control plants, which shows that *LEA3* gene has potential for application in developing drought resistance and salt tolerance for alfalfa breeding. The improvement of salt tolerance was also the aim of Bao et al. [25] where the transgenic co-expression of tonoplast Cation/ H^+ antiporter and H^+ -pyrophosphatase genes from the xerophytic plant Zygophyllum xanthoxylum increased the growth and salt tolerance of genetically modified alfalfa plants under field conditions. The use of transgenesis to introduce genes into alfalfa to confer stress tolerance outside of the range that is presented within sexually compatible species offers the potential to greatly increase the adaptation of cultivated alfalfa in stressed and degraded environments in China.

The recent advent of transcriptomics and next generation sequencing technologies offers the potential to identify genes involved in stress tolerance on a broader scale than previous technologies. Deep sequencing of the transcriptome of alfalfa was used recently to identify 5605 differentially expressed genes in the crown buds of cv. Zhaodong when grown under naturally occurring freezing stress in northern China [26]. These studies may identify further candidates for deployment using transgenic or genome editing technologies.

Due to various constraints, the further development of transgenic alfalfa in China has not been widely carried out. Although progress and achievements have been made, most are still at the laboratory experiment phase. Currently there are no transgenic alfalfa cultivars developed and adopted in practice.

3.6. Molecular Marker Technology

In stress resistance breeding, Yang et al. [27] used *M. sativa* L. cv. Zhongmu No. 1 and salt sensitive alfalfa as materials to screen from and build up the basis for research on salt tolerance

genes for molecular marker development in alfalfa, using the Random Amplified Polymorphism (RAPD) technique. In disease resistance breeding, Gui et al. [28] used RAPD technique and Bulked Segregation Analysis to study molecular markers linked to resistance genes against brown spot disease in five Medicago species, and selected eight random primers which are able to indicate polymorphism between resistant and susceptible materials in more than three species simultaneously. Wei [29] used RAPD, Simple Sequence Repeat (SSR), and Inter Simple Sequence Repeat (ISSR) molecular marker and field experiments to study DNA fingerprints and genetic diversity of 84 alfalfa lines. Xu and Jia [30] regenerated somatic hybrid plants between alfalfa and sainfoin by protoplast fusion and culture, and extracted DNA from leaf material from regenerants of hybrid tissue; they also analyzed the recombination of the genetic materials by RAPD and Southern hybridization techniques. Yu et al. [31] combined SSR and Expressed Sequence Tag derived SSR (EST-SSR) molecular marker techniques to construct molecular marker profiles of alfalfa germplasm. Su et al. [32] established a tetraploid F2 mapping family in a hybrid between subsp. sativa and susbsp. falacata, and constructed a genetic map of this population. Fu and Pauls [33] used 20 imported alfalfa accessions and demonstrated that RAPD markers could determine the relationship between alfalfa varieties. SSR marker technologies have been used to characterize and contrast the genetic diversity within and between cultivars and landraces of Chinese origin with those from other geographic regions, thereby providing insights into the nature of population structure and variation that could be used in a genomics assisted breeding program [34] Again, there have been no bred varieties of alfalfa registered in China using these technologies to date.

3.7. Space Breeding

Space breeding, also called space technology breeding or spaceflight breeding, takes advantage of space environment which has been known to induce physiological changes in plants and heritable mutations, although the mechanisms through which these mutations occur are not fully understood [35,36]. Space breeding has been performed in China since 1987 with recoverable spacecraft allowing the recovery of plant material. Space breeding has resulted in new cultivars of rice, wheat, and soybean, and although the efforts have focused on major food crops approximately 1% of the seed accessions in China's space breeding program have been pastures [35]. Ren [37] studied alfalfa seed carried by recoverable satellite using Fourier Transform (FT)-Raman spectroscopy, which showed that the content of DNA and Ca^{2+} increased and that of sugar and lipid decreased compared with the control seed on the ground. Zhang [38] analyzed leaf microstructure of four alfalfa varieties grown from seeds carried by Shenzhou-3 spacecraft in comparison with control plants, and found that leaf thickness and palisade tissue thickness of the varieties are much greater than that of the control plants, with their leaf protuberance degree being much less than the control plants. In addition, the cell structure tightness of the four alfalfa varieties was different from the control plants. These mutations may have influenced its resistance performance, and could be used for further resistance breeding. Zhang [39] studied the space mutation of cv. Longmu 803 and cv. Zhaodong with their seeds carried by recoverable satellite. The results indicated that the types of alfalfa chromosome aberration produced by space mutation were mainly the formation of micronuclei. Visible mutations occurred in mitotic cell chromosomes due to space mutation effects.

Space breeding technology has been widely used in the breeding of other crops; however, there is no alfalfa variety registered on a national scale. Nevertheless, there are several varieties registered in provinces or autonomous regions of China.

4. Problems and Challenges

Although China has made considerable achievements in alfalfa breeding, there is room for improvement compared with other countries, and the overall level of alfalfa breeding is still low and cannot meet the requirements of development in grassland agriculture, grassland animal husbandry, and ecological reconstruction. The main problems and challenges are discussed below.

4.1. Slow Breeding Cycle

The number of varieties bred in China is relatively low, and the progress of resistance breeding is slow. Up to date, only 36 bred Alfalfa varieties have been registered by the National Committee for the Examination and Approval of Herbage Variety in China, compared with 192 alfalfa varieties registered in the USA in 2015. The breeding target is more focused on yield in China, and overwintering rates has also become one of the priority breeding targets due to northern China being one of the major regions of *M. sativa* plantation. Hence, the target characteristics of bred varieties include high yield and high overwintering rates, and to a lesser degree, cold tolerance and fall dormancy. In disease and insect resistance, the main focus is on pathogen identification and field investigation of disease conditions [40,41], disease resistance identification methods, and the influence of environment on alfalfa resistance performance. There are few varieties that have been specifically bred for disease resistance, insect resistance, and drought tolerance, and almost no varieties for herbicide resistance.

4.2. Breeding Methods

At present, alfalfa varieties registered in China are mainly bred with conventional breeding methods. These methods are simple and widely adopted, and take advantage of excellent germplasm resources in different ecological regions to develop new varieties suitable for regional conditions. However, these methods normally take a long time to develop cultivars that are used on farms. The application of genetic engineering and other modern biotechnology in alfalfa breeding has been put in place in China for only 20 years, and research is currently focused on target gene cloning, construction of expression vectors, and related laboratory experiments with the commencement of some field trials.

4.3. Low Adoption Rate of Bred Varieties

The supply chain for seed production, distribution, and marketing of seed is less well developed in China than some other countries where specialized breeding companies are often involved and responsible for this process. In China, however, alfalfa breeding resources are mainly centered in universities and research institutes. As a result, the breeding targets are not well placed to meet the market demands, and breeding research is disconnected with business development and without effective involvement from industries, thereby leading to poor adoption of new varieties [42]. Largely due to the slow process of seed production of new varieties, most enterprises choose to purchase imported varieties, further reducing the adoption rates of newly bred varieties in China.

5. Opportunities and Further Research

In recent years, the collection of germplasm resources, innovation of breeding theory and technology, and molecular biology assisted breeding have played an important role in accelerating alfalfa breeding in China. These experiences confirmed that we could learn from the experience of developed countries and take integrated approaches in enhancing alfalfa breeding in China.

5.1. Strengthening the Research and Utilization of Alfalfa Germplasm Resources

Many developed countries give great emphasis on the collection, preservation, research, and utilization of germplasm resources. China covers a large territory with very diverse ecological environments due to the influence of latitude, elevation, and topography. This makes the country rich and unique in alfalfa germplasm resources, including conventional local and wild types, improved varieties, and new breeding materials such as alfalfa mutants, all of which form the basis of alfalfa breeding. In addition to the development of breeding methods and the adoption of new technology, a critical step for the success of modern breeding programs is to widely collect and make use of the right germplasm resources. Due to poor awareness in germplasm protection, overgrazing, and excessive exploitation, some excellent forage germplasm resources have been lost. Lack of desirable germplasm

resources for screening and breeding has seriously affected the process and results for developing new varieties. Therefore, it is imperative to learn from this experience, conduct further research and identification of existing germplasm in a systematic way, screen materials with desirable characteristics, and conduct research in an innovative manner [43].

5.2. Improving Alfalfa Breeding Theory

In the developed world, great efforts have been made to develop the basic theory of breeding. Forage breeding in China is not only behind developed countries, but also behind crop breeding domestically. This is largely due to lack of advanced forage breeding theory and technology. In order to make a breakthrough in alfalfa breeding, we need to strengthen research on forage breeding theory and techniques; otherwise, slow breeding cycle/process will be unavoidable.

5.3. Enhancing the Exploration of New Techniques in Alfalfa Breeding

Conventional breeding methods are basic methods to breed new variety all over the world and will continue to play an important role in alfalfa breeding in China in the near future. With the rapid development of science and technology, molecular technology has been used to improve been widely used in alfalfa breeding worldwide. Transgenic technology has been used to improve the resistance of alfalfa to cold, drought, salinity, alkalinity, disease, herbicides, and insect pests. Molecular technology has been widely used in assisted alfalfa breeding and germplasm introgression research, genetic linkage map, germplasm identification, and genetic diversity studies. The combination of conventional breeding techniques with modern breeding techniques can help to break through some of the constraints and speed up the breeding cycle, which is critical for improving alfalfa breeding technology in China.

5.4. Conservation of Distinctive and Special-Purpose Alfalfa Materials

Alfalfa breeding in China has the following characteristics: broad cultivation area, complex ecological conditions, and a wide range of uses in production. According to local conditions and uses, specific alfalfa varieties can be selected and cultivated. For example, in germplasm collection and application in western China, research should focus on germplasms that are resistant/tolerant to drought, cold, and saline soils of sandy texture. In these regions, new varieties that are able to prevent wind erosion and fix sandy soils, and to conserve water and soil resources could be the major breeding targets for alfalfa. On the other hand, improved animal production through high forage production and better forage quality could also be a high priority target. Therefore, selection and breeding of special-purpose varieties that fit both the environment/soil constraints and production requirements for various feeding systems, such as hay and silage, and different growing seasons are necessary to address regional production and sustainability issues [2]. Strategies must be worked out on a regional basis to achieve specific needs.

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