

DISCIPLINE SYSTEM OF ALFALFA BIOLOGY: FROM MULTIOMICS TO BIOLOGICAL BREEDING

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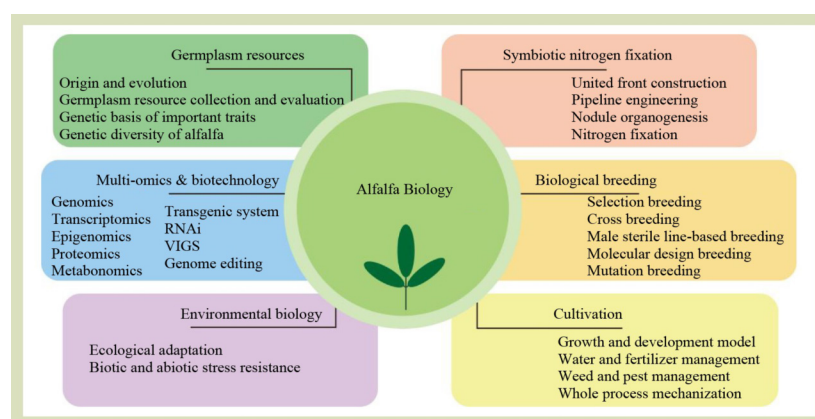
KEYWORDS

alfalfa, discipline system, forage biology, lucerne, *Medicago sativa*

HIGHLIGHTS

- This review systematically raises the subject concept of alfalfa biology.
- The discipline of alfalfa biology has been divided into six major sections.
- The recent advances from the perspective of discipline system have been reviewed.

GRAPHICAL ABSTRACT



ABSTRACT

Alfalfa (*Medicago sativa*) is the main leguminous forage crop with great ecologic and economic value. The research of alfalfa in various fields has exploded, but has not been included in a systematic framework. This paper summarizes the status of global alfalfa research over the past 10 years, raise the subject concept of alfalfa biology, and review the recent advances from the perspective of discipline system as germplasm resources, multiomics and biotechnology, environmental biology, symbiotic nitrogen fixation, biological breeding and cultivation. This paper proposes the key unsolved scientific and technical issues in alfalfa biology, and hope to appeal the research interest of more plant scientists and to promote the development of alfalfa industry.

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1 INTRODUCTION

Alfalfa (*Medicago sativa*), known as the king of forage, is the main cultivated member of the genus *Medicago* (Fabaceae), composing of the basis of dairy and husbandry^[1,2]. It has been widely grown all over the world on about 30 Mha, providing high-quality feed for cattle, horses and other animals^[1]. Alfalfa has rich nutritional value, especially high protein content, which can be up to 25%. Its fiber composition is also suitable

for ruminant health, with appropriate rate of digestion. Alfalfa can regenerate rapidly after cutting, so that cultivation of alfalfa can provide attractive economic benefits. It also has the capability of symbiotic nitrogen fixation with rhizobia, which can reduce N fertilizer application and have a positive effect on ecological environment. However, alfalfa production still faces numerous challenges, such as suitable cultivar breeding and selection, environmental limitations, coordinated farming systems, etc.^[1,3]. A better understanding of alfalfa from the

macro to the micro scales will help solve these problems and enhance productivity. Though research advances on numerous aspects have been made in alfalfa and its related species, and several review articles have competently summarized various specific aspects^[3-7], it is necessary to study alfalfa biology holistically and systematically for better understanding and further utilization. This paper proposes the subject concept of alfalfa biology, dissect the discipline of alfalfa biology according to the recent research progress, and put the remaining key scientific and technical issues forward to promote the development of alfalfa research and industry.

2 GLOBAL ALFALFA RESEARCH STATUS OVER THE LAST DECADE

Global alfalfa research has thrived over the last decade. From the statics of published paper, the US led alfalfa research with the most publications and citation frequency from 2012 to 2016, and China ranked second (Table 1). However, China has surpassed the United States on numbers of publications and citation frequency from 2017 to 2021, suggesting a great improvement on the overall level of alfalfa research in China (Table 2). It also indicates that alfalfa research has been highly valued, which might be driven by food safety^[9]. The US Department of Agriculture is the major contributor to alfalfa research, with the most publications from 2012 to 2021

(Table 3 and Table 4). However, the citation frequency of publications from Chinese Academy of Sciences has greatly improved, suggesting a high quality of these publications (Table 4). Also, China Agricultural University ranked third on the numbers of publications and citation frequency from 2017 to 2021 (Table 4). In general, China has become the core force on alfalfa research, and Chinese research institutes have achieved considerable outputs.

3 COMPOSITION OF ALFALFA BIOLOGY

Alfalfa biology is not only concerned with the typical developmental characteristics of the species, but also its relationship with the environment and human farming practices. Consequently, here the discipline has been divided into the following six sections: germplasm resources, multiomics and biotechnology, environmental biology, symbiotic nitrogen fixation, biological breeding and cultivation (Fig. 1).

3.1 Germplasm resources

Medicago sativa includes several subspecific taxa with diploid or tetraploid genome, consisting of the diverse gene pool^[5].

Table 1 National or regional distribution and metrics of alfalfa related studies from 2012 to 2016 ordered by citation frequency

Country code (ISO 3166-1)	Publications ($\times 10^2$)	Citations ^a ($\times 10^3$)	Documents cited ^b (%)	CNCI ^c
USA	11.96	25.544	95.2	1.03
CHN	6.63	13.791	96.4	1.03
CAN	3.39	7.352	96.8	1.19
AUS	2.75	5.553	98.6	1.02
FRA	1.98	4.980	93.9	1.18
ESP	2.44	4.973	96.3	1.09
DEU	1.87	4.783	96.3	1.14
GBR	1.21	4.302	99.2	1.55
ITA	1.84	3.997	97.3	1.06
IRN	2.11	3.052	91.5	0.75
NLD	0.81	2.776	98.8	1.60
NZL	0.95	2.314	99.0	1.30
CHE	0.96	1.593	92.7	0.96
JPN	0.93	1.454	97.9	0.82
ARG	1.06	1.393	98.1	0.56

Note: ^a Rankings are based on citation numbers. ^b Percentage of documents that have been cited at least once. ^c Category normalized citation impact^[8].

Table 2 National or regional distribution and metrics of alfalfa related studies from 2017 to 2021 ordered by citation frequency

Country code (ISO 3166-1)	Publications ($\times 10^2$)	Citations ^a ($\times 10^3$)	Documents cited ^b (%)	CNCI ^c
CHN	14.63	11.034	78.7	1.05
USA	13.67	9.295	77.5	1.02
CAN	3.53	2.849	81.6	1.30
AUS	3.26	2.295	85.3	1.20
ESP	2.31	2.158	84.0	1.18
ITA	2.19	2.001	84.5	1.30
DEU	2.36	1.912	86.0	1.07
FRA	1.95	1.446	80.5	0.89
IRN	2.54	1.302	75.2	0.73
GBR	1.48	1.275	85.1	1.73
BRA	1.66	1.105	78.9	0.98
CHE	1.33	0.979	75.2	0.98
KOR	0.96	0.839	84.4	1.31
MEX	1.45	0.803	71.0	0.56
NLD	0.56	0.769	89.3	1.63

Note: ^a Rankings are based on citation numbers. ^b Percentage of documents that have been cited at least once. ^c Category normalized citation impact^[8].

Table 3 Research institution distribution and metrics of alfalfa related studies from 2012 to 2016 ordered by citation frequency

Research institution	Publications ($\times 10^2$)	Citations ^a ($\times 10^3$)	Documents cited ^b (%)	CNCI ^c
United States Department of Agriculture	3.18	5.770	92.8	1.03
Chinese Academy of Sciences	1.49	3.709	97.3	1.11
Agriculture & Agri Food Canada	1.64	3.131	97.6	1.13
France's National Research Institute for Agriculture, Food and Environment (INRAE)	1.22	3.057	95.9	1.24
Cornell University	0.75	2.786	97.3	1.36
Consejo Superior de Investigaciones Científicas	1.26	2.664	96.8	1.08
University of California System	0.85	2.527	100	1.32
Centre National de la Recherche Scientifique	0.72	2.456	98.6	1.36
Nanjing Agricultural University	0.65	2.307	100	1.67
University of Wisconsin System	0.96	2.201	95.8	1.14
University of Wisconsin Madison	0.90	1.947	95.6	1.10
University of Minnesota System	0.71	1.835	91.6	1.18
University of Minnesota Twin Cities	0.70	1.829	91.4	1.19
Chinese Academy of Agricultural Sciences	1.01	1.612	96.0	0.85
Noble Research Institute	0.42	1.543	95.2	1.42
Northwest A&F University	0.62	1.528	98.4	1.25
China Agricultural University	0.95	1.452	94.7	0.92
Commonwealth Scientific & Industrial Research Organisation	0.57	1.299	100	1.12
University of Western Australia	0.54	1.205	100	1.04
Islamic Azad University	0.36	1.040	75.0	0.75

Note: ^a Rankings are based on citation numbers. ^b Percentage of documents that have been cited at least once. ^c Category normalized citation impact^[8].

Table 4 Research institution distribution and metrics of alfalfa related studies from 2017 to 2021 ordered by citation frequency

Research institution	Publications ($\times 10^2$)	Citations ^a ($\times 10^3$)	Documents cited ^b (%)	CNCI ^c
Chinese Academy of Sciences (CAS)	2.69	2.756	80.3	1.20
United States Department of Agriculture	3.41	2.017	76.8	0.89
China Agricultural University	2.16	1.705	83.3	1.20
Nanjing Agricultural University	1.26	1.356	86.5	1.40
Consejo Superior de Investigaciones Científicas	1.01	1.311	91.1	1.33
Agriculture & Agri Food Canada	1.72	1.265	79.7	0.99
Chinese Academy of Agricultural Sciences	1.88	1.146	83.5	0.93
Northwest A&F University	1.20	1.129	80.8	1.26
University of California System	1.22	1.099	86.9	1.12
France's National Research Institute for Agriculture, Food and Environment (INRAE)	1.25	1.078	83.2	0.95
University of Chinese Academy of Sciences, CAS	1.09	1.041	78.9	1.36
Lanzhou University	1.53	0.913	69.9	1.01
State University System of Florida	0.72	0.710	80.6	1.82
University of Florida	0.68	0.698	80.9	1.90
University of Western Australia	0.63	0.634	82.5	1.22
Cornell University	0.59	0.615	81.4	1.08
Institute of Soil & Water Conservation, CAS	0.54	0.595	81.5	1.54
University of Wisconsin System	0.97	0.590	79.4	1.13
University of Wisconsin Madison	0.90	0.574	78.9	1.19
University of California, Davis	0.72	0.555	84.7	1.02

Note: ^a Rankings are based on citation numbers. ^b Percentage of documents that have been cited at least once. ^c Category normalized citation impact^[8].

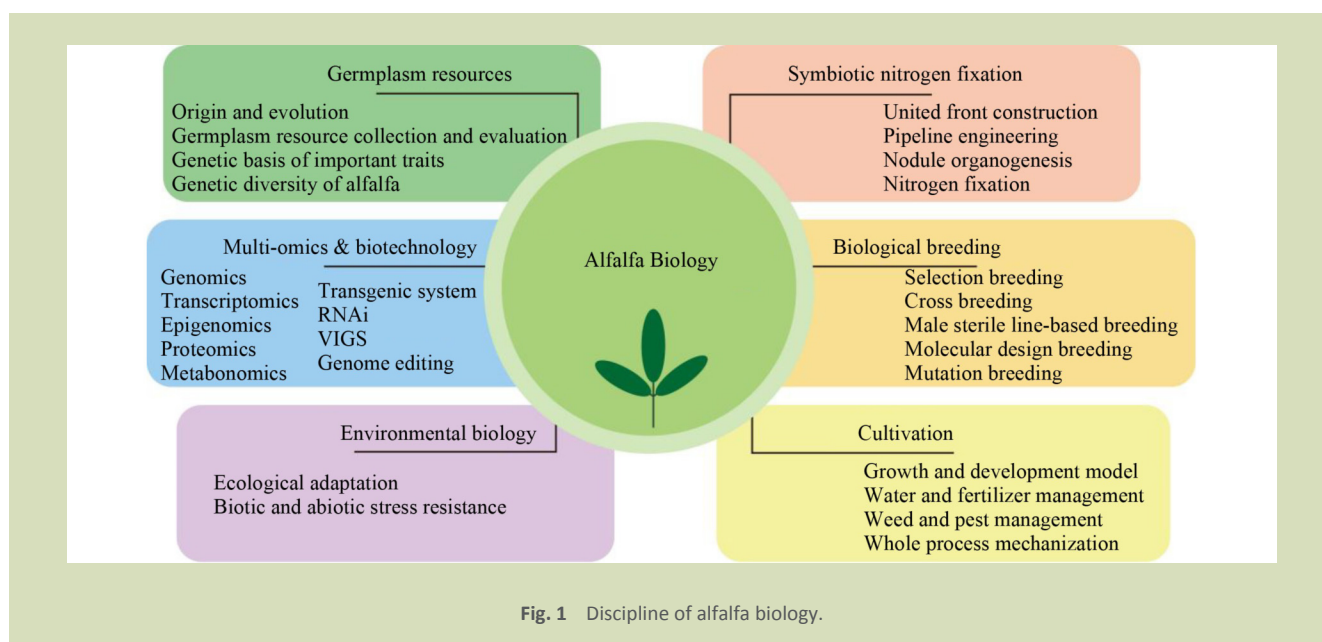
Among the species complex, cultivated alfalfa is a perennial outcrossing autotetraploid with high heterozygosity. Alfalfa and its related species germplasm resources are valuable for studies on the origin and evolution, excellent traits exploitation and genetic diversity maintenance.

The origin and evolution of alfalfa has been an intriguing subject for a long time. Based on germplasms resources and historical research and recent emerging genomics information, it has been a consensus that the genus *Medicago* originated in Mediterranean Basin, but the precise center of origin is still a matter of debate^[4]. The evolutionary trajectory of alfalfa remains unclear as well, though some researches considered subsp. *glomerata* as the ancestral taxa of the *M. sativa* complex, which is still lack of molecular evidence. Detailed discussion has been well reviewed by Şakiroğlu and İlhan^[10].

Related research institutes have been devoted to germplasm collection and restoration and further shared platform construction for decades. There are over 21,000 alfalfa

accessions in worldwide collections and over 47,000 *Medicago* spp. accessions other than *M. sativa*^[5]. The US Department of Agriculture National Plant Germplasm System (NPGS), the South Australian Research and Development Institute and the Russian Nikolai I. Vavilov Research Institute of Plant Industry contribute over 55% of the total alfalfa accessions, though the degree of redundancy among these collections has not been determined^[5].

For further efficient utilization of alfalfa and its related species germplasm resources, several platforms have been set up for accessibility. For example, the NPGS GRIN-Global descriptor site integrates multiple important descriptor traits of partial germplasms, especially the representative core collection, which provides useful evaluation information for the next phase of analysis of associated loci of agronomic traits^[11,12]. However, there are still a large amount of germplasms lack of phenotypic information, which is difficult to collect by standardized methods. The unified phenotypic test and description with well reproducibility and regional adaptability



is the major problem for germplasm resource evaluation.

Morphological and molecular markers have been widely adopted to assess genetic diversity, to analyze population structure, and to narrow down excellent agronomic traits-associated loci^[13–15]. With the development of new generation molecular markers from RFLP to SNP, the assessment becomes extensive and accurate^[11,13,16–19]. Unlike grain crops, alfalfa maintains a rich genetic diversity after a long period of cultivation and domestication, which ensures the stability and sustainability of the species and provides a precious gene pool for genetic improvement^[4,20]. For further breeding, the major challenge is to screen and isolate the target loci by functional verification and to introduce it into cultivars effectively through genetic manipulation.

3.2 Multiomics and biotechnology

As genome sequencing and other omics techniques are booming over recent decades, the connections between the molecules and the whole, individuals and the environment have been widely established. The related diploid model plant, *Medicago truncatula* A17, is the first published complete reference assembly in the genus *Medicago*^[21]. However, the whole genome sequencing and assembly was impeded in alfalfa because of its more complicated genome background and distinct physiologic features like outcrossing and partial self-incompatibility^[22,23]. Over the past 3 years, alfalfa genomics finally made breakthroughs with successful assembly of high-quality reference genome^[11,22,24–26]. There have been three complete tetraploid assemblies including XinJiangDaYe,

Zhongmu No. 1 and Zhongmu-4, which are all elite cultivars in China^[11,24,26]. The whole genomes of other related *Medicago* spp., including *M. polymorpha* and *M. ruthenica*, have also been sequenced and assembled, providing genome information for diverse evolution directions over the long-term^[27–29]. However, the accurate haploid genome assembly in alfalfa is still unresolved, which hinders pan-genome research and leaves a gap between the extensive phenotypic variations and genetic basis.

Aside from the whole genome sequencing, multiple omics approaches have been widely applied to study the functional genomics^[30]. Transcriptome assay has been one of the most commonly used methods because of its convenience and relatively low cost particularly over the past 10 years^[31]. Proteomics and metabonomics have also been involved, especially in biotic and abiotic stress research^[32–37]. However, these high-throughput experiments generated a substantial amount of data lack of effective interpretations, which should be updated with the complete alfalfa reference genome. In addition, it is difficult to identify the specific functional alleles by current methods because of autotetraploidy, while different alleles of the same gene may have different functional contribution for polyploids. Therefore, multiomics should be developed in synergy to uncover the complicated functional genome of alfalfa.

Biotechnology platform in alfalfa has been established over recent decades^[7,22]. Though a large number of techniques have been applied in other crops with ease, the species-specific optimization in alfalfa is necessary for its popularization and

application. Stable transgenic technique has been achieved in a number of specified genotypes such as Regen-SY4D, R2336, Baoding, Zhongmu No. 1 and XinJiangDaYe^[24,38,39], and the efficiency of transformation can be up to 80%. The transgenic technique has been used to generate two commercial cultivars with the excellent traits of herbicide resistance and reduced lignin, respectively^[7]. Stable transformation mediated RNA interference (RNAi), Virus Induced Gene Silencing (VIGS), and genome editing offers more flexible approaches to genetic manipulation in alfalfa. In particular, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 and its derivative system for genomic DNA editing has been an ideal breeding tool^[40]. The systematic optimization with endogenous genetic components from the related model, *M. truncatula*, improve its work efficiency in alfalfa^[41–43]. However, the identification of detailed mutation type is not easy because of its ploidy. An intelligent discrimination method is needed for the desired mutant screening. It is promising to introduce novel techniques in alfalfa, which is of great benefit to both theoretical and applied research.

3.3 Environmental biology

Alfalfa has been distributed worldwide and has evolved diverse environmental adaptation. As a perennial, its interaction with the environment can be divided into two cases: the elastic growth state with annual environmental changes and the resistant state under environmental biotic and abiotic stress. For the former state, for example, fall dormancy is a specific trait that reflects ecological adaptation. The decelerated growth and increased decumbency occurring in autumn is related to the genotypes of alfalfa as a result of evolution^[44]. Though dormancy happens in most perennials in winter to avoid injury caused by extreme cold, alfalfa takes precautions by fall dormancy in the relatively mild conditions of autumn, which sacrifices the autumn yield to some extent for farmers. It has been discussed for a long time how the relationship of fall dormancy and winter survival^[44–46]. Genetic analysis of segregating populations showed that fall dormancy is not linked with winter hardiness, which suggested a possibility to improve fall growth and winter hardiness at the same time^[46]. Recently, several genome-wide association studies of some natural populations screened the candidate genomic regions associated with fall dormancy and targeted a few candidate genes, but more evidence on the molecular level is needed to confirm the exact relationship between the complicated trait and these candidate genes^[11,26,47]. Transcriptomic and proteomic assays were also applied in research on fall dormancy for candidates screening^[48–50]. However, no clear conclusion could be made on how fall dormancy is regulated

and how the phenotypic gradient is generated in different genetic backgrounds.

For stress resistance, alfalfa and its relatives have also evolved great capacity to tolerate a range of biotic and abiotic stresses. However, further improving stress resistances of cultivated alfalfa is an important breeding target^[51], particularly in China, where the forage should not encroach on the land for food crops, which means that the cultivation conditions could not be ideal enough^[9]. The environmental stresses include drought, cold, salinity, pests and diseases. Since transcription factors are often actively involved in stress response and resistance regulation, series of studies conducted transcriptome assays on abiotic and biotic stress tolerance to screen the potential resistance genes^[31,51–54]. For example, the China Agricultural University (CAU) research team discovered that a lipid-anchored NAC transcription factor screened from the stress-related transcriptional profiling is translocated into the nucleus and activates Glyoxalase I expression in responding to drought stress^[55]. Recently, the CAU team found that this molecular mechanism might not be specific to drought stress, but also universal in other oxidative stress. Another example is the dehydrin MtCAS31(cold acclimation-specific 31), which was screened from the transcriptional data of the related model plant *M. truncatula*, enhances drought tolerance by overexpression to promote autophagic degradation of the cargo protein MtPIP2;7^[56,57]. Research on stress biology, especially to elucidate the detailed molecular mechanism, will contribute to further precise breeding and avoid introducing adverse traits.

3.4 Symbiotic nitrogen fixation

Alfalfa, as a legume forage, has the capability of symbiotic nitrogen fixation by interaction with rhizobia, which reduces the need for nitrogen fertilizers and benefits the ecological environment^[58]. Symbiotic nitrogen fixation is systematic engineering formed by the coevolution of legumes and rhizobia, consisting of the following stages.

The first stage is the interaction between plant root cells and rhizosphere free rhizobia including information and material exchange^[59], which could be called united front construction. Both the host plant and the rhizobia determine the symbiotic relationship at this stage, which might be dominant by the host over the evolutionary process. Though it has been reported that legume roots exude flavonoids as signals to rhizobia, other functional molecules may also participate in this stage^[59]. Small RNAs derived from rhizobia appropriate the host RNA-interference machinery and silence the host-expressed target genes to enhance nodulation^[60]. The reverse regulation

mechanism of the host-derived small RNAs on rhizobia has not been reported, which also has some possibilities to exist. In addition, the free small RNAs is not stable, so that a corresponding loading system also needs to be equipped^[61]. Thus, there are still numerous unknowns in the intelligent recognition system between the host and its symbiotic partner.

The second stage is the establishment of infection threads, which could also be figuratively described as pipeline engineering. Infection threads are plant-made pipeline-like structure, where rhizobia pass through and then infect the nodule primordia^[62]. Though several relevant components have recently been reported^[63–65], the chemical nature of infection threads and the polar extension of the tubular cells remains largely undetermined.

The third stage is nodule organogenesis, which is similar to factory design and construction. A recent study suggested that the SHR-SCR module specifies the cortical cell fate to initiate de novo nodule organogenesis^[66], which just likes siting the nodule factory and laying the foundation. Plant hormone signaling significantly contributes in this stage, for example, MtCRA2 (COMPACT ROOT ARCHITECTURE2) works at the nodes of auxin and ethylene signaling pathways to balance root and nodule development under N-deficient conditions^[67]. Another study showed that the master regulator NODULE INCEPTION (NIN) needs to be correctly processed for activating genes involved in the symbiosome development^[68], the process of which likes appointing the managers to recruit workers and assign them to production lines. However, the construction process and functional zoning of the nodule factory is largely unknown. The CAU research group has conducted single-cell RNA profiling of the mature nodule to elucidate the differentiation trajectories of the various nodule cells (unpublished data).

The fourth stage is nitrogen fixation, which is the core business of the specific organ. There have been numerous researches on the nitrogen assimilation and fixation^[69]. In addition to the main production line, the support system like microenvironment maintenance, energy supply, and assimilation product loading and export should not be neglected as well. A recent study showed that NIN-Like Protein (NLP) transcription factors regulate leghemoglobin genes to maintain the appropriate oxygen level^[70]. Efficient nitrogen fixation requires the coordination of mainline and branch line, the molecular mechanism of which needs to be further clarified. There are still many important scientific problems in symbiotic nitrogen fixation, which have both scientific significance and application value.

3.5 Biological breeding

Although multiple breeding approaches have emerged in many crops, recurrent phenotypic selection is still the most adopted method in alfalfa genetic improvement^[6,71]. The crossbreeding takes a substantial amount of time and cannot aggregate some excellent traits flexibility. With the development of alfalfa genomics, selection breeding assisted with molecular markers can shorten the selection cycle and can, in theory, reduce the breeding cost^[23]. Recently, genomic selection based on genotyping-by-sequencing has showed great potential on biomass yield, forage quality, abiotic stress tolerance and disease resistance^[72–76]. However, its practical application relies on precise marker data with further verification especially based on its autotetraploidy and complicated heredity.

Crossbreeding has also been used to generate alfalfa cultivars, especially by cross-pollination between subspecies. Some Chinese registered cultivars, for example, Caoyuan No. 1, Caoyuan No. 2, and Gannong No. 1, were bred by crossing the tetraploid subsp. *sativa* and the diploid subsp. *falcata*^[71]. However, a mature cultivar needs to be stable by going through multiple generations of backcrossing and selection, facing the same dilemma as standard crossbreeding.

Male sterile line-based breeding is a complete approach to generate real hybrids. Though it has been widely used in breeding and seed production of rice, maize and other crops, the application in alfalfa has been hindered. The first difficulty is the generation and maintenance of stable male sterile lines. Natural alfalfa male sterile lines have been discovered since the 1950s^[77–80], and used for hybrid generation since the early 1970s^[81,82]. To date, one of the most successful hybrid alfalfa seed companies, Dairyland Seed Corporation (US), has developed a series of commercial cultivars based on the cytoplasmic male sterility-based hybridization system called msSUNSTRA^[83]. However, screening of male sterile lines and its corresponding maintainer lines is time consuming and labor intensive, and the stability of fertility requires long-term observation. The CAU research group has adopted the reverse genetic strategy and used the genome editing tool to target *MsNPI* (*No Pollen 1*) in the genetic background of a Chinese landrace to generate the genic male sterile alfalfa and its maintainer lines^[42]. T0 generation of male sterile lines and the maintainer lines have been crossed to obtained a transgene-free male sterile line, which can be used as the female parent in hybrid seed production. Another difficulty for heterosis utilization is the cultivation of pure lines as parents^[83]. Alfalfa has the features of partial self-incompatibility and inbreeding depression, the molecular basis of which is still unknown. Recently, Wang et al. provided a strategy for creating pure lines

on the related diploid model, *M. truncatula*. A haploid inducer was created by genome editing of *DMP*, which could induce haploid genome elimination when pollinating another plant and generate haploid progeny^[84]. The haploid progeny has the potential to recover its ploidy, and the homozygous double-haploid could be obtained by then. However, whether the strategy works in alfalfa is totally unknown.

Molecular design breeding combines a variety of biotechnology for comprehensive trait improvement. For example, the CAU research group has co-downregulated *C3H* (coumarate 3-hydroxylase) and *HCT* (hydroxycinnamoyl transferase) by RNAi to increase cellulose content for the improvement of efficiency in biofuels production^[85]. Another example is that co-expression of bacteria-derived aspartate kinase and adenylsulfate reductase increases sulfur amino acid levels and improves nutritional value^[86]. The CAU group also used the CRISPR/Cas9 editing tool to obtain semidwarf and prostrate growth habits by targeting *MsGA3ox1* in tetraploid alfalfa^[43]. The flexible genetic manipulation for target trait improvement relies on gene function verification. Generally speaking, the biotechnology-based molecular-designed breeding is timesaving, but may face the policy risks to arising from safety concerns for genetically modification organisms.

Space mutation breeding has become more widely used with the development of space technology^[71]. Several Chinese cultivars like Zhongtian series were bred in this approach. Spatial mutagenesis has the possibility to generate rare and excellent traits, but long-period selection is indispensable due to its randomness.

Alfalfa breeding still faces many challenges. The innovation of breeding methods should be combined with basic research on desired agronomic traits, where there are still many gaps.

3.6 Cultivation

Alfalfa cultivation is not only field management, but also part of the biological system. Cultivation problems are the driving

force for the genetic improvement of alfalfa. It is necessary to set up standardized growth and development model of alfalfa and to design the ideotype suitable for cultivation measures and ecological location. Though the concept of ideotype has been proposed for over half a century^[87], it is much more complicated in perennial forage crops. The decomposition of yield components in alfalfa is not unitized, since all the aerial parts contribute to its yield. It is still a long way to go to establish an adaptable model.

In addition to cultivate as pure stands, alfalfa can also be rotated or intercropped, and the reasonable model requires a balanced relationship with companion crops, which brings both advantages and challenges^[88–91]. During planting, besides elite cultivar selection, water and fertilizer management, weed and pest management are also important for an optimal harvest^[1,3,92–94]. In addition, whole process mechanization is necessary for alfalfa production as well^[3], which requires the erect growth habit and great lodging resistance. The combination of mechanical innovation and breeding innovation will promote the convenience and stability of alfalfa cultivation.

4 CONCLUSIONS AND PERSPECTIVES

In recent years, considerable research has led to exciting discoveries and advances in various fields of alfalfa biology and development. Several review articles have focused on these aspects and made valuable comments and summaries. As the social and economic value of alfalfa industry is being improved, the paper has emphasized the concept of alfalfa biology, which is becoming a complex subject and worthy of further systematic research. There remains numerous challenging scientific questions for improved understanding of alfalfa biology, the answers to these questions will not only satisfy scientific curiosity, but also contribute to crop and livestock production. I encourage more plant scientists to devote their careers to research on alfalfa biology, as a worthy model system of forage crops.

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Compliance with ethics guidelines

Tao Wang declares that he has no conflicts of interest or financial conflicts to disclose. This article does not contain any studies with human or animal subjects performed by the author.

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