

The Impact of Genomic Studies on Alfalfa Crop Improvement

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Abstract Alfalfa (*Medicago sativa*) is a crucial forage crop globally, contributing significantly to livestock feed and sustainable agriculture. This study explores the profound impact of genomic studies on alfalfa crop improvement. By analyzing recent advancements in genomic technologies, such as whole-genome sequencing, marker-assisted selection, and genome-wide association studies (GWAS), this study highlights their role in enhancing alfalfa's agronomic traits, including yield, disease resistance, and stress tolerance. The integration of genomics with traditional breeding methods has accelerated the development of superior alfalfa cultivars, promising increased productivity and resilience in various environmental conditions. This study synthesizes findings from multiple studies to provide a comprehensive understanding of how genomic insights are reshaping alfalfa breeding programs and fostering agricultural sustainability. The study also discusses challenges and future directions in leveraging genomics for alfalfa improvement, emphasizing the need for continued research and collaboration in this field.

Keywords Alfalfa crop improvement; Genomic studies; Marker-assisted selection; Whole-genome sequencing; Agronomic traits enhancement

1 Introduction

Alfalfa (*Medicago sativa* L.), often referred to as the “Queen of Forages”, is a perennial legume widely cultivated for its high nutritional value and versatility as hay, silage, and pasture. It plays a crucial role in sustainable agriculture due to its ability to fix atmospheric nitrogen, thereby improving soil fertility and reducing the need for synthetic fertilizers (Jiang et al., 2022). Alfalfa is a significant forage crop globally, contributing over 58 million metric tons of hay, silage, and pasture annually in the United States alone (Hawkins and Yu, 2018). Its importance extends beyond agriculture, as it is also used in the production of industrial and pharmaceutical proteins, renewable energy sources, and novel compounds (Kumar et al., 2018).

The genetic complexity of alfalfa, being an autotetraploid and highly heterozygous species, has historically posed challenges for traditional breeding methods. However, recent advancements in genomic technologies have revolutionized alfalfa breeding. Techniques such as genotyping-by-sequencing (GBS) and single nucleotide polymorphism (SNP) markers have enabled the construction of high-density genetic linkage maps, facilitating quantitative trait locus (QTL) mapping and genome-wide association studies (GWAS) (Li et al., 2014; Zhang et al., 2020; Jiang et al., 2022). These tools have been instrumental in identifying genetic markers associated with desirable traits, such as flowering time, which is crucial for optimizing yield and adaptability (Zhang et al., 2020; Jiang et al., 2022). Moreover, genomic selection (GS) methods, which utilize statistical models to predict breeding values based on genotypic information, have shown promise in accelerating breeding gains (Hawkins and Yu, 2018).

This study explores the impact of genomic studies on the improvement of alfalfa crops. It reviews the current state of genomic technologies and their application in alfalfa breeding, analyzes the progress made in identifying genetic markers and QTLs associated with key agronomic traits, evaluates the potential of genomic selection and other advanced breeding techniques in enhancing alfalfa crop performance, and discusses the challenges and future directions in the genomic improvement of alfalfa. By synthesizing findings from recent research, this study

aims to provide a comprehensive overview of how genomic studies are transforming alfalfa breeding and contributing to the development of superior alfalfa cultivars.

2 Advances in Alfalfa Genomics

2.1 Sequencing technologies and genome assembly

Recent advancements in sequencing technologies have significantly contributed to the genomic studies of alfalfa (*Medicago sativa*), a crucial forage crop. The development of high-quality reference genomes has been a pivotal step. For instance, a de novo assembled 816 Mb chromosome-level haploid genome sequence for “Zhongmu No.1” alfalfa was reported, which includes 49 165 annotated genes and provides a comprehensive genomic resource for alfalfa research (Figure 1) (Shen et al., 2020). Additionally, the use of PacBio circular consensus sequencing, Illumina short-read sequencing, and high-throughput chromosome conformation capture technology has enabled the assembly of a 2.738 Gb allele-aware chromosome-level genome, consisting of 32 super-scaffolds and unplaced contigs, with 164 632 protein-coding genes annotated (Lyu, 2020). These advancements in sequencing and assembly technologies have laid a solid foundation for further genomic studies and breeding programs in alfalfa.

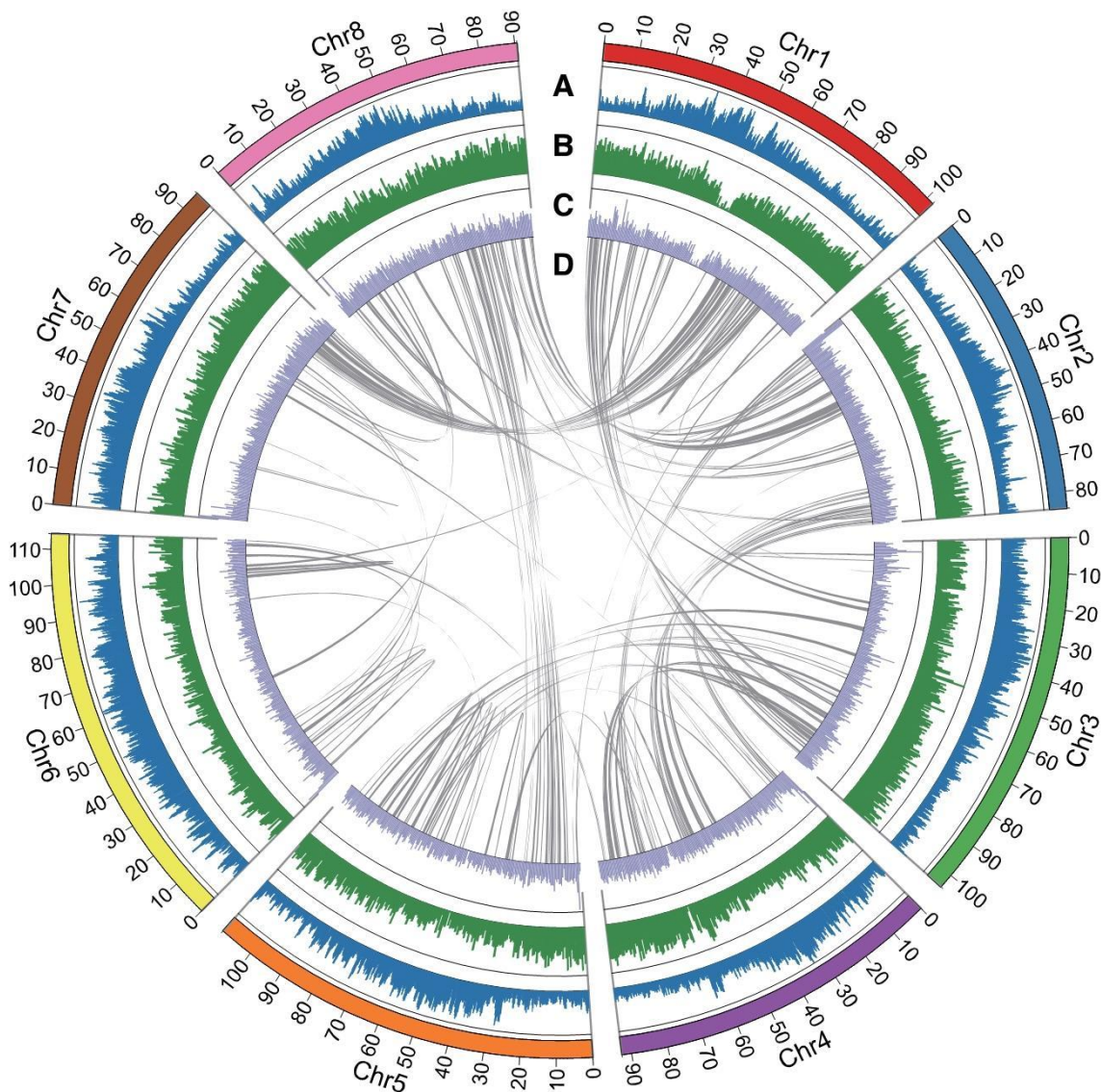


Figure 1 Distribution of Genomic Features within the Alfalfa “Zhongmu No.1” Genome (Adopted from Shen et al., 2020)
 Image caption: (A-C) Circular representation of the GC content (A), gene density (B), and LTR density (C) of genome regions (100 kb for each window). (D) Collinear gene blocks in the “Zhongmu No.1” genome (Adopted from Shen et al., 2020)

The study of Shen et al. (2020) presents a comprehensive circular visualization of the genomic features within the Alfalfa “Zhongmu No.1” genome. The outermost ring (A) displays the GC content, providing insight into the guanine-cytosine pair density across different regions of the genome. The second ring (B) illustrates the gene density, highlighting the distribution and concentration of genes within each chromosome segment. The third ring (C) shows the Long Terminal Repeat (LTR) density, indicating the presence and frequency of these retrotransposons in the genome. The innermost connections (D) represent collinear gene blocks, which are segments of the genome that share a conserved order of genes. These visualizations collectively offer a detailed overview of the genomic architecture, revealing regions of high gene concentration, GC content, and LTR density, as well as conserved gene order across the genome, crucial for understanding genetic organization and function in alfalfa “Zhongmu No.1”.

2.2 Annotation and functional genomics

The annotation of the alfalfa genome has revealed a wealth of genetic information that is crucial for understanding its complex traits. For example, the annotation of the “Zhongmu No.1” genome identified 49 165 genes, providing insights into the genetic diversity and population structure of alfalfa (Shen et al., 2020). Functional genomics studies have also identified key genes associated with important agronomic traits. For instance, the *MsFTa2* gene, a Flowering Locus T homolog, has been linked to fall dormancy and salt resistance, showing up-regulated expression in salt-resistant germplasm. Moreover, the development of efficient genome editing protocols, such as CRISPR-Cas9, has enabled the targeted modification of specific genes, such as the *Phytoene desaturase* gene and PALMATE-LIKE PENTAFOLIATA1 (PALM1), to improve traits like biomass and nutritional value (Lyu, 2020). These functional genomics approaches are essential for the precise manipulation of the alfalfa genome to enhance its agronomic performance.

2.3 Comparative genomics and phylogenetics

Comparative genomics and phylogenetic studies have provided valuable insights into the evolutionary history and genetic relationships of alfalfa. The divergence of alfalfa from its close relative, *Medicago truncatula*, approximately 8 million years ago, has been elucidated through genomic analyses (Shen et al., 2020). Additionally, the assembly of a chromosome-scale genome of the diploid progenitor of autotetraploid alfalfa, *Medicago sativa* spp. *caerulea*, has revealed significant differences in genome size and gene content compared to *M. truncatula*, highlighting the evolutionary events such as chromosomal fissions and fusions that have shaped the alfalfa genome (Li et al., 2020). These comparative studies are crucial for understanding the genetic basis of alfalfa’s unique traits and for identifying potential targets for breeding programs.

In summary, the advancements in sequencing technologies, genome assembly, annotation, functional genomics, and comparative genomics have significantly enhanced our understanding of the alfalfa genome. These genomic resources and insights are instrumental in developing improved alfalfa varieties with enhanced agronomic traits, ultimately contributing to the sustainability and productivity of alfalfa as a forage crop.

3 Genetic Diversity and Germplasm Resources

3.1 Exploration of genetic diversity in alfalfa

Genetic diversity is a cornerstone for crop improvement, providing the raw material for breeding programs. In alfalfa (*Medicago sativa* L.), significant efforts have been made to explore and characterize genetic diversity. The resequencing of core germplasms has revealed high genetic diversity and weak population structure, which is crucial for breeding programs aiming to enhance agronomic traits (Shen et al., 2020). Additionally, genomic population analysis has shown extensive gene flow from wild to cultivated alfalfa, further enriching the genetic pool available for breeding (Shen et al., 2020).

3.2 Utilization of germplasm collections

Germplasm collections serve as reservoirs of genetic diversity that can be harnessed for crop improvement. Effective utilization of these collections is essential for increasing genetic gain and addressing challenges such as

climate change and food security. Characterizing extensive germplasm collections can serve dual purposes: as reference populations for genomic selection (GS) models and as sources of desirable genetic variants for incorporation into elite cultivars (He and Li, 2020). The integration of high-throughput genotyping and phenotyping technologies can further enhance the utility of germplasm collections in breeding programs (He and Li, 2020).

3.3 Marker-assisted selection and breeding

Marker-assisted selection (MAS) and genomic selection (GS) are powerful tools for accelerating breeding programs. MAS has been successfully employed to introgress favorable alleles associated with drought resilience and forage productivity into elite alfalfa germplasm, demonstrating significant yield improvements under water-limited conditions (Singh et al., 2022). Genomic selection, which uses genome-wide markers to predict breeding values, has shown promise in improving complex traits such as biomass yield and forage quality (Annicchiarico et al., 2015; Li et al., 2015; Biazzi et al., 2017). The development of high-quality genomic resources, including SNP markers and reference genomes, has facilitated the application of these advanced breeding strategies in alfalfa (Li and Brummer, 2012; Hawkins and Yu, 2018; Medina et al., 2021).

In summary, the exploration of genetic diversity, effective utilization of germplasm collections, and the application of marker-assisted and genomic selection are pivotal in enhancing alfalfa crop improvement. These strategies collectively contribute to the development of superior alfalfa cultivars with improved yield, quality, and resilience to environmental stresses.

4 Key Traits Targeted by Genomic Studies

4.1 Forage quality and yield

Forage quality and yield are critical traits in alfalfa breeding, as they directly impact the nutritional value and productivity of the crop. Genomic studies have identified several genetic markers and candidate genes associated with these traits. For instance, genomic prediction (GP) methods have been used to estimate breeding values for various agronomic and quality traits, such as plant height, flowering date, and biomass yield, with prediction accuracies ranging from low to moderate (Jia et al., 2018). Additionally, genome-wide association studies (GWAS) have identified specific single nucleotide polymorphisms (SNPs) linked to forage quality traits, including leaf-to-stem ratio, protein content, and fiber digestibility (Biazzi et al., 2017). These findings suggest that genomic selection (GS) can be effectively applied to improve forage quality and yield in alfalfa breeding programs (Li et al., 2015).

4.2 Abiotic stress tolerance

Abiotic stress tolerance, particularly to drought and salinity, is another key target for genomic studies in alfalfa. Soil salinity and water deficit are major challenges that limit alfalfa production. Genomic analyses have identified several SNP markers and candidate genes associated with salt tolerance and drought resistance. For example, GWAS and GS have been used to identify markers linked to salt tolerance, with machine learning models achieving high prediction accuracies for yield under salt stress (Figure 2) (Medina et al., 2020; Medina et al., 2021). Similarly, studies have identified genetic loci associated with biomass yield under water deficit conditions, highlighting the polygenic nature of drought resistance and the potential for marker-assisted selection (MAS) to develop drought-tolerant alfalfa cultivars (Yu, 2017; Lin et al., 2020).

The study of Medina et al. (2021) illustrates the optimization of genomic selection (GS) models for biomass yield under salt stress using various statistical methods. Panel (a) compares the accuracy and computation time of different GS models, revealing that support vector machine (SVM) has the highest accuracy but also requires the longest computational time. Panel (b) highlights the importance of specific single nucleotide polymorphisms (SNPs) in predicting biomass yield, as derived from the SVM model. Panel (c) shows the Pearson's correlation for SNP weights, comparing variable importance values from SVM and random forest (RF) against different GWASpoly models, indicating varied levels of correlation among the methods. Panel (d) evaluates the accuracy of

genomic best linear unbiased prediction (GBLUP) and weighted GBLUP (WGBLUP) models, demonstrating that WGBLUP models, especially those incorporating SNP weights from SVM and RF, achieve higher accuracy. These insights are crucial for improving predictive performance in GS models.

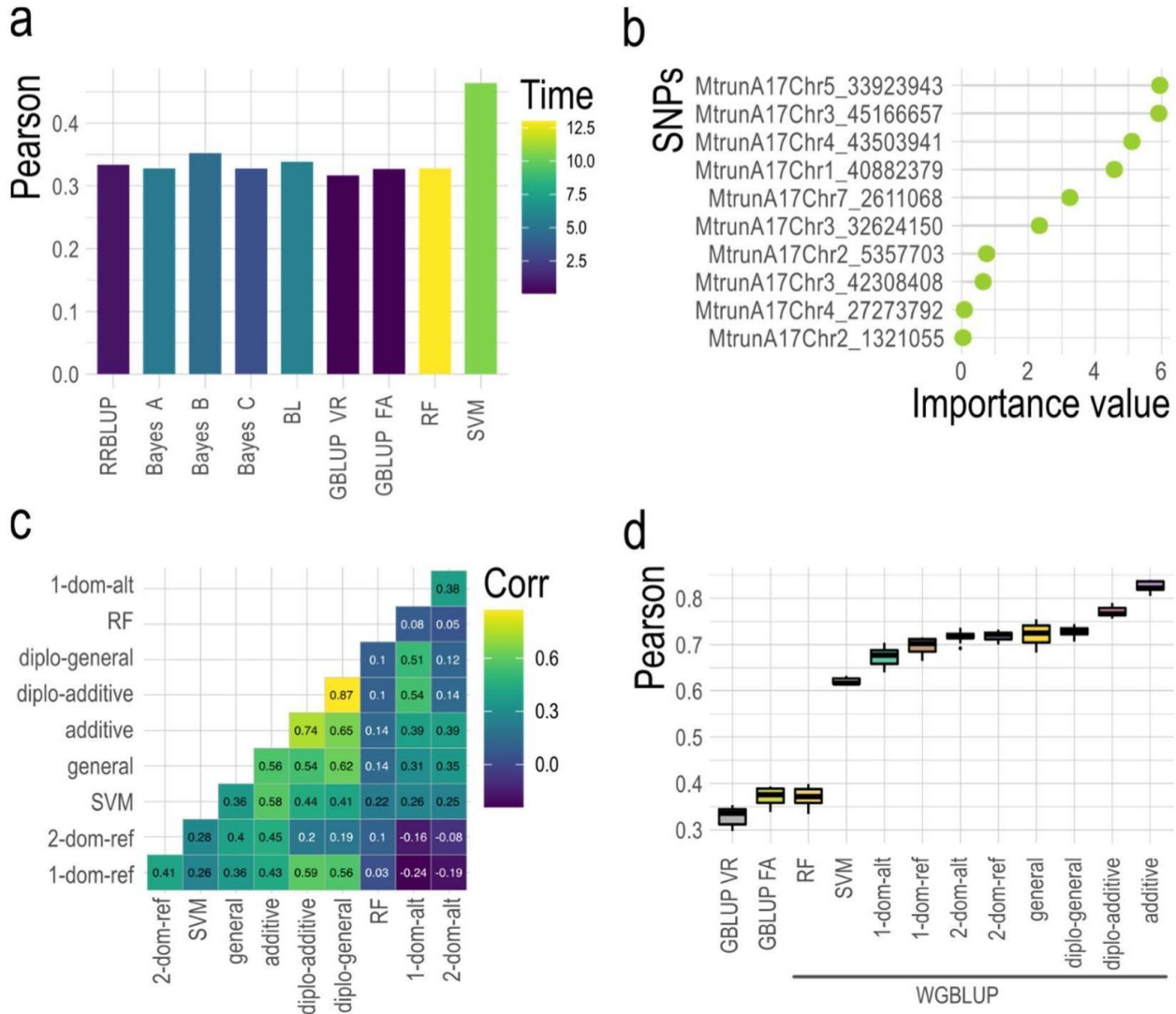


Figure 2 Optimization of GS models (Adopted from Medina et al., 2021)

Image caption: (a) GS model accuracy measured as Pearson's correlation after 10-fold cross-validation for biomass yield under salt stress. Computing time was measured as system time in seconds to run one cross-validation. (b) Example of variable importance values derived from SVM for 10 randomly chosen SNPs. (c) Pearson's correlation for 6 796 SNPs weights obtained by variable importance (SVM, RF) or by $-\log_{10}$ p-values of different GWASpoly models. (d) Accuracy of GBLUP (GBLUP VR and GBLUP FA) and WGBLUP models. Accuracy was measured 10 times using Pearson's correlation with 10-fold cross-validation. SNP weights for WGBLUP were obtained from variable importance values (SVM, RF) or $-\log_{10}$ p-values of different GWASpoly models. RRBLUP, best linear unbiased prediction using ridge-regression; BL Bayes LASSO; GBLUP, genomic best linear unbiased prediction; VR, VanRaden G matrix; FA, full autotetraploid G matrix; RF, random forest; SVM, support vector machine; WGBLUP, weighted GBLUP; 1-dom-alt and 1-dom-ref, simplex dominant models; 2-dom-alt and 2-dom-ref, duplex dominant models; diplo-general, diploidized general; diplo-additive, diploidized additive (Adopted from Medina et al., 2021)

4.3 Biotic stress resistance

Biotic stress resistance, including resistance to pests and diseases, is crucial for maintaining alfalfa crop health and productivity. Genomic studies have focused on identifying genetic markers and candidate genes associated with resistance to various biotic stresses. For instance, the use of high-throughput sequencing and gene editing techniques has facilitated the identification and improvement of genes involved in biotic stress responses (Figure

3) (Hrbáčková et al., 2020). These advancements have led to the development of alfalfa cultivars with enhanced resistance to pests and diseases, thereby improving overall crop resilience and yield.

The study of Wang et al. (2022) outlines the potential future applications of CRISPR/Cas technology in addressing both biotic and abiotic stresses in plants. It categorizes various stress factors into abiotic and biotic groups. Abiotic stress includes challenges such as water stress (drought, flooding, salinity), temperature stress (high and low temperatures), biochemical stress (herbicides, competition), nutrient stress (increased CO₂, nutrient-poor soil), and heavy metal stress. Biotic stress encompasses threats from pests (arthropods, mammals, nematodes, birds) and pathogens (fungal, bacterial, viral, oomycete). The diagram highlights that CRISPR/Cas technology can be utilized to improve plant traits, manage gene expression regulation, and understand host-pathogen interactions. By targeting specific genes associated with these stress responses, CRISPR/Cas can enhance plant resilience, growth, and productivity, making it a powerful tool in agricultural biotechnology for developing stress-tolerant crop varieties and improving overall agricultural sustainability.

In summary, genomic studies have significantly contributed to the improvement of key traits in alfalfa, including forage quality and yield, abiotic stress tolerance, and biotic stress resistance. The identification of genetic markers and candidate genes through GWAS, GS, and other genomic approaches has provided valuable insights and tools for alfalfa breeding programs, ultimately leading to the development of more productive and resilient alfalfa cultivars.



Figure 3 Future applications of CRISPR/Cas in plants against the biotic and abiotic stress (Adopted from Wang et al., 2022)

Image caption: CRISPR/Cas represents the future of genome editing technology and the potential use of the CRISPR/Cas system in various disciplines under biotic and abiotic stresses of agriculture. With the maturity of genome editing (GE) technology and the development of new GE tools, the application of CRISPR/Cas is becoming more and more extensive. CRISPR/Cas can now achieve gene knockout, knock-in, and knock-up in plants, replacing a single base to cause amino acid changes, etc. Therefore, CRISPR/Cas can be used to modify key genes of biotic and abiotic stresses, improving crop growth and development and coping with various environmental stresses to create more germplasm resources that meet human needs (Adopted from Wang et al., 2022)

5 Genomic Tools and Techniques

5.1 QTL mapping and association studies

Quantitative Trait Loci (QTL) mapping and association studies have been pivotal in identifying genomic regions associated with key agronomic traits in alfalfa. Family-based QTL mapping has successfully identified major QTLs related to agronomic traits, although these are often located in relatively large genomic regions (Li and Brummer, 2012). The integration of high-throughput sequencing technologies and advanced bioinformatics tools has facilitated the identification of numerous single nucleotide polymorphisms (SNPs), which are being developed into markers for fine mapping of quantitative traits and genome-wide association studies (GWAS) (Li and Brummer, 2012). GWAS has confirmed the polygenic control of quality traits and identified several SNPs linked to important traits such as stem protein content and leaf acid detergent lignin (ADL) (Biazzi et al., 2017). These advancements enable more precise marker-assisted selection (MAS) and the potential for improved breeding strategies (Singh et al., 2022).

5.2 Genomic selection and prediction models

Genomic selection (GS) leverages genome-wide molecular markers to predict the breeding values of individuals, thereby enhancing breeding efficiency. GS has shown promise in alfalfa breeding, particularly for complex traits like biomass yield. Studies have demonstrated moderate prediction accuracies for biomass yield, with values ranging from 0.34 to 0.66 depending on the population and environmental conditions (Li et al., 2015). The use of genotyping-by-sequencing (GBS) and various statistical models, such as Support Vector Regression (SVR) and Ridge Regression BLUP, has been explored to optimize prediction accuracy (Annicchiarico et al., 2015). Additionally, integrating machine learning models and GWAS-derived marker importance values has further increased prediction accuracies for traits like yield under salt stress (Medina et al., 2021). These approaches suggest that GS can significantly accelerate genetic gains in alfalfa breeding programs (Hawkins and Yu, 2018; Medina et al., 2021).

5.3 CRISPR and other genome editing technologies

Genome editing technologies, particularly CRISPR-Cas9, have revolutionized crop research, including alfalfa. The development of a chromosome-level genome assembly for alfalfa has provided a robust reference for genome editing efforts. Researchers have successfully used CRISPR-Cas9 to target specific genes, such as the Phytoene desaturase gene, resulting in mutant plants with desirable traits like dwarfism and altered leaf morphology. Another study demonstrated the editing of the PALMATE-LIKE PENTAFOLIATA1 (PALM1) gene, producing plants with more leaflets, which could potentially enhance biomass and nutritional value (Lyu, 2020). These advancements highlight the potential of genome editing to rapidly introduce beneficial traits into alfalfa cultivars, thereby accelerating breeding programs and improving crop performance.

6 Case Studies and Applications

6.1 Successful breeding programs utilizing genomic data

Recent advancements in genomic technologies have significantly impacted alfalfa breeding programs. One notable example is the use of genotyping-by-sequencing (GBS) and single nucleotide polymorphism (SNP) markers to enhance breeding efficiency. For instance, a study demonstrated the application of GBS in identifying SNP markers associated with biomass yield, which were then used to develop genomic selection (GS) models. These models showed prediction accuracies ranging from 0.34 to 0.66, indicating the potential of GS to accelerate genetic gains in alfalfa breeding programs (Li et al., 2015). Another study highlighted the use of SNP chips and statistical models to generate genomic estimated breeding values (GEBV), which facilitated the selection of superior plants at a young age, thereby speeding up the breeding process (Hawkins and Yu, 2018).

6.2 Examples of improved alfalfa varieties

The integration of genomic data has led to the development of several improved alfalfa varieties. For example, the “Zhongmu No.1” alfalfa variety was developed using a high-quality, chromosome-level genome sequence. This variety exhibited high genetic diversity and traits such as fall dormancy and salt resistance, which were linked to

specific genomic regions (Shen et al., 2020). Additionally, genomic selection has been employed to improve forage quality traits, such as leaf-to-stem ratio and protein content, resulting in varieties with enhanced nutritional value (Biazzi et al., 2017). Another study focused on *Verticillium* wilt resistance, identifying multiple QTLs associated with disease resistance, which can be used to develop resistant alfalfa varieties (Yu et al., 2017).

6.3 Translational genomics and field performance

Translational genomics has played a crucial role in bridging the gap between laboratory research and field performance. For instance, genomic prediction models developed using GBS data have shown moderate to high prediction accuracies for various agronomic traits, such as plant height, flowering date, and biomass yield. These models have been validated in multiple environments, demonstrating their robustness and applicability in real-world breeding programs (Jia et al., 2018). Furthermore, the use of machine learning models and GWAS-assisted approaches has increased the prediction accuracies for complex traits like yield under salt stress, thereby enhancing the selection of superior alfalfa lines based on their GEBVs (Medina et al., 2021). These advancements underscore the potential of translational genomics to improve field performance and accelerate the development of high-yielding, stress-resistant alfalfa varieties.

7 Challenges and Future Directions

7.1 Technical and methodological challenges

The advancement of genomic studies in alfalfa crop improvement faces several technical and methodological challenges. One significant issue is the complexity of alfalfa's genetic background, being an autotetraploid species, which complicates the identification and manipulation of genes associated with desirable traits (Volenc et al., 2002; He et al., 2022). Additionally, the accuracy of genomic selection (GS) models can vary significantly depending on environmental factors and the specific traits being targeted. For instance, prediction accuracies for biomass yield have been reported to range from 0.11 to 0.70, indicating a need for more robust models and methods (Li et al., 2015; He et al., 2022). The lack of a high-quality reference genome for alfalfa has also been a limiting factor, although recent efforts have produced a chromosome-level genome sequence that could mitigate this issue (Shen et al., 2020). Furthermore, the integration of high-throughput sequencing technologies and advanced bioinformatics tools is still in its nascent stages, requiring further refinement to enhance the efficiency and accuracy of genomic selection (Li and Brummer, 2012; Hawkins and Yu, 2018).

7.2 Integrating multi-omics data

The integration of multi-omics data, including genomics, transcriptomics, proteomics, and metabolomics, presents both opportunities and challenges for alfalfa crop improvement. Multi-omics approaches can provide a comprehensive understanding of the molecular mechanisms underlying complex traits, such as stress tolerance and biomass yield (Hrbáčková et al., 2020). However, the integration of these diverse datasets requires sophisticated computational tools and models to analyze and interpret the data effectively. The development of systems biology approaches that can integrate multi-omics data to construct predictive models for complex traits is still in progress (Yang et al., 2021). Additionally, the variability in data quality and the need for standardized protocols across different omics platforms pose significant challenges. Despite these hurdles, the potential for multi-omics to enhance our understanding of gene networks and their regulatory mechanisms in alfalfa is immense, and continued efforts in this area are crucial for future crop improvement.

7.3 Ethical and regulatory considerations

The application of genomic technologies in alfalfa breeding also raises several ethical and regulatory considerations. The use of genetic engineering and gene editing techniques, such as CRISPR/Cas9, to introduce or modify traits in alfalfa must be carefully regulated to ensure safety and public acceptance. There are concerns about the potential environmental impact of genetically modified alfalfa, including gene flow to wild relatives and non-target effects on ecosystems. Regulatory frameworks need to be established to address these concerns and to ensure that genetically modified alfalfa varieties are thoroughly tested for safety and efficacy before commercial release (Hrbáčková et al., 2020). Additionally, ethical considerations related to the ownership and control of

genetic resources and the potential socioeconomic impacts on smallholder farmers must be addressed. Ensuring equitable access to the benefits of genomic technologies and fostering public trust through transparent and inclusive decision-making processes are essential for the sustainable development of alfalfa crop improvement (Volencic et al., 2002).

In conclusion, while genomic studies hold great promise for enhancing alfalfa crop improvement, addressing the technical, methodological, ethical, and regulatory challenges is crucial for realizing their full potential. Continued research and collaboration among scientists, policymakers, and stakeholders will be key to overcoming these challenges and achieving sustainable advancements in alfalfa breeding.

8 Concluding Remarks

The impact of genomic studies on alfalfa crop improvement has been profound, as evidenced by the extensive research conducted in recent years. Key findings from these studies highlight the significant advancements in understanding the genetic basis of important agronomic traits and the development of genomic tools that facilitate more efficient breeding programs. For instance, the *de novo* assembly of a high-quality, chromosome-level genome sequence for “Zhongmu No.1” alfalfa has provided a valuable reference for genetic research and breeding efforts. Additionally, genomic population analyses have revealed high genetic diversity and extensive gene flow, which are crucial for the continued improvement of alfalfa varieties.

The implications of these findings for alfalfa crop improvement are substantial. The identification of candidate genes associated with traits such as flowering time, salt resistance, and root development can lead to the development of alfalfa varieties with enhanced performance under various environmental conditions. Moreover, the application of genomic selection (GS) and genome-wide association studies (GWAS) has shown promise in accelerating breeding gains by predicting breeding values and identifying markers associated with desirable traits. These genomic tools enable breeders to make more informed selections, thereby increasing the efficiency and effectiveness of breeding programs.

Looking to the future, several research priorities emerge. First, there is a need to refine and validate the genomic prediction models to ensure their accuracy and reliability across different environments and populations. Additionally, further exploration of the genetic basis of complex traits, such as biomass yield and forage quality, will be essential for developing superior alfalfa varieties. Finally, integrating genomic data with advanced phenotyping techniques and bioinformatics tools will enhance our understanding of genotype-environment interactions and facilitate the development of alfalfa varieties that are resilient to changing climatic conditions.

In conclusion, genomic studies have significantly advanced our understanding of alfalfa genetics and provided valuable tools for crop improvement. Continued research in this field will be crucial for addressing the challenges of sustainable agriculture and ensuring the long-term productivity and resilience of alfalfa crops.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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