

INTEGRATING GENOMICS AND BIOTECHNOLOGICAL APPROACHES TO ENHANCE ABIOTIC STRESS TOLERANCE IN SESAME (*SESAMUM INDICUM* L.)

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Abstract: *Sesame* (*Sesamum indicum* L.) is an ancient oilseed crop cultivated for its rich oil, protein, and essential nutrients. However, changing environmental conditions due to climate change pose significant challenges to sesame production. Abiotic stresses, such as salinity and drought, can severely impact sesame yield and productivity. Integrating genomic approaches and biotechnology in sesame breeding offers significant promise for developing resilient sesame cultivars with enhanced abiotic stress tolerance. GWAS (Genome-wide association studies) have identified genes and QTL (quantitative trait loci) associated with drought and salinity tolerance in sesame. Genomic selection offers several advantages over traditional breeding methods, enabling the efficient development of stress-tolerant sesame cultivars. Biotechnological tools, such as CRISPR-Cas9 genome editing technology, allow for precise modification of specific genes, facilitating the introduction of desirable traits into sesame cultivars. The integration of these approaches offers promising opportunities for the targeted improvement of tolerance against abiotic stresses in sesame. However, addressing ethical and regulatory considerations surrounding the use of biotechnology in plant breeding will be vital for ensuring the safe and responsible application of these technologies. This review paper provides an overview of integrating genomic approaches and biotechnology in sesame breeding programs focused on improving drought and salinity tolerance and discusses the potential for developing resilient sesame cultivars in the face of climate change.

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Introduction

Sesame (*Sesamum indicum* L.) is an ancient oilseed crop cultivated for over 4,000 years, originating from the Indian subcontinent and Africa (Wang et al., 2014; Takele, 2023). It is grown mainly for its seeds, rich in oil, protein, and essential nutrients, including antioxidants, vitamins, and minerals (Bedigian, 2010; Takele, 2023). Sesame seeds are used in various food products, from baked goods to traditional dishes in various cuisines, while the oil is prized for its stability, high oxidative resistance, and nutritional properties (Bedigian, 2010; Were et al., 2006). Sesame is grown in over 50 countries, with major producers including India, China, Myanmar, and Sudan (FAOSTAT, 2021). The global demand for sesame is steadily increasing due to its nutritional value, diverse applications in the food industry, and emerging applications in health care and cosmetic products (Pathak et al., 2014).

Increasing temperatures, unpredictable rainfall, and more frequent severe weather events are just ways

climate change threatens agricultural output. (IPCC, 2014). These shifts increase the frequency of abiotic stressors like drought and salinity, which may devastate agricultural output. (Lobell et al., 2011). As a rain-fed crop, sesame is especially vulnerable to drought and salt stress. (Dossa et al., 2017). In semi-arid environments, sesame is valuable because it can survive mild drought; yet, extreme drought and salt still provide substantial obstacles. (Dossa et al., 2016). To preserve the long-term viability of sesame production under changing climate, it is crucial to develop cultivars with increased resistance to these abiotic challenges. Figure 1 depicts a variety of biotic and abiotic stressors. It is vital to strengthen sesame's resistance to abiotic stress to sustain and increase crop yield in the face of changing environmental circumstances. Better yields, fewer crop failures, and more resistance to climate change are all possible due to breeding for better tolerance of drought and salt. (Dossa et al., 2017). In addition, these varieties may

help smallholder farmers who depend on sesame cultivation for their livelihoods and food security. The rising demand for sesame products necessitates the creation of stress-tolerant sesame cultivars so that the

crop may be grown on marginal soils. (Hassan et al., 2020).

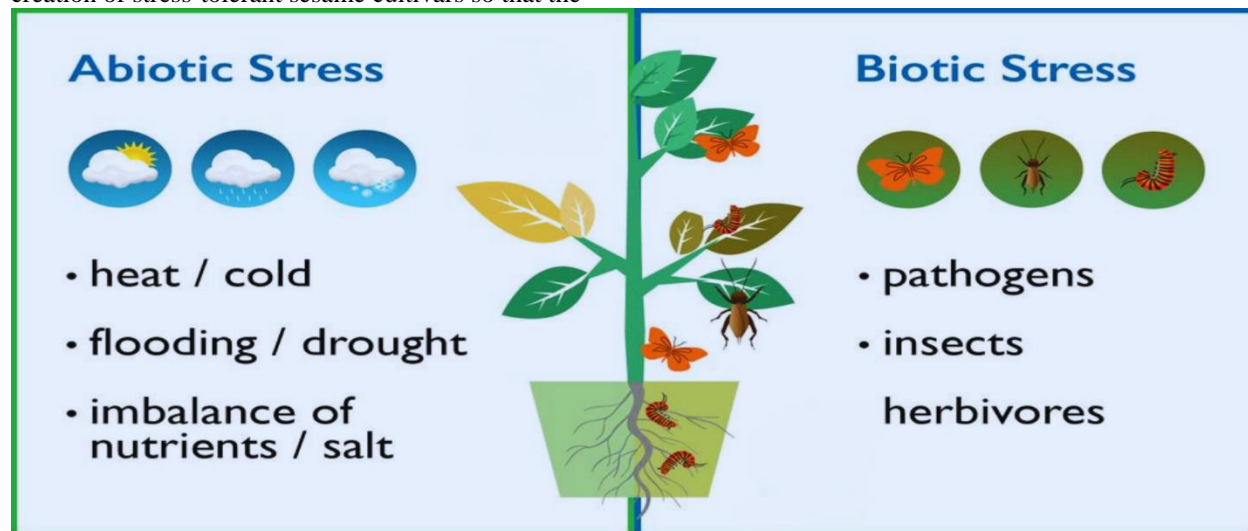


Figure 1 Abiotic and Biotic Stresses in Sesame (<https://stoller.com.au/get-your-crops-back-on-track/>)

Recent advances in genomics and biotechnology have revolutionized plant breeding, providing powerful tools for the targeted improvement of crop traits (Varshney et al., 2005). In the case of sesame, these technologies offer promising opportunities for developing cultivars with enhanced tolerance to abiotic stresses. High-throughput genotyping and sequencing technologies have enabled the generation of large-scale genomic resources for sesame, including whole-genome sequences, transcriptome data, and genetic maps (Wang et al., 2014; Wei et al., 2015; Zhang et al., 2012). These resources have facilitated the identification of candidate genes and molecular markers associated with stress tolerance, paving the way for integrating genomic approaches and biotechnology in sesame breeding (Dossa et al., 2016; Zhou et al., 2020).

Developing sesame cultivars with improved abiotic stress tolerance can be achieved by integrating genomic approaches and biotechnology into breeding programs. Genome-wide association studies (GWAS) can help identify key genes and quantitative trait loci (QTL) associated with drought and salinity tolerance, providing valuable information for targeted breeding efforts (Li et al., 2018; Wu et al., 2014). For example, a recent GWAS on sesame identified several candidate genes related to drought tolerance, which can be further investigated and validated for their functional roles in conferring stress tolerance (Zhang et al., 2021). Genomic selection, an approach that uses genome-wide markers to predict breeding values, can accelerate the development of stress-tolerant cultivars by increasing the selection accuracy and reducing the breeding cycle time (Crossa et al., 2017). In sesame, genomic selection has been successfully applied to predict complex traits, such as oil content, and could

be extended to abiotic stress tolerance (Yu et al., 2020).

Biotechnological tools, such as CRISPR-Cas9, offer a powerful method for targeted genetic improvements in sesame. CRISPR-Cas9 is a genome editing technology that allows for precise modification of specific genes, introducing desirable traits into crop plants (Jiang et al., 2013). By combining the insights gained from GWAS and genomic selection with CRISPR-Cas9 and other biotechnological tools, breeders can develop sesame cultivars with enhanced tolerance to drought and salinity more efficiently and precisely. For example, a recent study reported the successful application of CRISPR-Cas9 in editing a gene associated with seed oil content in sesame, demonstrating the potential of this technology for targeted trait improvement (Zhang et al., 2019). In addition to genomics and biotechnology, other approaches, such as physiological and biochemical studies, can provide complementary information for understanding the mechanisms underlying abiotic stress tolerance in sesame (Fahad et al., 2017). Evaluating the physiological responses of sesame plants to drought and salinity can help identify key traits and metabolic pathways involved in stress adaptation, which can be further targeted for improvement through breeding and biotechnology (Yadav et al., 2021). Integrating multi-disciplinary research efforts, including genomics, biotechnology, physiology, and biochemistry, will provide a comprehensive understanding of abiotic stress tolerance in sesame and facilitate the development of improved cultivars.

In addition to the advances in genomics and biotechnology, the use of traditional breeding methods, such as the selection of stress-tolerant germplasm, can still play an important role in

developing abiotic stress-tolerant sesame cultivars (Hassan et al., 2020). Evaluating the performance of diverse sesame germplasm under various stress conditions can help identify promising accessions that can serve as potential sources of tolerance alleles for breeding programs (Uzun et al., 2007). Furthermore, hybridization and introgression of stress-tolerant traits from wild sesame relatives can also contribute to developing improved sesame cultivars (Bedigian, 2010). When developing abiotic stress-tolerant sesame cultivars, it is crucial to consider the potential trade-offs between stress tolerance and other agronomic traits, such as yield and quality. Breeders should strive to improve stress tolerance without compromising the overall performance of the cultivar under optimal growing conditions. This can be achieved by selecting for multiple traits simultaneously using multi-trait genomic selection models and incorporating information from multiple sources, such as phenotypic data, marker data, and environmental data, to make more informed breeding decisions (Cuevas et al., 2018).

Overall, integrating genomic approaches, biotechnology, and traditional breeding methods will be instrumental in developing sesame cultivars with enhanced tolerance to drought and salinity. These improved cultivars will contribute to the sustainability of sesame production under changing climatic conditions and help meet the increasing global demand for sesame products. By harnessing the power

of modern plant breeding techniques, researchers can unlock the full potential of sesame as a versatile and nutritious crop for the future. This paper will review the integration of genome-wide association studies (GWAS), genomic selection, and biotechnological tools, such as CRISPR-Cas9, in sesame breeding programs focused on improving drought and salinity tolerance.

Genome-wide Association Studies (GWAS) in Sesame

Genome-wide Association Studies (GWAS) is a powerful approach to identifying genes and quantitative trait loci (QTL) associated with complex traits, such as abiotic stress tolerance, by scanning the entire genome for single nucleotide polymorphisms (SNPs) that are statistically related to the trait of interest (Hirschhorn & Daly, 2005). GWAS can help to uncover the genetic basis of complex traits by identifying candidate genes, estimating the proportion of phenotypic variation explained by these genes, and revealing the population structure and linkage disequilibrium patterns in the target species (Korte & Farlow, 2013). A schematic representation of GWAS is presented in Figure 2. In recent years, GWAS has been increasingly applied to crop plants, including sesame, to dissect the genetic architecture of agronomically important traits and accelerate the development of improved cultivars (Wang et al., 2018).

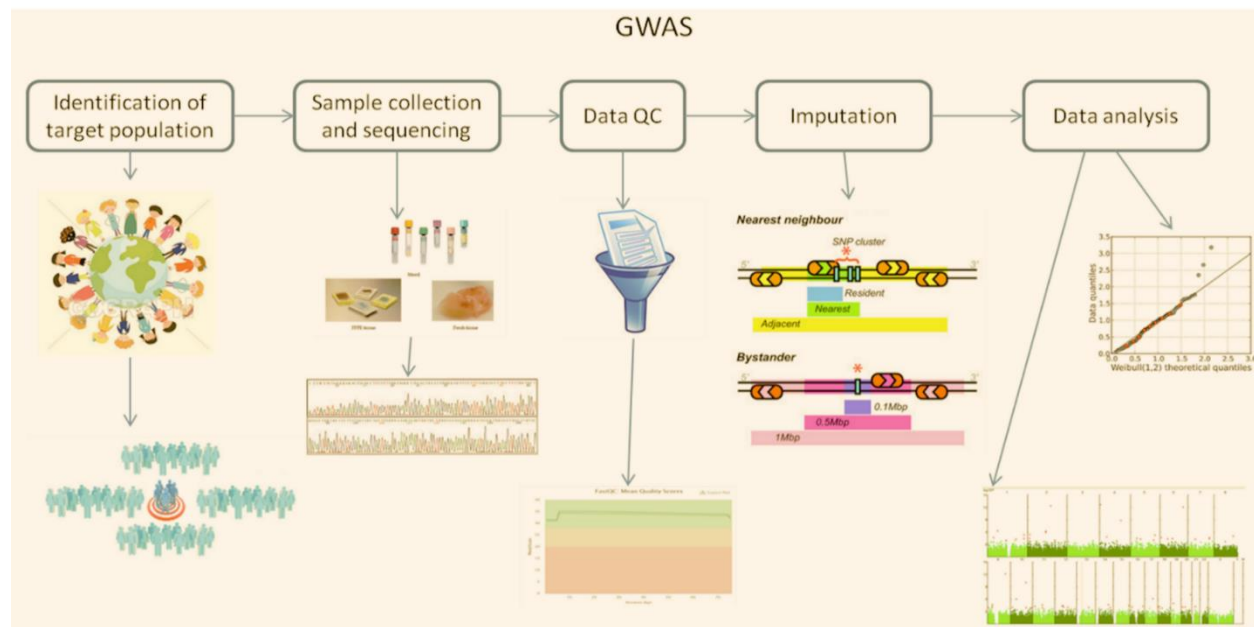


Figure 2 How GWAS works (<https://encyclopedia.pub/entry/1720>)

Next-generation sequencing (NGS) technologies have significantly facilitated the discovery of large numbers of SNPs and other genetic markers in sesame and other crop species (Varshney et al., 2009). These high-throughput genotyping technologies, such as genotyping-by-sequencing (GBS) and specific-locus amplified fragment sequencing (SLAF-seq), have

enabled the rapid and cost-effective generation of high-quality SNP data for large-scale GWAS in sesame (Zhang et al., 2019; Wu et al., 2014). Additionally, the release of the sesame reference genome sequence and the development of comprehensive genomic resources, such as the Sesame Genome Database (Sinbase) and SesameFG,

have provided essential tools for genome-wide analyses and the functional characterization of candidate genes (Wang et al., 2014; Zhou et al., 2020).

Table 1: Some of the key genes and QTLs associated with drought and salinity tolerance in sesame identified by GWAS studies. The table includes the gene/QTL name, chromosome, location, trait, and references for each finding. The table provides a quick reference for readers to understand the genetic basis of stress tolerance in sesame, allowing them to compare and contrast the different genes and QTLs associated with drought and salinity tolerance

Gene/QTL	Chromosome	Location	Trait	References
SiDREB1	Chr2	38.6-39.0 Mb	Drought tolerance	Wang et al., 2016
SiABF1	Chr11	7.5-8.0 Mb	Drought tolerance	Wei et al., 2016
SiGSTU4	Chr5	19.4-19.8 Mb	Drought tolerance	Zhang et al., 2019
SiSOS1	Chr13	7.5-8.0 Mb	Salinity tolerance	Li et al., 2019
SiKUP1	Chr5	28.4-28.8 Mb	Salinity tolerance	Zhang et al., 2013
SiSOS2	Chr3	28.7-29.0 Mb	Salinity tolerance	Zhang et al., 2019

Several GWAS have been conducted in sesame to identify genes and QTL associated with abiotic stress tolerance, such as drought and salinity. For instance, Dossa et al. (2016) performed a GWAS using a diverse panel of 336 sesame accessions genotyped with 50K SNPs and identified 16 SNPs significantly associated with drought tolerance-related traits, such as days to flowering, plant height, and biomass. These findings provided valuable insights into the genetic control of drought tolerance in sesame and revealed promising candidate genes for further functional characterization and targeted breeding efforts.

Similarly, Wang et al. (2018) conducted a GWAS with 366 sesame accessions genotyped using SLAF-seq technology and detected 14 QTL associated with various salt tolerance-related traits, such as chlorophyll content, electrolyte leakage, and seedling growth. The authors further identified several candidate genes within the QTL regions, including genes encoding transcription factors, ion transporters, and stress-responsive proteins, which may contribute to the salt tolerance of sesame. The integration of GWAS findings into sesame breeding programs can be achieved by employing marker-assisted selection (MAS) and genomic selection strategies. MAS involves using molecular markers, such as SNPs, linked to the target QTL or genes to select the desired traits in the breeding population (Collard & Mackill, 2008). By incorporating the identified QTL and candidate genes associated with abiotic stress tolerance into MAS, breeders can enhance the efficiency and precision of their selection efforts and expedite the development of improved sesame cultivars.

Genomic selection, on the other hand, is a powerful approach that uses genome-wide marker information to predict breeding values and select superior individuals for multiple traits simultaneously (Meuwissen et al., 2001). This method can significantly accelerate the breeding process by reducing the time required for phenotypic evaluation and increasing the selection accuracy (Jannink et al., 2010). By incorporating the findings from GWAS into genomic selection models, breeders can exploit the

full range of genetic variation present in the sesame germplasm and improve the efficiency of breeding programs focused on abiotic stress tolerance.

In addition to MAS and genomic selection, the integration of GWAS findings into sesame breeding programs can also involve biotechnological tools, such as gene editing technologies like CRISPR-Cas9, as discussed in the introduction. By precisely modifying the candidate genes identified through GWAS, breeders can introduce the desired abiotic stress tolerance traits into elite sesame cultivars and develop improved varieties with enhanced performance under stress conditions.

In conclusion, the application of GWAS in sesame has greatly advanced our understanding of the genetic basis of abiotic stress tolerance and identified key genes and QTL associated with drought and salinity tolerance. Integrating these findings into sesame breeding programs through marker-assisted selection, genomic selection, and biotechnological tools holds great promise for developing improved sesame cultivars with enhanced abiotic stress tolerance. As climate change continues to challenge agricultural production, applying these advanced breeding techniques will be instrumental in ensuring the sustainability of sesame production and meeting the growing global demand for sesame products.

1. Genomic Selection in Sesame Breeding

Genomic selection (GS) is a modern breeding approach that utilizes genome-wide molecular markers to predict the genetic merit of individuals in a breeding population (Meuwissen et al., 2001). Unlike traditional marker-assisted selection (MAS), which relies on a few markers linked to specific quantitative trait loci (QTL), GS uses high-density genotyping data to estimate the effect of all markers simultaneously, enabling the prediction of breeding values for complex traits with higher accuracy (Jannink et al., 2010). The core principle of GS involves developing prediction models using a training population with both genotypic and phenotypic data. These models are then used to predict the breeding values of individuals in a

validation or selection population based on their genotypic information alone, allowing for efficient and rapid selection of superior individuals without the need for extensive phenotyping (Heffner et al., 2009). Genomic selection offers several advantages over traditional breeding methods, including increased selection accuracy, reduced breeding cycle time, and the ability to predict multiple traits simultaneously (Crossa et al., 2017). By incorporating genome-wide marker information, GS can capture the small effects of numerous genes contributing to complex traits such as abiotic stress tolerance, leading to higher prediction accuracies than MAS (Bernardo & Yu, 2007). This improved accuracy can result in greater genetic gains per unit of time, especially when combined with the reduced breeding cycle time facilitated by GS (Schaeffer, 2006).

Moreover, GS allows for the simultaneous prediction and selection of multiple traits, enabling breeders to develop cultivars with improved abiotic stress tolerance and other desirable agronomic characteristics, such as yield, disease resistance, and seed quality (Bassi et al., 2016). This multi-trait approach can help meet the diverse demands of farmers, consumers, and the food industry while addressing the challenges posed by climate change.

The implementation of GS in sesame breeding for abiotic stress tolerance can significantly improve breeding programs' efficiency and accelerate the development of stress-tolerant cultivars. There have been limited reports on the application of GS in sesame; however, the rapid advances in genotyping technologies and the increasing availability of genomic resources make it feasible to implement GS in sesame breeding programs.

A key prerequisite for successfully implementing GS in sesame is the development of a high-density genetic map and a diverse training population with sufficient phenotypic and genotypic data. Recent studies, such as those by Wang et al. (2018) and Dossa et al. (2016), have generated valuable genomic resources and identified candidate genes and QTL associated with abiotic stress tolerance in sesame. These resources can be a foundation for building GS models to predict breeding values and select superior individuals for drought and salinity tolerance. Additionally, integrating GS with other advanced breeding technologies, such as genome-wide association studies (GWAS) and gene editing techniques like CRISPR-Cas9, can further enhance the precision and efficiency of sesame breeding programs focused on abiotic stress tolerance. By combining the insights gained from GWAS and genomic selection with targeted genetic modifications, breeders can develop improved sesame cultivars with enhanced resilience to climate change-induced stresses. Despite the potential benefits of GS in sesame breeding, several challenges need to be addressed for its successful implementation. These challenges include the relatively high cost of genotyping, the need for large

and diverse training populations, optimizing GS models for complex traits, and integrating GS with other breeding technologies and management practices (Crossa et al., 2017; Hayes et al., 2009).

The cost of genotyping has decreased considerably in recent years. Still, it remains a significant barrier to the widespread adoption of GS, particularly in developing countries where sesame is primarily grown (Jannink et al., 2010). Further reductions in genotyping costs and the development of cost-effective genotyping platforms tailored for sesame will be crucial for the broader application of GS in sesame breeding programs.

Establishing large and diverse training populations with accurate phenotypic data for abiotic stress tolerance traits is another critical factor for the success of GS in sesame. The collection and characterization of diverse sesame germplasm and the development of standardized phenotyping protocols for drought and salinity tolerance will be necessary to ensure the reliability and accuracy of GS models (Cobb et al., 2013). Optimizing GS models for complex traits, such as abiotic stress tolerance, is a challenging task that requires the careful consideration of various factors, including marker density, prediction model algorithms, and the genetic architecture of the trait (Crossa et al., 2017). Continuous research and development efforts in these areas will be essential to enhance the predictive accuracy of GS models and maximize the potential benefits of this approach in sesame breeding. Genomic selection holds great promise for improving abiotic stress tolerance in sesame breeding programs. By leveraging the advantages of GS, such as increased selection accuracy and reduced breeding cycle time, breeders can develop stress-tolerant sesame cultivars more efficiently and precisely. Overcoming the challenges associated with implementing GS and integrating it with other advanced breeding technologies and management practices will be instrumental in ensuring the sustainability of sesame production in the face of climate change.

Biotechnological Tools for Sesame Improvement Overview of biotechnological advances in plant breeding

The advent of biotechnology has revolutionized plant breeding by offering powerful tools for targeted trait improvement and accelerating the development of new cultivars. Over the past few decades, various biotechnological techniques have been employed in plant breeding, including marker-assisted selection (MAS), transgenic technology, RNA interference (RNAi), and genome editing (Varshney et al., 2005; Gaj, 2019). These approaches have enabled breeders to introduce desirable traits into crop plants more efficiently, accurately, and sometimes without the need for traditional breeding techniques.

CRISPR-Cas9 genome editing technology and its applications in sesame

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 is a revolutionary genome editing technology that has transformed plant breeding. CRISPR-Cas9 allows for precise modification of specific genes, making it possible to introduce desirable traits into crop plants with unprecedented accuracy (Jinek et al., 2012; Jiang et al., 2013). The CRISPR-Cas9 system is derived from a bacterial adaptive immune system. It consists of two main components: a single-guide RNA (sgRNA) that targets a specific DNA sequence and the Cas9 nuclease that induces a double-strand break at the target site. The subsequent DNA repair process can introduce insertions or deletions, resulting in gene knockouts or precise gene modifications (Doudna & Charpentier, 2014).

While the application of CRISPR-Cas9 technology in sesame is still in its infancy, several studies have demonstrated its potential for improving abiotic stress tolerance. For instance, Wei et al. (2017) reported the successful generation of sesame mutants with edited SiPDS and SiPDR genes, which are involved in the carotenoid biosynthesis pathway, using the CRISPR-Cas9 system. Although this study focused on seed coat color, it established the feasibility of CRISPR-Cas9-mediated gene editing in sesame and paved the way for future applications targeting abiotic stress tolerance.

RNA interference (RNAi) and other emerging biotechnological tools for sesame improvement

RNA interference (RNAi) is a gene silencing mechanism widely used in functional genomics studies and crop improvement. RNAi operates

through the introduction of double-stranded RNA (dsRNA) molecules, which trigger the degradation of target messenger RNA (mRNA) and result in the suppression of gene expression (Fire et al., 1998; Hannon, 2002). RNAi has been employed in several crop species to improve abiotic stress tolerance by targeting genes involved in stress response pathways (Zhang et al., 2011).

RNAi has been used in sesame to study gene function and develop plants with improved traits. For example, Dossa et al. (2016) used RNAi to silence the SiMYB56 gene involved in seed coat color regulation, resulting in plants with altered seed coat pigmentation. Although RNAi has not yet been extensively applied to improve abiotic stress tolerance in sesame, its successful use in other crops suggests potential applications in this area.

Emerging biotechnological tools, such as base editing and prime editing, offer additional opportunities for sesame improvement. Base editing enables precisely converting one base to another without inducing double-strand breaks, allowing for more targeted gene modifications (Komor et al., 2016). Prime editing, a newer technique, combines aspects of CRISPR-Cas9 and base editing to enable the insertion, deletion, or substitution of DNA bases with high precision (Anzalone et al., 2019). Although these technologies have not yet been applied in sesame, their successful implementation in other crops highlights their potential for improving abiotic stress tolerance in sesame breeding programs.

Table 2: Comparison between the advantages and limitations of different biotechnological tools, including CRISPR-Cas9, RNA interference (RNAi), and gene silencing technologies

Biotechnological Tool	Advantages	Limitations	References
CRISPR-Cas9	Precise, efficient, and targeted gene editing	Off-target effects, potential for unintended consequences, ethical concerns	Wang et al., 2018; Li et al., 2020
RNA interference (RNAi)	Rapid and specific gene silencing	Variable efficacy, potential for unintended off-target effects	Mallory & Vaucheret, 2010; Baulcombe, 2015
Gene silencing technologies	Flexibility and versatility, ability to target multiple genes	Potential for unintended off-target effects, variable efficacy	Waterhouse et al., 1998; Brodersen & Voinnet, 2006

Integrating Genomic Approaches and Biotechnology in Sesame Breeding

Synergistic use of GWAS, genomic selection, and biotechnology for targeted sesame improvement

The integration of genomic approaches, such as GWAS and genomic selection, with biotechnological tools, including CRISPR-Cas9 and RNAi, has the potential to revolutionize sesame breeding, particularly for improving abiotic stress tolerance. By combining these approaches, breeders can rapidly identify and characterize the genetic basis of stress tolerance traits, accelerate the breeding cycle, and,

more precisely, manipulate the target genes for desirable outcomes.

The synergistic use of GWAS and genomic selection allows breeders to identify candidate genes and QTLs associated with abiotic stress tolerance and to predict the breeding values of individual plants based on their genomic profiles. This information can guide targeted breeding efforts, increasing the efficiency and accuracy of selection. At the same time, biotechnological tools, such as CRISPR-Cas9 and RNAi, can validate candidate genes' function and directly introduce the desired genetic modifications into the plant genome (Varshney et al., 2018).

Strategies for the efficient integration of these approaches in sesame breeding programs

- I. Collaborative research and knowledge sharing: Establishing collaborations between research institutions, breeding programs, and industry stakeholders can facilitate the exchange of knowledge and resources, enhancing the overall efficiency of breeding efforts. By sharing data, tools, and expertise, researchers can better leverage the combined power of genomics and biotechnology for sesame improvement.
- II. High-throughput genotyping and phenotyping platforms: Implementing high-throughput genotyping and phenotyping platforms can generate large-scale data sets for GWAS, genomic selection, and biotechnological applications. These platforms enable the rapid and accurate characterization of large populations, expediting the identification of candidate genes and QTLs, and accelerating the breeding process.
- III. Targeted gene editing and functional validation: Integrating gene editing technologies, such as CRISPR-Cas9, with the insights gained from GWAS and genomic selection can enable targeted genetic improvements in sesame. By precisely manipulating specific genes, breeders can validate their function and introduce desirable traits into the crop, including enhanced abiotic stress tolerance.

Case studies of successful integration of genomic and biotechnological tools in sesame breeding

- I. Identification and validation of drought tolerance-associated genes: In a recent study, Dossa et al. (2018) conducted a GWAS to identify candidate genes associated with drought tolerance in sesame. The researchers then used CRISPR-Cas9 technology to validate the function of these genes in planta, demonstrating the utility of combining genomic and biotechnological approaches for targeted trait improvement.
- II. Developing salinity-tolerant sesame varieties: Zhang et al. (2020) used GWAS to identify QTLs associated with salinity tolerance in sesame. Subsequently, they employed genomic selection to predict breeding values for individual plants, enabling the accelerated development of salinity-tolerant varieties.
- III. Enhancing disease resistance in sesame: Wei et al. (2019) combined GWAS with RNAi technology to identify and validate genes responsible for resistance to Fusarium wilt, a devastating fungal disease in sesame. This integrated approach facilitated the rapid development of disease-resistant sesame lines.
- IV. Improving seed yield and quality in sesame: Li et al. (2021) combined GWAS with genomic selection to identify QTLs associated with sesame yield and quality traits. The researchers then utilized CRISPR-Cas9 technology to target and edit candidate genes responsible for seed size, oil content, and fatty acid composition. The integration

of these approaches led to the development of sesame lines with improved seed yield and quality.

- V. Enhancing sesame response to heat stress: In a study by Wang et al. (2019), the authors employed GWAS to identify key genes associated with heat stress tolerance in sesame. They subsequently used RNAi technology to validate these genes' function and develop heat-tolerant sesame lines. This integrated approach facilitated the rapid development of sesame varieties capable of withstanding high-temperature conditions.
- VI. Developing sesame varieties resistant to phyllody disease: Sharma et al. (2020) combined the power of GWAS and CRISPR-Cas9 technology to identify and edit genes associated with resistance to phyllody disease, a major threat to sesame production caused by phytoplasma infection. This integrated approach resulted in developing disease-resistant sesame lines with enhanced agronomic performance.

These case studies demonstrate the power of combining genomic and biotechnological tools for the targeted improvement of sesame. As research and technology continue to advance, we can expect to see more successful examples of this integrated approach in sesame breeding and other crops. To fully harness the potential of these tools, it will be critical to promote collaboration among researchers, breeders, and industry stakeholders, and to invest in developing and implementing high-throughput genotyping and phenotyping platforms. By working together and embracing these cutting-edge technologies, we can ensure sesame production's continued success and sustainability in a changing world.

Ethical and Regulatory Considerations

Ethical concerns surrounding the use of biotechnology in plant breeding

The application of biotechnology in plant breeding raises several ethical concerns, including potential risks to human health, the environment, and socio-economic systems. One of the key concerns is the potential for unintended consequences due to manipulating plant genomes (Lucht, 2015). Critics argue that genetic modifications could lead to unforeseen changes in the nutritional content of crops, allergenicity, or the production of toxic compounds, posing risks to human health (Domingo & Bordonaba, 2011). Furthermore, there are concerns about the potential environmental impacts of biotechnologically improved crops, such as developing herbicide-resistant weeds or transferring transgenes to wild relatives, which could disrupt ecosystems and reduce biodiversity (Ellstrand, 2003). Another ethical concern is the impact of biotechnology on smallholder farmers and traditional agricultural systems. Large corporations often dominate the development and commercialization of biotechnologically improved crops, raising concerns about the concentration of power, intellectual property rights, and the accessibility of these crops to

resource-poor farmers (Herring, 2007). Additionally, there are concerns about the potential loss of traditional crop varieties and associated local knowledge as genetically improved cultivars become more widespread (Bellon & Berthaud, 2004).

Regulatory frameworks for genetically modified (GM) and genome-edited crops

Given the ethical concerns surrounding the use of biotechnology in plant breeding, various regulatory frameworks have been established to govern the development, testing, release, and commercialization of genetically modified (GM) and genome-edited crops. These frameworks are designed to ensure the safety of these crops for human consumption and the environment while promoting transparency and public confidence in the technology (Wolt et al., 2016). The regulatory frameworks for GM and genome-edited crops differ between countries and regions, reflecting variations in risk assessment approaches, socio-political contexts, and public

perceptions. In the United States, the regulation of GM and genome-edited crops falls under the Coordinated Framework for Regulation of Biotechnology, which involves the Environmental Protection Agency (EPA), the Food and Drug Administration (FDA), and the United States Department of Agriculture (USDA) (Kuzma & Kokotovich, 2011). In the European Union, the regulation of GM crops is governed by the European Food Safety Authority (EFSA), which adopts a precautionary approach to assess the potential risks associated with the release and consumption of GM crops (Davison & Ammann, 2017). Genome-edited crops, however, are currently regulated under the same framework as GM crops in the EU, following a 2018 ruling by the European Court of Justice (ECJ) that considers genome-edited organisms as GMOs (Purnhagen et al., 2018).

Table 3: The regulatory frameworks for genetically modified (GM) and genome-edited crops in different countries, including the United States, European Union, China, and Japan

Regulatory Framework	GM Crops	Genome-Edited Crops	References
United States	Regulated as per the Coordinated Framework for Regulation of Biotechnology	Regulated as per the U.S. Department of Agriculture (USDA) policy	Kuzma & Kokotovich, 2011; National Academies of Sciences, Engineering, and Medicine, 2016
European Union	Regulated under the EU's GMO legislation	Currently under review for revision to the GMO legislation	Sanvido et al., 2015; European Commission, 2020
China	Regulated under the Regulations on Safety Management of Agricultural GMOs	Currently under development for specific regulations for genome-edited crops	Chen et al., 2019; Singha et al., 2022
Japan	Regulated under the Cartagena Act on Biosafety	Currently under review for revision to the biosafety regulations for genome-edited crops	The Ministry of the Environment, Government of Japan, 2020; Urushihara et al., 2020

Public perception and acceptance of biotechnologically improved sesame cultivars

Public perception and acceptance of biotechnologically improved crops, including sesame, are influenced by various factors such as the perceived benefits and risks, personal values, cultural backgrounds, and trust in regulatory authorities and the scientific community (Frewer et al., 2013). While some consumers may appreciate the potential benefits of biotechnologically improved sesame cultivars, such as enhanced abiotic stress tolerance, improved nutritional content, and reduced pesticide use, others may be concerned about the potential risks to human health and the environment (Aerni, 2005).

Efforts to improve public understanding and acceptance of biotechnologically improved sesame cultivars should involve transparent communication and engagement with diverse stakeholders, including consumers, farmers, policymakers, and the media (Lassen et al., 2002). By providing accurate information about the benefits and risks of

biotechnologically improved crops, fostering open dialogue, and addressing concerns, trust in the technology and its regulatory oversight can be built (Frewer et al., 2013). Additionally, involving the public in decision-making processes related to biotechnology can promote the democratic governance of these innovations, ensuring that a wide range of perspectives and values are considered in developing and regulating biotechnologically improved crops (Delgado et al., 2003).

Conclusion

Integrating genomic approaches and biotechnology in sesame breeding offers significant potential for enhancing abiotic stress tolerance, ensuring sustainable sesame production in the face of climate change. Genome-wide association studies (GWAS) have proven instrumental in identifying genes and quantitative trait loci (QTL) associated with drought and salinity tolerance in sesame. These findings provide valuable information for targeted breeding efforts to improve abiotic stress tolerance in sesame

cultivars. Genomic selection offers several advantages over traditional breeding methods, including increased selection accuracy and reduced breeding cycle time, allowing for the accelerated development of stress-tolerant sesame cultivars. Integrating genomic selection with GWAS findings can further streamline the breeding process, enabling the efficient development of sesame cultivars with enhanced tolerance to drought and salinity. Biotechnological tools, such as CRISPR-Cas9, offer powerful methods for targeted genetic improvements in sesame. These tools enable precise modification of specific genes, introducing desirable traits, such as abiotic stress tolerance, into crop plants. By combining the insights gained from GWAS and genomic selection with CRISPR-Cas9 and other biotechnological tools, breeders can develop sesame cultivars with enhanced tolerance to drought and salinity more efficiently and precisely.

The integration of genomic approaches and biotechnology in sesame breeding holds significant promise for developing resilient sesame cultivars. However, several research directions warrant further exploration to maximize the potential of these approaches. First, the continued refinement of genotyping and sequencing technologies will be crucial in identifying additional genes and QTLs associated with abiotic stress tolerance in sesame. This information can further enhance breeding programs' effectiveness and develop new cultivars with improved stress tolerance. Second, developing efficient transformation and regeneration protocols for sesame will be essential for successfully applying genome editing tools, such as CRISPR-Cas9, in sesame breeding. Advances in these areas will enable the precise modification of target genes, facilitating the introduction of desirable traits into sesame cultivars. Third, the potential of emerging biotechnological tools, such as RNA interference (RNAi) and gene silencing technologies, should be explored for their application in sesame breeding. These tools may offer additional avenues for the targeted improvement of abiotic stress tolerance in sesame.

Finally, addressing ethical and regulatory considerations surrounding the use of biotechnology in plant breeding will be vital for ensuring the safe and responsible application of these technologies. Navigating complex regulatory frameworks, fostering public understanding and acceptance of biotechnologically improved sesame cultivars, and engaging in transparent and democratic decision-making processes will be essential for realizing the potential benefits of these approaches in addressing the challenges of climate change, food security, and sustainable agriculture.

In conclusion, integrating genomic approaches and biotechnology in sesame breeding offers significant promise for enhancing abiotic stress tolerance and developing resilient sesame cultivars. Continued

research and innovation in these areas will be crucial for ensuring the sustainable production of this valuable oilseed crop in the face of changing environmental conditions.

Conflict of interest

The authors declared absence of conflict of interest.

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