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Enhancing Heterosis in Sorghum Using Male Sterile Lines

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Abstract This study aims to enhance heterosis in sorghum by utilizing male sterile lines, including the evaluation of the combining ability and heterosis of various sorghum lines, to identify hybrids that exhibit superior performance in terms of grain yield, biomass production, and other agronomic traits. The study revealed significant genotype, location, and genotype × location interaction effects for most traits, indicating mixed performances of the tested genotypes, which allows for further selection. Both general combining ability (GCA) and specific combining ability (SCA) effects were significant for most traits, including grain yield and anthracnose resistance, suggesting the involvement of both additive and non-additive gene actions. Hybrids derived from cytoplasmic male sterile lines exhibited higher brix, greater stalk yield, and higher sugar content compared to their male-fertile counterparts. Additionally, hybrids showed significant positive heterosis for grain Fe and Zn concentrations, which is crucial for biofortification efforts. The study identified several high-GCA effect germplasm, such as AMP418, AMP431, AMP434, AMP443, AMP495, AMP496, and the male line X097, which hold promise for accelerating the genetic improvement of sorghum for dual-purpose use. The findings underscore the potential of using male sterile lines to enhance heterosis in sorghum. The identified superior hybrids and parental lines can be utilized in breeding programs to improve grain yield, biomass production, and nutritional quality, thereby contributing to food security and biofuel production.

Keywords Sorghum; Heterosis; Male sterile lines; Combining ability; Grain yield; Biomass; Biofortification; Cytoplasmic male sterility

1 Introduction

Heterosis, also known as hybrid vigor, is a phenomenon where hybrid offspring exhibit superior traits compared to their parents. This includes increased yield, disease resistance, and stress tolerance, making it a critical component in crop improvement strategies. The genetic basis of heterosis involves complex interactions such as dominance, overdominance, and epistasis, which contribute to the enhanced performance of hybrids (Fujimoto et al., 2018; Hochholdinger and Baldauf, 2018; Paril et al., 2023). The development of high-yielding hybrid cultivars is essential to meet the growing global food demands, especially in the face of challenges posed by climate change and limited arable land (Chen et al., 2020; Paril et al., 2023).

Sorghum (*Sorghum bicolor*) is a vital cereal crop, particularly in arid and semi-arid regions of the world. It is the fifth most important cereal crop globally, providing food, fodder, and biofuel. Sorghum's resilience to harsh environmental conditions, such as drought and high temperatures, makes it a crucial crop for food security in many developing countries. Its versatility and adaptability have led to its widespread cultivation across Africa, Asia, and the Americas (Li et al., 2015; Hashimoto et al., 2021).

The production of hybrid seeds relies heavily on the use of male sterile lines, which prevent self-pollination and facilitate cross-pollination. Male sterility can be induced through genetic or cytoplasmic means, and it is a valuable tool in hybrid breeding programs. The use of male sterile lines simplifies the hybrid seed production process, ensuring the purity and uniformity of the hybrid seeds. This is particularly important in crops like sorghum, where hybrid vigor can significantly enhance yield and other agronomic traits (Li et al., 2015; Chen et al., 2020).

This study hopes to develop high-yielding and resilient sorghum hybrids by leveraging the genetic mechanisms of heterosis and employing advanced breeding techniques. It will focus on identifying and combining genes associated with heterosis and optimizing the use of male sterile lines to maximize hybrid performance. The goal is to significantly enhance sorghum productivity, contributing to global food security.

2 Male Sterility in Sorghum

2.1 Types of male sterility

Male sterility in sorghum can be broadly categorized into two types: genetic male sterility (GMS) and cytoplasmic male sterility (CMS).

Genetic male sterility (GMS): This type of male sterility is controlled by nuclear genes. For instance, the nuclear male sterile (NMS) trait in sorghum is caused by mutations in nuclear genes, as demonstrated by the ms8 mutant, which is a result of a single recessive nuclear gene mutation (Xin et al., 2017).

Cytoplasmic male sterility (CMS): CMS is caused by the interaction between the cytoplasmic genome and nuclear genes. It is widely used in hybrid breeding programs. Different CMS systems, such as A1, A2, A3, and others, have been identified in sorghum. The A1 CMS system is the most commonly used in commercial hybrid seed production (Kishan and Borikar, 1989; Choe et al., 2023).

2.2 Mechanisms and genetic basis of male sterility

The mechanisms underlying male sterility in sorghum involve complex interactions between nuclear and cytoplasmic genes.

Genetic male sterility (GMS): In the case of the ms8 mutant, male sterility is due to defective tapetum development, which leads to the arrest of pollen formation. This mutation is stable across different environments, making it a valuable tool for breeding (Xin et al., 2017).

Cytoplasmic male sterility (CMS): The CMS trait is caused by specific interactions between mitochondrial and nuclear genes. For example, the A1 CMS system involves the interaction of mitochondrial genes with nuclear restorer genes (*Rf* genes). The *Rf* genes, such as *Rf1* and *Rf2*, are responsible for restoring fertility in CMS plants. The *Rf2* gene has been mapped to chromosome SBI-02 and is associated with a pentatricopeptide repeat (PPR) gene family member (Jordan et al., 2010; Narkhede et al., 2022). Additionally, environmental factors can influence the expression of fertility-restoring genes, as seen in the 9E CMS system, where high water availability can up-regulate these genes (Elkonin et al., 2015).

2.3 Previous research on male sterile lines in sorghum breeding

Research on male sterile lines in sorghum has significantly contributed to the development of hybrid varieties.

Nuclear male sterile lines: The ms8 mutant is a well-characterized nuclear male sterile line that has been shown to be stable across various environments. This line is useful for hybrid breeding and genetic studies (Xin et al., 2017).

Cytoplasmic male sterile lines: The A1 CMS system is the most extensively used in commercial hybrid seed production. Studies have identified various restorer genes and their interactions with CMS lines. For instance, the Rf2 gene has been fine-mapped, and its role in fertility restoration has been elucidated (Jordan et al., 2010; Narkhede et al., 2022). Additionally, genetic analyses have shown that CMS systems like A2 and A3 can be utilized for practical breeding programs (Kishan and Borikar, 1989).

Marker development: Recent advancements include the development of DNA markers to distinguish between fertile and sterile cytoplasms, facilitating marker-assisted selection in breeding programs (Figure 1). For example, InDel markers have been developed to identify CMS-S and CMS-N cytotypes in sorghum (Choe et al., 2023).

3 Importance of Hybrid Breeding in Sorghum

3.1 Benefits of hybrid sorghum varieties

Hybrid sorghum varieties offer several significant advantages over traditional pureline varieties. One of the primary benefits is increased yield. Studies have shown that hybrids can produce greater stalk yield due to taller plants with greater stem diameter, which translates to higher biomass and grain yield (Makanda et al., 2010; Pfeiffer et al., 2010; Sandeep and Biradar, 2020) (Table 1). Additionally, hybrids often exhibit improved disease resistance and stress tolerance, which are crucial for maintaining productivity in varying environmental conditions (Makanda et al., 2010; He et al., 2020). For instance, the development of cytoplasmic male-sterile lines has enabled the creation of hybrids with higher sugar content in stems, which is beneficial for syrup and ethanol production (Pfeiffer et al., 2010).

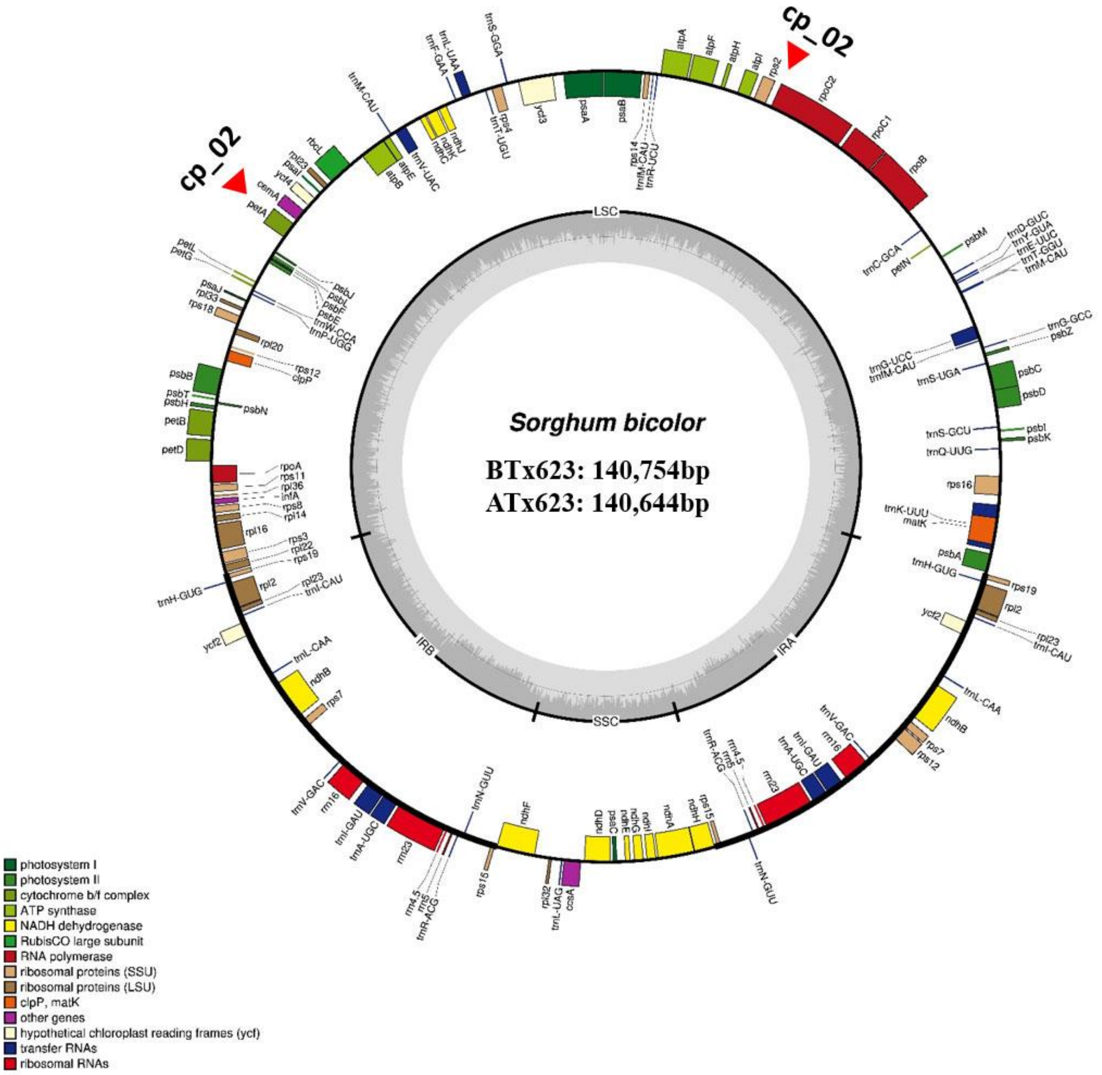

Figure 1 The complete chloroplast genome map of *Sorghum bicolor* in the male sterile (ATx623) and maintainer line (BTx623) (Adopted from Choe et al., 2023)

Image caption: The genes inside and outside the circle are transcribed in the clockwise and counterclockwise directions, respectively. Genes belonging to different functional groups are represented using different colors. Thick lines indicate the extent of the inverted repeats (IRa and IRb) that separate the genomes into small single-copy (SSC) and large single-copy (LSC) regions. The red-filled red triangles indicate the region of insertion and deletion (InDel) markers (Adopted from Choe et al., 2023)

3.2 Challenges in traditional breeding approaches

Traditional breeding approaches in sorghum face several challenges. One major issue is the limited genetic diversity available in pureline varieties, which restricts the potential for significant yield improvements and disease resistance (Makanda et al., 2010). Moreover, traditional breeding is time-consuming and labor-intensive, often requiring multiple generations to achieve desired traits. The reliance on natural pollination processes also introduces variability and unpredictability in breeding outcomes (Hanafi et al., 2022). These challenges underscore the need for more efficient breeding techniques, such as the use of male sterility systems to facilitate hybrid seed production (Song et al., 2020; Wan et al., 2021).

3.3 Role of male sterility in hybrid seed production

Male sterility plays a crucial role in the production of hybrid sorghum seeds. The use of cytoplasmic male-sterile (CMS) lines allows for controlled cross-pollination, ensuring that the resulting seeds are hybrids (Pfeiffer et al., 2010; He et al., 2020; Sandeep and Biradar, 2020). This method eliminates the need for manual emasculation, significantly reducing labor and increasing the efficiency of hybrid seed production. Additionally, male sterility systems can be combined with modern breeding strategies to create novel cultivation models that enhance grain yield and ensure sustainable agriculture (Wan et al., 2021). The CMS system has been successfully utilized in various crops, including sorghum, to exploit heterosis and improve overall crop performance (Makanda et al., 2010; Pfeiffer et al., 2010; He et al., 2020).

Table 1 Average heterosis and range of heterosis with number of heterotic crosses of parents and F1s in desirable directions in respect of quantitative traits in rabi sorghum (Adopted from Sandeep and Biradar, 2020)

Sl. No.	Characters	Average heterosis (%)	Range of heterosis (%) over				No. of crosses with significant heterosis in desired direction over			
			Better parent	Standard check			Better parent	Standard check		
				M 35-1	BJV 44	PKV Kranti		M 35-1	BJV 44	PKV Kranti
1	Days to 50% flowering	-2.87	-30.65 to -0.81	-25.86 to 6.03	-25.51 to 6.49	-29.22 to1.23	47	34	34	43
2	Plant height (cm)	6.36	-10.05 to 34.31	-18.27 to 10.99	-20.34 to 8.18	-22.21 to 5.64	17	08	05	02
3	Number of leaves	2.21	-38.46 to 40.00	-33.33 to 16.67	-33.33 to 16.67	-38.46 to 7.69	05	03	03	00
4	Panicle length (cm)	1.88	-47.45 to 26.41	-21.56 to 82.16	-33.54 to 54.33	-36.64 to 47.15	01	32	23	14
5	Panicle width (cm)	-12.07	-41.61 to 6.33	-35.94 to 4.15	-53.20 to -23.91	-53.67 to -24.67	02	00	00	00
6	Primaries Panicle-1	11.03	-39.87 to 19.62	-34.48 to 39.31	-48.65 to 9.19	-50.78 to 4.66	05	19	01	00
7	Panicle weight (g)	12.56	-51.52 to 51.95	-46.88 to 69.13	-72.21 to -11.50	-72.47 to -12.33	14	20	00	00
8	Grain yield per plant (g)	4.07	-58.03 to 18.47	-52.61 to 52.41	-75.16 to -20.13	-74.96 to -19.47	02	13	00	00
9	100 seed weight (g)	40.61	-26.25 to 43.88	-23.38 to 83.12	-26.25 to 76.25	-30.59 to 65.88	14	21	19	09

4 Development of Male Sterile Lines

4.1 Criteria for selecting male sterile lines

The selection of male sterile lines in sorghum is based on several key criteria. Firstly, the lines must exhibit complete and stable male sterility to ensure no self-pollination occurs, which is crucial for hybrid seed production. Additionally, the selected lines should possess desirable agronomic traits such as high yield potential, disease resistance, and adaptability to various environmental conditions. For instance, cytoplasmic male-sterile (CMS) lines like A3 cytoplasmic male-sterile 'Dale' and 'Wray' have been noted for their higher brix and greater stalk yield compared to their male-fertile counterparts, making them suitable candidates for hybrid development (Pfeiffer et al., 2010). Furthermore, the lines should be compatible with a wide range of restorer lines to facilitate the production of diverse hybrids (Mengistu et al., 2020).

4.2 Breeding strategies for developing and maintaining male sterile lines

Breeding strategies for developing and maintaining male sterile lines in sorghum involve several approaches. One common method is the use of cytoplasmic-genic male sterility (CGMS) systems, where male sterility is controlled by both cytoplasmic and nuclear genes. This system allows for the easy maintenance of male sterile lines by crossing them with maintainer lines that possess the same cytoplasm but are male fertile. For example, the use of

A3 cytoplasmic male-sterile lines in combination with their male-fertile counterparts has been effective in producing high-yielding hybrids (Pfeiffer et al., 2010). Another strategy involves the use of genic male sterility (GMS) systems, where male sterility is controlled by nuclear genes alone. This approach can be enhanced by modern techniques such as CRISPR/Cas-mediated gene editing to create targeted mutations that result in male sterility (Nadeem et al., 2021). Additionally, marker-assisted selection can be employed to identify and select for male sterility genes, as demonstrated in wheat breeding programs (Yang et al., 2021).

4.3 Examples of successful male sterile lines in sorghum

Several successful male sterile lines have been developed and utilized in sorghum breeding programs. For instance, the A3 cytoplasmic male-sterile lines 'Dale', 'Wray', 'Sugar Drip', and N100 have been used to create hybrids with higher brix and greater stalk yield, which are advantageous for syrup and ethanol production (Pfeiffer et al., 2010). Another example includes the hybrids derived from cytoplasmic genic male sterile lines such as AKMS 22A x RSSV 9 and BJ 3A x RSSV 9, which have shown superior performance in terms of sweet sorghum quality traits (Indhubala et al., 2010). These examples highlight the potential of male sterile lines in enhancing heterosis and improving the overall productivity of sorghum.

5 Enhancing Heterosis through Male Sterile Lines

5.1 Mechanism of heterosis and how male sterility contributes to hybrid vigor

Heterosis, or hybrid vigor, refers to the phenomenon where hybrid offspring exhibit superior qualities compared to their parents. This can include increased yield, improved resistance to diseases, and better adaptability to environmental stresses. The underlying mechanisms of heterosis involve complex genetic interactions, including dominance, overdominance, and epistasis, which result in enhanced performance of hybrids (Kibalnik, 2019).

Male sterility, particularly cytoplasmic male sterility (CMS), plays a crucial role in facilitating heterosis. CMS is a maternally inherited trait that prevents the production of functional pollen, thereby necessitating cross-pollination. This ensures that the resulting seeds are hybrids, combining the genetic material of two different parent lines. The use of CMS lines in sorghum breeding has been shown to enhance hybrid vigor by promoting genetic diversity and combining desirable traits from both parents (Kim and Zhang, 2018; Song et al., 2020).

5.2 Crossbreeding experiments to maximize heterosis

Crossbreeding experiments are essential to identify the best parental combinations that maximize heterosis. In sorghum, various studies have employed line × tester mating designs to evaluate the performance of hybrids. For instance, a study involving the crossing of eight restorer lines with five CMS lines in a line × tester design revealed significant heterosis for traits such as plant height, panicle length, and grain yield (Chauhan and Pandey, 2021). Another study conducted in East Africa used 36 pairs of male sterile lines and 42 restorers to generate 121 experimental hybrids. The hybrids were evaluated across multiple locations, and significant heterosis was observed for traits like grain yield and plant height (Ringo et al., 2015).

These experiments highlight the importance of selecting genotypically superior parents and evaluating their performance in different environmental conditions to identify the best hybrid combinations. The use of CMS lines in these experiments ensures that the resulting hybrids exhibit enhanced vigor and productivity.

5.3 Case studies or examples of enhanced heterosis in sorghum hybrids using male sterile lines

Several case studies have demonstrated the effectiveness of using male sterile lines to enhance heterosis in sorghum hybrids. For example, a study on sweet sorghum hybrids using CMS lines found that male-sterile hybrids had higher brix (sugar content) and greater stalk yield compared to their male-fertile counterparts. The hybrid A3 N100 × Dale showed positive heterosis for brix, indicating its potential for increased syrup or ethanol production (Pfeiffer et al., 2010).

Another study on forage sorghum hybrids revealed significant heterosis for traits such as plant height, leaf length, and green fodder yield. The best crosses, such as ICSA 94 × Pant Chari-4 and ICSA 94 × SDSL92111, exhibited superior performance in terms of both quantitative and qualitative traits (Agarwal and Shrotria, 2005).

In East Africa, hybrids like ICSA11 × S35 showed up to 81.90% average heterosis for grain yield, demonstrating the potential of CMS lines to produce high-yielding hybrids suitable for the region's cultivation systems (Ringo et al., 2015).

6 Genetic Tools and Techniques

6.1 Use of molecular markers for identifying and selecting male sterile traits

Molecular markers have become indispensable in the identification and selection of male sterile traits in sorghum breeding programs. For instance, the use of SSR markers has been demonstrated to effectively map male sterility genes in crops like wheat, which can be adapted for sorghum. In wheat, a recessive gene for male sterility was mapped to a specific chromosome region using SSR markers, facilitating marker-assisted selection (Yang et al., 2021). This approach can be similarly applied to sorghum to enhance the precision and efficiency of breeding programs aimed at developing male sterile lines.

6.2 Application of genetic engineering and CRISPR technology

Genetic engineering and CRISPR technology offer powerful tools for the manipulation of male sterility traits in sorghum. These technologies allow for the precise editing of genes responsible for male sterility, thereby accelerating the development of male sterile lines. For example, CRISPR/Cas9 can be used to knock out specific genes that control fertility, creating stable male sterile lines that are essential for hybrid seed production. The integration of these advanced genetic tools can significantly enhance the heterosis potential in sorghum by ensuring the consistent production of high-quality hybrids (He et al., 2020; Chauhan and Pandey, 2021).

6.3 Integration of genomic selection in breeding programs

Genomic selection (GS) is a cutting-edge approach that leverages genome-wide markers to predict the breeding values of individuals, thereby accelerating the selection process. In sorghum, GS can be integrated into breeding programs to improve the efficiency of selecting male sterile lines and their corresponding hybrids. Studies have shown that combining ability and heterosis for yield and other agronomic traits can be effectively assessed using genomic selection, which helps in identifying superior parental combinations for hybrid development (He et al., 2020; Kanbar et al., 2020; Mengistu et al., 2020). This integration not only speeds up the breeding cycle but also enhances the accuracy of selection, leading to the development of high-yielding and resilient sorghum hybrids.

7 Agronomic and Economic Benefits

7.1 Improvement in yield and quality traits through enhanced heterosis

The use of male sterile lines in sorghum breeding has demonstrated significant improvements in both yield and quality traits through enhanced heterosis. Studies have shown that hybrids derived from cytoplasmic male sterile lines exhibit superior performance in terms of grain yield and biomass production. For instance, hybrids tested with 34 cytoplasmic male sterile lines and 3 restore lines showed grain yields ranging from 0.3 to 14.0 t/ha and aboveground biomass yields from 9.6 to 109.9 t/ha, indicating a substantial heterosis effect (He et al., 2020). Additionally, hybrids such as AKMS 22A x RSSV 9 and BJ 3A x RSSV 9 have been identified for their superior sweet sorghum quality traits, which are crucial for high biomass and ethanol yield (Indhubala et al., 2010). The exploitation of heterosis in these hybrids not only enhances yield but also improves other agronomic traits such as plant height, productive tiller rate, and harvest index, which are positively correlated with grain and biomass yield (He et al., 2020; Sandeep and Biradar, 2020).

7.2 Cost-effectiveness and scalability of using male sterile lines in hybrid seed production

The use of male sterile lines in hybrid seed production is both cost-effective and scalable. The development of hybrids using cytoplasmic male sterile lines eliminates the need for manual emasculation, thereby reducing labor costs and increasing efficiency in seed production. For example, the use of thermosensitive genic male sterile (TGMS) lines in rice has shown potential for economic hybrid seed production, which can be translated to sorghum breeding programs (Shukla and Pandey, 2007). Furthermore, the scalability of this approach is evident from the successful production of 121 experimental hybrids using a line × tester mating design, which were evaluated across multiple locations, demonstrating the feasibility of large-scale hybrid seed production (Ringo et al., 2015). The significant general combining ability (GCA) and specific combining ability (SCA) effects observed in these studies indicate that both additive and non-additive gene actions are important, making the breeding process more predictable and efficient (Makanda et al., 2010; Mengistu et al., 2020).

7.3 Market demand and adoption of hybrid sorghum varieties
The market demand and adoption of hybrid sorghum varieties are driven by their superior agronomic performance and resilience to environmental stresses. High-yielding sorghum genotypes with farmer-preferred traits and resistance to diseases such as anthracnose are key factors in their adoption (Mengistu et al., 2020). The development of hybrids with high grain Fe and Zn concentrations also addresses micronutrient malnutrition, making these hybrids more attractive to farmers and consumers (Gaddameedi et al., 2020). In sub-Saharan Africa, the adoption of hybrid cultivars has been shown to enhance productivity and food security, with hybrids displaying up to 285% standard heterosis for grain yield (Makanda et al., 2010). The positive correlation between grain yield and traits such as head length and number of leaves per plant further supports the market potential of these hybrids (Makanda et al., 2010). Overall, the enhanced heterosis achieved through the use of male sterile lines not only improves yield and quality traits but also meets the market demand for high-performing and nutritionally superior sorghum varieties.

8 Challenges and Limitations
8.1 Genetic and environmental challenges in maintaining male sterility
Maintaining male sterility in sorghum presents several genetic and environmental challenges. One significant genetic challenge is the stability of male sterility genes across different environmental conditions. For instance, the cytoplasmic male-sterility (CMS) system, while effective, can be influenced by environmental factors such as temperature and photoperiod, which may affect the expression of sterility (Song et al., 2020). Additionally, the genetic background of the plant can influence the stability of male sterility, as seen in other crops like soybean and wheat, where specific genetic deletions or mutations are required to maintain sterility (Nadeem et al., 2021; Yang et al., 2021). Environmental stresses such as drought and nutrient deficiencies can also impact the expression of male sterility, potentially leading to partial fertility and complicating breeding efforts (Pfeiffer et al., 2010).

8.2 Limitations of current breeding techniques
Current breeding techniques for enhancing heterosis in sorghum using male sterile lines face several limitations. Traditional CMS systems, while useful, often have low resource utilization rates and can be environmentally sensitive, limiting their effectiveness (Song et al., 2020). Moreover, the development of male-sterile lines through conventional breeding is time-consuming and labor-intensive, requiring multiple generations to stabilize the trait (Indhubala et al., 2010). The reliance on specific genetic backgrounds for maintaining sterility also limits the genetic diversity that can be utilized in breeding programs, potentially reducing the overall adaptability and resilience of the resulting hybrids (Yang et al., 2021). Furthermore, the integration of modern biotechnological tools such as CRISPR/Cas9 for creating male-sterile lines is still in its nascent stages and requires further refinement and regulatory approval (Song et al., 2020; Nadeem et al., 2021).

8.3 Strategies to overcome existing challenges
To overcome these challenges, several strategies can be employed. One approach is the development of more robust male-sterile lines through advanced genetic techniques such as CRISPR/Cas9, which allows for precise editing of sterility genes and can create stable male-sterile mutants without introducing foreign DNA (Song et al., 2020; Nadeem et al., 2021). Another strategy is the use of dominant genic male sterility (DGMS) technology, which can produce hybrids with high yield potential and better adaptability to environmental stresses (Wan et al., 2021). Additionally, combining CMS with genic male-sterility systems can provide a more stable and flexible breeding platform, as demonstrated in hybrid rice technology (Song et al., 2020). Enhancing the genetic diversity of male-sterile lines by incorporating diverse genetic backgrounds can also improve the resilience and performance of hybrids under varying environmental conditions (Indhubala et al., 2010; Pfeiffer et al., 2010). Finally, integrating marker-assisted selection (MAS) can expedite the breeding process by allowing for the early identification of male-sterile lines, thereby reducing the time and resources required for developing new hybrids (Yang et al., 2021).

9 Conclusion and Future Prospects

The research on enhancing heterosis in sorghum using male sterile lines has demonstrated significant potential for improving various agronomic traits. Studies have shown that hybrids derived from cytoplasmic male-sterile lines exhibit higher brix, greater stalk yield, and improved juice yield compared to their male-fertile counterparts. Additionally, the use of male sterile lines has been effective in increasing grain yield and other yield-related traits, as well as enhancing resistance to diseases such as anthracnose. These findings underscore the importance of male sterile lines in sorghum breeding programs aimed at developing high-yielding, disease-resistant hybrids with superior quality traits.

Future research should focus on exploring new male sterile lines to further enhance genetic diversity and heterosis in sorghum. This includes the identification and utilization of diverse germplasm and landraces to create novel hybrid combinations. Additionally, there is a need to investigate the genetic mechanisms underlying heterosis and combining ability for key traits such as grain yield, biomass, and nutritional content. Enhancing the genetic diversity of male sterile lines and their corresponding restorers will be crucial for sustaining long-term breeding success and adaptability to various environmental conditions.

The development of superior sorghum hybrids through the use of male sterile lines has significant implications for global food security and agricultural sustainability. High-yielding and disease-resistant hybrids can contribute to increased sorghum production, thereby providing a reliable food source in regions prone to food insecurity. Moreover, the improved biomass and sugar content in sweet sorghum hybrids can support biofuel production, offering a sustainable alternative to fossil fuels and contributing to energy security. Overall, the advancements in sorghum breeding using male sterile lines hold promise for enhancing agricultural productivity and sustainability on a global scale.

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Conflict of Interest Disclosure

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

References

Agarwal M., and Shrotria P., 2005, Heterosis and inbreeding depression in forage sorghum [*Sorghum bicolor* (L.) Moench], Indian Journal of Genetics and Plant Breeding, 65(01): 12-14.

Chauhan P., and Pandey P., 2021, Studies on heterosis, residual heterosis and inbreeding depression for yield and related traits in sorghum (*Sorghum bicolor* L.), Agricultural Science Digest, 42(1): 53-57.
https://doi.org/10.18805/AG.D-5249

Chen G., Zhou Y., Kishchenko O., Stepanenko A., Jatayev S., Zhang D., and Borisjuk N., 2020, Gene editing to facilitate hybrid crop production, Biotechnology Advances, 46: 107676.
https://doi.org/10.1016/j.biotechadv.2020.107676

Choe M., Kim J., Nabi R., Han S., and Cho K., 2023, Development of InDels markers for the identification of cytoplasmic male sterility in *Sorghum* by complete chloroplast genome sequences analysis, Frontiers in Plant Science, 14: 1188149.
https://doi.org/10.3389/fpls.2023.1188149

Elkonin L., Gerashchenkov G., Domanina I., and Rozhnova N., 2015, Inheritance of reversions to male fertility in male-sterile sorghum hybrids with 9E cytoplasm male sterility induced by environmental conditions, Russian Journal of Genetics, 51(3): 251-261.

Fujimoto R., Uezono K., Ishikura S., Osabe K., Peacock W., and Dennis E., 2018, Recent research on the mechanism of heterosis is important for crop and vegetable breeding systems, Breeding Science, 68(2): 145-158.
https://doi.org/10.1270/jsbbs.17155

Gaddameedi A., Phuke R., Polavarapu K., Gorthy S., Subhasini V., Jagannathan J., and Are A., 2020, Heterosis and combining ability for grain Fe and Zn concentration and agronomic traits in sorghum [*Sorghum bicolor* (L.) Moench], Journal of King Saud University-Science, 32(7): 2989-2994.
https://doi.org/10.1016/j.jksus.2020.08.003

Hanafi S., Cherkaoui S., Kehel Z., Sanchez-Garcia M., Sarazin J., Baenziger S., and Tadesse W., 2022, Hybrid seed set in relation with male floral traits, estimation of heterosis and combining abilities for yield and its components in wheat (*Triticum aestivum* L.), Plants, 11(4): 508.
https://doi.org/10.3390/plants11040508

Hashimoto S., Wake T., Nakamura H., Minamiyama M., Araki-Nakamura S., Ohmae-Shinohara K., Koketsu E., Okamura S., Miura K., Kawaguchi H., Kasuga S., and Sazuka T., 2021, The dominance model for heterosis explains culm length genetics in a hybrid sorghum variety, Scientific Reports, 11(1): 4532. https://doi.org/10.1038/s41598-021-84020-3

He S., Tang C., Wang M., Li S., Diallo B., Xu Y., Zhou F., Sun L., Shi W., and Xie G., 2020, Combining ability of cytoplasmic male sterility on yield and agronomic traits of sorghum for grain and biomass dual-purpose use, Industrial Crops and Products, 157: 112894. https://doi.org/10.1016/j.indcrop.2020.112894

Hochholdinger F., and Baldauf J., 2018, Heterosis in plants, Current Biology, 28(18): R1089-R1092. https://doi.org/10.1016/j.cub.2018.06.041

Indhubala M., Ganesamurthy K., and Punitha D., 2010, Heterosis for quality attributes in sweet sorghum hybrids using cytoplasmic genic male sterile lines, Madras Agricultural Journal, 97(10/12): 309-311.

Jordan D., Mace E., Henzell R., Klein P., and Klein R., 2010, Molecular mapping and candidate gene identification of the *Rf2* gene for pollen fertility restoration in sorghum [*Sorghum bicolor* (L.) Moench], Theoretical and Applied Genetics, 120(7): 1279-1287. https://doi.org/10.1007/s00122-009-1255-3

Kanbar O.Z., Kanbar A., and Shehab S., 2020, Combining ability and heterosis for some yield traits in sorghum (*Sorghum bicolor* L. Moench) using (line×tester) design, Journal of Plant Production, 2(8): 1009-1016. https://doi.org/10.21608/jpp.2011.85633

Kibalnik O., 2019, Hybrid vigour effect in sorghum selection, Bulletin of NSAU (Novosibirsk State Agrarian University), (2):15-24. https://doi.org/10.31677/2072-6724-2019-51-2-15-24

Kim Y., and Zhang D., 2018, Molecular control of male fertility for crop hybrid breeding, Trends in Plant Science, 23(1): 53-65. https://doi.org/10.1016/j.tplants.2017.10.001

Kishan A., and Borikar S., 1989, Genetic relationship between some cytoplasmic male sterility systems in sorghum, Euphytica, 42(3): 259-269. https://doi.org/10.1007/BF00034462

Li X., Li X., Fridman E., Tesso T., and Yu J., 2015, Dissecting repulsion linkage in the dwarfing gene *Dw3* region for sorghum plant height provides insights into heterosis, Proceedings of the National Academy of Sciences, 112(38): 11823-11828. https://doi.org/10.1073/pnas.1509229112

Makanda I., Tongoona P., Derera J., Sibiya J., and Fato P., 2010, Combining ability and cultivar superiority of sorghum germplasm for grain yield across tropical low-and mid-altitude environments, Field Crops Research, 116(1-2): 75-85. https://doi.org/10.1016/J.FCR.2009.11.015

Mengistu G., Shimelis H., Laing M., Lule D., and Mashilo J., 2020, Combining ability and heterosis among sorghum (*Sorghum bicolor* [L.] Moench) lines for yield, yield-related traits, and anthracnose resistance in western Ethiopia, Euphytica, 216(2): 33. https://doi.org/10.1007/s10681-020-2563-6

Nadeem M., Chen A., Hong H., Li D., Li J., Zhao D., Wang W., Wang X., and Qiu L., 2021, *GmMs1* encodes a kinesin-like protein essential for male fertility in soybean (*Glycine max* L.), Journal of Integrative Plant Biology, 63(6): 1054-1064. https://doi.org/10.1111/jipb.13110

Narkhede G., Mehtre S., Thakur N., Ingle K., S., V., and Deshpande S., 2022, Genetics of fertility restoration in A_1 cytoplasmic genetic male sterility (CGMS) systems in sorghum (*Sorghum bicolor* L. Moench), bioRxiv, 2022: 825963. https://doi.org/10.31783/elsr.2022.825963

Paril J., Reif J., Fournier-Level A., and Pourkheirandish M., 2023, Heterosis in crop improvement, The Plant Journal, 117(1): 23-32. https://doi.org/10.1111/tpj.16488

Pfeiffer T., Bitzer M., Toy J., and Pedersen J., 2010, Heterosis in sweet sorghum and selection of a new sweet sorghum hybrid for use in syrup production in Appalachia, Crop Science, 50(5): 1788-1794. https://doi.org/10.2135/CROPSCI2009.09.0475

Ringo J., Onkware A., Mgonja M., Deshpande S., Rathore A., Mneney E., and Gudu S., 2015, Heterosis for yield and its components in sorghum ('*Sorghum bicolor*' L. Moench) hybrids in dry lands and sub-humid environments of East Africa, Australian Journal of Crop Science, 9(1): 9-13.

Sandeep N., and Biradar B., 2020, Extent of heterosis for grain yield and its contributing traits in rabi sorghum [*Sorghum bicolor* (L.) Moench], Journal of Pharmacognosy and Phytochemistry, 9(2): 949-959. https://doi.org/10.22271/phyto.2020.v9.i2p.10979

Shukla S., and Pandey M., 2007, Combining ability and heterosis over environments for yield and yield components in two-line hybrids involving thermosensitive genic male sterile lines in rice (*Oryza sativa* L.), Plant Breeding, 127(1): 28-32. https://doi.org/10.1111/J.1439-0523.2007.01432.X

Song S., Wang T., Li Y., Hu J., Kan R., Qiu M., Deng Y., Liu P., Zhang L., Dong H., Li C., Yu D., Li X., Yuan D., Yuan L., and Li L., 2020, A novel strategy for creating a new system of third-generation hybrid rice technology using a cytoplasmic sterility gene and a genic male-sterile gene, Plant Biotechnology Journal, 19(2): 251-260. https://doi.org/10.1111/pbi.13457

Wan X., Wu S., and Li X., 2021, Breeding with dominant genic male-sterility genes to boost crop grain yield in post-heterosis utilization era, Molecular Plant, 14(4): 531-534. https://doi.org/10.1016/j.molp.2021.02.004

Xin Z., Huang J., Smith A., Chen J., Burke J., Sattler S., and Zhao D., 2017, Morphological characterization of a new and easily recognizable nuclear male sterile mutant of sorghum (*Sorghum bicolor*), PLoS ONE, 12(1): e0165195.
https://doi.org/10.1371/journal.pone.0165195

Yang W., Li Y., Sun L., Shoaib M., Sun J., Wang D., Li X., Liu D., Zhan K., and Zhang A., 2021, Genetic mapping of *ms1s*, a recessive gene for male sterility in common wheat, International Journal of Molecular Sciences, 22(16): 8541.
https://doi.org/10.3390/ijms22168541