Review Article

The Role of Wide Hybridization in Crop Improvement: Advances, Challenges, and Future Prospects: A Comprehensive Review

ABSTRACT

Wide hybridization, the crossing of genetically distant species or genera, has emerged as a pivotal strategy in crop improvement, offering the potential to introduce desirable traits and enhance genetic diversity. This review examines the advancements, challenges, and future prospects of wide hybridization in crop breeding. Wide hybridization has facilitated the incorporation of traits such as disease resistance, abiotic stress tolerance, and improved nutritional profiles into various crops. These hybrids can often express hybrid vigour, leading to increased yield and robustness. However, the process presents significant challenges, including reproductive barriers, hybrid sterility, and genomic incompatibilities. Advances in molecular biology and biotechnology, such as marker-assisted selection, genomic sequencing, and embryo rescue techniques, have played a crucial role in overcoming these barriers, making wide hybridization more feasible and effective. Additionally, the review highlights case studies of successful wide hybridization efforts in key crops like wheat, rice, and legumes, displaying the tangible benefits and contributions to food security and agricultural sustainability. Future prospects focus on the integration of cutting-edge technologies like CRISPR-Cas9 and genomic selection to further streamline the process and enhance the precision of trait incorporation. The collaboration between traditional breeding techniques and modern biotechnological approaches holds promise for addressing the growing challenges in global agriculture. This review underscores the importance of continued research and innovation in wide hybridization to unlock its full potential and drive the next generation of crop improvement strategies, ensuring resilient and productive agricultural systems in the face of climate change and population growth.

Keywords: *Disease Resistance, Stress Tolerance, Marker-Assisted Selection, CRISPR-Cas9, Genomic Incompatibilities.*

1. INTRODUCTION

Agricultural sustainability and productivity are paramount in addressing global food security challenges exacerbated by factors like climate change, population growth, and dwindling natural resources. Traditional breeding methods have played a crucial role in crop improvement, yet they often face limitations in addressing the complexities of modern agriculture. Wide hybridization, a breeding technique involving the crossing of genetically distant species or genera, has emerged as a pivotal strategy to enhance crop performance by incorporating desirable traits from diverse genetic backgrounds [1-2]. This introduction provides a comprehensive overview of wide hybridization, its significance, the challenges it poses, and the modern tools and techniques that are revolutionizing this field [3]. Agricultural sustainability and productivity are crucial for addressing global food security challenges amidst climate change, population growth, and dwindling natural resources. While traditional breeding methods have been instrumental in crop improvement, they often encounter limitations in tackling the complexities of modern agriculture. Wide hybridization, involving the crossing of genetically distant species or genera, stands out as a pivotal strategy to enhance crop performance by incorporating desirable traits from diverse genetic backgrounds [4]. This approach offers a promising avenue to broaden the genetic base of crops, introduce novel traits, and develop resilient varieties capable of withstanding environmental stresses [48]. As wide hybridization continues to gain traction in crop improvement efforts, the integration of modern tools and techniques holds immense potential to overcome existing challenges and unlock new opportunities for sustainable and productive agriculture.

Wide hybridization not only enriches the genetic diversity of crops but also serves as a valuable tool for breeders to address specific challenges such as disease resistance, abiotic stress tolerance, and nutritional quality improvement [5]. By crossing genetically distant species or genera, breeders can access a broader spectrum of genetic variation, facilitating the development of crop varieties tailored to meet the evolving needs of farmers and consumers. Moreover, wide hybridization offers a pathway to explore unexplored genetic resources, including wild relatives and landraces, which harbor valuable traits that can enhance the resilience and adaptability of cultivated crops to changing environmental conditions. However, wide hybridization also presents inherent challenges, including reproductive barriers, genomic incompatibilities, and issues related to genetic stability and fertility of hybrids. Overcoming these challenges requires innovative approaches and the integration of modern tools such as molecular markers, genomic sequencing, and genome editing technologies [6]. By addressing these hurdles, breeders can maximize the potential of wide hybridization to develop crop varieties with improved performance, resilience, and sustainability, ultimately contributing to global food security and agricultural sustainability in the face of mounting challenges.

Achieving wide crosses in cereals presents significant challenges, encompassing traditional pollination methods and the transfer of specific genes between species. Wheat (*Triticum aestivum* L.), pearl millet (*Pennisetum glaucum* L.), maize (*Zea mays* L.) [50], and rice (*Oryza sativa* L.) are among the main cereals explored in this context [7]. Breeders have long aimed to introduce genes into economically vital cultivated crops to enhance resistance against biotic and abiotic stresses such as diseases, drought [45], and high aluminum levels, while also improving storage protein quality and maintaining yield improvements [8]. Despite the enrichment of cultivated varieties with high-yield, quality, and resistance genes, the widespread cultivation of only a few high-yielding genotypes in large areas leads to the rapid adaptation of new pathotypes [51-52]. This widespread practice also results in the loss of many valuable land races of cereals. Land races and closely related species serve as crucial sources of novel germplasm for breeders. Many accessions in collections worldwide are utilized in "wide crosses" to introduce desirable traits. Moreover, hybrids resulting from crosses between species spanning wide taxonomic boundaries within the Gramineae family have been reported. Examples include *Oryza sativa X Pennisetum spp., Saccharum officinarum X Sorghum bicolor, O. sativa X S. bicolor*, and *Triticum aestivum*. These wide crosses offer avenues for incorporating valuable genetic diversity and novel traits into cultivated cereals [9], thereby enhancing their resilience and adaptability to changing environmental conditions.

Wide hybridization plays a pivotal role in enhancing genetic variation in cultivated tomato (*Lycopersicon esculentum*). This technique has been particularly instrumental in improving tomato's resilience to various biotic stresses [10]. Recent advancements in tomato breeding have capitalized on wide hybridization by utilizing wild tomato genotypes to develop pseudo-diploid hybrids of *Solanumlycopersicoides* and *L. esculentum*. Through this approach, monosomic alien addition lines and diploid individuals carrying beneficial attributes inherited from wild relatives can be developed. Wild species such as *S. lycopersicoides* have contributed numerous desirable traits to tomato via wide hybridization. Mutants of *S. lycopersicoides* exhibit traits like white anthers, bifurcate inflorescence, day length sensitivity, frilly leaves, rugose leaf surface, fimbriate leaves, lacinate leaves, and various isozyme variants [11]. Additionally, a single dominant gene responsible for high anthocyanin content has been identified, albeit its expression is influenced by environmental factors such as sunlight. Furthermore, purple spotting in fruits is another characteristic observed in hybrids. In recent efforts, crosses between *S. rickii* and tomato have been pursued. However, hybrids obtained from pseudo-diploid crosses exhibit pistillate flowered traits. Diploid hybrids of *L. esculentum* × *S. rickii,* although sterile with reduced female fertility and compatibility with *L. esculentum*, have paved the way for the development of amphidiploid hybrids [12]. These hybrids serve as valuable sources of variation, offering not only morphological diversity but also novel horticultural attributes. The integration of attributes from wild species like *S. lycopersicon* into tomato breeding programs underscores the significance of wide hybridization in augmenting genetic variation and enhancing the resilience of cultivated crops.

2. THE PROMISE OF WIDE HYBRIDIZATION

Wide hybridization stands as a cornerstone in the quest for developing improved crop varieties capable of withstanding the multifaceted challenges posed by climate change and increasing global food demands. By incorporating genes from genetically distant species or genera, wide hybridization significantly enhances genetic diversity, allowing the development of crops with superior traits such as disease resistance, abiotic stress tolerance, and enhanced nutritional quality. These attributes are essential for ensuring agricultural sustainability and addressing global food security.

Overcoming Barriers with Modern Tools: The successful manipulation of alien chromatin introduced into cereals through wide crosses exemplifies the potential of this approach.

Recombination induced by the removal of controls on homologous pairing, chromatin breakage and repair induced by ionizing irradiation, and the use of cell culture to facilitate gene transfer are innovative strategies employed to overcome the inherent challenges of wide hybridization. For instance, the transfer of stripe rust resistance genes from *Aegilops comosa* to wheat highlights the effectiveness of these techniques in developing disease-resistant varieties [13].

Role of Advanced Biotechnological Tools: Advances in molecular biology, genomics, and biotechnology have significantly enhanced the feasibility and efficiency of wide hybridization. Techniques such as CRISPR-Cas9 genome editing, genomic sequencing, marker-assisted selection (MAS), genomic selection, somatic hybridization, and embryo rescue offer unprecedented precision and efficiency in trait introgression and hybrid development [14]. These advancements not only accelerate the breeding process but also enable the creation of crop varieties tailored to specific environmental conditions and market demands.

Ensuring Future Agricultural Sustainability: Continued innovation and collaboration are crucial for unlocking the full potential of wide hybridization. The synergy between plant breeders, geneticists, biotechnologists, and agronomists is essential for optimizing breeding strategies, overcoming existing challenges, and unlocking new opportunities for enhancing crop productivity, sustainability, and resilience [15]. The collaboration fosters the development of crop varieties that are not only high-yielding and nutritionally superior but also capable of adapting to evolving agricultural challenges.

Realizing the Full Potential of Wide Hybridization: Wide hybridization will remain a vital strategy for meeting global food security demands. By harnessing the untapped potential of wild relatives and unexplored genetic resources, breeders can discover novel traits and develop resilient crop varieties [16]. This approach addresses the immediate challenges of modern agriculture and contributes to long-term environmental sustainability and economic viability. Through continued innovation and collaboration, the full potential of wide hybridization can be realized, ensuring a sustainable and food-secure future.

Integration into Modern Breeding Programs: The integration of wide hybridization into modern breeding programs is facilitated by the application of advanced tools and techniques that streamline the breeding process and enhance the success rates of hybridization efforts. By utilizing these methods, breeders can efficiently introduce desirable traits into economically important crops, ensuring their resilience against biotic and abiotic stresses, and improving overall crop performance [17].

Broadening Genetic Diversity for Future Resilience: Wide hybridization plays a critical role in broadening the genetic base of crops, which is crucial for developing resilience against emerging pests and diseases, adapting to changing environmental conditions, and ensuring long-term sustainability in agriculture [18]. The introduction of genes from wild relatives and genetically distinct species enriches the genetic pool, providing a foundation for developing crop varieties that can thrive in diverse and challenging environments.

In summary, wide hybridization, complemented by modern biotechnological advancements, stands as a pivotal strategy for enhancing crop resilience, productivity, and nutritional quality. By addressing the challenges posed by climate change and global food demands, wide hybridization contributes significantly to agricultural sustainability and food security. Through continued innovation, collaboration, and the integration of advanced techniques, the full potential of wide hybridization can be unlocked, ensuring a robust and resilient agricultural future.

3. DOUBLE HAPLOID PRODUCTION IN WHEAT-MAIZE CROSSING SYSTEM

The method of producing double haploid (DH) wheat plants through a wheat x maize crossing system is a robust technique that involves several meticulous steps (Fig. 1) [19]. This process, as described by Khan and Ahmad, begins with the emasculation of F1 spikes of wheat, which are then pollinated using fresh or cryo-preserved maize pollen.

3.1 Pollination and Hormone Treatment

Fresh maize pollen is more effective for haploid production compared to cryo-preserved pollen, although the latter is useful when synchronization of flowering between wheat and maize is problematic. Once pollinated, the wheat spikes are placed in a tiller culture medium containing sucrose, sulfuric acid, and auxin hormones [49]. Auxin application is critical for seed setting in this system, with various hormones like 2,4-dichlorophenoxyacetic acid (2,4-D), indole acetic acid (IAA), naphthalene acetic acid (NAA), Zeatin, GA3, 6-benzyl aminopurine (BA), and kinetin being used in different concentrations [20]. Among these, 2,4-D at a concentration of 100 mg/L has shown superior results in embryo formation. The pollinated spikes are submerged in a tiller culture medium containing 2,4-D at 20°C for 14-16 days post-pollination. This treatment enhances seed setting and embryo formation, with additional treatments like spraying 2,4-D combined with 120 mg/L AgNO3 being beneficial for durum wheat [21].

3.2 Embryo Rescue and Culture

Embryo rescue is performed 14-16 days after pollination. Since wheat x maize embryos lack endosperm, these embryos must be cultured on a nutritional medium such as full-strength MS medium, half-strength MS medium, or B5 basal medium with agar. These embryos are maintained in vitro for 3-5 weeks under controlled conditions of light intensity, relative humidity, and temperature. A light intensity of 10,000 Lux is optimal for proper growth, as lower light intensities hinder pollen tube formation during embryo development [22]. Additionally, a temperature range of 21-26°C and relative humidity of 60-65% are ideal for the regeneration of plants from rescued embryos. Relative humidity below 60% can adversely affect this process.

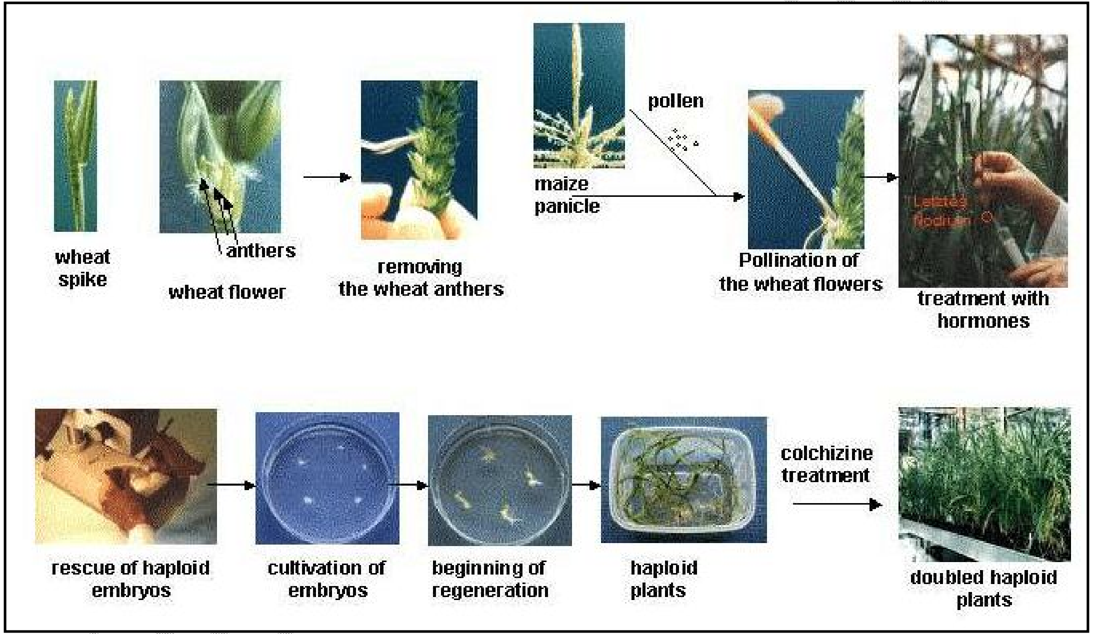

Fig. 1: Procedure for doubled haploid plant production by wheat x maize hybridization [19]

4. THE SIGNIFICANCE AND ADVANTAGES OF WIDE HYBRIDIZATION

Wide hybridization offers several significant advantages in crop improvement:

1. Genetic Diversity and Novel Traits: By crossing genetically distant species or genera, wide hybridization introduces new genetic variations into crop breeding programs. These variations are essential for developing crops with improved traits such as disease resistance, pest tolerance, abiotic stress resilience, and enhanced nutritional quality.Wide hybridization, a breeding technique that involves the crossing of genetically distant species or genera, is instrumental in introducing new genetic variations into crop breeding programs [23]. This infusion of genetic diversity is critical for the development of crops with enhanced traits, including disease resistance, pest tolerance, abiotic stress resilience, and improved nutritional quality. By combining the genetic material from different species or genera, wide hybridization opens up avenues for incorporating novel traits that may not be present within the gene pool of a single species, thereby enriching crop varieties with valuable characteristics to address evolving agricultural challenges. In addition to broadening the genetic base of crops, wide hybridization fosters the manifestation of hybrid vigour, also known as heterosis, in offspring. Hybrids resulting from wide hybridization often exhibit superior qualities compared to their parents, including increased yield, faster growth rates, and greater overall robustness [24]. This phenomenon underscores the potential of wide hybridization as a powerful tool in crop improvement efforts, offering a pathway to develop resilient and high-performing crop varieties capable of meeting the demands of a changing agricultural landscape.

2. Hybrid Vigour (Heterosis): Hybrids resulting from wide hybridization often exhibit heterosis, also known as hybrid vigour. This phenomenon leads to offspring with superior qualities compared to both parents, including increased yield, faster growth rates, and greater overall robustness.Wide hybridization stands as a promising avenue for enhancing crop resilience to climate change. By crossing genetically distant species or genera, this breeding technique introduces new genetic variations into crop breeding programs [25]. These variations are instrumental in developing crops with improved traits such as disease resistance, pest tolerance, abiotic stress resilience, and enhanced nutritional quality. Furthermore, hybrids resulting from wide hybridization often exhibit hybrid vigour, known as heterosis, leading to offspring with superior qualities compared to their parents, including increased yield, faster growth rates, and greater overall robustness [26]. Embracing wide hybridization offers a pathway to develop resilient crop varieties capable of thriving in the face of changing climatic conditions, thereby contributing to global food security and agricultural sustainability.

3. Expanding the Genetic Base: Wide hybridization broadens the genetic base of crops by incorporating genes from wild relatives and genetically distinct species. This is crucial for developing resilience against emerging pests and diseases, adapting to changing environmental conditions, and ensuring long-term sustainability in agriculture.Wide hybridization plays a pivotal role in expanding the genetic base of crops by incorporating genes from wild relatives and genetically distinct species [27]. This process is essential for developing crop varieties with increased resilience against emerging pests and diseases, as well as adapting to changing environmental conditions. By broadening the genetic base, wide hybridization contributes to the long-term sustainability of agriculture by equipping crops with the genetic diversity needed to withstand evolving challenges. Embracing wide hybridization offers a promising pathway to enhance crop resilience and ensure the continued productivity and viability of agricultural systems in the face of dynamic environmental pressures [28].

5. CHALLENGES OF WIDE HYBRIDIZATION

Despite its potential benefits, wide hybridization presents several challenges:

1. Reproductive Barriers: Genetic differences between species can result in reproductive barriers such as differences in chromosome number, structural differences in chromosomes, and pre- and post-fertilization incompatibilities [29]. These barriers hinder successful hybridization and can prevent the production of viable offspring. Reproductive barriers pose significant challenges to wide hybridization efforts. These barriers arise due to genetic differences between species and can manifest as differences in chromosome number, structural variations in chromosomes, and pre- and post-fertilization incompatibilities. Such barriers hinder the successful hybridization process and can ultimately prevent the production of viable offspring. Overcoming these reproductive barriers is essential for the successful implementation of wide hybridization strategies and requires innovative approaches and techniques to navigate the complexities of interspecific breeding. By addressing these challenges, researchers can unlock the full potential of wide hybridization, paving the way for the development of novel crop varieties with enhanced traits and improved resilience to environmental stresses.

2. Hybrid Sterility: Even when hybrids are successfully produced, they often suffer from sterility, limiting their utility in breeding programs. Sterility issues arise due to genetic incompatibilities and meiotic irregularities between the parental genomes. Even when wide hybridization successfully produces hybrids, they often face issues of sterility, which significantly limit their utility in breeding programs. Sterility problems stem from genetic incompatibilities and meiotic irregularities between the parental genomes of the hybrid offspring [30]. These genetic differences can disrupt the normal processes of meiosis, leading to abnormalities in gamete formation and ultimately resulting in sterile hybrids. Overcoming sterility issues is crucial for harnessing the full potential of wide hybridization in crop improvement efforts. Innovative techniques such as embryo rescue and somatic hybridization may offer solutions to mitigate sterility problems, enabling the development of fertile hybrids with desirable traits for sustainable agriculture. Addressing sterility challenges is essential for realizing the benefits of wide hybridization and advancing crop-breeding programs towards enhanced resilience and productivity.

3. Genomic Imbalances: Hybrids resulting from wide hybridization may experience genomic imbalances, where the combination of divergent genomes leads to developmental issues, reduced fitness, and viability problems in the offspring [31].Hybrids resulting from wide hybridization may encounter genomic imbalances, characterized by the combination of divergent genomes from genetically distant parent species or genera. These imbalances can give rise to developmental issues, reduced fitness, and viability problems in the offspring. The discordance between the parental genomes can disrupt normal gene expression patterns and regulatory mechanisms, leading to aberrant development and compromised fitness in the hybrid progeny. Overcoming genomic imbalances is essential for maximizing the success of wide hybridization efforts and ensuring the production of viable and high-performing hybrids. Innovative approaches, such as genomic selection and advanced breeding techniques, may offer strategies to mitigate genomic imbalances and optimize the performance of hybrids derived from wide hybridization [32].

4. Complex Breeding Processes: Wide hybridization involves complex breeding processes that require extensive resources, expertise, and specialized techniques to overcome reproductive barriers. These processes can be time-consuming and labour-intensive, further complicating the implementation of wide hybridization strategies.Wide hybridization encompasses complex breeding processes that necessitate significant resources, expertise, and specialized techniques to surmount reproductive barriers [33]. Overcoming these barriers can be challenging and requires meticulous planning and execution. The intricate nature of wide hybridization processes, coupled with the need for specialized techniques, renders them time-consuming and labour-intensive. These factors further compound the challenges associated with implementing wide hybridization strategies in crop improvement programs. Despite the complexities involved, advancements in molecular biology, genomics, and biotechnology offer promising avenues for streamlining wide hybridization processes and increasing their efficiency.

6. MODERN TOOLS AND TECHNIQUES IN WIDE HYBRIDIZATION

Advances in molecular biology, genomics, and biotechnology have significantly enhanced the feasibility and efficiency of wide hybridization. Several modern tools and techniques are instrumental in overcoming traditional challenges associated with this method:

1. Marker-Assisted Selection (MAS): MAS utilizes molecular markers linked to desirable traits to facilitate the selection of superior hybrids. This technique accelerates the breeding process by identifying favourable genetic combinations at the seedling stage, reducing the time and resources required for field trials.Advances in molecular biology, genomics, and biotechnology have revolutionized the field of wide hybridization, significantly enhancing its feasibility and efficiency [34]. One key advancement is Marker-Assisted Selection (MAS), a technique that utilizes molecular markers linked to desirable traits to expedite the selection of superior hybrids. By identifying favourable genetic combinations at the seedling stage, MAS accelerates the breeding process, reducing the time and resources needed for field trials. This approach not only enhances the precision of hybrid selection but also allows breeders to focus their efforts on promising candidates, maximizing the likelihood of success in crop improvement programs. The integration of MAS into wide hybridization strategies represents a significant step forward in overcoming traditional challenges associated with this method and holds great promise for advancing crop breeding efforts towards the development of resilient and high-yielding varieties.

2. Genomic Sequencing: High-throughput genomic sequencing provides detailed insights into the genetic makeup of parent species and hybrids. This information is crucial for understanding genetic compatibility, identifying beneficial alleles, and designing strategies to overcome reproductive barriers.Genomic sequencing, another powerful tool in modern crop breeding, offers detailed insights into the genetic makeup of parent species and hybrids. Through high-throughput sequencing techniques, researchers can decipher the entire genetic code of organisms, allowing for a comprehensive understanding of their genetic composition. This information is invaluable for several aspects of wide hybridization. Firstly, it aids in assessing genetic compatibility between parent species, helping breeders identify suitable candidates for hybridization. Secondly, genomic sequencing enables the identification of beneficial alleles associated with desirable traits, providing valuable guidance for trait introgression in hybrid offspring [35]. Lastly, genomic data facilitates the design of targeted strategies to overcome reproductive barriers by pinpointing genetic regions responsible for compatibility issues. By leveraging genomic sequencing, breeders can make informed decisions, accelerate the development of successful hybrids, and unlock the full potential of wide hybridization in crop improvement programs.

3. Embryo Rescue: Embryo rescue is a tissue culture technique used to save embryos from hybrid crosses that would otherwise abort due to incompatibility. By cultivating hybrid embryos in vitro, researchers can grow plants that would not survive through conventional breeding methods.Embryo rescue is a vital tissue culture technique employed in wide hybridization to salvage embryos from hybrid crosses that would otherwise perish due to genetic incompatibility [36]. By cultivating these hybrid embryos in vitro, researchers can bypass the barriers to natural development, ensuring the survival and growth of plants that would not thrive through conventional breeding methods. This technique is particularly valuable in overcoming reproductive barriers encountered in interspecific or intergeneric crosses, where embryo abortion is a common occurrence. Embryo rescue not only facilitates the production of viable hybrids but also allows breeders to expand the genetic diversity of crop populations by incorporating genes from genetically distant species or genera. By harnessing the potential of embryo rescue, researchers can overcome one of the major hurdles in wide hybridization and unlock new avenues for crop improvement, ultimately contributing to the development of resilient and high-performing crop varieties for sustainable agriculture.

4. Somatic Hybridization:Somatic hybridization involves the fusion of protoplasts (cells without cell walls) from different species to create hybrid cells. These hybrid cells can then be regenerated into plants, bypassing some of the reproductive barriers associated with sexual hybridization [37].Somatic hybridization is a ground-breaking technique in crop breeding that entails the fusion of protoplasts, cells devoid of cell walls, from distinct species to generate hybrid cells. These hybrid cells have the potential to be regenerated into plants, circumventing certain reproductive barriers typically encountered in sexual hybridization processes. By bypassing these barriers, somatic hybridization offers a promising alternative for the creation of interspecific or intergeneric hybrids. This technique not only facilitates the incorporation of desirable traits from genetically distant species or genera but also enables the development of novel genetic combinations that may not be achievable through traditional breeding methods. Somatic hybridization holds immense potential for expanding the genetic diversity of crop populations and accelerating the development of resilient and high-performing crop varieties capable of addressing the evolving challenges of modern agriculture [38].

5. CRISPR-Cas9 Genome Editing: CRISPR-Cas9 is a revolutionary tool for precise genome editing. It allows for the targeted modification of specific genes, enabling the correction of genetic incompatibilities and the introduction of beneficial traits with high precision. This technique holds great promise for enhancing the efficiency and success rate of wide hybridization.CRISPR-Cas9 genome editing represents a ground breaking advancement in crop breeding, offering precise and targeted modification of specific genes [39]. This revolutionary tool enables researchers to correct genetic incompatibilities and introduce desirable traits with unprecedented accuracy. By precisely editing the genome, CRISPR-Cas9 holds the potential to overcome the challenges associated with wide hybridization, such as genetic incompatibilities and reproductive barriers. By precisely targeting and modifying genes involved in hybridization, CRISPR-Cas9 can enhance the efficiency and success rate of wide hybridization efforts. This technique opens up new avenues for crop improvement, allowing breeders to develop resilient and high-yielding crop varieties with tailored genetic characteristics. The integration of CRISPR-Cas9 genome editing into wide hybridization strategies represents a significant leap forward in crop breeding, offering unparalleled precision and potential for innovation in agriculture.

6. Genomic Selection:Genomic selection utilizes genome-wide markers to predict the performance of hybrids based on their genetic profiles. This approach enables the selection of the best candidates for breeding, improving the accuracy and efficiency of hybridization programs.Genomic selection is a cutting-edge approach in crop breeding that harnesses genome-wide markers to predict the performance of hybrids based on their genetic profiles [40]. By analyzing extensive genomic data, researchers can identify markers associated with desirable traits and use this information to select the best candidates for breeding. This innovative approach enhances the accuracy and efficiency of hybridization programs by enabling breeders to make informed decisions about which hybrids to prioritize for further development. By leveraging genomic selection, breeders can expedite the breeding process, maximize genetic gain, and develop crop varieties with superior traits tailored to meet specific agricultural needs. The integration of genomic selection into wide hybridization strategies represents a significant advancement in crop breeding, offering unprecedented precision and efficiency in the development of resilient and high-performing crop varieties for sustainable agriculture.

7. Haploid Plant Production:The process of haploid plant production involves several meticulous steps, beginning with the manual emasculation of florets two days prior to pollination [41] (Fig. 2A). Fresh maize pollen, collected at 15-minute intervals, is applied to the emasculated florets using a brush (Fig. 2B). The following day, each oat pistil receives one drop of 50 mg/L or 100 mg/L 2,4-D water solution (Fig. 2C) to stimulate embryo development. After three weeks, the enlarged ovaries, or caryopses without endosperms, are surface-sterilized using a sequential treatment of 70% ethanol (1 minute), 2.5% calcium hypochlorite (7 minutes), and 0.1% mercuric chloride (1 minute), followed by three washes with sterile water. The embryos are then isolated (Fig. 2D) and cultured on 190-2 medium at 21 ± 2°C with 16 hours of light at an intensity of 60 µmol m^{-2} s^{-1}. The developed haploid plants are initially grown on MS medium (Fig. 2E). Subsequently, they are transferred to a moist perlite substrate (Fig. 1F) before being moved to soil for acclimatization to ex vitro conditions (Fig. 2G). The double haploid (DH) line plants are then planted individually into 3 dm^3 pots with a diameter of 16 cm, filled with a mixture of horticultural soil and sand (2:1 v/v). Acclimatization is conducted under controlled glasshouse conditions to ensure optimal growth. Seeds are typically harvested after 5-6 months. The efficiency of this haploid production method is demonstrated by calculating the number of haploid embryos obtained per hundred pollinated florets [42].

8. Chromosome Doubling:To double the number of chromosomes in oat seedlings, colchicine treatment is applied when the seedlings reach the 4-5 leaf stage. The process involves immersing the seedlings in a 0.1% colchicine solution (Fig. 2H) for 7.5 hours at a temperature of 25°C and a light intensity of 80-100 µmol m^{-2} s^{-1}. After the colchicine treatment, the plant roots are thoroughly rinsed with running water for 48 hours to remove any residual colchicine. Following this rinsing step, the treated seedlings are then planted singly into pots for further growth (Fig. 2I) [41].

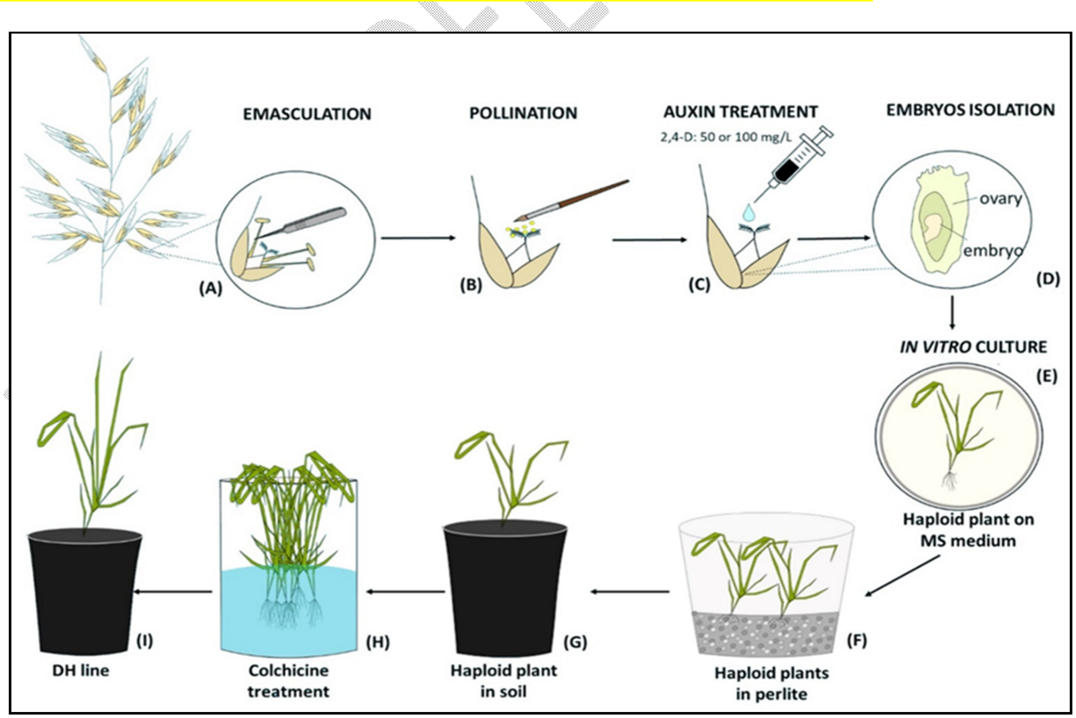

Fig. 2: Diagram of the production of oat doubled haploid lines through the wide crossing method [41]

(A) Floret emasculation, **(B)** floret pollination with maize pollen, **(C)** 2,4-D application to the pistil, **(D)** embryo isolation, **(E)** in vitro culture of embryos on MS medium and their conversion into haploid plants, **(F)** haploid plants grown in perlite, **(G)** haploid plant acclimatization in soil, **(H)** chromosomes doubling by colchicine treatment, **(I)** DH plant grown in soil until maturity

7. FUTURE ASPECTS OF WIDE HYBRIDIZATION IN CROP IMPROVEMENT

The future of wide hybridization in crop improvement holds immense promise, driven by advancements in technology and a growing understanding of genetic diversity. One key aspect is the continued exploration and utilization of wild relatives and unexplored genetic resources to expand the genetic base of cultivated crops. Harnessing the untapped potential of these genetic reservoirs can lead to the discovery of novel traits and the development of resilient crop varieties capable of withstanding evolving biotic and abiotic stresses. Furthermore, the integration of modern tools and techniques such as genomic selection, CRISPR-Cas9 genome editing, and high-throughput phenotyping will revolutionize wide hybridization efforts [43]. These technologies offer unprecedented precision and efficiency in trait introgression and hybrid development, accelerating the breeding process and enabling breeders to tailor crops to specific environmental conditions and market demands.

Additionally, interdisciplinary collaborations between plant breeders, geneticists, biotechnologists, and agronomists will be vital for advancing wide hybridization in crop improvement. By pooling expertise and resources, researchers can overcome existing challenges, optimize breeding strategies, and unlock new opportunities for enhancing crop productivity, sustainability, and resilience in the face of emerging agricultural threats [44]. Overall, the future of wide hybridization in crop improvement holds great potential to address the multifaceted challenges of modern agriculture, ensuring food security, environmental sustainability, and economic viability for future generations. Through continued innovation and collaboration, wide hybridization will remain a cornerstone of crop breeding efforts, driving transformative changes in global agriculture.

Advancements in Genetic Exploration and Utilization: The future of wide hybridization in crop improvement hinges on the continued exploration and utilization of wild relatives and unexplored genetic resources. Leveraging these genetic reservoirs can lead to the discovery of novel traits and the development of resilient crop varieties capable of withstanding evolving biotic and abiotic stresses.

Integration of Modern Technologies: The integration of modern tools and techniques such as genomic selection, CRISPR-Cas9 genome editing, and high-throughput phenotyping is poised to revolutionize wide hybridization efforts [45]. These technologies offer unprecedented precision and efficiency in trait introgression and hybrid development, accelerating the breeding process and enabling breeders to tailor crops to specific environmental conditions and market demands.

Interdisciplinary Collaborations: Interdisciplinary collaborations between plant breeders, geneticists, biotechnologists, and agronomists will be crucial for advancing wide hybridization in crop improvement [46]. By pooling expertise and resources, researchers can overcome existing challenges, optimize breeding strategies, and unlock new opportunities for enhancing crop productivity, sustainability, and resilience in the face of emerging agricultural threats.

Driving Transformative Changes: Overall, the future of wide hybridization in crop improvement holds great promise to address the multifaceted challenges of modern agriculture. Through continued innovation and collaboration, wide hybridization will remain a cornerstone of crop breeding efforts, driving transformative changes in global agriculture to ensure food security, environmental sustainability, and economic viability for future generations [47].

8. CONCLUSION

Wide hybridization stands as a powerful tool in the quest for improved crop varieties capable of withstanding the multifaceted challenges posed by climate change and global food demands. By incorporating genes from genetically distant species or genera, this technique enhances genetic diversity, enabling the development of crops with superior traits such as disease resistance, abiotic stress tolerance, and improved nutritional quality. The integration of modern tools and techniques, such as genomic selection, CRISPR-Cas9 genome editing, and high-throughput phenotyping, is progressively dismantling the barriers to wide hybridization. These advancements offer unprecedented precision and efficiency in trait introgression and hybrid development, accelerating the breeding process and enabling the creation of crop varieties tailored to specific environmental conditions and market demands. The future of wide hybridization in crop improvement is promising, driven by continued exploration and utilization of wild relatives and unexplored genetic resources. By harnessing the untapped potential of these genetic reservoirs, breeders can discover novel traits and develop resilient crop varieties capable of adapting to evolving agricultural challenges. Interdisciplinary collaborations between plant breeders, geneticists, biotechnologists, and agronomists will be essential for optimizing breeding strategies, overcoming existing challenges, and unlocking new opportunities for enhancing crop productivity, sustainability, and resilience. Wide hybridization will remain a cornerstone of crop breeding efforts, driving transformative changes in global agriculture. Through continued innovation and collaboration, the full potential of wide hybridization can be realized, ensuring a sustainable and food-secure future. This approach not only addresses the immediate challenges of modern agriculture but also contributes to long-term environmental sustainability and economic viability, making it a vital strategy for meeting the global food security demands of the future.

REFERENCES

1. Kashyap A, Garg P, Tanwar K, Sharma J, Gupta NC, Ha PT, Bhattacharya RC, Mason AS, Rao M. Strategies for utilization of crop wild relatives in plant breeding programs. Theoretical and Applied Genetics. 2022;135(12):4151-67.
2. Acquaah G. Conventional plant breeding principles and techniques. Advances in plant breeding strategies: Breeding, biotechnology and molecular tools. 2015:115-58.
3. Chen L, Xu J, Sun X, Xu P. Research advances and future perspectives of genomics and genetic improvement in allotetraploid common carp. Reviews in Aquaculture. 2022;14(2):957-78.
4. Bohra A, Kilian B, Sivasankar S, Caccamo M, Mba C, McCouch SR, Varshney RK. Reap the crop wild relatives for breeding future crops. Trends in Biotechnology. 2022;40(4):412-31.
5. Ali N, Rahman IU, Badakshi F, Tariq MJ, Mujeeb-Kazi A. Ensuring sustainable food security: Exploiting alien genetic diversity in wheat breeding for adaptation to emerging stresses. InClimate Change and Food Security with Emphasis on Wheat 2020 Jan 1 (pp. 31-42). Academic Press.
6. Nerkar G, Devarumath S, Purankar M, Kumar A, Valarmathi R, Devarumath R, Appunu C. Advances in crop breeding through precision genome editing. Frontiers in genetics. 2022 Jul 14;13:880195.
7. Rajasekharan PE, Kumar GA. Genetic Resources of Cereal Crops. Cereal Crops: Genetic Resources and Breeding Techniques. 2023 Jun 21:107.
8. Rao GJ, Reddy JN, Variar M, Mahender A. Molecular breeding to improve plant resistance to abiotic stresses. Advances in plant breeding strategies: agronomic, abiotic and biotic stress traits. 2016:283-326.
9. Hodnett GL, Norton SL, Ohadi S, Bagavathiannan MV, Rooney WL. Wide Hybridization and Utilization of Wild Relatives of Sorghum. Sorghum in the 21st Century: Food–Fodder–Feed–Fuel for a Rapidly Changing World. 2020:65-99.
10. Gascuel Q, Diretto G, Monforte AJ, Fortes AM, Granell A. Use of natural diversity and biotechnology to increase the quality and nutritional content of tomato and grape. Frontiers in plant science. 2017;8:250157.
11. Saeed A, Fatima N. Wild germplasm: shaping future tomato breeding. InWild Germplasm for Genetic Improvement in Crop Plants 2021 Jan 1 (pp. 201-214). Academic Press.
12. Bajaj YP. Plant protoplasts and genetic engineering II. Springer Science & Business Media; 2012 Dec 6.
13. Kashyap A, Garg P, Tanwar K, Sharma J, Gupta NC, Ha PT, Bhattacharya RC, Mason AS, Rao M. Strategies for utilization of crop wild relatives in plant breeding programs. Theoretical and Applied Genetics. 2022;135(12):4151-67.
14. Stanton Gelvin DN. Theme 4-New Plant Breeding Techniques. In Vitro Cellular & Developmental Biology-Plant. 2018;54(1):S84-106.
15. Dwivedi SL, Goldman I, Ceccarelli S, Ortiz R. Advanced analytics, phenomics and biotechnology approaches to enhance genetic gains in plant breeding. Advances in agronomy. 2020;162:89-142.
16. Saini P, Saini P, Kaur JJ, Francies RM, Gani M, Rajendra AA, Negi N, Jagtap A, Kadam A, Singh C, Chauhan SS. Molecular approaches for harvesting natural diversity for crop

improvement. Rediscovery of genetic and genomic resources for future food security. 2020:67-169.

17. Hafeez U, Ali M, Hassan SM, Akram MA, Zafar A. Advances in breeding and engineering climate-resilient crops: A comprehensive review. International Journal of Research and Advances in Agricultural Sciences. 2023;2(2):85-99.
18. Naqvi RZ, Siddiqui HA, Mahmood MA, Najeebullah S, Ehsan A, Azhar M, Farooq M, Amin I, Asad S, Mukhtar Z, Mansoor S. Smart breeding approaches in post-genomics era for developing climate-resilient food crops. Frontiers in Plant Science. 2022;13:972164.
19. Hussain B, Khan MA, Ali Q, Shaukat S. Double haploid production in wheat through microspore culture and wheat x maize crossing system: an overview. Int J Agron Vet Med Sci. 2013;6:332-44.
20. Ghosh SN, Tarai RK, Ahlawat TR. Plant growth regulators in tropical and sub-tropical fruit crops. CRC Press; 2022 Jun 1.
21. Ghose AK, Abdullah SN, Md Hatta MA, Megat Wahab PE. In vitro regeneration of stevia (Stevia rebaudiana Bertoni) and evaluation of the impacts of growth media nutrients on the biosynthesis of steviol glycosides (SGs). Agronomy. 2022;12(8):1957.
22. Kasperbauer MJ, Wilson HM. Anther culture Introduction Media preparation Haploid plant production. Technical Bulletin. 1979(1586):33.
23. Bohra A, Kilian B, Sivasankar S, Caccamo M, Mba C, McCouch SR, Varshney RK. Reap the crop wild relatives for breeding future crops. Trends in Biotechnology. 2022;40(4):412-31.
24. Calvo-Baltanás V, Wang J, Chae E. Hybrid incompatibility of the plant immune system: an opposite force to heterosis equilibrating hybrid performances. Frontiers in Plant Science. 2021;11:576796.
25. Bohra A, Kilian B, Sivasankar S, Caccamo M, Mba C, McCouch SR, Varshney RK. Reap the crop wild relatives for breeding future crops. Trends in Biotechnology. 2022;40(4):412-31.
26. Landge R, Patil K, Patil P, Salunkhe H. Molecular basis of heterosis: A review. Pharma Innov. J. 2022;11:426-34.
27. Kashyap A, Garg P, Tanwar K, Sharma J, Gupta NC, Ha PT, Bhattacharya RC, Mason AS, Rao M. Strategies for utilization of crop wild relatives in plant breeding programs. Theoretical and Applied Genetics. 2022;135(12):4151-67.
28. Zenda T, Liu S, Dong A, Duan H. Advances in cereal crop genomics for resilience under climate change. Life. 2021;11(6):502.
29. Bala M, Rehana S, Singh MP. Self-incompatibility: a targeted, unexplored pre-fertilization barrier in flower crops of Asteraceae. Journal of Plant Research. 2023;136(5):587-612.
30. Dedukh D, Majtánová Z, Marta A, Pšenička M, Kotusz J, Klíma J, Juchno D, Boron A, Janko K. Parthenogenesis as a solution to hybrid sterility: the mechanistic basis of meiotic distortions in clonal and sterile hybrids. Genetics. 2020;215(4):975-87.
31. Fishman L, Sweigart AL. When two rights make a wrong: the evolutionary genetics of plant hybrid incompatibilities. Annual review of plant biology. 2018;69:707-31.
32. Xu Y, Ma K, Zhao Y, Wang X, Zhou K, Yu G, Li C, Li P, Yang Z, Xu C, Xu S. Genomic selection: A breakthrough technology in rice breeding. The Crop Journal. 2021;9(3):669-77.
33. Labroo MR, Studer AJ, Rutkoski JE. Heterosis and hybrid crop breeding: a multidisciplinary review. Frontiers in Genetics. 2021;12:643761.
34. Hasan N, Choudhary S, Naaz N, Sharma N, Laskar RA. Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes. Journal of Genetic Engineering and Biotechnology. 2021;19(1):128.
35. Liu W, Zhang Y, He H, He G, Deng XW. From hybrid genomes to heterotic trait output: Challenges and opportunities. Current opinion in plant biology. 2022;66:102193.
36. Amiteye S. In Vitro Embryo Rescue Techniques and Applications in Hybrid Plant Development. InAdvanced Crop Improvement, Volume 2: Case Studies of Economically Important Crops 2023 Sep 8 (pp. 419-456). Cham: Springer International Publishing.
37. Begna T. Review on somatic hybridization and its role in crop improvement. J Biol Agric Healthc. 2020;10(11).
38. Arumugam T, Hatta MA. Improving coconut using modern breeding technologies: Challenges and opportunities. Plants. 2022;11(24):3414.
39. Mushtaq M, Ahmad Dar A, Skalicky M, Tyagi A, Bhagat N, Basu U, Bhat BA, Zaid A, Ali S, Dar TU, Rai GK. CRISPR-based genome editing tools: Insights into technological breakthroughs and future challenges. Genes. 2021;12(6):797.

40. Sinha D, Maurya AK, Abdi G, Majeed M, Agarwal R, Mukherjee R, Ganguly S, Aziz R, Bhatia M, Majgaonkar A, Seal S. Integrated genomic selection for accelerating breeding programs of climate-smart cereals. Genes. 2023;14(7):1484.
41. Juzoń K, Warchoł M, Dziurka K, Czyczyło-Mysza IM, Marcińska I, Skrzypek E. The effect of 2, 4-dichlorophenoxyacetic acid on the production of oat (*Avena sativa* L.) doubled haploid lines through wide hybridization. PeerJ. 2022 Jan 31;10:e12854.
42. Tadesse W, Sanchez-Garcia M, Tawkaz S, Baum M. Doubled haploid production in wheat. InAdvances in breeding techniques for cereal crops 2019 Aug 8 (pp. 93-116). Burleigh Dodds Science Publishing.
43. Lamichhane S, Thapa S. Advances from conventional to modern plant breeding methodologies. Plant breeding and biotechnology. 2022;10(1):1-4.
44. Bakala HS, Singh G, Srivastava P. Smart breeding for climate resilient agriculture. InPlant breeding-current and future views 2020 Dec 30. IntechOpen.
45. Paudel P, Pandey MK, Subedi M, Paudel P, Kumar R. Genomic approaches for improving drought tolerance in wheat (*Triticum aestivum* L.): A Comprehensive Review. Plant Archives. 2024;24(1):1289-300.
46. Gonal B, Doggalli G, Kumar B, Bhushan S, Surekha S, Malathi G, Singh L. Exploring the Future of Plant Breeding: Advancements and Challenges. International Journal of Plant & Soil Science. 2023;35(24):49-55.
47. Anderson JA, Ellsworth PC, Faria JC, Head GP, Owen MD, Pilcher CD, Shelton AM, Meissle M. Genetically engineered crops: importance of diversified integrated pest management for agricultural sustainability. Frontiers in bioengineering and biotechnology. 2019 Feb 20;7:24.
48. Tutlani A, Kumar R, Kumari S, Chouhan S. Correlation and path analysis for yield and its phenological, physiological, morphological and biochemical traits under salinity stress in chickpea (*Cicer arietinum* L.). International Journal of Bio-resource and Stress Management. 2023;14(Jun, 6):878-90.
49. Reddy B, Kumar B, Kumar R, Thota H. Analysis of Heterotic Potential for Yield and Its Contributing Traits in Wheat (*Triticum aestivum* L.). International Journal of Environment and Climate Change. 2023;13(9):388-400.
50. Nisar S, Rashid Z, Touseef A, Kumar R, Nissa SU, Faheem J, Angrez A, Sabina N, Shabeena M, Tanveer A, Amal S. Productivity of fodder maize (*Zea mays* L.) SFM-1 under varied sowing dates and nitrogen levels. International Journal of Bio-resource and Stress Management. 2024;15(Jan, 1):01-12.
51. Rathore M, Yellanki Pravalika RK, Tutlani A, Aggarwal N. Enhancing seed quality and insect management in wheat (*Triticum aestivum* L.) through optimization of storage treatments with natural and chemical compounds. Plant Archives. 2024;24(1):26-36.
52. Santhoshini A, Dubey N, Avinashe HA, Thonta R, Kumar R. Inheritance Studies in Segregating Population of Bread Wheat (*Triticum aestivum* L.). International Journal of Environment and Climate Change. 2023;13(9):277-87.