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SHAPING CROPS THROUGH TIME: FROM CLASSICAL BREEDING TO CRISPR

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ABSTRACT

Plant breeding has been a crucial **e**ndeavor throughout human history, enabling the development of improved crop varieties to meet the ever-growing demands for food, feed, fiber, and bioenergy. Over the past century, significant strides have been made in plant breeding techniques, evolving from conventional methods to more precise and efficient molecular breeding approaches. Conventional plant breeding methods, which primarily relied on phenotypic selection and cross-breeding, have contributed to the creation of numerous improved crop varieties. However, these approaches often required extended breeding cycles and involved complex genetic backgrounds, leading to limited progress in addressing complex traits and specific genetic targets. The advent of molecular biology and biotechnology has revolutionized plant breeding, giving rise to molecular breeding techniques. Molecular breeding incorporates various methodologies, including marker-assisted selection (MAS), quantitative trait loci (QTL) mapping, genomic selection (GS), and gene editing techniques like CRISPR-Cas9. By identifying and utilizing genetic markers associated with desirable traits, breeders can accurately predict and select plants carrying these traits, reducing the time and resources required for breeding. Furthermore, QTL mapping and GS enable the identification of multiple genes influencing complex traits, allowing for more comprehensive trait improvement. In combination with gene editing techniques, it is now possible to precisely modify specific genes to achieve desired traits, thereby opening up new opportunities for crop enhancement. Breeders can now access vast repositories of genomic information, aiding in the identification of genetic resources and selecting parental lines with the highest potential for desired trait combinations. Despite these remarkable advancements, challenges persist in applying molecular breeding universally. Limited accessibility to advanced technologies, ethical concerns surrounding gene editing, and potential unintended consequences require continuous assessment and regulation. In conclusion, advancements in plant breeding from conventional to molecular breeding have provided unprecedented opportunities to enhance crop productivity, resilience, and nutritional quality. As these technologies continue to mature, their responsible and equitable implementation will be critical to achieving sustainable food security for the growing global population.

KEYWORDS: Conventional methods, Molecular breeding, Gene editing, Trait improvement, Resilience

1. INTRODUCTION

In a world where agricultural productivity is crucial for the survival and development of societies, plant breeding emerges as a timeless practice, shaping the course of history (Qaim, 2020). Plant breeding is the process of selectively breeding plants to develop new varieties with desirable traits (Breseghello & Coelho, 2013**)**. For centuries, dedicated breeders have selectively bred plants, aiming to develop new varieties with desirable traits that can withstand the challenges of nature and meet the growing demands of a burgeoning population. From ancient civilizations to modern times, plant breeders have sought traits like improved yield, resistance to pests and diseases, adaptability to different climates, and enhanced nutritional value (Chakraborty & Newton, 2011; Henry, 2020; Begna, 2021). In the early days, the selection process relied on phenotypic traits - visible characteristics that could be observed and chosen for propagation. But as the scientific world progressed, so did the art of plant breeding. The advent of molecular

techniques brought a revolution in the field, enabling breeders to delve into the plants' genotypic traits - the hidden genetic codes that determine their characteristics (Moose & Mumm, 2008). This breakthrough allowed them to precisely select plants with the desired traits, accelerating the development of new and improved varieties.

One fascinating method that emerged from this scientific leap was mutation breeding. By exposing plants to radiation or chemicals, breeders could induce mutations that occasionally resulted in desirable traits (Novak & Brunner, 1992). This method opened up new possibilities, but it was still largely based on chance, requiring multiple attempts to achieve the desired results. Then came the era of genetic engineering, where breeders could go beyond the boundaries of conventional breeding by introducing foreign DNA into a plant's genome (Chawla, 2011). This breakthrough allowed them to create entirely new traits that were difficult, if not impossible, to achieve through traditional methods (Adlak et al., 2019). Despite its enormous potential, genetic engineering stirred controversies due to political and moral concerns in various countries.

Nevertheless, the combined power of conventional breeding and genetic engineering has led to remarkable advancements in agriculture. High-yielding and disease-resistant crop varieties have been developed, significantly boosting agricultural productivity worldwide. Yet, the ever-increasing demand for food challenges breeders to further push the boundaries of what is achievable. As the global population surges, the need for a substantial increase in food production becomes evident. Conventional breeding, coupled with improved agricultural practices, has managed to raise food production by 32 metric tonnes per year (Fedoroff, 2010). However, this falls short of the ambitious goal set by the Declaration of the World Summit on Food Security, which strives to achieve a 70% increase in food production by 2050 (Tester and Langridge, 2010). As climatic changes intensify, stress management for crops becomes increasingly crucial. Conventional breeding approaches, though historically dominant, face difficulties in tackling the intricate networks of plant response mechanisms (Sinclair, 2011). The complexity of plant responses to stress and the pressing need for yield improvement demand novel and innovative techniques.

Transgenic technology, with its potential to impart specific stress-resistance traits to crops, emerges as a viable solution. Yet, the controversial nature of genetic modification continues to impede its widespread adoption in many countries Chantre Nongpiur et al., 2016). Despite the progress made, challenges persist. The responses to salt and drought stress remain elusive, demanding continued research and development. New techniques that isolate and characterize genes responsible for yield improvement and stress management become imperative for overcoming these hurdles. In this ever-evolving landscape of agriculture, plant breeding stands as an enduring endeavor, continuously shaping the fate of humanity. Through the integration of traditional wisdom and cutting-edge science, breeders strive to create a world where bountiful harvests and resilient crops meet the needs of the present and secure the future for generations to come.

2. Conventional Plant Breeding methods

The history of plant breeding dates back to ancient times when farmers first began domesticating crops for their use (Kingsbury, 2011; Purugganan, 2019). In the 19th century, Gregor Mendel's experiments on pea plants led to the discovery of the basic principles of genetics (Smykal et al., 2016). In the early 20th century, plant breeders began using hybridization techniques to create new and improved plant varieties (Hoskin & Higgie, 2011). In the 1940s and 1950s, plant breeding was revolutionized by the discovery of the double helix structure of DNA and the development of genetic engineering techniques. Conventional breeding involves crossing two plants with desirable traits to produce offspring with a combination of both traits (Priyadarshan, 2019). Conventional plant breeding refers to the process of breeding plants using traditional breeding methods such as selecting desirable traits, crossing different varieties or species, and selecting the offspring with the desired traits (Acquaah, 2015). This process involves the manipulation of the genetic variation naturally present in plants to create new plant varieties. The breeding process can take several years or even decades to develop a new variety that is stable and has the desired traits.

However, the introduction of planting material across regions poses a significant risk of introducing harmful pathogens, which can severely affect crop health and yield. To mitigate this risk, the NBPGR plays a crucial role in phytosanitary measures by inspecting and intercepting potential plant pathogens in introduced materials. The accompanying diagram illustrates (**Figure 1**) some of the key pathogens that have been intercepted by NBPGR, emphasizing the importance of rigorous quarantine protocols in safeguarding agricultural biodiversity and ensuring the success of plant breeding programs.

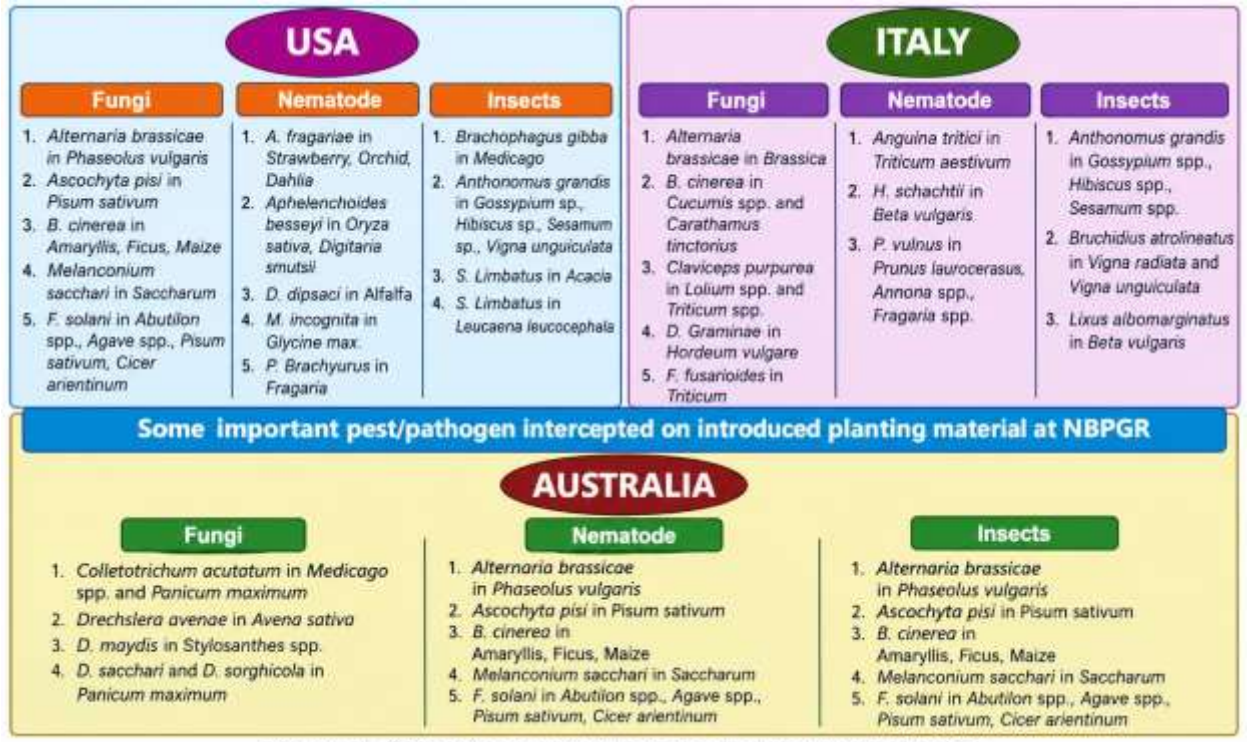


Figure 1. Key pathogens that have been intercepted by NBPGR

2.1 Selection

Phenotypic Selection involves visually evaluating and selecting plants based on their observable traits, such as plant height, yield, disease resistance, or fruit quality (Troyer, 2007; Heffner et al., 2010). Breeders choose plants with desirable traits and use them as parents for the next generation. Variation is a fundamental requirement for effective selection in plant breeding (Kuckuck et al., 2020). Variation refers to the existence of differences among individuals within a population for the traits of interest (Westneat et al., 2015). Without sufficient variation, selection cannot occur as there would be no basis for choosing superior individuals for further breeding (Moose and Mumm, 2008). Breeders often maintain diverse breeding pools or germplasm collections that contain a wide range of genetic resources. These collections serve as repositories of genetic variation that can be utilized for breeding programs. By accessing and incorporating diverse germplasm into breeding populations, breeders can introduce novel variation and expand the genetic base for selection.

Phenotypic selection is extensively applied in plant breeding across various crops to improve specific traits. For instance, in maize breeding, phenotypic selection is crucial for improving yield and disease resistance (Barker et al., 2015). Breeders visually evaluate plants for traits such as plant height, ear size, kernel number, and grain weight to select high-yielding varieties (Prasanna et al., 2020). Additionally, phenotypic selection is employed to identify maize plants with resistance to diseases like maize streak virus (Abalo et al., 2009) or fungal infections such as Fusarium ear rot (Mesterhazy et al., 2012; Hung and Holland, 2015). By selecting wheat plants with desired phenotypes, breeders have developed high-yielding and stress-tolerant wheat varieties suitable for different growing conditions (El-Ameen et al., 2013). Furthermore, in rice selection is also employed to identify resistance to diseases such as blast and bacterial leaf blight or tolerance to abiotic stresses like drought and salinity (Uphoff et al., 2015).

However, plant breeder's selection processes have been shown to increase genetic differentiation but often at the cost of genetic diversity. This loss of diversity has been observed in plant populations undergoing different types of selection, such as domestication in the pre-systematic plant breeding era and pedigree selection (both half-sib and full-sib) during the systematic plant breeding era. These selection methods, while effective in promoting desired traits, can inadvertently lead to the reduction of genetic diversity within breeding populations. Maintaining genetic diversity is crucial for the long-term sustainability and adaptability of cultivated crops (Rauf et al., 2010; Lommen et al., 2017)

2.2 Hybridization

Throughout history, botanists and geneticists have heavily depended on the genetic diversity that arises from crossbreeding plants belonging to the same species. Nevertheless, the available genetic diversity within species populations is insufficient, thereby necessitating the exploration of favorable traits in distantly related or even unrelated plants using hybridization methods (Mwangangi et al., 2019). Hybridization is a fundamental technique in

plant breeding that involves the controlled crossing of two genetically distinct parent plants to create offspring with desired traits. This process is often used to combine desirable traits from different parental lines and generate new, superior plant varieties. The success of hybridization depends on careful selection of parental lines (**Figure 2**). Breeders choose parent plants with complementary traits that they wish to combine in the offspring. These traits can include characteristics like disease resistance, yield potential, quality attributes, or adaptability to specific environments. The parental lines should have distinct genetic backgrounds to maximize the potential for genetic recombination and the expression of novel traits in the hybrid offspring. Cui et al. (2000) showed that genetic diversity of Chinese soyabean was high due to careful selection of unrelated parents while constituting transgressive segregation. Interspecific hybridization offers a way to broaden the genetic base by incorporating novel traits from different species. These methods have implications for genetic diversity in plant breeding. Balancing intraspecific and interspecific hybridization is important for maintaining a diverse and adaptable crop population (Rauf et al., 2010).
Hybridization, although a valuable technique in plant breeding, is not without its challenges and potential problems. One of the main issues is the occurrence of incompatibility between parent plants, which can hinder successful crossbreeding (Lafon-Placette and Kohler, 2016). Genetic barriers, differences in ploidy levels, or incompatible reproductive structures can impede the formation of viable hybrids (Vallejo-Marín and Hiscock, 2016). Overcoming these obstacles requires careful selection of suitable parent plants and the utilization of techniques like embryo rescue or chromosome doubling (Jansky, 2006; Dempewolf et al., 2017). Maintaining the genetic purity of hybrid crops is also a significant concern. Furthermore, hybridization can be a time-consuming and labor-intensive process. It requires careful planning, controlled pollination, and extensive field evaluations to identify superior hybrids. Additionally, the cost associated with hybrid seed production and the need for specialized knowledge and resources may pose challenges for smaller-scale breeders or farmers.

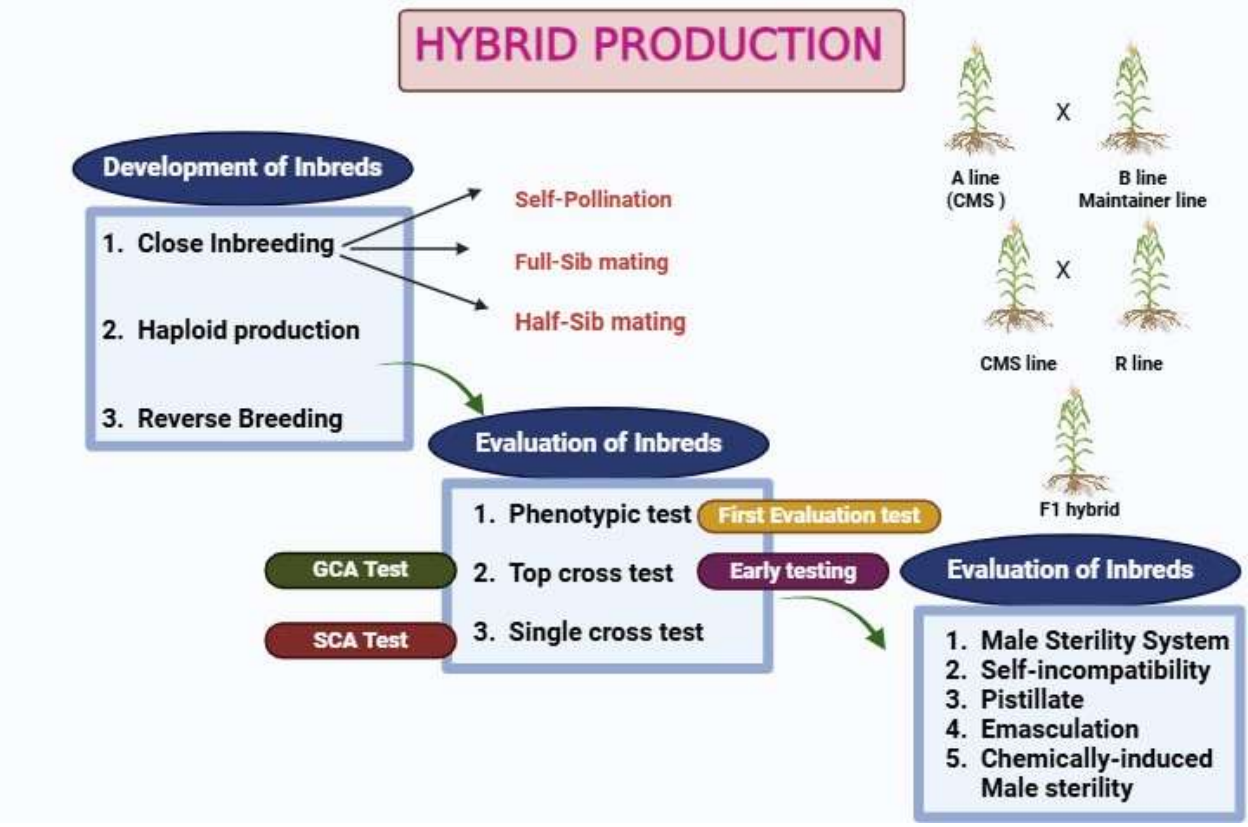


Figure 2. Steps involved in production of hybrids

2.3 **Mutation Breeding**

Mutation breeding is a technique used to induce mutations in plant DNA to create new plant varieties (Sigurbjornsson, 1983; Ahloowalia & Maluszynski, 2001). This technique involves exposing plants to radiation or chemicals that cause changes in their DNA (Bado et al., 2015). Mutations can lead to the creation of new traits that may be desirable, such as improved yield or disease resistance (Oladosu et al., 2016; Raina et al., 2016). The mutated plants are then screened to identify those with desirable traits, and the selected plants are further bred to develop new plant varieties. Mutation breeding has been used to develop over 3,000 new plant varieties, including crops such as rice (Rutger, 1992; Baloch et al., 2003), wheat (Njau et al., 2005) and barley (Ohnoutkova, 2019). Mutation breeding has played in enhancing

rice varieties globally, resulting in improved yield, disease resistance, abiotic stress resistance and other desirable characteristics. One significant contribution of mutation breeding in rice is the induction of thermo-sensitive genic male-sterile (TGMS) mutants. The PL-12 mutant in Japonica rice, controlled by a single recessive gene, has played a vital role in the production of hybrid rice varieties (Maruyama et al., 1991). The combination of γ-rays, EMS and SA was also used in Basmati rice mutagenesis (Siddiqui and Singh, 2010). The combination of different doses promoted decreases in plant height, panicle size, panicle number and in the weight of 100 seeds. Tolerance to iron toxicity was obtained, fast neutron irradiation induced changes in the FRO1 gene, responsible to increase tolerance and higher iron accumulation in grains (Ruengphayak et al., 2015). Wheat mutants exhibited higher diseases resistance (Septoria tritici blotch, tan spot, yellow rust, and leaf rust) as well as better grain quality parameters (thousand kernel weight, grain protein content, test weight, gluten content, and wet gluten content (Hassine et al., 2022). Additionally, draught tolerant mutants are developed due to mutation (Njau et al., 2005). The availability of knock-out mutants and the ease of genetic transformation have made mutation tremendously significant in assessing the functions of individual drought-tolerant genes (Saif-u-Malook et al., 2015). The study conducted by Emrani et al. (2013) sheds light on the significant role of mutation in maize improvement. By subjecting maize cultivars to different levels of gamma radiation, the researchers induced mutations in the plants, leading to observable changes in various traits related to germination and growth. Through their research, it was demonstrated that different levels of gamma radiation had a direct impact on the growth characteristics of maize, including root length, shoot length, shoot diameter, germination percentage, and germination rate. The highest levels of root and shoot growth were observed on the third and fourth days at the 400 and 600 cGy levels of irradiation. These findings highlight how mutations induced by gamma radiation can create variations in maize traits, leading to potential improvements in crop performance.

Several studies have demonstrated the successful application of mutation breeding to enhance the nutritional characteristics of crops. Pearl millet is an important staple crop in regions where malnutrition, particularly zinc deficiency, is prevalent. Studies highlighted the use of mutation breeding to increase zinc content in pearl millet. By applying gamma radiation, researchers generated mutant lines with significantly higher zinc concentration in the grain (Kugbe et al., 2015; Vinoth & Ravindhran 2017). Mutation breeding has been instrumental in enhancing various traits in rice. Notable achievements include modifications in amylose content, enhancing starch resistance, developing high-lysine genotypes, fortifying grains with iron and zinc, reducing toxic elements like arsenic and cadmium, as well as decreasing anti-nutritional compounds such as phytic acid (Da Luz et al., 2020; Das et al., 2020). Tran and Ho (2017) conducted a study on breeding new aromatic rice varieties with high iron content using a combination of gamma radiation and hybridization.

Conventional plant breeding methods, while foundational in crop improvement, are not without their limitations. The process is often time-consuming and labor-intensive, requiring multiple generations of crossbreeding and selection (Ayan et al., 2022). Genetic diversity within crop species can be limited, hindering the introduction of novel traits. Complex inheritance patterns and the presence of linkage drag pose challenges in targeting specific traits efficiently (Scheben et al., 2017). In addition, addressing recessive traits and overcoming genetic barriers or incompatibilities can be difficult. Few drawbacks of mutation breeding include unpredictability and the low efficiency of obtaining desirable mutations. Additionally, the lack of precision in introducing genetic changes and the potential for genetic load are concerns. Conventional breeding lacks precision, making it challenging to control the precise introduction of desired genes or genetic changes. Furthermore, the long breeding cycles associated with conventional methods can delay the development and release of new crop varieties. Overcoming these limitations necessitates the integration of modern techniques, such as molecular breeding and biotechnology, to complement and enhance conventional breeding efforts **(Figure 3**).

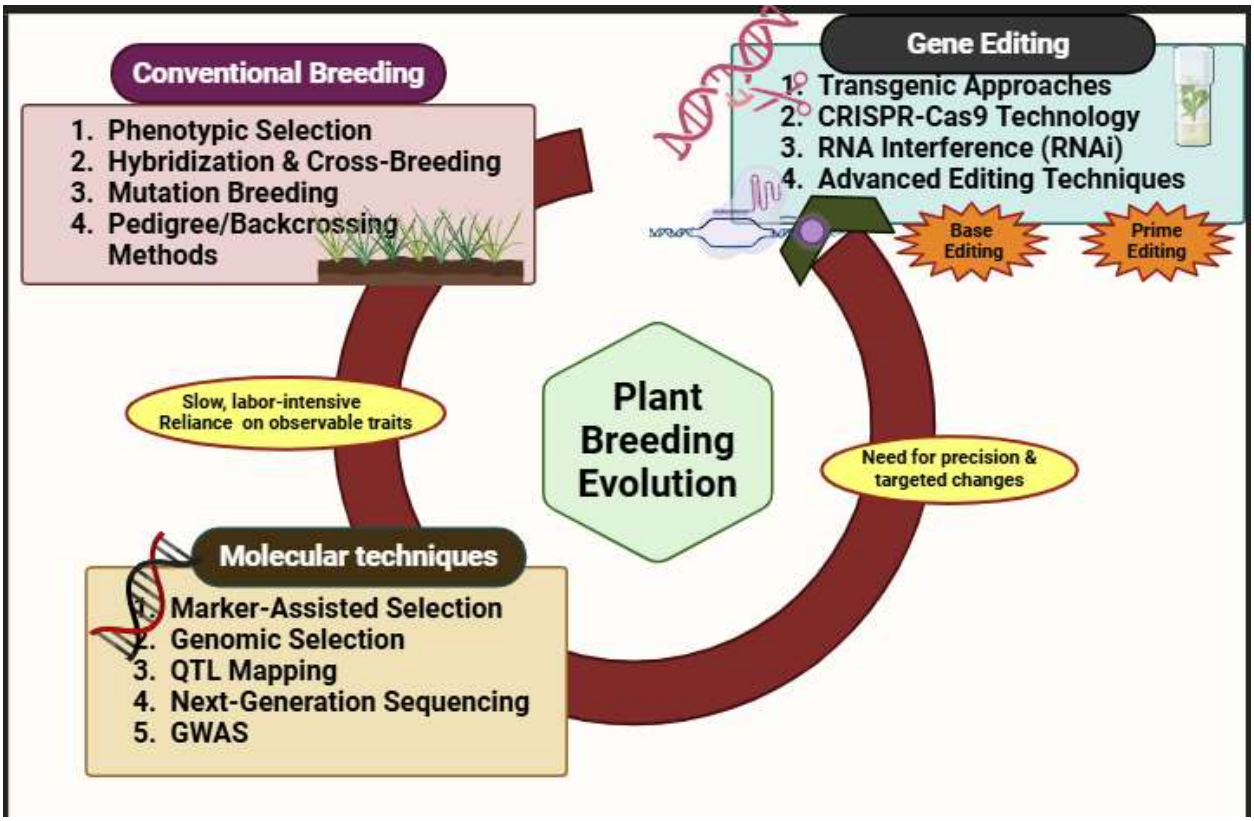


Figure 3. Transition in plant breeding methods over time

3. Biotechnology Advancements

3.1 Genetic Engineering

Genetic engineering involves the manipulation of plant DNA using biotechnology to create new plant varieties with desirable traits. This technique involves inserting genes from one organism into another to create plants with specific traits such as improved pest resistance or higher yields (Abdallah et al., 2015; Kumar et al., 2020). Genetic engineering allows for the precise targeting of specific genes, making it possible to create plants with specific traits quickly. However, there are concerns about the potential risks of genetic engineering, such as unintended effects on the environment or human health (Maghari & Ardekani, 2011). Biotechnology has the potential to play an important role in herbicide resistance management in weeds by enabling the development of new herbicides, as well as new weed control strategies. One approach is the development of herbicide-tolerant crops through genetic engineering. These crops are designed to be resistant to specific herbicides, which allows for more targeted weed control and can help reduce the development of herbicide resistance in weeds (Westwood et al., 2018).

Another approach is the use of RNA interference (RNAi) technology to target specific genes in weeds that are involved in herbicide resistance (Jagtap et al., 2011). This technology involves the use of small RNA molecules that can bind to and silence specific genes, making the weed more susceptible to herbicides. Biotechnology can also be used to develop new herbicides that are less prone to resistance. For example, researchers can use genetic engineering to create herbicides that target multiple genes in the weed, making it less likely for the weed to develop resistance to the herbicide. Overall, the role of biotechnology in herbicide resistance management is promising, but it is important to consider the potential risks and limitations associated with this technology. It is crucial to carefully evaluate the potential benefits and risks of any biotechnology-based approach to weed management and to ensure that it is used in a responsible and sustainable manner (Shaner & Beckie, 2014), Sahoo et al. (2022) and Tuli et al. (2022).

3.2 Marker-Assisted Breeding

MAS is a breeding strategy that utilizes molecular markers to aid in the selection of individuals with desired traits. By genotyping plants or seeds, breeders can quickly identify individuals that carry the target marker associated with the trait of interest. MAS enables the early selection of superior genotypes, reducing the time and resources required for conventional phenotypic selection methods. In Marker-Assisted Backcrossing, molecular markers are used to track and introgress specific target traits from a donor parent into an elite recipient parent. This approach accelerates the transfer of desirable genes while minimizing the genetic linkage drag associated with conventional backcrossing. Marker-Assisted Recurrent Selection (MARS) combines the advantages of traditional recurrent selection with

molecular marker information. The markers assist in the selection of individuals with favorable alleles at multiple loci, allowing for the simultaneous improvement of multiple traits within breeding populations. Marker-assisted breeding is a technique that involves the use of molecular markers to identify desirable traits in plants (Singh & Singh, 2015). This technique involves identifying markers linked to specific genes associated with desirable traits, such as disease resistance or drought tolerance (Khan et al., 2017). Plants can be screened for these markers, allowing breeders to select plants with the desired traits more efficiently. This technique has been used to develop new plant varieties in crops such as maize, wheat, and soybean (Ribaut & Ragot, 2007; Pandurangan et al., 2021; Kim et al., 2020). Molecular markers play a crucial role in marker-assisted breeding by providing a means to identify and track specific regions of the genome associated with desired traits.

Several types of molecular markers are commonly used in marker-assisted breeding programs. Although different marker systems have been developed over time including Restriction Fragment Length Polymorphisms (RFLPs), Amplified Fragment Length Polymorphisms (AFLPs), Simple Sequence Repeats (SSRs), Single Nucleotide Polymorphisms (SNPs) etc. SNPs are the most abundant type of genetic variation in genomes. SNPs are single base pair differences that can occur throughout the genome and can be easily genotyped using high-throughput sequencing or genotyping platforms. The widespread use of SNPs in marker-assisted breeding is driven by their abundance, high-throughput genotyping capabilities, co-dominant inheritance, association mapping potential, and transferability across diverse genetic backgrounds (Gupta et al., 2008, Gorlapalli et al. (2022)). These qualities make SNPs the marker of choice for many breeding programs aiming to enhance the efficiency and precision of trait selection and introgression.

The integration of molecular markers into breeding programs has revolutionized the field of plant breeding by providing valuable tools for efficient trait selection, genetic mapping, and germplasm improvement. The incorporation of molecular markers offers several advantages and has become an integral component of modern breeding strategies. Here, we explore the key aspects of integrating molecular markers into breeding programs.

3.3 QTL Mapping and Marker-Trait Association Studies

Molecular markers are extensively used in quantitative trait locus (QTL) mapping and marker-trait association studies. By genotyping mapping populations, researchers can identify genomic regions associated with specific traits of interest. This information helps in understanding the genetic basis of traits and facilitates marker-assisted selection (MAS) by targeting these genomic regions. QTL mapping has been widely employed in various crops for crop improvement, leading to significant advancements in developing improved varieties with desirable traits. QTL mapping has been extensively utilized in crops to enhance traits such as yield, disease resistance, abiotic stress tolerance, and grain quality (.

For instance, QTL mapping has been instrumental in identifying genomic regions associated with yield-related traits like grain size, grain weight, and panicle architecture (Barik et al., 2023). It has also facilitated the improvement of disease resistance against pathogens such as blast, bacterial blight, false smut and sheath blight (Andargie et al., 2018). A study was carried out to identify novel quantitative trait loci (QTLs) from an indica variety Zenith. It resulted that introducing qBK1z or pyramiding with other previously identified QTLs could provide effective genetic control of bakanae disease in rice (Li et al., 2021). Additionally, QTL mapping has aided in enhancing abiotic stress tolerance, including drought, salinity, and submergence tolerance, leading to the development of stress-resilient rice varieties (Raj and Nadarajah 2022; Toulotte et al., 2022). QTL mapping has played a role in enhancing nutritional traits in maize, including provitamin A content and protein quality (Maqbool et al., 2018). QTL mapping has contributed to improving abiotic stress tolerance in wheat, including drought and heat stress (Kamal et al., 2019). Through QTL mapping, genomic regions associated with traits such as seed size, oil content, protein content, and resistance to diseases like soybean cyst nematode and Phytophthora root rot have been identified.

3.4 Genomic Selection (GS):

Genomic Selection (GS) has revolutionized the field of plant breeding by leveraging dense marker data and advanced statistical models to predict the breeding values of individuals based on their genotypic information (Heffner et al., 2009). Through the incorporation of genome-wide marker coverage, GS empowers breeders to select individuals with the highest genetic potential for multiple traits, even in the absence of phenotypic data (Crossa et al., 2010). This approach has proven particularly effective in crops with long breeding cycles or traits that are difficult to measure directly (Lorenzana & Bernardo, 2009), Kale et al. (2021) and Srilatha et al. (2019).

Genetic diversity and population structure within crop germplasm collections play a critical role in plant breeding, and molecular markers have become indispensable tools in assessing these aspects (Thachuk et al., 2009). Marker-based diversity analysis aids in the selection of diverse parental lines for breeding programs and facilitates the identification of potentially valuable alleles for trait improvement (Gupta et al., 2019). Moreover, molecular markers contribute to the development of core collections and the identification of duplicate or mislabeled accessions, streamlining germplasm management efforts (Ortiz et al., 2010). The emergence of high-throughput genotyping technologies and sophisticated statistical modeling has paved the way for genomic prediction methods (Lorenzana & Bernardo, 2009). Genomic prediction integrates marker information with phenotypic data to estimate the breeding value of individuals, enabling early-stage selection and more accurate prediction of performance (Garrick et al., 2009).

In breeding programs aimed at developing cultivars with specific traits, molecular markers play a pivotal role (Collard & Mackill, 2008). For instance, markers linked to disease resistance, abiotic stress tolerance, quality traits, or nutritional enhancement are identified, allowing breeders to expedite the selection and introgression of desired traits into elite cultivars (Varshney et al., 2005). With the integration of cutting-edge molecular technologies and statistical methodologies, the field of plant breeding has witnessed unprecedented progress, propelled the development of superior crop varieties and contributed significantly to global food security (Cobb et al., 2019). These molecular breeding approaches continue to evolve and hold immense promise for addressing future agricultural challenges.

3.5 Transcriptomic Analysis

Transcriptomics, a key branch of omics sciences, has gained significant recognition in crop improvement due to its critical role in examining the complete set of transcripts (Pietu et al., 1999). It encompasses various transcript types, such as messenger RNAs (mRNAs), microRNAs (miRNAs), and diverse long noncoding RNAs (lncRNAs). Given the intricate nature of plant responses to stresses, conventional and transgenic breeding approaches have achieved only limited success in enhancing tolerance to stresses like salinity or drought (Flowers, 2004; Yamaguchi and Blumwald, 2005). Modern transcriptomics employs high-throughput techniques, enabling the detailed examination of transcript expression across various physiological and pathological states, thereby deepening insights into transcriptome-phenotype interactions. Numerous genes conferring tolerance to abiotic and biotic stresses have been identified at the transcriptional level in various crops. Analysis of stress-inducible gene transcripts has led to the discovery of many such genes, facilitating the development of transgenic plants with improved stress tolerance (Umezawa et al., 2006).

3.6 Association Mapping

In recent decades, association mapping a high-resolution approach grounded in linkage disequilibrium has shown immense promise for dissecting complex genetic traits (Liu et al., 2016; Cockram et al., 2010). The process involves several key steps: (i) selection of an association panel; (ii) genotyping the panel; (iii) phenotyping the panel; and (iv) statistical analysis (Gupta et al., 2014; Bangarwa et al., 2020). Factors influencing the statistical power of association studies include population size, effect size of target alleles, marker density, linkage disequilibrium decay rate between markers and target alleles, phenotyping and genotyping errors, and the chosen significance threshold (Breseghello et al., 2006; Ritland, 2005). Compared to traditional QTL mapping in biparental populations, population-based association mapping offers advantages through access to greater genetic diversity and a broader context for marker-trait associations (Abdurakhmonov and Abdukarimov, 2008). It typically employs individuals from germplasm collections or natural populations (Kraakman et al., 2004). Advances in high-throughput genotyping and sequencing technologies suggest widespread future adoption in crop plants. Association mapping serves as an effective means to identify novel genes or QTLs associated with biotic and abiotic stress tolerance in major field crops (Pushparajan et al., 2011; Beena et al., 2021).

4. Recently Developed Techniques

4.1 Next-Generation Sequencing (NGS)

The demand for sequencing larger numbers of samples at reduced costs drove the emergence of next-generation sequencing technologies (Vlk and Repkova, 2017). NGS has transformed DNA sequencing, accelerating genome sequencing efforts via efficient, versatile, and economical platforms, including Roche 454 sequencing, Illumina, SOLiD, Ion Torrent, and Oxford Nanopore technology (Huang et al., 2013). Its capacity to identify numerous single-nucleotide polymorphisms (SNPs) and insertions/deletions (InDels) underscores its value in crop improvement (Varshney et al., 2009). By pinpointing extensive SNP loci across genomes, NGS elucidates the genetic foundations of phenotypic variations among cultivars in response to biotic and abiotic stresses (Qi et al., 2011; Papworth et al., 1996). NGS has revolutionized molecular marker discovery, identification, and mapping of causal mutations in a timely and cost-effective manner. With rapid, low-error sequencing, trait-specific molecular markers can now be generated in large quantities, even without reference genomes (Trebbi et al., 2011; Cortés et al., 2011).

4.2 TILLING and Eco-TILLING

Functional genomics increasingly emphasizes gene function elucidation. Reverse genetic methods are commonly employed to assess phenotypes arising from sequence data. Techniques such as site-directed mutagenesis, gene knockout, RNAi, and transposon tagging often require transgenic approaches, which may be impractical for certain plant species (An et al., 2005; Burch-Smith et al., 2004). TILLING (Targeting Induced Local Lesions IN Genomes) represents a non-transgenic, high-throughput reverse genetics method applicable to any mutagenizable plant species, irrespective of pollination mode, ploidy, or genome size (McCallum et al., 2000a). It targets SNPs and/or InDels in specific genes within mutagenized populations (Gilchrist et al., 2006). Requiring prior DNA sequence knowledge, TILLING leverages mismatch endonucleases to detect induced mutations, yielding allelic series of silent, missense, nonsense, and splice-site mutations for functional analysis (Till et al., 2004). TILLING has proven effective and efficient for functional genomics across diverse plant species, enabling swift mutation detection in target genes from mutagenized populations. Applications span crops like wheat, rice, maize, and sorghum for evaluating mutations in

selected genes (De-Kai et al., 2006). EcoTILLING, a TILLING variant, detects natural genetic variation in populations and has successfully identified SNPs, including rare alleles, in plants (Comai et al., 2004).

4.3 CRISPR/Cas9

CRISPR is a natural immune system of Streptococcus pyogenes against viruses. With the first evidence of its efficacy for genome editing in 2012 in mammalian cells, the CRISPR/Cas9 gene editing technology has transformed research in animal and plant biology (Jinek et al., 2012). Cas9 protein cleaves out viral genome to prevent it from hijacking bacterial genome and causing disease, while CRISPR array saves a copy of the invading virus genome in the bacterial genome to thwart future viral attack. CRISPR/Cas9 falls under the bacterial-specific Class II division of the CRISPR/Cas system, which is further segmented into Classes I through III. The prerequisite for the CRISPR cleavage methodology involves i) A 20 nucleotide short synthetic gRNA sequence that binds to the target DNA, ii) An enzyme called Cas9 that cleaves three to four bases after the protospacer adjacent to motif (Jinek et al., 2012). In the past five years, CRISPR/Cas9 approaches have been employed more frequently to modify plant genomes than ZFNs/TALENs, which is a reflection of how simple they are to use (Jaganathan et al., 2018). To far, approximately 20 crop species have adopted the CRISPR/Cas9 method of gene editing for a variety of features, including yield improvement and stress management (Ricroch et al., 2017).

Table 1. Overview of Crop Improvement: Traits, Breeding Methods, and Milestones

S.No.	Crop	Trait Improved	Breeding Method Used	Year	Reference
1	Rice	Improved plant architecture	Domestication	2008	Jin et al. (2008)
2	Wheat	Rust resistance	Mutation breeding (Lr34 SNPs)	2009	Krattinger et al. (2009)
3	Rice	Reduced seed shattering	Domestication (SHA1, SH4, qSH1)	2006	Li et al. (2006)
4	Maize	Increased ear rank number	Domestication (zfl2 gene)	2006	Bomblies and Doebley (2006)
5	Wheat	Increased grain yield	Hybrid breeding	2019	Basnet et al. (2019)
6	Rice	Increased grains per panicle	Domestication (FZP gene)	2018	Huang et al. (2018)
7	Wheat	Powdery mildew resistance	TILLING (mutation detection)	2017	Acevedo-Garcia et al. (2017)
8	Rice	Drought tolerance	Marker-Assisted Selection (MAS)	2015	Malik and Malik (2015)
9	Maize	Rough dwarf disease resistance	QTL Mapping	2020	Xu et al. (2020)
10	Soybean	High oleic acid content	Genome Editing (CRISPR)	2019	Wang et al. (2019)
11	Wheat	Semi-dwarfism	Haplotype-Based Breeding	2022	Beyene et al. (2022)
12	Rice	Disease resistance	Genome Editing (CRISPR)	2018	Li et al. (2018)

13	Mustard	Higher yield	Genetic Engineering (DMH-11)	2023	Pental, 2023
14	Rice	Bacterial blight resistance	Marker-Assisted Selection (MAS)	2018	Collard & Mackill, (2008
15	Soybean	Cyst nematode resistance	Marker-Assisted Selection (MAS)	2018	Collard & Mackill, (2008
16	Maize	Quality protein enhancement	Marker-Assisted Selection (MAS)	2018	Collard & Mackill, (2008
17	Rice	Blast resistance	Marker-Assisted Selection (MAS)	2018	Collard & Mackill, (2008
18	Wheat	Bread-making quality	Genetic Engineering	2018	Collard & Mackill, (2008

Conclusion

In conclusion, each of these breeding techniques has its advantages and disadvantages, and the choice of technique depends on the specific goals of the breeding program. Conventional breeding and mutation breeding are slower processes, but they do not involve the manipulation of genes in the same way as genetic engineering. Marker-assisted breeding allows breeders to select plants with desirable traits more efficiently, while genetic engineering allows for the precise targeting of specific genes to create new plant varieties.

Future research

Future research in plant breeding will likely focus on developing crops that are more resilient to climate change, pests, and diseases. Additionally, there will be a growing demand for crops with improved nutritional value, and research will focus on developing plants with enhanced nutrient profiles. The use of machine learning and artificial intelligence in plant breeding is also likely to become more prevalent, enabling breeders to analyze large amounts of genomic data and identify plants with desirable traits more efficiently.

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