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AMALGAMATION OF RECENT EFFORTS IN PLANT BREEDING & BIOTECHNOLOGY

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PREFACE

The field of plant breeding and biotechnology has witnessed unprecedented advancements in recent years, driven by the growing need to meet the demands of a rapidly expanding population, evolving climate conditions, and a heightened focus on sustainability. This book, Amalgamation of Recent Efforts in Plant Breeding and Biotechnology, seeks to provide an insightful overview of the latest innovations, research, and practical applications in these domains.

The fusion of traditional plant breeding techniques with modern biotechnological approaches has opened new avenues for improving crop yields, enhancing resistance to pests and diseases, and fostering climate resilience. This synergy has not only accelerated crop improvement efforts but also provided tools for the development of novel traits in plants, with profound implications for global agriculture and food security.

In this volume, experts from various disciplines have contributed their research and findings, covering a broad range of topics—from genomic editing techniques and gene silencing to the integration of molecular markers and bioinformatics tools in breeding programs. Special emphasis has also been placed on the ethical considerations, regulatory frameworks, and the potential impact of biotechnological innovations on biodiversity and environmental conservation.

As editors, we are proud to present this compendium of knowledge, which we believe will serve as a valuable resource for researchers, academicians, students, and practitioners in the fields of plant science, biotechnology, and agricultural sciences. We hope that the insights presented in these chapters will inspire continued research and development, as well as collaborative efforts, to harness the full potential of plant breeding and biotechnology for a more sustainable and food-secure future.

We extend our heartfelt gratitude to all the contributors who have made this book possible through their expertise and dedication. We also wish to acknowledge the collaborative spirit of the academic and research communities that continue to push the boundaries of plant science.

Editors

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HAPLOTYPE BREEDING

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Abstract:

Haplotype breeding is a cutting-edge method that leverages sets of alleles located on the same chromosome to enhance crop improvement, offering greater precision and efficiency over traditional approaches. Enabled by advancements in genome sequencing and bioinformatics, this technique allows breeders to identify genetic variants associated with complex traits, such as disease resistance, yield, and quality. By analysing haplotypes, breeders gain insights into the genetic architecture of these traits, leading to more informed selections and higher accuracy in genomic predictions. The method has been successfully applied to crops like rice, wheat, and maize. In rice, haplotypes linked to yield, disease resistance, and stress tolerance have been identified. In wheat, key genetic regions associated with quality traits, disease resistance, and stress tolerance have been pinpointed. For maize, haplotypes related to nitrogen use efficiency, drought resistance, and flowering time have been utilized in breeding programs. Technological advancements, including next-generation sequencing (NGS), long-read sequencing, and bioinformatics tools such as BEAGLE, SHAPEIT, and MACH, have been crucial for resolving haplotypes in crops. Machine learning and AI are increasingly helping breeders predict complex traits more accurately, further enhancing the efficiency of haplotype breeding. However, challenges remain, particularly in accurately identifying and phasing haplotypes in complex genomes. Ethical and regulatory concerns must also be addressed. Yet, future innovations in sequencing technologies, bioinformatics, and AI are expected to overcome these hurdles and boost the potential of haplotype breeding. As agriculture faces climate change, population growth, and the demand for sustainable practices, haplotype breeding offers a

pathway to developing crop varieties that are more resilient, high-yielding, and environmentally sustainable, paving the way for precision agriculture

Keywords: SNP, Hapmap and GWAS

Introduction:

A haplotype is a set of alleles for several polymorphisms (including SNPs, insertions/deletions, and other markers or variations) located on the same chromosome, inherited together with a minimal chance of concurrent recombination. (Stram *et al.,* 2017, Garg *et al.,* 2021). Unlike traditional breeding methods that select based on individual markers or traits, haplotype breeding captures the essence of genetic blocks, providing a more accurate representation of genetic variation, inheritance patterns, and trait expression.

Plant breeding has placed a greater focus on haplotype comprehension because to advancements in genome sequencing and bioinformatics. They improve the breeding process by assisting in the identification, selection, and incorporation of beneficial genetic areas that enhance crop nutrition, disease resistance, and yield. Breeders may use complete advantageous genomic areas instead of just single markers by concentrating on haplotypes, which will guarantee more stable and long-lasting benefits in crop varieties.

The haplotypes are defined/assigned in three principal ways:

a) Using a number of haplotypes within a particular chromosomal sequence,

b) It is assessed by r^2, which is the pairwise LD between the jointly inherited markers that demonstrate a lack of evidence for historical recombination. (Pritchard *et al.,* 2001 and Liu *et al.,* 2019)

c) By assembling SNPs with varying or fixed chromosomal lengths. When it comes to determining the haplotypes in the genomic/chromosome areas, LD-based methods are more effective (Huang *et al.,* 2007).

With the advent of the human genome sequence, the idea of finding haplotype tag SNPs (htSNPs) within haplotype-based blocks was first proposed with the objective of reducing the number of markers required to capture meaningful information within a genomic area (Daly *et al*., 2001). It was proposed that creating a "hapmap" would be essential to comprehending human disorders (Couzin, 2002). But as Gabriel *et al.,* (2002) pointed out, it was quickly discovered that knowledge of the underlying genetic structure of different subpopulation groupings was essential and the concept of common haplotypes emerged.

Crop plants are subjected to a wide range of biotic and abiotic stresses that impede normal growth and cause significant reductions in yields across the globe. These stresses present a critical challenge for agricultural scientists working to ensure food security, particularly as the global human population continues to grow. The development of climate-smart, high-yielding, and nutritious crop varieties is essential in addressing this challenge. While conventional breeding methods have achieved notable success in enhancing crop productivity, particularly through the development of high-yielding varieties, the need to accelerate crop improvement programs remains urgent. This is especially true for complex traits such as yield under stressful conditions.

The complexity of these agriculturally important traits makes traditional breeding approaches less effective. Many conventional experimental populations suffer from limited genetic diversity, low recombination rates, and poor resolution, hampering the identification of crucial genetic regions linked to these traits. Genome-wide association studies (GWAS) have emerged as a powerful tool to overcome these challenges, offering higher resolution and allelic richness for dissecting complex traits. With the availability of cost-effective and high-density genotyping platforms, breeding populations can now be screened more efficiently, enabling better estimation of breeding values through genomic selection. Genome-wide association studies (GWAS) using a variety of genotypes and populations in plants have become an important tool for revealing genetic variation. (Ersoz *et al.*, 2007).

NGS-based genotyping methods, including genotyping-by-sequencing, restriction site-associated DNA sequencing, and whole-genome resequencing, have facilitated the large-scale genotyping of germplasm collections for use in GWAS and GS. However, the single-nucleotide polymorphisms (SNPs) used in these analyses come with certain limitations, such as their biallelic nature and the prevalence of rare alleles. Additionally, linkage drag, where genetic regions associated with the SNPs affect traits without directly being responsible for them, complicates the interpretation of SNP data (Poland *et al.*, 2012).

Haplotype-based approaches have gained attention to increase the resolution of candidate genomic regions. Haplotypes refer to specific combinations of DNA markers or nucleotides that are inherited together from polymorphic sites within the same chromosomal segment. By considering haplotypes rather than individual SNPs, researchers can overcome some of the challenges posed by linkage drag and the limitations of biallelic markers.

Haplotypes in Crop Genomes

In plants, haplotypes play a critical role in understanding genetic variation and evolutionary processes (Flint-Garcia *et al.,* 2003). Crop genomes undergo recombination events during sexual reproduction, which reshuffle alleles and create new haplotype combinations. Over generations, recombination shapes haplotype blocks — regions of the genome where certain SNPs tend to be inherited together (Ersoz *et al.,* 2007).

Recombination hotspots, where the frequency of recombination is high, fragment haplotypes, while areas with lower recombination maintain larger haplotype blocks. This reshuffling is significant for crop evolution and breeding, as it allows for the introduction of new traits like disease resistance or stress tolerance. (McVean *et al.,* 2004)

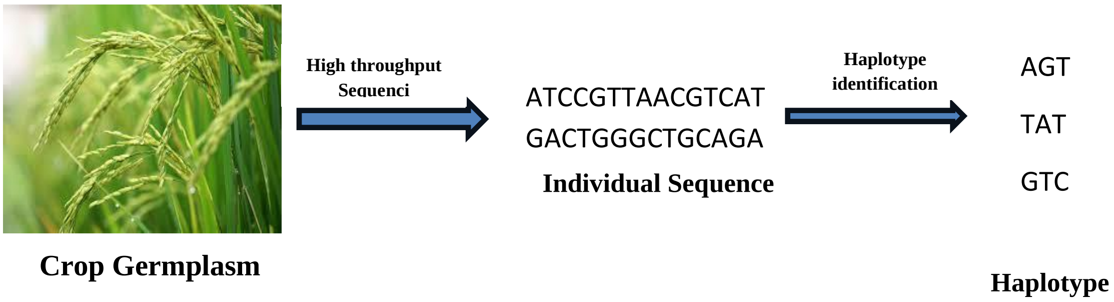


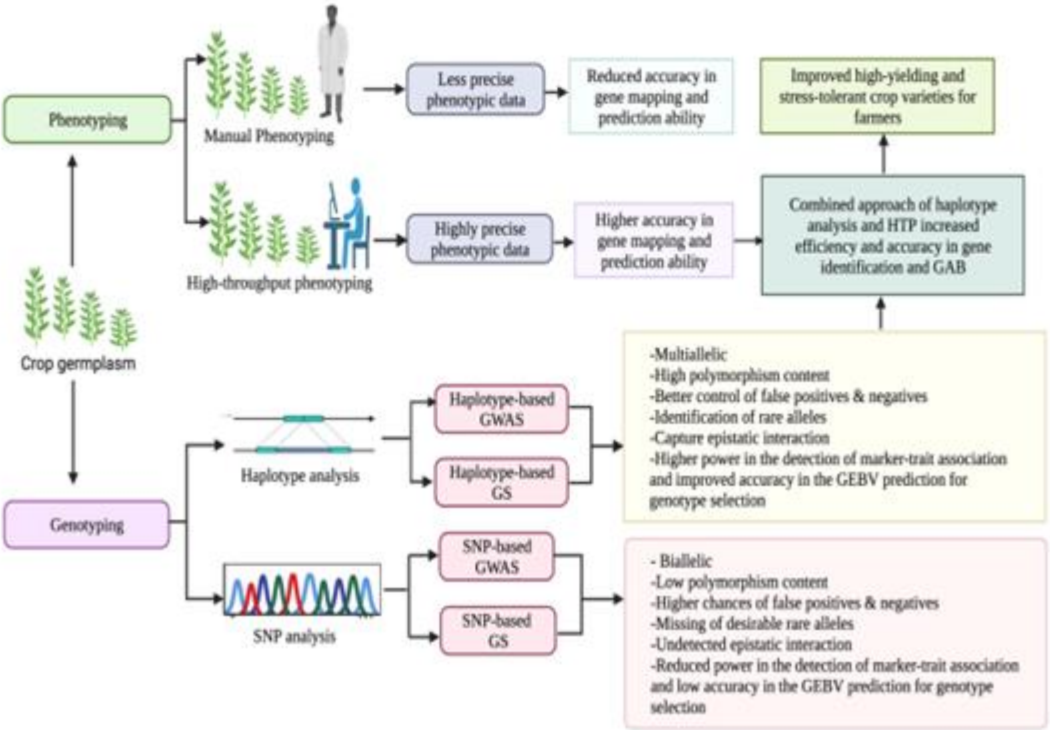
Fig. 1 Formation and haplotypes from haploid sequences

Mapping and Identifying Haplotypes in Crops

The identification of haplotypes in crop genomes has been revolutionized by high-throughput sequencing technologies like next-generation sequencing (NGS). With NGS, researchers can analyze entire genomes to detect SNPs and identify haplotypes. (Weigel *et al*., 2010) Bioinformatics tools, such as Haploview, PLINK, and SHAPEIT, assist in the reconstruction of haplotypes from sequencing data. These tools allow scientists to identify haplotype blocks and analyze their association with traits of interest in crops. (Barrett *et al*., 2005)

Linkage disequilibrium (LD) is key in haplotype mapping. LD refers to the non-random association of alleles at different loci, and its extent helps define haplotype blocks. In crops like rice, wheat, and maize, LD can vary across the genome, influenced by factors such as population history, mating systems, and recombination rates. (Flint-Garcia *et al*., 2003, Huang *et al*., 2010) Comparative analysis of haplotypes across species reveals common patterns of genetic variation and offers insights into how selective breeding and natural selection shape genomes. For instance, studies comparing the haplotypes of maize and rice have identified regions of the genome associated with drought tolerance and yield improvement.

Genome-Wide Association Studies (GWAS) and haplotype analysis are powerful tools in haplotype breeding, allowing researchers to identify genetic variants associated with specific traits and understand the genetic architecture of complex diseases. GWAS involves scanning the entire genome to identify single nucleotide polymorphisms (SNPs) associated with a particular trait or disease.



SNP mining and haplotype generation are used to determine genome estimated breeding values and find marker-trait relationships (GWAS). (Bhat *et al.* 2021)

GWAS in Haplotype Breeding

GWAS has been widely used in haplotype breeding to identify genetic variants associated with complex traits such as disease resistance, yield, and quality. For example, a GWAS study in soybean identified several SNPs associated with resistance to powdery mildew disease (PMD) (Sang *et al*., 2023). Another study in wheat identified SNPs associated with resistance to stripe rust disease (Tibbs Cortes *et al*., 2021).

GWAS has several advantages in haplotype breeding, including:

- **High resolution**: GWAS can identify genetic variants associated with specific traits at a high resolution, allowing for the identification of causal variants.
- **Whole-genome analysis**: GWAS involves scanning the entire genome, allowing for the identification of genetic variants associated with complex traits.
- **High-throughput**: GWAS can be performed using high-throughput sequencing technologies, allowing for the analysis of large numbers of samples.

Haplotype Analysis in Haplotype Breeding

Haplotype analysis is a powerful tool in haplotype breeding, allowing researchers to identify specific combinations of alleles at multiple loci associated with a particular trait or disease. Haplotype analysis can be used to identify haplotype blocks, which are regions of the genome where genetic variants are inherited together.

Haplotype analysis has several advantages in haplotype breeding, including:

- **Identification of causal variants**: Haplotype analysis can identify causal variants associated with specific traits or diseases.
- **Understanding genetic architecture**: Haplotype analysis can provide insights into the genetic architecture of complex traits, allowing for the identification of genetic variants associated with specific traits.
- **Marker-assisted selection**: Haplotype analysis can be used to identify genetic markers associated with specific traits, allowing for marker-assisted selection in breeding programs.

Software Tools for GWAS and Haplotype Analysis

Several software tools are available for GWAS and haplotype analysis, including:

- **TASSEL**: A software tool for GWAS and haplotype analysis that allows for the identification of genetic variants associated with specific traits.
- **PLINK**: A software tool for GWAS and haplotype analysis that allows for the identification of genetic variants associated with specific traits.
- **SHAPEIT**: A software tool for haplotype analysis that allows for the identification of haplotype blocks and the reconstruction of haplotypes.

Marker-Assisted Selection (MAS) vs. Haplotype-Based Selection

Marker-assisted selection (MAS) has traditionally been used in plant breeding to select for specific traits using molecular markers like SNPs. However, MAS is limited because it often targets single loci, which may not fully capture the complexity of traits influenced by multiple genes.(. Yousef and Juvik, 2001.)

Haplotype-based selection offers a solution by considering the co-inheritance of multiple linked traits. This approach enables breeders to select for combinations of alleles across the genome that collectively contribute to traits like yield, disease resistance, or stress tolerance. This is especially valuable in breeding programs for crops like wheat and maize, where polygenic traits (controlled by multiple genes) are common.

Genomic Selection (GS) with Haplotypes

Genomic selection (GS) involves predicting the genetic potential of individuals based on genome-wide markers. Incorporating haplotypes into GS models can increase the accuracy of genomic predictions, as haplotypes capture more genetic variation than individual SNPs. Studies have shown that integrating haplotypes into GS for crops like rice and maize improves the precision of selecting varieties with desirable traits, such as nitrogen use efficiency and drought tolerance. (Heffner *et al*., 2009.)

Haplotype-Based Breeding (HBB) and Haplotype-Assisted Genomic Selection

Developing stress-tolerant crop varieties with improved yield potential is a major challenge for breeders, especially in the face of global climate change. Haplotype-based breeding (HBB) and haplotype-assisted genomic selection (GS) are innovative approaches that leverage the power of haplotypes to enhance the accuracy and efficiency of breeding programs.

Implementation of Haplotypes in Crop Improvement

Haplotypes can be used in two approaches: retrospective and prospective. The retrospective approach involves identifying favorable haplotypes that have been selected for during the long-term selection process, while the prospective approach involves re-sequencing a large collection of ancestral and wild germplasm to identify novel haplotypes with a broader range of genetic variation.

Haplotype-Assisted GS

Haplotype-assisted GS uses haplotypes to improve the accuracy of GS models. GS is a method that uses genome-wide markers to predict the performance of individuals based on their genetic makeup. Haplotype-assisted GS involves fitting haplotypes with statistically significant associations to phenotypes as fixed effects in GS models, capturing local high-order allelic interactions and population structure.

Advantages of Haplotype-Assisted GS

Haplotype-assisted GS has several advantages, including:

- Improved prediction accuracy

- Increased selection gain
- Better capture of LD and genomic similarity

Applications of Haplotype Breeding in Major Crops

Haplotype Breeding in Rice (*Oryza sativa*)

Rice, one of the most important global crops, has seen significant advances through haplotype-based breeding. Haplotypes linked to key agronomic traits, such as yield, disease resistance, and stress tolerance, have been identified. For example, the Sub1A gene haplotype has been linked to flood tolerance in rice, leading to the development of rice varieties that can survive prolonged submergence during floods (Xu *et al.,* 2006).

Haplotype Breeding in Wheat (*Triticum aestivum*)

In wheat, haplotype analysis has been instrumental in identifying genetic regions associated with quality traits, disease resistance (such as rust resistance), and stress tolerance. As climate change threatens global wheat production, haplotypes linked to heat, and drought tolerance are becoming increasingly valuable. By selecting wheat varieties with favourable haplotypes, breeders can develop crops better suited to changing environments. (Reynolds *et al* .2012)

Haplotype Breeding in Maize (*Zea mays*)

Maize is another major crop where haplotype breeding has accelerated trait improvement. Haplotypes associated with nitrogen use efficiency, drought resistance, and flowering time have been identified and used in breeding programs. By focusing on haplotypes rather than individual SNPs, breeders can make more informed selections and accelerate the development of maize varieties that meet the needs of farmers in diverse environments (Cooper *et al.,* 2014).

Technological Innovations Driving Haplotype Breeding

Next-Generation Sequencing (NGS) and Long-Read Sequencing

Advancements in sequencing technologies, particularly NGS, have been crucial for resolving haplotypes in crops. NGS allows for high-throughput sequencing of entire genomes, enabling the identification of haplotype blocks. Additionally, long-read sequencing technologies, such as those developed by Pacific Biosciences and Oxford Nanopore, provide more accurate haplotype phasing by sequencing larger segments of DNA, which reduces the ambiguity in identifying haplotypes (Goodwin *et al*., 2016).

High-Density Genotyping Arrays

Genotyping arrays, such as the Illumina Infinium platform, enable the detection of SNPs and haplotypes across large crop populations. These high-density arrays offer a cost-effective solution for haplotype detection in large-scale breeding programs. For example, genotyping arrays have been used in rice and wheat to identify haplotypes associated with yield and disease resistance

Bioinformatics and Data Analytics Tools

Bioinformatics tools play a critical role in haplotype-based breeding. Software such as BEAGLE, SHAPEIT, and MACH enable haplotype phasing, while tools like TASSEL and GAPIT allow for the integration of haplotype data into association studies. Furthermore, the application of machine learning and artificial intelligence (AI) in genomic data analysis is helping breeders predict complex traits with greater accuracy, making haplotype-based breeding more efficient. (Browning *et al.,* 2007).

Challenges and Future Prospects in Haplotype Breeding

Challenges in Haplotype Identification and Phasing

One of the main challenges in haplotype breeding is the accurate identification and phasing of haplotypes, especially in complex genomes with high levels of recombination. Recombination hotspots, rare alleles, and the presence of structural variations can complicate haplotype identification. Current phasing algorithms may struggle to resolve haplotypes in regions of the genome with low LD or high genetic diversity, limiting their usefulness in breeding programs.

Ethical and Regulatory Concerns

As with other genomic technologies, the use of haplotypes in breeding raises ethical and regulatory concerns. The potential for unintended consequences, such as the introduction of unfavourable genetic traits, must be considered. Additionally, regulations surrounding biotechnology, especially in countries with strict laws on genetically modified organisms (GMOs), may impact the adoption of haplotype-based breeding (Kuzma *et al.,* 2011).

Conclusion:

Haplotype breeding is poised to revolutionize plant breeding by allowing for more precise, efficient, and effective selection of desirable traits. As the agricultural sector grapples with challenges posed by climate change, population growth, and the need for sustainable practices, haplotype breeding presents an avenue for developing crop varieties that are resilient, high-yielding, and sustainable.

Future advances in sequencing technologies, bioinformatics, and machine learning will likely further enhance the power of haplotype breeding, paving the way for precision agriculture on a global scale.

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RECENT DEVELOPMENTS IN BREEDING FOR BIOTIC RESISTANCE

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Abstract:

The rapid global population growth and increasing per capita income, particularly in developing nations like India, have heightened pressure on agriculture to expand growing areas and increase yields. Climate change further complicates this by introducing new challenges. Biotic stresses such as insect infestations, weed proliferation, and diseases caused by various pathogens are recurring issues that affect yield stability. Traditional breeding methods focusing on major genes have led to boom-and-bust cycles, necessitating constant cultivar replacements or chemical interventions. Sustainable control of diseases and pests requires a shift towards durable resistance based on minor, additive genes, combined with other desirable traits. Understanding pathogen epidemiology and utilizing genetic resistance have significantly mitigated disease epidemics in recent decades. The long-term goal remains improving crop resilience to biotic stresses through breeding programs, considering mechanisms like nonhost resistance and gene-for-gene interactions. However, breeding strategies must also address specificity of defence mechanisms, durability of resistance, and explore alternative approaches like mutation breeding and molecular interventions. These efforts aim to ensure stable food production while minimizing environmental impact.

Keywords: Biotic, Stress, Plant Breeding, Molecular Breeding, Genome Editing

Introduction:

In an era marked by burgeoning global population growth and the consequential strain on agricultural resources, the imperative to enhance food production while ensuring environmental sustainability has never been more pressing. As the world braces for a projected increase of over 1,000 million people within the next four decades, the agricultural industry faces a paramount challenge: to optimize crop yields in a manner that is both environmentally sustainable and economically viable. As per FAO, 16.6 per cent of the Indian population is undernourished (FAOSTAT, 2023).

At the forefront of this challenge lies the critical issue of biotic stresses, particularly the damage inflicted by insect pests, which is estimated to range from 35% to 100% globally. While synthetic insecticides have historically played a significant role in boosting food production, their adverse environmental and health impacts underscore the urgent need for alternative solutions.

The total global potential loss due to pests varies significantly among crops, ranging from approximately 50% for wheat to over 80% for cotton. Estimated losses for specific crops are 26–29% for soybean, wheat, and cotton, and 31%, 37%, and 40% for maize, rice, and potatoes, respectively. Overall, weeds cause the highest potential loss at 34%, while animal pests and pathogens account for losses of 18% and 16%, respectively. For example, in maize production, biotic stresses result in global losses of about 10.9% from diseases, 14.5% from insects, and 13.1% from weeds. Additionally, a 10% postharvest loss brings the total estimated loss for maize to 48.5% (García-Lara and Serna, 2016).

Recent decades have witnessed remarkable strides in the realm of biotechnology, notably the advent of transgenic crops engineered for enhanced resistance to biotic stresses. A milestone in this trajectory was the development of genetically modified tobacco plants over two decades ago, which expressed entomotoxic proteins from the bacterium *Bacillus thuringiensis* (*Bt*), conferring resistance against major insect pests.

Subsequent releases of transgenic crops, such as Bollgard cotton in India, have further underscored the potential of biotechnological interventions in bolstering crop resilience. However, the emergence of secondary pests underscores the evolving nature of this challenge, necessitating continual innovation in breeding strategies.

Beyond genetic modification, a multifaceted approach encompasses the manipulation of endogenous defence mechanisms within plants, as well as the integration of novel insecticidal proteins derived from diverse sources. Moreover, cutting-edge techniques like RNA interference offer promising avenues for precise pest control.

The urgency of addressing biotic stresses stems not only from their direct impact on crop yields but also from their role in exacerbating the broader food security crisis. With over 42% of potential global crop yield lost to biotic stresses, the imperative to mitigate their impact cannot be overstated.

Against the backdrop of escalating environmental challenges, from climate change to regulatory constraints on chemical pesticide use, breeding for tolerance and resistance to biotic stresses emerges as a linchpin in securing future food production. By harnessing genetic resistance mechanisms, breeders aim to confer lasting protection against pests while minimizing reliance on chemical interventions.

This chapter sets the stage for a comprehensive exploration of recent developments in breeding strategies aimed at combating biotic stresses. From elucidating the genetic and molecular underpinnings of plant defence mechanisms to evaluating novel breeding approaches, the ensuing discourse aims to illuminate pathways toward sustainable agricultural resilience in the face of mounting global challenges. Due to space constraints and editorial guidelines, this overview focuses on selected biotic stresses. Cereals are excluded from this discussion as extensive resources are readily available. For more detailed information, readers are encouraged to consult the following materials- Fritsche-Neto and Borém (2012) and Shanker and Shanker (2016).

Pulses

Fungal Disease Resistance

In the realm of agricultural research, scientists embarked on a journey to fortify pulse crops against various fungal diseases, striving to enhance their resilience and productivity. Among these endeavours, chickpea emerged as a focal point, with efforts concentrated on combating fusarium wilt (FW) and Ascochyta blight (AB).

Through Marker-Assisted Backcrossing (MABC), two promising introgression lines, 'Super annigeri 1' and 'JG 74315-14', were crafted, integrating genomic segments from WR 315 into the genetic makeup of Annigeri 1 and JG 74, respectively. This infusion bestowed upon them resistance to the insidious FW Mannur *et al.*, (2019). Meanwhile, Deokar *et al.*, (2019) navigated the genetic landscape of chickpea, charting a high-density genetic map to explore the terrain of Ascochyta blight resistance. Their expedition led them to unearth eight QTLs nestled within chromosomes 2, 3, 4, 5, and 6, offering potential avenues for bolstering AB resilience.

In a groundbreaking stride, Jha *et al.*, (2021) pioneered the first association mapping (AM) endeavour targeting FW resistance in chickpea. Leveraging Simple Sequence Repeats (SSR) markers, they pinpointed three significant marker-trait associations, namely CESSR433, NCPGR21, and ICCM0284, heralding a new era in the quest for FW-resistant cultivars.

Elsewhere in the legume kingdom, mung bean breeding witnessed a surge in innovation, as Witsarut *et al.*, (2019) melded enhanced breeding lines resilient to Cercospora leaf spot (CLS) and powdery mildew (PM). Their meticulous crosses yielded

BC_2F_1 plants boasting a remarkable 85 to 100% recovery rate of the desired traits, promising a bright future for mung bean cultivation. Pea, too, joined the fray against fungal foes, as Wu *et al.*, (2021) homed in on a strategic chromosome 4 region harbouring partial resistance against Aphanomyces root rot isolate Ae-MDCR1. Their discovery unveiled a potential stronghold against this formidable adversary, offering hope to pea farmers battling root rot.

Lentil, with its own array of challenges, found solace in hybridization endeavours orchestrated by Gela *et al.*, (2021a). By melding the wild relative *Lens ervoides* with the LR-59-81 cultivar, they birthed an advanced BC population teeming with resistance to anthracnose (*Colletotrichum lentis*) and Stemphylium blight (*Stemphylium botryosum*), bolstering lentil's defences against these menacing pathogens. Further fortifications against lentil anthracnose were unveiled by Gela *et al.*, (2021b), who identified two robust QTLs nestled within chromosomes 3 and 7. Their discovery, accompanied by phenotypic variations ranging from 20.1% to 31.2% and 8.3% to 18.4%, illuminated new avenues for combating this persistent menace. Delving deeper into lentil's molecular arsenal, Khorramdelazad *et al.*, (2018) and Garcia *et al.*, (2019) unravelled the intricate dance of defence mechanisms triggered by Ascochyta infection. Their insights into the up-regulation of structural defence-related genes, jasmonic acid pathway, and lignin biosynthesis pathway in resistant genotypes shed light on lentil's innate resilience against fungal incursions.

In the realm of common bean breeding, the efficacy of pyramided lines against anthracnose disease was scrutinized. Genes *Co-4*2 and *Co-5* showcased broad-spectrum resistance against various races, while *Co-43* and *Co-9* conferred resistance to specific races. Interestingly, pyramids containing *Co-4*2 + *Co-5* + *Co-9* exhibited the lowest disease severity, highlighting the potential synergistic effects of combining multiple resistance genes (Kiryowa *et al.*, 2021).

Across the legume spectrum, from chickpea to lentil, from FW to AB, the battle against fungal adversaries rages on. Yet, armed with genetic insights and innovative breeding strategies, researchers stand poised to turn the tide in favour of resilient, bountiful harvests.

Viral Disease Resistance

Dasgupta *et al.*, (2021) embarked on a quest into the molecular intricacies of mungbean resistance, unravelling the genetic symphony orchestrated by WRKY, NAC, and

MYB transcription factors in fortifying against *mungbean yellow mosaic virus* (MYMV). Their RNA-seq-based transcriptome analysis of PMR-1 (resistant) and Pusa Vishal (susceptible) unveiled a tale of resilience, as PMR-1 defiantly upregulated defence genes like peroxidase and lipoxygenase while downregulating viral susceptibility factors.

Meanwhile, on the frontier of genetic engineering, Talakayala *et al*., (2022) wielded the CRISPR/Cas9 system as a molecular scalpel, carving precise edits into the MYMV genome of mung bean. By targeting key viral genes- replicase enzyme (AC1) and coat protein (AV1) encoding genes, they ushered in a new era of viral management, paving the way for multiplex strategies capable of taming multiple viral adversaries simultaneously.

Urd bean varieties VBN 9 and VBN 10 emerged as beacons of resistance, boasting immunity against a myriad of viral afflictions including MYMV, *urd bean leaf crinkle virus*, *leaf curl virus*, and powdery mildew. Furthermore, VBN 3, a new cowpea variety, has been introduced, showcasing resilience against *bean common mosaic virus*, rust, and anthracnose. Similarly, VBN 4, a mung bean variety, has been unveiled, displaying a moderate level of resistance to MYMV (NPRC Vamban, 2019).

Patwa *et al*., (2021) explored a tapestry of 422 differentially expressed miRNAs orchestrating a delicate balance of resistance against MYMIV in *Phaseolus vulgaris*. These tiny regulators emerged as guardians of genetic integrity, steering the fate of crucial transcription factors in the battle against viral intrusion.

Insect-Pest Resistance

In the verdant fields of agricultural research, a symphony of discovery unfolds, illuminating pathways to enhanced crop resilience and productivity. Lentil, a humble legume, stands fortified against the ravages of pests and diseases, thanks to the resilience of genotypes like ILL 9924, RL 83, ILL 10856, ILL 6458, and RL 67, which boast higher grain yields and resilience to *Aphis craccivora*. Notably, lentil varieties with green or yellowish-green foliage and slightly pubescent leaves serve as a natural barrier against aphid infestations, offering a shield to the precious crop (Neupane *et al*., 2020). The International Institute of Tropical Agriculture (IITA) unveils a trio of lentil accessions - TVu6464, TVu1583, and TVu15445 - heralded as beacons of resistance to *Aphis craccivora*. These resilient varieties boast low sucrose content and elevated levels of kaempferol and quercetin, underscoring the role of secondary metabolites in plant defence mechanisms (Togola *et al*., 2020).

In the realm of comparative proteomic analysis, Ngugi-Dawit *et al*., (2021) shed light on the intricate dance between plants and pests. Through tandem mass tag proteomics, insights emerge into the molecular arsenal deployed by *Cajanus scarabaeoides* against the notorious *Helicoverpa armigera*. Increased levels of secondary metabolite precursors, antioxidants, and phenylpropanoid pathway components emerge as key players in the plant's defence strategy.

Common bean stands stalwart against the onslaught of spider mites, as revealed by transcriptomic studies unravelling the genetic underpinnings of defence mechanisms (Hoseinzadeh *et al*., 2020).

In the world of peas, the battle against aphids (*Acyrthosiphon pisum*) rages on, with genetic studies illuminating the path to resistance. Barilli *et al*., (2020) uncover QTLs associated with tolerance to aphid damage, derived from the resilient *P. fulvum* accessions. The discovery of the major-effect quantitative trait locus, *ApRVII*, on Linkage Group VII, heralds a breakthrough in pea aphid resistance, further bolstered by subsequent genome-wide association studies (Olliver *et al*., 2022; Rahman *et al*., 2023).

Through QTL analysis and marker mapping, the genetic locus conferring resistance to bruchids is pinpointed to linkage groups 7 and 5. Fine mapping efforts culminate in the identification of candidate genes *VrPGIP1* and *VrPGIP*2, unveiling the molecular guardians of bruchid resistance in peas (Schafleitner *et al*., 2016; Chotechung *et al*., 2016; Kaewwongwal *et al*., 2017).

Bacterial Disease Resistance

In the verdant fields of pea cultivation, a quiet battle rages beneath the soil's surface. The innocuous-looking legume faces a formidable adversary in the form of *Pseudomonas syringae* pv. *pisi*, a seedborne bacterium capable of wreaking havoc on pea crops. Rodda *et al*., (2015) found that peas can be impacted by as many as eight strains of this seedborne bacteria. Hunter *et al*., (2001) have pinpointed and charted resistances specific to each strain, while Sudheesh *et al*., (2015) have reported additional genetic factors influencing resistance. Notably, *P. abyssinicum* variants exhibit resistance to all strains, including strain 6, either fully or partially. Elvira-Recuenco *et al*., (2001) have detailed that this notable resistance in *P. abyssinicum* is controlled by a major recessive gene and several accompanying modifiers.

Meanwhile, in the realm of common beans, a similar narrative unfolds. The cultivar 'Redwolaita' stands as a beacon of hope against two notorious adversaries: angular leaf spot (ALS) and common bacterial blight (CBB). Through the pioneering work of Rezene *et al*., (2019), this cultivar has been fortified with resilience, incorporating three distinct genes – including *Phg*-2 for ALS resistance and two others for CBB resistance. Such rapid strides in breeding owe their success to the rich Recombinant Gene Pool (RGP) present within common bean populations, as elucidated by Rezene *et al*., (2019).

Nematode Resistance

Batieno *et al*., (2016) integrated QTLs for drought tolerance, striga resistance, and resistance to root-knot nematodes (RKN) from a Burkina Faso cowpea cultivar into intercropping varieties. From the BC_3F_1 generation, six families were chosen after screening for desired traits to produce seeds for further breeding.

In the cowpea gene pool, resistance against RKN has been traced back to a single dominant gene, *Rk*, which effectively combats avirulent RKN strains. Moreover, the discovery of new resistance genes, *Rk*2 (with dominant effects) and *rk3* (exhibiting recessive and additive effects), within the cowpea gene pool has addressed the challenge posed by virulent RKN strains. This breakthrough has spurred initiatives to enhance the genetic diversity of resistance in elite cowpea cultivars grown worldwide (Ndeve *et al*., 2019).

In India, efforts to combat *Meloidogyne incognita* (RKN) have led to the identification of resistance sources within *Vigna unguiculata* germplasms, specifically EC724523, EC723686, and EC725122. These discoveries signify a concerted global endeavour to fortify cowpea cultivars against the diverse threats posed by nematode populations, ensuring sustainable crop yields in various regions.

Weed Management

Broomrapes, belonging to the Orobancheae family, are parasitic plants that infest the roots of various crops, posing a significant threat to agricultural productivity. Among the most notorious and widespread species affecting peas is *Orobanche crenata* (Rubiales *et al*., 2012).

Galili *et al*., (2021) embarked on a project to breed resistant chickpea cultivars against broomrape infestation. They utilized an ethyl methane sulfonate (EMS) mutant population derived from the F01 variety (Kabuli type). Through their efforts, they identified a mutant line (CCD7M14) that displayed remarkable resistance to both *Phelipanche aegyptiaca* and *O. crenata*. The resistance mechanism was attributed to the mutant's inability to produce strigolactones (SLs), which are compounds that stimulate broomrape seed germination. Sequence analysis identified a point mutation in the Carotenoid Cleavage Dioxygenase 7 (CCD7) gene, responsible for encoding key enzymes in SL biosynthesis. This mutation resulted in a premature stop codon in CCD7, effectively disrupting SL production.

In a more recent study, Delvento *et al*., (2023) utilized a different Recombinant Inbred Line (RIL) population to identify three QTLs associated with the field response to *O. crenata* infection. This led to the development of three KASP markers linked to these QTLs, offering valuable tools for breeding programs aimed at enhancing broomrape resistance in chickpeas.

Oilseeds

Fungal Disease Resistance

Researchers have identified potential resistance genes, including LLSR1 and LLSR2 for late leaf spot (LLS), and LR1 for leaf rust (LR), through genetic mapping and pooled sequencing methods (Pandey *et al*., 2024). These genes are crucial for combating fungal diseases, which can cause substantial yield loss and reduce fodder quality in groundnut crops. Moreover, the demand for high oleic acid content in groundnuts is increasing due to its benefits such as improved shelf life and health advantages.

Innovative genomic technologies have been instrumental in enhancing crop sustainability, particularly in oilseed brassicas. Techniques such as enriching the nucleotide-binding leucine-rich repeat receptor (NLR) repertoire, over-expressing resistance (R) genes, and suppressing avirulent genes through RNA interference and CRISPR-Cas systems have shown promise in conferring resistance against pathogens (Anupriya *et al*., 2020; Arora *et al*., 2019). Given the absence of resistance against Alternaria, exploring resistance genes in non-host plants and transferring them to oilseed Brassica species could be a valuable strategy for resistance breeding. For instance, the transgenic line BjV5 of *B. juncea*, which carries the *MPK3* gene, has demonstrated enhanced resistance against Alternaria blight, highlighting the potential role of *MPK3* in plant defence mechanisms (Tasleem *et al*., 2017).

Quantitative resistance (QR), characterized by minor-effect genes and resulting in a range of phenotypic values, offers a promising avenue for sustainable disease management. Unlike classic Mendelian segregation, QR involves multiple genes, reducing the selective pressure on pathogens and potentially prolonging resistance effectiveness. Incorporating QR alongside known resistance genes, such as *Rlm1*, *Rlm4*, and *Rlm7* in the Brassica-blackleg pathosystem, has shown to mitigate blackleg symptoms in field conditions. Cultivars with both QR and R-genes exhibit more stable resistance across diverse environments and over time (Huang *et al*., 2018).

Fu *et al*., (2020) employed a genome-wide association study (GWAS) in canola and identified 32 and 13 SNPs associated with blackleg resistance in Canadian and Chinese accessions, respectively. These SNPs were located on various chromosomes, including A03, A05, A08, A09, C01, C04, C05, and C07. Notably, potential SNPs on chromosome A08 were linked with resistance to 12 *L. maculans* isolates, with 25 resistance gene analogues (RGAs) identified within this genomic region.

Chittem *et al*., (2020) utilized transcriptome analysis to uncover key pathways and genes involved in the interaction between *S. sclerotiorum* and *B. napus*. Drawing from knowledge of WRKY transcription factors in *Arabidopsis thaliana*, they elucidated the roles of BnWRKY11 and BnWRKY70 in *B. napus*. Targeted genome editing has emerged as a powerful tool for both basic research and crop enhancement. The CRISPR/Cas9 system, known for its simplicity and efficacy, is widely employed for targeted genomic editing in plants. By employing Cas9 enzymes and sgRNAs to induce nucleotide-specific mutations in these genes, Sun *et al*., (2018) observed increased resistance to *S. sclerotiorum* in mutant lines of BnWRKY70, while lines overexpressing BnWRKY70 exhibited susceptibility to Sclerotinia (Sun *et al*., 2018).

Bacterial Disease Resistance

Hussain *et al*., (2021) employed protein sequences from 49 cloned disease resistance (R) genes, known to confer resistance against fungal and bacterial diseases in Brassicaceae species, to predict cloned disease resistance gene homologs (CDRHs). This approach aimed to enhance the understanding of disease resistance mechanisms in economically significant Brassicaceae crops like Brassica, Camelina, and Raphanus species.

Qi *et al*., (2022) emphasized the importance of fine mapping QTLs associated with bacterial wilt resistance to facilitate marker-assisted breeding for disease-resistant varieties. Utilizing a recombinant inbred population consisting of 521 lines, they constructed a high-density genetic linkage map and identified QTLs for bacterial wilt resistance through restriction-site-associated DNA sequencing in groundnut. This effort contributes to the development of strategies to combat bacterial wilt, a significant threat to groundnut production.

Viral Disease Resistance

Various methods have been employed to enhance virus resistance in soybeans, including conventional breeding and transgenic techniques, with a particular focus on combating the *Soybean Mosaic Virus* (SMV), the primary viral threat to soybean crops. One transgenic approach, the Pathogen Derived Resistant (PDR) method, targets the transformation of the Coat protein (CP) gene into soybeans to tackle SMV (Furutani *et al*., 2006). Another strategy involves cloning the HC-Pro gene of SMV in both sense and antisense orientations within a vector, generating small interfering RNA (siRNA) upon transformation into soybean plants, aiding in the suppression of SMV. More recently, efforts have shifted towards targeting the *SMV P3* gene, which holds promise in conferring tolerance not only to SMV but also to other potyviruses (Yang *et al*., 2018).

In addition to SMV, Yellow Mosaic Disease (YMD) poses a significant challenge to soybean production in South Asia, mainly caused by *Mungbean yellow mosaic virus* (MYMV) and *Mungbean yellow mosaic India virus* (MYMIV). Transgenic soybeans have been developed to resist MYMIV, with one approach targeting the *Rep* (Replication associated) gene using an antisense RNA strategy. Another research group in India aimed at the CP (coat protein) gene of MYMIV utilizing the short hairpin RNA (shpRNA) strategy (Kumari *et al*., 2018).

Furthermore, mutation breeding and crosses have been employed to develop high-yielding recombinant lines resistant to diseases and pests in soybeans. For instance, varieties such as PK-564 and SL-742 resistant to *yellow mosaic virus*, DSb12 resistant to rust, and multiple disease and pest-resistant BARC mutant selections and high-yielding varieties have been developed through single, double, and three-way crosses. An experiment conducted by Badigannavar and Bhad (2020) focused on studying the genetic variation at three SMV resistance loci in a set of SMV-resistant and susceptible Indian soybean genotypes using 13 mapped SSR markers. This research aids in the selection of suitable parents for breeding programs aimed at SMV resistance.

In two independent studies, the YMV resistance gene in soybeans has been identified on two distinct chromosomes, namely chromosome 17 and chromosome 18. Utilizing a set of 22 soybean genotypes, consisting of 8 YMV-resistant and 14 YMV-susceptible genotypes, Badigannavar and Bhad (2020) screened a total of 52 SSR markers (26 markers each from chromosome 17 and chromosome 18). The SSR marker GMHSP179, located on chromosome 17, effectively distinguished between YMV-susceptible and -

resistant soybean genotypes. This identified marker holds promise for facilitating the breeding of YMV-resistant soybean cultivars through marker-assisted selection.

In another study, 27 predominantly homozygous *B. napus* accessions, comprising doubled-haploid (DH) or inbred lines, were evaluated for resistance/susceptibility to TuYV (*Turnip Yellow Mosaic Virus*). Researchers identified a major QTL for TuYV resistance on chromosome A04 (qTUYVA4) in the Yudal cultivar. This QTL was significantly associated with TuYV resistance, explaining between 18% and 36% of the phenotypic variation. The dominance of TuYV resistance was confirmed through phenotyping two BC_1 populations. Notably, the only genetic resource for TuYV resistance in Brassica identified thus far originates from the re-synthesized *B. napus* line ‘R54’. This study represents the first instance of QTL mapping for TuYV resistance in natural *B. napus* (Hackenberg *et al*., 2020).

Nematode Resistance

The *rhg1-b* allele of soybean is extensively utilized for resistance against soybean cyst nematode (SCN), which is the most economically damaging pathogen affecting soybeans in the United States. Through gene silencing experiments, it was revealed that genes located within a 31-kilobase segment at rhg1-b, including an amino acid transporter, an a-SNAP protein, and a WI12 (wound-inducible domain) protein, collectively contribute to the resistance (Cook *et al*., 2012; Rahman *et al*., 2023).

In soybeans, the enzyme salicylic acid methyl transferase (GmSAMT1) catalyses the conversion of salicylic acid to methyl salicylate. Overexpression of GmSAMT1 in transgenic soybean hairy roots has been shown to confer resistance against SCN. This suggests that GmSAMT1 overexpression could potentially provide broad resistance against multiple SCN races, making it applicable for commercial production (Lin *et al*., 2016; Rahman *et al*., 2023).

St-Amour, V (2020) conducted a QTL analysis on the SCN-resistant soybean accession PI 494182 (MG 0). Using a recombinant inbred lines (RILs) population (‘Costaud’ × PI 494182) segregating for SCN resistance, challenged with SCN (*H. glycines* [HG] type 0), and genotyped via genotyping-by-sequencing (GBS), a genetic map was constructed, leading to the identification of six resistance QTL. To combat widespread nematode infestations, Meinhardt *et al*., (2021) proposed gene pyramiding using different soybean resistant genes against nematodes derived from PI 88788, PI 437654, PI 468916, and PI 567516C.

Insect-Pest Resistance

In a study by Hill *et al.*, (2004), examination of 1530 soybean cultivars between 2001 and 2002 led to the identification of *Rag1*, a single dominant gene conferring antibiosis, as the initial member of several *Rag* genes (resistance to *Aphis glycines*). Soybean genotypes harbouring *Rag* resistant genes have been identified as a means to combat aphid infestations, with *Rag1*–*Rag5* providing resistance against aphids. Furthermore, recessive resistance mechanisms have been uncovered in soybean crops as a defence against aphid attacks (Bales *et al.*, 2013; Rahman *et al.*, 2023). The utilization of *Bt* soybean exemplifies the successful application of transgenic technology for insect resistance (Rahman *et al.*, 2023; Transformation, 1996).

Historically, interspecific hybridization has been widely employed in the Brassica genus to introduce beneficial traits into crops (reviewed by Katche *et al.*, 2019; Quezada-Martinez *et al.*, 2021). Hence, the transfer of valuable insect resistances from related species into *B. napus* through resynthesis and interspecific hybridization is a strategy worthy of consideration to address this issue (Quezada-Martinez *et al.*, 2021). Notably, resistance to mustard aphid (*Lipaphis erysimi*) has recently been successfully introgressed from the wild species *B. fruticulosa* (F genome) into *B. juncea* (2n = AABB) (Agrawal *et al.*, 2021).

Around the same period when many other *Bt* crops were developed, the first *Bt* oilseed rape lines were also created, demonstrating efficacy in controlling the diamondback moth and the cabbage looper (*Trichoplusia ni*). However, despite their development, these *Bt* oilseed rape lines were not commercialized (Hervé, 2018).

Recent advancements in agrobiotechnology have revolved around harnessing RNA interference (RNAi), a cellular mechanism that effectively suppresses gene expression (knockdown) in target organisms. This mechanism holds significant promise for enhancing crop protection, whether by administering double-stranded (ds) RNA directly to crop plants through spraying, known as spray-induced gene silencing (SIGS), or by integrating constructs into transgenic plants encoding dsRNA (Chung *et al.*, 2021; Liu *et al.*, 2020).

Glucosinolates (GSLs) serve as a defence mechanism in plants against pathogens and herbivorous insects. There is a strong desire to bolster pest and disease resistance by elevating leaf GSL content while maintaining low levels in the seeds of *Brassica napus*, a globally significant oil crop. Through DNA polymorphism analysis and gene expression studies, the candidate gene *BnaA03g40190D* (*BnaA3.MYB28*) has been validated. This gene is associated with high leaf/low seed GSL content and accounts for a substantial portion (30.62%) of total leaf GSL variation in low seed GSL panels. Importantly, it has been observed that this gene remains polymorphic and is not fixed during the breeding of double-low rapeseed varieties (Liu *et al*., 2020).

Weed Management

Beversdorf *et al*., (1987) developed the first triazine-resistant canola variety by transferring the cytoplasm of triazine-resistant mustard interspecifically to oilseed rape. This transfer was achieved through a technique commonly known as back-crossing, accompanied by selection for chromosome number. The resulting variety, 'OAC Triton,' marked the emergence of triazine-resistant canola varieties and is showing promising acceptance in commercial markets.

Various methods have been employed to engineer crop varieties with herbicide resistance (HR). These methods include altering the target sites of herbicides to prevent binding, enhancing enzymes that deactivate or break down herbicides within plants, and modifying plant physiology to limit the herbicide's access to molecular target sites. Among these methods, metabolic inactivation or degradation is primarily utilized as a natural mechanism of crop resistance to selective herbicides (Kole *et al*., 2010).

The discovery of a soybean mutation conferring resistance to a broad spectrum of sulfonylurea (SU) herbicides would significantly broaden weed control options for soybean farmers. Through seed mutagenesis using N-nitroso-N-methylurea and ethyl methanesulfonate, followed by selection for resistance to chlorsulfuron, Hussain *et al*., (2021) and Sebastia *et al*., (1989) successfully produced a soybean mutant exhibiting high resistance to both postemergence and preemergence applications of various SU herbicides. Pyramiding multiple genes has enabled the development of rapeseed introgression lines with resistance to clubroot and herbicides, high oleic acid content, and early maturity (Wang *et al*., 2023).

Vegetables

Mechanisms of multiple diseases resistance (MDR) operate at three distinct levels within the genetic framework: the genome level, the loci level, and the gene level.

At the genome level, MDR plays a crucial role in the evolutionary dynamics and agricultural significance of plant pathogens. For instance, European potato crops face onslaughts from at least a dozen major diseases and pests (Jellis, 1992), while wheat plants frequently contend with multiple pathogens simultaneously (Gurung *et al.,* 2012).

Similarly, legumes are susceptible to numerous diseases across various taxa, often experiencing multiple infections concurrently (Nene, 1988). The resistance to multiple diseases holds immense value for both plant pathologists and breeders.

Germplasm screening, a standard practice in crop improvement programs, involves evaluating collections for resistance to multiple diseases. The prevalence of lines exhibiting MDR varies considerably across studies and even within the same host-pathogen systems. Nonetheless, significant correlations in resistance across germplasm collections are often observed, even if individual lines with high MDR are scarce.

Multi-environmental trials offer an alternative approach, assessing the stability of resistance to multiple pathogen species and genera under varied field conditions. For instance, a study on faba bean tested 43 accessions for reactions to two diseases (Villegas-Fern´andez *et al.,* 2011).

Screening of wild relatives of crops is another avenue pursued to identify broader-spectrum resistance genes beyond those found in cultivated varieties. This method aims to capture highly effective forms of MDR from wild or cultivated crop relatives.

Evaluation of structured populations involves assessing patterns of genetic relatedness among individuals of the same species. To discern MDR, typical methods involve making crosses and studying segregation patterns in the offspring. For instance, resistance conferred by a dominant gene with substantial effect (e.g., an R gene) segregates in a 3:1 ratio in an F_2 population, whereas resistance mediated by multiple genes with smaller effects results in a continuous phenotypic distribution.

MDR at the loci level involves understanding the correlation between QTLs and variations in quantitative traits within a population. The process, known as QTL mapping, entails constructing linkage maps and conducting analysis to identify QTLs associated with different resistances on plant genomes. By mapping QTLs for various resistances in different populations of a given plant species, researchers can evaluate the spatial relationships among them within the chromosomal context. Meta-analysis, which rigorously integrates QTLs from multiple mapping studies, further elucidates the overall architecture of MDR. The colocalization of QTLs associated with different diseases offers suggestive evidence for MDR loci.

At the gene level, MDR involves recognizing conserved signals crucial for plant defence. Pathogens can disrupt these recognition pathways, undermining basal resistance provided by pattern-triggered immunity (PTI). Genetic variation in plants may confer MDR by altering the perception of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) or by affecting the inhibition of PRRs by pathogen effectors. Single-gene MDR can also occur when plant resistance (R) genes recognize effectors from multiple pathogens. For instance, the tomato *Pto* gene detects distinct effector molecules from different pathogens, conferring resistance against both *Pseudomonas* and *Ralstonia*.

Hormone signalling pathways are crucial for initiating defence responses once threats are recognized. Signalling molecules such as salicylate, jasmonate, ethylene, and abscisic acid, as well as crosstalk between these pathways, play roles in responding to biotic and abiotic stresses. Loci affecting hormone pathways are implicated in known resistance QTLs. For example, natural variation in the potato *aosi*2 gene, affecting jasmonic acid production, is suspected to underlie resistance QTLs against *Phytophthora infestans* and *Erwinia carotovora*.

Cell death mechanisms, particularly the hypersensitive response (HR), are important defences against biotrophic pathogens but can facilitate infection by necrotrophic pathogens. Genes involved in cell death contribute to MDR; for instance, the barley *mlo* gene provides resistance to several biotrophs (Buschges *et al.,* 1997). Lesion mimic mutants, which spontaneously develop lesions resembling an HR or pathogen-induced lesions, are useful for studying cell death and its impact on MDR (Neuffer and Calvert, 1975). Variation in cell death can also affect resistance; deficiencies in HR can confer resistance to necrotrophs but susceptibility to biotrophs. *Arabidopsis* mutants deficient in hypersensitive response, although more susceptible to *P. syringae*, were much more resistant to the necrotrophic fungi *Botrytis cinerea* and *S. sclerotiorum.*

Antimicrobial peptides (AMPs) are another defence mechanism produced by plants. These peptides have broad antimicrobial activity and are essential components of plant defence. These compounds, found in high concentration in plant seeds and cell walls, have been shown to inhibit a diverse array of fungi and oomycetes, as well as a more limited number of bacteria *in vitro.* Although AMPs have been used as transgenic sources of resistance, their role in underlying resistance QTLs is less well-documented.

Understanding the genetics of disease resistance is fundamental for selecting appropriate breeding methods to transfer resistance effectively. Breeding methods for MDR can be classified into conventional and non-conventional approaches.

Conventional methods include the pedigree method, backcross method, and recurrent selection. Non-conventional methods encompass MABC for gene pyramiding and various genetic engineering techniques such as hybrid stacking, re-transformation, co-transformation, and multi-gene cassette transformation.

In the context of climate change, achieving durable resistance poses a significant challenge. In tomato, major pests and diseases include Tomato Leaf Curl Disease (ToLCD), late blight, and Root-Knot Nematodes (RKN). Kumar *et al*., (2019) utilized integrated phenotypic and molecular screening methods to identify elite gene-pyramided tomato breeding lines. Among 100 evaluated lines, 51 exhibited resistance to ToLCD, 49 to late blight, and 17 to RKN. Notably, 13 lines demonstrated resistance against all three diseases in various combinations, holding promise for resistance breeding programs and gene pyramiding efforts.

Hanson *et al*., (2016) developed a three-parent cross, coded CLN3241, aimed at creating tropically adapted, multiple disease-resistant tomato lines. This cross involved parental lines with homozygosity for various resistance genes against bacterial wilt, tomato yellow leaf curl disease, *tomato mosaic virus*, fusarium wilt, and gray leaf spot pathogens. The development of CLN3241 involved an integrated approach, combining conventional breeding techniques with molecular marker-assisted selection and gene pyramiding strategies.

Bhardwaj *et al*., (2020) developed Kufri Karan (SM/00-42) through selection from a cross between Cruza 27 and HB/83-39. Cruza 27, obtained from CIP, Peru, exhibits moderate resistance to foliar and tuber blight, wart immunity, and resistance to PVX, PVY, and hopper burn. HB/83-39, an indigenous breeding line, inherits good tuber traits and resistance to late blight from its parental lines.

Sulu *et al*., (2022) conducted screening and validation of three molecular markers for disease resistance in eggplant. They used 53 genotypes to analyse resistance against *Ralstonia solanacearum*, *F. oxysporum* f.sp. *melongenae*, and *Verticillium dahliae*. Three markers were utilized: an SNP marker for the *Ve* gene, a SCAR marker for the *Fomg gene*, and a CAPS marker for the *ERs1* gene. Highly pathogenic isolates of *F. melongenae* and *V. dahliae* were employed for classical testing.

Ozkayank *et al*., (2014) employed molecular markers to pyramid multiple resistance genes against *Potato Virus Y* (PVY), *Tomato Spotted Wilt Virus* (TSWV), and *Pepper Mild Mottle Virus* (PMMoV) in pepper. They developed a new pepper line resistant to PVY, TSWV, and PMMoV by integrating three resistant genes (*Pvr 4*, *Tsw*, and *L4*) into the sweet Charleston pepper line 'Y-CAR' using marker-assisted gene pyramiding.

In a study by Khan *et al.*, (2014), *Agrobacterium*-mediated re-transformation was conducted in stem cuttings of in vitro grown marker-free transgenic potato variety May Queen. This involved introducing the chitinase (*ChiC*) gene from *Streptomyces griseus* along with the *wasabi defensin* (*WD*) gene from *Wasabia japonica*. The aim was to pyramid two disease-resistant genes against Fusarium wilt and Early blight caused by *F. oxysporum* and *A. solani*, respectively. The co-expression of *chitinase* and *wasabi defensin* in transgenic potato plants conferred increased resistance against fungal pathogens, enhancing broad-spectrum antifungal activity. However, further investigation is needed to assess resistance against other fungal and bacterial pathogens.

Conclusions and Perspectives:

In the ever-evolving landscape of agricultural science, each discovery and breakthrough weaves a new thread into the rich tapestry of genetic resilience, ensuring that the bounty of the harvest endures for generations to come. Transgenic technology emerges as a beacon of hope, epitomized by *Bt* crops. These marvels not only transformed global crop production but also brought about a profound change in the lives of farmers, especially in India. As the battle against pest's rages on, another front opens in the realm of disease management. To combat these challenges, scientists delve into the intricate world of plant defence mechanisms. They seek solace in the genes of non-host plants, blending them with the resilience of crops. RNAi-based control emerges as a silent guardian, warding off threats while maintaining environmental harmony. Undeterred, researchers embrace the power of genetic mapping and high-throughput sequencing, illuminating the path toward pest and disease-resistant crops. By identifying and characterizing key genes and QTLs associated with resistance, scientists pave the way for the development of robust cultivars. Techniques like genome-wide association mapping (GWAS), QTL-seq, MutMap, and ChIP-seq offer precise insights into the genetic architecture of resistance traits. Simultaneously, transcriptomic analyses such as RNA-seq, alongside protein profiling, unravel the molecular mechanisms underpinning plant resilience. These approaches identify candidate genes and pathways involved in pathogen resistance, which are then validated through functional studies. Metabolomics and metabolite profiling further enrich the understanding of biochemical responses to biotic stresses. The integration of genetic and genomic resources with advanced biotechnological tools becomes crucial for developing biotic stress-resistant cultivars. Transgenic technologies exploit genetic variability beyond the primary and secondary gene pools, overcoming cross ability barriers. Emerging techniques like induced gene-specific DNA methylation and epigenome editing generate novel epigenetic alleles for various biotic stresses. Speed breeding, or rapid generation advancement protocols, emerges as a potent technology for accelerating genetic gains in crop breeding, achieving up to four to five breeding cycles per year. This approach significantly shortens breeding times, expediting the development of resistant cultivars. Despite the availability of numerous tools and technologies, their judicious use remains challenging. Nevertheless, integrating next-generation genomics and phenomics in crop improvement programs holds immense potential for enhancing productivity by developing biotic stress-resistant cultivars. Concentrated efforts in proteomic and transcriptomic analyses are necessary to elucidate disease and pest resistance mechanisms at the molecular level, ultimately facilitating the editing or modification of crop genomes to develop biotic stress resilient varieties.

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GENOMIC ASSISTED BREEDING

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Abstract:

Genomics has revolutionized plant breeding by providing powerful tools to decode genetic traits and expedite the development of improved crop varieties. This chapter synthesizes insights from discussions on genomics tools and their applications in enhancing agricultural productivity. Next-Generation Sequencing (NGS) has enabled rapid and comprehensive genome analysis, facilitating the identification of genes crucial for traits like disease resistance and yield potential in crops. Marker-Assisted Selection (MAS) and genomic selection (GS) have leveraged genetic markers such as SSRs and SNPs to enhance breeding efficiency and precision. These tools are instrumental in selecting plants with optimal genetic profiles, accelerating breeding cycles and improving crop resilience to biotic and abiotic stresses. The integration of genomics with traditional breeding methods has transformed crop improvement strategies, enabling breeders to predict and manipulate genetic traits with unprecedented accuracy. Ongoing advancements in sequencing technologies, bioinformatics, and phenomics promise to further unravel genetic complexities and broaden the scope of trait improvement in crops. Integrating genomics with other omics disciplines, such as metabolomics and epigenomics, offers opportunities to optimize crop traits related to nutritional quality, environmental sustainability, and climate resilience.

Keywords: Genomic Tools, Sequencing Technologies, Bioinformatics, Genomic Selection

Introduction:

Plant breeding has been incredibly effective in creating crops and types that have aided in the formation of modern society from the beginning of plant domestication, approximately 10,000 years ago. Using traditional pre-genomics from the middle of the 20th century, the advancement of current cultivars by means of scientific breeding techniques has significantly increased the production of most main crops. Using both naturally occurring and mutant-induced genetic diversity, as well as effectively selecting the most advantageous genetic combinations through suitable breeding techniques, have been key components of plant breeding's success over the past century. In this regard, the phenotypic evaluation has served as an essential basis for both the selection procedures employed and the assessment and identification of genetic variations presence in the genotypes.

In recent years, the field of genomics and its application to plant breeding have grown rapidly. Most of the difficulties are being resolved by a new breed of plants called genomics-based plant breeding, which is the result of combining traditional breeding methods with genomic tools and approaches. Developing more successful varieties of plants is vital in this new plant breeding context, as per the Agriculture and Food Organization, for the 'greener revolution' that will feed the growing world population while conserving natural resources. classical genetics revolutionized plant breeding at the beginning of the 20th century, genomics is leading to a new revolution in plant breeding at the beginning of the 21th century. Genomics approaches are particularly useful when dealing with complex traits which are mostly associated with the quantitative characters, as these traits usually have a multi-genic nature and easily affected by the environment. However, more and more breeders are incorporating genomic techniques into conventional breeding tactics as scientists link advantageous agricultural features to genetic variants. Scientists can now find critical genes linked to crucial crop features like higher yields, resilience to drought, or resistance to pests thanks to developments in computational biology and plant biotechnology. Through expedited breeding programmes or precise genome editing, the advantageous features can be incorporated into current crop lines. Thus, the use of genomic techniques is making it easier to find and identify favourable alleles that have a minor influence but have often gone unreported and are not part of the gene pool used in breeding.

History of Genomics in Plant breeding

Early history (1910 – 1971): Discovery of DNA

In 1871, Friedrich Miescher published a paper that identified the presence of nuclein and associated proteins in the nucleus of cells. This is what we now call DNA and forms the base of the genomics field. In 1904, Walter Sutton and Theodor Boveri gives the Chromosome Theory of Heredity.

Albert Kossel was awarded the Nobel Prize in Physiology and Medicine in 1910 for the discovery of the five nucleotide bases, which are:

- Adenine (A)
- Cytosine (C)
- Guanine (G)
- Thymine (T)
- Uracil (U)

1950-1968: Development of knowledge about DNA

In 1950, Erwin Chargaff developed the pairing of the adenosine, cytosine, guanine and thymine nucleotide bases. He discovered that there were always equal amounts of thymine and adenine or cytosine and guanine.

In 1952, Alfred Hershey and Martha Chase demonstrated with a series of experiments that DNA, not protein, is responsible for carrying genetic traits that may be inherited by using ^{35}S and ^{32}P radioactive molecule. James Watson and Francis Crick discovered the double helix structure of DNA in 1953 and got the noble prize.

In 1961, Marshall Nirenberg and Har Gobind Khorana led the research team that identified what is now known as the "codon" of DNA. This codon correlates to a specific amino acid. It is when amino acids combine, during translation, that particular proteins are made. They won the Nobel Prize for Physiology and Medicine in 1968 for deciphering the genetic code, alongside Robert Holley, who sequenced the first transfer RNA (tRNA) molecule.

1977 – Present: Sequencing of DNA

In 1977, Frederick Sanger developed a sequencing technique for DNA to sequence the first complete genome, called phiX174 virus, which opened the doorway to the possibility in the field of genomics. In 1980, he won the Nobel Prize with Wally Gilbert and Paul Berg for pioneering DNA sequencing methods.

In 1983, Dr. Kary Mullis developed polymerase chain reaction (PCR) which create a significant devolvement in the genomics. In 1985, Alec Jeffreys developed a method to profile DNA by counting the number of repeating DNA sequences at specific regions of the genome.

In 1995, the first bacterium genome sequence, *Haemophilus influenza,* was completed. 1996, the genome of yeast, *Saccharomyces cerevisiae*, was completed. Dolly the Sheep, who was the first cloned animal was also born at this time. In 1998, John Sulston and Bob Waterston published the genome of the nematode worm, *Caenorhabditis elegans.* In 2000 complete sequwnce of *Ardiopsis thaliana* was published followed by Rice in 2005.

In 1990, the Human Genome Project was started. The first chromosome to be sequenced as part of this research in 1999 was chromosome 22. When the experiment was finished in 2003, it was determined that humans have between 20,000 and 25,000 genes.

Advancements in genome-wide expression studies give breeders knowledge about the genetic bases of complex characteristics. TILLING and EcoTILLING are two genomic techniques that enable the screening of mutant and germplasm collections for allelic variations in target genes. The creation of high density genetic maps and the genome-wide identification of markers suitable for high-throughput genotyping platforms, such as SSRs and SNPs, are two applications for which resequencing of genomes is highly helpful. Studying genetic diversity is made easier by all of these methods and resources, which is crucial for managing, improving, and using germplasm. Additionally, they make it possible to identify markers associated with genes and QTLs by a variety of methods, such as fine genetic mapping, association mapping, and bulked segregant analysis (BSA). These new markers are used for marker assisted selection, including marker assisted backcross selection, ‘breeding by design’, or new strategies, like genomic selection. In conclusion, advances in genomics are providing breeders with new tools and methodologies that allow a great leap forward in plant breeding, including the ‘superdomestication’ of crops and the genetic dissection and breeding for complex traits. These new markers are employed in novel approaches such as genomic selection, "breeding by design," and marker assisted backcross selection. New techniques and approaches made possible by genomics research are enabling plant breeders to make significant progress in the field. Examples of these include the "superdomestication" of crops, genetic dissection, and breeding for complex traits.

Genomics Tools and Techniques

The field of plant breeding has been revolutionized by genomics tools and techniques, allowing for the precise and efficient creation of new plant varieties. Utilizing high-throughput DNA sequencing, marker-assisted selection, and advanced genome editing technologies, these tools expedite the identification and incorporation of beneficial traits. Core methods such as next-generation sequencing enable rapid genome analysis, while CRISPR-Cas9 facilitates targeted genetic modifications. Genomic selection uses genome-wide markers to predict breeding outcomes, enhancing the development of crops with superior yield, disease resistance, stress tolerance, and nutritional value. These advancements are transforming agricultural practices and food production, leading to more resilient and productive crops.

1. DNA Sequencing

Next-Generation Sequencing (NGS): This technology enables rapid sequencing of entire genomes, allowing for the identification of genetic variations associated with desirable traits. NGS provides high-throughput data, making it possible to sequence multiple genomes simultaneously, significantly speeding up the breeding process.

Whole Genome Sequencing (WGS): WGS offers a comprehensive view of the entire genome, which is crucial for identifying mutations and structural variations. By sequencing the entire genetic makeup of a plant, breeders can pinpoint specific genes or regions responsible for beneficial traits, leading to more targeted breeding strategies.

RNA Sequencing (RNA-seq): RNA-seq analyzes gene expression patterns, providing insights into how genes are regulated at the transcriptome level. This technique helps in understanding the functional aspects of the genome, including how certain traits are expressed and regulated, which is essential for improving plant performance and resilience.

2. Marker-Assisted Selection (MAS)

Simple Sequence Repeats (SSRs): Also known as microsatellites, these are repeating sequences of 2-6 base pairs of DNA. They are highly polymorphic, making them excellent markers for genetic studies. SSRs are used to track the inheritance of genes associated with desirable traits in breeding programs.

Single Nucleotide Polymorphisms (SNPs): These are single base pair variations in the genome and are the most common type of genetic variation. SNPs are highly abundant and useful for genetic mapping and association studies. They help breeders identify specific regions of the genome associated with traits of interest.

Amplified Fragment Length Polymorphism (AFLP): This is a PCR-based method used to detect genomic polymorphisms. AFLP is used to generate a large number of markers for genetic mapping and diversity studies. It is useful for identifying genetic variations even in species with little prior genetic information.

3. Genomic Selection

Genomic Selection (GS) is an advanced breeding technique that utilizes genome-wide markers to predict the breeding value of individuals for complex traits. This method significantly accelerates the breeding cycle compared to traditional selection methods.

Predicting Breeding Value: In GS, a large number of genetic markers spread across the genome are used to predict the performance of plants for various traits. This prediction is based on the combined effect of all markers, allowing for the estimation of an individual's breeding value—the potential to pass on desirable traits to the next generation. This is especially useful for complex traits controlled by many genes, such as yield, disease resistance, and drought tolerance.

Integration of Statistical Models: GS integrates sophisticated statistical models with high-throughput genotyping data. These models, such as Best Linear Unbiased Prediction (BLUP) and genomic BLUP (GBLUP), utilize information from both phenotypic data (observable traits) and genotypic data (genetic markers) to make accurate predictions. Advanced machine learning techniques are also increasingly being employed to improve the predictive accuracy of these models.

High-Throughput Genotyping: High-throughput genotyping technologies, such as SNP arrays and NGS, enable the rapid and cost-effective collection of genetic marker data from a large number of individuals. This extensive genotypic data forms the basis for building and validating predictive models in GS.

4. Genetic Mapping and QTL Analysis

Genetic mapping and QTL (Quantitative Trait Loci) analysis are essential techniques in plant breeding for identifying genomic regions associated with specific traits. QTL mapping identifies sections of the genome linked to quantitative traits, such as yield or drought tolerance, by analyzing the correlation between genetic markers and phenotypic variation in a structured population. Linkage mapping, a foundational method in QTL analysis, uses recombination frequencies between markers to determine their relative positions on chromosomes, providing a genetic map. This method requires a controlled breeding population and is effective for mapping traits with clear inheritance patterns. Association mapping, also known as linkage disequilibrium mapping, extends this approach to diverse, naturally occurring populations by examining the statistical association between markers and traits. This method leverages historical recombination events and can provide higher resolution maps than traditional linkage mapping. Together, these techniques enable breeders to pinpoint genetic factors underlying important agricultural traits, facilitating marker-assisted selection and the development of improved plant varieties.

5. Transcriptomics and Proteomics

Transcriptomics and proteomics are critical tools in understanding the functional dynamics of genes and proteins in plants. Microarrays are a powerful transcriptomic technology that allows researchers to analyze the expression of thousands of genes simultaneously. By comparing gene expression profiles under different conditions or in different plant varieties, scientists can identify genes associated with specific traits, such as disease resistance or drought tolerance. This high-throughput analysis provides a comprehensive view of the transcriptome, offering insights into how genes are regulated and how they interact with each other to influence phenotypic traits.

Proteomics, on the other hand, focuses on the study of the protein content of a plant. Since proteins are the functional molecules that execute the instructions encoded by genes, proteomics provides a deeper understanding of the actual biological processes occurring within the plant. Techniques such as mass spectrometry and two-dimensional gel electrophoresis are used to identify and quantify proteins, as well as to study their modifications and interactions. By analyzing the proteome, researchers can gain insights into the functional expression of genes, the mechanisms underlying specific traits, and how these proteins respond to environmental changes. Together, transcriptomics and proteomics offer a comprehensive picture of gene function and regulation, aiding in the development of plants with enhanced traits.

6. Metabolomics

Metabolomics is the large-scale study of small molecules, known as metabolites, within plants. These metabolites are the end products of cellular processes and provide a snapshot of the biochemical activity occurring in a plant at a given time. By analyzing metabolite profiles, researchers can gain insights into the biochemical pathways that contribute to specific traits, such as flavor, nutritional content, stress responses, and growth rates. Advanced analytical techniques, such as gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-mass spectrometry (LC-MS), are used to identify and quantify metabolites, revealing the complex network of metabolic interactions within the plant.

Understanding these biochemical pathways is crucial for improving plant breeding and crop production. Metabolomics can help identify key metabolites that are indicators of desirable traits, enabling breeders to select plants with optimal metabolic profiles. Additionally, it can uncover how plants adapt to different environmental conditions, providing valuable information for developing varieties that are more resilient to stresses such as drought, pests, and diseases. By integrating metabolomics with genomics, transcriptomics, and proteomics, researchers can obtain a holistic view of plant biology, paving the way for the development of superior crop varieties with enhanced performance and sustainability.

Genomics Data and Resources

Genomic data and resources are foundational elements in modern plant breeding and biotechnology, providing comprehensive information on the genetic makeup of various plant species. These resources include extensive databases such as GenBank, Ensembl Plants, and the Plant Genome Database, which store vast amounts of genomic sequences, annotations, and related genetic information. Advanced bioinformatics tools enable the analysis and interpretation of this data, facilitating the identification of genes and genetic variants associated with important traits. Additionally, public repositories and collaborative platforms like the 1000 Plant Genomes Project and the International Wheat Genome Sequencing Consortium provide access to high-quality genomic datasets, fostering global collaboration and accelerating research efforts. By leveraging these genomic resources, researchers and breeders can make informed decisions, enhancing crop improvement programs and addressing agricultural challenges more effectively.

Bioinformatics encompasses a wide range of databases, tools, and educational resources essential for genomic and biological data analysis. Key databases include GenBank for nucleotide sequences and Ensembl for genome annotations across species. Tools like Bioconductor facilitate the analysis of high-throughput genomic data, while platforms such as Galaxy enable reproducible and collaborative research workflows. Educational resources from NCBI, Coursera, and edX offer courses ranging from introductory to advanced bioinformatics topics, catering to researchers, educators, and students seeking to leverage computational approaches in biological research and data interpretation. These resources collectively support advancements in genomics, transcriptomics, proteomics, and beyond, crucial for understanding biological systems and accelerating scientific discoveries.

Application of Genomics in Plant Breeding

Genomics has revolutionized plant breeding by providing powerful tools that enhance our understanding of crop genetics and accelerate the development of improved varieties. Next-Generation Sequencing (NGS) has enabled rapid and cost-effective whole-genome sequencing, crucial for identifying genes linked to important agronomic traits like drought tolerance and disease resistance in crops such as wheat (Varshney *et al*., 2018). Whole Genome Sequencing (WGS) complements this by offering a comprehensive view of genetic variations, aiding in the discovery of mutations and structural variations that influence traits such as yield and nutrient content (Pont *et al*., 2019).

Marker-Assisted Selection (MAS) utilizes genetic markers like Simple Sequence Repeats (SSRs) and Single Nucleotide Polymorphisms (SNPs) to guide breeding decisions, expediting the development of cultivars with enhanced traits such as disease resistance in rice (Thomson, 2014; Septiningsih *et al*., 2015). Genomic Selection (GS) takes breeding a step further by integrating genomic data with statistical models to predict the breeding value of plants for complex traits like yield and stress tolerance, revolutionizing breeding strategies in crops like maize (Crossa *et al*., 2017). CRISPR-Cas9 genome editing technology offers precise tools for modifying specific genes, enhancing traits such as fruit shelf life in tomato and resistance to diseases like soybean mosaic virus in soybeans (Zhang *et al*., 2018; Li *et al*., 2020). Moreover, conservation efforts focused on understanding and preserving plant genetic diversity, particularly in crops like maize, are essential for developing resilient varieties that can thrive in changing environmental conditions (Lu *et al*., 2020). Overall, genomics continues to play a pivotal role in shaping the future of agriculture by enabling more efficient and sustainable crop improvement strategies to meet global food security challenges.

Genomics has also ushered in a transformative era in plant breeding through diverse applications such as Transcriptomics, Proteomics, Metabolomics, Bioinformatics, Phenomics, and Epigenomics. Transcriptomics involves deciphering RNA transcripts to understand gene expression dynamics under varying conditions, aiding in the selection of traits like drought tolerance in maize through RNA sequencing (Zhang *et al*., 2021). Meanwhile, Proteomics explores the complete set of proteins to unravel biochemical pathways crucial for enhancing nutritional quality in crops, exemplified by studies improving fruit traits in tomatoes (Wang *et al*., 2017). Metabolomics complements these efforts by analyzing metabolites to uncover pathways influencing traits such as flavor and nutrient content, pivotal in enhancing crop quality and consumer satisfaction, as seen in tomato breeding (Moco *et al*., 2018).

Bioinformatics plays a pivotal role in managing vast genomic and transcriptomic data, employing advanced computational tools like machine learning to predict gene functions and regulatory networks. These tools have been instrumental in identifying stress tolerance mechanisms in staple crops such as rice and wheat, highlighting their role in accelerating breeding programs (Sarkar *et al*., 2020). Phenomics encompasses comprehensive trait analysis at the whole-plant level, leveraging technologies like high-throughput phenotyping to rapidly assess traits such as drought tolerance and nutrient efficiency in crops like maize, thereby informing breeding decisions (Trachsel *et al*., 2020). Finally, Epigenomics explores inherited changes in gene expression not caused by alterations in DNA sequence, crucial for understanding and enhancing traits like stress resilience and yield stability in major crops such as maize and *Arabidopsis* (Li *et al*., 2021). Together, these multidimensional genomic approaches underscore their collective impact on developing resilient, nutritious, and sustainable crops essential for addressing global food security challenges amidst environmental uncertainties.

Although Genomics has revolutionized plant breeding by providing tools to understand genetic traits and accelerate crop improvement. Despite its transformative potential, several challenges limit its widespread application. One significant hurdle is the complex architecture of traits, often influenced by multiple genes and environmental factors. This complexity makes it challenging to predict and manipulate traits accurately using genomics alone. Additionally, while genomic technologies can identify genetic markers associated with traits, understanding the functional roles of genes and their interactions within biological pathways remains a major gap. This limits the precision of genetic manipulation in breeding programs. Genetic and phenotypic diversity among crop varieties also poses challenges. Genomic tools must account for this variability to develop universally applicable breeding strategies that work across different genetic backgrounds and environmental conditions. Moreover, the high cost of genomic technologies, including sequencing and computational analysis, presents a barrier, particularly for researchers and breeders in developing countries or smaller institutions. The integration of genomics into traditional breeding methods requires significant investment in infrastructure and expertise, further complicating widespread adoption.

Ethical and regulatory considerations also impact the adoption of genomic technologies in plant breeding. Issues related to intellectual property rights, biodiversity conservation, and public acceptance of genetically modified organisms (GMOs) require careful navigation. Regulatory frameworks vary globally, influencing the commercialization and deployment of genetically improved crops. Addressing these challenges demands collaborative efforts, technological advancements, and supportive policies to ensure that genomics continues to drive sustainable agricultural innovation while addressing global food security challenges.

Conclusion:

In conclusion, while genomics has significantly advanced plant breeding by unraveling genetic complexities and accelerating the development of improved crop varieties, several challenges remain to be addressed. The intricate architecture of traits, influenced by both genetic and environmental factors, necessitates continued research to enhance our understanding of gene function and interaction. Bridging these gaps will enable more precise genetic manipulation and tailored breeding strategies that meet evolving agricultural needs. Moreover, the integration of genomics with traditional breeding methods remains crucial. This approach ensures that genomic insights translate into practical improvements in crop performance, resilience, and nutritional quality. It requires investment in infrastructure, capacity building, and collaborative partnerships to democratize access to genomic tools and empower breeders globally.

The ethical and regulatory landscape surrounding genomics in agriculture also demands attention. Clear guidelines and policies are essential to navigate issues such as intellectual property rights, biodiversity conservation, and public perception of genetically modified organisms. By addressing these concerns transparently and responsibly, stakeholders can foster trust and facilitate the sustainable deployment of genomic innovations in agriculture.

Looking ahead, the future prospects of genomics in plant breeding are promising. Advances in technology, such as improved sequencing techniques, high-throughput phenotyping, and bioinformatics tools, will continue to enhance our ability to decode and manipulate the genetic blueprint of crops. This will enable breeders to develop varieties that are not only high-yielding and resilient to biotic and abiotic stresses but also nutritious and environmentally sustainable.

Furthermore, genomics holds potential beyond traditional breeding goals. It offers opportunities to explore and harness genetic diversity for novel traits, such as enhanced nutritional profiles, flavor profiles, and climate resilience. Integrating genomic data with other omics approaches, such as metabolomics and epigenomics, will provide a more holistic understanding of plant biology and adaptation mechanisms.

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SPEED BREEDING

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Abstract:

A fundamental worry for scientists worldwide is crop improvement in the face of a rapidly changing environment and an expanding human population. The rate at which current crop enhancement programs are moving forward is not keeping up with the need for food. Today, speed breeding is widely employed as a technique to sustain several crop generations annually and reduce generation time. In order to increase breeding efficiency, scientists are now employing an integrated strategy that combines genetic engineering, plant breeding, and speed breeding. By reducing the length of breeding cycles for the improvement of food and industrial crops, speed breeding is a promising strategy for attaining nutritional security and sustainable agriculture. With the use of speed breeding, plant breeders can increase crop yield by modifying the temperature, duration, and intensity of light to promote plant growth. It initiates growth and reproduction far earlier than usual by turning on the photosynthetic process with an artificial light source that is kept on all the time. Numerous technologies, such as re-domestication, genomic selection, high throughput phenotyping, genotyping, and marker-assisted selection, can be combined with speed breeding to enable plant breeders to adapt to a changing climate and expanding human population.

Keywords: Speed Breeding, Phenotyping, Re-Domestication, Genomic Selection, MAS

Introduction:

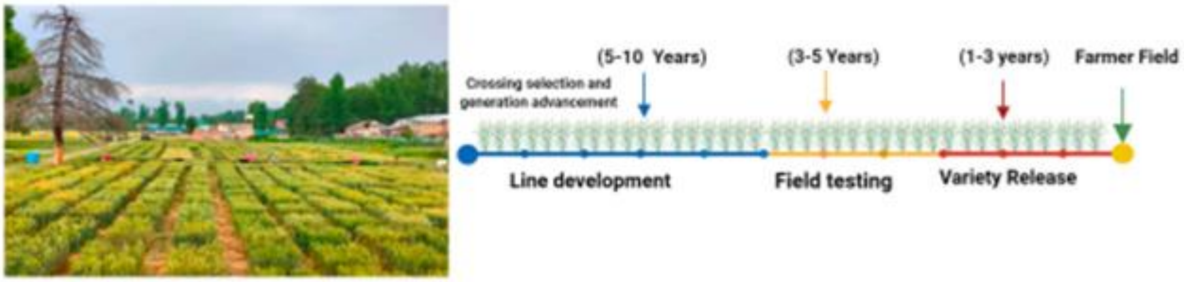
In order to breed a new crop variety using a conventional method, complementary parental genotypes with desired features must be chosen. Next, crossings must be made, and superior progenies must be advanced through selection and advancement in order to release candidate cultivars that satisfy market demands (Shimelis and Laing, 2012). Higher yield potential, improved nutritional quality, and increased resistance to biotic and abiotic stressors are notable breeding objectives in agricultural cultivar improvement programs (Breseghello and Coelho, 2013; Tester and Langridge, 2010). In order to identify the best performing and stable candidate cultivars, the following steps must be taken: (a) choosing desirable parents with complementary traits to combine; (b) involving the selected parents in crosses and the progenies' development; (c) selecting and genetically advancing the best progenies based on target traits; (d) selecting the best progenies for screening in multiple target production environments; and (e) registering the cultivar and distributing seeds to growers (Shimelis and Laing, 2012). Most crop cultivar development programs employ these traditional breeding techniques. In the absence of an integrated pre-breeding program, conventional breeding methods can take over ten years to develop and release an improved variety (Ahmar *et al.*, 2020; De La Fuente *et al.*, 2013).

In order to feed everyone on the globe, crop yields must rise; nevertheless, this is a challenging challenge due to shifting environmental factors. Earth is getting warmer and drier due to climate change, commonly referred to as the "climate catastrophe" (FAO, 2019). The rate at which crops are improving now will not be enough to feed the world's expanding population by 2050. Higher, steadier, and sustainable crop production is required in the face of drought stress, which causes significant losses in crop yields.

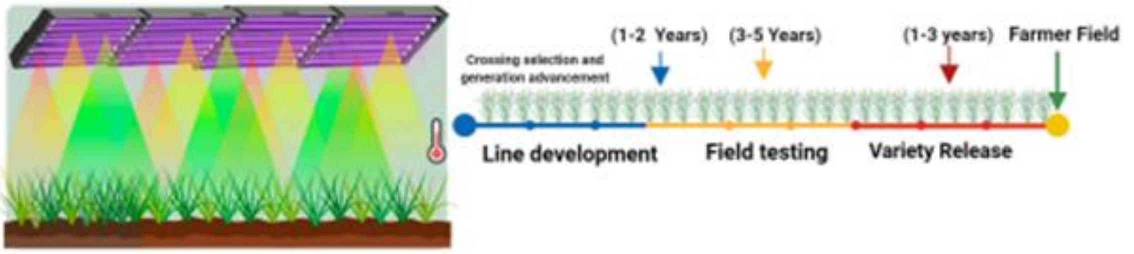
Since the 1940s, single-seed descent (Brim *et al.*, 1966; Goulden *et al.*, 1939) and shuttle breeding (Borlaug *et al.*, 1968) have been used in plant breeding to alter the rate of plant lifecycle turnover. More recently, scientists have extended the duration of the plant lifespan by manipulating controlled-environment (CE) growth conditions. The term "speed breeding" (SB) is commonly used to refer to methods that increase cycle turnover (Ghosh *et al.*, 2018).

Speed breeding is a recent development that significantly shortens the period of many crops' breeding cycles. It was inspired by NASA's trials to grow wheat in space, which involves shining continuous light on the crop to encourage early reproduction in the plants. Additionally, it speeds up the growth of inbred lines by utilizing regulated temperature and an extended photoperiod to create six generations of wheat annually. It has long been known that plants can grow and develop more quickly when artificial electric lamps are used (Pfeiffer *et al.*, 1926; Wheeler *et al.*, 2008).

In order to go as soon as possible to the next breeding generation, speed breeding is a suite of procedures that involves manipulating the environmental conditions under which crop genotypes are produced in order to speed up flowering and seed development. The technique's quick generation advancement saves breeding time and costs. To reduce the breeding cycle and make efficient use of resources, speed breeding can incorporate a variety of selection techniques, including clonal selection, marker-assisted selection (MAS), single plant selection (SPS), single pod descent (SPD), single seed descent (SSD), and single plant selection (SPS) (Hickey *et al*., 2017; Samineni *et al*., 2019; Watson *et al*., 2018). Approximately three to nine generations are produced year using speed breeding, as opposed to one to two generations annually through traditional selection techniques (Ghosh *et al*., 2018; Ochatt *et al*., 2002). Thus, homozygous and stable genotypes may be quickly developed by speed breeding, and it can also speed up generation advancement, which speeds up the creation and release of novel cultivars (Watson *et al*., 2018). Furthermore, for multiple trait selection, high-throughput phenotyping approaches and MAS work well with speed breeding technologies. Compared to traditional methods, speed breeding offers a number of benefits, such as transgenic pipelines, pyramiding traits, and faster backcrossing.



(a) Timeline of conventional breeding



(b) Timeline of Speed Breeding

Figure 1: timelines of varietal development with (a) conventional breeding; (b) speed breeding (source: Samantara ***et al.,*** 2022**)**

Comparing Alternative Breeding Approaches with Speed Breeding

Traditional breeding methods cannot produce new plant types due to the lack of progress in plant genome development. To get around this obstacle in plant breeding techniques, molecular markers have been used since the 1990s to identify superior hybrid lines (Dreher *et al*., 2000). To improve plant phenotypic for a particular desired trait, plant breeders must use artificial selection and breeding of this given attribute. Breeders typically concentrate on diploid or diploid-like traits (like tomatoes and maize) as opposed to polyploidy traits (like potatoes and alfalfa), which have more intricate genetics. Instead of using crops that reproduce only once a year or perennial plants that reproduce only once every few years, breeders prefer to use crops with shorter reproductive cycles, which allow for the production of multiple generations in a single year and speed up artificial breeding of desired phenotypes (Stetter *et al.*, 2016).

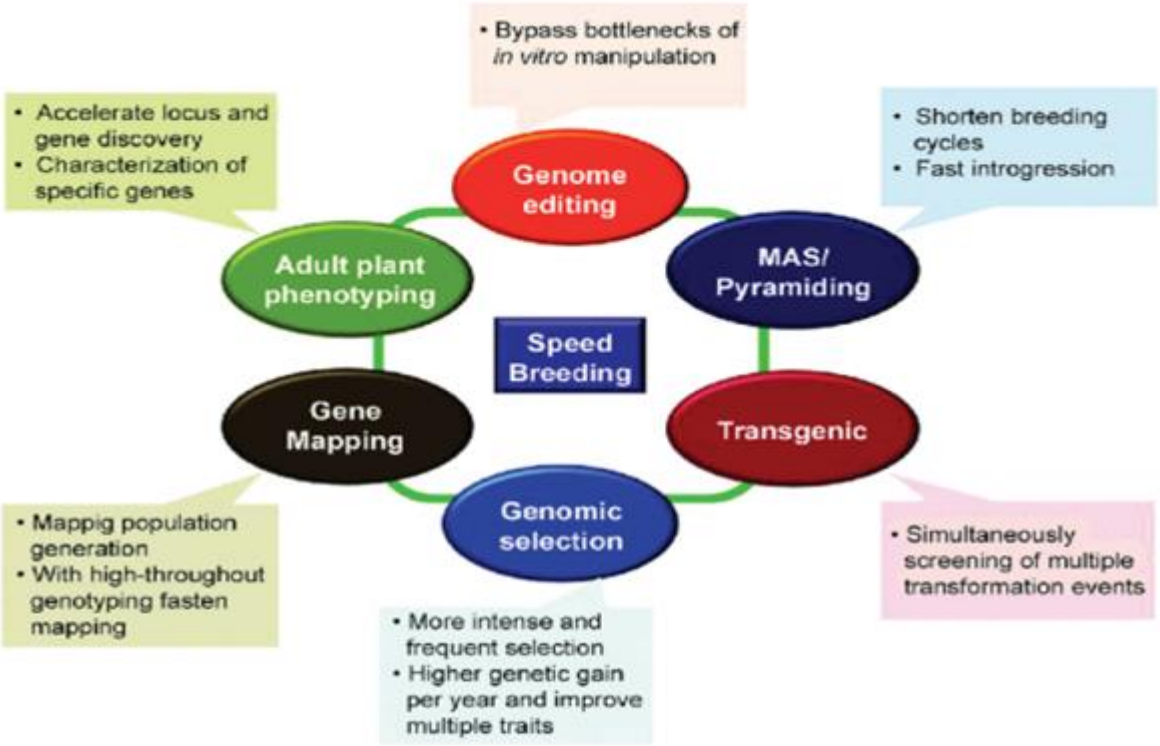


Figure 2: speed breeding coupled with other breeding methodologies

(Source: Begna, 2022)

Advantages of Speed Breeding Methods

1. **Quick creation of homozygous lines to speed up breeding**
    - The photoperiod regime is manipulated
    - Control of the temperature range
    - Control of soil moisture
    - Plant population density
    - Changing the amount of carbon dioxide
    - Hormones, organ tissue culture, and plant nutrition
2. **Adaptability to selection techniques**

    To pick genotypes that produce the best results, conventional selection techniques like bulk, mass, recurrent, pedigree, and pure line selection need a genetically stable plant population. Because these techniques necessitate lengthy rounds of inbreeding and selection, they are not recommended for rapid breeding. The best selection techniques compatible with rapid breeding are:

- **Single seed descent method**

  In order to proceed to the F3–F4 generations using the SSD selection approach, a sizable number of F1 plants (50–100) must produce between 2000 and 3000 F2 plants (Priyadarshan, 2019). In order to achieve optimal yield, plants are grown under ideal field circumstances at the F5 generation, which enables the selection of superior F6 genotypes and their advancement using a head-to-row technique. For the preliminary yield trials (F7) and yield trials (F8–F10), superior lines/rows are chosen. The best lines (F11–F12) are then offered as new cultivars.

  With single seed descent (SSD), one seed from each F2 plant is kept and these individuals are passed down to the following generation to continuously inbreed segregating populations until homozygous populations are achieved. Every produced inbred line can be traced back to an F2 plant (Fehr, 1991). According to Yan *et al.,* (2017), the time required to produce inbred lines using SSD is similar to that of the doubled haploid (DH) approach. The SSD selection approach has the benefit of requiring less labor and growing space for the processing of earlier generations. Progeny under high-density plantings in small nurseries, growth chambers, or greenhouses can be advanced with its help (Arbelaez *et al*., 2019; Funada *et al*., 2013). The drawback of SSD is that compared to pure line, pedigree, and recurrent selection techniques, it produces a greater number of poor progenies. Urrea and Singh (1994) discovered that inbred lines developed using the SSD approach produced lower overall mean seed yields in common beans than lines developed using pedigree and mass selection.

- **Single pod descent method**

  Using the single pod descent (SPD) approach, one pod rather than a single seed is chosen for each F2–F4 plant. In comparison to SSD selection, SPD has a better likelihood of retaining every F2 plant in the subsequent generations because most legume crops have many seeds per pod. A further benefit of SPD is its ability to pick pods early on, allowing for the advancement of a smaller population. To ascertain the effectiveness of SPD for the crop and trait being selected for under speed breeding, preliminary trials are necessary.

- **Single plant selection method**

  By gathering all of the seeds from each chosen plant, the single plant selection (SPS) approach progresses every F2 plant. As a result, the following generation will progress from plant to row. In order to create introgression lines (ILs) in barley within two years, a modified backcross strategy has been implemented, utilizing the SPS method (Hickey *et al*., 2017). Unlike SSD and SPD, the SPS uses a smaller population to inform the early selection of plants.

Challenges of Speed Breeding

One effective way to quicken traditional breeding programs is through the application of speed breeding techniques. But the technique needs knowledge, complementary and efficient plant phenomics facilities, suitable infrastructure, and ongoing funding for research and development (Shimelis *et al*., 2019). The following are the most frequent obstacles to using fast breeding:

- A deficiency of skilled breeding technicians and plant breeders
- Insufficient facilities
- Unreliable sources of energy and water for operations that are sustainable

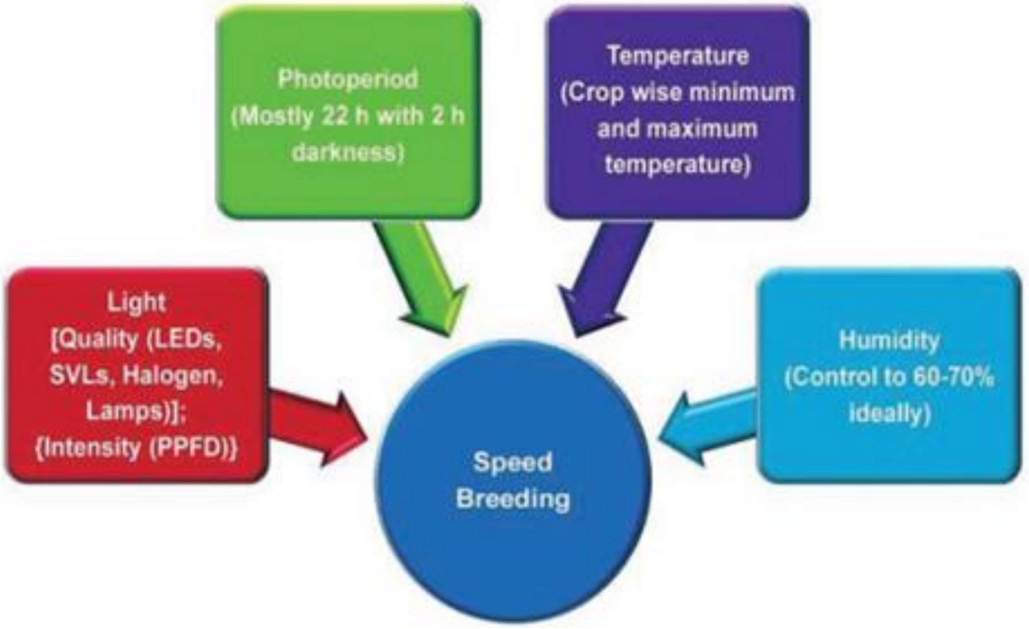


Figure 3: Key altering factor in speed breeding (Source: Begna, 2022**)**

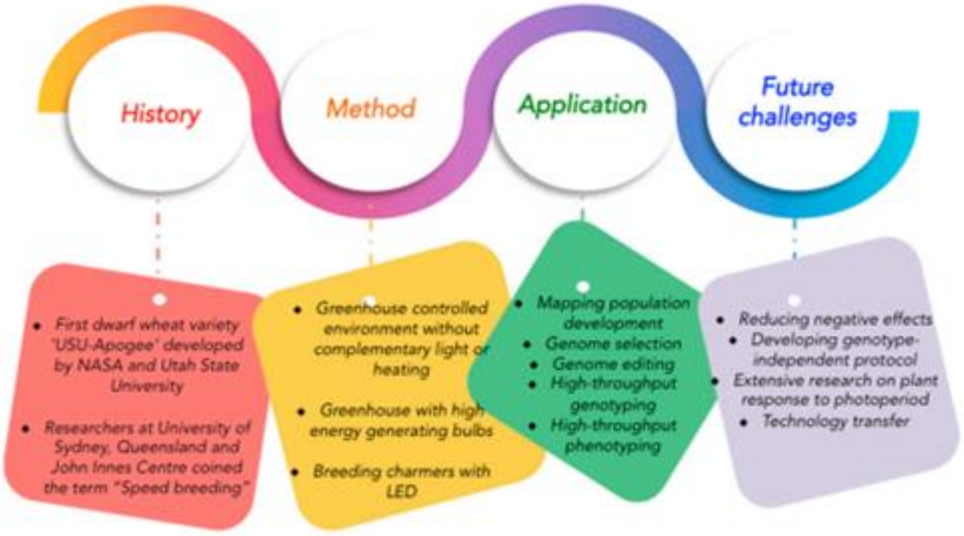


Figure 4: Retrospect, current methods, applications and challenges of speed breeding (Source: Samantara *et al.,* 2022)

Conclusion:

Through the creation of disease-resistant varieties, the reduction of salt sensitivity in crops, and adjustments to the duration, intensity, and temperature-controlled zone of light, speed breeding is a process that can be utilized to boost agricultural productivity. Through speed breeding, the photosynthetic process is enhanced, leading to accelerated crop development. This method makes it possible to release multiple generations of the same crop more quickly than with traditional breeding. By reducing the generation time, speed breeding is a novel method for quickly developing new long-day plant cultivars.

Plant breeders can produce superior crop types more quickly thanks to this technology. Efficient procedures that minimize labor and facilities losses are essential for incorporating speed breeding into a crop improvement program. Additionally, elite genotypes and lines with unique features, like increased yield and superior nutritional attributes along with biotic and abiotic stress tolerance, can be more effectively selected by combining speed breeding with traditional, MAS, and GE breeding procedures. The SSD, SPD, and SPS procedures are the most suitable selection techniques compatible with speed breeding. However, a shortage of qualified plant breeders and plant breeding specialists, as well as inadequate infrastructure and dependable water and electricity sources, limit the use of speed breeding in many developing nations, particularly in public plant breeding programs. Additionally, there is currently insufficient funding and policy support from the government to start and maintain speed breeding in public plant breeding programs.

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BIOINFORMATIC IN PLANT BREEDING

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Abstract:

The integration of bioinformatics and computational biology into plant sciences is revolutionizing research by accelerating discoveries and offering new biological insights. Initially, bioinformatics focused on creating databases to store vast biological data, such as nucleotide and amino acid sequences, and developing tools to access and analyse this data. Advances in sequencing technology now allow plant biologists to study the genetic architecture of various species, including their proteomes and metabolomes, providing a foundation for functional understanding. In plant breeding, bioinformatics has enabled the identification of genetic factors that influence crop performance, improving resistance to pathogens and crop quality. Genome projects are underway for over 60 plant species, including key crops like maize, rice, and wheat. Rice, with its small genome, serves as a model for genomic research, facilitating advancements in other cereal crops. For wheat, bioinformatics tools have revealed complex genome rearrangements, enhancing yields through genome-wide association studies (GWAS). Similarly, maize research benefits from databases like Maize GDB, which help improve yields by linking genotype-phenotype data. Bioinformatics also aids in understanding plant stress responses, essential for improving breeding strategies and predicting how plants will adapt to environmental changes. The use of next-generation sequencing (NGS) and computational tools has advanced research on plant-pathogen interactions and molecular adaptations to stress. Additionally, machine learning and deep learning techniques are enhancing genotype-phenotype predictions and phenotyping processes. Bioinformatics is crucial for advancing plant breeding, improving crop yields, and addressing environmental challenges. Its ongoing integration into plant research holds great promise for enhancing agricultural practices, developing disease-resistant crops, and ensuring global food security.

Keywords: Genetically Modified Organisms, Transgenic and Next-Generation Sequencing

Introduction:

The integration of bioinformatics and computational biology into plant biology is significantly accelerating scientific advancements in life sciences. The main objective is to facilitate the discovery of new biological insights and to establish a comprehensive perspective to identify unifying principles in biology. At the onset of the "genomic revolution," a key focus in bioinformatics was developing and maintaining databases to store biological data, including raw nucleotide and amino acid sequences. Creating these databases required addressing design challenges and developing sophisticated interfaces for researchers to access existing data and submit new or updated data.

Sequencing technology has enabled plant biologists to uncover the genetic architecture of various plant and microorganism species, including their proteomes, transcriptomes, metabolomes, and metabolic pathways. Sequence analysis is a fundamental method in modern science to obtain the complete genome sequences of DNA, RNA, and proteins from an organism. Whole genome sequencing allows scientists to determine the organization of different species and serves as a foundation for understanding their functionality. Comprehensive sequence data includes both coding and non-coding regions, which are essential precursors for functional genes that determine unique organism traits. The sequences encompass all regions such as exons, introns, regulatory elements, and promoters, resulting in a vast amount of genomic information. The advent of next-generation sequencing (NGS) and other omics technologies for plant genomics is leading to the revelation of more plant genomes. To manage this extensive data, bioinformatics development and implementation enable scientists to systematically capture, store, and organize the information in databases.

Bioinformatics in Plant Breeding

Plants are fundamental to life on Earth, generating the oxygen we breathe, contributing to our nutrition and health, and supporting diverse ecosystems. Over centuries, humans have selectively bred plant varieties to enhance traits favourable for agriculture, improving the quality and yield of crops compared to their wild counterparts. Despite these advances, improving complex traits like resistance and quality simultaneously has been challenging. However, the advent of genomics has revolutionized plant breeding by expanding the scale and precision of genetic research. Genomics offers detailed insights into the genetic factors influencing plant performance, allowing for systematic analysis of the biological processes that underlie pathogen resistance and crop quality. This analysis, facilitated by specialized software and large databases, falls under the domain of plant bioinformatics. (1,2)

There are currently genome initiatives underway for over 60 plant species. Economically, the most significant of these are the major feed crops, including maize, rice, wheat, sorghum, barley, soybean, and alfalfa. Due to the large size of several of these genomes, caused by autopolyploidization and the expansion of repetitive DNA, whole genome sequencing is often impractical. Therefore, comparative genome methods are being utilized instead. However, because rice and maize have relatively small genomes and are vital to the agricultural economies of developed countries, their complete genome sequences have been prioritized. (9)

Rice

Since the first production of transgenic rice in 2000, there has been a significant acceleration in crop genome sequencing projects, driven by advancements in technology and the rapid growth of genetically modified organisms (GMOs). (3,4,5) One of the most well-known products in rice biotechnology is golden rice, engineered to produce β-carotene (pro-vitamin A) to combat vitamin A deficiency. The World Health Organization (WHO) has identified vitamin A deficiency as a major public health issue, leading to childhood blindness in half a million children annually. (4) Vitamin A is crucial for vision development, growth, cellular differentiation, and immune function; its deficiency can result in blindness, anaemia, and weakened immunity in children. (6)

Rice, being the first crop genome to be sequenced, serves as an ideal model for genomic studies and the improvement of other species due to its small genome size and diploid nature. This makes it an excellent reference for other cereal crops with larger genomes, such as maize and wheat. The complete genome sequences of two rice subspecies, japonica and indica, were reported by Song *et al.,* in 2005, providing a foundation for molecular studies and plant breeding research. (7)

Recent advancements in bioinformatics have made it possible to align sequences between large, complex genomes of other crops using rice genomic data. Programs like BLAST and FASTA enable rapid sequence searches in databases, providing the best possible alignments and calculating alignment scores to measure homology between sequences from related species. (8)

Wheat

Wheat, along with rice and maize, is among the most widely grown and consumed crops, providing over 60% of the calories and protein in our daily diets. To meet the demands of a growing human population, it is essential to advance wheat research and breeding to significantly increase wheat yields by 2050. Despite its critical role, enhancing wheat production has been challenging due to the complex nature of the wheat genome, which is highly repetitive and large, making it difficult to achieve a fully sequenced reference genome.

Recent advancements in next-generation sequencing (NGS) platforms and bioinformatics tools have revealed extensive structural rearrangements and complex gene content within the wheat genome. These innovations have transformed wheat genomics, improving wheat yields and its adaptability to diverse environments. NGS platforms enable the rapid detection of DNA markers from vast genome data in a short time, revolutionizing allele discovery and genotype-by-sequencing (GBS).

The availability of a high-quality reference genome for wheat in databases facilitates sequence comparisons between wheat and other species, aiding in the identification of homologous genes. Additionally, the advancements in sequencing technologies, including high-throughput genotyping and increased read lengths, combined with biological databases, support the rapid development of novel algorithms for analyzing the complex wheat genome. For example, genome-wide association studies (GWAS) are used to quickly screen raw data and identify specific genomic regions associated with agronomic traits. This approach enables the testing of multiple genetic variants across the genome to study genotype-phenotype associations, thereby facilitating crop improvement through genomic selection and genetic modification. (10,11).

Maize

Maize, a globally significant crop, not only has a broad range of economic uses but also serves as a genetic model for studying the genotype-to-phenotype relationship in plant genomics. Its high genetic diversity makes maize particularly promising for yield improvement to meet the needs of a growing population. However, despite its economic and genomic importance, generating a complete genome sequence for maize has been challenging due to the substantial structural variation (SV) within its genome.

The advent of next-generation sequencing (NGS) techniques has enabled rapid de novo genome sequencing and the generation of vast amounts of genomic and phenomic data for several crops, including maize. (12,13) Improved integration of data across multiple genome assemblies is essential to understand the phenotype-genotype connection, which is crucial for enhancing maize yield and quality.

Today, user-friendly online databases such as qTeller, MaizeDIG, and MaizeMine facilitate the comparison and visualization of genotype-phenotype relationships. MaizeGDB, a comprehensive database for maize, provides access to data on genes, alleles, molecular markers, metabolic pathways, and phenotypic images, all of which are valuable for maize research. MaizeMine, a data mining resource under MaizeGDB, accelerates genomic analysis by enabling researchers to customize their data for downstream analysis. MaizeDIG is a genotype-phenotype database that allows users to link genotypes with their phenotypic expressions through images. (13)

According to Cho *et al*., tools like MaizeDIG enhance the visualization of the gene-phenotype relationship via image searches. These tools, by integrating and visualizing high-quality data, enable quick prioritization of phenotypes of interest, which is crucial for advancing plant breeding efforts.

Bioinformatics for Studying Stress Resistance in Plants

Understanding plant stress responses is crucial for improving agricultural breeding efforts and predicting the fate of natural plants under changing abiotic conditions, especially in the era of continuous climate change. Plant stress responses can be categorized into biotic and abiotic types. Biotic stress refers to negative impacts caused by living organisms such as viruses, fungi, bacteria, insects, nematodes, and weeds, while abiotic stress includes factors like extreme temperatures, drought, flooding, salinity, and radiation, all of which significantly affect crop yields.

Advances in next-generation sequencing (NGS) technologies and other powerful computational tools have enabled extensive molecular studies of plant stress responses by facilitating whole-genome and transcriptome sequencing. The vast amount of plant genome data obtained from these technologies allows researchers to investigate the correlations between the molecular structure of organisms and their environmental adaptations.

Biotic and Abiotic Stress Management

Understanding how plants and crops respond to stressful environments is essential for ensuring their growth and development and avoiding significant yield penalties caused by harsh conditions. Therefore, bioinformatic tools are crucial for studying and analysing plant transcriptomes in response to biotic and abiotic stress. Applying these tools to plant and crop genomes benefits the agricultural community by enabling the search for desired genes across different species' genomes and elucidating their functions.

Genome databases play a vital role in storing and mining large and complex plant genome sequences. In addition to data storage, some genome databases can perform gene expression profiling to predict gene expression patterns at the transcript level in cells or tissues. Using in silico genomic technologies, researchers can identify disease resistance genes, enzymes, and their respective transcription factors that play roles in defence mechanisms against stress. (14)

For instance, Xu *et al.,* conducted large-scale transcriptome sequencing of chrysanthemum plants to study dehydration stress. They developed an online database called the Chrysanthemum Transcriptome Database (http://www.icugi.org/chrysanthemum) to store and distribute transcriptome sequences and analysis results among the research community. With the aid of various protein databases, researchers can predict the biochemical pathways and kinase activities of chrysanthemums in response to dehydration stress. Xu *et al.,* also identified 306 transcription factors and 228 protein kinases as important upstream regulators in plants encountering various biotic and abiotic stresses. (14)

Bioinformatics Approaches to Study Resistance to Plant Pathogen

One of the major challenges in modern agriculture, given the growing global population, is crop loss due to diseases. Studying plant pathogens is crucial for understanding plant diseases, including pathogen identification, disease etiology, disease resistance, and economic impact. Plants defend themselves through a complex system against various pathogens, including insects, bacteria, fungi, and viruses. Plant-pathogen interactions involve the detection of pathogen-derived molecules, such as proteins, sugars, and polysaccharides, by pattern recognition receptors (PRRs) within the plants. Once these molecules are recognized, signal transduction occurs, and the plant's immune system responds through different pathways involving various genes.

According to Schneider *et al*., the development of molecular plant pathology can be divided into three eras. The first era, from the early 1900s to the 1980s, focused on disease physiology. The second era concentrated on molecular plant genetics, emphasizing one or a few genes of bacterial pathogens. The third era, starting in 2000, began with plant genomic studies and the sequencing of the first complete genome of a bacterial pathogen, Xylella fastidiosa. Recent advances in DNA sequencing technologies have allowed researchers to study plant immune systems at the genomic and transcriptomic levels. (15) Genomics has unveiled the complexity of phytopathogens, providing detailed information about plant-pathogen interactions.

The application of various bioinformatics tools has given researchers a clearer understanding of plant-pathogen interactions in terms of transcriptomics and proteomics, facilitating the engineering of disease resistance in plants.

Bioinformatics Can Be Applied to Breed Germplasm with High Yield and Quality

Bioinformatics can be utilized in crop breeding to enhance yield and quality. (Figure-1) (16) By analysing genes related to seed germination, seedling growth, and reproductive yield, and through targeted manipulation of these genes, crops can be improved. For instance, the adaptability, yield, and quality of rapeseed (*Brassica napus*) have been genetically enhanced through breeding efforts. Additionally, bioinformatics can be employed to determine the optimal leaf angle for maximizing photosynthetic rates, thereby creating plants with ideal leaf angles to increase the accumulation of organic matter.

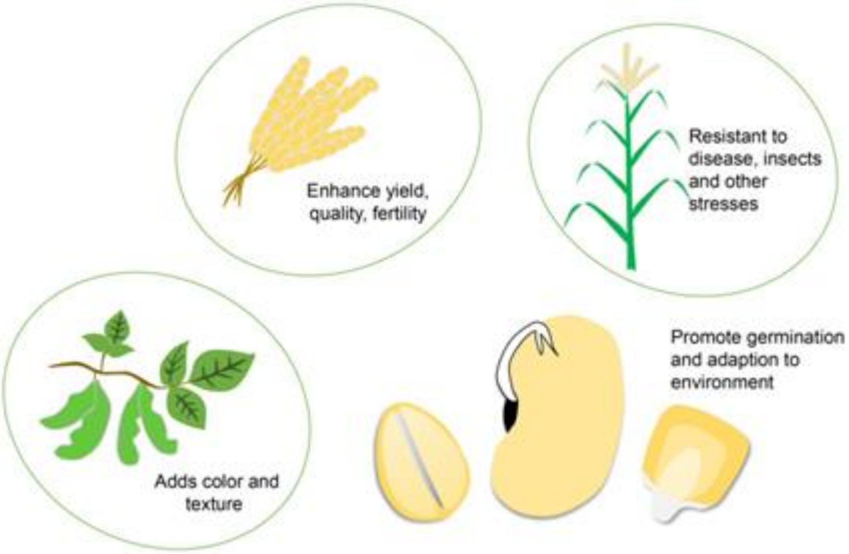

Figure 1: Breeding indicators that can be improved using bioinformatics. Breeding aims to integrate various indicators such as yield, quality, fertility, disease resistance, insect resistance, collapse resistance, as well as salt resistance and adaptability to adverse environments such as drought, waterlogging, high temperature, low temperature, and salinity to achieve superior varieties.

Accurate Prediction of Experimental Results and Transgenic Phenotypes

In plant research, genotype-phenotype prediction has traditionally relied on statistical methods. For instance, autoregressive (AR) and Markov chain (MCMC) models have been used to predict plant growth trends using the Normalized Difference Vegetation Index (NDVI). (17) However, the application of machine learning to genotype-phenotype prediction offers significant advantages. Machine learning can distinguish between different types of genomic regions and predict the location of genomic crossovers, thus extending its application to population genetics.

Plant breeders increasingly utilize genomic selection, which involves selecting favourable alleles at specific loci. This process requires mapping and localizing quantitative trait loci (QTL) to describe the genetic architecture of traits and identify the causal alleles. For example, QTLs have been identified in durum wheat for traits such as protein grain content, high grain yield, disease resistance, and quality traits. In Chrysanthemum, QTL analysis has identified loci controlling flower colour, flowering time, ray floret number, and disc floret number. Similarly, seven QTLs affecting tuber shape have been detected in potatoes (*Solanum tuberosum*). In maize, QTLs have been described for insect resistance and multiple drug resistance, which relates to disease resistance research. QTLs for thrips resistance in pepper (*Capsicum annuum*) and for the genetic basis of cooking time and protein concentration in dried beans (*Phaseolus vulgaris* L.) have also been reported.

While machine learning methods for QTL localization are still limited, they are primarily used for pre-screening. Alternatively, deep learning has been successfully applied to plant phenotype identification. For example, a Convolutional Neural Network (CNN) has been used to detect and classify spikes and spikelet in wheat images, aiding in the study of plant development.

Multiple Genes can be Merged to Analyse their Roles in Various Resistance

Ab initio methods are a crucial research area in bioinformatics, with many prediction algorithms and corresponding procedures having been developed and applied. Unlike homology-based comparison methods, ab initio prediction methods rely on the statistical characteristics of coding regions and gene signals to predict gene structures.

The introduction of exogenous DNA into plant genomes has significantly advanced both basic and applied plant research. Plant genome engineering can be utilized to modify plant metabolism, produce desired metabolites, and improve crop traits. Various transformation methods and strategies have been developed to enable the simultaneous production of multiple plant- or non-plant-derived recombinant proteins in transgenic plant hosts.

Future research on enhancing plant stress resistance should focus on integrating multiple approaches, such as introducing multiple genes simultaneously into transgenic plants. One alternative to stacking multiple genes in transgenic plants is the use of iterative or serial transformation strategies. These involve introducing genes of interest one at a time through successive rounds of transformation or through the sexual crossing of transgenic lines, each carrying different transgenes, to combine them in the same genetic background. (18) For example, genes involved in osmoprotectant biosynthesis have been co-expressed with other stress resistance-related genes, such as ion transporters and transcription factors.

The Role of Model Organisms

Over the past century, research on a select few organisms has significantly advanced our understanding of numerous biological processes. This is because many biological aspects are similar across most organisms, but it is often easier to study certain aspects in specific organisms. These extensively studied organisms are referred to as model organisms because they possess characteristics that make them ideal for laboratory study. The most popular model organisms offer significant advantages for experimental research, such as rapid development, short life cycles, small adult sizes, easy availability, and tractability. They become even more valuable when many scientists work on them, allowing for a large amount of information to be derived, which provides valuable data for understanding normal human or crop development, gene regulation, genetic diseases, and evolutionary processes.

Comparing the genome sequences of rice and Arabidopsis suggests that extensive, but complex, patterns of synteny will be a useful feature in plant genomics. Medicago (alfalfa), a true diploid, plays a crucial role in fixing soil nitrogen and is a major component of forage diets. Other grasses and legumes are subjects of extensive EST sequencing and high-resolution genetic map construction, including radiation hybrid mapping, to leverage the expected pervasive synteny within these families. Web sites established by individual research groups integrate research efforts globally. Useful sites include the UK CropNet, the U.S. Agricultural Research Service's site, and organism-specific resources such as MaizeDB. These sites aim to link seed stock and genetic resources to virtual data on linkage and mapping, supported by search engines and increasingly sophisticated relational databases.

In the 1980s, it became clear that significant investments in studying various plants, such as corn, oilseed rape, and soybean, were diluting efforts to fully understand the basic properties of all plants. Scientists realized that the ambitious goal of completely understanding plant physiology and development could best be achieved by focusing on a model plant species. Fortunately, because all flowering plants are closely related, sequencing the genes of a single representative plant species provides much knowledge about all higher plants. Similarly, discovering the functions of proteins in a model species offers insights into the roles of proteins in all higher plants.

Arabidopsis thaliana has become the universally recognized model plant for study. This small flowering plant belongs to the Brassica family, which includes broccoli, cauliflower, cabbage, and radish. Although non-commercial, it is favoured among basic scientists because it develops, reproduces, and responds to stress and disease similarly to many crop plants. Systematic studies of Arabidopsis are expected to provide significant advantages for basic research in genetics and molecular biology, illuminating many aspects of plant biology that are valuable to agriculture, energy, environment, and human health. Several reasons have made Arabidopsis the preferred organism for basic studies of the molecular genetics of flowering plants. (19)

Conclusion:

In the era of big data, bioinformatics offers both opportunities and challenges for its application in agriculture. Developing and learning more bioinformatics tools will help integrate existing bioinformation resources, supporting effective breeding and plant resistance analysis.

Food production systems are under immense pressure due to the growing human population. Many ecosystems are already overexploited, making it impossible to meet the increasing food demand by expanding arable land. According to the Food and Agriculture Organization (FAO), only 10% of future agricultural production growth will come from expanding acreage, while the remaining 90% must come from yield increases. Genomics technology has provided significant support for breeders, enabling them to develop new varieties that are more adaptable and higher yielding, thereby continuously improving the seed replacement rate.

The era of bioinformatics, initiated by next-generation sequencing (NGS), has revolutionized experimental design in molecular biology, significantly contributing to scientific knowledge and various agricultural applications. Data from different research areas support the co-development and advancement of molecular knowledge through extensive efforts, with bioinformatics as the driving force. The organization, detection, integration, and sharing of data are facilitating multidisciplinary interactions, expanding resources, and disseminating common methods. Bioinformatics is transforming agricultural practices and production by providing knowledge and tools to enhance product quality and develop strategies to counteract environmental stresses, diseases, and pests. As bioinformatics continues to evolve, its integration into plant research holds great promise for the future.

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TOLERANCE OR RESISTANCE BREEDING: PATH FORWARD FOR TO TACKLE ABIOTIC STRESS

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Abstract:

Abiotic stress, encompassing drought, salinity, extreme temperatures and soil pollution, poses significant challenges to global agriculture, resulting in substantial yield loss and threatening food security. The long-term goal of enhancing crop tolerance to abiotic stress is crucial for breeders. The global population is expected to grow by 1.8 billion by 2030 and 2.5 billion by 2050. Therefore, food demand is projected to rise by 50% by 2030 and potentially double by 2050. This necessitates a 70% increase in world food production with significant emphasis on the developing world. Advances in breeding techniques, particularly genomics, have accelerated the development of crop varieties with enhanced stress tolerance. Recent studies highlight the role of genetic, epigenetic, transcriptomic and metabolomic mechanisms in enabling crops to adapt to unfavourable conditions. Innovative applications such as genome-wide association studies (GWAS) and marker-assisted selection (MAS) have proved effective in identifying quantitative trait loci (QTL) linked to abiotic stress tolerance. Furthermore, the integration of multi-omics approaches has facilitated a comprehensive understanding of the underlying biological pathways paving the way for the design of robust, climate-resilient crop varieties. Collectively, these advancements highlight the essential need for continued research and innovation in breeding strategies to combat the escalating impacts of climate change on agriculture.

Keywords: Abiotic Stress, Genomics, Marker-Assisted Selection (MAS), Genome-Wide Association Studies (GWAS), Climate Resilience, Multi-Omics Approaches, Food Security

Introduction:

Anthropogenic activities such as increased emissions and deforestation, is responsible for ecological imbalance. Due to climate change, land degradation, pollution and declining water quality agriculture is negatively impacted (Springmann *et al.,* 2018). Abiotic stress refers to the adverse effects imposed by non-living environmental factors such as heat, cold, drought, salinity, waterlogging, heavy metal toxicity, nutrient deficiency and oxidative stresses. In India, 67% of agricultural land is rainfed, frequently facing varying degrees of drought. Although 33% of the cropped area is irrigated, it still suffers from environmental stresses such as extreme temperatures (Shanker and Venkateswarlu 2011). These stresses significantly impede agricultural productivity by adversely influencing plant physiology and metabolism (Tester and Langridge, 2010; Witcombe *et al.,* 2008). Drought, salinity, and temperature extremes greatly affect the geographical distribution of crops and restrict their productivity. Around 40% of the global land area is affected by drought and 7% by salinity, making these the primary environmental factors for crop losses (Trenberth *et al.,* 2014). Climate change has heightened the frequency of adverse events, often resulting in the simultaneous occurrence of multiple abiotic stresses, which exacerbates their negative impacts. Addressing crop losses caused by environmental stressors is crucial to meet the growing food demand.

Given that agricultural productivity is not keeping pace with the rising population demand, this presents a significant challenge. Researchers focusing on stress tolerance are vital, not only because agricultural production must meet the rising demand from limited resources but also due to potential climate changes making agriculture even more challenging. Tolerance and resistance breeding are pivotal strategies in agriculture aimed at enhancing crop resilience to drought, salinity and extreme temperatures. Tolerance refers to a plant's capability to endure and thrive despite adverse environmental conditions, while resistance often pertains to the plant's ability to withstand specific stressors or reduce their impact. These two approaches can be employed synergistically to ensure robust crop performance in the face of an ever-changing climate.

This chapter explores genetic resources and the evolution of breeding strategies for improving abiotic stress resistance in plants. Tracing the development from conventional methods to cutting-edge techniques. Initially, traditional breeding approaches that rely on selection, hybridization and random mutation methods were utilised for stress tolerance. The discussion progresses to the incorporation of advanced genetic and genomic tools, including marker-assisted selection (MAS) and genetic modification, which have significantly expanded the scope of trait improvement. Recent innovations, such as genome editing technologies (CRISPR/Cas9) and oligonucleotide-directed mutagenesis (ODM) represent a transformative shift in breeding practices. These modern techniques allow precise alterations of stress-responsive genes, leading to enhanced tolerance to drought, salinity and other environmental challenges. This chapter explores aspects of abiotic stresses such as definitions, importance, conventional as well as modern breeding practices and advanced techniques. The chapter aims to provide a comprehensive understanding of how targeted breeding can enhance crop resilience in the near future.

Types of Abiotic Stresses

Abiotic stress are environmental factors which are unfavourable to plant growth. These stresses can cause lasting damage to plants, including stunted growth, disrupted metabolism, decreased yield and altered genetic behaviour, which can result in mutations in the progeny (Zaidi *et al*., 2014; Bhat *et al*., 2020). Each abiotic stress triggers specific physiological, biochemical and molecular response in the plants. Understanding the different types of abiotic stresses (figure 1) and their specific effects on plants (figure 2) is crucial for developing strategies to enhance crop resilience and ensure sustainable agricultural productivity.

1. Drought: Major abiotic stress, characterized by deficiency of moisture in the soil. It leads to reduced water availability for plants. Drought can be classified into four distinct categories. Meteorological drought, which occurs in regions experiencing less rain than usual. Hydrological drought is a result of a lengthy meteorological drought, which lowers the amount of water available, especially in surface and groundwater levels. Drought in agriculture, which lowers soil moisture and affects crop failures and the world's food supply. Drought on a socioeconomic scale, which causes disruptions in the supply and demand of different commodities (Heim, 2002). Stomatal closure, reduced photosynthesis, impaired nutrient absorption and wilting are the effect of drought on plants (Cattivelli *et al.,* 2008).
2. Salinity Stress: Excess Na^+ and Cl^- ions disrupts osmotic balance and causes ion toxicity. High salt concentrations in the soil hinders uptake of water and nutrients by plants. Common effects on plant are leaf scorch, reduced growth and chlorosis due to ion toxicity (Negrão *et al.,* 2017).
3. Heat Stress: Elevated temperatures can cause protein denaturation and impair reproductive success in plant. Reduced yield, flower drop and heat-induced osmotic stress are observed (Bita *et al.,* 2013).
4. Cold Stress: Low temperatures can lead to frost damage and metabolic disruptions in function. Cellular damage, reduced growth rates and potential plant mortality effects plant growth (Sanghera *et al.,* 2011).

5. Flooding and Waterlogging: Excessive water in the soil can lead to oxygen deficiency in roots, hampering plant respiration. It leads to root rot, reduced nutrient uptake and overall plant stress.
6. Heavy Metal Stress: Accumulation of heavy metals (like lead, cadmium and arsenic) in soil can be toxic to plants and disrupt physiological processes. It Inhibits root growth, reduces chlorophyll production and impairs nutrient metabolism.

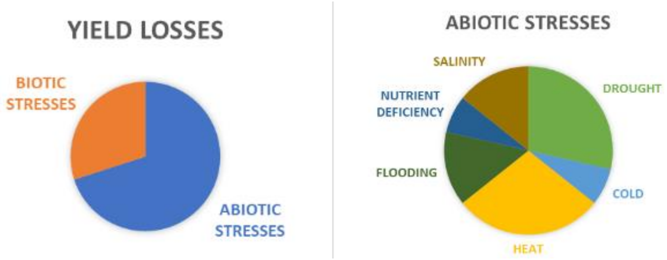


Figure 1: Yield losses in crop plants due to different stresses (Meena *et al*2016)

Mechanism of Plant Response

Plants have evolved a range of physiological, biochemical and genetic pathways to cope with abiotic stresses. Drought conditions, in particular, have led to the evolution of specific adaptive strategies in plants (Zhang *et al.,* 2018). Some species from Cactaceae and Agavaceae families, have developed physical traits such as thick waxy cuticles and long root systems for drought avoidance. Others, like ephemeral plants, complete their life cycle rapidly during favourable conditions to escape drought. True desiccation-tolerant plants, resurrection plants, can survive long periods without water, resuming growth rapidly when rehydrated (Bartels and Hussain, 2011).

Moreover, drought stress can reduce photosynthesis by inducing stomatal closure and inhibiting electron transport chain. However, plants with C4 carbon assimilation or crassulacean acid metabolism (CAM) minimize photorespiration and improve CO2 harvest under such conditions (Ilyas *et al.,* 2021). Adaptive root plasticity, increases water uptake in drought conditions. Hence, physiological responses include the modulation of stomatal conductance to regulate water loss, adjustment of photosynthetic pigments to optimize light capture and alteration of root architecture to enhance water and nutrient uptake (Condon, 2020).

Furthermore, plants maintain cellular homeostasis, protect cellular structures and ensure the continuation of vital metabolic processes under stress. A key biochemical response is the accumulation of compatible solutes like proline, glycine betaine and sugars. These osmolytes, known as osmoprotectants, stabilize cell membranes, protect protein structures and neutralize toxic compounds under stress conditions. For instance, proline and glycine betaine are synthesized in specific pathways and accumulate in plants like maize, rice, spinach and sugar beet to mitigate the effects of drought, salinity and cold stresses (Blum, 2017; Zulfiqar *et al.,* 2020).

Abiotic stresses such as drought, cold, heat and salinity trigger the production of reactive oxygen species (ROS) like hydrogen peroxide (H_2O_2) and superoxide (O_2-), which are highly toxic and can cause severe damage to cell membranes, proteins and DNA potentially leading to cell death. To mitigate oxidative damage, plants activate antioxidant defense mechanisms that include enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), peroxidase (POD), catalase (CAT) and glutathione reductase (GR). Additionally, sugars play a role in quenching ROS and contribute to stress tolerance by being strategically localized within specific cellular compartments or near membranes. Enhancing ascorbate levels in crops, either through increased biosynthesis, improved recycling or modulation of regulatory factors, can boost their tolerance to abiotic stress (Sachdev *et al.,* 2021).

Plants combat heat stress, particularly during summer and midday, by accumulating heat-shock proteins (HSPs). These molecular chaperones, including HSP100, HSP90, HSP70 and others, prevent and repair protein misfolding and aggregation. Overexpression of HSPs can partially alleviate heat stress, but engineering the expression of upstream regulators, such as heat-shock transcription factors (HSFs), offers broader stress tolerance. Additionally, plants produce low molecular weight proteins known as late embryogenesis abundant (LEA) proteins. These proteins, rich in glycine and lysine, protect against subcellular damage from drought, salinity, and cold stress by stabilizing membranes and preventing protein unfolding. LEA genes are activated through both ABA-dependent and ABA-independent pathways, as seen in Arabidopsis genes RD29A and RD29B (Lim *et al.,* 2015).

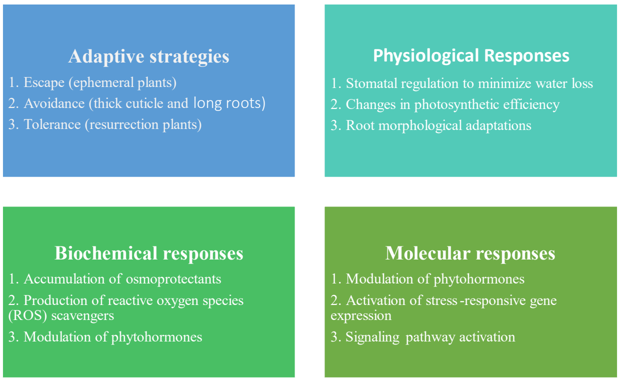
Water transport is crucial for plant growth and aquaporins regulate water movement across cell membranes under both normal and stressful conditions. These proteins, part of the major intrinsic protein (MIP) superfamily, also transport small solutes and gases. Salt stress disrupts plant growth by due to the accumulation of Na^+ and Cl^- ions. Plants manages by either extruding excess Na^+ from cells or compartmentalizing it in vacuoles. Key transporters, such as the HKT1 and ATK1 symporters for K^+ uptake and the

SOS1 antiporter for Na^+ extrusion maintains ion balance, enabling plants to tolerate salinity (Yepes-Molina *et al.,* 2020).

Abiotic stress triggers complex signalling cascades in plants, integrating hormonal and environmental cues into multistep phosphorelay pathways. These pathways are initiated by membrane or intracellular sensors that cause shifts in intracellular Ca^{2+} levels and the production of secondary messengers like inositol phosphate (IP) and reactive oxygen species (ROS). This leads to the activation of various protein kinases, such as calcium-dependent protein kinases (CDPKs) and mitogen-activated protein kinases (MAPKs), which phosphorylate specific transcription factors to regulate stress-responsive genes. Key plant hormones, including abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), cytokinins (CK) and ethylene (ET) interact with transcription factors like MYB, WRKY and DREB to modulate stress responses. ABA plays a central role, particularly in response to drought and salinity, by regulating root growth and stomatal closure. JA also contributes by protecting cells from the toxic effects of salt and drought stresses (Khan *et al.,* 2018; Mostofa *et al.,* 2018).

Recent advances highlight the role of novel plant growth regulators and small molecules in stress signalling. For example, 5-aminolevulinic acid (ALA) enhances stress tolerance by promoting antioxidant synthesis, while nitric oxide (NO) and melatonin act as signalling molecules that improve stress responses and overall plant health. Epigenetic modifications, such as DNA methylation and histone acetylation, also play a crucial role in regulating gene expression under stress conditions. These modifications, along with long non-coding RNAs (lncRNAs) and microRNAs (miRNAs), fine-tunes the plant response to environmental challenges (Rhaman *et al.,* 2021; Arnao *et al.,* 2019).

Rhizosphere microorganisms play a crucial role in enhancing plant growth and abiotic stress tolerance. These microbes interact with plants to modulate phytohormone levels and gene expression, leading to the accumulation of osmolytes, nutrients and antioxidants, as well as the activation of proton transport and ion compartmentalization pathways. Plant growth-promoting rhizobacteria (PGPR) can produce phytohormones like indole-3-acetic acid (IAA) and cytokinins, which stimulate root and shoot growth. PGPR also enhance drought tolerance by increasing ABA levels, which boosts osmoprotectant production, K+ accumulation and ROS scavenging. Additionally, PGPR containing 1-aminocyclopropane-1-carboxylate (ACC) deaminase reduce ethylene levels and promote growth under salt stress. Soil microbes like Trichoderma and arbuscular mycorrhizal (AM) fungi further mitigate stress by improving root growth, membrane stability, water and nutrient uptake, and photosynthesis through the activation of aquaporins and membrane transporters and the accumulation of osmolytes (Singh *et al.,* 2022).



Adaptive strategies
1. Escape (ephemeral plants)
2. Avoidance (thick cuticle and long roots)
3. Tolerance (resurrection plants)

Physiological Responses
1. Stomatal regulation to minimize water loss
2. Changes in photosynthetic efficiency
3. Root morphological adaptations

Biochemical responses
1. Accumulation of osmoprotectants
2. Production of reactive oxygen species (ROS) scavengers
3. Modulation of phytohormones

Molecular responses
1. Modulation of phytohormones
2. Activation of stress-responsive gene expression
3. Signaling pathway activation

Figure 2: Types of Plant Responses to Abiotic Stresses (Villalobos-López *et al.,* 2022)

Genetic Resources for Abiotic Stress Resistance

Abiotic stress resistance in crops is derived from various genetic sources including landraces, wild relatives, cultivated varieties and advanced breeding materials. These sources offer valuable traits for breeding programs aimed at improving crop resilience to challenging environmental conditions such as drought, salinity and extreme temperatures.

1. Cultivated Varieties: Many existing high-yielding varieties and breeding materials already exhibit significant genetic variation for abiotic stress tolerance, particularly for drought and salinity. Breeders often prioritize these sources because they generally present fewer challenges in breeding programs compared to other genetic resources. Utilizing these improved cultivars allows for the relatively straightforward enhancement of stress resistance traits.
2. Landraces: Landraces, which are traditional crop varieties that have adapted to specific environmental conditions, are important sources of abiotic stress resistance. These varieties have evolved under natural selection pressures in their native environments, making them valuable for breeding programs. However, breeding with landraces can be challenging due to the presence of undesirable traits linked to the stress resistance genes. Therefore, breeders typically explore landraces only after exhausting the genetic variability available in elite breeding materials and cultivated varieties.
3. Wild Relatives: Wild species and progenitors of cultivated crops are critical reservoirs of genes for abiotic stress tolerance. These species have naturally adapted to various environmental stresses and often carry unique alleles that are no

longer present in modern cultivated varieties. However, incorporating stress tolerance traits from wild relatives into crops can be complicated due to issues such as cross-incompatibility, hybrid inviability, and the linkage drag. Despite these challenges, wild relatives are invaluable for introducing novel resistance traits into cultivated crops. For example, wild species like *Aegilops* and *Agropyron* have been utilized as sources of drought and salinity resistance in wheat, while *Porteresia coarctata* has been used for salinity resistance in rice. In sugarcane, *Saccharum spontaneum* provides resistance to both drought and salinity. Similarly, wild relatives of sunflower, such as *Helianthus argophyllus* and *Helianthus debilis*, have been identified as sources of drought and salinity tolerance. These wild species offer a diverse genetic pool that breeders can tap into to develop crops better suited to withstand abiotic stresses.

4. Mutant Populations: Another valuable source of abiotic stress resistance comes from mutant populations, which are generated through the application of mutagens like gamma radiation or EMS. These mutations can lead to the creation of new alleles associated with stress tolerance. Techniques like TILLING (Targeting Induced Local Lesions IN Genomes) are used to identify mutants with desirable traits, providing a platform for functional genomics and breeding of stress-resilient crop varieties. For instance, TILLING has been successfully employed in crops like wheat, rice, and soybean to enhance their tolerance to abiotic stresses.

Breeding for Abiotic Stress Resistance

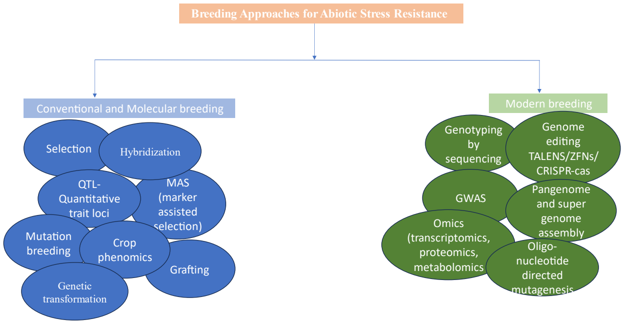


Figure 3: Breeding for Abiotic Stress Resistance

Conventional and Molecular breeding

Conventional approaches have been pivotal in developing crop varieties that can tolerate abiotic stress. This process leverages natural genetic variation found in germplasm collections, wild relatives and landraces to improve stress tolerance. Selection is based on evaluating genotypes under stress conditions in either field trials or controlled environments. Here, we explore the primary conventional breeding methods (as in figure 3) used to enhance abiotic stress tolerance.

Hybridization and Selection

Hybridization involves crossing two genetically distinct parent plants to create progeny with a mix of their traits. This method aims to combine desirable attributes such as high yield and abiotic stress resistance into a single genotype. The effectiveness of hybridization depends on selecting parents with complementary traits and significant genetic diversity, which increases the likelihood of obtaining superior offspring. Various selection methods, including pedigree selection, bulk selection and single seed descent are employed to identify the best-performing individuals under stress conditions.

Mutation Breeding

Mutation breeding uses physical or chemical mutagens to induce random genetic mutations in crop species. The resulting mutants are screened for traits like enhanced abiotic stress tolerance, and promising individuals are selected as breeding parents. This approach can generate novel alleles and traits not present in existing germplasm. However, because mutations are random, most are either deleterious or neutral with respect to the trait of interest. Therefore, large mutant populations and efficient screening methods are necessary to identify beneficial mutations.

Marker-Assisted Selection (MAS)

Marker-assisted selection (MAS) utilizes molecular markers, specific DNA sequences linked to genes or quantitative trait loci (QTLs) controlling desirable traits. MAS allows breeders to select for traits indirectly by identifying these markers, which can significantly reduce the need for extensive phenotyping under stress conditions. The success of MAS hinges on the availability of high-quality markers linked to target traits and requires constructing genetic linkage maps and mapping QTLs for stress tolerance. MAS enhances selection accuracy, shortens breeding cycles and enables the selection of traits that are challenging or costly to phenotype directly (Oladosu *et al.,* 2019).

Integration of Molecular and Phenotypic Approaches

Modern crop breeding incorporates molecular markers like RFLP, SSR, AFLP, RAPD and SNP, which facilitate the development of trait-linked markers. The process of generating these markers involves sequencing segregating populations and using reliable phenotyping methods. MAS can reduce the time and resources required for breeding by focusing on genotypic information rather than extensive phenotypic evaluation. This method is particularly useful for traits with low to moderate heritability and high selection intensity.

Quantitative Trait Loci (QTLs) and Phenomics

Abiotic stress tolerance and yield are often governed by multiple genes, or polygenes, regulated by environmental factors. These quantitative traits are linked to specific loci known as quantitative trait loci (QTLs). Advances in genomics have allowed for the cloning of important QTLs and the shift from single-marker to interval mapping using multiple marker-based approaches. Comprehensive QTL information is available through public databases, aiding plant breeding efforts (Liu *et al.,* 2021).

Crop phenomics is an emerging field that utilizes high-throughput technologies to gather extensive phenotypic data on crop morphology, structure and physiological status. These technologies are crucial for accelerating genetic gains in breeding programs. However, integrating and analysing large datasets from diverse sensors remains a challenge for optimizing breeding strategies (Zhao *et al.,* 2019).

Grafting

Grafting is a specialized form of asexual plant propagation where a portion of one plant (the scion) is joined with another plant (the rootstock). The two parts grow as a single plant with a unified vascular system. Typically, the scion and rootstock come from different plant varieties, allowing the combination of their desirable traits. The grafting process involves establishing tissue connections at the grafting points, leading to dynamic cell division that forms a callus and common cell wall, and finally results in a unified vascular system. Grafting is extensively used for propagating important fruit crops such as apples, avocados, peaches, citrus fruits, apricots, cherries, plums, and almonds, as well as vegetable crops (Tsaballa *et al.,* 2021).

The trans-grafting method is a notable advancement, combining traditional grafting techniques with genetic engineering. This method involves grafting a non-genetically modified scion onto a genetically modified rootstock. The scion benefits from the traits conferred by the transgenes in the rootstock, but the resultant fruits do not contain the transgene, thereby avoiding genetic modification in the end product. Another innovation in grafting is micrografting, which involves grafting small shoot apices or lateral buds onto decapitated rootstock seedlings in vitro. This technique allows for precise control and potential improvements in grafting success rates.

While the molecular mechanisms underlying grafting signaling are not yet fully elucidated, recent studies suggest that plant hormones, proteins, epigenetic modifications and various types of RNA play roles in mediating the changes observed in the scion. These insights are crucial for optimizing grafting techniques and improving plant resilience to stress factors (Vidoy-Mercado *et al.,* 2021).

Plant Transformation

Plant genetic transformation involves introducing foreign genes into plants, creating genetically modified organisms (GMOs) with new traits. These transgenics, integrates and expresses these genes within the host plant. Techniques for transformation include:

1. Agrobacterium-Mediated Transformation: This method uses *Agrobacterium tumefaciens* to transfer DNA into plant cells. The Ti plasmid carries the genes into the plant genome. This method is efficient but varies in success depending on plant species and cultivar.
2. Biolistic Transformation (Particle Bombardment): This technique involves shooting DNA-coated gold or tungsten particles into plant cells using high pressure. Unlike Agrobacterium, biolistic does not depend on the plant receptivity and can integrate multiple copies of DNA. It is especially useful for rapid, transient expressions and can target both nuclear and organellar genomes.

Transformation techniques offer a way to introduce only specific genes of interest, minimizing the transfer of unwanted genes. Alternatives like intragenesis and cisgenesis use genetic material from closely related species or varieties and avoid inserting additional sequences (Holme *et al.,* 2013).

Recent advances include tools like the GoldenGate and GoldenBraid systems for efficient DNA assembly and various methods have been developed to facilitate the design and construction of transgenes. The integration of Agrobacterium and plant totipotency has revolutionized plant biotechnology, allowing for precise genetic modifications in a wide range of crop species (Sarrion-Perdigones *et al.,* 2013).

Modern Plant Breeding Techniques

Genotyping-by-Sequencing (GBS) and Omics

Genotyping-by-sequencing (GBS) is a powerful tool for simultaneous marker discovery and genotyping. It benefits from high-throughput, high-resolution and cost-effective DNA sequencing. GBS has made it possible to identify numerous single nucleotide polymorphisms (SNPs) linked to desirable traits, which can be utilized in marker-assisted breeding through genome-wide association studies (GWAS) and quantitative trait locus (QTL) mapping. This technique supports a wide range of applications, including genomic selection (GS), diversity studies and epigenetic research. The GBS process involves digesting genomic DNA with restriction enzymes, ligating it with adaptors and performing PCR amplification before sequencing. This approach is limited by sequencing errors and depth.

With the advent of new genome sequencing technologies, the concept of pangenomes has emerged. Pangenomes encompass the entire genetic diversity of a species, including core and variable genes. Super-pangenomes, which compile data from numerous species. It provides a more comprehensive view and facilitate the development of markers for GWAS and gene discovery, aiding in the improvement of traits like abiotic stress tolerance (Danilevicz *et al.,* 2020).

Additionally, transcriptomic data from pan-transcriptome analyses which compares different transcriptomes within a species, helps identify genes involved in stress responses and secondary metabolite production. Combining data from pangenomes, transcriptomes and QTLs has been effective in pinpointing genes for heat stress tolerance in crops like rice. The integration of various "Omics" approaches (genomics, transcriptomics, metabolomics, proteomics, and phenomics) through systems biology is crucial for understanding complex traits. By combining multiple data types, researchers can gain a holistic view of how biological systems interact with their environment to produce phenotypic outcomes (Zhou and Liu, 2022).

Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs)

Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) were pioneering technologies in the genome editing field. ZFNs, the first generation of genome-editing tools, leverage chimeric nucleases to create double-strand breaks (DSBs) in DNA. They are based on the Cys2-His2 zinc finger domain, which binds to specific DNA sequences and are coupled with the FokI endonuclease. Although ZFNs offer high specificity and efficiency in genome editing, their design and production are complex and costly (Miglani *et al.,* 2020).

Following ZFNs, TALENs have also proved crucial to genome editing. They are made up of a nuclease that causes DSBs at particular DNA locations and a DNA-binding domain that may be customised. Notable applications of TALENs included the development of the first crop with a modified genome and their part in the first human medicine to be effective. Through the aid of certain repeat-variable di-residues (RVDs), they attach to DNA through a core repeat domain where each repetition matches a single nucleotide. This makes it possible to precisely customise the specificity of DNA binding. TALEs employ tandem repeats to bind DNA and control host plant gene expression. They were first discovered in the plant pathogen Xanthomonas. TALENs are often used in pairs to introduce DSBs, which are then repaired either by non-homologous end joining (NHEJ) or homologous recombination (HR). In addition to genome editing, TALENs are useful in the study of epigenetics, gene activation, repression, and modifying the genomes of organismellar cells (mitoTALENs and cpTALENs).

The development of high-throughput cloning techniques like Golden Gate and Gibson assembly has facilitated the creation of custom TALEs, overcoming some of the design challenges associated with ZFNs. Despite their complexity, TALENs offer versatile and effective genome-editing capabilities (Becker and Boch 2021).

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)

The discovery of a distinct adaptive immune mechanism in prokaryotes, such as Escherichia coli, led to the development of the CRISPR-Cas system, which marks a major biological advance over the past twenty years. Using RNA-guided nucleases known as CRISPR-associated (Cas) enzymes, this mechanism, found in around 50% of bacterial genomes and 90% of archaeal genomes, enables these organisms to recall and fight against viral infections. This method gave rise to the CRISPR-Cas9 system, which has been widely used to modify the genomes of many different species, including plants. Components of CRISPR-Cas system consists of the tracrRNA, crRNA and Cas9 nuclease. The two nuclease domains (RuvC and HNH) of the bilobed protein Cas9 cleaves the target and non-target DNA strands respectively. A PAM sequence (usually NGG) next to the crRNA-target sequence is necessary for the recognition of the target DNA. The target DNA is recognised and bound to by the Cas9-tracrRNA-crRNA complex, resulting in DSBs that can be repaired by homologous recombination (HDR), alternative end-joining (alt-EJ) or nonhomologous end-joining (NHEJ) (Jinek *et al.,* 2012).

Recent Developments in CRISPR-Cas Systems

1. Single Guide RNA (sgRNA): A synthetic RNA chimaera that combines tracrRNA and crRNA into a single molecule, simplifying the CRISPR-Cas system for more efficient genome editing.

2. CRISPR-Cas12a and Donor Templates: CRISPR-Cas12a (Cpf1) creates double-strand breaks (DSBs) with sticky ends, facilitating accurate DNA repair when donor templates are used.
3. Base Editing: This technique enables precise nucleotide modifications without creating DSBs. The main types include:
   - Adenine Base Editors (ABEs): Convert adenine-thymine (A-T) base pairs to guanine-cytosine (G-C).
   - Cytidine Base Editors (CBEs): Convert cytosine-guanine (C-G) base pairs to thymine-adenine (T-A).
   - RNA Editing: Tools like REPAIR and RESCUE modify nucleotides at the RNA level, offering transient and reversible gene editing.
4. Prime Editing: A method for targeted DNA sequence alterations without DSBs. It uses a fusion protein consisting of reverse transcriptase and a nickase Cas9, along with a prime editing guide RNA (pegRNA).
5. Epigenome Editing: This approach alters DNA or RNA methylation patterns to modulate gene expression, providing a way to control gene activity without changing the underlying DNA sequence.
6. Tissue Culture-Free Editing: This method involves removing meristems and introducing CRISPR constructs via Agrobacterium, allowing new meristems to carry the genome modifications into the next generation without the need for tissue culture.
7. Tissue-Specific Knockout (CRISPR-TSKO): Enables precise gene editing in specific tissues or cell types, providing targeted functional studies.
8. CRISPR-SKIP: Uses cytidine base editors to induce exon skipping by mutating intron-exon boundaries, which affects RNA splicing and can modulate gene function.
9. CRISPR Start-Loss (CRISPR-SL): Disrupts gene expression by modifying the start codon (ATG) using base editors, thereby preventing the initiation of translation.
10. DNA-Free Genome Editing: This technique utilizes pre-assembled Cas9/sgRNA ribonucleoproteins (RNPs) to edit genes without integrating transgenes into the genome, minimizing concerns over GMO regulations.

Oligonucleotide-Directed Mutagenesis (ODM)

ODM uses oligonucleotides with specific mismatches to guide repair machinery of cell to incorporate desired mutations without integrating the oligo itself into the genome.

ODM enables the creation of custom single-nucleotide polymorphisms (SNPs) without transgenic modifications, making it an attractive option for plant breeding. It has been used to edit the acetohydroxyacid synthase (AHAS) gene in tobacco and maize, conferring herbicide resistance. ODM was used to develop SU Canola™, an herbicide-tolerant variety launched in the USA (2015) and Canada (2017) as a non-GMO crop. ODM has potential applications in chloroplast gene modifications. Ongoing research explores the use of nanoparticles to enhance this process (Sauer *et al.,* 2016).

These advancements in CRISPR-Cas systems and ODM highlight the expanding toolkit available to researchers for precise and efficient genetic modifications in plants.

Comprehensive Overview of Abiotic Stress Tolerance Mechanisms in Rice and Wheat

Using conventional, molecular and cutting-edge methods, major global crops for food security have been greatly enhanced in terms of their ability to withstand abiotic stress. These improvements are outlined in Tables 1 and 2 for rice and wheat, respectively.

Table 1: Abiotic Stress Tolerance in Rice

Breeding Strategy	Genes/Varieties/QTLs	Trait	References
Mutation Breeding (Gamma Irradiation)	MK-D-2, MK-D-3, MR219-9, MR219-4	Drought tolerance	Abdul *et al.,* 2012; Hallajian *et al.,* 2013; Soe *et al.,* 2016; Efendi *et al.,* 2017
MAS and QTLs	DRO1	Deep rooting and Drought	Choudhary *et al.,* 2019
QTLs	Saltol	Salinity tolerance	Singh *et al.,* 2018
QTLs	TT1, qPSL^{ht}4.1, qSTIPSS9.1	Thermotolerance, Spikelet fertility and spikelet sterility	Li *et al.,* 2015
GWAS	LOC_Os10g34840	Cold tolerance	Xiao *et al.,* 2018
QTLs/Genes	SUB1, qTIL1, qTIL12, qNEI12, qLEI12	Submergence tolerance	Choudhary *et al.,* 2019
Genes	IR64-Sub1, qDTY12.1, qDTY3.1	Multiple abiotic stress tolerance	Mohd Ikmal *et al.,* 2021

Overexpression	PcCFR, PEPC, PPDK, NADP-ME	Photosynthesis-Related Genes	Yadav and Mishra 2020
Osmolyte-Related Genes	ADC, DSM2, OsOAT, OsTPS1, TPSP, P5CS	Enhances tolerance to drought, cold and salinity	Oladosu *et al.,* 2019
Transcription Factors	ABF3, AP37, OsbZIP23, OsMYB48-1, SNAC1, ONAC045, DREB1A, OsWRKY11	Tolerance to multiple abiotic stresses	Darwish *et al.,* 2021
Transgenics (Overexpression and knockout)	hsp101, mtHsp70, OsWRKY11, ZFP	Heat Tolerance	Zafar *et al.,* 2018
CRISPR-Cas9 Edited	OsPIN5b, OsPYL9, OsSAPK2, GS3, OsAnn3, OsMYB30 OsPYL9, OsERA1,OsSRL1,OsSRL2, DST and OsmiR535	Cold, drought and salinity tolerance	Kumar *et al.,* 2020
Effective Microbes	*Bacillus haynesii, Pseudomonas putida* and *Trichoderma harzianum*	Microbial inoculants improving growth under drought, salinity and flooding conditions	Joshi *et al.,* 2020; Singh *et al.,* 2020

Table 2: Abiotic Stress Tolerance in Wheat

Breeding Strategy	**Genes/Varieties/QTLs**	**Traits**	**References**
Varieties and landraces	Aka Komugi, Creole, Triticum boeoticum, Kauz, Ningchun 47, Nesser, NI-5439, WH-1021, HD-2733, Chakwal-86	Drought tolerance traits	Khadka *et al.,* 2020; Barakat *et al.,* 2015; Malik *et al.,* 2015; Goel *et al.,* 2020

Wild Relatives	Agropyron elongatum, Aegilops umbellulata	Drought and salt tolerance	Placido *et al.,* 2013
Mutation Breeding	Binagom-1 and L-880	Salinity tolerance	Jankowicz-Cieslak *et al.,* 2017
Transgenics or Overexpression /Genes	P5CS, mtlD, BADH, TaFER-5B Ferritin, SeCspA, HVA1, AISAP, TdPIP2, TaPYL4	Drought, salinity and other abiotic stresses	Jeyasri *et al.,* 2021; Maghsoudi *et al.,* 2018
Photosynthesis-Related Genes /Overexpression	PEPC, PPDK, 1-FEH w3	Drought, high-temperature tolerance and increased yield in transgenic wheat	Qin *et al.,* 2016
Transcription Factors Overexpression/ modulation	SNAC1, TaNAC69, TaWRKY2, AtWRKY30, TaERF3, AtDREB1A, GmDREB1, TaDREB3, TaCBF5L, HaHB4, AtHDG11, TaSHN1, TabZIP2, TaNF-YB4	Improved tolerance to drought, salinity, and low temperature	Gao *et al.,* 2018; Cui *et al.,* 2019; Baillo *et al.,* 2019
Post-Translational Regulation/over expression	AtOTS1, TaPEPKR2, TaCIPK23	Drought, osmotic and heat stress tolerance through post-translational modifications	Le Roux *et al.,* 2019; Zang *et al.,* 2018
Genome Editing (CRISPR-Cas9)	TaDREB2, TaERF3	Drought tolerance	Kim *et al.,* 2018
Effective Microbes	*Azospirillum brasilenseSp245, Azotobacter chrocoocum(E1)* and *Bacillus amyloliquefaciens5113*	Microbial inoculants used to mitigate abiotic stress in wheat	Kumar and Verma 2018; Etesami and Maheshwari 2018

Conclusions and Future Perspectives

The creation of climate-resilient cultivars or climate-smart crops, is crucial for ensuring sustainable food and energy supplies in the face of climate change. However, the challenge lies in the fact that yield and abiotic stress tolerance traits are often unlinked, making it difficult to select for both traits through traditional breeding methods. Advanced approaches, such as omics technologies and site-directed mutagenesis, offer the potential to enhance stress tolerance in high-yielding lines or to simultaneously select for these traits using these cutting-edge techniques.

Key considerations include:

- **Genome/Epigenome Editing:** Modifying key multi-stress-responsive genes or transcription factors at the genomic or epigenomic level has been demonstrated to enhance tolerance to various stressors. The use of CRISPR/Cas9 for the simultaneous editing of multiple structural and regulatory genes holds promise for developing multi-stress-resilient crops.
- **Organellar DNA Repair Systems:** Altering the expression of genes involved in organellar DNA damage repair can promote more efficient mutagenesis, increase genetic diversity, and improve tolerance to reactive oxygen species (ROS) and oxidative stress.
- **Post-Transcriptional and Post-Translational Regulation:** Future research should emphasize post-transcriptional and post-translational regulators, including the diverse types of long non-coding RNAs (lncRNAs) and the recently discovered glycoRNAs, by integrating multiple omics approaches such as PlantOmics with genome-wide association studies and pan-genomic/pan-transcriptomic strategies.
- **Plant Phenomics:** The application of plant phenomics can accelerate the breeding of stress-resilient cultivars, including their wild relatives, under real field conditions.
- **Signalling Cross-Talk:** Understanding the complex cross-talk among diverse atmospheric and soil abiotic factors—including drought, salinity, nutrient deficiency, and various types of environmental stress—can help target breeding efforts more effectively.
- **Sugar Signalling Pathways:** Given the role of sugar signalling in abiotic stress responses, targeting these pathways may help mitigate the negative feedback effects

of sugars on photosynthesis, leading to stress-tolerant phenotypes and increased crop yields.

- **Resurrection Plants and Microbiomes:** There is significant potential to learn from resurrection plants and their associated microbiomes, which are tolerant to extreme abiotic stresses.
- **Multi-Stress Experiments:** Conducting multi-stress experiments in the laboratory, considering variable stress intensity, timing, and recovery, will provide insights into photosynthesis and growth under stress.
- **Microbiome Enrichment:** Enhancing the seed and soil microbiomes with effective microbe-based inoculants can contribute to the integrated management of crops, helping them withstand multiple stressors.
- **Integration of Molecular Tools:** The integration of all available molecular tools is essential for developing climate-smart crops that do not compromise yield or require additional land.

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EPIGENETICS IN PLANT BREEDING

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Abstract:

Epigenetics, the study of heritable changes in gene function without altering the DNA sequence, is increasingly important in plant breeding and development. Key epigenetic mechanisms, such as DNA methylation and histone modifications, regulate gene expression and are influenced by environmental factors like temperature, light, and nutrient availability. These modifications can be inherited through mitosis or meiosis, contributing to heritable phenotypic variations that extend beyond traditional genetic diversity.

Epigenetic variations are critical in regulating plant traits such as flowering time, yield, disease resistance, and stress tolerance. For instance, DNA methylation controls flowering in *Arabidopsis thaliana* and enhances maize yield by improving photosynthesis-related genes. Histone modifications, like acetylation, promote plant defence mechanisms. These processes provide new opportunities for crop improvement through marker-assisted selection and advanced techniques like CRISPR-Cas9, enabling precise control over gene expression.

The growing global population and shifting dietary preferences pose significant challenges for agriculture, requiring a 70% increase in food production by 2050. Traditional breeding strategies are no longer sufficient. Epigenetics offers a solution by expanding the genetic variation available for breeding, helping plants adapt to environmental stressors through epigenetic reprogramming. Epigenetic priming can also enhance broad-spectrum resistance in crops, providing a fast and cost-effective means of boosting stress tolerance without altering genetic diversity.

Incorporating epigenetic diversity into breeding programs addresses genetic erosion caused by traditional breeding methods, which rely on a limited set of genotypes. Epigenetic modifications reduce this pressure, improving crop resilience while maintaining diversity. Moreover, epigenetics may ease public concerns about genetically modified organisms (GMOs) since it does not alter the DNA sequence directly.

Overall, epigenetics offers a promising framework for enhancing crop traits, improving disease resistance, and supporting sustainable breeding practices in response to global food demand and environmental changes

Introduction:

The study of heritable features, or a persistent alteration in cell function, that occur without alterations to the DNA sequence is known as epigenetics in biology (Brenner *et al.,* 2009). Epigenetics is a crucial aspect of plant breeding, as it plays a significant role in the regulation of gene expression and plant development (Kawakatsu & Ecker, 2019). Epigenetic variations, which are inherited or uninherited effects that occur beyond the DNA sequence of an individual, can influence plant traits and phenotypes (Matzke & Mosher, 2014). These variations can be induced by environmental factors, such as temperature, light, and nutrient availability, and can be inherited through mitosis or meiosis

Epigenetic mechanisms, including DNA methylation and histone modifications, can affect gene expression and plant development. DNA methylation, which involves the addition of a methyl group to cytosine residues in DNA, is a crucial epigenetic mechanism that regulates gene expression in plants (Kawakatsu & Ecker, 2019). For example, DNA methylation has been shown to play a key role in the regulation of flowering time in Arabidopsis thaliana (Berner *et al*., 2012). Histone modifications, which involve the addition of various chemical groups to histone proteins, also play a key role in regulating gene expression (Matzke & Mosher, 2014). Histone modifications have been implicated in the regulation of plant defence responses, with histone acetylation promoting the expression of defence-related genes (Alvarez-Venegas *et al*., 2006). In epigenetics, characteristics that are "on top of" or "in addition to" the traditional (DNA sequence based) genetic method of inheritance are implied by the Greek prefix epi- (ἐπι- "over, outside of, around"). In 2015. Rutherford, A. Epigenetics typically deals with alterations to gene expression regulation that are not reversed by cell division. (Maggert, 2015) Such effects on physiological and cellular phenotypic features could be a byproduct of normal development or the outcome of external impacts. The goal of traditional plant breeding is to collect many desirable and variable alleles to potentially improve desired features. Epigenetics has several applications in plant breeding, including crop improvement, marker-assisted selection, and epigenome editing. Epigenetic variations can be used to improve crop traits, such as yield, disease resistance, and drought tolerance. For example, epigenetic variations have been used to improve the yield of maize by increasing the expression of genes involved in photosynthesis (Li *et al*., 2015). Epigenetic markers can be used to identify genes associated with desirable traits, allowing breeders to select for these traits more efficiently (Kumar *et al*., 2017). Epigenome editing techniques, such as CRISPR-Cas9, can be used to introduce specific epigenetic modifications into crops, allowing for more precise control over gene expression (Liu *et al*., 2019).

In addition to its applications in crop improvement, epigenetics also has implications for our understanding of plant evolution and development. Epigenetic variations can influence the expression of genes involved in plant development, leading to changes in plant morphology and architecture (Kawakatsu & Ecker, 2019). For example, epigenetic variations have been shown to influence the expression of genes involved in root development in Arabidopsis thaliana (Duan *et al.,* 2017). Epigenetics also has implications for our understanding of plant responses to environmental stress, with epigenetic variations influencing the expression of genes involved in stress responses.

Meeting the Needs for Novel Features

Over about 10 000 years of agricultural history, crops have been developed to feed more than seven billion people. The world population is expected to come close to 10 billion by 2050, and a 70% food gap between 2006 and 2050 is estimated. A sustained average annual increase in crop production of 44 million metric tons per year for 40 years is required to meet this demand. At the same time, peoples' diets are changing. Producing gluten free products, plant-based protein, and dairy-free products are a few examples of the new growing nutrition demands. Previous plant breeding strategies, which led to the green revolution in the 1960s–1970s by introducing new strains of rice and other crops, are not promising now with the rate of annual yield saturated at about 1%–2%. The projected food demand, along with the complexity imposed by global climate changes and changing global diet, challenges the effectiveness and efficiency of the current agricultural and breeding strategies; subsequently, breeders are obliged to consider new characteristics for selecting elite crop variation for breeding, which intensifies the demand for a wider source of variation. During adaptation, plants naturally use both genome sequence-dependent (genetic) and -independent (epigenetic) variations to maximize the heritable phenotypic variations to cope with environmental perturbations. Epigenetic changes, such as DNA (de) methylation, histone modification, and chromatin remodelling, by reprograming the transcriptome during plant development and in response to environmental conditions, can widen sources of phenotypic variation (Gallusci *et al.,* 2017) for breeders to utilize.

A Clarific Choice Among Elite Crop Varieties

For a precise selection of elite crop varieties, breeders need to know the sources underlying the formation of phenotypic diversity. Genetic diversity alone is unable to explain the observed inheritance of phenotypic diversity. For example, phenotypic variability of Brassica oleracea (Salmon *et al*., 2008) and the variation of crucial traits for attractiveness to pollinators, i.e., floral scent and morphology, in *Brassica rapa* are explained by epigenetic variations (Kellenberger *et al*., 2016). The phenotype of increased resistance and decreased negative effect of stresses in primed plants pre-exposed to stress conditions are also phenotypic evidence of epigenetic changes. Wheat drought priming at the vegetative stage improves carbon assimilation and nitrogen-use efficiency so the occurrence of drought and heat stresses and their combination at later productivity growth stages results in less yield penalty (Liu *et al*., 2017).

Broad-Spectrum Induced Resistance

Nowadays, the need for broad-spectrum resistance of crops is more urgent than ever due to the steadily increasing trend in the severity and frequency of the simultaneous occurrence of different biotic and abiotic stresses driven by global climate change, which can potentially cause annual yield losses at billions of dollars worldwide. For example, in canola drought tolerant epilines, genes responsive to salt, osmotic, abscisic acid, and drought stresses expressed differentially, confirming the involvement of the canola epigenome in regulating responses to various abiotic stresses (Verkest *et al.,* 2015). Resistance in Arabidopsis to *Pseudomonas syringae* pv tomato DC3000 bacteria is also increased due to previous exposure to heat, salinity, or cold stresses driven by an epigenetic-dependent mechanism (Singh *et al*., 2014). These observations show epigenetic marks might be a key factor in inducing broad spectrum resistance/tolerance to both biotic and abiotic stress into the crops.

Less Gene Erosion

The main kinds of selection used in breeding programs to discover desired agronomic features are natural and artificial genetic variability. Genetic erosion, a permanent decrease of genetic diversity caused by this breeding selection process based on the use of a restricted set of genotypes (Gallusci *et al.,* 2017). For example, just nine types account for 96% of the pea crop farmed in the United States. Due to their limited genetic foundation, modified cultivars are more susceptible to sudden changes in climate as well as new pests and diseases. In addition, intense breeding can result in unintentional disappearance of desirable traits. Maize domestication is an example when a mutation in the acyl-CoA:diacylglycerol acyltransferase (DGAT) gene caused the elimination of healthy oleic acid from oil production (Palmgren *et al*., 2015). The addition of epigenetic diversity as a source of variation will better explain the source of observed phenotypes and will increase the accuracy of the breeding selection process. When epigenetic modifications can produce desirable phenotypes, enforced selection of specific gene/s is not required. This reduces the pressure of basing selection on only genetic diversity (Gallusci *et. al.,* 2017) and results in less genetic erosion.

Harmony Among Essential Agronomic Characteristics

Utilizing epigenetic variation, plants may precisely and timely rewire their transcriptome to balance crucial agronomic parameters. For instance, in rice, epigenetic modulation can counteract the yield penalty and gene-mediated resistance to blast disease (Deng *et al*., 2017). According to Raju *et al.,* (2018), in soybeans, epigenetic processes can mitigate the effects of environmental factors and produce production stability in a variety of settings. Abiotic stresses have a significant impact on fertilization, one of the most important stages in the formation of seeds in maize. Research indicates that epigenetic processes connected to stress and pollination can control each other similarly. (Begcy and Dresselhaus, 2018). An innovative breeding approach to more effectively manage the damage caused by stressors during seed development toward yield stability may be the characterization and application of epigenetic pathways.

A Different Way to Control Widespread Damage

The era of epigenetic epidemiology, the study of the association between epigenetic variation and the risk of disease, is increasing in medical research. In humans, for example, the role of epigenetic changes in allergen susceptibility and immunity development has been proven, which can lead to arresting or reversing an allergy epidemic (Prescott and Saffery, 2011). Similarly, in plants, there are several pathogens and host traits associated with disease epidemics that may be regulated epigenetically. The occurrence of plant disease epidemics depends on both environmental conditions and plants susceptibly. The environmental conditions are also a key regulatory factor of epigenetic marks, which themselves. Epigenetic Diversity Contribution to Plant Breeding Schemes. The eight contributions listed in the green circle correspond to the section headings in the text. can alter plants' vulnerability to pathogens. Plant epigenetic epidemiology, by investigating the association of epigenetic variations with disease epidemics, may lead to a revolution in managing plants disease epidemics.

The Time and Cost Money Saved

By creating a wide range of induced resistance without affecting genetic variety, priming through the application of epigenetic memory offers a quick and affordable way to increase plants' resistance against novel and severe future shocks. Li *et al.,* (2019) used a cycle of mild drought and re-watering treatment to show how DNA methylation contributes to increased drought tolerance in primed rice seedlings. This emphasizes how epigenetic differences may benefit breeding initiatives.

Ease Public and Producer Acceptance

All crop enhancement strategies must have the financial and non-financial backing of the public, farmers, and the government to be implemented successfully at the DNA level. The difficulties in getting the public to accept items that have undergone epigenome editing may be lessened by the fact that this process does not alter the genome sequence. Conversely, epigenetic-dependent phenotypes are not exclusively reliant on DNA sequence, which poses a challenge in studying their transgenerational behaviour because of their reliance on the mode of plant proliferation (sexual versus clonal). Because of the possibility of erosion during meiosis, histone PTMs—post-translational modifications—are especially helpful for clonally propagated crops like potatoes. Compared to other epigenetic marks, DNA methylation marks exhibit greater stability following both mitosis and meiosis (Gallusci *et al*., 2017). Variations in epigenetics

It's also hard to find heritable epialleles. Natural heritable epialleles are a valuable source of variety, but their production may not keep up with the demands of breeding operations. A number of techniques, including epigenome editing, have been used recently to bring about variety (Gallusci *et al*., 2017; Springer and Schmitz, 2017). Inducing heritable transgenerational gene silence in plants using RNA-directed DNA methylation (RdDM) has been made possible by the effective use of RdDM in potato epigenetic markings (Kasai *et al*., 2016). A boost has been provided to the application of epigenome editing by 1310 Molecular Plant 12, 1309–1311, October 2019 © The Author 2019. The CRISPR-Cas9 method was discovered by Molecular Plant Comment; yet, creating the most effective EpiEffector and determining the ideal arrangement of chromatin changes.

Conclusion:

Epigenetics represents a frontier in plant breeding, offering novel opportunities to enhance crop traits beyond traditional genetic methods. By regulating gene expression without altering the DNA sequence, epigenetic mechanisms like DNA methylation and histone modifications contribute to plant development, stress resistance, and yield improvements. As the global population grows and climate change poses new challenges, the demand for innovative breeding techniques has never been greater. Traditional methods have reached their limits in boosting crop productivity, necessitating new approaches like epigenome editing and marker-assisted selection.

Incorporating epigenetic diversity into breeding programs can help breeders address pressing issues such as broad-spectrum resistance to biotic and abiotic stresses, as well as the mitigation of genetic erosion caused by intensive breeding. This can lead to more sustainable and resilient crops capable of withstanding environmental perturbations. Additionally, epigenetic approaches are more acceptable to the public as they do not directly alter the genome sequence, potentially easing concerns around genetically modified organisms.

Epigenetics thus offers a cost-effective and efficient way to enhance agricultural productivity while maintaining genetic diversity. With advances in techniques like CRISPR-Cas9, the precision of epigenetic modifications can be further refined, leading to improved crop performance and stability in response to environmental changes. As the field progresses, the integration of epigenetic principles into plant breeding will be pivotal in meeting future food demands while ensuring sustainable agricultural practices.

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Amalgamation of Recent Efforts in Plant Breeding & Biotechnology

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