Understanding Modern Techniques and Tools to Revolutionize Plant Breeding for Sustainable Crop Improvement

Muhammad Ahtisham and Zainab Obaid

University of Agriculture Faisalabad, Faisalabad, Pakistan

Correspondence to: Muhammad Ahtisham, ahtishamislam10@gmail.com

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ABSTRACT

The modern era of plant breeding is experiencing a shift from conventional to modern breeding methods, driven by the integration of these modern tools for rapid and sustainable crop improvement. Marker-assisted selection (MAS) helps breeders in the precise selection of traits at the genomic level, hence accelerating crop improvement and improving the efficiency of selection. For quick generational advancement, speed breeding is a great technique, as it significantly reduces the crop maturity cycle with manipulation of photoperiod, temperature, CO_2 concentration, and other factors, hence boosting the process of crop improvement. Big data provided by high-throughput phenotyping enables breeders in precise assessment of large plant populations in breeding programs. CRISPR/Cas9 has also emerged as a great tool for precise editing of a specific region of the genome allowing targeted improvement in plants. Tools such as Artificial intelligence and machine learning have a great potential for analyzing big data produced by these modern tools and in trait prediction as well. The review tends to provide a detailed understanding of these modern tools and their application in plant breeding.

The integrated use of these modern plant breeding tools such as MAS, speed breeding, high-throughput phenotyping, CRISPR/Cas, and artificial intelligence/machine learning with conventional breeding holds the potential to address the problem of future food security, ensuring sustainable crop improvement to feed future generations.

Keywords: Marker-assisted selection, Speed breeding, High-throughput phenotyping, CRISPR-Cas9, Artificial intelligence in plant breeding

Introduction

Owing to severe climatic conditions, farmers worldwide are facing crop yield losses. To feed the ever-growing population of 10 billion by 2050, we must increase the genetic improvement of crops by twofold to overcome these future food challenges. With the ever-increasing demand for food in the future, the current crop cultivars and farming practices will not be able to meet the future food demand.[1] The yield increase in most of the crop varieties is not enough to cope with the population growth rate globally.[2] As conventional breeding methods rely mostly on phenotypic selection and are comparatively slow, the continuously increasing pressure of food due to the growing population has resulted in the development of modern breeding techniques for crop improvement, such as CRISPR/Cas9 genome editing, high-throughput phenotyping (HTP), and marker-assisted selection.[3] Modern plant breeding tools such as CRISPR/Cas9, haploid induction, de novo domestication, and speed breeding integrated with genomic selection are enabling scientists to produce high-yielding and climate-resilient crops in a comparatively short period.[4]

Modern plant breeding tools can be used to transfer genes that are otherwise difficult to transfer using conventional breeding methods, especially to transform genes for disease resistance and bio-fortification, hence allowing rapid crop improvement.[2] Just as the green revolution resulted in increasing the yield of crops in the twentieth century, modern molecular tools are helping plant breeders to produce high-yielding varieties for sustainable food and agriculture. Integration of these modern tools especially in developing countries can be valuable for sustainable crop improvement in these countries.[5]

Despite all these benefits of modern tools, conventional breeding has stayed as an indispensable link between variation in crops and the development of new crop varieties.[6] However, one of the major drawbacks of conventional breeding is its over-reliance on phenotypic selection which involves the selection of plants based on their morphological appearance. Due to this reliance, conventional breeding has struggled to incorporate many genes of agronomic importance and has narrowed the genetic bases of the crops due to continuous phenotypic selection.[7] Keeping in view the need for integration of modern plant breeding tools with conventional breeding, the review tends to explore different modern plant breeding techniques. The review provides a comprehensive overview of the use of genomic selection with molecular markers, high-throughput phenotyping, speed breeding, genome editing, and artificial intelligence as modern tools for sustainable crop improvement in the future.

Modern Tools and Crop Improvement

Genomic Selection/Marker-Assisted Selection

Since the emergence of PCR in 1990 and the first molecular marker system, restriction fragment length polymorphism (RFLP), developed in 1980, marker-assisted selection (MAS) has revolutionized plant breeding by improving the overall precision and efficiency of trait selection using DNA markers. These markers are evolving the conventional breeding, especially for complex trait selection.[8] Many molecular markers have been developed and utilized in genetic improvement in plant breeding all over the world due to their ease of use and affordability.[9]

Molecular markers are classified as dominant or co-dominant based on the nature of gene action and also classified based on the nature of the detection system. Markers that are detected using PCR are called PCR-based molecular markers and the ones that are detected by hybridization are called hybridization-based

molecular markers.[9] There are many marker systems used all over the world but we have discussed the ones that are most commonly used and find broader application in plant breeding.

Hybridization-Based Molecular Markers

These markers are used to detect specific DNA sequences by employing a probe complementary to the DNA sequence and are used mostly in gene identification, mapping, and chromosomal studies, and these markers are comparatively more laborious in contrast to PCR markers.[10]

RFLPIt is the earliest and most well-established marker system used in 1974 for the first time. These are co-dominant markers. They are used to detect polymorphism in the length of DNA fragments after being cut down by restriction enzymes. The fragments of DNA generated by restriction enzymes are then separated on the gel electrophoresis and then transferred to the membrane for labeling using the probe complementary to the specific target sequence, and later the hybridization pattern of the probe is observed on the X-ray film. RFLPs have historically been used in plant breeding and genetic mapping but now are being replaced by PCR-based marker systems.[11]

PCR-Based Molecular Markers

In PCR-based molecular marker systems, specific portion of the DNA is amplified using specific complementary primers. The most commonly used PCR-based markers are as follows:

Randomly Amplified Polymorphic DNA (RAPD)

As the name shows in the RAPD marker system, the short arbitrary primers are used to amplify the random portions of DNA without knowing the prior understanding of the DNA sequence, these are dominant markers and are used for the assessment of genetic diversity in populations but there is an issue of reproducibility of RAPD results due to random use of primers and its sensitivity to PCR.[12]

Short-Sequence Repeats (SSRs)

They are also called micro-satellites and are co-dominant markers. These are short tandem repeats of 1–6 bases in length, and they are highly polymorphic due to a high rate of mutation. Due to its highly polymorphic nature, these markers are very useful in genome mapping population studies, testing, and linkage analysis.[13]

Amplified Fragment Length Polymorphism (AFLPs)

This marker system is a combination of RAPD and RFLP as it combines restriction enzyme breakdown of DNA in small fragments followed by selective PCR amplification. AFLPs are dominant markers and can be used for genetic mapping, population genetics, and in biodiversity studies with highly reproducible results.[14]

Single Nucleotide Polymorphisms (SNPs)

SNPs are abundant forms of variations at the genomic level, This marker system is based upon a variation of single base pair in DNA sequence, making them most informative and abundant among all markers. SNPs are also co-dominant types of markers and are identified using PCR. They are commonly used in genome-wide association studies, MAS, and population genetics due to their high number in the genome.[15]

Variable Number of Tandem Repeats (VNTRs)

This marker system was first developed for the mammalian genome but holds promise in plant breeding today. This marker system is based upon polymorphism due to a VNTR spread throughout the whole genome of organisms. They are classified based upon the size of repeats as micro-satellites of 1–5 bases and mini-satellites of 10–50 bases and are co-dominant (Table 1).[8,16]

Marker-assisted selection in plant breeding offers the potential to improve overall selection efficiency at the early stages of the crop, hence reducing the size of the plant population. However, most of the marker loci are still non-additive and are subjected to the environment, linkage, and pleiotropy. Hence, the efficiency of marker system depends upon a number of factors, and it often becomes difficult to predict genetic gain and response to selection.[17] This predictability of genetic markers depends upon inherent repeatability, linkage with the gene (trait) of interest, and map position.[18]

Speed Breeding

Traditional varieties developed through conventional breeding require the selection of favorable genotypes

Table 1 | Overview of genetic markers discussed in review

Marker	Type	Inheritance	Polymorphism	Applications	References
RFLP	Hybridization-based	Co-dominant	Moderate to high	Genomic mapping, gene identification	Botstein et al.[11]
RAPD	PCR-based	Dominant	Moderate	Genetic diversity, population studies	Williams et al.[12]
AFLP	PCR-based	Dominant	High	Genetic mapping, population genetics, biodiversity studies	Vos et al.[14]
SSR	PCR-based	Co-dominant	High	Genetic mapping, population genetics	Tautz[13]
SNP	PCR-based	Co-dominant	Very high	Genome-wide association studies, population genetics, marker-assisted selection	Brookes[15]
VNTR	PCR-based	Co-dominant	High	Forensic analysis, population genetics	Nakamura et al.[16]

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followed by crossing and several generations of selection to produce varieties of commercial importance.[19] After initial crosses of the parents in the plant breeding program, space, time, and resources are required during a long period of selection and generational advance, speed breeding offers a solution to this problem by reducing the time required for the development of cultivars.[20] In conventional breeding, the slow rate of generational advancement is due to the genetic nature of the crop cycle as most cereals and legumes take 3–6 months to mature, and variable climatic patterns such as low rainfall, high temperatures, and variable day length allow only one cycle of crop per year.[21] This duration of the crop breeding cycle can be reduced using advanced breeding tools such as speed breeding.[22]

Speed breeding is a technique in which the crop cycle is shortened by manipulating different environmental conditions to induce the plant to flower and to produce seed for the completion of the breeding cycle as soon as possible. The method offers a great solution to save resources and time required for each cycle of breeding and can be integrated with modern and conventional breeding methods such as MAS, single seed, pod or plant descent, and clonal selection.

Manipulation of Photoperiod

Each crop species has its specific photoperiodic requirement for the initiation of flowering and seed setting as well.[23] It is very crucial to apply an optimum level of light to artificially induce flowering in plants.[20] The source of light that emits light within the range of 400–700 nm (which is photosynthetically active radiation) and intensity of 60–650 μmol/m^2/s has been used in many crops such as wheat, canola, barley, and soybean.[24] An 18-hour light cycle with a 6-hour dark cycle is ideal for quick vegetative and reproductive growth while keeping stress minimal in lentils and chickpeas.[25] Additionally, an extended duration of photoperiod of 22 hours under light and 2 hours in dark using red-blue lights in a ratio of 5:3, respectively, significantly improves the growth and reduces the flowering time for lentils (36–41 days) and (24–42) days in chickpeas (Figure 1).[26]

Manipulation of Temperature

Temperature also plays a very crucial role in speed breeding as it induces flowering and forces the plants toward maturity.[27] The optimum temperature of around 22°C during the light cycle and cooler temperatures typically 18–20°C during the dark cycles allows plants to efficiently transition through different growth phases, hence reducing the duration of the breeding cycles.[22]

CO_2 and Drought

CO_2 supplementation chambers where the level of CO_2 > 400 ppm also tend to shorten the life cycle of soybean plants from 102 to 132 days to 70 days only. CO_2-enriched environment aided with appropriate use of light and temperature accelerates growth and flowering in plants allowing five generations of soybeans per year.[28] In crops such as wheat, pearl millet, and barley, drought stress is also commonly used to induce early flowering.[29]

Hence with manipulation of these factors such as light intensity, temperature, and other relevant factors speed breeding is enabling the researcher to boost up their breeding programs by completing multiple breeding cycles within a single year.[22] It is a great modern

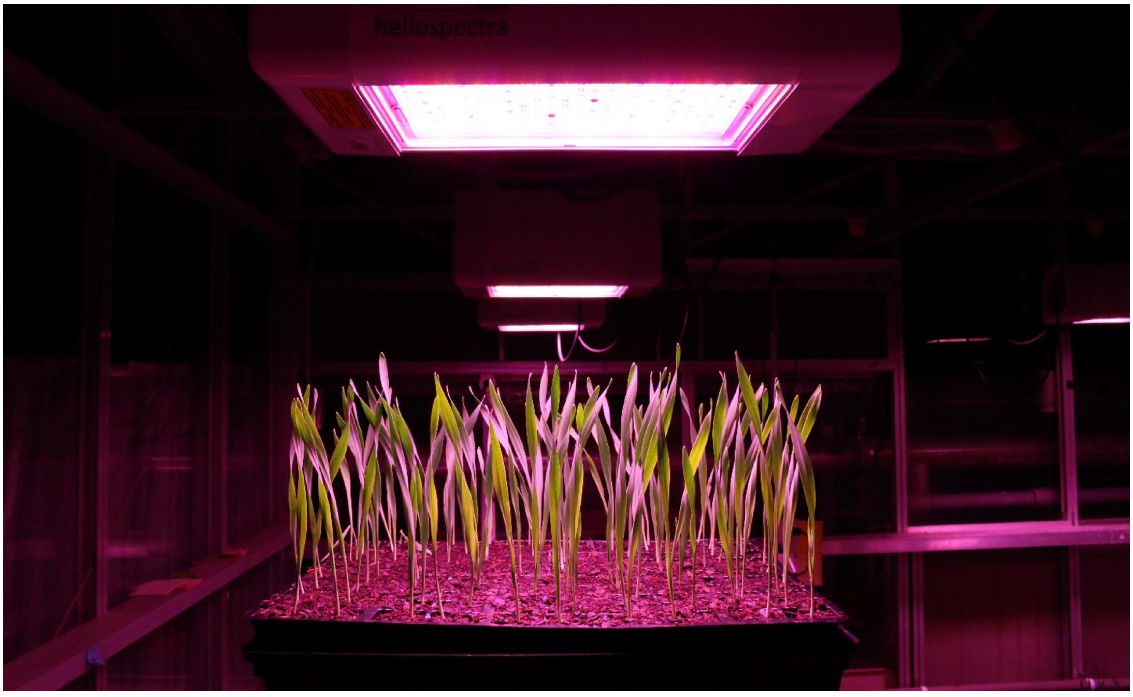

Fig 1 | Speed breeding setup with LED lighting. Attribution: Queensland Alliance for Agriculture and Food

tool for revolutionizing and speeding up the process of sustainable crop improvement.

High Throughput Phenotyping (HTP)

Conventional methods of field phenotyping limit our ability to analyze complex quantitative traits such as yield, drought tolerance, and heat tolerance. HTP is crucial for advancement in the field of plant breeding, especially for field analysis of these quantitative traits. Recent advancements in the fields of remote sensing and data processing have improved the overall efficiency of high-throughput phenotyping. With the integration of automation and efficient data management, HTP can improve the efficiency for analysis of these complex traits revolutionizing plant breeding in the future.[30] As plant breeders want the phenotyping of a large number of populations to identify the best progenies for sustainable crop improvement, with advancement in HTP, breeders now have access to affordable genomic data that enables the mapping of thousands of recombinant inbred lines for phenotyping.[31]

HTP Field-Based Phenotyping and Simulation Modeling Tools

HTP field-based phenotyping can be done using simulation modeling tools, which can help in the estimation of traits such as rooting capacity and canopy conductance. While simulation proximal sensing systems can assist with recording data for spectral reflectance, plant architecture, and canopy temperature, the development of new sensor and imaging technologies can help us in future for field-based phenotyping even for large breeding programs.[32]

Proximal/remote sensing methods are non-destructive and non-invasive.[32] They are mostly based on data recorded from near/visible infrared and far infrared radiations emitted from the surface of the crop.[33] Proximal/remote sensing techniques can be employed for in situ screening against different breeding objectives such as yield, and adaptation to biotic and abiotic stresses.[34] Along with proximal sensing techniques, analyses of plant samples for stable isotopes can also provide information about field phenotyping. For example, for breeding aimed to improve yield potential and drought stress, the carbon isotope δ13C is a very good indicator in plant dry matter and has a strong association with yield of the crop.[35]

HTP has emerged as a modern and effective tool enabling efficient data collection and analysis for traits such as yield or response to biotic and abiotic stress. Integration of HTP with other modern tools and conventional breeding can help in the future to improve the efficiency of the breeding programs, hence contributing to sustainable crop improvement in the future.

CRISPR/Cas9 and Genome Editing Technology

Conventional plant breeding has been extremely successful in producing high-yielding crop varieties. The process of domestication has mostly relied on diversity within populations for plant breeding, and lack of diversity has been a limiting factor in crop improvement.[36] The thousands of years of controlled evolution of crops through plant breeding have resulted in the fixation of major portions of the genomes of the crops consequently leading to a reduction in genetic variability, making improvement of crops difficult. Although mutation breeding is helping to create favorable variation by induction of random mutations using physical or chemical mutagens.[37] The CRISPR/Cas system has a great potential to induce genetic diversity in breeding enabling breeders to modify multiple targets simultaneously in the genome with high efficiency, this ability enables the rapid combination of multiple traits of interest into a single plant within one generation.[36]

Mechanism

The CRISPR/Cas9 system consists of two main components: the Cas9 protein, which acts as a molecular scissor, and a guide RNA (gRNA), which guides the Cas9 protein to a specific site in DNA.[38] Once the binding site is recognized by the Cas9-gRNA complex, it binds to the site and cleaves that specific portion of DNA, consequently triggering the endogenous repair mechanism primarily non-homologous end joining or in some cases homology-directed repair. This repair results in insertions or deletions in that part of the gene leading to disruption of the gene function (Figure 2).[39]

The ability of CRISPR/Cas to edit genomes by creating targeted mutation is helping researchers introduce targeted sequence-specific diversity across plant genomes. The adaptability, reliability, and versatility of using the CRISPR/Cas9 technology make it an exceptional tool for genetic enhancement in crops through gene knockout, knockdown, modification of gene action, and the building of high-throughput mutant libraries.[40] The simplicity of the CRISPR/Cas9 system makes it a great tool for plant breeders to improve crops rapidly and hence avoid long cycles of selection and crop improvement.[41]

Artificial Intelligence and Machine Learning

Artificial intelligence (AI) is the field focused on enabling machines and computer systems to think and act in ways resembling humans.[42] The ability of AI to handle big data analysis and pattern recognition makes it an ideal tool for plant breeding.[43]

Omics technologies are a set of interdisciplinary fields used to analyze extensive data in various fields such as genomics, proteomics, metabolomics, transcriptomics, and phenomics. A broad range of HTP omics technologies have been employed in plant breeding to accelerate breeding programs and to improve overall efficiency.[44] These advanced technologies produce big data on the genetic architecture of the plants. This data is used for crop improvement, and to analyze this complex data, plant breeders rely on AI, machine learning (ML) tools, high-performance computing, and bioinformatics tools. The use of big data coupled with ML tools and AI can revolutionize the field of plant breeding.[45] This big data produced by omics technologies helps plant breeders find the most productive

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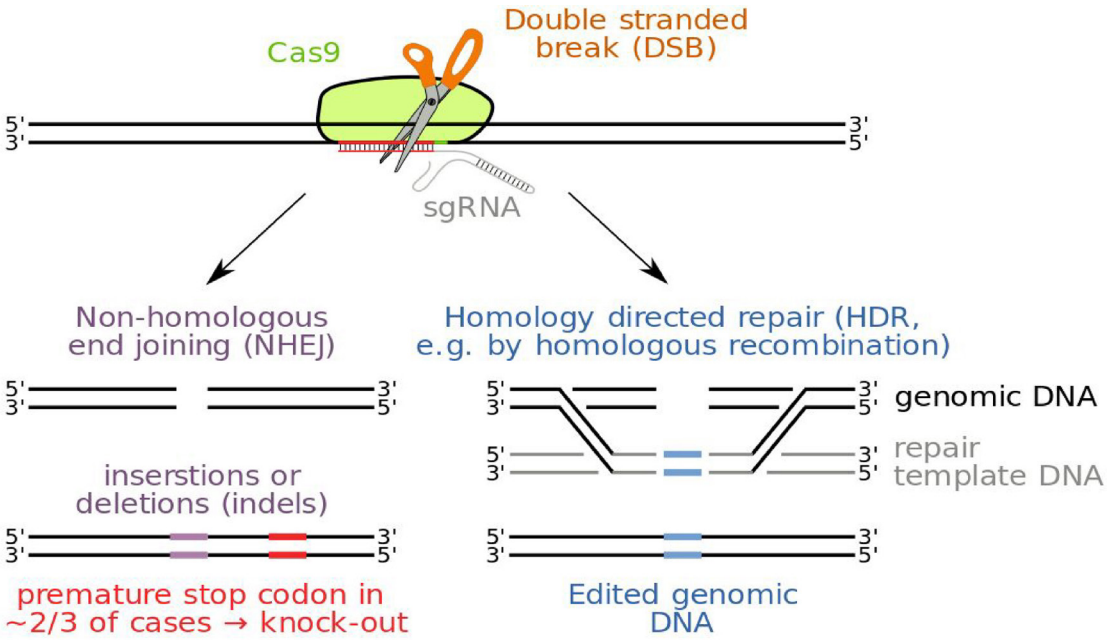

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Fig 2 | Mechanisms of DNA repair following CRISPR-CAS9 induced double-stranded breaks

experiments and areas of plant breeding where additional data is required by analyzing the volume of this data.[46]

Moreover, the use of ML in pre-breeding, regional selection, and MAS can assist plant breeders in enhancing genetic diversity and can boost the overall developmental process of climate-resilient cultivars.[47] Various studies have highlighted the potential use of AI in biochemical data analysis to understand the biology of plant stress, and AI has proven to be effective in predicting the genomic crossovers in paternal and maternal maize plants, hence helping researchers to find genomic regions with higher mutation rates.[48] Similarly, AI was used to predict the expression patterns of important genes and cis-regulatory in maize and *Arabidopsis thaliana*.[49]

Hence, AI and ML learning are great tools especially when it comes to analyzing big data produced by modern plant breeding tools such as omics technologies and HTP. AI and machine learning have great potential to revolutionize plant breeding, and AI holds great promise in future by making crop improvement quick and efficient.

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