"Enhancing Crop Improvement through Synergistic Integration of Advanced Plant Breeding and Proximal Remote Sensing Techniques"

Abstract:

To accelerate crop improvement with enhance adaptability to changing climatic conditions and meeting the ever-increasing global food demand requires urgent action. To achieve this, we must employ advanced molecular breeding techniques, such as marker-assisted selection, marker-assisted backcrossing, genomic selection, genome editing, and targeted mutation. However, these approaches demand the screening of large populations to identify potential genes and genotypes. Genetic information derived from genotyping requires extensive phenotyping to gain insights into the complex genetic mechanisms governing crucial traits in crops. Unfortunately, a significant bottleneck lies in the absence of high-throughput plant phenotyping methods that can rapidly and cost-effectively facilitate data-driven genotype selection in plant breeding. This limitation hampers our ability to understand the genetics of complex polygenic traits, especially those related to yield and stress tolerance, such as drought, heat resistance, and nutrient efficiency. Traditional phenotyping methods, reliant on trained experts, are slow, expensive, labor-intensive, subjective, and often require destructive sampling. In this context, the integration of proximal remote sensing with molecular breeding approaches has emerged as a promising strategy to accelerate crop improvement programs. Proximal remote sensing technologies, including RGB imaging, thermal imaging, hyperspectral imaging, multispectral imaging, and fluorescence imaging, offer a non-destructive and rapid collection of detailed phenotypic data, providing valuable insights into various plant traits at different growth stages. High-throughput phenotyping platforms, such as Conveyor-Type Indoor, Benchtop-Type Indoor, Unmanned Aerial Platform (UAP), and Manned Aerial Platform (MAP), utilize a combination of the aforementioned remote sensing technologies. These platforms have shown significant potential for advancing the efficiency of phenotyping processes. This review article aims to explore the integration of proximal remote sensing and molecular breeding approaches, showcasing how this synergistic approach can expedite crop improvement efforts. By emphasizing the benefits, challenges, and future prospects of this integrative approach, we hope to pave the way for sustainable and productive agriculture, ensuring food security in the face of changing environmental conditions.

Keywords: Molecular breeding, Marker Assisted Selection, proximal remote sensing, Thermal imaging, High-throughput phenotyping platforms.

Introduction:

The challenges faced by global crop production due to the increasing human population and the impact of climate change, urbanization, soil degradation, water shortages, and pollution (Tilman *et al*., 2011; Prosekov *et al*., 2018; Breene, 2022) ensuring food security in the face of these challenges necessitates the development of new crop varieties that exhibit higher yield potential and stress tolerance. This is where plant breeding plays a crucial role, with a focus on creating varieties resistant or tolerant to major plant diseases affecting modern agriculture. Fortunately, modern plant breeders have access to cutting-edge technologies that aid in generating large numbers of superior new varieties for selection. These technologies include advances in genomics, doubled haploid technology, rapid cycling, and molecular breeding (Phillips 2010, Poland 2015). Molecular breeding, including techniques like Marker-Assisted Selection (MAS), Marker-Assisted Backcrossing (MABB), Genome wide association mapping (GWAS), Genomic Selection, and Targeted mutation via genome editing, enables the discovery and generation of new genetic variability for improved crop traits.

However, a significant bottleneck hindering the rapid selection of improved varieties is the time-consuming and labor-intensive process of screening large populations, such as mapping populations and mutants. High-throughput genetic improvement breeding requires precise, cost-effective, and quick assessment of phenotypic expressions in the field (Bilder *et al*., 2009; Araus *et al*., 2014; Ghanem *et al*., 2015; Sankaran *et al*., 2015; Tardieu *et al*., 2017). Plant phenotyping, which involves the measurement of various visible characteristics and physiological traits of plants at both the single plant level and canopy scale, is essential for the success of molecular breeding efforts (Hickey *et al*., 2019).

Traditional phenotyping methods often rely on the visual assessment of crop vigor and other abiotic stresses by trained experts, but they are slow, costly, laborious, and not easily scalable to large areas and numerous varieties. Additionally, these methods may involve destructive sampling and pose the risk of damaging fully developed canopies (Furbank *et al*., 2011; Dhondt *et al*., 2013; Anthony *et al*., 2017).

The advent of advanced molecular breeding techniques has increased the demand for high-throughput, accurate, repeatable, and novel phenotyping methods. Image-based phenotyping tools, capable of imaging thousands of plants or plots within a few hours with high accuracy, are meeting this demand (Fahlgren *et al*., 2015). There is also a growing need for non-destructive, timely, and repeatable phenotyping methods, particularly for studying senescence dynamics and measuring novel phenotypes like the leaf-to-panicle ratio (LPR) and canopy occupation volume (COV) (Tao *et al*., 2022).

In this review, we explore various molecular breeding approaches and advanced proximal remote sensing techniques and their integration for the rapid development of climate-resilient crop varieties. Remote sensing involves collecting spectral readings based on the interaction between incoming radiation and target objects, resulting in characteristic signatures of reflected light. These signatures are used to calculate spectral indices, providing information on the light absorption properties of plants at specific wavelengths (Mullan, 2012; Zarco-Tejada *et al*., 2013). Recent advancements in remote sensing technologies and data processing have made it possible to apply these techniques in both field and controlled growing conditions (Swain and Zaman, 2012; Araus and Cairns, 2014), offering a rapid and non-destructive approach to plant screening (White *et al*., 2012). Integrating high-throughput phenotyping through remote sensing tools, along with the ability to account for environmental factors, will significantly improve selection efficiency in plant breeding (Sankaran *et al*., 2015).

The use of genomic technologies in the investigation of crop genetics:

The critical role of genotyping in plant breeding is essential, as it is essential to have a diverse pool of plant individuals with varying genetic variations. During the early days of Mendelian genetics, genotyping primarily relied on phenotypic variations, which limited the selection of target traits for plant breeding. However, the introduction of the polymerase chain reaction (PCR) method revolutionized genotyping technology, offering superior PCR-based molecular markers such as random amplification length polymorphism (RAPD) and amplified fragment length polymorphism (AFLP). These marker types became popular choices for many studies due to their cost-effectiveness and the fact that they did not require nucleotide sequence information, although their reproducibility across different populations posed challenges.

Among the PCR-based markers, simple sequence repeat (SSR) markers stood out as an alternative that was both cheap and very useful. They are found in large numbers in the genomes of plants. The development of expressed sequence tags (ESTs), which capture actively expressed genes, synergized with SSRs, enhancing their power as genetic markers.

The most recent advancement in molecular markers is single nucleotide polymorphisms (SNPs), which are theoretically unlimited in plant genomes. While scientists initially favored SSRs over SNPs in the 1990s, early NGS technologies have made SNPs the primary choice in many breeding studies due to their high flexibility, speed, and cost-effectiveness (Kim *et al*., 2016; Chung *et al*., 2017). SNP markers have the potential to be universally used for genotyping from different sources, enabling integrated analysis across different species due to certain levels of similarities in nucleotide sequences.

In cases where desirable alleles cannot be found, breeders often investigate mutant lines, either artificially induced or naturally occurring. For facilitating the identification of mutant alleles, genomic tools known as Target-Induced Local Lesions in Genomes (TILLING) (Till *et al*., 2003) or Ecotype TILLING (EcoTILLING) (Comai *et al*., 2004) prove to be valuable. These methods have been successfully applied to major crops such as rice (Kadaru *et al*., 2006), wheat (Wang *et al*., 2008), barley (Caldwell *et al*.,2004), and maize (Weil *et al*.,2007).

The continuous advancement of breeding techniques has significantly accelerated the pace of genetic enhancement (Phillips 2010). Farmers and plant breeders have historically selected desired plants based on phenotypes, even before the discovery of DNA and molecular markers. Crop breeding involves a numbers game: the more crosses and conditions used for selection, the greater the chance of finding superior varieties. Therefore, efficient and specific phenotyping of vast numbers of lines becomes essential for plant breeders to easily identify the best progeny. Improving breeding productivity is crucial to meeting future needs, and high-throughput genotyping has played a pivotal role in this regard. The technology has enabled the creation of thousands of recombinant inbred lines for phenotyping large mapping populations and diversity panels (McMullen *et al*., 2009). Despite the focus of molecular breeding strategies, such as marker-assisted selection and genomic selection, on genotypic information-based choices, phenotypic information remains essential (Jannink *et al*., 2010). Similarly, phenotyping is crucial for classifying promising events in transgenic experiments (Gaudin *et al*., 2013; Saint *et al*., 2012). Successful phenotyping is likely to be necessary to capitalize on advances in traditional, molecular, and transgenic breeding and ensure effective genetic enhancement of crops. There is an increasing demand for efficient phenotyping methods across various domains, as phenotypes are robust predictors of important biological traits such as disease and mortality (Houle *et al*., 2010). Molecular biologists and breeders emphasize that advanced molecular techniques can only be truly valuable in breeding if the collection of quantitative traits is based on reliable phenotyping techniques (Araus *et al*., 2008).

In this review, we provide an overview of important advanced molecular techniques like marker-assisted selection, marker-assisted backcross breeding, Genome-wide association mapping, genomic selection, and next-generation Sequencing (NGS), which facilitate the study of genetic diversity and are crucial for germplasm management, enhancement, and utilization.

Marker-Assisted Selection:

Marker-assisted selection, which involves selecting a trait based on genotype using associated markers instead of relying solely on the phenotype. This method helps with several breeding activites, such as identifying cultivars, measuring genetic diversity and purity, choosing parents, figuring out which parts of the genome are being chosen, and studying heterosis (Collard *et al*., 2008). The identification of plants carrying specific genes, or QTLs, is primarily determined by their genotype, which is achieved through the analysis of molecular (DNA-based) markers (Ramalingam *et al*., 2017). Remarkably, marker-assisted selection holds immense promise for the development of BLB disease-resistant rice varieties, as demonstrated by various studies (Oladosu *et al*., 2018; Das *et al*., 2015; Samuel *et al*., 2019).

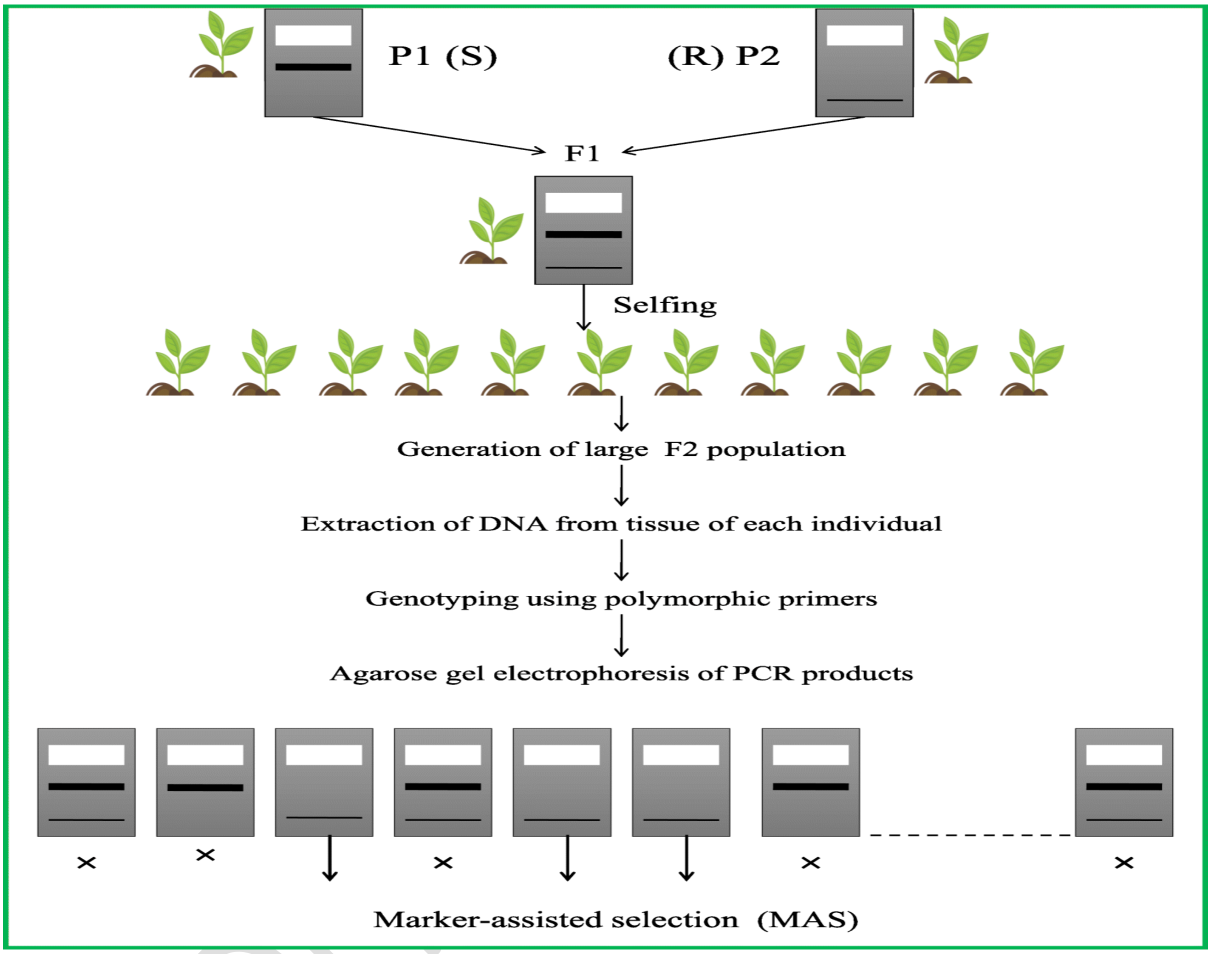

Figure1: marker assisted selection

Next-Generation Sequencing Technologies:

DNA marker identification perspectives have moved from fragment-based polymorphism identification to sequence-based single nucleotide polymorphism (SNP) identification owing to the availability of whole genome sequences (WGS). While WGS technologies based on Sanger sequencing were once time-consuming, costly, and limited to providing information on individual targets, the emergence of next-generation sequencing (NGS) technologies and powerful computational pipelines has revolutionized the process.

The utilization of NGS has drastically reduced the cost of whole genome sequencing, enabling the discovery, sequencing, and genotyping of thousands of markers in a single step, which was previously unattainable (Stapley *et al*., 2010). This has opened up new possibilities for next-generation plant breeding, as NGS serves as a powerful tool to rapidly detect numerous DNA sequence polymorphisms within a short timeframe. Consequently, NGS technologies are gaining widespread acceptance in the field of crop breeding. Moreover, many NGS-based marker discovery techniques now allow for SNP discovery and genotyping simultaneously, further expediting the entire process (Singh *et al*., 2022). Because of this breakthrough, WGS technologies are no longer limited by how long they take and how much they cost. This makes NGS a key tool for large breeding populations and finding specific genes in agriculture.

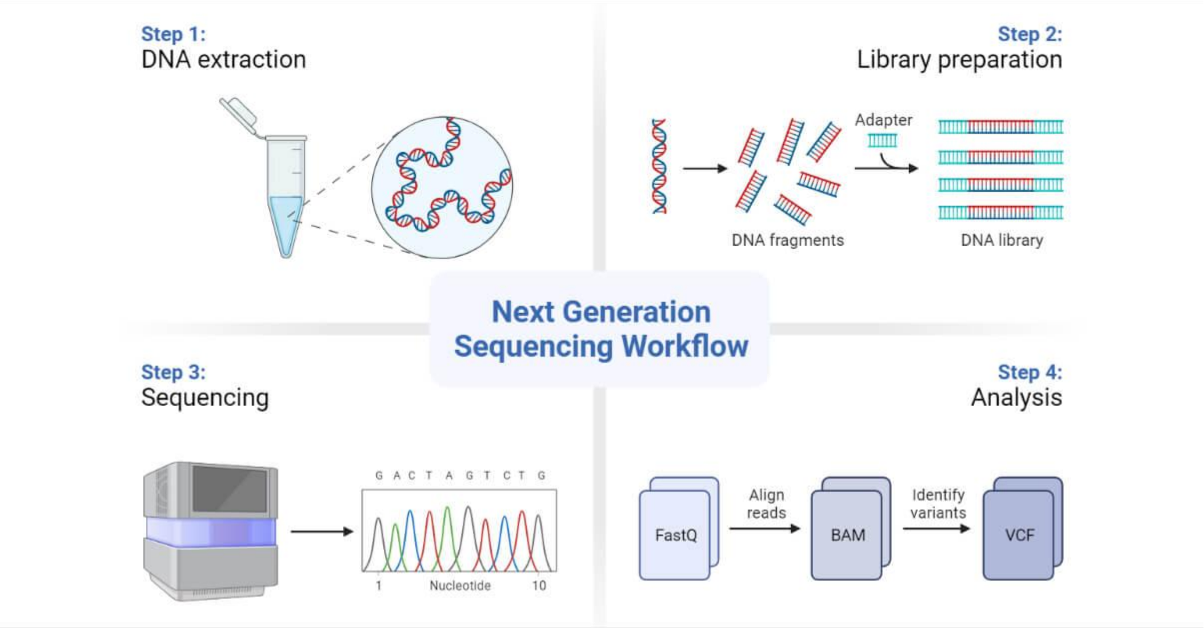

Figure2: workflow of next generation Sequencing

Genome-Wide Association Studies (GWAS):

GWAS aims to detect differences in the allele frequency of genetic variants among ancestrally similar individuals who exhibit phenotypic variations. While GWAS can consider copy-number variants or sequence variations in the genome, single-nucleotide polymorphisms (SNPs) are the most commonly studied genetic variants in this context (Uffelmann *et al*., 2021).

GWAS, based on linkage disequilibrium (LD), proves to be an effective approach for detecting marker trait associations (MTAs) using whole genome-wide variants, especially for complex quantitative traits in various crop species (Pasam *et al*., 2012; Saini *et al*., 2022). However, conducting GWAS successfully requires careful assessment of the population structure of the diversity panel to establish genetic relatedness among individuals and minimize the risk of false associations (Korte and Farlow, 2013; Sul *et al*., 2016). Additionally, the reliability of GWAS results depends on the use of a sufficiently large number of markers to capture the genetic variability adequately.

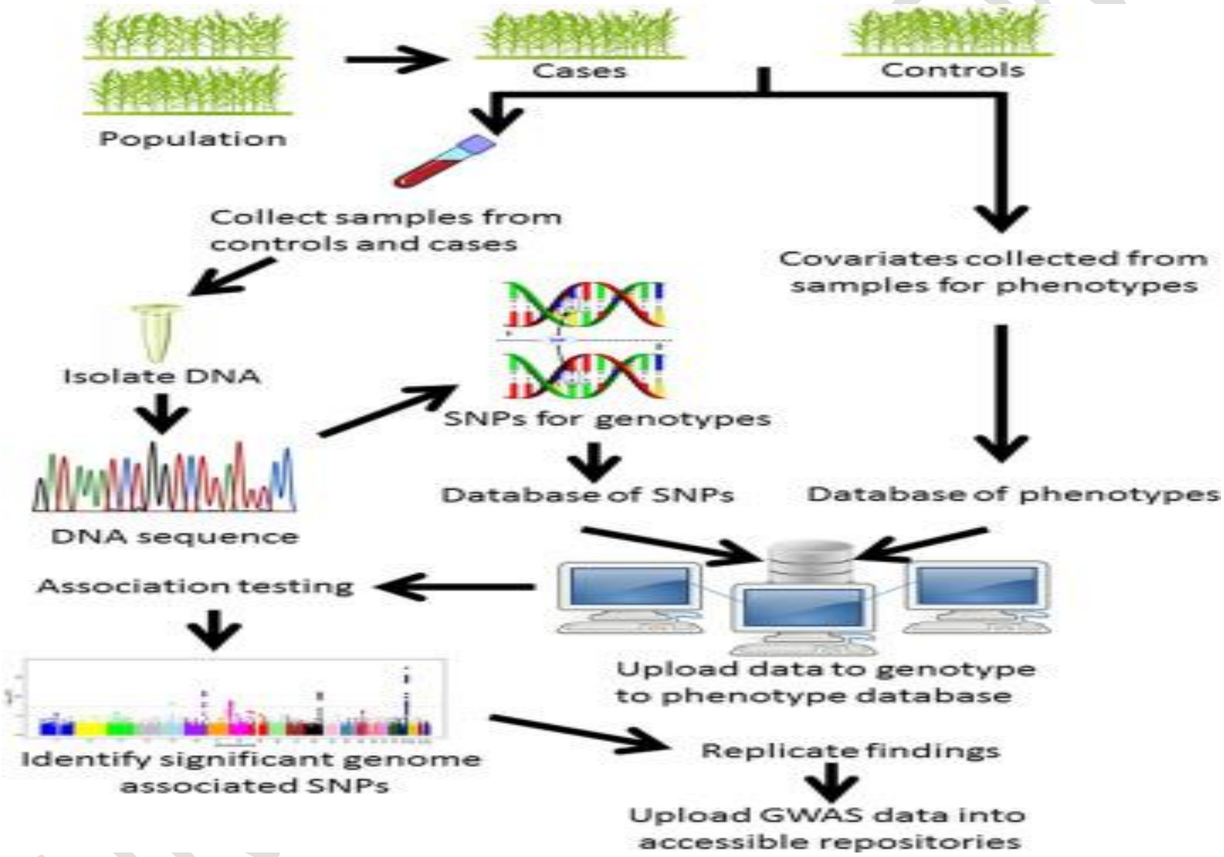

Figure3: Different step of GWAS

Genomic Selection for Predictive Breeding:

Genomic selection represents an extension of marker-assisted selection (MAS) in plant breeding. MAS predicts the genotypic values of individuals (Lande and Thompson 1990) based on the effects of a few selected markers, which facilitates indirect selection. In contrast, genomic selection takes advantage of all markers without the need for significance testing. Consequently, in genomic selection, the predicted genomic values replace the marker score used in MAS (Heffner *et al*., 2009; Jannink *et al*., 2010; Zhao *et al*., 2015).

Genomic selection (GS) is a promising approach that leverages molecular genetic markers to design novel breeding programs and develop marker-based models for genetic evaluation. It offers significant opportunities to increase the genetic gain of complex traits per unit time and cost in plant breeding (Bhat *et al*., 2016). GS estimates the genetic value of individuals based on a large set of marker information distributed across the entire genome, rather than relying on a limited number of markers as in MAS. The GS method creates a prediction model using genotypic and phenotypic data from a training population (TP), which is then used to derive genomic estimated breeding values (GEBVs) for all individuals in the breeding population (BP) based on their genomic profiles (Meuwissen *et al*., 2001). Researchers recognize the potential of genomic selection as a powerful tool in agriculture to accelerate breeding progress and enhance the development of improved crop varieties with desirable traits.

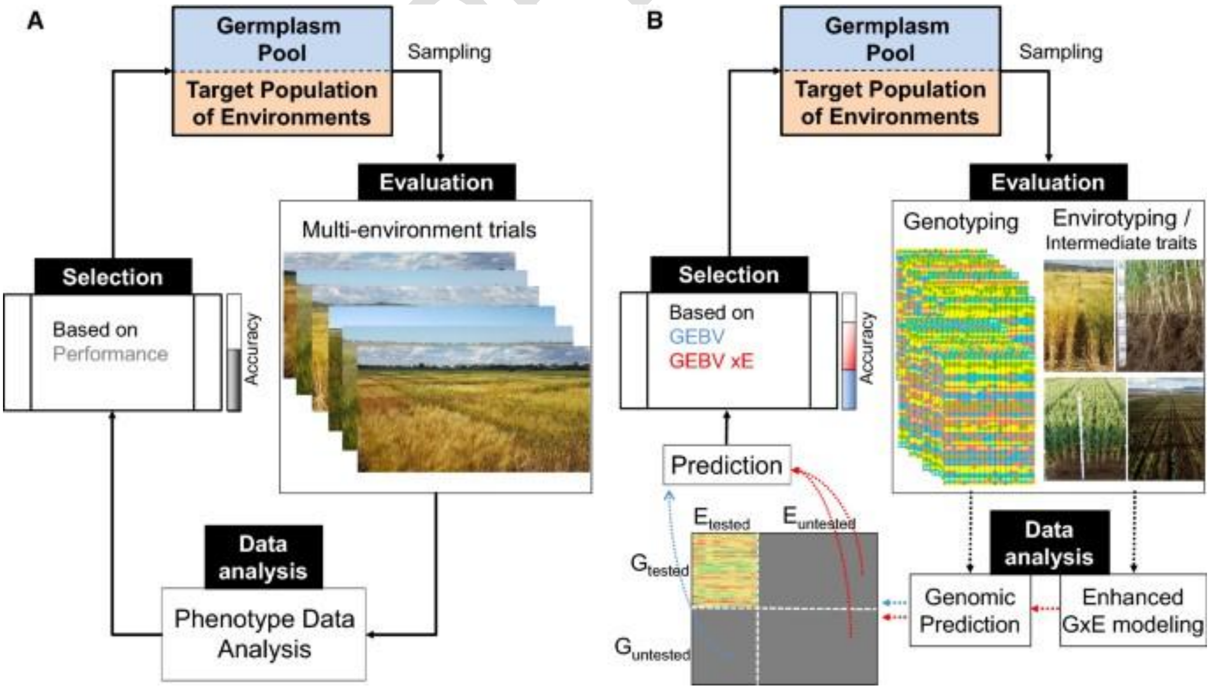

Figure 4: Showing the different steps of genomic selection (GS)

Different Proximal Remote Sensing Techniques for High Throughput Phenotyping:

In the pursuit of genetic improvement, plant phenotyping plays a vital role. Thanks to recent progress in proximal remote sensing and the development of new sensors and computer science applications, cost-effective high-throughput phenotyping (HTP) is now achievable (Montes *et al*., 2007; Araus and Cairns, 2014; Coppens *et al*., 2017). HTP enables the collection of time-series measurements that monitor a crop's development throughout its life stages and its responses to the environment. This wealth of information includes gene function, gene activation, and gene network interactions at various stages of plant growth and in response to environmental stimuli (Montes *et al*., 2007). Consequently, plant breeders can now perceive light interception, biomass accumulation, and response to drought stress as dynamic traits rather than fixed points in time (Montes *et al*., 2007).

Primarily employ ground-based proximal sensing approaches, canopy temperature (CT) has proven to be strongly linked to plant performance, especially under stress, as it is closely associated with water status and stomatal conductance (Blum *et al*., 1982; Berliner *et al*., 1984; Amani *et al*., 1996). Additionally, the Normalized Difference Vegetation Index (NDVI) allows us to estimate relative crop biomass at different growth stages (Babar *et al*., 2006, Kumawat *et al*., 2023) and assess factors like nitrogen deficiency and crop senescence rate (Babar *et al*., 2006; Olivares-villegas *et al*., 2007; Tattaris *et al*., 2016).

Thermal imaging for stress detection and monitoring:

Thermal sensing or imaging stands out as one of the most potent tools for phenotyping, especially when studying traits associated with water stress responses (Jones, 2004, Pachori *et al*., 2023). This approach capitalizes on the fact that surfaces experience cooling due to evaporation, causing their temperatures to decrease in correlation with the evaporation rate. Consequently, any stomatal closure resulting from drought stress will be apparent as a warmer temperature. By detecting emitted infrared radiation in the thermal infrared range (8 to 12 mm) using thermographic and infrared cameras, we can create false color images where each pixel represents the temperature value of the object being measured (Mahlein, 2016). This enables utilization of thermal imaging effectively in quantifying stomatal closure.

However, akin to spectral reflectance sensing, one of the primary challenges in thermal sensing is interference from various factors such as background effects, sunlight, rainfall, or wind speed. The temperature of the soil, for instance, tends to be significantly warmer than that of the transpiring canopy. To overcome this hurdle, specialized techniques are required to obtain a pure signal from the canopy alone. Some of these techniques involve overlaying multispectral images and extracting shaded or sunlit canopy temperatures (Jiménez *et al*., 2011; Wang *et al*., 2010; Jones, 2002). Additionally, the use of thresholding has also been proposed as an effective method (Jones, 2002). Several automated or semi-automated approaches have been put forward for extracting canopy temperature data from different experimental plots (Wang *et al*., 2010).

RGB-imaging:

RGB-imaging in plant breeding as a valuable tool for evaluating morphological traits. The utilization of digital cameras has become pivotal due to their user-friendly nature, allowing for easy handling and quick capture of RGB (red, green, and blue) digital images. These images play a crucial role in detecting, identifying, and quantifying both biotic and abiotic stresses. Over time, there have been remarkable improvements in the technical parameters of these handheld devices. Factors such as light sensitivity of the photo sensor, spatial resolution, and optical and digital focus have undergone significant enhancements on an annual basis, as outlined by Mahlein in 2016.

Multispectral reflectance sensors:

Spectral sensors play a crucial role in research, and they can be categorized based on their spectral resolution, spatial scale, and type of detector (imaging or non-imaging sensor systems). Among the earliest spectral sensors developed were the multispectral sensors, which assess the spectral information of objects using several relatively broad wavebands. For instance, multispectral imaging cameras typically capture data in the red (R), green (G), and blue (B) wavebands, along with an additional near-infrared (NIR) band.

The optical properties of leaves are complex and involve various interactions. These properties encompass light transmission through a leaf, light absorption by leaf chemicals (such as pigments, water, sugars, lignin, and amino acids), and light reflection from internal leaf structures or directly from the leaf surface. Consequently, the reflectance of light from plants depends on multiple biophysical and biochemical factors. In the visible range (VIS, 400 to 700 nm), leaf pigment content significantly influences the reflectance. The near-infrared reflectance (NIR, 700 to 1,100 nm) depends on the leaf structure, internal scattering processes, and the absorption by leaf water. Additionally, the short-wave infrared (1,100 to 2,500 nm) is influenced by the composition of leaf chemicals and water (Carter and Knapp, 2001).

Higher yield among cultivars is correlated with a greater green area per square meter and a higher NDVI around the heading or anthesis stages, regardless of drought or irrigated conditions. Our stepwise multi-linear regression analysis has confirmed that green area per square meter and NDVI are among the most significant traits contributing to grain yield (GY) under drought conditions (Nehe *et al*., 2021). These insights are invaluable for the ongoing efforts to enhance agricultural productivity and adapt to varying environmental conditions.

Hyperspectral Reflectance Sensors:

The advancement of modern hyperspectral sensors now cover an extensive spectral range, spanning from 350 to 2,500 nm, and offer the potential for narrow spectral resolution below 1 nm (Steiner *et al*., 2008). Unlike non imaging sensors, which average spectral information over an area, hyperspectral imaging sensors provide both spectral and spatial data for the imaged object. The resulting hyperspectral data is represented as large matrices, with spatial x- and y-axes, and spectral information as reflectance intensity per waveband in the third dimension, z (Mahlein *et al*., 2012b).

Spectral reflectance is an effective means of deriving valuable information about canopy biochemistry, including water content, stem soluble carbohydrates, and pigments like chlorophyll, carotenoids, and xanthophylls. While broadband multispectral sensors can be utilized for this purpose, narrow-band or hyperspectral sensors provide more accurate information (Hilker *et al*., 2009; Tejada *et al*., 2005; Dreccer *et al*., 2014).

Fluorescence imaging:

Chlorophyll fluorescence parameters serve as essential tools for estimating variations in the photosynthetic activity of plants. Chlorophyll fluorescence imaging instruments, commonly classified as active sensors, employ LED or laser light sources to assess photo- synthetic electron transfer (Bauriegel *et al*., 2014). This approach proves valuable in studying differences in photosynthetic activity resulting from both biotic and abiotic stresses across the leaf area (Thakur *et al*., 2023). Furthermore, the combination of fluorescence imaging with image analysis techniques has demonstrated utility in discerning and quantifying fungal infections (Konanz *et al*., 2014).

Despite its advantages, one drawback of current chlorophyll fluorescence imaging systems is the necessity for strict plant preparation protocols, making it challenging to implement in regular agricultural greenhouses or field environments. Consequently, ongoing research has focused on extracting fluorescence parameters from sun-induced reflectance in the field, which holds promising potential for assessing plant diseases at the canopy or field level (Rossini *et al*., 2014).

LiDAR and Time of Flight sensor:

Various sensors are available to provide crucial 3D structural information of crops and vegetation. Among these, LiDAR stands out as the most well-known and extensively utilized sensor for 3D canopy reconstruction (Eitel *et al*., 2011; Sanz *et al*., 2013). Leveraging laser technology, LiDAR has proven effective in rapidly mapping Leaf Area Index (LAI) and estimating plant area density profiles within wheat canopies (Chéné *et al*., 2012).

However, for simpler alternatives, there are other sensors with slightly reduced spatial resolution or range compared to LiDAR. These options include depth cameras based on time-of-flight (Klose *et al*., 2011), ultrasonic sensors, and even consumer-grade gaming interfaces like the Microsoft Kinect. Surprisingly, these alternative sensors have also found utility in characterizing various types of canopies (Li *et al*., 2020). It is essential to explore and utilize a diverse range of sensors to gather comprehensive 3D structural information for crop monitoring and analysis.

Sensors for assessing plant biomass and plant architecture:

Plant architecture and biomass is crucial for assessing health status and detecting diseases at both individual plant and field scales. Various advanced technologies, such as stereo cameras, 3D laser scanners, ultrasonography, and densitometry, hold promise in providing valuable insights into plant biomass and architecture (Busemeyer *et al*., 2013; Paulus *et al*., 2013; Wahabzada *et al*., 2015b).

By employing these technologies, it becomes possible to automatically determine single plant organs and extract volume information, such as the measurement of wheat panicles. Remarkably, this volume data exhibits a strong correlation with the actual thousand kernel weight, a crucial parameter in assessing grain quality. Photogrammetric techniques also find application in detecting wheat kernels infected with Fusarium spp., offering a potential solution for disease detection.

Stereo cameras and 3D laser scanners further enrich the data by capturing color and reflectance intensity information, which can be harnessed for disease detection through image and reflectance analysis, respectively (Paulus *et al*., 2014). These innovative technologies presents exciting opportunities for precise plant health assessment and disease monitoring in crops, enabling timely interventions and informed decision-making for improved agricultural productivity.

Advancements in Proximal remote sensing platforms and technologies:

Recent years have witnessed the advent of diverse High-throughput phenotyping platforms (HTPP) that have revolutionized plant breeding (Kim *et al*., 2020). These advanced platforms leverage novel sensors, image analysis, robotics, and remote-sensing data to enable rapid and accurate evaluation of numerous agricultural traits. By adopting HTP, plant breeders can efficiently handle large volumes of phenotypic data and effectively match them with the vast amount of genotypic information, facilitating more comprehensive and insightful analyses. The integration of HTP with genomics data paves the way for more informed and efficient breeding decisions, leading to the development of improved crop varieties that can address agricultural challenges and enhance overall productivity.

Conveyor-Type Indoor HTPP:

We utilize a conveyor-type HTPP system, which operates in the "plant-to-sensor" mode. This system involves transporting potted plants into an imaging room using a conveyor, where cameras are strategically placed on the top and sides of the darkroom. The process is fully automated, with the computer controlling an automatic door that allows the plants to pass through for imaging. After imaging, the plants are returned to their original growth positions. To facilitate data acquisition, the plants may also be rotated during the imaging process. This setup effectively eliminates interference from ambient light, ensuring accurate measurements. Furthermore, halogen lamps are used to provide consistent and controlled illumination during the imaging procedure. Ge *et al*. (2016) employed RGB and hyperspectral imaging rooms to analyze the growth, leaf water content and water-use dynamics of maize crop.

Benchtop-Type Indoor HTPP:

The importance of accurately measuring phenotypic traits that can be affected by environmental factors such as temperature and wind, particularly for small plant species with delicate stems. To achieve precise measurements without disturbing the plants, the benchtop HTPP system operates in a "sensor-to-plant" mode. In this setup, the imaging head is equipped with multiple sensors and mounted on a computer-controlled mechanical arm. The mechanical arm automatically locates the position of the growing plant and collects its phenotypic data directly at the site where it is situated. By keeping the plants stationary while the sensors move around them, we can ensure reliable and undisturbed data acquisition.

The benchtop HTPP also boasts other valuable features. It includes a precisely controlled irrigation and weighing system, ensuring the plants receive the required amount of water while accurately monitoring any changes in their weight. Additionally, the system incorporates supplemental light sources to provide controlled illumination, maintaining consistent growth conditions for the plants throughout the phenotyping process (Li *et al*., 2021).

Unmanned Aerial Platform (UAP):

The UAP serves as an aerial platform, equipped with various on-board sensors, a GPS unit, and an inertial measurement unit (IMU), a battery, and a crucial gimbal for stabilizing pitch and roll motion. This integration allows the UAP to collect phenotypic data at the plant canopy scale. To ensure high-precision geographic positioning of plots, the use of Ground Control Points (GCPs) and calibration boards is essential. The successful phenotyping of plants with UAPs depends on the characteristics of the unmanned aerial vehicle (UAV) and the properties of the deployed sensors (Sankaran *et al*., 2015). UAPs can be classified into two main types based on their most distinguishing feature: multi-rotor and fixed-wing UAVs.

UAV-based phenotyping offers a higher degree of resolution, crucial for detecting subtle changes in plant responses, such as those caused by disease infection, heat and drought stress, or mineral deficiencies. UAV-based phenotyping operates at the plot level, providing instantaneous records of single or multiple plots, making it highly applicable to plant breeding (Araus and Cairns, 2014; Chapman *et al*., 2014).

Manned Aerial Platform (MAP):

The Multispectral Aerial Phenotyping (MAP) system is typically adapted from either a manned helicopter or a fixed-wing aircraft. This adaptation involves the installation of a phenotype acquisition kit, which can be housed in a cargo pod or directly affixed to the aircraft's step using a bracket. During operation, a passenger or passengers are responsible for evaluating the captured images and providing real-time feedback on their quality to the pilot. This feedback is communicated via a video monitor located within the cockpit. The phenotypic equipment used in this study comprises sensors, a GPS unit, gyroscopes, and inertial measurement units (Li *et al*., 2020).

Integration of proximal remote sensing techniques and advance molecular techniques:

Challenges in phenomics for the upcoming years, particularly in understanding the interplay between genotype and environmental influences that shape phenotypes. One promising approach is the utilization of multi-omics analysis to unravel the intricate spatio-temporal regulatory networks governing crucial agronomic traits (Yang *et al*., 2020a). While significant progress has been made in gene discovery based on phenotypic variation, there is still a long way to go, especially with the adoption of PRS-derived phenomics (Furbank *et al*., 2019). The concept of "genetic gain" remains fundamental in our quantitative genetics and breeding endeavors, signifying the incremental improvement achieved through artificial selection over time (Araus *et al*., 2018).

To maximize genetic gain and expedite breeding cycles, we aim to integrate various phenotyping approaches with modern breeding techniques such as marker-assisted selection (MAS), QTLs, and GWAS (Xiao *et al*., 2022). Recent studies have explored using hyperspectral traits, RGB images combined with KASP markers, and CT scans to investigate genetic variation at different organizational levels, from populations to cells/tissues (Sun *et al*., 2019; Nehe *et al*., 2021; Zhang *et al*., 2021). Additionally, time-series phenotyping has shed light on the genetic basis of dynamic plant phenotypes (Campbell *et al*., 2019).

Collaboration between high-throughput phenotyping and functional genomics has been instrumental in identifying novel genetic variants, ultimately expediting precision breeding and cultivating crops while bridging the gap between genomics and phenomics (Grzybowski *et al*., 2021; Araus and Kefauver, 2018; Singh *et al*., 2019). Another important aspect of the genomics and phenomics combination is predicting phenotypes based on genetic variation, which holds great potential for guiding gene editing and achieving smart breeding (G2P) (Yang *et al*., 2014; Ma *et al*., 2018).

In the era of breeding 4.0, where phenomics, genomics, bioinformatics, and biotechnology converge, researchers have gained insights into diverse molecular mechanisms that govern phenotype formation through DNA expression (Wang *et al*., 2020b). By correlating molecular phenotypes with organism-wide traits, we can further uncover genetic loci associated with plant phenotypes and establish a comprehensive information flow model from DNA to phenotypic traits (Salon *et al*., 2017). These models can help explain the causal relationship between genetic variants and phenotypic variation, leading to the removal of deleterious alleles and the introduction of beneficial ones, significantly accelerating the crop improvement process (Rodriguez-Leal *et al*., 2017; Wang *et al*., 2020b). Through continuous advancements in remote sensing technologies and interdisciplinary collaborations, we can revolutionize agriculture by harnessing the power of genomics and phenomics to create more resilient and productive crop varieties.

Challenges and consideration:

Data Integration and Analysis Challenges:

The advancements molecular breeding techniques, which have contributed to a vast array of genetic variability on a large scale. To fully leverage this potential for plant breeding programs, it is imperative to establish strong connections between genotypic data and phenotypic traits. This linkage allows us to utilize the genetic information effectively in our efforts to improve crop varieties. In this endeavour, the analysis of a substantial number of plants becomes essential to identify specific genes or QTLs (Quantitative Trait Loci) of interest. However, handling such complex datasets requires advanced bioinformatics tools and algorithms (Grosskinsky *et al*., 2015, Hickey *et al*., 2019). These computational resources play a crucial role in processing and extracting meaningful insights from the vast amount of genetic and phenotypic information.

Scaling Up to Large Breeding Populations:

The importance of scalable high-throughput phenotyping techniques, especially when dealing with large breeding populations. To identify potential mutant alleles and transgressive segregants for closely linked genes or QTLs, breeders need to screen a substantial number of plants with high accuracy. Therefore, there is a pressing need for phenotyping methods that can efficiently handle this increased workload (Araus *et al*., 2014). Achieving scalability in phenotyping requires embracing automation and robotics to streamline the phenotyping process and reduce the labor and time involved (Fahlgren *et al*., 2015). By automating repetitive tasks and employing robotic systems, we can significantly increase the throughput and accuracy of phenotyping, making it feasible to analyze a large number of plants or plots efficiently.

Ethical Considerations and Intellectual Property Rights:

The ethical considerations and intellectual property rights issues that accompany the adoption of high-throughput phenotyping techniques in our field. The increasing use of molecular breeding methods, such as genome editing technologies, has brought forth important questions about the regulation and oversight of genetically modified crops (Montes *et al*., 2007). To foster public trust and ensure transparency, it is crucial to address concerns related to the development and deployment of high-throughput phenotyping methods. Open communication and clear dissemination of information about the processes and outcomes of these techniques are essential in gaining public acceptance and support.

Adoption and Implementation in Breeding Programs:

The successful adoption and implementation of high-throughput phenotyping in breeding programs come with certain challenges that must be addressed. To effectively utilize and interpret the phenotypic data generated through advanced technologies, it is essential to provide training and equip breeders and researchers with the necessary skills and knowledge (Grosskinsky *et al*., 2015).

Bridging the gap between technology development and practical application in breeding programs requires strong collaboration between different experts. Plant breeders, phenotypic and genotypic data scientists, and bioinformaticians must work together to ensure seamless integration and meaningful utilization of high-throughput phenotyping data (Araus *et al*., 2014). Moreover, it is crucial to consider the cost-effectiveness and practicality of high-throughput phenotyping methods to encourage their widespread adoption by breeders. Making these technologies accessible and economically viable will facilitate their integration into breeding pipelines (Hickey *et al*., 2019).

Conclusion and Future Perspectives:

Plant Phenotyping (PP) has become a bottleneck technology for high-throughput breeding and a key valve for increasing yield production. Our era has witnessed tremendous advances in PRS and PP. Strengthening the spatial and temporal consistency of PRS data The plant phenotype involves comprehensive traits (e.g., biomass) that also show spatio-temporal changes with plant growth and development owing to the co-regulation of genomics and the environment (Dowell *et al*., 2010). PRS enables high throughput, high precision, and multi-dimensional phenotyping, benefiting from various available and affordable sensors and platforms. Some considerations are recommended to improve phenotypic data quality. First, choose an appropriate spectral, spatial, and temporal resolution based on the phenotypic targets. Second, standardize data collection processes to ensure comparability and improve processing efficiency by following standards published by international organizations (Liping, 2003; Kresse, 2010). More importantly, because of the increasing need for repeatable phenotyping, data sharing, and interdisciplinary collaboration, a more serious challenge for PRS-based phenotyping is maintaining the spatio-temporal consistency of multisource data. PRS usually has comparability problems between different sensors due to their different working settings and spatio-temporal resolutions (Aasen *et al*., 2018). Therefore, it is necessary but challenging to improve the spatio-temporal consistency of PS-based phenotyping.

Specific PRS technologies (sensor and platform types), phenotypic research fields, working environments, species, and traits as a bridge for multi-omics research, PRS-based PP involves multi-dimensional data acquisition, processing, and modelling, which can be used to accelerate multi-omics studies to identify new genetic loci, screen plants, and accelerate breeding. The strategy of developing drought-tolerant wheat varieties depends on understanding and identifying below-ground and above-ground traits for drought tolerance, together with the use of marker-assisted selection.

By integrating proximal remote sensing techniques with genomic approaches, researchers and breeders can enhance their understanding of crop genetics, improve selection efficiency, and accelerate the development of improved varieties. This review article provides valuable insights into the current status, challenges, and future directions of this integrative approach, offering guidance for researchers and practitioners aiming to leverage the power of proximal and remote sensing.

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