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Identification of Novel Genes and New Breeding Techniques for Crop Improvement

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Abstract

The global population is expanding rapidly, projected to reach 10 billion by 2050. This necessitates an increased focus on producing sufficient grains to address global food and nutritional security. To effectively tackle future food security challenges, it is crucial to explore new genetic resources to identify potential novel genes for enhancing the yields. The exploration of crop wild relatives serves as a solution for discovering novel genes, with next-generation sequencing technologies facilitating efficient gene identification in major crop and wild species to enhance crop improvement. The CRISPR/Cas9 system has emerged as the predominant tool for crop breeding, while speed breeding methods offer additional avenues for the rapid development of new crop varieties. These techniques have become powerful resources, poised to advance plant breeding and contribute to a second green revolution. Their application is vital in meeting the food demands of a rapidly growing global population, especially in the face of ever-changing climate conditions.

Keywords:
Climate change, food security, novel genes, speed breeding, wild relatives

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1. Introduction

Agriculture holds the key to meet the food security and cater the needs of the ever-growing population at global level. Numerous plant species are available as source of food but, human population mostly rely on only 20 species for 90% of our plant food such as wheat, rice, maize, etc. Hence, the plant productivity has to be doubled for the yield enhancement in case of major food crops. However, various biotic and abiotic stress factors are causing severe yield reduction. In order to meet the challenges of food security, there is a need for the second green revolution in which the advanced biotechnological and genomics tools hold great promise in reducing yield gaps and improving plant productivity. Over the last three decades, significant progress has been seen in crop genetic improvement programmes targeting increased tolerance/resistance to environmental stresses to enhance the crop yield through the efforts of plant breeders. But to face the future challenges of global

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food security under variable climate conditions and the continuously growing population, attention needs to be paid to explore new genetic resources for identification of potential novel genes for crop improvement. The crop wild relatives (CWRs) are the potential sources for identification of novel genes that could be utilized in crop improvement programmes. The wild species are expected to have novel beneficial alleles that have been lost from cultivated species during the process of domestication. Novel alleles/genes can be easily transferred from the wild species to cultivated species using various biotechnological techniques. Different modern biotechnological techniques such as plant tissue culture, genetic engineering, genome sequencing and gene editing help in identification and understanding the function of the important genes for further utilization in the crop improvement programmes.

2. Utilization of Wild Relatives for Crop Improvement

Wild relatives have a strong contribution in crop improvement and are the rich sources of genes for nutritional properties, and biotic and abiotic stresses in crops. The beginning of the human civilization was associated with plant domestication process in which humans used wild species of different crops grown from generation to generation to fulfil their basic needs. Later on this domestication process resulted in the reduction of diversity in closely linked loci. Due to reduction of genetic diversity at key loci in modern crops, a meagre scope is left for developing novel varieties with improved traits due to lower content of gene pool in their genome. Moreover, continuous selection for desirable traits during domestication made cultivated crop species prone to biotic and abiotic stresses as compared to the wild plant species.

Hence, the wild species are expected to have the novel beneficial alleles that have been lost from cultivated species during the process of domestication. The wild species can function as a reservoir of novel alleles/genes for stress resistance/tolerance and quality traits. In general, wild species can tolerate various biotic and abiotic stresses under different ecologies. Over the last three decades, the availability of advanced biotechnological techniques has significantly facilitated plant breeding for efficient utilization of wild relatives. These advanced technologies including NGS and high-throughput phenotyping (HTP) are playing an important role in accelerating the process of gene discovery in wild species.

3. High throughput Techniques

3.1. High-throughput phenotyping (HTP)

It involves gathering phenotypic data using non-invasive imaging and spectroscopy techniques. It employs high-performance computing equipment to swiftly analyze plant growth activities and physiological status. In contrast to conventional phenotyping methods, HTP enables the concurrent acquisition of data for multiple traits in large populations and allows dynamic observation of plants at various growth stages

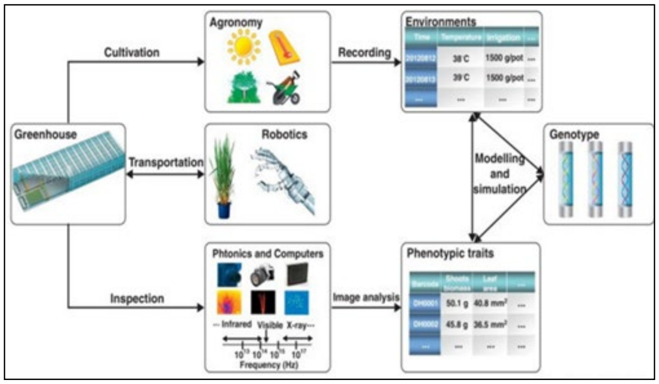

Figure 1: An Overview of High-throughput Phenomics in Plants (Kumar and Pandey, 2020)

3.2. NGS and TGS techniques

Advances in DNA sequencing technologies such as NGS (Next Generation Sequencing) and TGS (Third Generation Sequencing) enable the efficient utilization of sequencing in identification of novel alleles/genes in wild species to be used in crop improvement with variable levels of gene expression. Transcriptome analysis (RNA-Seq) of wild and cultivated plant species can be used to determine the differentially expressed genes (DEGs) which can provide an insight into novel alleles/genes present in wild species (Brozynska et al., 2016). RNA-Seq analysis can be used to understand genomic basis of adaptation of the wild species in varied environments.

3.3. Biotechnological tools for transferring useful traits to cultivated species

Diverse advanced breeding techniques, such as marker-assisted recurrent selection, genomic selection, and speed breeding, play a crucial role in facilitating the transfer of desirable genes from wild relatives to cultivated species. Additionally, genome editing technologies have emerged as powerful tools for precisely modifying crop genomes at specific sites. The efficiency and cost-effectiveness of genome modification methods, have led to its widespread application in crop breeding.

3.3.1. Marker assisted breeding (MAB)

Molecular markers play a crucial role in selection of desirable offsprings resulting from parental crosses during early developmental stages. Both Marker Assisted Selection (MAS) and backcross breeding can be extensively employed to transfer major genes, creating superior lines with enhanced tolerance to biotic and abiotic stress. In case of rice, substantial efforts have been directed towards introgression of genes and major Quantitative Trait Loci (QTLs) governing submergence, drought, and salinity tolerance, as well as resistance to bacterial blight, sheath blight, and blast. This aims to develop stress-tolerant improved cultivars that exhibit increased yield and productivity. Marker-assisted recurrent selection (MARS) is another approach that has been reported in various crops, including rice, wheat, barley, soybean, cotton, pea, and sunflower and is extremely useful for developing durable resistance in crops.

3.3.2. Genomic selection (GS)

In addition to MAS, genomic selection (GS) has the potential to capture multiple QTLs/genes widely distributed with minor additive effects as it utilizes the large-scale DNA markers dispersed throughout the genome to develop superior germplasm lines.

Genomic selection utilizes DNA-based markers on a training population, where both genotypes and phenotypes are expressed to predict superior genes with desired traits. The initial group of genotypes, referred to as the training population, is identified and phenotyped. Subsequently, a regression model is developed to predict Genomic Estimated Breeding Values (GEBVs) for individuals that were not phenotyped. GEBVs are determined by summing the effects of genetic markers or haplotypes across the entire genome, encompassing all quantitative trait loci (QTL) influencing variations in the trait (Ibeagha-Awemu and Khatib, 2017).

GS has been widely adopted in breeding programs to improve selection accuracy, minimize phenotyping, reduce cycle time, and increase genetic gains. This approach was successfully utilized in different crops like rice, wheat, maize, barley, oilseeds, pulses and other horticultural crops for the improvement of abiotic and biotic stresses, quality and yield attributing traits. For example, identification of blast-tolerant lines in rice, stem rust and drought-tolerant high-yielding lines in maize, improved yield under drought in chickpea, and improved productivity in superior hybrids of rice were developed by researchers utilizing GS.

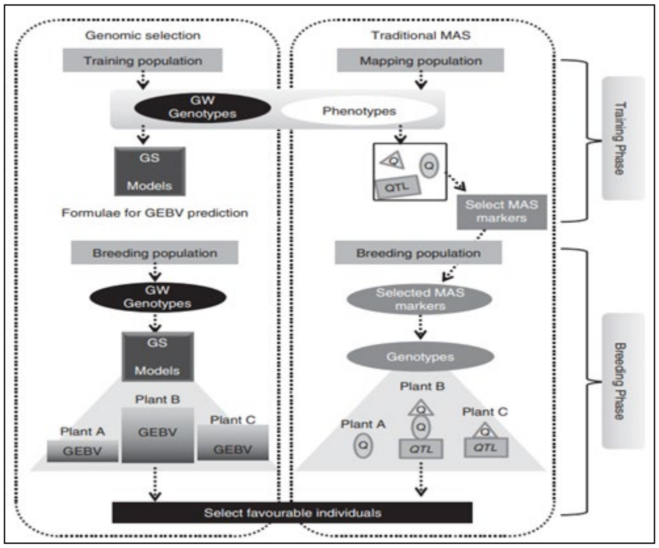

Figure 2: Genomic Selection and Marker Assisted Selection (Nakaya and Isobe, 2012)

3.3.3. Speed breeding

Is a new and exciting technique in plant breeding that promises to develop new crop varieties faster, offering new options for food security. It is a combination of methods to accelerate the speed of crop breeding. It uses optimum light intensity (22 h light, 22°C day/17°C night, and high light intensity), temperature and day length to increase the rate of photosynthesis, and shorten the generation time, leading to quicker development of new varieties. This approach can achieve six generations per year for crops like wheat, barley, chickpea, and canola (Hickey et al., 2019). The approach named Speed GS (combining speed breeding with genomic selection) is gaining popularity among the breeders for achieving higher genetic gain per cycle, especially for traits with low heritability.

3.3.4. Transgenic or genetically modified (GM) crops

Gene cloning and isolation have facilitated the removal of target genes from any genome, that can then be transformed into any other genome for its expression.

An illustrative instance of this approach is the expression of the ‘Cry’ gene from Bacillus thuringiensis in plants.

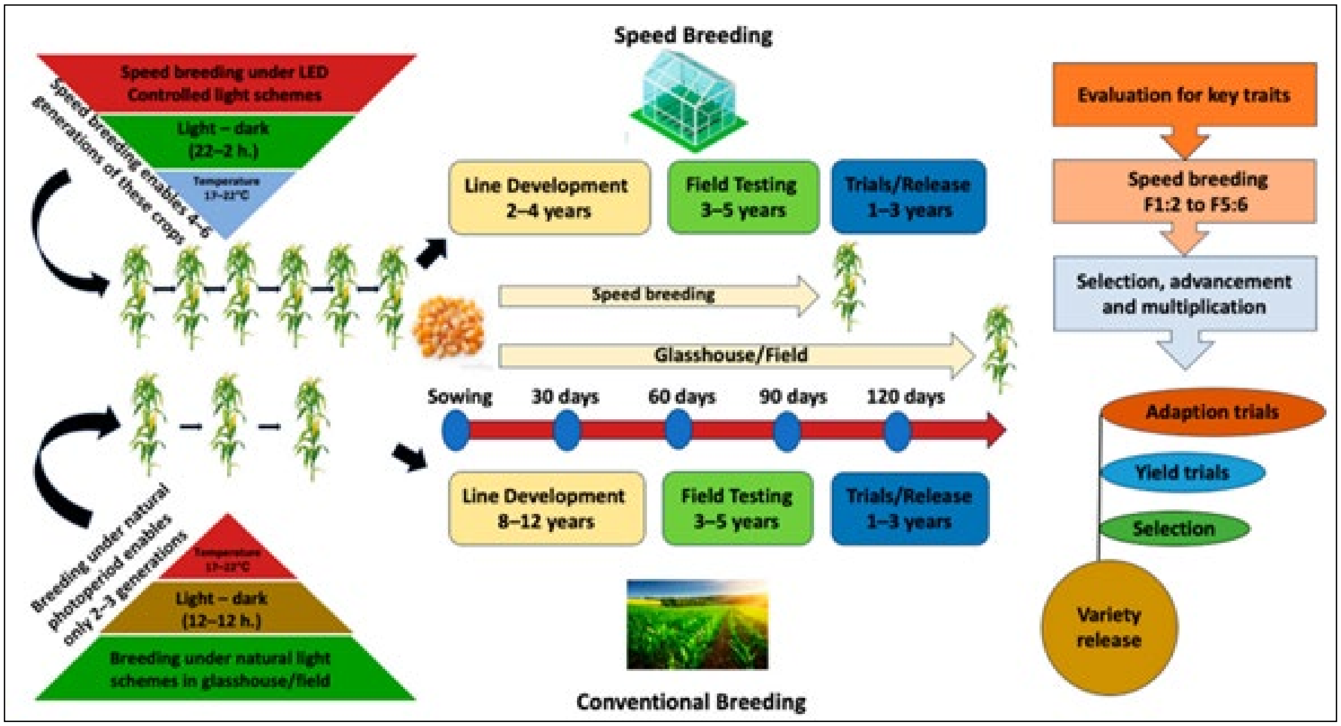

Figure 3: Speed Breeding Technique of crops (Potts et al., 2023)

Similarly, genes encoding DREB (dehydration-responsive element-binding) proteins frequently isolated from one species and expressed in another are used for enhancing the tolerance to different abiotic and biotic stresses. Moreover, these expression and overexpression strategies play a vital role in elucidating gene functions, utilizing Next-Generation Sequencing (NGS) technologies

3.3.5. Gene and genome editing

The application of genetic engineering in plants raises numerous ethical and legislative concerns associated with environmental safety and health. These issues have constrained the development, commercialization, and adoption of genetically modified (GM) crops in numerous countries worldwide. In the past two decades, the integration of sequence-based knowledge with advanced biotechnologies has facilitated the emergence of new plant breeding techniques (NBT). These techniques aim to transfer beneficial alleles into elite varieties and expedite crop improvement while gaining widespread public acceptance. Among these techniques, gene-editing (GE) technology utilizes the CRISPR/Cas9 system to enable the introduction of genetic variations with a high degree of precision and specificity, limited to the targeted gene of interest. GE technologies also offer the potential for multiplexing, enabling the simultaneous induction of targeted mutations in multiple genes. Given its efficiency and perceived safety, GE has the capacity to hasten the generation of plant varieties with desired traits, overcoming many objections associated with transgenesis.

Significant advancements have been achieved in the development of crops resistant to diseases and tolerant to abiotic stresses, exhibiting enhanced yield, improved nutritive value, and extended shelf life. The notable characteristics of genome-editing technology contributing to its widespread adoption in crop breeding within a relatively short span of less than a decade due to its precise modification of the target genome, the absence of foreign DNA in genome-edited plants, and

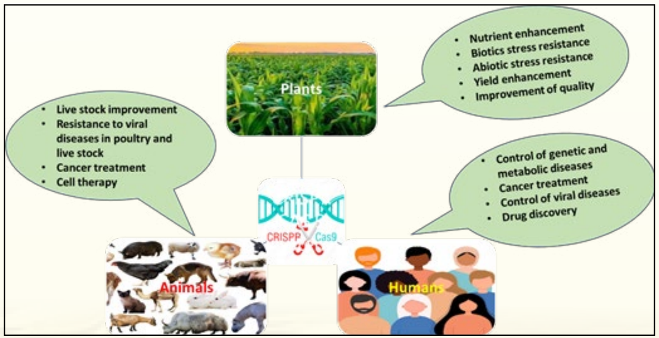

Figure 4: Applications of CRISPR CAS 9 technology

the expeditious and cost-effective nature of genome modification methods.

Conclusion

Molecular breeding and genetic engineering techniques have emerged as powerful avenues for improving crop plants for several agronomic attributes. Similarly, speed breeding methods provide more options for crops in situations where more crop cycles are needed for rapid development of new varieties. With the ultimate aim of reaching the farming community across the globe, the molecular breeding technologies will undoubtedly advance our understanding about trait-based manipulation and in designing new improved crop varieties for achieving food security and sustainability.

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