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Biotechnology Enabled Genetic Improvement of Pea (*Pisum sativum*): From Genomic Tools to Commercial Cultivar Development

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The pea (*Pisum sativum* L.) is a significant cool-season legume that is valued for its role in sustainable cropping systems, nutritional value, and capacity to fix nitrogen. Despite its importance, a limited genetic base, vulnerability to biotic and abiotic stresses, and the complexity of polygenic traits have limited pea genetic improvement. Breeding of pea has been revolutionized with the advent of biotechnology, which has enabled the breeder to make the desired changes selectively, quickly, and as needed. Quality reference genomes, dense molecular markers, quantitative trait loci (QTL), and genome-wide association studies (GWAS) are genomic resources that have been utilized in unraveling complex traits and identifying desirable alleles. Among the methods used to accelerate cultivar development is high-throughput phenotyping based on the application of molecular breeding, such as marker-assisted selection (MAS), marker-assisted backcrossing (MABC), or genomic selection (GS), and speed breeding. Transgenic systems, including genetic engineering and others, have introduced novel characteristics (e.g., disease and insect resistance), and the system using genome editing, particularly CRISPR/Cas, can be utilized in conjunction with previously unheard-of accuracy in editing properties of yield, stress, and quality. These biotechnological tools, in combination with traditional breeding, have led to the introduction of pea cultivars that are high-yielding, resistant, and contain nutritionally enhanced properties. Pangenomics, artificial intelligence, and synthetic biology are some of the upcoming technologies that will strive to enhance pea in the future to contribute to food security in the world, as well as climate-adaptive agriculture. The majority of reported yield, stress-tolerance, and nutritional-enhancement improvements are, however, pegged on published QTL/GWAS relations and experimental validation in regulated or multi-location field trials, whereas others are projected potential based on genomic resources and a marker-based breeding pipeline.

Keywords: *Pisum Sativum*, Improvement of Pea, Biotechnology, Molecular Breeding, Genome Editing, Genomic Selection, Cultivar Development, Functional Genomics.

Introduction:

Importance of Pea in Global Agriculture:

One of the most significant cool-season grain legumes, which is grown globally, is Pea (*Pisum sativum* L.), which is highly significant for its nutritional, agronomic, and ecological importance. Pea seeds are a good source of plant protein that generally has around 20–25 percent protein, and thus are an important addition to human diets, particularly among vegetarians and the poor [1]. Besides protein, peas contain dietary fiber, complex carbohydrates, vitamins (folate and vitamin C), and essential micronutrients (iron, zinc, and magnesium). This nutritional mix aids their purpose in the correction of malnutrition and enhancement of dietary variety [2].

In addition to human consumption, peas play an important role in world food security and sustainable production. They are consumed popularly as animal feed and as more raw material for plant-based protein foods. This biological nitrogen fixation helps minimize the reliance on synthetic nitrogen fertilizers, cut production costs, and reduce environmental pollution [3]. Peas serve to increase soil fertility, improve the soil structure, and disrupt the disease and pest cycles when they are part of the crop rotation systems, thus benefiting the crop of cereals that follows. These facts contribute to the fact that pea cultivation is especially relevant in environmentally sustainable and climate-smart agriculture [4].

Genetic Improvement Challenges in Pea:

In spite of its significance, genetic enhancement of the pea is faced with a number of constant obstacles. The genetic basis of the cultivated pea is rather limited, which can be viewed as one of the most significant constraints due to domestication bottlenecks and needs to select certain agronomic traits intensively. This reduces genetic diversity to implement new alleles to improve yield, stress tolerance, and quality [5].

The pea is also vulnerable to various forms of biotic stress conditions. Ascochyta blight and powdery mildew are some of the key yield-limiting biotic stresses, and the prevalent abiotic stresses in semi-arid regions are drought and terminal heat stress, which often result in a yield loss of 20–50% depending on severity and site.

An increasing threat in changing climatic conditions is represented by abiotic stresses, especially drought, heat stress, salinity, and cold. genotype × environment (G×E) interactions also lead to yield instability that makes breeding and related activities more complex, particularly in rainfed land and marginal production regions. The conventional breeding methods, though effective in the production of better varieties, are usually time-consuming and less productive with complex and polygenic traits related to stress tolerance and yield stability [6].

Transition from Classical Genetics to Biotechnology:

Pea holds a special place in the history of genetics as it has been used as the paradigm organism to investigate Gregor Mendel's laws of inheritance. Classical genetics in pea has helped in gaining a lot of knowledge in genetic segregation, genetic linkage, and inheritance patterns. Nevertheless, until the late 20thcentury, pea improvement was based mainly on traditional breeding strategies like selection and hybridization with relatively little adoption of advanced biotechnological approaches, which are usually time-consuming and less productive for improving with complex and polygenic traits related to stress tolerance and yield stability [6].

Modern biotechnology has led to new opportunities to overcome the restrictions of classical methods. The development of molecular markers, genomics, transcriptomics, and genome editing has revolutionized the strategies of crop improvement in significant crops. In the case of pea, The implementation of biotechnology in breeding represents a significant advancement in modernizing pea improvement programs, building on classical genetics [7]. According to recent comparative breeding experiments, biotechnology-aided breeding (MAS and GS) has been found to enhance the accuracy of the selection process and the duration of the breeding cycle by 30–50% when compared to phenotype-based selection, particularly when the trait of interest is polygenic (yield stability and drought tolerance) [8].

Objectives and Structure of the Review:

The overall goal of this review is to systematically draw together current developments in genetic enhancement of pea using biotechnology, and to emphasize how the tools of genomic technology have evolved to commercial cultivar development. This is due to the need to bring about productivity, resilience, and sustainability of pea amidst agricultural and climatic changes that necessitate the concentration on biotechnology.

Research Questions and Objectives of the Review:

Research questions are:

To summarize genomic resources (reference genome, markers, pangenome).
To assess molecular breeding tools (MAS, MABC, GS).
To emphasize the transgenic and genome-editing advancements using evidence.
To examine the cultivar development pipeline and adoption restrictions.
To mention regulatory, socio-economic, and ethical factors.

This review is also organized in such a way that it further talks about genomic resources and molecular tools created to optimize the pea first, then there is a discussion of marker-assisted breeding, genomic selection, transgenic regimes, and genome editing technologies. The following parts discuss innovations in phenotyping, combining omics, regulatory issues, and success case studies that result in cultivar release. This review will act as a good resource in the future betterment of *Pisum sativum* through offering a comprehensive overview of the issue, and can be used by researchers, breeders, and policymakers to gain a complete understanding of the issue and henceforth work towards improving the crop.

Genetic Resources and Germplasm Diversity in Pea:

Origin, Domestication, and Evolution:

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Global Germplasm Collections:

To safeguard the genetic diversity of the peas and facilitate their use in the breeding process, today there are enormous germplasm depositories all over the globe. Great numbers of cultivated varieties, landraces, and wild relatives of pea are held in large international and national gene banks. One of the most widespread and different collections of pea belongs to the International Center of Agricultural Research in the Dry Areas (ICARDA), and is particularly interested in the material adapted to semi-arid conditions and the Mediterranean climate. This library has been exploited as a valuable resource in the breeding of resistant varieties of pea to stress [11].

Being a part of the United States, the USDA National Plant Germplasm System (NPGS) keeps a comprehensive set of peas, on which the basic research is based, and which is the foundation of breeding programs. Similarly, accessions of pea genetically diverse and of historical interest are also held in a number of European gene banks, such as Russia, France, and the United Kingdom. In Russia, particularly in the Vavilov Institute, special material is held based on early centers of diversity, as a reminder of the study of crop in the world [12].

In view of the sheer size of the collections of germplasm, core and mini-core collections have been developed in order to make such collections more accessible and useful. A core collection is a part of the accessions that best acquires the maximum genetic diversity with minimum redundancy, and a mini-core collection is a part of a core collection that is further reduced to manageable sets that can be easily assessed. These collections enable high-throughput phenotyping, genotyping, and trait discovery, and consequently enable the discovery of useful alleles in breeding to occur at a much faster rate [13].

Utilization of Genetic Diversity in Breeding:

The proper exploitation of genetic diversity is also one of the pillars of sustaining pea improvement. Pre-breeding strategies have been vital in closing the gap between the conserved genetic resources and elite lines. Pre-breeding: Desired traits of wild relatives or landraces, or unadapted germplasm, are identified, characterized, and introgressed to produce breeding material that is easier to utilize by breeders. Characteristics that are usually targeted in pre-breeding are resistance to major gene-based illness, drought, and heat stress tolerance, and better efficiency in utilizing.

Linkage drags, reproductive barriers, and a lack of agronomic performance of a donor material are some of the reasons why the introgression of novel traits of wild species into cultivated pea is often difficult to achieve. Nevertheless, the use of molecular markers and genome tools has greatly enhanced the effectiveness and accuracy of introgression breeding. Breeders can use the techniques of marker-assisted backcross and genomic-assisted selection to follow desirable alleles with limited transfer of undesirable regions of the genome. Consequently, it is possible to see genetic diversity preserved in world germplasm banks being more and more translated into measurable benefits in yield stability, resilience, and adaptability [14].

Within the framework of biotechnology-based enhancement, strategic utilization of pea genetic resources holds the key to diversity on which contemporary genomic and biotechnological technologies work. It is important to harness this diversity in order to come up with strong and high-yielding pea pods that can satisfy the future agricultural and nutritional needs.

Genomic Resources and Tools in Pea Improvement:

The rapid development of genomic technologies has made pea (*Pisum sativum* L.) a classical genetic model to be a genomics-enabled crop. The introduction of genome sequence, molecular markers, and functional genomics tools has also made it possible to dissect complex traits and hasten cultivar development more than ever before. This section provides an overview of the most important genomic resources and tools that support the contemporary improvement of pea [15].

Pea Genome Sequencing and Assembly:

The genome of pea is large and complicated, and is estimated to be between 4.3 and 4.5 Gb, as opposed to other legumes, which have relatively large genomes. The overabundance of repetitive DNA, especially transposable elements that make up over 80 percent of the genome, is observable as the main reason behind such a genome size. The repetitive high content of DNA minimizes the regions of unique genomic content, and this can restrict the development of markers within repetitive regions and the genetic mapping. Thus, SNP markers that are based on gene-rich euchromatic regions are favorable to use as linkage markers, in GWAS, and in breeding [16]. Previously, this complication has slowed down the rate of whole-genome sequencing and assembly projects, and slowed the development of high-quality reference genomes of crops such as rice or soybean [17].

Another major development in the genomics of legumes was the release of the first high-quality reference genome of pea. Recent improvements in long-read sequencing technology (e.g., PacBio and Oxford Nanopore), optical mapping, and next-generation sequencing (NGS) have made enabled longer, more accurate chromosome-scale assemblies. Refined and re-annotated gene models have been developed. Since that time, repeats have been sorted out, and subsequent functional annotation of coding and non-coding regions has been performed. They have now been viewed as a beginning point in gene discovery and marker development and comparative genomics, based on these reference genomes [18].

Molecular Markers and Genetic Mapping:

The key to genomics-assisted breeding in pea is molecular markers. Initial genetic analyses were based on morphological markers and isozymes, although these were slowly supplanted by DNA-based markers that are more resolvable and reproducible. Microsatellites or simple sequence repeats (SSRs) were one of the earliest commonly used markers in pea. The SSRs are co-dominant, highly polymorphic, and can be used in diversity analysis, linkage mapping, and cultivar identification [19].

Single-nucleotide polymorphism (SNP) has emerged as the system of choice of the marker because of the abundance and genome-wide occurrence, which has been enabled by the introduction of high-throughput sequencing. SNP arrays and genotyping-by-sequencing (GBS) systems have made it possible to genotype populations of peas on large-scale basis at a relatively low cost. INDELs (insertion-deletion polymorphisms) also supplement SNPs by offering extra variation that can be used to perform fine mapping and marker-based selection.

These are the markers of which several genetic linkage maps have been built, utilizing bi-parental populations, including recombinant inbred lines (RILs) and doubled haploids. A combination of information from varied groups has created high-density consensus maps, which reconcile markers amongst investigations and genetic histories. The accuracy of mapping traits is enhanced by such maps, and the transfer of marker-trait associations across breeding programs is made easier [20]. The comparison of the most used molecular markers should be done to advise the breeders on the most suitable marker system in terms of cost-effectiveness, throughput, reproducibility, and applicability in different pea germplasm. Early marker systems like the SSRs are still useful in genetic diversity analysis and identification of the cultivars because of their high polymorphism and co-dominant inheritance. Nonetheless, the emergence of high-throughput genotyping schemes has changed the trend of breeding schemes in modern times, with SNP-based markers taking the center stage, as these offer genome-wide genotyping information and are most appropriate in GWAS, QTL mapping, and genomic selection. Genotyping-by-sequencing (GBS) has improved the efficiency of marker discovery and genotyping and has created thousands of SNPs at relatively low cost, but the lack of required data and bioinformatics means may restrict its everyday application in breeding programs. The InDel markers are also used to aid fine mapping and validation studies, especially when used in conjunction with SNP datasets. In general, the marker system selection is determined by breeding goals, access to resources, and scale of the genotyping work, and SNP-based systems are taking over the contemporary pea enhancement pipelines through their resource-effectiveness and overall genome-wide intensity, as shown in Table 1.

Quantitative Trait Loci (QTL) Mapping:

Numerous agronomically useful traits, such as yield, stress, and disease resistance, are qualitatively inherited and regulated by several genes of small effects in pea. QTL mapping has thus been a very vital technique for observing the genome regions related to these complex traits. With the help of linkage maps and phenotypic data of structured populations, many QTLs have been traced on the yield-related traits like seed size, pod number, biomass, and harvest index [21].

One of the areas of QTL mapping in pea has been disease resistance. There are QTLs, which confer resistance to major disease like ascochyta blight, powdery mildew, rust, and root rot, which have been reported in various genetic backgrounds. Likewise, tolerance to abiotic stresses (such as drought, heat, and salinity) has also had QTLs, which are also generally highly interactive with the environment [22]. A number of powdery mildew and Ascochyta blight QTLs have been confirmed across numerous genetic backgrounds and environments, and can therefore be used as markers in MAS programs. There are, however, a number of abiotic stress

QTLs that are still environment-specific and yet should be further validated before they can be incorporated in routine breeding [23].

Although QTL mapping has proven to be very informative, it has a constraint in terms of the resolution based on the size of the mapping populations and the number of recombination events. However, the QTLs discovered using the linkage analysis have served as useful targets in marker-assisted selection (MAS) and have created the foundation on which more sophisticated genomic methods have been based.

Table 1. Comparison of widely used pea breeding and genetic mapping molecular marker systems in pea (*Pisum sativum* L.), with reference to their relative cost, throughput, level of polymorphism, and their use in various breeding and genetic mapping uses.

Marker Type	Cost	Throughput	Co-dominant	Best Use
SSR	Medium	Low	Yes	diversity studies
SNP array	Medium–High	Very High	Yes	GWAS/GS
GBS	Low–Medium	High	Yes	diversity + mapping
InDel	Medium	Medium	Yes	fine mapping

Genome-Wide Association Studies (GWAS):

Genome-wide association studies (GWAS) are a complementary study to conventional QTL mapping. GWAS takes advantage of natural variation in genes that occurs in different germplasm collections, such as landraces, breeding lines, and wild accessions. GWAS permits high-resolution locus mapping of complex traits by associating genome-wide marker data with phenotypic variation [24].

GWAS has been effectively used in the identification of loci related to flowering, seed characteristics, components of yield, and resistance to biotic and abiotic stresses in pea. Among the benefits of GWAS over bi-parental QTL mapping, one must mention the power of this technique to record historic recombinations, which results in higher mapping resolution and more accurate localization of candidate genes. Also, GWAS can be used to test many alleles at a locus simultaneously, which can inform about the allelic diversity which can provide information on the allelic diversity and its functional importance [25].

Nevertheless, GWAS must be considered with great caution in terms of population structure and relatedness to prevent false positives. To minimize false positives, mixed linear models (MLM), principal component correction, and kinship matrices are frequently used in GWAS in pea. Multi-year, multi-location, and cross-validation enhance the reliability of marker-trait associations. The increase in the development of statistical models and dense SNP datasets has made a big difference in enhancing the strength of GWAS in pea. Combined with linkage mapping and functional genomics, GWAS can be effective in discovering traits and performing allele mining.

Functional Genomics Approaches:

Functional genomics is an intermediate between genotype and phenotype, which explains the regulatory networks and gene functions. One of the most common methods currently employed in pea to study the patterns of gene expression in tissues, developmental stages, and stressful situations is transcriptomics, especially through RNA sequencing (RNA-seq). The RNA-seq analysis has discovered the versatile genes that are differentially expressed during disease resistance, nodulation, flowering, and stress responses and that can be used as candidate genes to be functionally confirmed and in breeding [26].

In addition to transcriptomics, there are proteomics and metabolomics that provide extra information on biology. Proteomic studies determine post-translational modifications and protein-protein interactions, which cannot be captured solely by transcriptomics. metabolomics measures the products of cellular activities and connects genetic variation with chemical pathways that determine food characteristics such as nutritional value, flavor, and stress adaptation to stress. Though these have not been as advanced in pea as in major crops, these two approaches, combined with genomic and phenotypic data, have a lot of potential [27] As an example, RNA-seq data have been used together with QTL mapping to identify possible resistance genes against Ascochyta blight, and metabolomics profile has been used to assist in the selection of seeds with improved nutritional qualities, including improved amino acid and micronutrient content.

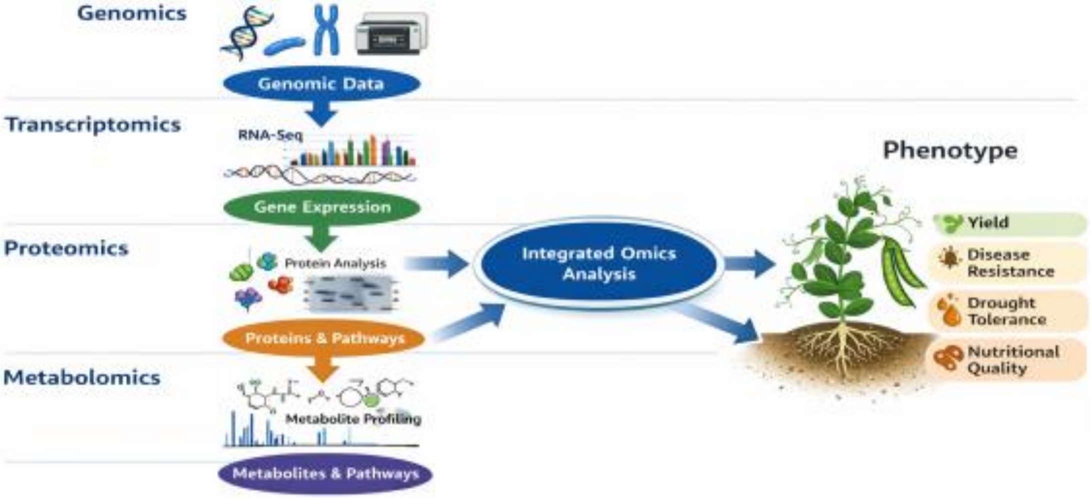

Figure 1. An interlocking omics system that describes the correlation between genomics, transcriptomics, proteomics, metabolomics, and phenotypic characteristics.

Together, the increase in genomic resources and tools has completely transformed strategies of pea improvement. Reference genomes and dense marker systems to GWAS and functional genomics allow the fine dissection of complex traits, and help to move towards genomics-aided and biotechnology-enabled cultivar development. The combination of these tools and breeding pipelines is critical in achieving sustained genetic returns in *Pisum sativum*, both in the present and future, under agricultural pressure.

Molecular Breeding and Marker-Assisted Improvement:

The molecular breeding has brought a revolution to pea (*Pisum sativum* L.) breeding through a combination of both the genomic and the traditional breeding techniques. With the use of DNA markers, high-throughput genotyping, and predictive models, it is possible to select desirable alleles with high precision and develop improved cultivars with better yield, quality, and stress resistance. The significant methodologies of molecular breeding are identified in this section; they are marker-assisted selection (MAS), marker-assisted backcrossing (MABC), genomic selection (GS), and speed breeding with high-throughput phenotyping. MAS and MABC can shorten the breeding cycle by 2-3 generations, and GS has demonstrated the possibility of enhancing the selection gain per year by improving prediction accuracy and allowing early generation selection [28].

Marker-Assisted Selection (MAS):

The marker-assisted selection (MAS) is an advanced breeding strategy in which genetic markers have been linked to specific traits and used to choose individuals that possess the required alleles. Unlike conventional breeding, where breeders consider phenotypes only, MAS allows breeders to screen the traits at the seedling level without taking into consideration the environmental variability [29]. The overall MAS process comprises the identification of markers having a strong correlation with traits of interest, with QTL mapping or GWAS.

Mapping of markers and characters in diverse genetic backgrounds.

Genotype the breeding populations with the desired alleles to select those individuals with the preferred alleles.

Selected lines are then advanced in breeding programs after further cross and evaluation.

MAS has been developed successfully using pea to enhance resistance to major biotic stresses. In such a case, markers of genes controlling resistance against powdery mildew (Erysiphe pisi), ascochyta blight (Ascochyta pisi), and other genes have been used and proved successful in selecting resistant lines. Similarly, MAS has been used to improve the seed quality characteristics, e.g., protein levels and size of the seed, by selecting the genotypes with preferred alleles at an early stage. The efficiency of MAS reduces the breeding period and improves precision with regard to inclusion of traits, particularly those that are cumbersome to assay or phenotype [30].

Marker-Assisted Backcrossing (MABC):

The most specialized type of MAS is known as marker-assisted backcrossing (MABC), which is applied to introgress targeted genes of the donor parents into elite cultivars and eliminate the introduction of unwanted fragments of genomic material. It is particularly useful in introducing disease resistance or tolerance to stress genes in wild relatives or landraces to high-performing cultivars.

In pea, MABC has been effectively used in transferring powdery mildew and Fusarium wilt resistance genes into commercial varieties. As an example, introgression of the er1 gene that provides powdery mildew has been done into the susceptible lines high-yielding but susceptible lines with flanking SNP and SSR markers. Equally, Fusarium root rot (Fusarium oxysporum f. sp. pisi) resistance has been integrated into elite cultivars using MABC, and this has allowed the production of resistant lines without reducing the agronomic performance. The standard MABC process includes repeated backcrossing to the elite parent along with marker-assisted selection of the desired gene and background selection to reinstate the recurrent parent genome within a short period of time [31][32].

MABC decreases drag due to linkage and promotes the production of better cultivars. Its breeding success in pea shows the strength of the combination of molecular markers and classical breeding backcrossing techniques to solve a particular breeding problem.

Genomic Selection (GS):

Genomic selection (GS) is a modern molecular breeding method that forecasts the breeding worth of an individual using the genome-wide data of markers, without having to consider phenotypic assessment alone. In GS, the training population is genotyped at thousands of markers and phenotyped to be used to form prediction models. The model approximates effects of markers over the whole genome and produces genomic estimated breeding values (GEBVs) of individuals in the breeding population.

GS is especially helpful in polymorphic, complex traits, including yield, drought tolerance, heat stress tolerance, etc., where the more traditional methods of QTL-based selection prove less effective. GS provides the possibility to select the best genotypes early and correctly by addressing small-effect alleles that manifest throughout the genome. Preliminary research in the case of pea has indicated that GS could predict seed weight, the number of pods, and resistance to disease among other traits with high precision. The combination of GS and MAS or MABC may further increase the efficiency of selection, which leads to a powerful structure of rapid cultivar development [33][34].

Speed Breeding and High-Throughput Phenotyping:

One of the remarkable technologies that can be used to improve the acceleration of the process of pea improvement is high-throughput phenotyping and speed breeding. Controlled environment techniques like long photoperiod, temperature regulation, and growth chambers would allow one to generate numerous generations of pea each year and shorten the breeding cycle by a great deal. Speed breeding can reduce the normal breeding period from 7–10 years to 3–4 years, enabling the earlier deployment of improved varieties. The common speed breeding conditions of pea are: 20–22 h day photoperiod, 20–25 °C Day temperature, 15–18 °C night temperature, and regulated humidity conditions beneath LED growth chambers with a maximum of 4-6 generations annually, by genotype.

High-throughput phenotyping platforms complement molecular breeding by providing precise, rapid, and non-destructive assessment of plant traits. Digital imaging, hyperspectral sensors, and automated platforms can be used to measure large populations in terms of growth rate, biomass accumulation, flowering time, pod development, and stress responses. As an example, it is possible to note that thermal imaging is used to identify stress responses during drought, whereas the efficiency of photosynthesis and the health of plants can be studied with the help of chlorophyll fluorescence and multispectral imaging. When used with MAS, MABC, or GS, high- throughput phenotyping can help breeders have greater confidence in the precision of any genomic prediction and make improved decisions based on the selection of better lines.

A combination of speed breeding, high-throughput phenotyping, and molecular selection would offer a powerful platform for improving modern pea. This is in the way that cultivar development becomes more efficient and effective in that it reduces the time (breeding cycle) when it comes to breeding, increases the accuracy of the selection, and enables the rapid introgression of inherent complex traits [35].

The concept of molecular breeding via MAS, MABC, and GS with speed breeding and high-throughput phenotyping has transformed the mode of pea improvement radically. With MAS, one can be precise in the choice of particular attributes; with MABC, one can cross disease resistance, and GS can be used in order to facilitate the process of selecting complex polygenic attributes. Controlled environment methods and automated phenotyping systems further enhance breeding cycles and make them more accurate. Together, these molecular and technological advances have put pea breeding in a fast response to the global challenges of climate change, new diseases, and increasing food security demands that will enable production of robust, high-yielding, and improved nutritional breeds, as shown in figure 2.

Genetic Engineering and Transgenic Approaches:

Genetic modification and transgenic methodologies have emerged as an effective means of enhancing pea (*Pisum sativum* L.) with either new or novel traits that are challenging or time-consuming to obtain via traditional breeding technology. With these methods, biotic and abiotic stress resistance can be accurately improved, and potential herbicide tolerance can be achieved by the direct manipulation of genes. In this section, transformation methods, transgenic characteristics that have been attained in pea, and biosafety and regulation have been presented.

Transformation Techniques in Pea:

The most widely used technique in the transformation of pea to a foreign gene is Agrobacterium-mediated transformation. This method takes advantage of the natural capability of Agrobacterium tumefaciens to insert T-DNA into its plasmid into the plant genome, leading to stable genetic incorporation. The general sequence of action is that the infection of the explants (i.e., cotyledonary nodes, embryos, or hypocotyl segments) is done, the bacterium is then co-cultivated with the explant, transformed tissues are selected by using antibiotic or herbicide resistance markers, followed by regeneration of the whole plant through organogenesis or somatic embryogenesis [36].

Although pea has potential, its regeneration has long been a difficult process, as the tissue culture recalcitrance and low regeneration efficacy have historically impeded pea regeneration research. Genotype, the type of explant, the composition of the culture medium, and the hormonal balance are some factors that greatly determine the success of the transformation. Moreover, somaclonal variation that occurs as a result of the tissue culture may impact the stability and expression of transgenes. In an effort to address these drawbacks, investigators have streamlined explanation preparation, Agrobacterium strains, co-cultivation states, and regeneration procedures, resulting in progressive increases in the effectiveness of transformation in various pea varieties.

Another alternative to Agrobacterium-mediated transformation is the biolistic or particle bombardment technique. This technique introduces DNA-coated microprojectiles into plant cells and is less host genotype dependent, but has a high copy number insertion rate and gene silencing. Although each of the two methods has been used successfully in the research environment, Agrobacterium-mediated transformation is the method of choice since it is relatively simple, less costly, and has a propensity to insert into a single copy with a stable expression [37].

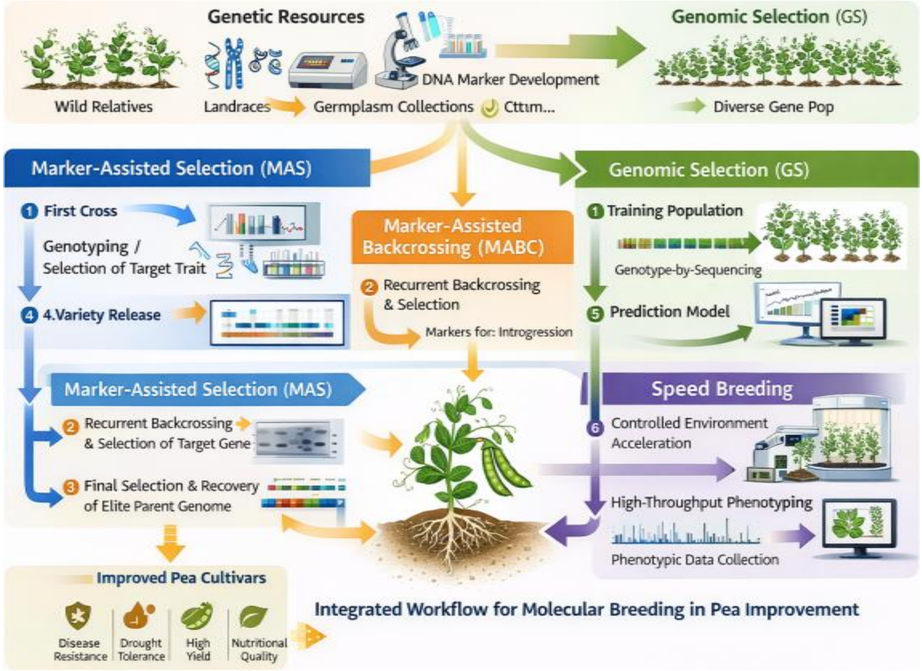

Figure 2. Integrated Molecular Breeding Strategies for Pea Improvement

Transgenic Traits Developed in Pea:

The genetic engineering of pea has been based mostly on the methods of improving resistance against biotic stresses, better tolerance against herbicides, and quality improvement prospects.

Insect Resistance: Transgenic lines of pea have been engineered that express genes of Bacillus thuringiensis (Bt) to offer resistance against major insect pests of pea, including pea weevils (Bruchus pisorum) and aphids. Bt (Bacillus thuringiensis) proteins function as insecticidal toxins that specifically target pest insects while exhibiting minimal toxicity toward non-target organisms and humans. The introduction of these features will minimize the use of chemical pesticides, cutting down costs of production and environmental effects.

Disease Resistance: A number of transgenic approaches have been made in the area of fungal and viral infections of pea, such as powdery mildew (Erysiphe pisi) and pea seed-borne mosaic virus. Increased resistance has been transferred to genes that encode pathogenesis-related proteins, antifungal peptides, or RNA interference (RNAi) constructs. Those methods are complementary to both traditional breeding and molecular marker-assisted methods, and provide solutions to diseases whose natural resistance is restricted or absent in the gene pool Grown out.

Herbicide Tolerance: The second characteristic that is being considered in transgenic pea is herbicide tolerance, which aims at making weed management easy and enhancing crop establishment. The selective application of herbicides can be introduced by the introduction of genes that encode acetolactate synthase (ALS) or Glyphosate- tolerant EPSPS enzymes, which do not harm the crop. This aspect is especially useful with intensive cropping systems in which weed management through manual means is cumbersome or unfeasible [38].

The stability of transgenes is usually tested by PCR confirmation, Southern blot, and expression assays (qRT-PCR/ELISA) up to several generations (T1-T3), and greenhouse and small field trials confirm the phenotypic efficacy. Along with this set of characteristics, research has been conducted to investigate metabolic engineering to enhance the quality of nutrition, such as increasing protein content in the seeds and supplementing them with vitamins. Most of these strategies, however, are still at the experimental phase because of technical, regulatory, and acceptance-level issues.

Biosafety and Regulatory Considerations:

The introduction of GM pea breeds and varieties of plants has been taken with much caution, both in terms of legislation laws and biosafety. Risk assessment involves an analysis of the environmental consequences, which can be negative, such as the presence of gene flow to wild relatives, non-target organisms, and the development of resistant pests or pathogens. The basis of food and feed safety tests is on allergenicity, toxicity, and nutritional equivalency to the traditional cultivars.

The transgenic peas' perception and acceptance by the people also constitute a critical role in the adoption of transgenic peas. The issues surrounding GM foods in society with regard to regulatory approvals and commercial release are, in most instances, culturally, ethically, and market-oriented. Development of trust between farmers, consumers, and policymakers will be necessary to create benefits, safety measures, and scientific evidence that will be communicated in a transparent way [39].

The world has a wide variety of regulatory measures on GM crops. In countries where biotech rules and regulations have been developed, such as the United States of America and certain European countries, there is a strict risk assessment and approval procedure that must be adhered to before commercial launch. Other areas have been very slow in adopting genetically engineered varieties of pea because in the regulatory system, they are not developed [40].

The potential of transgenic and genetic engineering for improving pea is enormous, as it brings the plant some properties that cannot be achieved through the natural breeding process. The use of Agrobacterium, complemented by optimized tissue culture conditions, enables stable gene insertion. Transgenic properties such as insect resistance, disease resistance, and herbicide tolerance can address major production challenges. Implementation of the technologies will require that biosafety, compliance, and social acceptance are well considered to enhance successful implementation. Genetic engineering, together with molecular breeding, genomics, and high-throughput phenotyping, would be used to hasten the development of better pea varieties that are highly productive, resilient, and nutritious.

Genome Editing Technologies in Pea Improvement:

With the introduction of genome editing technology, the future of improving pea (*Pisum sativum* L.) has begun, and it offers discrete and targeted technologies to edit the genes related to the essential agronomic characteristics. In contrast to traditional breeding or transgenic methods, genome editing enables easy modification of the target genes in the genome of the plant itself, by which new breeds can be bred in the short term to develop a better cultivar without the need to insert foreign DNA. This section will look at the CRISPR/Cas systems used in pea, the traits of interest that have been targeted in the genome editing, and the benefits of the genome editing system over the traditional transgenics, as well as the current developments and limitations in this crop.

CRISPR/Cas Systems and Variants:

The ease, efficiency, and versatility of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) have also contributed to their popularity in genome editing. The CRISPR/Cas9 system, which is developed on the foundation of Streptococcus pyogenes, is the most popular in the field of plant studies. It uses a guide RNA (gRNA) to position the Cas9 endonuclease at a specific genomic sequence that results in the formation of either double-strand breaks (DSBs) that may be repaired by the non-homologous end joining (NHEJ) repair process or by homology-directed repair (HDR), to generate desired mutations [41].

Other forms of CRISPR, other than Cas9, have also been explored for pea improvement. Cas12 (Cpf1) is able to have other PAM (protospacer adjacent motif) specificities and make staggered cuts at the DNA, which has the potential to make editing more precise and enable multiple genes to be edited. Cytosine and adenine base editors provide a safer and more predictable approach to editing functional alleles that may be made use of to induce single nucleotide mutation without creating DSBs. Another new technology is prime editing, which enables much more precise in-target insertions, deletions, and base replacements with off-target consequences that are inconsequential, but is currently in its early experimental stages in pea. CRISPR technology systems give the choice of designing and interfering with multiple genes at once, which allows them to modify complicated traits that are under control by polygenic networks.

Target Traits for Genome Editing:

Genome editing in pea has focused on the improvement of genotypes that are hard to manipulate in traditional breeding.

Yield Components: Some of the important predictors of yield are seed size, number of pods, and plant structure. It is possible to edit genes that regulate the flowering time and optimize the growth and partitioning of the nutrients, which will enable maximization of the productivity of the plants and their growth. One illustration is that by controlling the transcription factors or hormone regulation genes in seed development, seed weight and pod filling will be enhanced, and this will not influence the plant vigor. Multi-trait phenotyping, yield component analysis, and multi-location trials are conducted to control potential pleiotropic effects to make sure that the gains in stress tolerance do not affect the quality of the yield and the seed.

Disease resistance: Pea is susceptible to an onslaught of different biotic stressors, including powdery mildew, ascochyta blight, and Fusarium root rot. Genome editing may cause disruption or mutation, or introduction of gain-of-function mutations in susceptibility (S) genes or gain-of-function mutations in resistance (R) genes. Such specificity reduces the risk of linkage drag associated with conventional breeding and makes it possible to maintain the resistance with the accumulation of many alleles associated with defense.

Abiotic Stress Tolerance: The capacity to tolerate drought, heat, and salinity is also increasingly important in the changing climatic conditions. CRISPR/Cas system can be applied in the revision of genes, which are involved in the perception of stress and pathways, and adaptation to osmotic conditions. Indicatively, it is possible to enhance change in the transcription factors, or stress-responsive regulatory genes, to enhance drought or heat resistance without any adverse impact on growth or yield.

Advantages Over Transgenic Approaches:

There are several advantages of genome editing compared to traditional transgenic procedures.

Precision and Speed: CRISPR/Cas-based editing can perform targeted edits at a locus with minimal likelihood of causing accidental genetic modification and greater chances to refine specific phenotypes. Genome editing may result in creating a null mutant, allele replacement, or a less drastic base modification into the plant genome than transgenics, which may involve random insertion of foreign DNA.

Less Regulatory burden: In most regulatory jurisdictions, genome-edited crops containing no foreign DNA are considered differently in comparison with transgenic GMOs. This difference will be able to simplify the regulatory approval process, accelerate the creation of a commercial deployment, and reduce the spending on safety tests. It is an extremely desirable property in crops where small farmers, like pea are involved since regulation procedures are likely to be long and costly, hence discouraging its use.

Additionally, with genome editing, it is possible to rapidly test the candidate genes nominated by QTL mapping and GWAS and functional genomics, allowing them to undergo cycles of gene discovery and optimization of the trait phenotype.

Current Progress and Limitations in Pea:

There is a lot of potential in genome editing, and its application in peas is premature. **CRISPR/Cas -** -mediated editing of pea genes is not well described in the literature, which can be partially explained by the challenge in transforming and regenerating plants. The essential factors in achieving the high efficiency of the editing method are an optimization of delivery methods (such as Agrobacterium-mediated transformation or protoplast transfection), promoter selection, and guide RNA design.

Editing Efficiency: The available data indicate that efficiency is not uniform, and it typically varies under different conditions of the target genotype, target gene, and conditions of tissue culture. Improvement of the regeneration processes and the development of the delivery mechanisms are also of great concern to expand the genome editing in several pea varieties [42]. Reported editing efficiencies in legumes, range from 10–60% based on genotype and delivery method, and off-target risks are reduced with high-fidelity Cas variants and in silico gRNA screening with sequencing validation [43][44].

CRISPR/Cas systems are highly specific, with unwanted mutagenesis potentially occurring at sequences that partially complement the gRNA. Off-target mutations could also occur with a probability to affect the growth of plants or destabilize traits. Reduction in the off-target effects in pea is being done using computational prediction tools, variants of high-fidelity Cas, and careful design of gRNAs.

Despite these inadequacies, genome editing combined with the existing molecular breeding mechanisms, such as MAS, MABC, and genomic selection, can become a powerful framework to accelerate pea improvement. Genome editing can revolutionize the development of high-yielding, disease-resistant, and climate-resistant varieties of peas since it enables making specific changes on the targeted level, fast, and exact [45].

Genome editing systems, namely, CRISPR/Cas. and its variations, are very specific and versatile in improving peas, never tried before. Under controlled conditions, experimental CRISPR-edited pea lines that alter flowering-related genes and disease susceptibility loci have been reported, although on a large scale, field implementation has not been realized because of issues of transformation and regulatory obstacles. Genome editing complements conventional and molecular breeding by targeting yield components, disease resistance, and abiotic stress tolerance. Despite the remaining challenges that include efficiency of editing, transformation limitation, and off-target effects, the further development of the delivery mechanism, base editing, and functional genomics continues to expand the possibilities of genome editing in pea. As regulatory systems are developed, genome-edited pea varieties are likely to emerge as an actual standard of biotechnology-based crop improvement, leading to increased productivity, sustainability, and food security, as shown in fig.3.

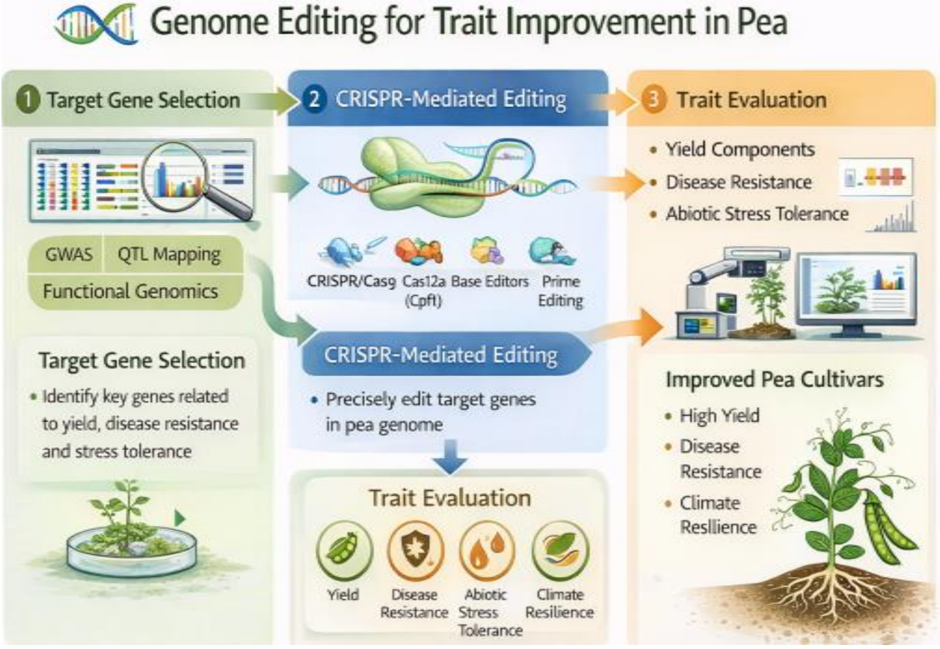

Figure 3. Genome Editing Approaches for Trait Improvement in Pea

From Laboratory to Field: Commercial Cultivar Development:

The ultimate target of the biotechnology-based pea (*Pisum sativum* L.) is to come up with commercial varieties that are high-yielding, drought resistant as well as possessing excellent nutritional value that meets the farmers and consumer's demand. The process of laboratory research and production to field-ready varieties encompasses a combination of molecular tools with conventional breeding systems, severe multi-environment examination, and strong seed systems. This part reveals the processes involved in the breeding of cultivars, effective examples of better varieties, and problems that influence adoption [46].

Integration of Biotechnology with Conventional Breeding:

Recently developed breeding pipelines in pea breeding are a combination of biotechnological interventions such as marker-assisted selection (MAS), genomic selection (GS), and genome editing in conjunction with traditional breeding approaches. Alternatively, in a typical breeding project, the elite germplasm is first filtered based on the desired traits using the model of molecular markers, QTL mapping, or genomic prediction. Crossbreeding of promising lines is then carried out, and selection of such lines is done based on the information, which is genotypic and phenotypic. This integration then facilitates the selection of the optimum genotypes at an early stage, and hence, less time and resources are required to select the optimum genotypes through the traditional means of phenotypic selection.

Advanced lines are then subjected to multi-location trials to evaluate performance across diverse environments. These experiments are needed to attain the interaction of the genotype and the environment that has particular importance, especially in pea, because it is sensitive to climate and soil conditions. It is so multi-location testing that the cultivars that will be selected are high-yielding, disease-resistant, and stress-tolerant to the changing conditions of the field; therefore, they will be reliable in performance for the farmers. Stacking of traits can also be done with biotechnology application to traditional breeding, where multiple desirable traits can be stacked in a single cultivar, like disease resistance, drought tolerance, and improved nutritional quality. This is a plan that enhances the overall worth of new varieties and adaptation degree to diverse agro-climatic areas.

Case Studies of Improved Pea Cultivars:

There are a few successful cases of the effects of biotechnology-based breeding on commercial pea varieties. Using MAS, disease-resistant varieties against fungal pathogens such as powdery mildew (Erysiphe pisi) and ascochyta blight (Ascochyta pisi) have been developed. Breeders have developed cultivars with certain resistance alleles of wild relatives or landraces to retain high production and limit the use of chemical fungicides, which will lead to lower production costs and less environmental impact. Genomic selection and QTL-assisted breeding have also been used in high-yielding cultivars. The use of genome-wide markers to select seed size, pod count, and plant architecture has enhanced the production of lines that have outperformed older varieties regarding productivity and efficiency in resource utilization [47]. The agronomic performance of biotechnology-enhanced cultivars has demonstrated a 10- 25 percent yield increase in response to disease pressure, and introgression of resistant crops has dramatically lowered the use of fungicides in certain production systems.

Another success is biofortified peas. Traditional breeding coupled with molecular markers has resulted in the production of varieties with improved protein levels, micronutrients of iron, zinc, and amino acid composition. Such cultivars play a nutritional role, especially in areas where legumes form a significant source of protein in the diet. These enhancements demonstrate the capability of modern breeding strategies to focus on agronomic performance and human health at the same time.

Seed Production, Intellectual Property, and Adoption:

Breeding is not the only factor that determines the success of the improved pea cultivars, but also the production and distribution of the seeds. Quality seed production guarantees purity of genetic composition, a high percentage of germination, and homogeneity, which are essential in the assurance of the results for farmers and their crops. There are complementary roles between the public and the private seed systems, with the public institutions on some occasions supplying the breeder and the foundation seed, and the large-scale multiplication and commercialization usually being advanced by the private seed companies [48].

The rights to intellectual property, such as plant variety protection and patents, have an impact on the use of biotechnology-based cultivars. Protecting new varieties will encourage investment in breeding and research, and may also limit access to smallholder farmers when the cost of seed is high. There should be a balance between IP protection and fair access so that a wide adoption is achieved.

Various factors drive farmer adoption, with awareness of better varieties, quality seed availability, affordability, agronomic information, and market demand being the key determinants with respect to farmer adoption. Demonstration trials and extension services are essential to inform the farmers of the advantages of new cultivars as well as promote adoption. The issues of adoption are especially acute in those regions where fragmented seed systems are observed or where access to the input markets is low. These barriers can only be dealt with through concerted action between breeders, seed companies, policymakers, and farmers.

The laboratory-to-field development of better pea genotypes is a good example where biotechnology is combined with traditional breeding systems, multi-location testing, and strong seed systems. Successful cultivars, such as disease-resistant, high-yielding, or biofortified, can provide the practical advantage of molecular instruments in resolving agronomic, nutritional, and environmental issues. Having proper seed production, intellectual property management, and farmer adoption are also important to ensure that genetic gains are translated into sustainable agriculture. With the growing momentum of biotechnological innovations, the creation of climate-resistant, nutritionally improved, and massively adapted pea species will become the key to worldwide food and nutritional security.

Regulatory, Ethical, and Socioeconomic Aspects:

Genetic modification and genome editing of pea (*Pisum sativum* L.) are under strict control of a complex system of national and international regulations. Regulatory frameworks, such as the Cartagena Protocol on Biosafety, EFSA, and those that the USDA has created, require rigorous risk assessment to establish the environmental safety, food and feed safety, and the risk of gene flow to wild relatives. The use of simplified regulatory pathways is occasionally used in the case of the genome-edited crops, which contain no foreign DNA, and this may impact the adoption and commercialization based on the country-differing regulations.

Ethical considerations are the most vital part of developing and applying the biotechnological interventions. These are concerns pertaining to the alteration of the plant DNA, equity concerning improved varieties, and environmental ramifications. This may be attained by openness, participation, and informed social interaction to address issues of interest and earn trust in biotechnology. There are significant socio-economic effects, particularly on the smallholder farmers, who constitute the larger proportion of the pea growers in most of the regions. Public-private partnerships, affordable seed pricing models, and farmer training, as well as supportive national policies, are needed in order to enable smallholder farmers to receive the benefits of biotechnology-driven pea improvement to achieve sustainable scaling. This is because, through biotechnology in cultivars, yield, strength, and nutrients can be increased to enhance food security and livelihood. Nonetheless, access can be blocked due to such factors as the price of seeds, intellectual property, and dependence on commercial seed system/industry. The adoption must be sustainable by establishing policies that will strike a balance between innovation, affordability, and equalized accessibility, where the technological advancement must be advantageous to both the producers and the consumers, in addition to causing environmental sustainability.

Future Prospects and Emerging Technologies:

The advanced technologies will be in front of the prospects of enhancing the pea (*Pisum sativum* L.). Pangenomics and haplotype-based breeding provide a more detailed insight into genetic diversity and offer a person an opportunity to choose favorable alleles. The breeding programs increase the application of artificial intelligence (AI) and machine learning to forecast features, perform genomic selection, and make faster decisions. Synthetic biology and gene stacking can result in a combination of numerous characteristics of elite cultivars, such as resistance to diseases and nutritional improvement. A combination of these will enable the creation of high-yielding, abiotic and biotic stress-resistant, and environment-friendly climate-smart types of peas that will satisfy the requirements of sustainable farming in a changing environment.

Conclusion:

Biotechnology has facilitated the bridging between classical breeding and modern precision genetic development, which has basically revamped the genetic improvement of pea (*Pisum sativum* L.). Genomic resources combined with high-quality reference genomes, molecular markers, QTL mapping, and genome-wide association studies have been involved in the identification of desirable alleles that lead to the existence of complex agronomic traits. The molecular breeding methods, such as marker-assisted selection, marker-assisted backcrossing, and genomic breeding, have enabled the rapid generation of high-yielding breeds that have enhanced the yield, stress resistance, and nutrition. Genetic engineering and transgenic, as well as the CRISPR/Cas-mediated genome editing has introduced new traits such as disease and insect resistance, and the CRISPR/Cas-mediated genome editing has allowed a certain degree of precision never achieved in the process of altering qualitative and quantitative traits. These technologies have successfully been translated into commercial cultivars, therefore indicating that biotechnology can play a role in addressing the issues affecting the world, including food security, climate change and malnutrition. New technologies such as pangenomics, artificial intelligence, and synthetic biology will probably be much more effective in improving efficiency in breeding, stacking of traits, and cultivar flexibility in the future. Ultimately, high-level genomics, molecular breeding, and biotechnological innovation have seen the development of pea as a breed among sustainable, tough, and nutritionally upgraded legume production on planet earth.

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