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Original Article

Unveiling Biotechnology Approaches to India's Orphan Legume Pulses Through Novel Gene Targeting for Agro-Economic Empowerment as Promotion for 21st-Century Super Foods

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Abstract *- Orphan legumes (underutilised pulses) are inherently climate-resilient, stress-tolerant, and nutrient-dense crops vital for future food security. Despite India hosting a vast gene pool (>1,150 species across 79 genera—including grass pea, moth bean, and horse gram), these species remain excluded from mainstream agricultural research and global commodity markets. Current literature addresses orphan legume diversity, major legume genetic modification, and global improvement strategies only as isolated domains. This review consolidates India's orphan legume genetic resources with the constraints hindering their adoption and the applicability of modern biotechnological tools. We synthesize the state of marker and genomic resources, alongside the available genetic modification toolbox—ranging from CRISPR/Cas9, base editing, and prime editing to CRISPR/Cas13, CRISPR-associated transposons, and nanoparticle delivery systems. Key adoption barriers are highlighted, including scarce genome sequencing data, anti-nutritional factors impacting palatability and digestibility, weak seed supply chains, and regulatory challenges. We conclude that positioning orphan legumes as mainstream 21st-century food sources requires integrated investments across genomic characterization, field-validated gene editing, seed systems, and policy frameworks. Precision gene editing offers a critical, though not standalone, mechanism for integrating these climate-resilient crops into sustainable agricultural systems.*

Keywords *- Orphan Legumes (Fabaceae), Underutilized Crops, Staple Food, Abiotic Stress, MAS, SNPs, DArT, ESTs, CRISP/Cas9, CRISPR/Cas12 and Pangenomics.*

1. Introduction

By 2050, there will be 9.7 billion people on the planet, as per the United Nations report [1], [2]. One of the biggest concerns is ensuring food security to feed the world's expanding population, especially in developing nations where the majority of people reside [3], [4]. In addition to competing with sprawling urban settlements for land and water, agriculture will have to serve on other important fronts as the twenty-first century approaches, such as adapting to and helping to mitigate climate change, the most pressing adverse condition at our doorstep. The four components of food security—availability, accessibility, use, and stability—will be impacted by climate change [5], [6]. Global warming (Figure 1) may cause developing nations' total potential agricultural productivity to drop by 9% to 21% by 2050, according to a discussion paper published by the FAO [6]. Sadly, a significant drop in agricultural productivity, including labor productivity, will expose the poorest areas [7] to the greatest level of food production volatility, increasing poverty and mortality rates [8]. Adverse climatic aspects, which are predicted to include a greater frequency of extreme heat (temperature and duration), short-term swings, seasonal oscillations, abrupt discontinuities, and long-term variations [9], will have a substantial influence on the food and agriculture sectors [10].

Despite a number of unknowns and uncertainties, there is increasing agreement that as new niches emerge and habitats become more conducive to their establishment and growth, ecological dynamics and equilibriums are likely to be impacted, and overall invertebrate pest diversity and abundance—and pest pressure—will rise. Additionally, research indicates that hosts and infections might be introduced in unfamiliar settings, posing new risks to aquaculture, livestock, and crop systems, as well as posing new difficulties that will require large financial and human resources to solve [12].

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2. Research Problem — Recent Production, Consumption, and Malnutrition Status

2.1. The Magnitude of the Issue

India is still the world's biggest producer and consumer of pulses. According to the Third Advance Estimate, total pulse output grew from 163.23 lakh tons in 2015–16 to 252.38 lakh tons (25.24 million tons) in 2024–25 [13], [14]. However, as a result of this expansion, India is now a net importer of pulses, bringing in 47.38 lakh tons in 2023–2024 to close a supply-demand deficit that is expected to increase to 32–33 million tons by 2030–2031 [15]. Even while per-capita pulse consumption is still much below the suggested levels, this disparity nevertheless exists. According to data from the national consumption expenditure survey, rural per-capita consumption is only 22–26 g/day, which is less than 70% of the recommended allowance. The largest deficiencies are found in the lowest expenditure deciles [16]. The Indian Council of Medical Research (ICMR) recommends a daily intake of 40 g of pulses per adult. National health data show the effects of this protein and micronutrient deficiencies. According to the 2025 UN State of Food Security and Nutrition in the World (SOFI) report, wasting afflicted 18.7% of Indian children under five, which is the greatest prevalence globally. According to FAO, IFAD, UNICEF, WFP, and WHO (2025), 37.4 million children under five were stunted [17]. Underweight prevalence is 32.1%, child stunting is 35.5%, and wasting is 19.3%, according to India's National Family Health Survey (NFHS-5, 2019–21) [18]. An estimated 203 million women nationwide suffer from anaemia, which has risen to 53.7% among women aged 15 to 49 (FAO, IFAD, UNICEF, WFP, and WHO, 2025) [17]. Nutrient-dense orphan legumes are particularly well equipped to bridge this gap because of their higher protein content and micronutrient density compared to cereal mainstays. These metrics portray the prevalence of malnutrition in India—often referred to as "hidden hunger"—as an issue of dietary quality and protein-micronutrient sufficiency rather than general food availability. The pulse base needs to be broadened beyond the few major legumes (black gram, pigeon pea, and chickpea) that presently account for the majority of production data and research funds in order to close this gap. Rather, a wider variety of climate-resilient, nutritionally similar, and regionally significant orphan legumes that are still mostly absent from government production reporting and cultivation-scale-up programs must be included [16].

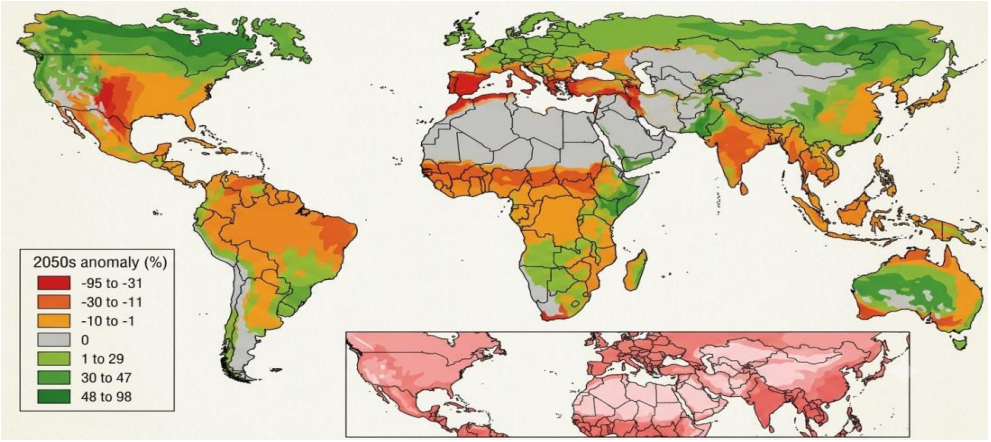


Fig. 1 Map showing the world regions expected to be the most impacted countries across the globe by the effects of climate change in 2050 (shown in red). Source: the CGIAR Research Program on Climate Change, Agriculture and Food Security (CCAFS)

2.2. *The Agricultural Background of Legumes*

2.2.1. Legumes in global and Indian Context

Because of its members' capacity to fix nitrogen symbiotically and their significance as a major source of plant protein, the Fabaceae (Leguminosae) family is the third biggest family of flowering plants and one of the most significant to agriculture in India and throughout the world [19]. Grown in nearly every agro-ecological zone, legumes constitute a disproportionate amount of the diets of those with low and intermediate incomes, who often substitute them for more expensive animal protein sources [20]. India is known as a centre of legume diversity, with individuals from 79 genera and about 1,150 species [21]. Of these, 719 are considered to have demonstrated nutritional value and commercial value. [22]. However, a much wider variety of regionally important legumes are mostly ignored, with the majority of India's pulse production and research coming from a small number of important pulses, including chickpea, pigeon pea, black gram, and green gram.

2.3. The Concept and characteristics of Orphan or Underutilised Crops

The labels used to characterize these neglected species—"orphan," "underutilized," "minor," or "neglected and underutilized species (NUS)"—reflect a shared history of scientific and commercial disinterest rather than any inherent agronomic weakness [23]. According to the International Plant Genetic Resources Institute (IPGRI, 2002), these crops are those that satisfy local nutritional and economic needs, particularly in developing countries, without receiving

comparable financing from governmental research institutions or private breeding programs. A number of agronomically important traits are shared by orphan crops [24]. These traits include adaptation to specific marginal agro-ecological niches, a strong connection to the traditional knowledge and cultural heritage of the area in which they are grown, a lack of formal documentation of agronomic practices, and an almost total absence of formal, quality-assured seed supply systems. The phrase "underutilized" or "orphan" refers to a species' disregard by both domestic and foreign scientific communities. Accordingly, reporters [25] claimed that to orphan species are typically categorized as crops that have minimal global significance, and this carelessness has worsened over time. A vicious circle of underinvestment has resulted from the same lack of research focus that kept these crops "orphaned", also preventing genetic improvements that may have increased their yield and competitiveness versus larger staples. Research networks have paid little attention to orphan crops, which are a varied group of small crops that are typically significant in their local economies but are not sold globally [26] . Private companies have conducted extensive research on these crops, which are used in developing nations to address protein and micronutrient shortages linked to the majority of dietary calories coming from rice (*Oryza* spp.) and wheat (*Triticum Aestivum*) [27]. Although they are essential food crops in many poor nations, orphan or underutilized crops—including orphan legume crops—have little economic significance in international markets [28], [29]. Traditional farmers, especially women, cultivate them primarily to provide households with high-nutrient food security [30]. These species tend to occupy specific niches in the local production and consumption systems, despite being widely diffused outside of their centres of origin. They are still little documented and receive insufficient attention from mainstream research and development projects, despite their significance for the subsistence of local inhabitants. It is challenging to pinpoint exactly which characteristics make a crop an orphan or underutilized crop, but they are frequently associated with the cultural heritage of their native regions, have little documentation regarding their use and cultivation, and are suited to particular agro-ecological niches and marginal land with inadequate or nonexistent formal seed supply systems[31].

Additionally, they can be utilized as animal feed and in other agricultural applications to help farmers with limited resources make money [32]. However, when compared to commodities like rice, corn (*Zea mays*), and wheat(*Triticum aestivum),* they have been overlooked by industry and the international scientific community due to their lack of economic significance [33]. Furthermore, this neglect has resulted in a decreased yield in terms of both quantity and quality due to the absence of genetic improvement. Only recently have scientists in developing countries actively taken on the challenges of studying orphan crops as a potential food crop for the twenty-first century, with the help of international organizations such as the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT).

High-yielding cultivars, chemical fertilizers, insecticides, and agricultural mechanization brought about an unparalleled achievement in food grain production during the green revolution[34]. As a result, quite a significant numbers of superior high-yielding varieties of cereal crops, including maize, rice, and wheat, were subjected to monoculture. This approach has ultimately led to the loss of advantageous alleles, especially those that provide resistance to biotic and abiotic stressors. India boasts a wealth of wild edible plant variety; about 600 species have been identified [35], and many more remain undiscovered. The genetic diversity of several significant species of local importance is still largely unknown, despite the fact that India is thought to be the center of variety for some cultivated crops[36]. The group of pulses and related plants known as legumes is a very important food crop because they are incredibly nutrient-dense sources of micronutrients and protein that can improve livelihoods and health, especially in impoverished nations [37], [38]. With 79 genera and 1152 species spread across different phytogeographic zones, the Indian gene centre is responsible for the great richness of legumes [21]. Accordingly, India is home to 719 commercially significant legume species with 18 significant nutritional statuses[39].

2.4. Nutritional and Phytochemical Significance

Orphan legumes are often more nutritious than major pulses, despite their marginalization. High-quality protein, dietary fiber, complex carbohydrates, vital micronutrients (zinc, iron, and folate), and bioactive phytochemicals (Figure 2) with proven pharmacological, antimicrobial, and antioxidant qualities are all found in them [40]. A number of species have additional therapeutic value beyond basic nutrition. For instance, compounds isolated from the seed of the faba bean (*Vicia faba*) have established pharmacological application in the management of Parkinson's disease [41], demonstrating that the value of these crops extends beyond their contribution of calories or protein into the functional food and nutraceutical domains. Because of their dual nutritional and phytochemical makeup, orphan legumes are increasingly characterized to as "super foods" or "smart foods" in both scientific and public discourse [32]. Orphan (underutilized) legumes represent an untapped natural reservoir of specialized secondary metabolites and bioactive constituents. Beyond conferring enhanced physiological tolerance to abiotic and biotic stresses, these compounds possess significant pharmacological potential for human health. Positioned as valuable nutraceuticals and functional food matrices, orphan legumes offer novel dietary interventions for managing non-communicable lifestyle diseases[42]. Furthermore, their unique biochemical profiles provide promising lead structures for drug discovery in vascular biology, hypoxia signaling, and oncology [43].

An unexplored natural source of specialized secondary metabolites and bioactive components is represented by orphan (underutilized) legumes. These substances have substantial pharmacological promise for human health in addition to providing improved physiological resistance to biotic and abiotic stressors. Orphan legumes are positioned as useful nutraceuticals and functional food matrices that provide innovative dietary strategies for the treatment of non-communicable lifestyle disorders [42]. Additionally, their distinct biochemical profiles offer intriguing lead structures for cancer, hypoxia signaling, and vascular biology drug discovery [43]. The grass pea (*Lathyrus sativus* L.) is one of the greatest choices for creating useful foods. Reports [44] stated that it is the only plant species known to produce substantial amounts of L-homoarginine, a non-proteinogenic amino acid that has vasoprotective properties, acts as a precursor for the synthesis of nitric oxide, and may help lower hypertension and associated cardiovascular risk factors. Nonetheless, the vast majority of stress-inducible phytochemicals generated by orphan legumes remain systematically understudied and biochemically uncharacterized [45]. Meanwhile, compared to major staple crops, multi-omics analysis of orphan legumes has historically lagged. For the majority of species, there are still few complete genomic, transcriptomic, metabolomic, and proteomic databases. Through pangenomic projects and high-throughput whole-genome sequencing, international consortiums like the African Orphan Crops Consortium (AOCC) have started to close this knowledge gap. To fully utilize their adaptive characteristics and nutritional architecture, however, increased funding for functional genomics and multi-omics integration is still necessary[46]. However, the pigeonpea (*Cajanus cajan* [L.] Millsp.) is a prime example of how omics-assisted breeding may turn an undervalued pulse into a common agricultural product. With the assembly and ongoing improvement of its reference genome, pigeonpea—often referred to as "poor people's meat" because of its high seed protein content—has entered the post-genomic age [47]. Pigeonpea, which is well-known for its remarkable resistance to drought, uses sensitive osmotic adjustment mechanisms, deeply penetrating taproot architecture, and a polycarpic flowering habit that modifies flower abortion under extreme water deficiency to conserve metabolic energy. Over 40,000 functional genes have been found in pigeonpea by modern comparative genomics and transcriptomics, several hundred of which form important regulatory networks underpinning adaptation to heat stress and drought. Global food security and climate-resilient crop engineering could greatly benefit from utilizing these multi-omic insights and genome-editing technologies [48].

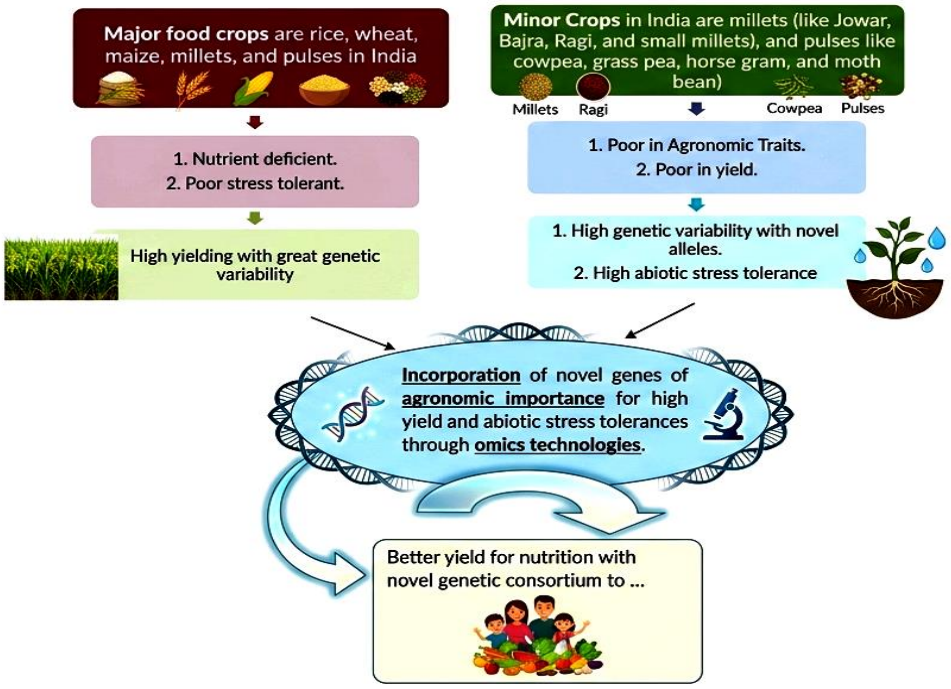


Fig. 2 India's primary and minor crops: a comparison of their differences and similarities for farming. The domestication and evolution of crops. Major crop domestication resulted in a significant increase in productivity but a corresponding decrease in stress tolerance. Conversely, orphan crops have consistently adapted to endure harsh environments, albeit at the expense of productivity. In the twenty-first century, minor crops can be elevated to the status of major sources of nutritional supplements through the use of omics and biotechnological approaches.

In an effort to find the novel gene or mechanism that can be used to improve stress tolerance in elite cultivars, scientists have recently begun investigating wild relatives of important crops [49]. As a result, gene banks, also known as seed vaults, are now a vital tool for plant scientists to conserve and study genetic resources. A comparatively underappreciated set of small crops known as "orphan crops" have been recognized as a valuable genetic resource for

significant features linked to stress tolerance and grain nutrients, in addition to being wild cousins of main crops (Figure 2). However, orphan crops' advantages for human nutrition and health, as well as their capacity to thrive on marginal soils and endure in difficult climates, were frequently overlooked [50].

In contrast, neither before nor during the green revolution were sufficient attempts or investments made to integrate orphan crops into the mainstream food chain [29]. Consequently, in terms of productivity, production, and preference, orphan crops have fallen behind major crops. It's interesting to note that orphan crops, sometimes referred to as "super foods" or "smart foods," have gained attention lately as alternative nutrient-rich diets [51]. In response to the unprecedented demand for orphan crops as food and genetic resources for agri-traits, several international research initiatives have been launched, such as Crops for the Future (CFF) (http://www.cffre search.org/) and the African Orphan Crops Consortium (AOCC) (http://afric anorp hancr ops.org/). Enhancing orphan crops and incorporating them into the main food chain are the goals of these programs[51]. The alarming global malnutrition situation, known as "hidden hunger", and the difficult global climate in recent years have forced the scientific community to revisit orphan crops in order to explore their genetic potential for abiotic stress tolerance and superior nutritional properties [52], [53].

Conversely, it has been shown that during the processes of domestication and yield-oriented breeding, the main cereal crops—such as rice and wheat—lose trait(s) or gene(s) linked to stress tolerance [51] and nutritional property [54]. In order to find superior traits that contribute to stress tolerance and nutritional qualities, orphan crops could be used as a quick resource. These traits could then be introduced into elite kinds of staple crops. This review has highlighted the importance of orphan crops as a genetic resource for improving abiotic stress tolerance in other significant staple crops, as well as the possibility of using orphan crops to speed up crop improvement programs because of recent advancements in genomics and phenotyping platforms. The work carried out at several international consortiums with the goal of leveraging the benefits of orphan crops for food security has also been critically evaluated [50].

2.5. Evolution of Crop Improvement Approaches

In the past, any crop, whether it was a major or an orphan, could be improved through traditional phenotypic selection and hybridization. It was challenging to prevent the co-transfer of unwanted linked genes, and it often took eight to twelve breeding generations to fix a desired characteristic [55]. The advent of DNA-based molecular markers beginning in the late 20th century allowed for marker-assisted selection. Initially, more precise tracking of genetic variation independent of phenotype was made possible by PCR-based methods such as Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), and Amplified Fragment Length Polymorphism (AFLP) [56]. Thanks to the subsequent decrease in sequencing costs, genotyping-by-sequencing techniques and sequencing-based markers (SNPs, DArT, ESTs) have enabled genome-wide association studies (GWAS) and genomic selection even in previously undiscovered species [57]. Targeted genome-editing technologies, such as Zinc-Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and mainly the CRISPR/Cas system, have recently shifted crop improvement from selection among existing genetic variation to the deliberate, site-specific introduction of desired variation [58]. CRISPR/Cas9 in particular has proven to be the most accessible and accurate of these tools, even though its application to orphan legumes is still relatively limited. This has already been shown in model and major legume species like *Medicago truncatula*, *Lotus japonicus*, cowpea, and soybean [59]. due to these species' reduced transformation and regeneration efficiency [60].

2.6. Genomic Resource Landscape of Orphan Legumes

The availability of a reference genome and functional gene annotation is essential for the effective implementation of marker-assisted and gene-editing techniques to any species [61]. Whole-genome sequencing and annotation are well progressed for key crops and specific orphan species that are prioritized by international consortiums, such as Crops for the Future and the African Orphan Crops Consortium [62].

However, genomic characterization is still incomplete or nonexistent for many of the India-specific orphan legumes covered in this review (Table 1). This limitation influences both the viability of gene-editing applications (more on this in the Gene Editing Tools section: Figure 13-22) and the future research priorities listed in the Conclusion. As explained later in this review, national and international genebank holdings, such as the National Bureau of Plant Genetic Resources (NBPGR) in India and the worldwide collections kept by ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), IITA (International Institute of Tropical Agriculture), and CIAT (Centro Internacional de Agricultura Tropical), represent an existing, underutilized foundation on which such genomic characterization could be built.

Together, these five dimensions—the agricultural importance of legumes, the particular traits that characterize their status as orphan crops, the nutritional case for their improvement, the technological trajectory of crop breeding, and the current landscape of genomic resources—provide the background that is required to comprehend the particular gap that this review addresses, which is described below.

Due in large part to the efforts of international consortiums like the African Orphan Crops Consortium (AOCC), whole-genome sequences for a variety of underutilized species are emerging at a rapid pace. As a result, many orphan legume crops are no longer being ignored by the molecular genomics revolution [63]. However, these species often continue to be functionally "orphaned" because of a persistent lack of infrastructure, financing, and specialized breeding programs. In order to unlock structural and functional genes that control abiotic stress tolerance, non-nodulating nitrogen/nutrient absorption pathways, and bioactive human health compounds, it is imperative to strategically allocate resources toward orphan legumes.

Thus building human capital and bio-informatic competence within local scientific communities while preserving long-term research infrastructure happens to be a critical obstacle that must be addressed in order to translate basic genomic datasets into practical agricultural improvement. Incorporating orphan crop research into higher education curricula (Course-based Undergraduate Research Experiences, or CUREs) can both accelerate scientific discovery and ease short-term bioinformatic workforce shortages, as demonstrated by recent institutional frameworks [64].

In the marama bean (*Tylosema esculentum* [Burchell] A. Schreiber), an unnodulating, highly drought-tolerant desert legume, for example, foundational genomic initiatives have effectively used Illumina and PacBio long-read platforms to produce robust whole-genome, chloroplast, and multipartite mitochondrial assemblies [65]. Certain cysteine proteases and cystatin inhibitors with different amino acid structures have been found through functional mining of these assemblies, providing potential candidate genes for crop protection and therapeutic biomedical applications. Additionally, examining the genetic foundations of the non-nodulating mycorrhizal symbioses of marama beans offers important insights into alternate, stress-resilient nutrient acquisition mechanisms in marginal soils [66].

Additionally, building Multi-Parent Advanced Generation Inter-Cross (MAGIC) populations throughout southern African landraces will provide a fundamental resource for high-resolution Multi-parent Mapping, Genome-Wide Association Studies (GWAS), and Genomic Selection (GS) in order to bridge discovery and application. These populations make it easier to isolate and fine-map the loci controlling drought resistance, seed composition, yield stability, and pod shattering. Integrating successive cohorts of undergraduate and graduate researchers guarantees project continuity, even if the multigenerational timescale inherent in population development necessitates ongoing funding [67]. The ultimate goal of this integrative educational and genomic framework is to provide resource-poor smallholder farmers with better, climate-resilient germplasm [68].

2.7. Genomics-Assisted Breeding, GWAS, QTL Mapping and Pangenomics

By making it possible to genetically dissect complex features that were previously challenging to quantify due to a lack of reference genomes, marker resources, and well-annotated germplasm, the quick creation of genomic resources is revolutionizing the improvement of orphan legumes. Genome-Wide Association Studies (GWAS) and Quantitative Trait Locus (QTL) mapping offer complementary methods for locating genomic areas and potential genes governing yield, nutritional quality, drought and salinity tolerance, and other adaptive features. While GWAS uses historical recombination across varied germplasm to obtain higher-resolution detection of marker–trait relationships, QTL mapping is especially helpful for identifying trait-associated genomic areas in biparental populations. By capturing genetic diversity, presence-absence variation, and structural changes that can be overlooked when using a single reference genome, the development of high-quality chromosome-scale genomes and pangenomes has further increased this potential. According to recent research, pangenomics can link naturally occurring genomic diversity with agronomic and adaptive features, making it easier to find advantageous alleles for breeding [69]. This makes pangenomics a crucial part of improving orphan crops. It is possible to further prioritize candidate genes and differentiate functional loci from statistically related markers by integrating GWAS and QTL data with transcriptomic, proteomic, and metabolomic datasets. For orphan legumes, where large phenotyped populations are frequently unavailable, genomic selection and machine-learning techniques provide chances to anticipate breeding values for complex, environmentally responsive characteristics [70]. Therefore, a strong framework for transitioning from the identification of marker-trait associations to the deployment of validated favorable alleles in climate-resilient orphan-legume breeding is provided by the integration of QTL mapping, GWAS, pangenomics, multi-omics, genomic selection, and high-throughput phenotyping.

2.8. Expanded MAGIC Population & Molecular Breeding Pipeline Section

One important strategy for modern orphan crop development is to create Multi-parent Advanced Generation Inter-Cross (MAGIC) populations across multiple geographical landraces to bridge the gap between fundamental genomic research and field-level implementation. MAGIC populations employ multi-way crossing methods (such as 4-, 8-, or 16-way crossings) to capture greater genetic variation and promote widespread recombination, as opposed to conventional biparental mapping populations. This high number of current and historical recombination events greatly enhances mapping resolution by lowering the Linkage Disequilibrium (LD) block constraints that may make it challenging to pinpoint

the causal locus in underutilized species [71]. MAGIC populations enable strong Genome-Wide Association Studies (GWAS) when paired with Whole-Genome Resequencing (WGRS) or high-density SNP genotyping tools. Quantitative Trait Loci (QTLs) and candidate genes controlling important domestication traits, like pod dehiscence, seed coat permeability, and growth habit, as well as complex agronomic traits, like osmotic adjustment, deep root system architecture, and yield stability under extreme drought and nutrient deficits, can be fine-mapped using GWAS pipelines [72]. Moreover, Genomic Selection (GS) pipelines provide a revolutionary paradigm change for highly polygenic characteristics governed by several small-effect QTLs that avoid strict GWAS significance thresholds. GS enables breeders to predict performance and carry out early-generation selection without requiring extensive, multi-environment field phenotyping in each breeding cycle by computing Genomic Estimated Breeding Values (GEBVs) from genome-wide marker profiles across a phenotyped training population [73]. In the end, high-throughput Marker-Assisted Selection (MAS), Marker-Assisted Backcrossing (MABC), and Forward Breeding assays (such as KASP or competitive allele-specific PCR markers) are created from candidate SNPs and structural variants found through GWAS and verified via functional genomics. By incorporating these molecular marker platforms into breeding programs for orphan legumes, generation intervals are significantly shortened, selection intensity is increased, and accelerates the release of high-yielding, climate-resilient, and biofortified cultivars to resource-poor smallholder farmers [61].

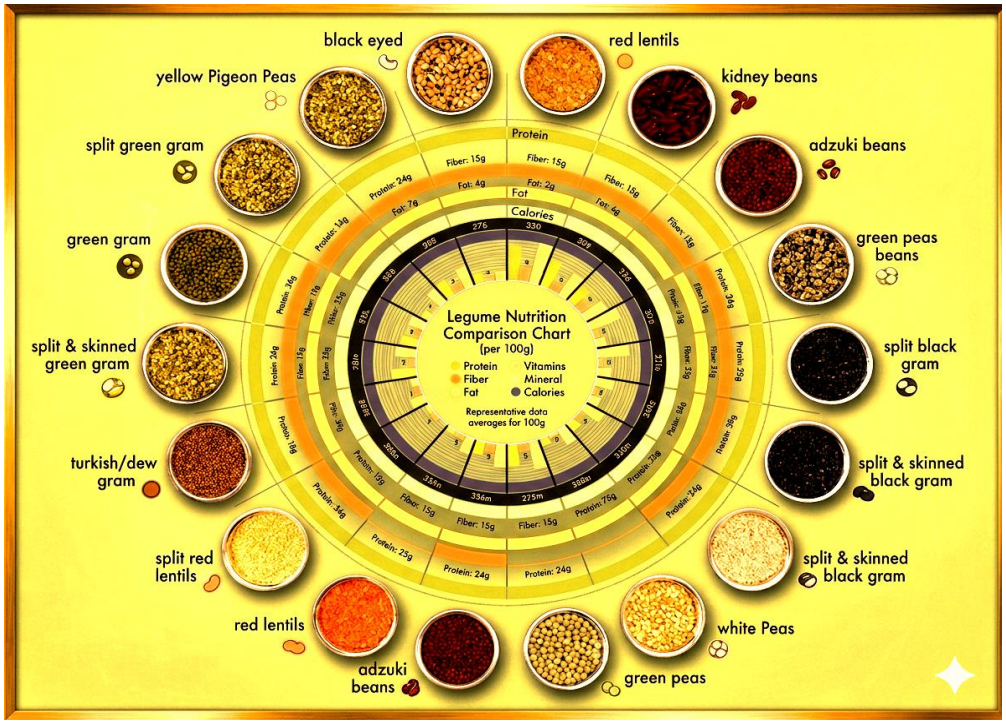


Fig. 3 comparative chart representing all the Indian Pulses with NUTRITION COMPARISON (per 100g)" (ref: https://ministryofcurry.com/indian-pulses/)

Table 1. List of Orphan legumes in India and their major uses

Orphan Legumes in India	Uses
• Cowpea (*Vigna unguiculata*): Subspecies (cultivated): *Vigna unguiculata* subsp. *unguiculata* • Cultivar Groups: Unguiculata, Biflora, Sesquipedalis, Textilis (Family Leguminoseae, (2n=22)	Important for both human and animal nutrition, with a wide range of uses in food, cosmetics, and pharmaceuticals.
Horse Gram (*Macrotyloma uniflorum*): 2n=20	Indigenous to South Asia, it is cultivated in unfavourable environmental conditions and is a valuable source of protein and other nutrients.
• Dolichos Bean (*Lablab purpureus*):	An ancient legume with potential for food and income generation,

(2n=22)	especially for those with limited access to animal protein.
Bambara Groundnut (*Vigna subterranea* (L.) Verdc.):2n=22	Known for its high nutritional value and resilience to drought.
Grass Pea (*Lathyrus sativus*): 2n=14	A drought-tolerant crop with a long history of cultivation, fantastic animal fodder, a good source of novel proteins with a stable karyotype, and a climate resilient crop
Moth Bean (Vigna aconitifolia): 2n=22	A drought-resistant legume with potential for food and fodder.
Rice Bean (*Vigna umbellata*): 2n=22	Native to the Indo-Chinese region, it has high nutritional potential and antioxidant activity.
Tepary Bean (*Phaseolus acutifolius*): 2n=22	A drought-tolerant legume with potential for food and fodder.
African Yam Bean (*Sphenostylis stenocarpa*): 2n=22	A lesser-known legume with potential for food and nutritional security.
Kersting's Groundnut (*Macrotyloma geocarpum*): 2n=20	A drought-tolerant legume with high protein content, mainly found in West Africa.
Jack Bean (*Canavalia ensiformis*): 2n=22	A legume with potential for food and fodder, found in the tropics and subtropics.
Lima Bean (*Phaseolus lunatus*): 2n=22	A versatile legume with potential for food and fodder.
Pigeon Pea (*Cajanus cajan*): 2n=22	It is a significant legume crop in India, ideal for semiarid areas, and an essential source of protein, especially in vegetarian diets.
Broad Bean, (*Vicia faba*), 2n=12	It is a good source of protein for North-Eastern India and Bihar. The ability of the faba bean plant to control atmospheric nitrogen eventually increases soil fertility. In addition to being inexpensive and a high source of protein, V. faba is well-known for being a covered crop because it stops soil erosion. The presence of polyphenols with strong antioxidant activity is the cause of its therapeutic qualities. It is applied and used as an ingredient to ease stiff limbs. Faba bean seeds are a source of L-DOPA, a precursor to dopamine. Parkinson's disease is treated using it. It possesses antioxidant properties as well. Additionally, it is utilized as a medication to treat Parkinson's disease.[32]

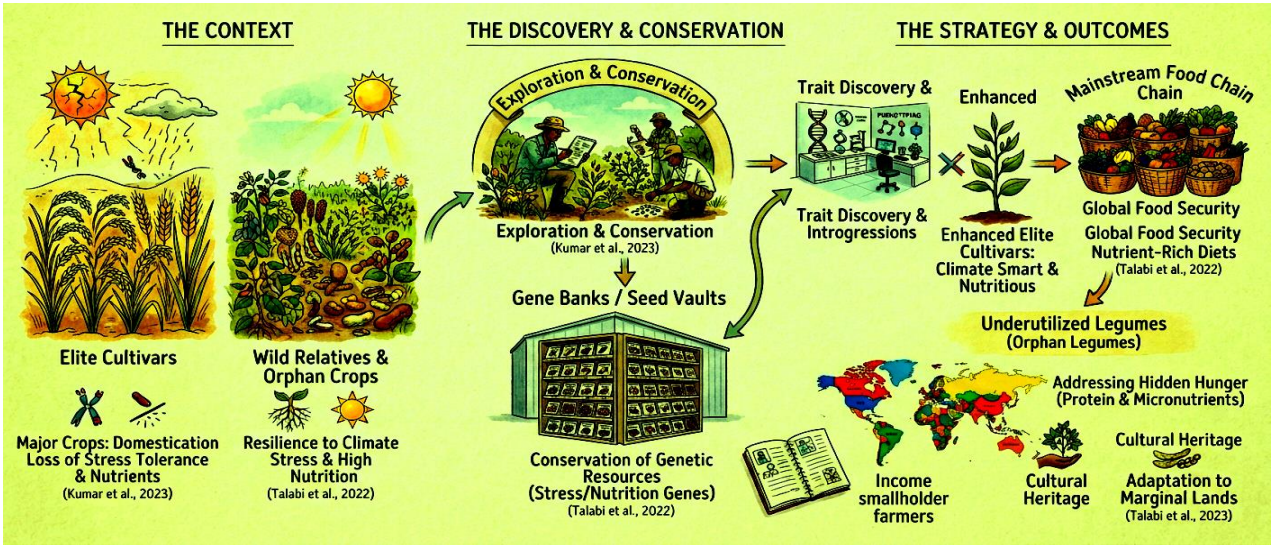


Fig. 4 The proposition of subjecting orphan legumes for better utilization as dietary supplements across the globe is shown as a framework for Integrating Underutilized and Orphan Legumes into Global Food Systems for Climate Resilience and Food Security.

By definition, underutilized legumes are a wide range of cultivated pulses with advantageous qualities, but because of supply and utility issues, they are not as important as major world crops like rice, maize, potatoes, and wheat. Despite their neglect, this group of crops has cultural values, useful agronomic, genetic, and biochemical features, and is characterized by diversity [29], [74]. In many developing nations, orphan legume pulses are essential because they help smallholder farmers make money and address protein and micronutrient deficiencies brought on by consuming a high-calorie diet made from the main crops that scientists and private companies have studied. Orphan legumes perform unique roles in conventional production and consumption systems, despite the fact that these crops may be widely dispersed outside of their original locations. Although many rural communities in sub-Saharan Africa (SSA) rely on underutilized legumes as staple crops, their economic potential for both regional and global markets has not been fully realized. As a result, they are neglected and lack genetic improvement, which lowers their quality and yield [29], [53]. Although it is difficult to identify precisely what qualities make a crop underutilized, orphan legumes are characterized by the following limitations: (i) they are often associated with the cultural heritage of their native regions; (ii) there is insufficient documentation of their cultivation and use; (iii) they are adapted to specific marginal land and agro-ecological niches; and (iv) there are no official seed supply systems.

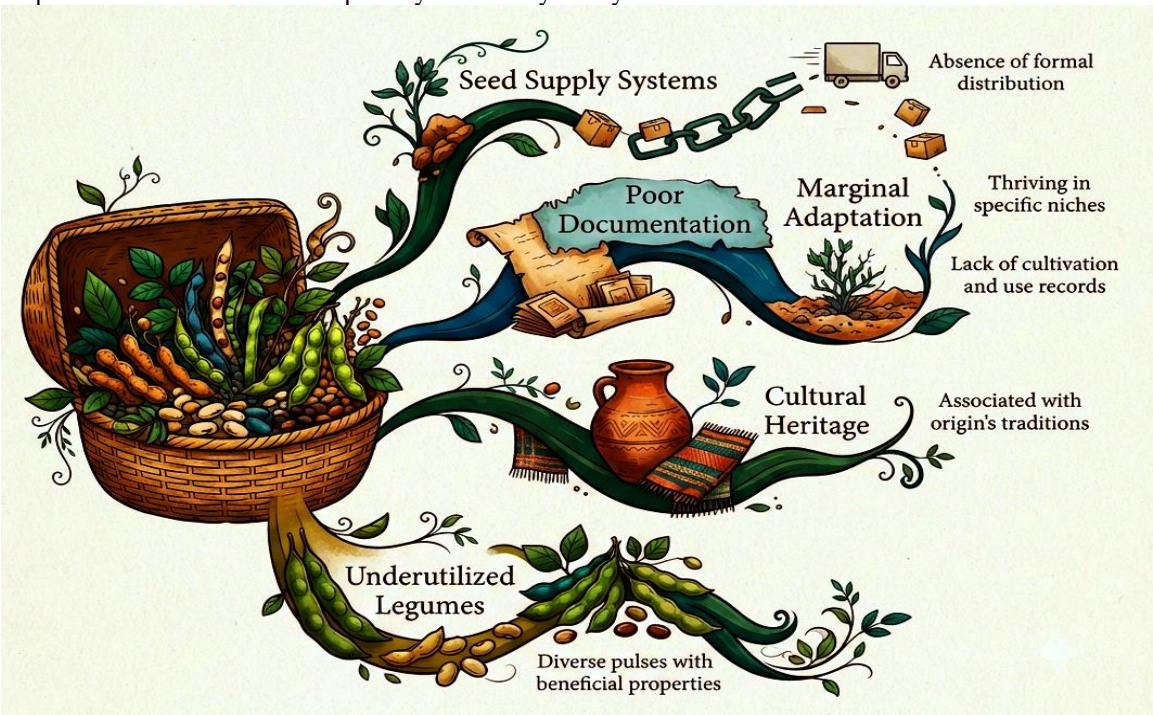


Fig. 5 The constraint components, i.e., Key Characteristics, Value, and Challenges of Underutilized Legumes and Orphan Legumes in Indian Agriculture

3. Comparison with Existing Research Findings

The review's corroborations both support and expand upon those found in relevant literature. Similar to [75], this review confirms that historical underinvestment in genetic improvement relative to major cereals and pulses is more responsible for the underutilization of orphan legumes than an innate agronomic deficiency. This pattern is confirmed here across a wider range of India-specific species than either of the previous studies examined. In a similar vein, the constraints found in this review—hard-seededness, anti-nutritional factor accumulation, and weak seed-supply systems -strongly resemble the obstacles documented by [32] for African orphan legumes, indicating that these constraints are not specific to the Indian context but rather structurally similar across regions, thereby reinforcing that solutions developed within international consortia (e.g., AOCC, CFF) may transfer meaningfully to Indian breeding programs. This review differs from earlier studies in the assessment of gene-editing readiness. Transgene-free RNP delivery, nanoparticle-mediated distribution, and prime editing have all independently decreased particular transformation-related barriers since that assessment, despite the fact that no single orphan legume species has yet integrated all three developments in a validated workflow. According to this review's synthesis of later, more recent research [76] came to the conclusion that transformation and regeneration efficiency continue to be the key barriers to CRISPR/Cas9 application in legumes. Similarly, the species-level comparison in our review demonstrates that this barrier is unevenly distributed, despite reportings [77] and [79] framing low genomic resources as the primary obstacle to orphan-crop gene editing generally: While crops like African

Yam Bean and Bambara Groundnut already have SNP-level genomic characterization adequate to support marker-assisted and GWAS-based approaches, several Indian orphan legumes discussed here, such as grass pea, rice bean, and moth bean, are still comparatively genomically uncharacterized. By narrowing down the general "genomic resource scarcity" narrative that was common in earlier assessments into a more specific, species-differentiated study goal, this result clearly informs the future research plan proposed in the Conclusion. Finally, the integration of India's tiered SDN-1/SDN-2/SDN-3 regulatory framework with specific gene-editing techniques discussed (Ethical, Biosafety, and Regulatory Considerations) in this review implies that Indian orphan legume improvement programs may have access to a more differentiated regulatory pathway than is typically believed in the larger orphan-crop literature [78] an implication not previously articulated in these terms, in contrast to reviews that only briefly address regulatory and consumer acceptance barriers [79].

3.1. Novelty of this Review

This review examines the potential of India's orphan legume pulses to transition from regionally significant crops to mainstream contributors to agro-economic and nutritional security, through the lens of modern gene-targeting and biotechnological approaches. Specifically, this review addresses three questions:

- which orphan legume pulses cultivated in India hold the greatest agronomic and nutritional promise, and what constraints currently limit their wider adoption;
- Which gene-editing and molecular breeding technologies are best suited to overcoming these constraints, given the genomic resources currently available for these species, and
- What socio-economic, regulatory, and consumer-acceptance factors will determine whether biotechnological improvement translates into practical, on-the-ground adoption.

3.2. Below is the systematic workflow and literature search methodology structured for this review on India's orphan legume pulses and biotechnological advancements.

[Phase 1: Identification & Database Search]
↓
[Phase 2: Screening & Selection Criteria]
↓
[Phase 3: Data Extraction & Categorization]
↓
[Phase 4: Synthesis & Thematic Mapping]

3.2.1. Phase 1: Database Search Strategy & Query Formulation

To systematically review underutilized Indian legume pulses, their climate-resilience mechanisms, omics profiles, and precision gene-editing interventions, the literature search workflow is defined across major academic databases (PubMed, Scopus, Web of Science, Google Scholar, and AGRICOLA):

Table 2. Search domains, key terms and target focus for literature review

Search Domain	Key Search Terms & Boolean Operators	Target Focus Areas
Target Crops	("Orphan legumes" OR "Underutilized pulses" OR "Vigna unguiculata" OR "Macrotyloma uniflorum" OR "Lablab purpureus" OR "Lathyrus sativus" OR "Vigna aconitifolia" OR "Vigna umbellata" OR "Vicia faba" OR "Phaseolus acutifolius")	Indian grain legume species and genotypes.
Biotechnology & Editing	("CRISPR-Cas9" OR "Base editing" OR "Prime editing" OR "PASTE" OR "CASTs" OR "Multiplexing" OR "RNP delivery" OR "Nanotechnology" OR "Marker-assisted selection" OR "GWAS")	Genetic engineering tools, viral vectors, and delivery platforms.
Agronomic Traits & Stresses	("Abiotic stress" OR "Drought tolerance" OR "Salinity" OR "Heavy metal toxicity" OR "Biofortification" OR "Antinutritional factors" OR "L-ODAP" OR "Hard-to-cook")	Physiological adaptations, cellular ROS scavenging, and nutritional barriers.
Genetic Resources	("NBPGR" OR "ICRISAT" OR "IITA" OR "Genebank accessions" OR "Germplasm diversity")	Ex-situ and in-situ conservation repositories. [80]

3.2.2. Phase 2: Eligibility and Screening Requirements

The following multi-tier inclusion and exclusion criteria are used to filter the articles that were found using the search queries:

Criteria for Inclusion

- Original empirical research, peer-reviewed publications, and comparative genomics studies that highlight underutilized Fabaceae species in India or around the world.
- Research assessing the physiological, biochemical, or molecular mechanisms that underlie resilience to abiotic stressors (heat, salt, heavy metals, and drought).
- Studies on transcriptomics, genome-wide association studies (GWAS), gene editing in legumes, and the

development of molecular markers (SNPs, SSRs, DArTseq).

- Reports on nutritional contents, bioactive secondary metabolites, and anti-nutritional elements (such as L-ODAP, phytates, tannins, trypsin inhibitors, and alkaloids).[21]

Exclusion Standards

- Why Research that solely focuses on major cereal crops (rice, wheat, and maize) without considering the context of gene transfer or comparative importance to orphan crops.
- Opinion pieces that are not subjected to peer review or studies that lack methodological validation in molecular or cytogenetic assays.

3.2.3. Phase 3: Data Extraction & Categorization

Five Functional Research Domains are used to Classify the Retrieved Literature

1. Germplasm & Taxonomic Mapping: cataloging accessions from national and international repositories (NBPGR, ICRISAT, IITA, CIAT) and examining genotype variability (e.g., low L-ODAP characteristics in 25 genotypes of Lathyrus sativus, flower color, leaf structure, and biomass).
2. Physiological and Biochemical Adaptations: Gathering information on osmotic adjustment (proline, sugar alcohols), root architecture (depth, hydraulic conductivity), antioxidant enzymatic pathways (SOD, CAT, APX), and rhizobial/mycorrhizal symbioses under stress.
3. Socio-Economic & Culinary Constraints: Recording yield disparities, the absence of official seed supply systems, market skepticism, and processing difficulties (hard-seededness, anti-nutritional elements).
4. Omics & Marker-Assisted Selection (MAS): combining genomic resources, high-throughput DArTseq SNP genotyping, QTL mapping, and comparative pangenomics using model legumes (Medicago truncatula, Glycine max).
5. Advanced CRISPR/Cas subvariants and delivery vectors are mapped using precision gene editing technologies, such as:

- Knockouts and knock-ins for Cas9 and Cas12a
- Prime editing (PE) and base editing (CBE/ABE)
- Site-specific Integration: CRISPR-Associated Transposons (CASTs) and the PASTE system
- Epigenetic editing: dCas9-fused histone modifiers and methyltransferases (CRISPR-on/off)
- Delivery methods include ribonucleoprotein (RNP) complexes, and nanocarriers (polymeric nanoparticles, carbon nanotubes) [69], [81]

3.2.4. Phase 4: Systematic Synthesis & Framework Integration

i. A comprehensive paradigm connecting Germplasm Discovery - Multi-Omics Profiling - Precision CRISPR/Cas Editing is created by combining data taken from the literature. Promoting orphan legumes as sustainable superfoods for climate-resilient agriculture through agro-economic mainstreaming [82].

ii. A clear empirical "Methodology" section outlining an experimental research design, protocols, or experimental controls is absent from the publication, according to the given copy. This paper is a thorough review piece instead. In order to suggest how contemporary biotechnological methods, particularly genome editing and high-throughput phenotyping, can be used to improve Indian orphan legumes, the methodological component focuses on collecting research and developing a strategic framework [82].

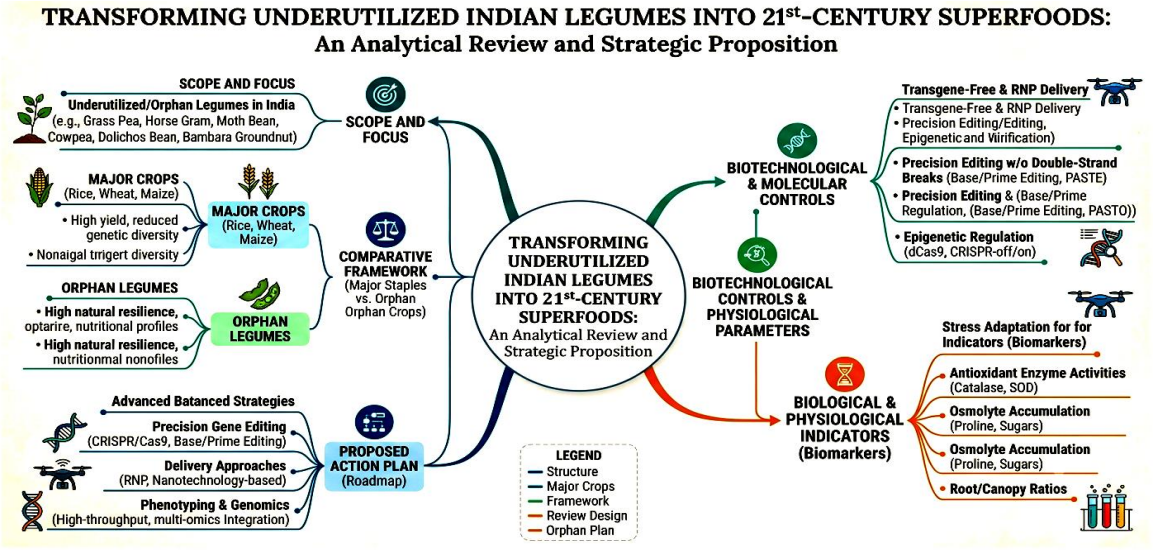


Fig. 6 Conceptual Framework and Strategic Roadmap for Transforming Underutilized Indian Legumes into 21st-Century Superfoods

3.3. Below is the comprehensive outflow of how the paper addresses its conceptual framework, study focus, and the proposed controls/mechanisms associated with the biotechnological strategies discussed*:*

3.3.1. Research Design (Conceptual & Review Framework)

Rather than an empirical laboratory trial, the study is designed as an analytical review and strategic proposition:

- Scope and Focus: The study focuses on underutilized/orphan legumes in India (e.g., *Lathyrus sativus* / grass pea, horse gram, moth bean, cowpea, Dolichos bean, Bambara groundnut, etc.).
- Comparative Framework: The authors contrast major staple crops (rice, wheat, maize) with orphan crops across evolutionary and agricultural dimensions:
  - *Major Crops:* High yield and productivity, but reduced genetic diversity and stress tolerance due to domestication bottlenecks and monoculture.
  - *Orphan Legumes:* Lower yield and quality due to historical scientific neglect, but high natural resilience to abiotic stresses (drought, salinity, heavy metals) and rich nutritional/phytochemical profiles.
- Proposed Action Plan: The study outlines a roadmap to elevate orphan legumes into "21st-century superfoods" using advanced biotechnological and omics interventions. Key strategies proposed include:
  - Precision Gene Editing: Deploying CRISPR/Cas9 along with subvariants (Base Editing, Prime Editing, CRISPR/Cas13, Epigenetic Editing)[51].
  - Delivery Approaches: Implementing RNP (Ribonucleoprotein) complex delivery, transgene-free editing, and nanotechnology-based delivery (using carbon nanotubes, lipid nanocarriers, or polymeric nanoparticles) to bypass regulatory hurdles and reduce off-target effects.
  - Phenotyping & Genomics: Integrating high-throughput phenotyping (ground-based, UAV, and satellite imaging) with molecular markers (SNPs, DArTseq, GBS) and multi-omics (transcriptomics, proteomics, metabolomics)[51].

3.3.2. Controls and Regulatory/Mechanistic Parameters

Because no empirical experiment was conducted in the paper, traditional experimental controls (such as negative/untreated controls or mock vectors) are not presented in the results section. However, the paper explicitly details the molecular mechanisms, biochemical parameters, and regulatory controls necessary for these biotechnological applications:

- Biotechnological & Molecular Controls:
  - Transgene-Free & RNP Delivery: The paper highlights pre-assembled CRISPR/Cas RNP complexes and transient expression systems to avoid permanent integration of foreign DNA, thereby mitigating off-target effects and regulatory strain.
  - Precision Editing without Double-Strand Breaks (DSBs): Promotes tools like Base Editors, Prime Editing, and PASTE (Programmable Addition through Site-Specific Targeting Elements) to execute precise substitutions or insertions without inducing damaging double-strand breaks.
  - Epigenetic Regulation: Mentions dead Cas9 (dCas9) fused with DNA methyltransferases or histone-modifying enzymes (CRISPR-off/CRISPR-on) to regulate or silence gene expression without altering the underlying DNA sequence.
- Biological & Physiological Indicators (Biomarkers):
  - The study discusses evaluating stress adaptation through specific biological markers and physiological controls, including antioxidant enzyme activities (catalase, superoxide dismutase, peroxidase), osmolyte accumulation (proline, sugars, betaine), root/canopy ratios, and chlorophyll concentrations under abiotic stress [83].

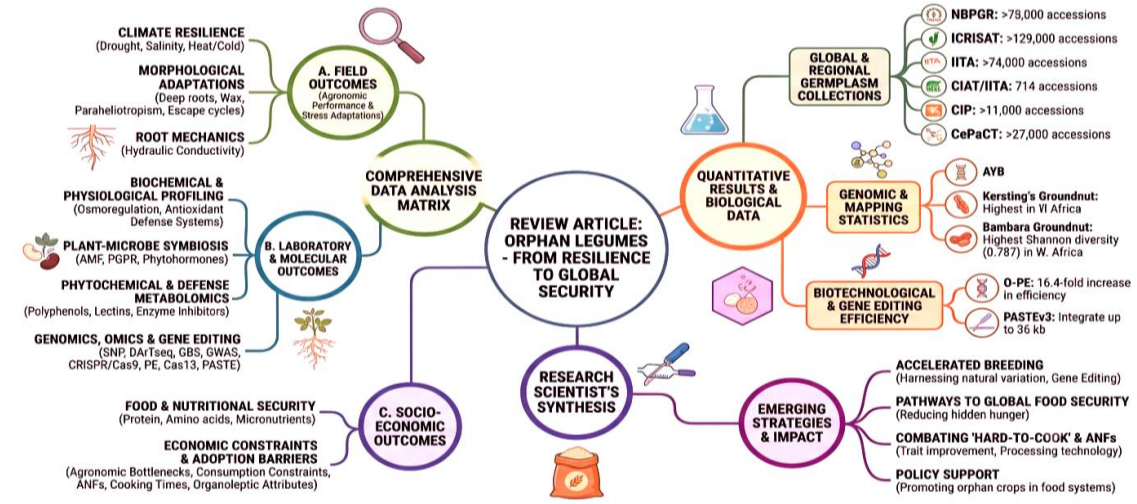


Fig. 7 Unlocking Underutilized Legumes: A Comprehensive Data Analysis and Synthesis Matrix for Climate Security

Based on the provided manuscript, here is the structured analysis of data, quantitative findings, and analytical methodologies across the Field, Lab, and Socio-Economic domains.

3.3.3. Comprehensive Data Analysis Matrix
Field Outcomes: Through Literature Reviews

- Stress Adaptations & Agronomic Performance:
- When major cereal crops fail due to harsh abiotic circumstances like drought, salt, heat/cold, heavy metals, and soil phytotoxicity, orphan legumes show greater climatic resistance.
- Morphological adaptations to avoid dryness include paraheliotropism, reduced canopy size, deep/profuse root structures, smaller leaf surface areas, increased epicuticular wax deposition, and quicker flowering/maturity cycles.
- Root Mechanics: Even in low soil moisture levels, efficient water absorption is made possible by enhanced root hydraulic conductivity, which is fueled by meta-xylem vessel distribution [84].

Laboratory & Molecular Outcomes: Through literature searches

- Physiological and Biochemical Profiling:
    - Osmoregulation: High levels of osmolytes, such as proline, alanine, sucrose, hexoses, mannitol, ononitol, sorbitol, and betaine derivatives, preserve cellular turgor and the integrity of the plasma membrane.
    - Antioxidant Defense Systems: Catalase (CAT), Superoxide Dismutase (SOD), Peroxidase (POD), Ascorbate Peroxidase (APX), and Dehydroascorbate Reductase are important enzymatic antioxidants that enhance the scavenging of Reactive Oxygen Species (ROS).
    - Plant-Microbe Symbiosis: ACC deaminase, chitinase, siderophores, organic acids, and phytohormones (auxins, gibberellins, cytokinins, and ABA) are produced by beneficial relationships with Arbuscular Mycorrhizal Fungi (AMF) and Plant Growth-Promoting Rhizobacteria (PGPR).

(a) Phytochemical & Defense Metabolomics

Natural immunity against microbial diseases and insect pests is provided by bioactive defensive chemicals such as lectins, polyphenols (flavonoids, saponins, and tannins), and enzyme inhibitors (trypsin, chymotrypsin, α-amylase, and cysteine protease inhibitors)

(b) Genomics, Omics & Gene Editing

- Genomic Resource Mapping: Evaluation of single-nucleotide polymorphisms (SNPs), DArTseq, ESTs, and high-throughput GBS for Genome-Wide Association Studies (GWAS) and QTL mapping.
- Precision Gene Editing Targets: Deployment of CRISPR/Cas9, base editors (CBE/ABE), Prime Editing (PE), CRISPR/Cas13, and PASTE systems targeting key agronomic loci (e.g., *Phytoene Desaturase* [PDS], flowering time, and photosensitivity genes) [85].

Socio-Economic Outcomes

- Food & Nutritional Security: To fight "hidden hunger" in low-income people, high doses of plant-based dietary protein, essential amino acids, micronutrients (iron, zinc, folates), carbs, dietary fiber, and essential fatty acids are recommended.
- Economic Constraints & Adoption Barriers:
    - Agronomic Bottlenecks: Low baseline production, irregular development, asynchronous flowering, vulnerability to breakage of seed pods, and absence of official seed supply channels.
    - Consumption Constraints: Anti-Nutritional Factors (ANFs) such as phytic acid, tannins, saponins, L-DOPA, and oligosaccharides; longer cooking times because of the "Hard-to-Cook" (HTC) phenomena; and unfavorable organoleptic characteristics (bitter tastes, astringency, off-flavors).

3.3.4. Quantitative Results & Biological Data: Through literature searches

- Global & Regional Germplasm Collections:
    - NBPGR (India): Holds 14,904 chickpea, 25,785 minor millet, and 26,395 sorghum accessions.
    - ICRISAT: Preserves >129,000 accessions across 144 countries.
    - IITA: Manages 15,000 cowpea, ~5,900 yam, and ~2,000 Bambara groundnut accessions.
    - CIAT & IITA: Maintain 5,965 cassava accessions.
    - CIP: Houses over 5,500 sweet potato accessions and 2,529 Andean roots/tubers accessions.
    - CePaCT: Retains ~1,300 taro accessions (~70% of global total).
- Genomic & Mapping Statistics:
- African Yam Bean (AYB): Quantitative trait loci (QTLs) for seed protein, oil, and starch content were effectively found by GWAS utilizing 2.48K quality SNPs (out of 3.6K SNPs obtained with DArTseq GBS).
- Kersting's Groundnut: 493 SNPs from 281 accessions showed 10.6% genomic alignment with reference genomes of mung beans and adzuki beans.
- Bambara Groundnut: Using 270 genotypes from five locations, diversity analysis revealed that West Africa had the highest mean gene diversity (0.478) and Shannon diversity index (0.787).
- Biotechnological & Gene Editing Efficiency
    - Optimized Prime Editors (O-PE): When used in plant cells, these editors had an average editing efficiency of 1.15%, which is 16.4 times higher than that of ordinary prime editor implementations.

- Large target DNA sequences up to 36 kb can be integrated into particular genomic loci using PASTE Systems (PASTEv3) without causing double-stranded DNA breaks (DSBs) [86].

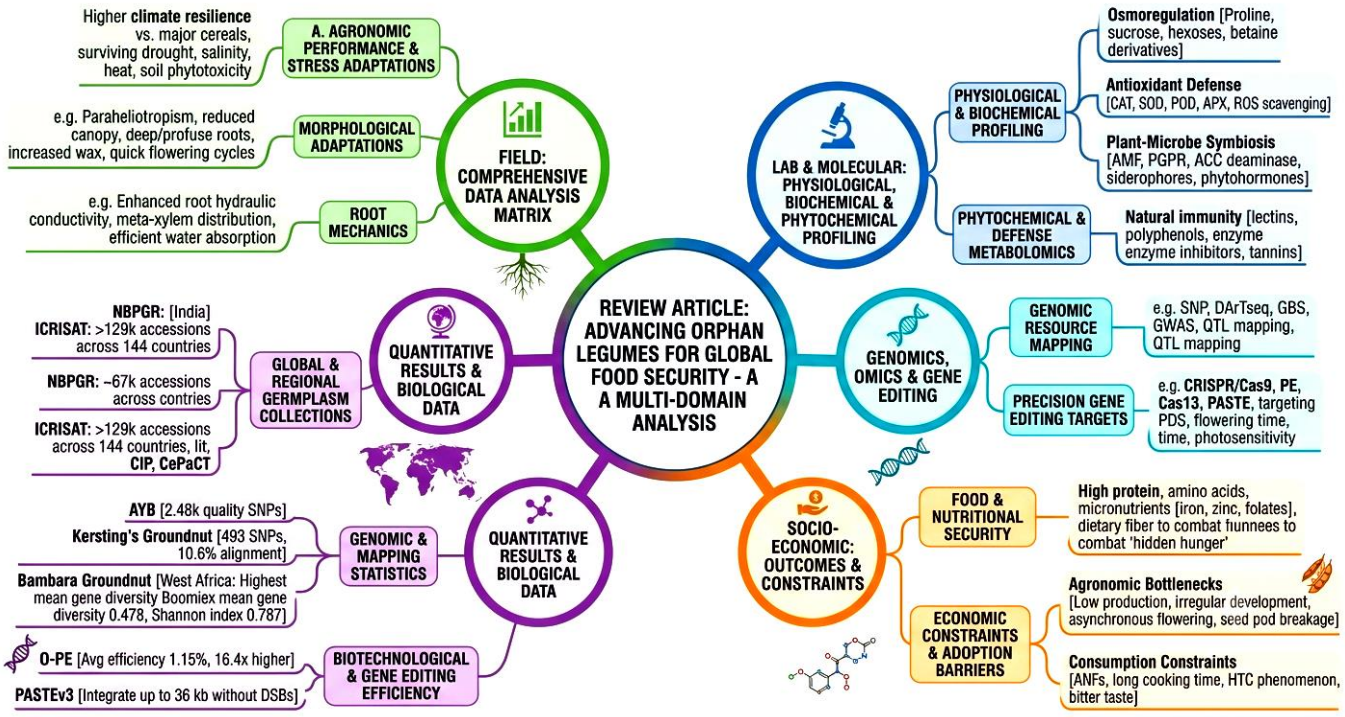


Fig. 8 From Germplasm to Field: A Comprehensive Review Architecture for Advancing Underutilized Legumes

3. Data Analysis Methods coupling Analytical Frameworks

Analytical Category	Methods & Methodologies Employed
Phenotyping & Field Analysis	High-throughput phenotyping platforms, Ground-Based Imaging, Unmanned Aerial Vehicle (UAV) aerial imaging, Satellite Remote Sensing, and Multi-location Agroclimatic Stability Matrices.
Cytogenetic & Morphological Analysis	Mitotic index assays, chromosomal aberration bioassays, testa color/morphology profiling, root length density assessments, and stomatal conductance/leaf anatomy measurements.
Biochemical & Physiological Profiling	Biomarker-guided ROS assaying, enzymatic antioxidant activity profiling (SOD, CAT, POD, APX), osmolyte quantification, and atomic/spectrophotometric chelating determinations.
Genomic & Molecular Breeding Methods	Simple Sequence Repeats (SSRs), Random Amplified DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Single Nucleotide Polymorphism (SNP) genotyping, DArTseq, Genotype-by-Sequencing (GBS), and Genome-Wide Association Studies (GWAS).
Multi-Omics & Computational Tools	Whole-Genome Sequencing (WGS), Transcriptomics, Proteomics, Metabolomics, Bioinformatic Knowledge Graphing, and Machine Learning predictive algorithms.
Precision Bioengineering Systems	CRISPR/Cas9, Cytidine & Adenine Base Editors (CBE/ABE), Prime Editing (PE/pegRNA), CRISPR/Cas13 RNA editing, Ribonucleoprotein (RNP) complex delivery, Nanoparticle-mediated delivery, CRISPR-Associated Transposons (CASTs), and Programmable Addition via Site-specific Targeting Elements (PASTE).

4. Summary: Precision Biotechnology corroborating Gene-Editing Arsenal for Orphan Legume Improvement

Tool / Technology	Mechanism of Action	Target Traits / Application in Legumes	Advantage over Traditional Approaches
CRISPR/Cas9	RNA-guided double-strand DNA cleavage & repair via NHEJ/HDR.	Knockout of undesirable traits (e.g., PDS gene, photosensitivity, extended flowering time).	Enables rapid *de novo* domestication and precise targeted gene editing.
Base Editors (CBE / ABE)	Single-nucleotide conversions (C-to-T or A-to-G) without DSBs.	Promoter editing (e.g., *SWEET* genes) for pathogen resistance and herbicide tolerance.	High specificity; eliminates risk of double-strand break side effects.
Prime Editing (PE)	Cas9 nickase fused with reverse transcriptase using pegRNA.	Precise search-and-replace base conversions, targeted insertions, and deletions.	Extremely high precision; achieved 1.15% efficiency (16.4-fold increase) in plant cells.
CRISPR/Cas13	Direct RNA-guided cleavage of single-stranded RNA targets.	Post-transcriptional viral RNA degradation and gene expression regulation.	Confers robust viral disease resistance without modifying host DNA genomes.
PASTE Integration	Fusion of nCas9, reverse transcriptase, and serine integrase via atgRNA.	Targeted site-specific integration of large genes at *attB*/*attP* attachment sites.	Allows insertion of large gene constructs (up to 36 kb) without double-strand breaks.
RNP / Nano-Delivery	Direct delivery of pre-assembled protein-gRNA complexes via nanoparticles.	Non-transgenic genome editing in protoplasts, calli, or floral tissues.	Bypasses traditional GMO regulatory frameworks and eliminates foreign DNA integration.[87]

4.1. Adaptability of Orphan Pulses under Adverse Environmental Conditions

Variety of specific adaptive responses [88], [89] allow them to be stress resilient to thrive and yield under harsh edaphic and agro-climatic conditions. To escape drought stress, the legume plants have evolved some effective strategies such as a deep and profuse root system, stomatal control, lowering of canopy size and duration, increase in wax deposition on the leaf surface and paraheliotropism, accelerated flowering and maturity, osmotic adjustment, raise in cell wall distensibility to sustain tissue turgor, augment in antioxidant systems to help cells from oxidative stress [90]. Testa color has been proposed as a phenotypic characteristic of the drought stress tolerance traits. Orphan legumes like rice beans, tepary beans, adzuki beans, and horse gram have been shown to be immune-resistant to a number of pests and illnesses in addition to being drought-tolerant.

Secreting some of the bioactive compounds, viz., polyphenols, alkaloids, enzyme inhibitors, lectins and peptides has been reported by many workers for conferring resistance against various pests and diseases in orphan legume pulses [91]. Polyphenols (flavonoids, saponins, and tannins) have anti-microbial mechanisms of action by destroying the integrity of the cell membrane as well as inhibiting the microbial metabolism by binding the essential minerals such as iron and Zn by chelating and obstructs the extracellular enzyme in microbes [92].

Lectins are thought to exert an antimicrobial activity against pathogens by destroying their membranes [93]. Inhibitors of enzymes such as protease inhibitors present in these legume pulses can inhibit the enzymatic activity of proteases secreted by invading pathogens [94]. Legume plants express protease inhibitors (e.g., trypsin, cysteine protease, and chymotrypsin inhibitors), lectins and α-amylase inhibitors as a defense against attack by insect pests [95].

The trypsin inhibitors inhibit blood-breaking serine proteases in the larval gut, impairing digestion and absorption of vital amino acids [96]. On the other hand, these inhibitors inhibit the proteolytic activity of various enzymes as well, thereby causing plant tissues to be indigestible and/or at least partially resistant to invasive insects and diseases [97].

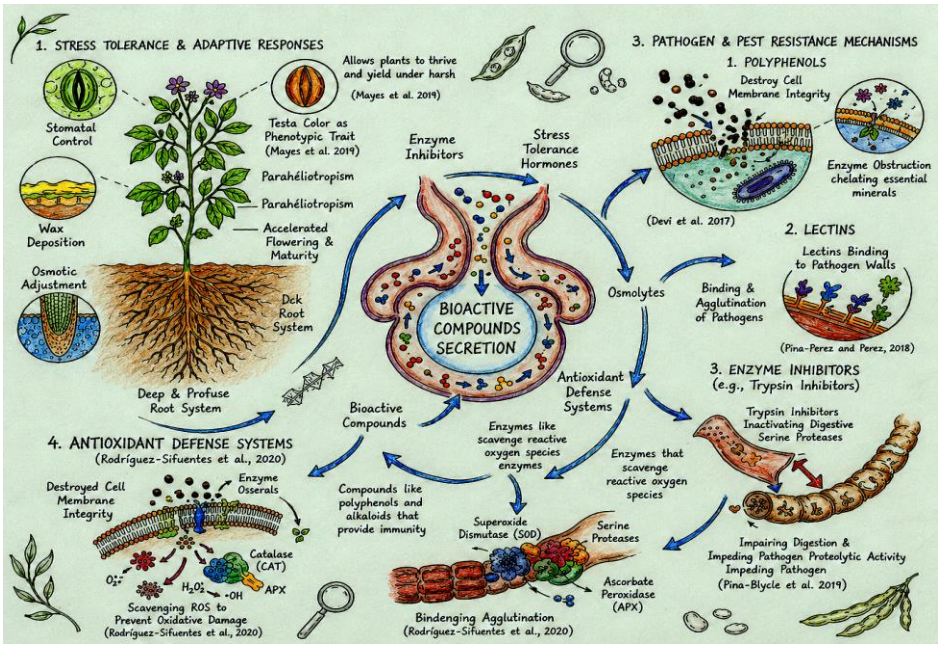


Fig. 6 Hidden defence mechanisms of underutilized orphan legumes in Indian Agriculture: Physiological, Biochemical, and Molecular Mechanisms of Stress Tolerance and Defense

Grass pea is one of the orphan legumes in India that can flourish in conditions such as salinity, phytotoxicity and heavy metal stress. Abiotic stress factors are growing at a rapid pace, and those plants that can survive such extreme conditions go through a variety of morphological, physiological, and biochemical changes to enhance their adaptations, which are compatible with evaluation through several biomarkers [98]. Stress tolerance-regulating hormones, such as abscisic acid, have been considered priming agents that control gene expression in plants to optimize physiological responses under aluminium toxicity [99]. They work by keeping out Na+ and/or Cl−ions and raising the concentration of chlorophyll and the root/canopy ratio. Additionally, by preserving a favourable water balance for the maintenance of photosynthetic activity, the modification in leaf anatomy prevents ion toxicity) [100]. Under stressful conditions, legumes collect osmolytes such as sugars (sucrose, hexose), sugar alcohols (mannitol, ononitol, and sorbitol), amino acids (proline, alanine, and others), betaine, and its derivatives. The solutes preserve the integrity of the plasma membrane, aid in the detoxification of heavy metals, shield enzymes from denaturation, and offer osmotic adjustment [101]. Additionally, legumes have strong antioxidant defense mechanisms that use enzymes such as catalase, dehydroascorbate reductase, superoxide dismutase, peroxidase, and ascorbate peroxidase to scavenge reactive oxygen species [102]. By fully comprehending the mechanisms of stress tolerance by orphan legumes, breeders can identify critical traits and responses to crop survival in harsh environments [99].

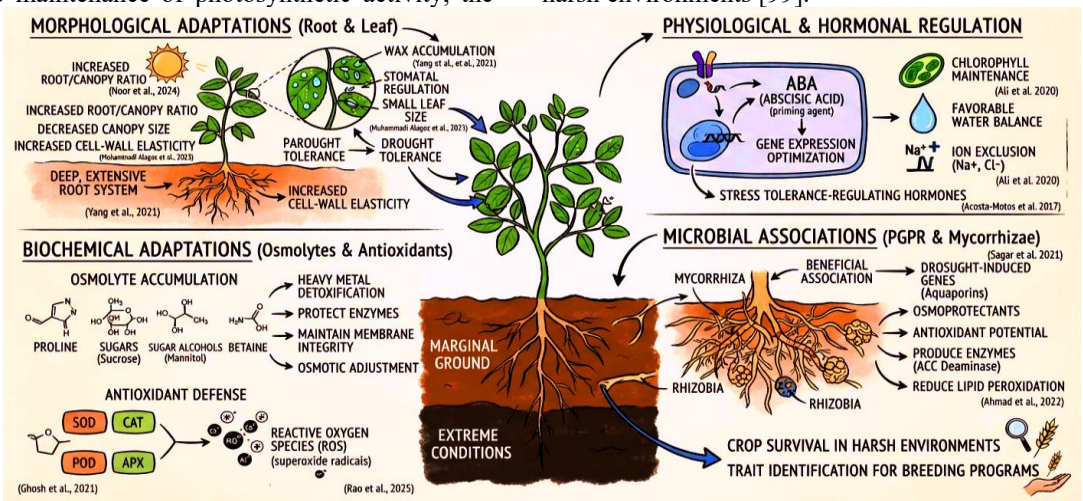


Fig. 7 Superiority of underutilized orphan legumes in terms of Morpho-Physio-Micorbial- and Biochemical characteristics: Multidimensional Adaptive Mechanisms Driving Stress Tolerance and Crop Survival in Extreme Conditions

By controlling drought-induced genes like aquaporins, preserving tissue water potential, decreasing stature and energy conservation [89], and building up osmoprotectants, the beneficial association of legumes with mycorrhizae and plant growth-promoting rhizobacteria may further increase tolerance. Along with producing siderophores, organic acids, and compounds that regulate plant growth (auxins, gibberellins, cytokinins, ABA, and ethylene), this may also reduce lipid peroxidation, increase antioxidant potential, and produce essential enzymes like ACC deaminase and chitinase [103]. Legumes have evolved effective defenses against drought stress, such as a deep and extensive root system, stomatal regulation, a reduction in canopy size and duration, an increase in wax accumulation on the leaf surface, paraheliotropism, accelerated flowering and maturity, osmotic adjustment, strengthened antioxidant systems to shield cells from oxidative damage, and increased cell-wall elasticity to maintain tissue turgidity [104].

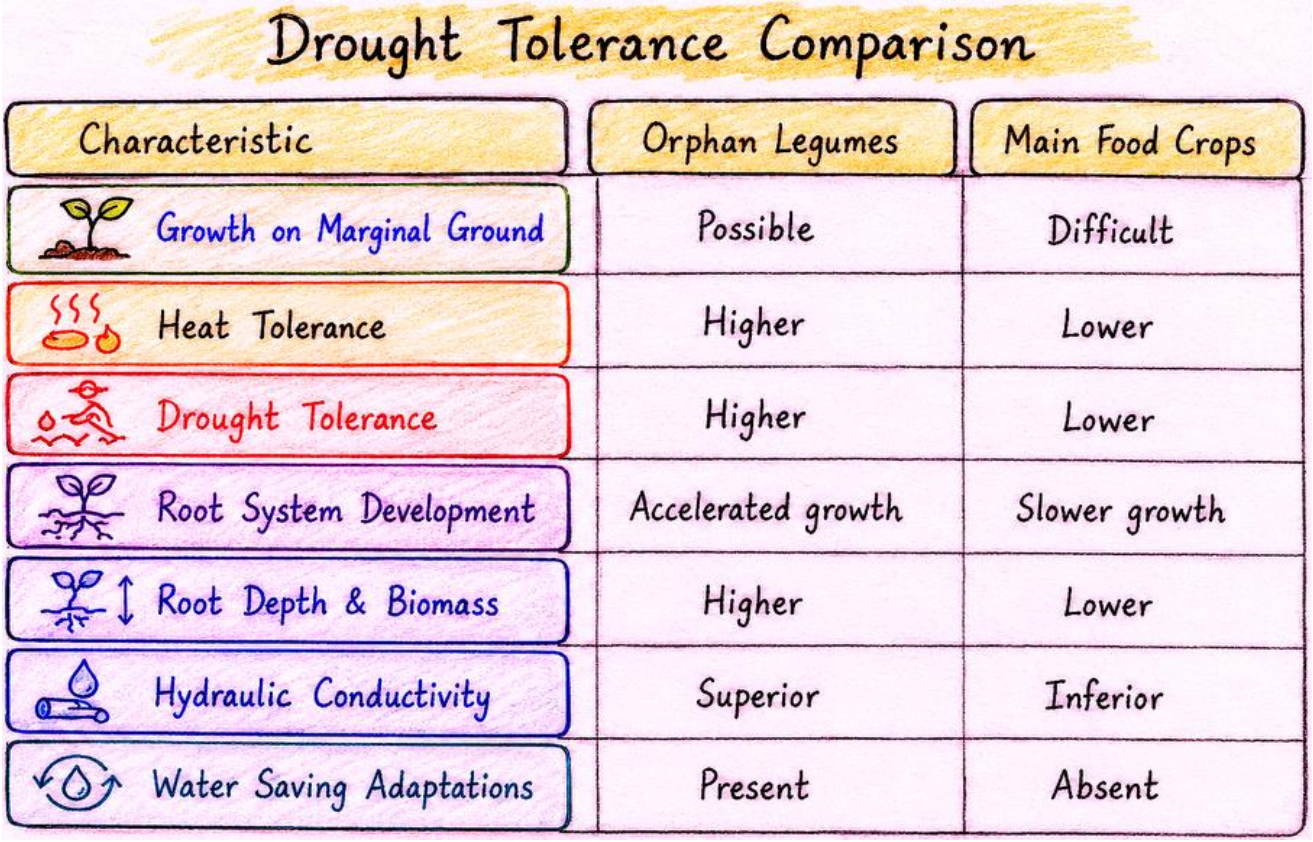


Fig. 8 Superiority of underutilized orphan legumes versus popular food crop cultivars in terms of drought tolerance in Indian Agriculture: Comparative Analysis of Drought Tolerance Characteristics Between Orphan Legumes and Main Food Crops

Because of their greater resistance to heat and drought, orphan legumes can flourish on marginal ground in hot, dry regions when rain-fed. In drought conditions and on marginal land, such growth is either impossible or extremely difficult to achieve with key food crop species [105]. Examining the reasons behind their high heat and drought tolerance is of interest in order to transfer certain genes that code for these unique characteristics to significant food crop species. Drought can alter a plant's morphology and physiology as adaptive processes. These drought responses include decreased leaf growth, stomata closure, a shift in the shoot to root biomass ratio, and modified root characteristics [106]. The performance of legumes under drought stress is closely correlated with the growth and expansion of the root system. When seed filling begins, root growth in legumes slows down after accelerating during the vegetative growth phase [107]. A more efficient extraction of any available soil moisture is made possible by deeper roots and higher biomass [108]. Root hydraulic conductivity, which depends on the diameter and distribution of the meta-xylem vessels, has also been shown to improve drought tolerance in legumes. For instance, the grain legume chickpea (Cicer arietinum) has a lower root length density than barley (Hordeum vulgare), but the legume may absorb water more effectively due to its better hydraulic conductivity. The largest root mass is found in the deepest soil profile in some tepary bean (Phaseolus acutifolius) lines that have deeper roots. These changes result in better absorption of water. According to [109], the bean's tiny leaves and decreased stomatal conductance are adaptive traits for water conservation.

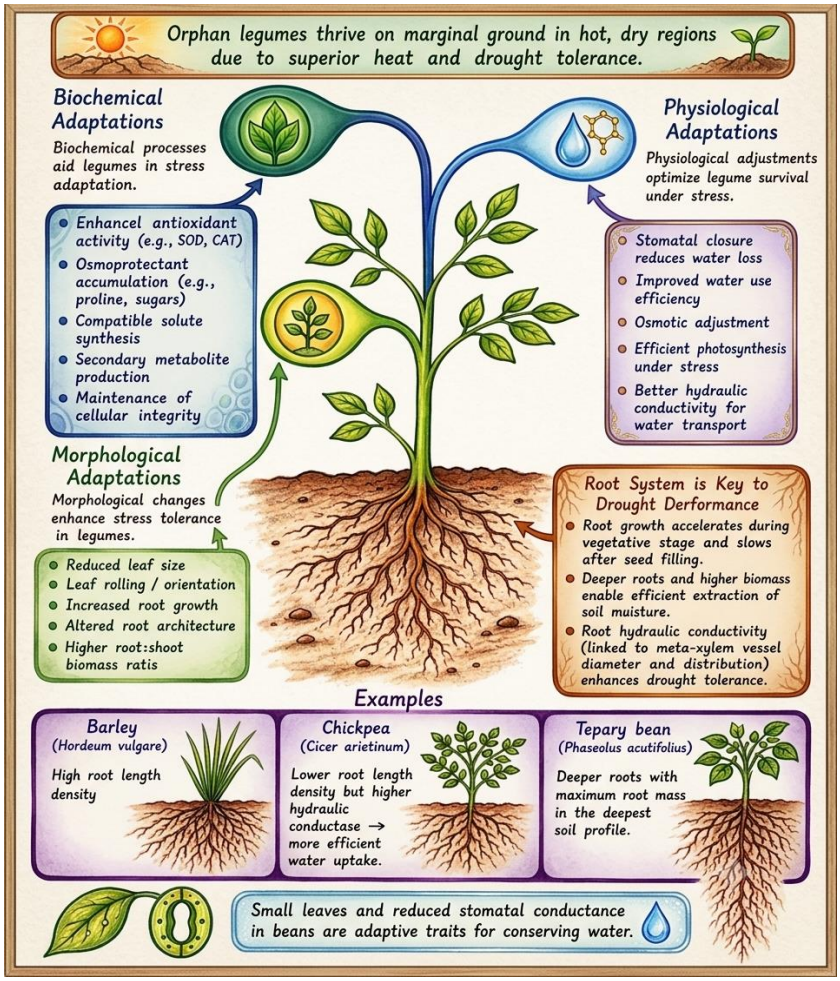


Fig. 9 Morpho-anatomical superiority of underutilized orphan legumes versus popular food crop cultivars in terms of drought tolerance in Indian Agriculture

4.2. Social Satisfactoriness and Consumption Precincts of Orphan Legumes

- Since prehistoric times, orphan legumes have been essential components of regional food systems in various agro-ecological areas [110]. They are also referred to as "foods of the poor" because their appeal is primarily limited to areas with limited resources [111].
- Several explanations for their lack of popularity among the general mass are consumption constraints (as discussed earlier), undesirable agronomic traits in existing landraces and varieties (long duration, low yield, indeterminate growth, asynchronous flowering habits, susceptibility to shattering, diseases and pests, etc.), high labour requirements and low economic values[110].
- Orphan legumes are produced with lower quality and quantity due to their lack of genetic improvement and the availability of high-quality seeds. The cultivation of these legumes is less profitable due to high labor costs and unfavorable marketing conditions. For these reasons, poor farmers have begun to replace orphan legumes with major staple crops in order to reap more financial rewards. Rich farmers have substituted them with cash crops or enhanced crops like soybeans and black gram [112].
- Despite the fact that orphan legumes offer a variety of nutritional and nutraceutical advantages, certain of the compounds they contain may restrict their use in everyday cooking. One of the primary restrictions is the presence of some bioactive substances in legumes, such as polyphenols, tannins, saponins, trypsin inhibitors, phytic acid, α-amylase inhibitors, HCN, and oligosaccharides, which lower the nutrients' bioavailability [113]. These substances are therefore frequently referred to as Antinutritional Factors (ANFs).
- According to reports [114], polyphenols are known to block proteolytic enzymes, which causes them to form insoluble complexes with dietary proteins and ultimately lower protein digestibility. The human body's absorption and utilization of macro and micronutrients like calcium, magnesium, zinc, and iron are impacted by phytic acid's binding effect. Additionally, it frequently forms

complexes with proteins and enzymes like amylases and proteases, which prevent proteolysis and impede food digestion and absorption [115].

- The Lipoxygenase Enzyme(LOX), saponins, and oligosaccharides that cause flatulence are frequently responsible for the astringency and off-flavor of legumes [116]. Quinolizidine alkaloids (lupanine, D-lupanine, sparteine, 3-hydroxylupanine, 13-hydroxylupanine, tetrahydrorombifoline, and 4-hydroxylupanine) have been associated with the bitter taste of Andean lupin grain [117].
- Once more, legumes including rice beans, adzuki beans, tepary beans, and bambara groundnuts are frequently referred to as "hard-to-cook," which lengthens cooking times and uses more fuel [118].
- To lessen these restrictions in legumes, genetic methods such as evaluating the genetic diversity of landraces, sophisticated breeding methods, etc., can be used [90].
- The large-scale acceptability of food crops by consumers is largely dependent on their organoleptic features and cooking attributes. Major legumes, such as chickpeas, lentils, and black gram, were preferred over orphan legumes based on taste [119]. Legumes, including rice beans, tepary beans, and Andean lupin, have been reported to have strange and disagreeable tastes that hinder their popularity [120].
- Hard-seeded legumes, including rice beans and bambara groundnuts, require more fuel and take longer to cook, which sometimes limits their utilization [121]. Indigestion or flatulence, toxic and allergic chemicals, and an unpleasant smell caused by compounds like tannins and saponins are other causes that lead to their abandonment, in addition to issues with cooking and taste[122]. In this sense, there are several underutilized forms of legume crops that have undesirable characteristics. Neglected and underutilized species could gain from sufficient attention and research funding to enhance their culinary and productive features, much like the success attained in the development of key legumes [75].
- A number of other factors, such as the color and appearance of the seeds, must also be carefully considered when enhancing these legumes for greater marketability. For instance, consumers prefer Andean lupin and bambara groundnut with cream or white seed coats [123].
- Tepary beans' seed coat wrinkles often give consumers the impression that the beans are from older harvests and hence require a longer cooking time [124]. Coordinated efforts are therefore required to make the species in question more attractive and competitive, like main legume crops, in order to promote their general acceptance. Encouraging research into the industrial potential of underused legumes and their usage as raw materials could help level the playing field for their economic position on the global market.

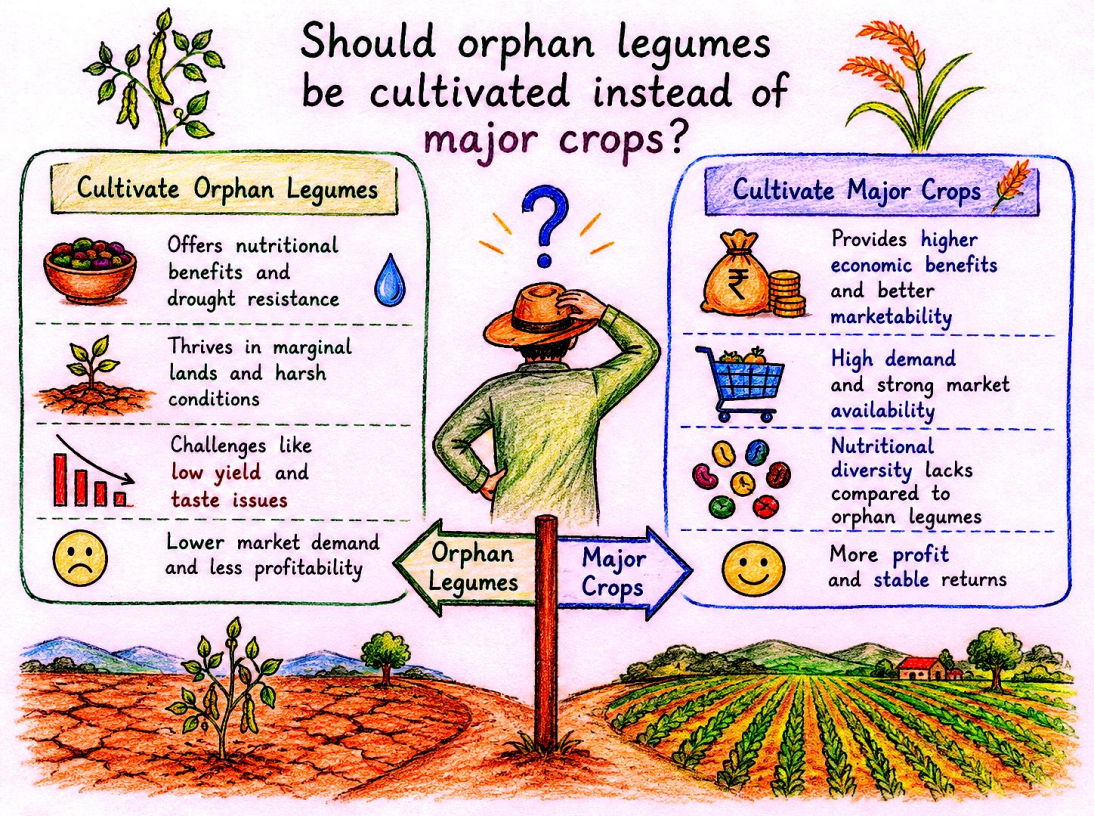


Fig. 10 Social and commercial dubiousness for acceptance of underutilized orphan legumes versus popular food crop cultivars n Indian Agriculture

4.3. But Orphan Crops are the Treasure Trove of Novel Genes for Future Plant Biotechnologists

During the green revolution, India achieved an unprecedented level of food grain production thanks to the introduction of high-yielding cultivars, chemical fertilizers, pesticides, and agricultural technologies [125]. But this resulted in the monoculture of a few elite, high-yielding varieties of cereal crops, such as wheat, rice, and maize. Over time, this method has resulted in the loss of beneficial alleles, particularly those that provide resistance to biotic and abiotic stresses. Researchers have lately started looking into wild relatives of significant crops in an attempt to identify the novel gene or mechanism that can be exploited to improve stress tolerance in elite cultivars [126]. As a result, gene banks, also known as seed vaults, are now a vital tool for plant scientists to conserve and study genetic resources.

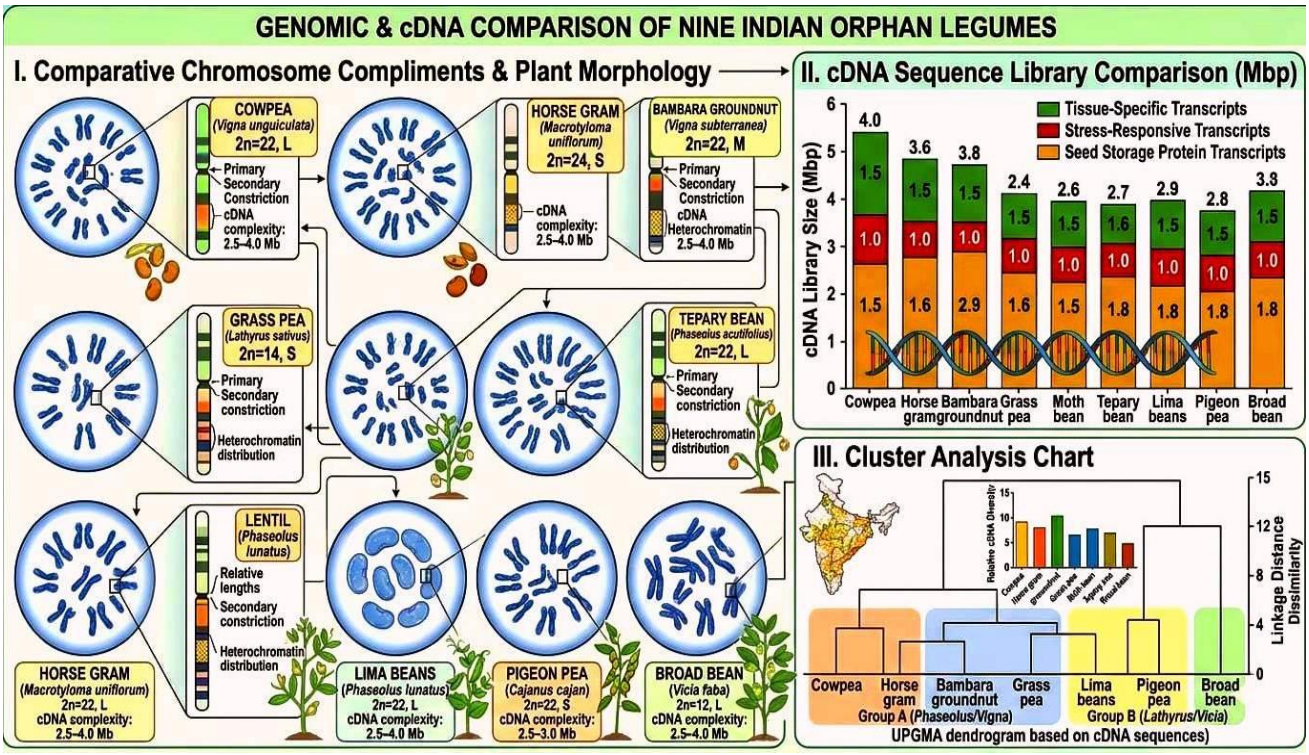


Fig. 11 Genomic blueprint of nine underutilized orphan of India

Strategic reserves for agricultural improvement, biodiversity preservation, and climate change adaptation are genetic resources. In addition to protecting the genetic information of wild and endangered species, their methodical collection and preservation offer diverse gene pools for agricultural breeding, improving crop output and stress tolerance.

- In India, the National Bureau of Plant Genetic Resources (NBPGR) has identified 14,904 chickpea accessions, 25,785 minor millets, and 26,395 sorghum accessions [127].
- Furthermore, 129,000 accessions spanning peanuts, chickpeas, pigeonpeas, minor millets, and sorghum have been gathered from 144 countries by the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) [128].
- With 15,000 cowpea accessions, the International Institute of Tropical Agriculture (IITA) has the largest and most varied collection in the world. They also have around 5,900 yam accessions and roughly 2,000 Bambara groundnut accessions [129]. The primary farmed varieties of cassava are covered by the 5,965 accessions that the International Centre for Tropical Agriculture (CIAT) and IITA have preserved, respectively [130].
- In addition to collecting 2,529 accessions of nine Andean roots and tubers (ARTCs), the International Potato Center (CIP) is home to the largest sweet potato genbank in the world, which includes over 5,500 accessions maintained ex-situ [131](/). The protection of particular crops is also greatly aided by certain regional organizations. For example, the largest collection of breadfruit germplasm in the world is maintained by the National Tropical Botanical Garden (NTBG) [132].
- About 70% of the world's Taro germplasm, or about 1,300 accessions, have been saved at the Centre for Pacific Crops and Trees (CePaCT)[133].

Furthermore, more than half of the world's Foxtail millet germplasm is preserved by the Chinese Crop Germplasm Resources Information System (CGRIS) [133], [135].

In India, different varieties of grasspeas are among the most popular orphan pulse crops. Other names for it include: khesari (Bangladesh); san lee do (China); fovetta (Cyprus); sabberi (Ethiopia); gisette (France); khesari dal, lang, chural, latri, lakhori, batura, tiwra (India); pisellobrettone (Italy); kheshari (Nepal); matri, mattra, kesari (Pakistan); almorta (Spain); gilban (Sudan); murdumuk (Turkey); and pharetta, garbanzo (Venezuela) (Campbell, 1997)[136]. Plants of the World Online, The current list of 184 taxonomically recognised names for the genus *Lathyrus* may be found at Royal Botanic Gardens, Kew, UK. The genus *Lathyru*s has over 160 annual and perennial species [137] and subspecies, which are separated into 15 divisions based on morphological traits [138]. The distribution of species in the genus *Lathyrus* in West Bengal, India, is depicted in Table 2 and Figure 8. The most often planted species for food and feed in Europe are *L. sativus*, *L. cicera*, and *L. hirsutus*, although *L. latifolius* and *L. odoratus* L. are lovely decorative plants.

Table 2. List of Genotypes of Lathyrus sativus L., cultivated in West Bengal, India, showing no active reports of neurolathyrism with very low L-ODAP concentrations

Sl.no.	Genotypes	Flowercolour	Leafstructure	Plantstand	Biomass	Plantheight
1.	Mahateora	Pink	Medium	Tall	Medium	Medium
2.	Ratan	Blue	Broad	Erect	Medium	Tall
3.	Nirmal	Blue	Medium	Erect	High	Tall
4.	BerhampurLocal	Blue	Narrow	Erect	Medium	Medium
5.	BankuraLocal	Blue	Narrow	Erect	Low	Dwarf
6.	KaikhaliLocal	Blue	Narrow	Erect	Medium	Tall
7.	NirmalMutant	Blue	Slender	Erect	Medium	Tall
8.	BK-10	Blue	Broad	Erect	High	Tall
9.	BK-3-6	Blue	Broad	Erect	High	Tall
10.	BK-10-2-1	Blue	Medium	Erect	Medium	Tall
11.	BK-11-3	Blue	Slender	Erect	Medium	Tall
12.	BK-2	Blue	Broad	Erect	High	Tall
13.	BK-27-1	Blue	Broad	Erect	High	Medium
14.	BK-37-2	White	Slender	Erect	Medium	Tall
15.	BK-28-1	Blue	Slender	Erect	Medium	Tall
16.	BK-7-1	Blue	Broad	Erect	Medium	Medium
17.	BK-20-5	Blue	Broad	Erect	Medium	Medium
18.	WBK-5	Blue	Slender	Erect	medium	Tall
19.	BK-35-1-1	White	Slender	Trailing	Medium	Medium
20.	BK-2-2	Pink	Broad	Erect	Medium	Tall
21.	BK-13-1	Blue	Broad	Erect	Medium	Tall
22.	BK2-46	White	Medium	Erect	Medium	Medium
23.	BK-14-1	Blue	Broad	Tall	High	Medium
24.	BK-14-8-3	Blue	Slender	Erect	Low	Dwarf
25.	BK-26-5	Blue	Medium	Erect	High	Tall

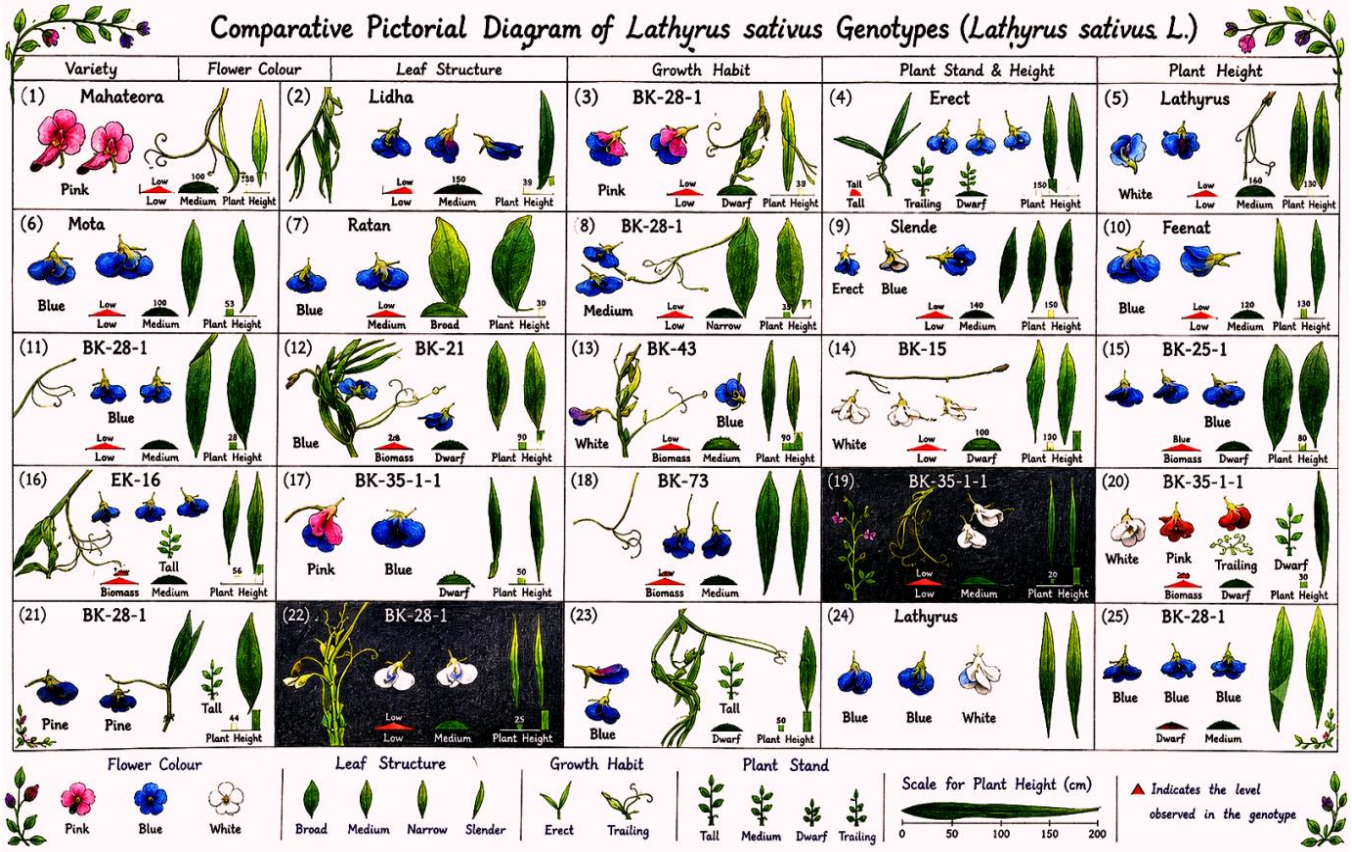


Fig. 12 Flower colour variation and variability of leaf and tendril structure of 25 wild and cultivated genotypes of Lathyrus sativus L., in India, showing superior germplasm variations for a novel gene pool. After [138]

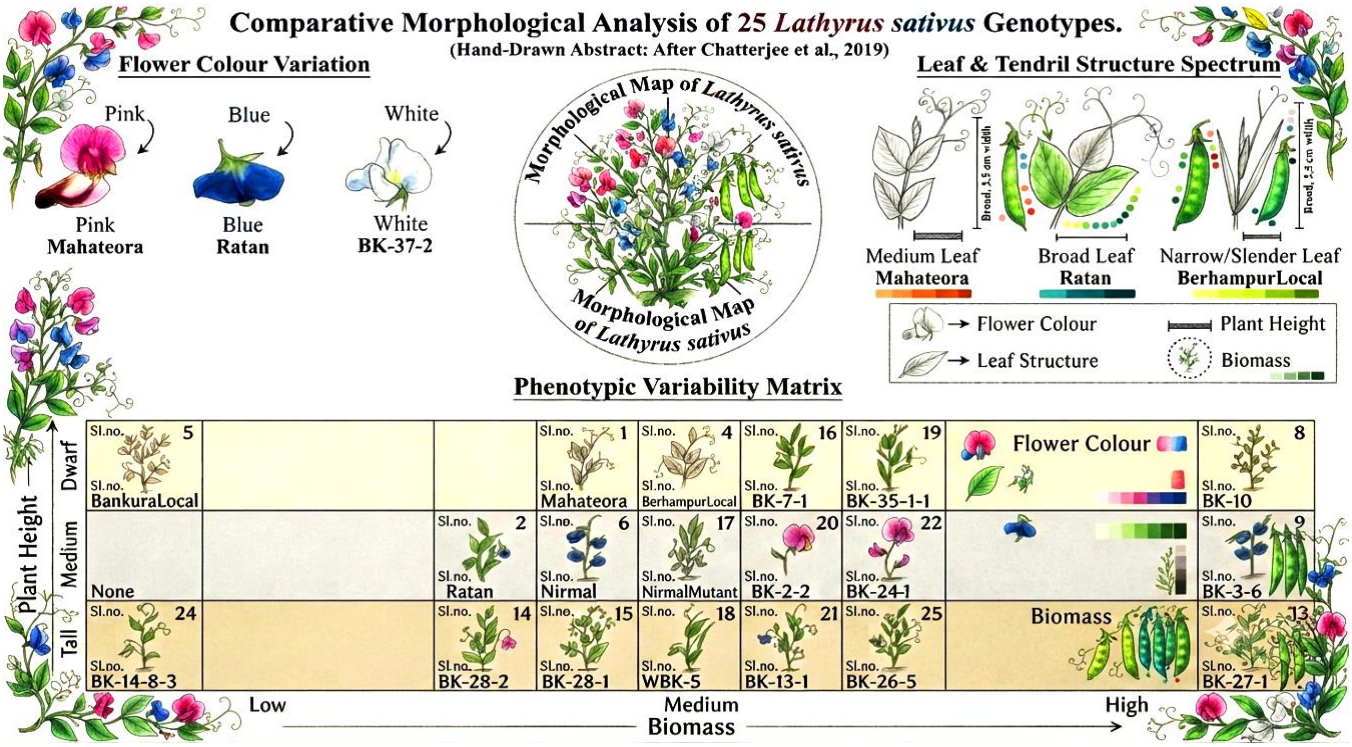


Fig. 13 Comparative Phenotypic variability matrix of 25 reported cultivars of *Larthyrus sativus* showing low to high morphomatix in terms of height and biomass coupled with distinction in flower colour, leaf and tendril structures. After[138]

Abiotic stressors are multigenic, meaning they affect several important cellular, metabolic, physiological, and molecular processes at the same time owing to multifactorial signalling cascades in crop plants [80], [81]. Salinity and desiccation interfere with the normal production of DNA, RNA, proteins, and other pathways such as glycolysis, the tricarboxylic acid cycle (TCA), hormone biosynthesis, photosynthesis, and others [139]. Normal cellular growth and development are slowed down by stressful conditions because they adversely affect a number of vital physiological and biochemical processes, such as the inhibition of chlorophyll biosynthesis, stomatal regulation, leaf senescence, and free radical formation. Furthermore, seed vigor, yield, quantity, and germination are all negatively impacted by these stresses. According to reports [140], the reproductive stage—which includes pollen fertility, anthesis, germination, seed establishment, etc.—is the most vulnerable to salt and desiccation stress. Consequently, by restricting the formation of root nodules and legume-rhizobium symbiosis, abiotic stressors also negatively impact the process of symbiotic nitrogen (N_2) fixation. The opening or closure of channels and the start of a signaling cascade, which necessitates modifications in calcium phosphorylation and ROS-induced PTMs, are how plants react to changes in their surroundings [141]. However, the processes by which changes in the cytosolic redox status are translated into specific responses are not entirely clear. The amount, type, and length of exposure, and subcellular localization of ROS in each cellular compartment are the primary determinants, especially from germinating root tips to the stem, hampering normal mitochondrial functioning, leading to apoptosis during seed germination stages. Understanding the physiological, phytochemical, cytogenetic, epigenetic, and cytological underpinnings of stress tolerance in grain legumes, such as grasspeas, has been the subject of numerous studies in recent years. One of the most important concerns for attaining food security and agricultural sustainability is increasing agricultural productivity in the face of changing climate conditions [141].

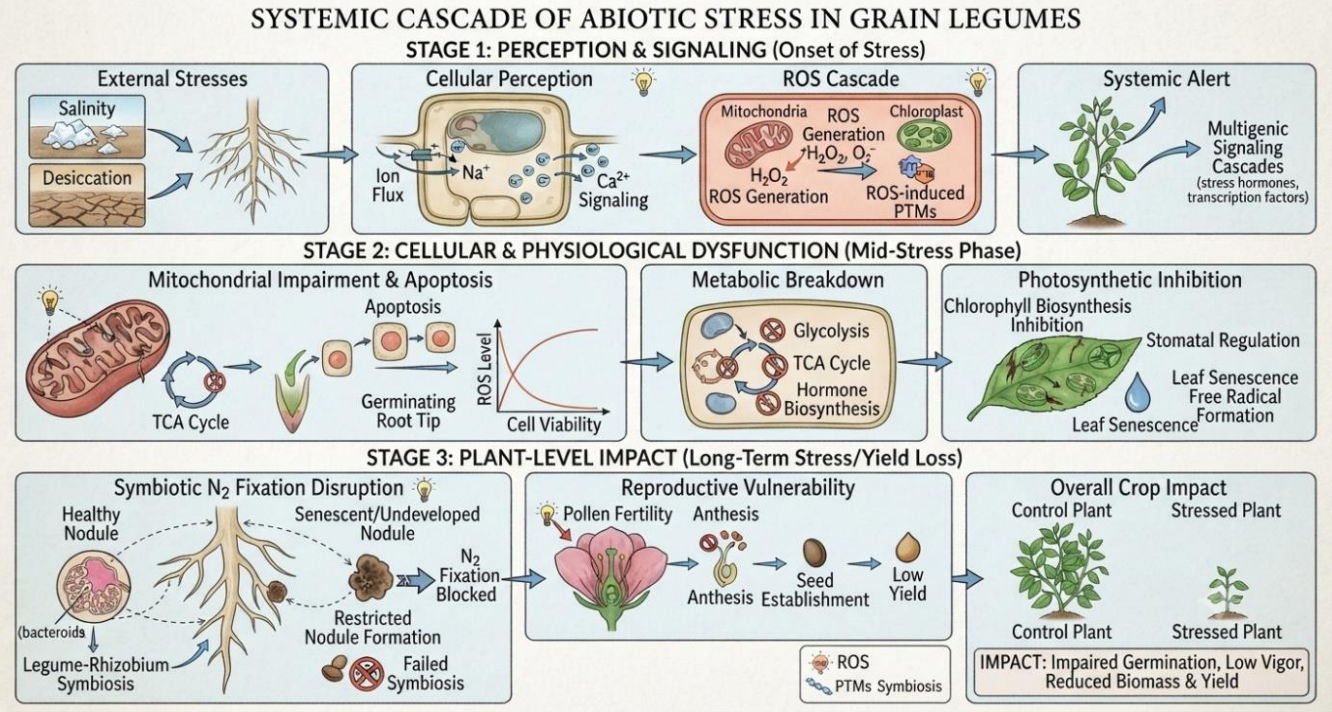


Fig. 14 Different abiotic stress factors (like salinity, heavy metal, heat, drought, etc.) that impact the physiology of legume crops

5. Molecular Markers Assisted Selection and Breeding Strategies of Orphan Legumes

The traditional technique of creating plants with enhanced characteristics depended on tedious and time-consuming procedures. Although other genes may be transferred during the process, it may take up to 8–12 generations to produce an enhanced crop with the desired feature. Therefore, a number of researchers have thought of using a different approach, such as contemporary biotechnology, to improve orphan legumes. But for genetic diversity studies and marker-assisted breeding of these neglected species, most of the research that has been done has relied on the use of DNA or molecular markers. For a specific DNA sequence in a population or gene pool, molecular markers are segments of DNA that show changes [32]. These segments can be used to identify polymorphism between various genotypes or alleles of a gene [142]. These molecular markers are genetically connected and can be passed down from one generation to the next since they are

brief nucleotide sequences that are placed close to the target gene's DNA sequence. Molecular markers are important for identifying genetic differences between individual creatures and evaluating the relationship across breeds because of the polymorphisms that result from changes in nucleotides or mutations within the genomic locus[143].

The genetic diversity in the germplasm of orphan legumes has been evaluated using a variety of molecular markers. Prior to the development of sequencing-based molecular markers like Single Nucleotide Polymorphism (SNP), Diversity Array Technology (DArT), and Expressed Sequence Tags (ESTs), which have yielded a wealth of valuable information for the improvement of orphan legumes, the first molecular markers were PCR-based Restriction Fragment Length Polymorphism (RFLP), Random Amplified DNA (RAPD), and Amplified Fragment Length Polymorphism (AFLP). Genotyping by sequencing may eventually replace the use of SSR and/or single-nucleotide polymorphisms for genetic diversity analysis and association mapping due to the greater simplicity and lower cost of next-generation sequencing technology for genetic improvement of wild relatives of orphan legume pulses throughout the arid and semi-arid regions of South East Asia, the Indian Subcontinent and Africa [144].

With the use of certain molecular markers, marker-assisted selection has enabled the discovery of Quantitative Trait Loci (QTLs) for a number of attributes in orphan legumes as well as the creation of codominant molecular markers and linkage maps for certain orphan crops. For these orphan legumes to be selected for genetic improvement, it is imperative that phenotypic and genotypic data be linked.

Of the 3.6 k SNPs produced using a high-throughput DArTseq genotype-by-sequencing SNP method, 2.48 K quality SNPs were used for the Genome-Wide Association Study (GWAS) in the AYB population. To enhance the protein, oil, and starch contents of AYB, they found Quantitative Trait Loci (QTL) for genes that might be helpful.

The DArTseqTM method was also used to genotype a population of 281 Kersting's groundnut accessions from Benin using 493 SNPs. It was discovered that approximately 10.6% of the SNPs were aligned to the reference genomes of mung and adzuki beans, suggesting that Kersting's groundnut and adzuki and mung beans have an evolutionary relationship[145], [146].

The genetic diversity and population structure of selected BG genotypes from Nigeria, Cameroun, West Africa, East Africa, and Southern Africa were studied by researchers [147] using DArTseq and SNP. Within the populations of the five areas, the analysis of the data showed that the West African region had the greatest Shannon diversity index (0.787) and the highest mean gene diversity (0.478) among the populations.

Additionally, it showed that, of the 270 BG genotypes examined, the genotypes in the United Kingdom's unknown origin population were more closely related to the Western Africa population (0.018) than to the Nigerian and Cameroonian populations (0.020). These populations were all grouped into a single subpopulation, which was the largest according to the population structure produced by the ADMIXTURE model. The application of gene technology and contemporary biotechnology has not received much attention.

The application of gene technology and contemporary biotechnology to improve the genetic makeup of orphan legumes has not received much attention. Even though modern biotechnology is viewed with suspicion by the public, it could be highly helpful for genetically enhancing orphan beans while keeping to biosafety regulations and norms [148].

A new generation of omics, including transcriptomics, proteomics, metabolomics, and genomics, has been made possible by advances in next-generation sequencing. These omics can improve crop improvement, expand the range of different germplasms, and offer crucial answers to these underutilized legumes [32]. Several crops' growth, senescence, yield, and reactions to environmental stressors have all been successfully explained by these multi-omics techniques [149]. Higher understanding of the biology of plant systems, gene expression, and the many metabolic systems in plants has been made possible by the use of omics technologies in conjunction with high-throughput next-generation sequencing platforms and bioinformatics [149]. The use of genomics to improve orphan legumes has begun as a result of the steadily declining cost of sequencing. Nevertheless, the application of omics to the characterisation of orphan legumes is still relatively new. This is due to the fact that the majority of these legumes' genomes have not yet been sequenced.

However, a number of organisations, including the African Orphan Crops Initiative, are working to close the gap by using genomics, transcriptomics, and proteomics to extract valuable information for the improvement of orphan legumes [150]. These efforts are focused on whole-genome sequencing of some orphan legumes. Genomic progress in other significant orphan legumes like cluster bean, Dolichos bean AYB, and winged bean, which are still undergoing sequencing, would be accelerated by comparative genomics using the sequenced genomes of legumes and model species like *Glycine max*, *Vigna unguiculata*, and *Medicago trunculata* [151].

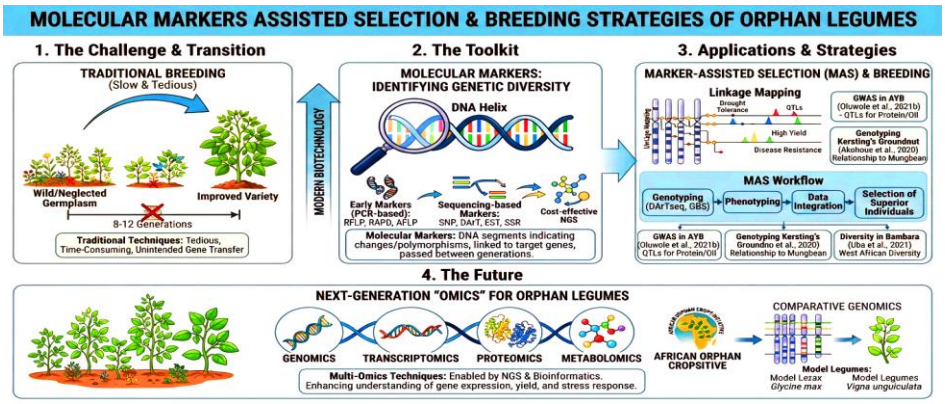


Fig. 7 Flow chart showing interrelationships between traditional vs modern omics approaches for enhancement of orgaph legume crops' breeding approaches

5.1. Molecular Breeding Techniques for the Improvement of Orphan Legumes

It is difficult to anticipate the phenotype based solely on the genotype because of the complicated link between the two. As the cost of obtaining nucleic acid sequence information has rapidly fallen, the amount of information required to conduct genomic-assisted breeding has continuously increased. It was widely accepted a few years ago that the implementation of genomic-assisted breeding would be made possible by genome sequencing [135]. It is widely recognized, therefore, that a single genome sequence only represents a portion of a species' variety. When land races—which make up the majority of orphan legumes—rather than well-established inbred lines make up the experimental material, this relationship becomes much more complex. It is more challenging to determine which of these land races' beneficial gene (or allele) combinations to target for selection and integration into new kinds. Furthermore, to guarantee that the same phenotype is truly being researched and that the assessment of the genotype and environment interaction can be completed effectively, a strong phenotyping platform is necessary [152].

Ongoing initiatives aim to include orphan crops in the global food basket alongside big crops. However, in addition to conventional plant breeders, biochemists, plant geneticists, botanists, and traditional farmers also need to find ways to expedite the improvement of orphan crops in order to remove the natural barrier that keeps them orphan[32]. The use of high-throughput phenotyping, such as Ground Based Imaging, Unmanned Aerial Vehicle (UAV) imaging, and satellite imaging, has shown promise in increasing the speed of genetic gain by the precise measurements of desired traits among thousands of field-grown plants within a short period of time [153]. Exploring the genomics of these orphan crops through conventional breeding, Genome-Wide Association Studies (GWAS), modern biotechnology, and computational technology will also hasten orphan crop improvement for wide acceptability and utilization [148]. Another tactic for speeding up orphan crop improvement is core collection development, which aims to provide the information needed to better the use of genetic resources in any crop improvement initiative. 76. Breeders no longer have to spend time estimating genetic diversity, assessing germplasm, and choosing parents to improve crops. Target allele mining is also made possible by the creation of a core collection [154].

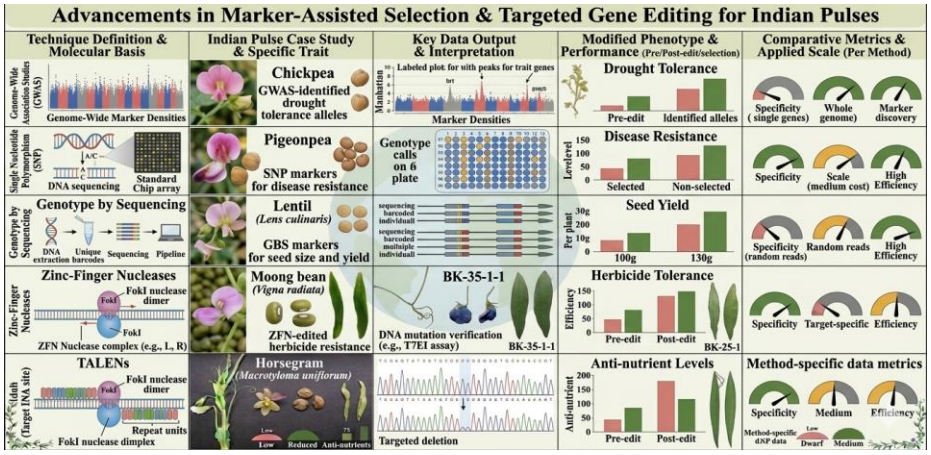


Fig. 8 Comparative chart showing interrelationships between Marker-assisted selection strategies for betterment of omics approaches for enhancement of Indian pulse crops' breeding approaches

The improvement of orphan crops can be accelerated by utilizing a number of recently established breeding techniques and technologies that allow for the quick identification and selection of genes underlying desired features, hence reducing the time for selection [155], [156]. Modern genomics and gene editing technologies, such as Zinc-Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas system), might boost the genomic improvement and production of orphan crops. However, these techniques rely on the sequencing data of the crop [157]. Using tools like Single Nucleotide Polymorphism (SNP) and Genotype by Sequencing (GBS), the genomes of many orphan crops will be sequenced to give the data needed to employ these modern technologies to accelerate crop improvement. These contemporary technologies can then be used to boost genes that have been found through sequencing [158]. Another way to speed up the improvement of orphan crops is to domesticate them. The crops may be improved and used more quickly as a result. Overall, a well-designed breeding program for orphan crops can help them improve quickly by utilizing all of these techniques [159].

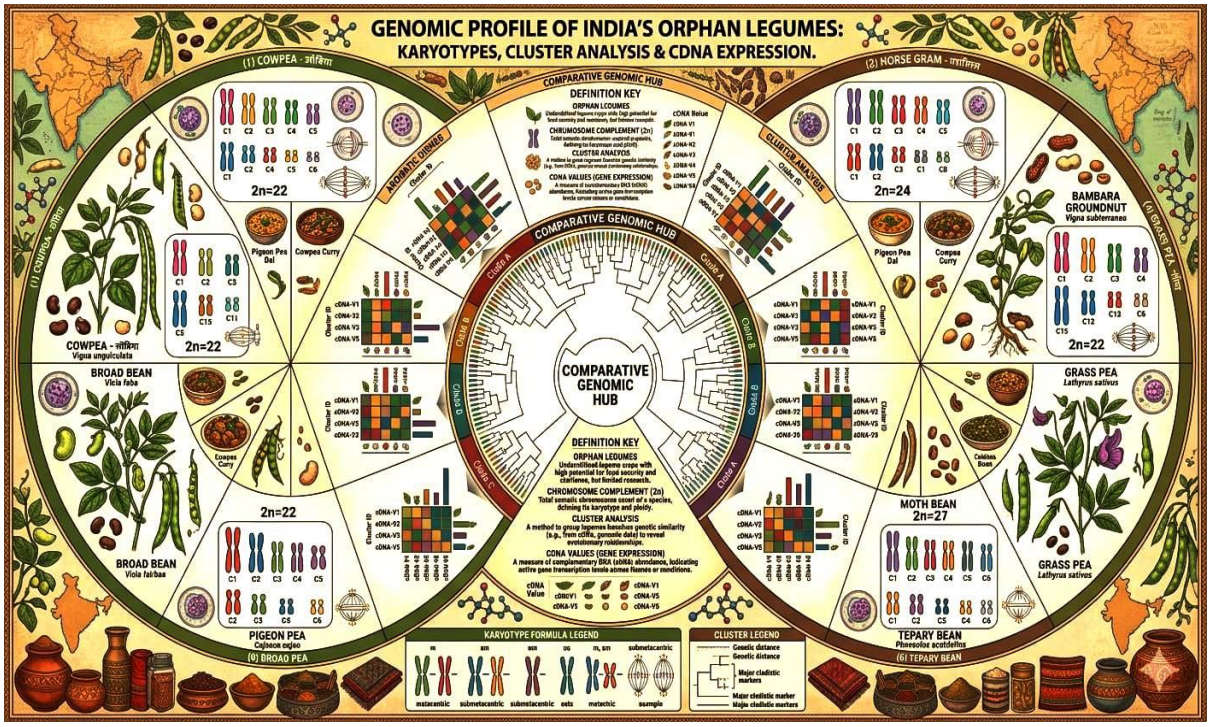


Fig. 9 comparative chart showing interrelationships between cytogenomic, C-DNA expression profiling versus cluster analysis employed in common Indian pulse crops' breeding approaches

5.2. Potential of Gene Editing Tools in Orphan Legumes

Gene editing entails the use of engineered nucleases to instigate cellular DNA repair pathways to make precise, site-directed alterations to an organism's genome [160]. Over the years, several genome editing techniques have been developed, including Zinc-finger Nucleases (ZFNs), Meganucleases, Transcription Activator-Like Effector Nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPR)- CRISPR-associated protein 9 (Cas9) and homing endonucleases [161]. However, CRISPR/Cas9-mediated gene editing has emerged as the most straightforward, adaptable, and precise strategy for genetic manipulation in plants [160], [162]. Although legumes' resistance to in vitro gene transfer, low transformation amenability, and regeneration efficiency have made the use of CRISPR technology extremely difficult, some progress has been made. Medicago truncatula, Lotus japonicus, cowpea, and soybean are among the legume crops that have successfully undergone CRISPR/Cas9-assisted gene editing [84], [160]. The technique's performance in these crops suggests that underutilized legumes could benefit from its cross-application [160]. Since the approach depends on functionally described target genes, comprehensive bioinformatic and genomic data are essential for crop improvement using this method [163]. The most often targeted gene in orphan legumes is primarily the Phytoene Desaturase (PDS) gene, which codes for an enzyme involved in carotenoid production [164]. The method of deleting flowering time and photoreceptor genes linked to photoperiod sensitivity in pigeon pea is also being tested by researchers at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT, 2020) [165].

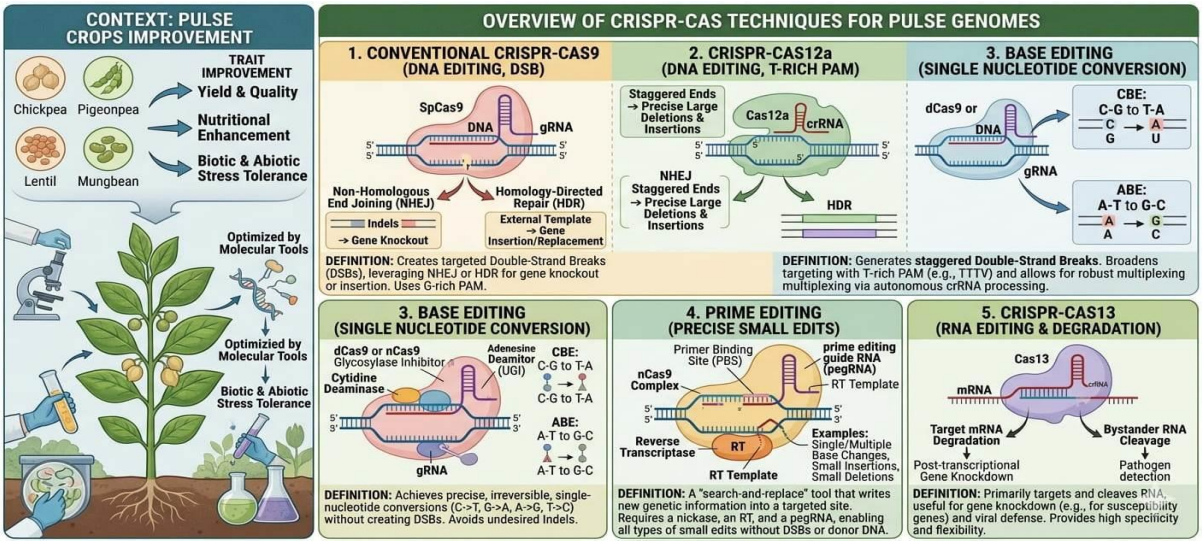


Fig. 10 Comparative overview of CRISPR-Cas techniques for genome editing approaches in common Indian pulse crops' breeding approaches

Disease resistance, salt tolerance, biomass output, grain yield and quality, and nutrient usage efficiency are further characteristics of orphan legumes that can be improved with the CRISPR/Cas system[166]. The first step is to develop an effective transformation process for guide RNA (gRNA) delivery and make entire genome sequences available in public databases, even if CRISPR offers an unmatched chance to enhance characteristics in underutilized legumes. The availability of whole-genome sequences and annotations of the underutilized legumes could speed up the deployment of CRISPR/Cas9 technology to enhance their commercial value and improve traits linked to photosensitivity, prolonged maturity period, and hard seed coat that cause long cooking times, even though the Bioscience Eastern and Central Africa International Livestock Research Institute (BecA-ILRI) has made significant progress in sequencing the entire genome of AYB [167]. Regulatory limitations continue to be a major problem despite the potential advantages of CRISPR technology for underutilized legumes [160]. The European Court of Justice recently ruled that, notwithstanding the product's lack of any foreign genes, targeted mutagenesis through genome editing should be governed by strict GMO laws [167]. In order to create a thorough framework for the integration of CRISPR-generated crops, scientists and policymakers must work together. To assess the performance of CRISPR-generated crops for qualities that might be harmed due to the loss of particular genes, extensive field studies are also necessary [167].

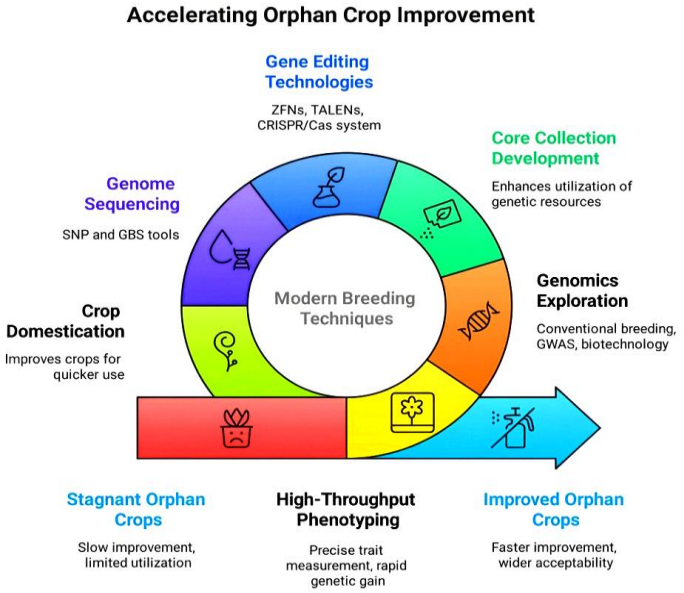


Fig. 11 Comparative overview and advantageous outcomes for the enhancement of modern breeding approaches in common Indian pulse crops

5.3. Plant Genome Editing Strategies

5.3.1. Multiplexing

In genome editing, multiplexing is the simultaneous targeting of various genes or genomic loci using a single construct that expresses multiple single guide RNAs (sgRNAs) in addition to a CRISPR-associated nuclease like Cas9. This method is especially useful for creating complex, polygenic features that are governed by interrelated genetic pathways since it allows for the coordinated alteration of several genes in a single phase. Researchers can create parallel edits, such as gene knockouts, insertions, or regulatory alterations, by creating sgRNAs that target distinct genes or regulatory regions. This speeds up the breeding process and lessens the need for repeated rounds of transformation and selection.

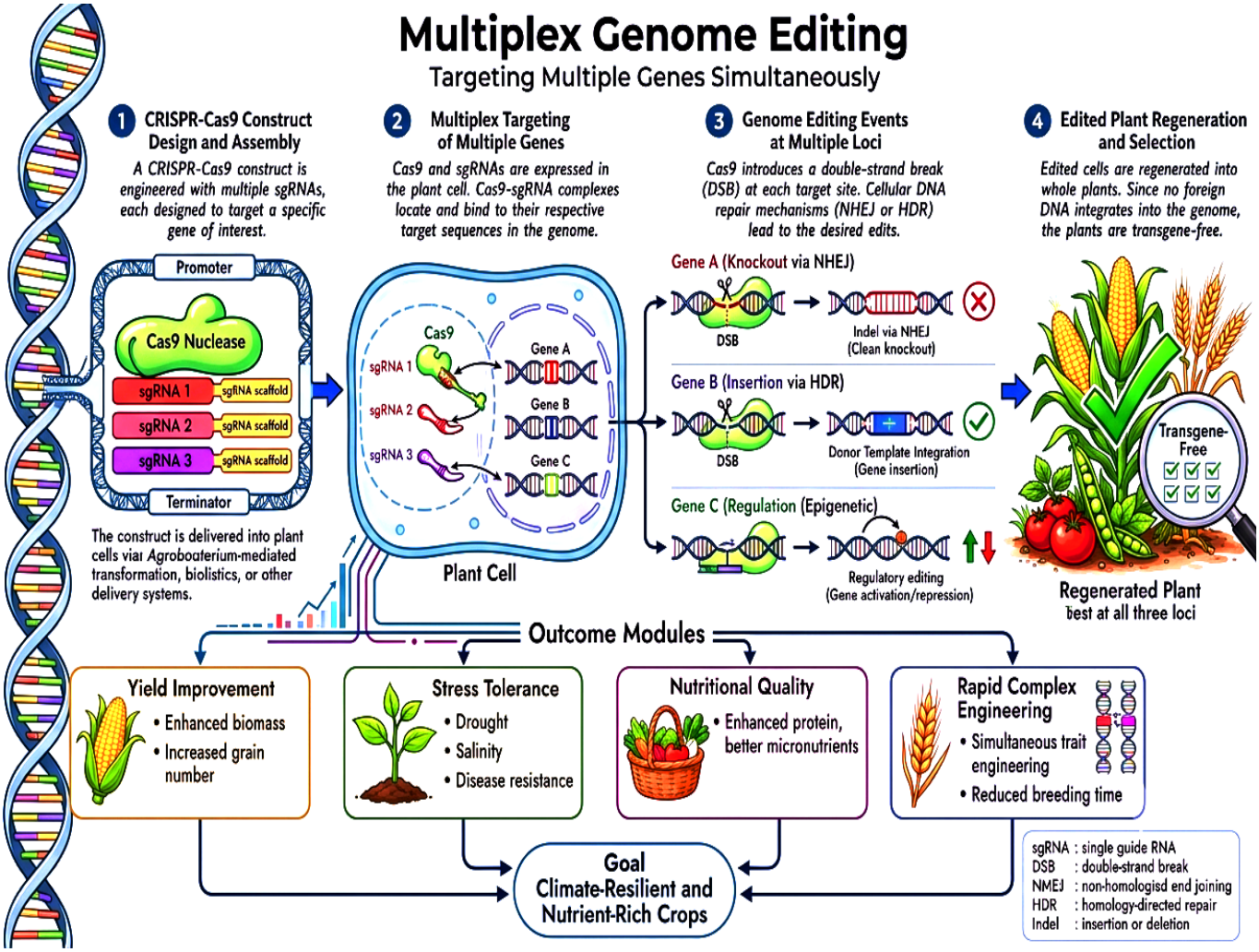


Fig. 12 Overview of multiplex genome editing approaches for enhancement yield in common Indian pulse crops

Multiplexing is very effective in crop development efforts when many genes regulate traits like yield, stress tolerance, and nutritional quality. Additionally, by enabling the analysis of gene networks and interactions, this approach improves accuracy and efficiency in functional genomics research[169]. All things considered, multiplex genome editing is a revolutionary technique for creating superior cultivars with stacked features quickly and affordably.

5.3.2. Transgene-Free Editing

In plant biotechnology, transgene-free genome editing is a cutting-edge method that introduces desired genetic alterations without causing foreign DNA to become permanently integrated into the host genome. Pre-assembled CRISPR/Cas Ribonucleoprotein (RNP) complexes or transient expression systems, in which the editing machinery is only momentarily present within the cell, are frequently used to do this. The Cas nuclease and guide RNA-containing RNP complexes enter plant cells directly, carry out precise genome editing, and are then broken down, leaving only the desired alteration. Because of this, the final modified plants are identical to those created by conventional breeding or natural mutations, but with much greater accuracy and efficiency [170].

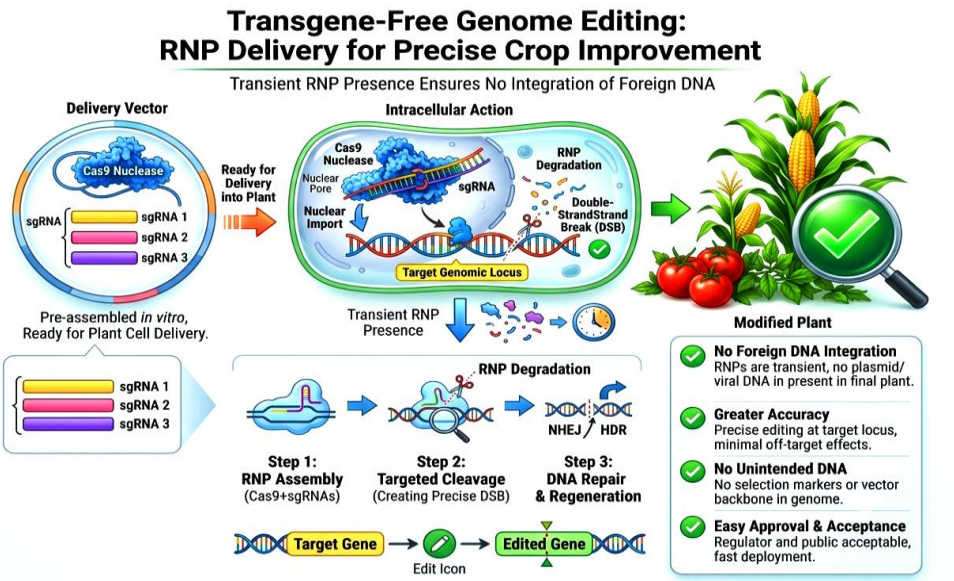


Fig. 13 Overview of Transgene-free genome editing approaches for enhancement yield in common Indian pulse crops

This strategy tackles societal concerns and regulatory issues related to Genetically Modified Organisms (GMOs) while also reducing the possibility of off-target impacts and unwanted genetic insertions. Therefore, transgene-free editing has enormous potential to speed up crop improvement while promoting wider acceptance and more efficient approval procedures for enhanced plant types [171].

5.3.2. Nanotechnology Delivery

A possible method for effectively and precisely introducing CRISPR components into plant and animal cells is the development of nanotechnology-based delivery systems. CRISPR-associated nucleases and guide RNAs are transported across biological barriers, such as the cell wall and plasma membrane, using manufactured nanoparticles (), such as carbon nanotubes, lipid-based nanocarriers, or polymeric nanoparticles. These nanoparticles allow for regulated release within the cell, selective delivery to particular tissues or organelles, and protection of the CRISPR components against degradation. Therefore, while avoiding cellular harm and lowering dependence on conventional transformation techniques like Agrobacterium-mediated transfer or biolistics, nanotechnology-mediated delivery can greatly increase editing efficiency [172].

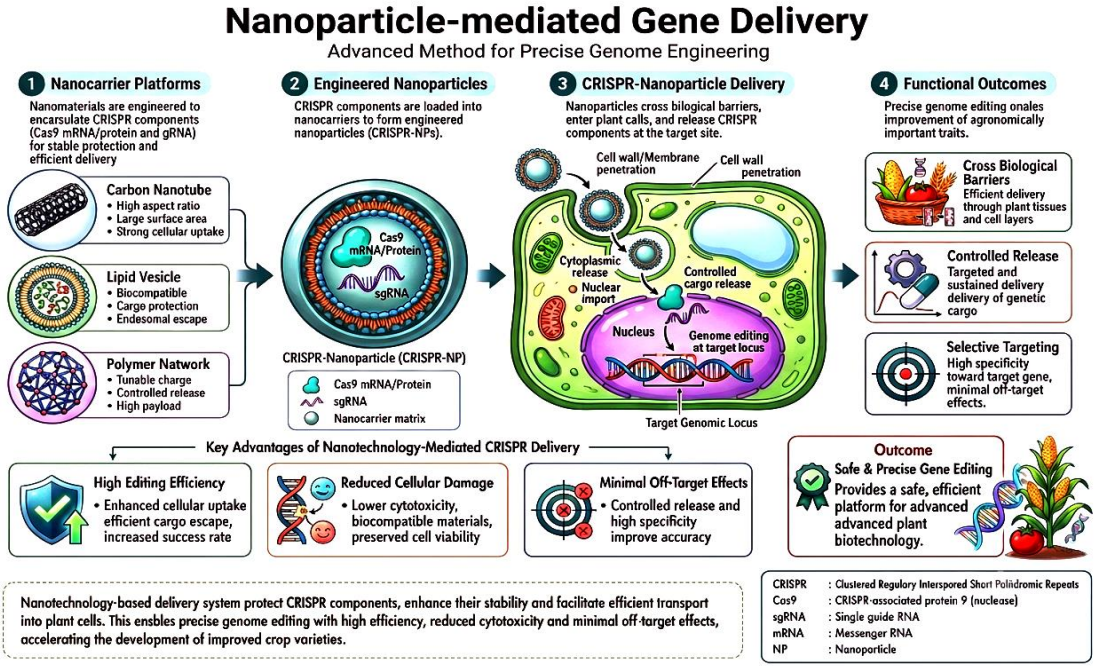


Fig. 14 Overview of Transgene-free genome editing approaches for enhancement yield in common Indian pulse crops

Crucially, by allowing the editing apparatus to operate temporarily and locally, this method can also reduce the possibility of off-target impacts.

As a result, CRISPR delivery systems powered by nanotechnology provide a highly adaptable and non-invasive platform for precise genome engineering, with significant promise to advance crop improvement and sustainable agricultural biotechnology.

5.3.3. CRISPR/Cas systems for Orphan Crop Breeding Improvement

CRISPR/Cas sub variant systems are transforming orphan crop breeding—scientifically and economically ignored plants critical for regional food security—by allowing for rapid, precise, and cost-effective genetic improvements. Unlike traditional breeding, which is time-consuming and labour-intensive, CRISPR technology facilitates. rapid *de novo* domestication, biotic/abiotic stress resistance, and nutritional enhancement [173].

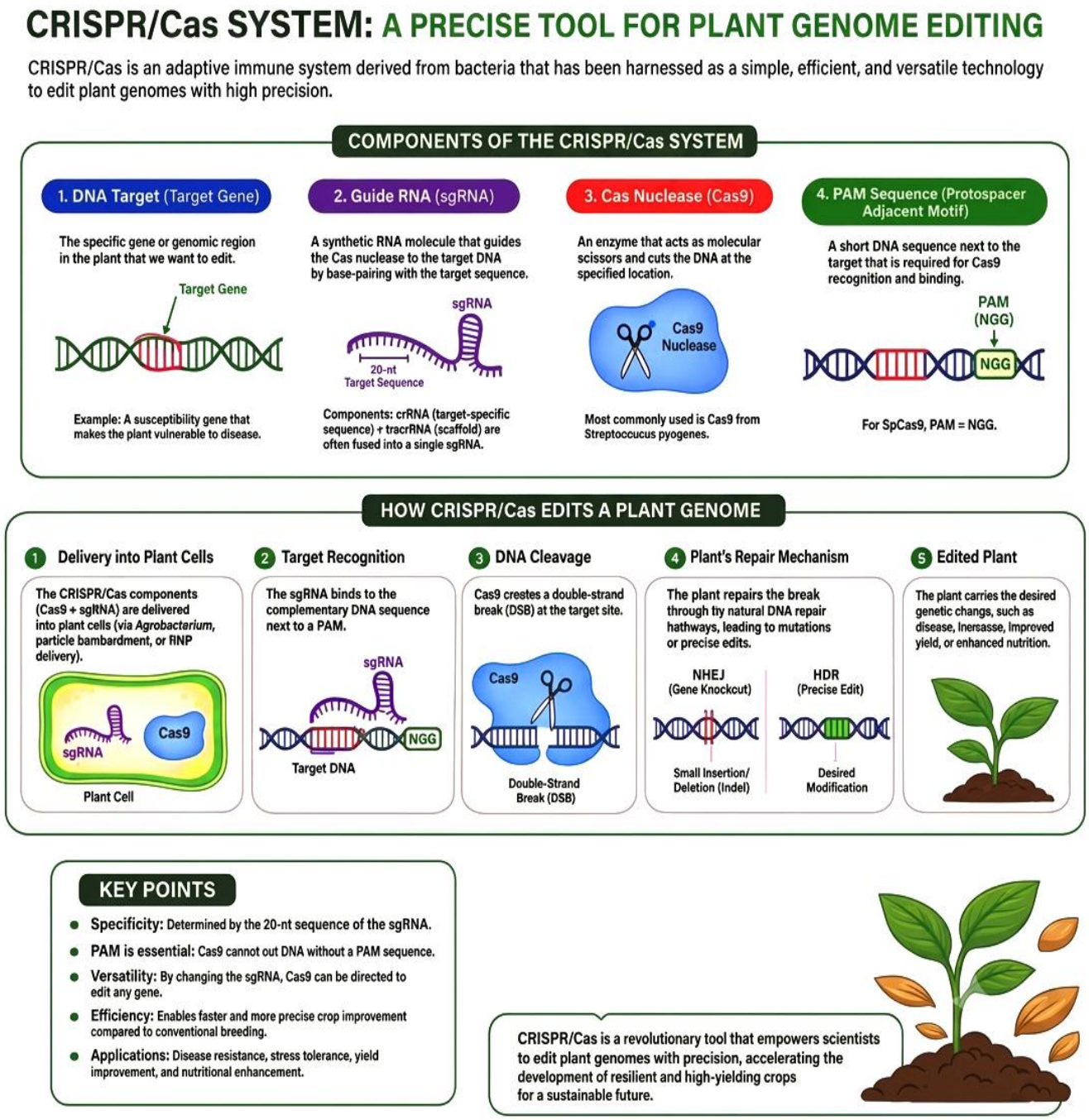


Fig. 15 Overview of CRISPR-Cas-based genome editing approaches for enhancement yield in common Indian pulse crops

5.3.4. Key CRISPR/Cas Sub-Variants and Systems for Precise Genome Editing in Orphan Legumes

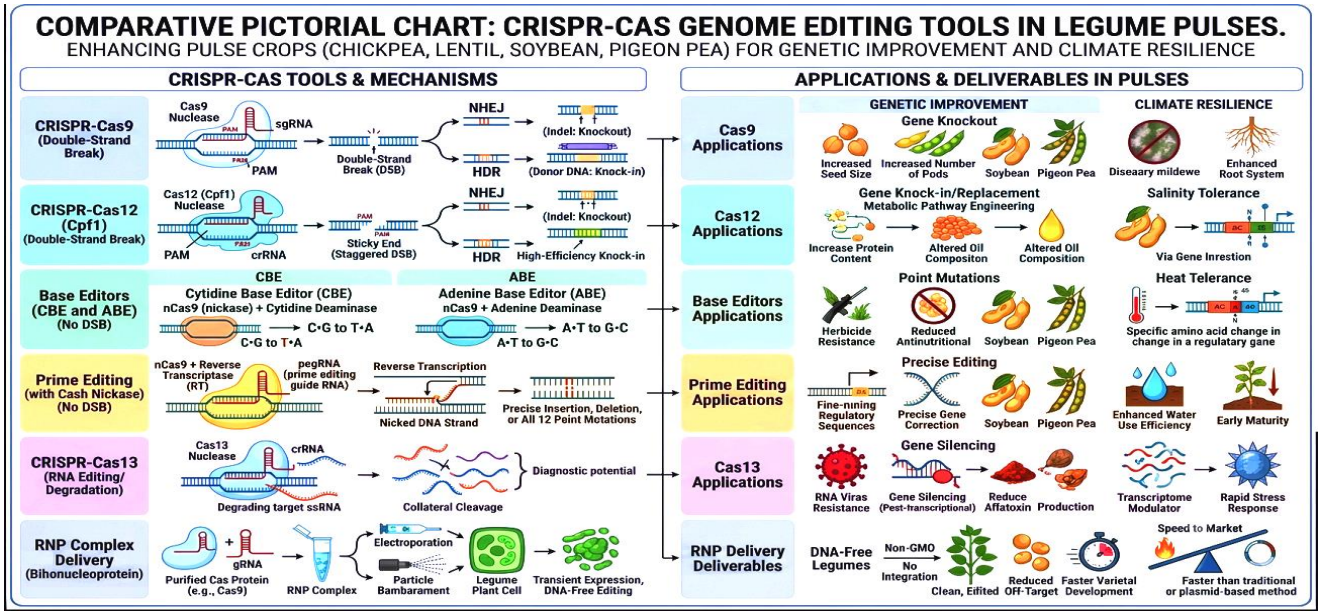


Fig. 16 Overview of CRISPR-Cas subvariants and their application-based genome editing approaches for enhancement yield in common Indian pulse crops

- CRISPR/Cas9: By focusing on important agronomic genes, this fundamental technology for gene knockouts and, with Homology-Directed Repair (HDR), precise insertions (knock-ins) allows for the quick domestication of wild species [173].
- Base Editors (CBE and ABE): These fusion systems are perfect for modifying particular regulatory regions (such as the SWEET gene promoters for bacterial resistance) or producing herbicide-tolerant lines because they enable precise, single-nucleotide conversions (C-to-T or A-to-G) without generating Double-Strand Breaks (DSBs) [174].
- Prime Editing: Usually utilizing an Agrobacterium-mediated system, Prime Editing (PE) in plants is a precise "search-and-replace" genome editing technique that permits targeted base substitutions, insertions, and deletions without causing Double-Strand Breaks (DSBs). This technique provides greater precision and fewer off-target effects than traditional CRISPR-Cas9 by using a Cas9 nickase coupled to a reverse transcriptase and a customized prime editing guide RNA (pegRNA) [175]
- CRISPR/Cas13: The CRISPR/Cas13 system represents a powerful advancement in genome engineering by specifically targeting RNA molecules rather than DNA, distinguishing it from more widely known CRISPR systems such as Cas9. Cas13 functions as an RNA-guided ribonuclease that binds to complementary single-stranded RNA sequences and induces their cleavage, enabling precise post-transcriptional regulation of gene expression. This unique property makes CRISPR/Cas13 particularly valuable for combating RNA viruses, which constitute a major class of plant and animal pathogens [176]. By designing guide RNAs that recognize viral RNA genomes or transcripts, Cas13 can selectively degrade viral RNA within host cells, thereby inhibiting viral replication and spread without altering the host genome. Additionally, its transient and reversible mode of action reduces the risk of permanent off-target genetic modifications. Consequently, CRISPR/Cas13 offers a promising, flexible, and efficient tool for developing virus-resistant crops and enhancing disease management strategies in agriculture [177].
- RNP Complex Delivery: DNA-free genome editing in plants is made possible by RNP complex delivery, which reduces off-target effects and regulatory loads by delivering pre-assembled CRISPR-Cas9/Cas12a protein and guide RNA. It works quite well for inserting instruments into plant protoplasts, calli, and embryos. PEG-mediated transfection, biolistics (gene gun), and nanoparticle delivery are important methods that enable accurate modifications across a variety of crop species[178], [179].

Therefore, additional CRISPR-Cas9 applications would emphasize and serve as more examples of emerging genome editing technologies. Future plant biologists will get new insights into the development of molecular breeding approaches in cash crops for biofortification versus crop production thanks to recent advancements in genome editing, which are broadening the potential for precise to large-scale DNA alterations.

6. Here, a few of these more Recent Strategies are Worth Highlighting

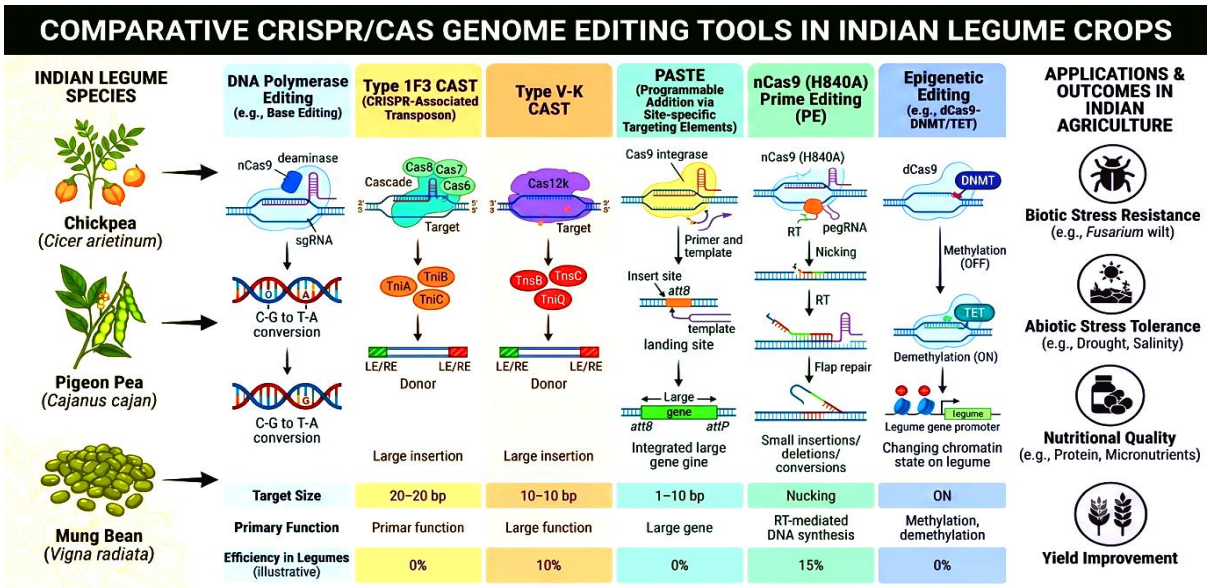


Fig. 17 Overview of CRISPR-Cas-based state-of-the-art genome-editing modifiers and their application-based genome editing approaches for enhancement yield in common Indian pulse crops

6.1. DNA Polymerase Editing (Click Editor)

This approach effectively introduces targeted modifications by fusing nCas9 (H840A) with DNA polymerase and using an endonuclease with single-stranded DNA templates. The nontarget strand is broken into a single strand by the structure of Cas9 nickase (H840A Cas9), and the nicked strand immediately polymerizes with a short-edited strand created by reverse transcriptase connected to Cas9 nickase. Prime Editing Guide RNA (pegRNA) contains the altered sequences of the Reverse Transcriptase (RT) template. Protospacer-Adjacent Motif (PAM) and Primer Binding Site (PBS) (Source: Lee et al., 2020) [180].

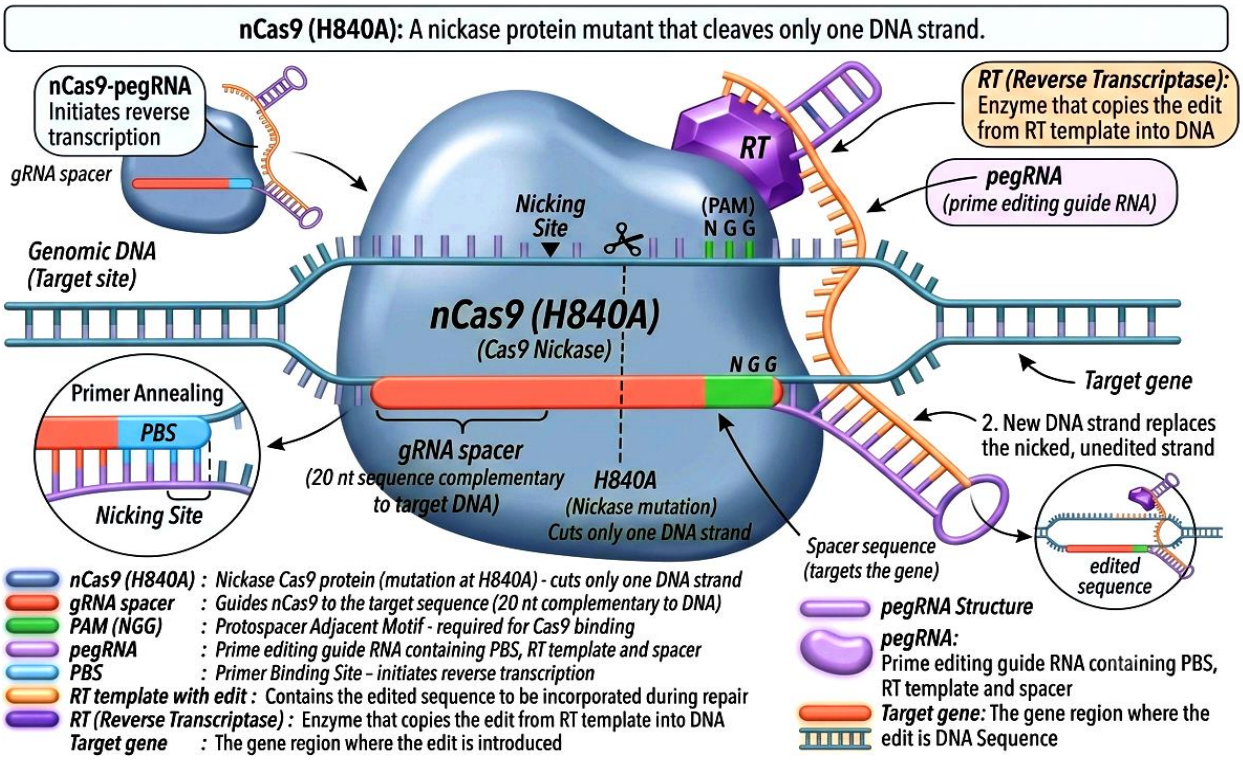


Fig. 18 Overview of nCas9(H840A) with DNA polymerase for enhancement yield in orphan legumes.

6.2. CRISPR-Associated Transposons (CASTs)

Through unique mechanical processes directed by CRISPR effectors and specialized transposase proteins, CRISPR-associated transposons (CASTs) accomplish site-specific genomic alterations. The "cut-and-paste" method used by the Type I-F3 CAST is non-replicative. The target site next to a protospacer adjacent motif (PAM) is identified by a multi-protein CRISPR complex (Cas5–8, Cas6, and Cas7) coupled to crRNA. TnsA and TnsB transposases cut the transposon DNA from the donor locus and insert it approximately 80–82 bp downstream of the PAM, while the auxiliary factor TniQ binds and activates TnsC ATPase filaments. The Type V-K CAST, on the other hand, uses a single Cas12k effector in conjunction with tracrRNA, crRNA, and the tiny protein S15 to carry out a "replicative" process. TnsB nicks a single strand of donor DNA without removing it, and ATP hydrolysis coupled with TnsC depolymerization mediates transposition ~60–66 bp downstream of the PAM (courtesy: Schmitz and Querques, 2024) [181].

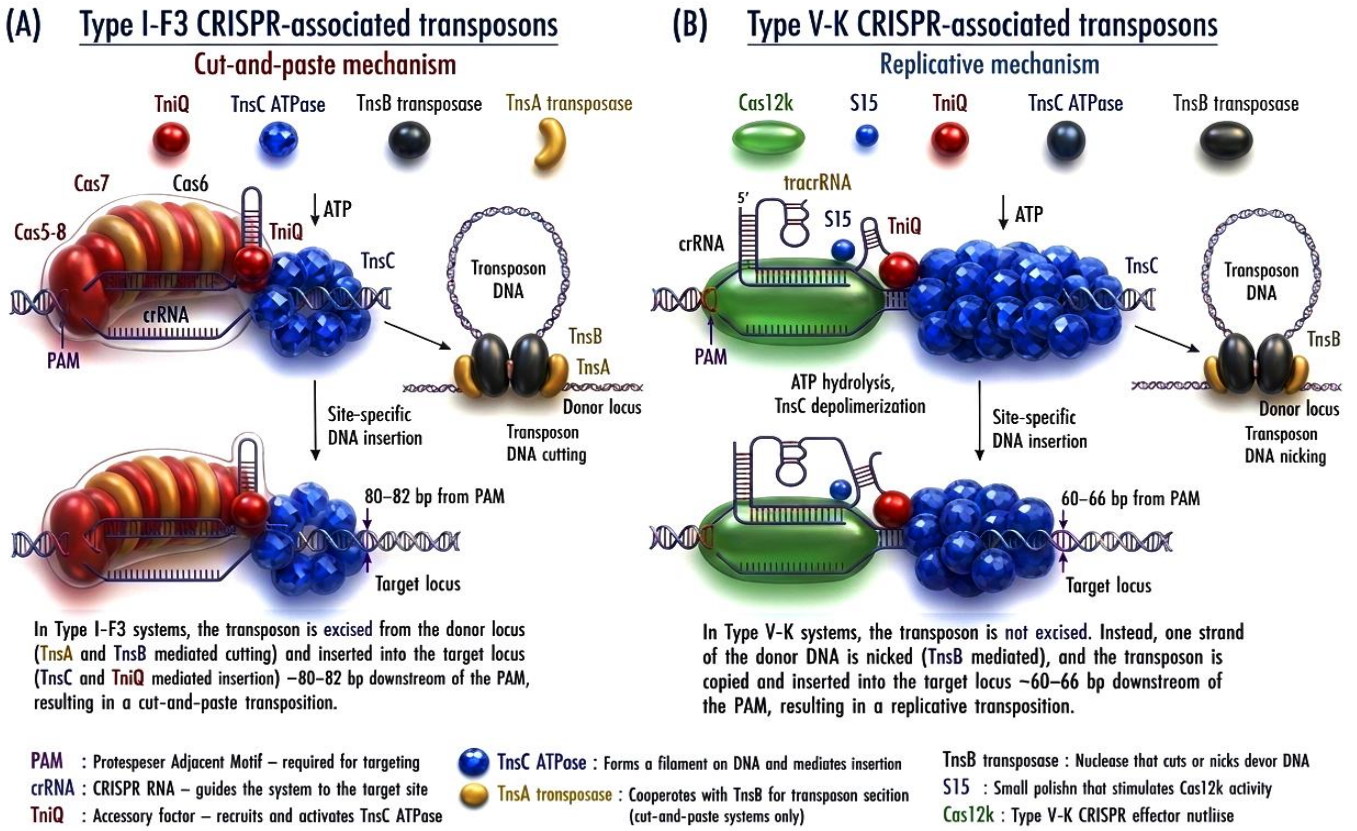


Fig. 19 Overview of differences in mode of action of Type I-F3 CAST and Type V-K CAST for genome editing in orphan legumes

6.3. Site-Specific Integration of Large Genes

Prime editing and site-specific recombinases have been effectively used to integrate big genetic constructs at specified genomic loci. Programmable Addition via Site-specific Targeting Elements (PASTE), which combines the integration efficiency of serine recombinases with the target specificity of CRISPR-Cas9, is a significant development in this field. PASTE works mechanistically by fusing a CRISPR-Cas9 nickase with a serine integrase and a Reverse Transcriptase (RT). The technique uses an Attachment Site-Containing Guide RNA (atgRNA) to insert a minimum recognition sequence (attB) into the target genome. Large, attP-flanked donor DNA segments are then specifically inserted into the modified attB site by the integrase. PASTEv3, an improved variation with an engineered Bxb1 integrase (BxbINT), optimized linkers, guide sequences, and atgRNA architecture, was the result of recent optimization efforts. PASTEv3 offers a reliable approach for locus-specific transgene delivery by accurately and efficiently integrating large genomic templates up to 36 kilobases [182].

Targeted insertion is the purpose of programmable addition through a site-specific targeting element (PASTE). In order to facilitate the integration of an attB site, a PASTE fusion complex consisting of Cas9 nickase (nCas9), Reverse Transcriptase (RT), and integrase is directed to a particular genomic locus by an attachment site-containing guide RNA (atgRNA). After identifying the attB site, the integrase integrates a sizable DNA fragment surrounded by attP sites without causing a double-strand break (DSB) [183].

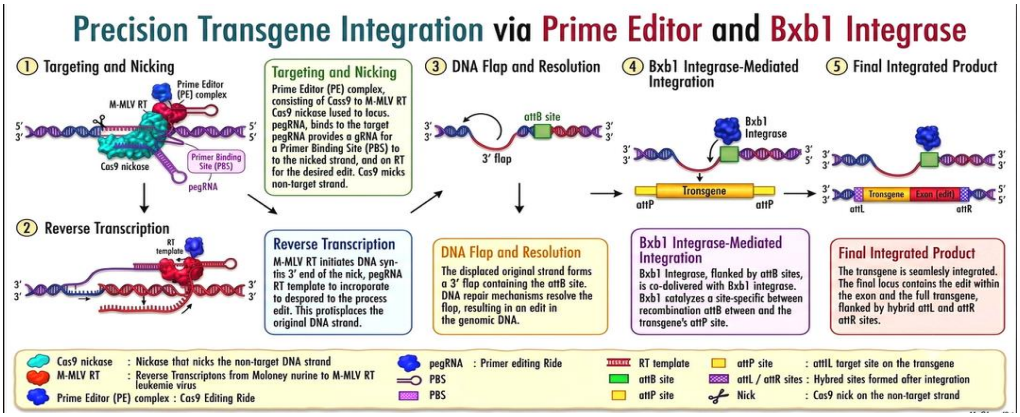


Fig. 20 Overview of the schematic mechanism of precision transgene integration mediated by Prime Editor and Bxb1 Integrase (twin PE/PASTE system) for genome editing in orphan legumes

6.4. RT-Based Editing: nCas9(H840A) is used with a non-LTR reverse transcriptase and RNA to generate a free 3' end, allowing reverse transcription of RNA and its insertion into the target DNA

When nCas9 (H840A) is combined with RNA and a non-LTR reverse transcriptase, it creates a free 3′ end that enables reverse transcription of RNA and its insertion into the target DNA: Prime Editor (PE), a versatile editing tool that can insert and remove any sequence and all 12-point mutations without DSB and a donor template, was recently reported. PE is made up of reverse transcriptase fused to nickase Cas9 (H840A) via a flexible linker (nCas9-RT) (the nCas9-RT uses a uniquely designed Prime Editing Guide RNA (pegRNA)). The intended changes are carried by the reverse transcriptase template (RT template), Primer Binding Site (PBS), and Single Guide RNA (sgRNA) in the pegRNA. PBS attaches to the 3′flap after nCas9 nicks the non-target strand during prime editing. PBS transcribes the RT template with the intended change after binding to the 3′flap and serving as a primer for RT [184]. The edit is permanently placed into the target spot after the flap is resolved during DNA repair and replication. Research capabilities are significantly improved by Prime Editor (PE), a flexible editor that permits the insertion and deletion of arbitrary sequences, all 12-point mutations without Double-Strand Breaks (DSB), and a donor template. PE comprises Reverse Transcriptase (RT), nickase Cas9 (H840A), and prime editing guide RNA (pegRNA). An optimized-prime editor (O-PE) for endogenous gene editing in Arabidopsis thaliana cells was found to have an average editing effectiveness of 1.15% in the 2022 study [185], which is 16.4 times higher than previously reported. Meanwhile, an increase in indels was observed when testing alternative reverse transcriptase, and it was found that nCas9(H840A) fused to non-functional reverse transcriptase was responsible for the increase. This work develops an efficient prime editor for plant cells.

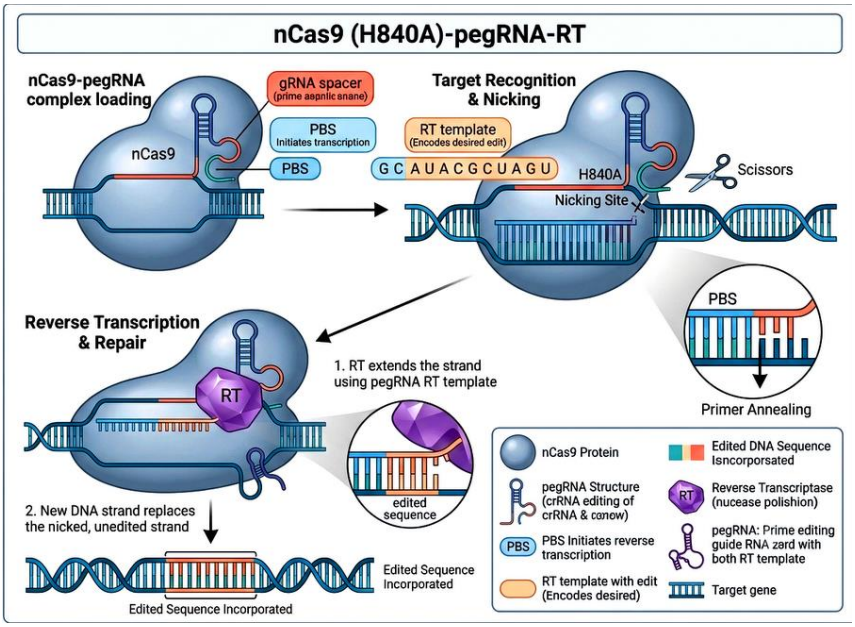


Fig. 21 Overview of nCas9(H840A) coupled with a non-LTR reverse transcriptase with an RNA insertion into the target DNA for genome editing in orphan legumes. [185]

6.5. Epigenetic Editing

It is of note that dCas9 can be fused with DNA methyltransferases or histone-modifying enzymes to silence genes (CRISPR-off) without altering DNA. Alternatively, dCas9 can recruit demethylases or transcriptional activators to restore gene expression (CRISPR-on)[186].

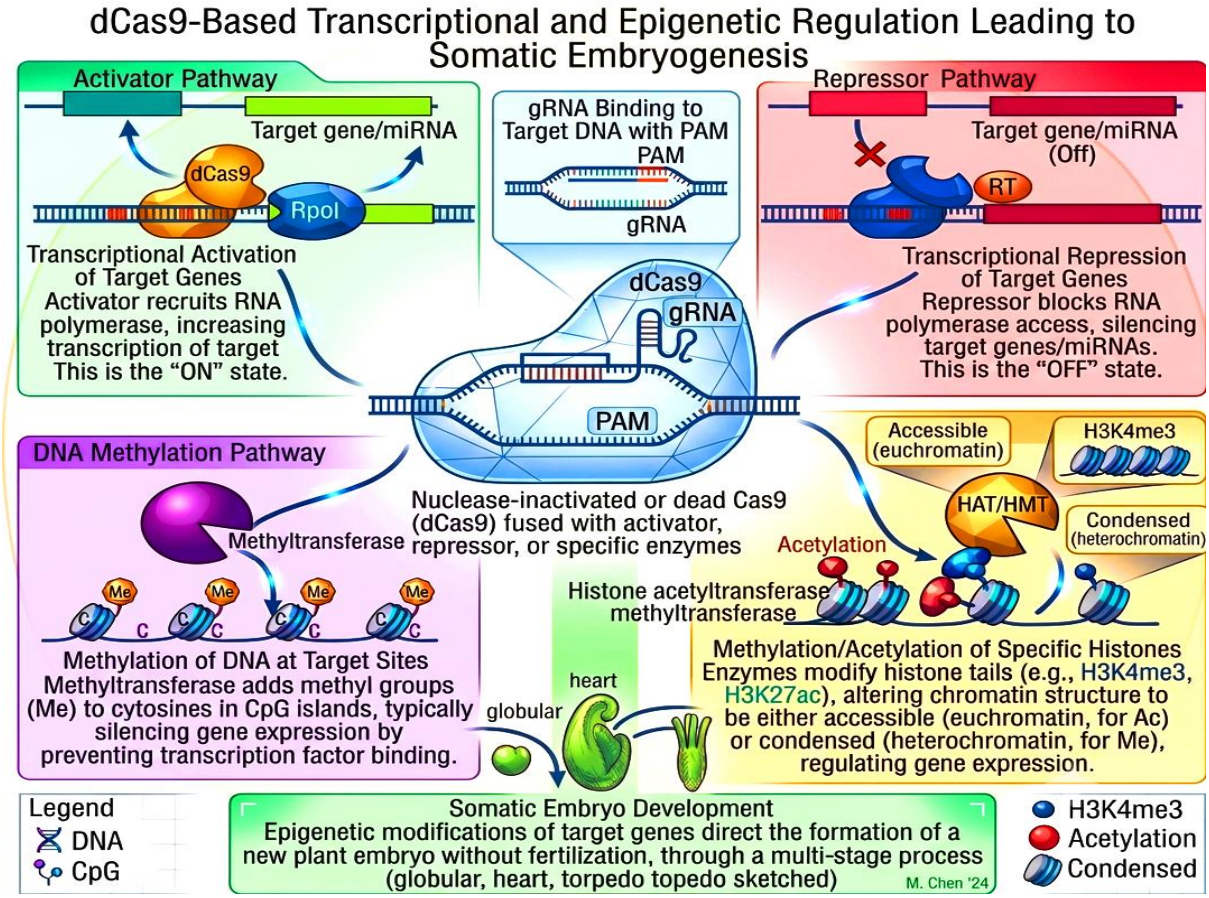


Fig. 22 Overview of Epigenetic Editing by dCas9 fused with DNA methyltransferases or histone-modifying enzymes to silence genes (CRISPR-off) without altering DNA for genome editing in orphan legumes

The CRISPR/Cas system controls the epigenetic modification of Somatic Embryo (SE) development in plants. The dead Cas9 (dCas9) protein fused with enzymes or regulators is guided to specific regions of the genome by guide RNAs (gRNA). A specific region of the plant genome can be methylated when dCas9 is paired with enzymes such as methyltransferase. Certain histone proteins may be methylated and acetylated by certain enzymes when combined with dCas9. miRNA or SE-related gene transcription may be regulated by the fusing of activator or repressor domains with dCas9. [187].

7. International efforts for improvement of the Orphan Legume Genome: Recent Developments

Because different research have used different sequencing technologies, filtering criteria, and annotation workflows, and because assembly size is not always equivalent to estimated genome size, the genome statistics should be interpreted carefully. For instance, the 2025 T2T lablab assembly is far more complete than its previous draft genome [188], whereas the original Bambara groundnut assembly was 535.05 Mb with 31,707 projected protein-coding genes.

- Lablab genome: For a 2026 evaluation, the lablab genome is especially notable. Its 460.76 Mb T2T assembly includes 29,280 predicted protein-coding genes and resolves all 11 chromosomes, including centromeres and telomeres. Furthermore, a 2025 pangenome-focused analysis discovered that about 7.7% of reads from eight accessions [189] did not map to the Highworth reference genome, highlighting the importance of non-reference sequencing for identifying potentially adaptive breeding variation.
- Grass pea: The 2024 chromosome-scale LS007 genome [190] for grass peas is a significant advancement; the assembly is 5.96 Gb, with 5.03 Gb anchored to seven pseudochromosomes. The whole-genome sequencing of 344 global lines in June 2026 came next, yielding >1.5 million SNPs and significantly bolstering the

population-genomic basis of this crop. I advise classifying the latter as a preprint rather than a peer-reviewed publication since it is a June 2026 preprint.

- The African yam bean; Another significant recent advancement is the African yam bean. With a N50 of 69.5 Mb and 31,614 projected protein-coding genes [191], the 2024 chromosome-scale genome offers a significantly enhanced platform for genetic mapping, marker creation, and genome-assisted breeding.
- The marama bean: From the standpoint of climatic resistance, the marama bean genome is especially significant. Using PacBio HiFi and Omni-C data, the 2025 study created a 558.78-Mb near-chromosome-level assembly and discovered two different genetic population groups [192]. This is significant because marama is a potentially significant genetic resource for dryland agriculture in the future due to its adaptation to harsh drought and high temperatures.
- Horse gram: There are currently 24,521 annotated genes in the 279.12-Mb horse gram genome. Significantly, ddRAD analysis of 40 germplasms yielded 3,942 high-quality SNPs when the genome was paired with transcriptome and germplasm sequencing. The resource base has been broadened by subsequent GWAS, such as drought studies employing 96 accessions and a 2024 study [193] that found 11 stable QTLs for yield-related characteristics and 143 marker–trait relationships.
- Rice bean: The transcriptome-only stage reported in earlier work has been significantly surpassed by rice beans. PacBio long reads and Hi-C were used to create a high-quality reference genome, and further research yielded a ~414-Mb assembly with 31,276 high-confidence genes [194]. The rice bean is a particularly intriguing candidate for genomics-assisted domestication, as population genomic analysis has since found genes linked to adaptability and yield.
- Winged bean: According to F_2 populations and DArTSeq-derived SNP maps, winged beans now have a 586.44-Mb pseudochromosome-level assembly with 96.2% complete BUSCOs [195]. Candidate areas for seed protein, plant architecture, and phytochemical characteristics have already been found thanks to the genome toolkit.
- Andean lupin (*Lupinus mutabilis*): A new genomic phase has also been reached by the Andean lupin (*Lupinus mutabilis*). 37,754 projected protein-coding genes make up the 620.0 Mb 2024 genome, which was created utilizing ONT, Illumina, and Hi-C techniques. Significantly, the study examined genes [196] associated with quinolizidine alkaloid concentration and seed protein, making this resource directly relevant to enhancing food safety and nutritional quality.

Table 4. Recommended classification of genomic-resource maturity

Resource category	Representative orphan legumes	Status
Advanced / rapidly approaching mainstream genomic breeding	Lablab, adzuki bean, rice bean, winged bean, tepary bean	Reference/chromosome-scale genomes + substantial SNP/QTL/GWAS resources [193],
Intermediate genomic development	Grass pea, horse gram, African yam bean, Bambara groundnut, Andean lupin	Good reference genomes and/or substantial marker resources, but limited pangenomes/functional validation[194]
Emerging genomic resources	Marama bean, yam bean, cluster bean	Genome available, but population-scale resources and breeding validation remain limited[196]
Transcriptome/marker-dominated resources	Moth bean	Strong transcriptomic information but limited high-quality reference/pangenomic resources[155]
Major genomic-resource gap	Bitter vetch	No reference genome or comprehensive assembled transcriptome available by June 2026[197], [198].

For certain orphan legumes that are crucial to the security of food and nutrition worldwide, genetic and genomic resources are accessible, with a focus on germplasm, reference genomes, molecular markers, QTL/GWAS, transcriptome resources, population genomics, and pangenomics. Because "genome sequenced" and "genomically enabled breeding" are not synonymous, this classification is very helpful. The 2025 orphan-crop pangenomics review highlights the need for pangenomics, sophisticated selection, and genome editing to convert genomic variety into crop development, as a single reference genome only captures a portion of species-wide variation.

7.1. Limitations for future research: Current Bottlenecks and Future Directions

Despite these advances, the broad implementation of novel gene targeting across orphan legumes faces two major limitations:

1. Recalcitrance to *In Vitro* Regeneration: Many underused legumes are still very resistant to tissue culture regeneration or typical Agrobacterium-mediated transformation [198]. To get over tissue culture bottlenecks, it is essential to use morphogenic developmental regulators (like Babyboom [BBM] and Wuschel [WUS2]) or viral delivery vectors (such as negative-sense RNA virus vectors).
2. Polygenic Complexity: Many climate-resilient and yield-related traits are multigenic; editing individual loci without disrupting integrated metabolic or stress-signaling networks [199] remains a challenge.
3. Transgene-Free Editing & Regulation: Delivering CRISPR/Cas ribonucleoproteins (RNPs) via biolistics or transient expression enables the production of non-transgenic, genome-edited lines, which face significantly reduced regulatory hurdles [200]globally compared to classical genetically modified organisms across the Indian Subcontinent (GMOs) [201].

7.2. Ethical, Biosafety, Legal, and Social Considerations in Orphan Legume Bioengineering

A complicated web of biosafety, socioeconomic, legal, and international regulatory factors governs the deployment of orphan legume engineering, which presents a revolutionary prospect for climate-resilient agriculture and global food security. To reduce ecological risks, such as off-target double-strand breaks in polyploid genomes, gene flow into wild landrace populations, and unintentional biochemical trade-offs from the knockout of anti-nutritional factors like L-ODAP [202], precision tools (such as base and prime editors), strong containment, and thorough metabolomic profiling are required[202]. Open-access frameworks like BiOS are also necessary in an effort to combat IP monopolies concentrated in the Global North, in addition to equitable benefit-sharing under the Nagoya Protocol and ITPGRFA to protect traditional knowledge and the rights of indigenous farmers. Last but not least, as global regulatory architectures shift from process-based to trait-based or tiered models (as evidenced by the US SECURE Rule, the EU's proposed NGT categories, India's SDN-1/SDN-2 exemptions, and emerging African polycentric guidelines), it is critical to establish streamlined, transgene-free approval pathways to expedite the public sector translation of gene-edited pulses for smallholder farming communities [203].

7.2.1. Biosafety & Ecological Integrity

Off-Target Mutations & Genomic Instability

- Mechanistic Vulnerabilities: Engineered nucleases like CRISPR/Cas9 introduce Double-Strand Breaks (DSBs) at non-target genomic sites with sequence homology. In complex or polyploid orphan legume genomes (e.g., *Vicia faba*, *Sphenostylis stenocarpa*), off-target cleavage risks disrupting vital metabolic networks or causing structural rearrangements [204].
- Next-Generation Mitigation: Modern tools like Cas12a, base editors, and prime editors eliminate the need for DSBs [205], significantly reducing off-target risks.
- Verification Protocols: Multi-generational whole-genome resequencing (WGS) paired with deep-learning off-target prediction models is increasingly required to confirm target specificity prior to open-field testing [206].

Gene Flow & Interspecies Hybridisation

- Ecological Hazards: Orphan legumes frequently share native centres of origin with wild relatives and landraces (e.g., *Vigna* spp. in Sub-Saharan Africa and South Asia). The escape of gene-edited traits—such as altered secondary metabolite profiles or abiotic stress tolerance—via pollen drift poses risks of wild gene pool contamination, loss of native agrobiodiversity [207], or the emergence of invasive "superweeds".
- Biological Containment: Mitigation strategies rely on maternal inheritance systems (chloroplast genome editing), genetic male sterility [208], and enforced spatial/temporal isolation buffers during field evaluations.

Toxicity, Allergenicity, and Secondary Metabolite Dynamics

- Biochemical Balance: Targeted knockout of anti-nutritional factors—such as β-L-oxalyl-α,β-diaminopropionic acid (L-ODAP) in *Lathyrus sativus*—must be monitored so as not to unintentionally upregulate [209] defense compounds (e.g., alkaloids, condensed tannins, or trypsin inhibitors) that provide natural immunity against field pests.
- Safety Evaluation: Mass spectrometry-based metabolomic profiling, standardized mammalian feeding studies, and sequence matching against WHO/FAO allergen databases are required to verify toxicological safety [210].

7.2.2. Socio-Economic, Equitable Access, & Social Issues

Intellectual Property (IP) Bottlenecks & Open Science

- Patent Concentration: Core CRISPR and precision bioengineering intellectual property remains concentrated within institutions in the Global North. Restrictive licensing costs threaten to prevent smallholder breeders [211](Nath et al., 2026) and public institutions in (LMICs) from deploying edited orphan crop varieties. Low- And Middle-Income Countries
- Equitable Frameworks: Open-access platforms (e.g., *BiOS* – Biological Open Source) and CGIAR-negotiated humanitarian licenses are being implemented to keep

gene-edited orphan crops in the public domain for smallholder farming communities [212].

Farmers' Rights & Traditional Knowledge Protection

- Benefit-Sharing Dynamics: Indigenous and local agricultural communities have developed, preserved, and selected orphan legume landraces over centuries. Commercialization or patenting of gene-edited derivatives without equitable benefit-sharing violates the Nagoya Protocol on Access and Benefit-Sharing [213] and the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA).
- Participatory Gene Editing: Integrating local farming communities into early-stage trait selection (e.g., targeting bush-type canopy architecture, synchronized flowering, or reduced cooking times) ensures that edited lines [214] align with traditional farming systems and local culinary preferences.

7.2.3. Regulatory & Legal Frameworks: Global Comparative Analysis

Global governance of gene-edited crops varies widely across jurisdictions, creating distinct operational pathways for orphan legume research, field release, and international trade.

Jurisdiction / Region	Regulatory Trigger & Model	Oversight Status for Site-Directed Nucleases (SDN-1 / SDN-2)	Implications for Orphan Legumes
United States *(USDA / EPA)*	**Product-based / Trait-focused** (*SECURE Rule*)	**Exempt from GMO oversight** if the edit could occur via conventional breeding (e.g., SDN-1 knockouts).	Accelerated path to commercial release (e.g., USDA deregulation of CRISPR bushy/synchronous cowpea and anti-lodging teff).[215], [216],[217]
European Union *(EC / EFSA)*	**Process-based** (*NGT Directive revisions*)	**Tiered Approach:** Category 1 NGTs (≤20 base modifications) face streamlined notification; Category 2 retains GMO status.	Reduces regulatory compliance costs for basic NGT orphan crops while maintaining strict monitoring for complex edits.[218], [219]
India *(DBT / MoEFCC)*	**Hybrid / Tiered Framework** (*SDN-1 & SDN-2 Exemption*)	**Exempted** from strict GMO biosafety rules (IBSC/GEAC) for SDN-1 and SDN-2 edits free of foreign DNA.	Encourages domestic public breeding programs for orphan pulses (*L. sativus*, *M. uniflorum*, *V. aconitifolia*).[220], [221]
African Union & Regional Bodies *(e.g., NBA Kenya, Nigeria)*	**Polycentric / Evolving**	**Case-by-case exemptions:** Early adopters (Kenya, Nigeria, Malawi) treat transgene-free SDN-1/2 edits as conventional crops.	Enables regional multi-location field trials for climate-resilient pulses (Bambara groundnut, early-flowering cowpea).[222], [223]

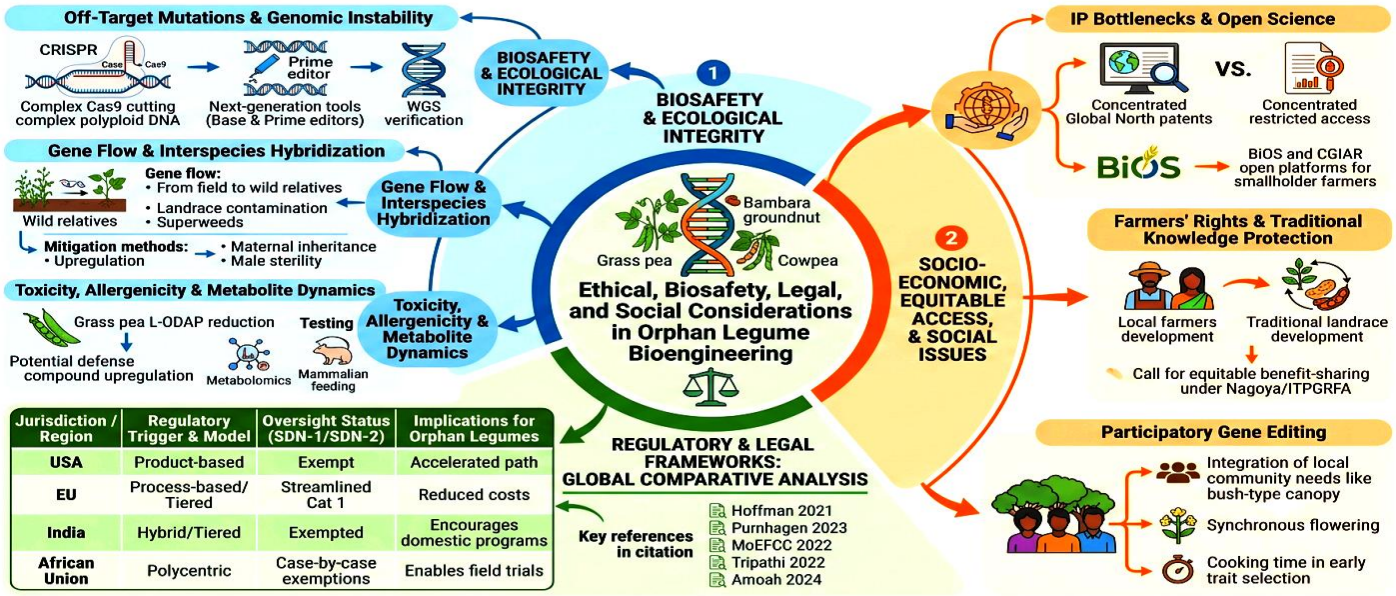


Fig. 23 The comparative regulatory figure consolidates several official policy documents and peer-reviewed regulatory syntheses published between 2021 and 2026. Each jurisdiction's classification and oversight mechanism is anchored in the following official frameworks and peer-reviewed studies

7.3. Specific References by Jurisdiction

7.3.1. United States (USDA / EPA - SECURE Rule)

Regulatory Status

Establishes exemptions under 7 CFR Part 340 for plants modified by genetic engineering [216] where the alteration could otherwise have been achieved through conventional breeding (e.g., SDN-1 single-base substitutions or knockouts in the absence of a repair template).

7.3.2. European Union (EC / EFSA - NGT Framework)

Regulatory Status

European Parliament adoption of New Genomic Techniques (NGT) proposals creating a tiered structure—Category 1 NGTs genetic modifications, [218] treated similarly to conventional breeding) versus Category 2 NGTs (retaining Directive 2001/18/EC GMO-style oversight).

7.3.3. India (DBT / MoEFCC - Guidelines for Genome-Edited Plants)

Ministry of Environment, Forest and Climate Change (MoEFCC) / Department of Biotechnology (DBT), Government of India. (2022)

The Indian government's Department of Biotechnology (DBT) and Ministry of Environment, Forests, and Climate Change (MoEFCC; 2022). Standard Operating Procedures (SOPs) for Regulatory Review of Genome-Edited Plants under SDN-1 and SDN-2 Categories and Guidelines for the Safety Assessment of Genome-Edited Plants, 2022. [220] .

Regulatory Status

SDN-1 and SDN-2 are exempt from regulations. Rules 7–11 of the Environmental Protection Act (1986) apply to some types of genome-edited plants that are free of foreign or exogenous DNA, transferring initial oversight to Institutional Biosafety Committees (IBSC).

7.3.4. African Union & Sub-Saharan Regional Bodies (Kenya NBA, Nigeria NBMA)

Regulatory Status

Case-by-case notification pathways implemented by pioneer regulatory authorities (e.g., Nigeria's National Biosafety Management Agency in 2020 and Kenya's National Biosafety Authority in 2022) to determine if SDN-1/SDN-2 products without foreign DNA [222] can be regulated as conventional varieties rather than GMOs[223].

8. Future Perspectives of Orphan Crop Improvements

As long-read systems have developed and DNA sequencing costs have continued to decline, genomics' contribution to the improvement of orphan legumes has intensified. In order to produce high-quality, near-chromosome-level assemblies for orphan legumes that were previously unavailable for genomic research, short-read Illumina data are now frequently combined with long-read PacBio HiFi sequencing and chromatin-based scaffolding techniques, such as Omni-C from Dovetail Genomics/Cantata Bio [224]. The marama bean (Tylosema esculentum) itself has recently been shown to exhibit this combination; 21.5 Gb of PacBio HiFi data assembled with HiCanu and Hifiasm and scaffolded with Dovetail Omni-C data produced a 558.78 Mb assembly with a N50 of 22.68 Mb, indicating that marama is an ancient allotetraploid with two distinct genome types assembled from 21.5 Gb of PacBio HiFi information using HiCanu and Hifiasm and scaffolded with Omni-C data from Dovetail Genomics using HiRise, resulting in a 558.78 Mb assembly with near chromosome-level continuity [191].

The rapid expansion of reference-genome resources for underutilized legumes beyond the major grain legumes is demonstrated by comparable Africa-led efforts that have produced chromosome-scale assemblies for other orphan legumes, such as lablab (Lablab purpureus), whose Africa-led plant genome collaboration produced a high-quality chromosome-scale assembly and re-sequenced cultivated and wild accessions to confirm two independent domestication events. Once a reference assembly is available, reduced-representation methods like genotyping-by-sequencing [225] can be used to further characterize the genome space at a comparatively low cost.

This method offers higher precision and lower cost than previous marker technologies for surveying genome-wide variation and does not strictly require a reference genome. However, over the past ten years, major grain legumes have seen tremendous advancements in reference genome sequencing and germplasm resequencing, while underutilized legumes continue to lag behind in the development of genetic resources [226].

This difference is especially significant for species that are essentially outcrossing, like the marama bean, which exhibits high intra-population diversity but low inter-population diversity, probably due to its primarily outcrossing mating system and restricted gene flow between populations. The ensuing heterozygosity, which is exacerbated by the polyploid genome structure of marama, will make it more difficult to find beneficial alleles for creating genomic selection strategies in this and other outcrossing orphan legumes [227].

Examining the expressed and functionally active parts of orphan legumes' genomes is a useful tactic for overcoming their genomic complexity and low molecular resources. The molecular toolkit for neglected and underutilized crops has significantly increased because to the quick development of short- and long-read sequencing, high-quality reference genomes, de novo transcriptome assembly, and enhanced genome annotation. Differentially Expressed Genes (DEGs), stress-responsive pathways, regulatory networks, and

potential genes linked to drought, salt, temperature extremes, and biotic stresses can all be found more easily with transcriptome sequencing. Transcriptional variation can now be understood in the context of larger allelic and structural diversity thanks to the increasing integration of these transcriptome resources with population genomics and pangenomics. Pangenomic methods are especially useful because they provide a more thorough framework for identifying genes and haplotypes linked to agronomic traits and environmental adaptation by capturing presence/absence variation and structural variants that might be overlooked by analyzes based on a single reference genome [228].

However, genotype, developmental stage, environmental factors, and tissue specificity must all be carefully taken into account when interpreting transcriptome responses. Roots, leaves, reproductive tissues, and other organs may exhibit significantly different stress-responsive transcriptional alterations, and bulk RNA-seq may mask responses in certain cell types. Therefore, differentiating core mechanisms of stress tolerance from secondary or consequential responses requires careful tissue selection and sampling at physiologically relevant phases.

Further chances to resolve cell-type-specific responses and regulatory networks are provided by the growing integration of transcriptomics with single-cell and spatial techniques. More generally, the integration of genomic and multi-omics datasets is highlighted in recent reviews as a crucial element of methods for improving next-generation orphan crops [43]. Crucially, a candidate gene and a stress-tolerance trait cannot be causally linked by differential gene expression alone. Instead of serving as the main factor determining tolerance, a transcript may be highly induced as a result of cellular damage.

Therefore, it is appropriate to combine transcriptome findings with physiological phenotyping, genome-wide association studies, genetic mapping, and functional validation. By enabling the mapping of transcriptomic, proteomic, metabolomic, and epigenomic information against a more representative catalogue of genomic variation, the growing availability of pangenomes offers a crucial basis for such integration. The identification of functional genes, regulatory components, and advantageous haplotypes underpinning complex stress-adaptation features can be enhanced by this integrated framework [69].

Because the proteome contains the functional products of gene expression and is more closely linked to cellular processes than transcript quantity alone, proteomics offers a crucial additional layer. Stress-responsive enzymes, transporters, signaling proteins, molecular chaperones, and elements of antioxidant and metabolic pathways that might not show a direct association with their corresponding transcript levels might be identified by comparative or quantitative proteomics. Recent developments show a progressive movement toward multi-layer molecular profiling rather than isolated transcriptome analyzes, even though proteomic and integrated multi-omics datasets in orphan legumes are still smaller than in major crops. The potential of genetic resources has already been demonstrated by comparative genomic studies of underused legumes, which have shown significant heterogeneity in gene content, gene-family expansion, and pathways linked to biotic and abiotic stress response, demonstrating the potential of genomic resources to support subsequent functional and multi-omics investigations[229].

The lack of genome references and annotated genomic resources for these species is a significant barrier to the genomics-based development of orphan crops. This has made it more difficult to identify the causative genes linked to desirable crop traits. To predict gene function from DNA or RNA sequencing information, with different may be helpful. Prioritizing functional variations found through Genome-Wide Association Studies (GWAS), RNA sequencing, and other genomic analyses might also be done using the predicted gene function output.

Lack of finance is one of the biggest obstacles to improving orphan crops and the corresponding increase in food security in countries that depend on these products. Due to their specialized habitats or restricted potential, most orphan crops will remain orphans, but many have the potential to become significant crops on a regional or even global scale with the right investment.

Since knowledge may be transmitted to closely related species, the growing wave of genomic technology could improve the performance of all crops. However, given the use of machine learning to maximize outcomes, the investment should be concentrated on crops with the most potential for development. Machine learning methods can lower the amount of data required for trait prediction and genomic feature identification in orphan crops by training on large crop datasets. However, to guaranty strong alignment with the training datasets, strategic data collection from orphan crops is necessary. The models can be adjusted over time to increase their specificity and accuracy when more datasets from orphan crops are produced. Furthermore, there is a case for international seed companies to support orphan crop improvement either directly or indirectly through technology exchange, as many of them currently do.

This is because understanding the genomic basis of traits in orphan crops could benefit the major crops through gene introgression and editing. Given that breeding and seed companies have a plethora of data from field trials and genome sequencing, increased funding from these businesses for improving orphan crops might significantly speed the application of machine learning. Giving machine learning

models access to a range of datasets would allow them to account for intra-species genetic variability and other factors affecting trait prediction outcomes. Investment diversification from genomics-based breeding to agronomy, processing, and marketing is required to enhance the performance of orphan crops. Purchasing a single data-generating technology is unlikely to provide sufficient outcomes and cross-disciplinary support.

Furthermore, limiting investment to a certain geographic area may not necessarily be the most effective strategy to speed up crop improvement, even while investing in the nations where orphan crops are mostly cultivated can take use of local knowledge and have major societal advantages. The efficacy and impact of such initiatives can be increased by a plan that incorporates the greatest technologies and knowledge available on a local and global scale. This is especially crucial for developmental tactics, which frequently call for powerful computers and specialized knowledge that are more affordable to obtain globally. A balanced approach is crucial given the limited financial resources available for improving orphan crops; low-cost traditional breeding, teaching, and marketing tactics may yield the most effective gains per dollar invested. After low-cost methods have been used, machine learning models can then take advantage of the generated knowledge.

Secondly, the whole consortium of orphan legumes worldwide is mostly unexplored but has the potential to become an important part of improved food and nutritional security. It is necessary to raise the awareness and traditional knowledge of these species on their use and cultivation from the level of insignificant farmers to a broad range of scientific methods. For a larger range of climate-resilient agricultural systems, genetic modification is crucial for the development of desirable traits, increased yield, and the selection of elite features.

In order to improve these legumes' production integration, product processability, propagation, and ex situ establishment ability—all of which could improve their economic standing in the worldwide market—intensive future research must concentrate on these qualities. Fortunately, the technical community is now encouraged to pursue more useful genetic research on new and additional orphan crops due to the affordable methods of genome sequencing and characterisation.

Additionally, the growth of crop plants is aided by the breakthroughs in statistical methods, speed breeding, and participatory improvement techniques, which change how these neglected crops are studied genetically. A wide range of in situ and ex situ selection must be emphasized for the application of both conventional and advanced breeding techniques to precisely harness their usability. Genetic research on orphan and other unique crops appears to be a crucial model for understanding crop evolutionary processes more broadly and directing future important crop evolution, in addition to supporting genetic breakthroughs. The bridging point of orphan crops between new and major crops can be used to examine genetic approaches for de novo domestications and major crop re-wilding. Cultivating orphan legumes is a challenging endeavor that calls for advanced techniques for rapid exploitation. However, the foundation of possible in addition to that the food and nutritional security, as well as a global network of biodiversity, will be a methodical approach and an increase in basic and practical research into grain legumes.

Thirdly, the potential for creating stable, high-yielding, and resistant lines of different legumes is great, as is the importance of underutilized legumes in human and animal diets, their role in lowering abiotic stress, fighting hunger and malnutrition, and achieving nutritional security. Additionally, the nutritional and anti-nutritional properties of these legumes help solve health issues by giving people critical nutrients through non-traditional but crucial dietary sources. The ability of these underutilized legumes to adapt to a variety of climates and avoid pest and disease issues by producing autoimmune secondary metabolites is their main advantage.

In addition to being a rich source of proteins, vitamins, minerals, lipids, fiber, and carbohydrates, underutilized legumes also offer a balanced diet in ideal amounts. In times of severe drought and famine, they also play a significant part in providing rural residents with new sources of income and employment. The government's lack of support for production and marketing, the underfunding of research on underutilized legumes, the over-reliance on staple cereals for food security, the lack of access to genetic materials of underutilized legumes, and the underutilized genetic diversity of underutilized legumes in breeding programs are some of the reasons why legumes are not becoming very popular despite having many agronomic and nutritional advantages over staple cereals. Given the nutritional and climatic significance, the cultivation and consumption of underutilized legumes must be promoted in order to achieve both food and nutritional security.

Additionally, providing high-quality seed material, marketing facilities, and raising consumer knowledge can improve the cultivation of underutilized legumes. Underutilized legumes can reduce poverty and improve food security, but selling them to customers is not always simple. One major barrier for underutilized legumes is that they are not as well-known or consumed as other significant crops like rice, wheat, or maize. Because people are less familiar with certain legumes, marketing and selling them may be difficult. The fact that underutilized legumes have a different taste, texture, and nutritional profile from more popular crops may turn off some consumers. However, there are many

opportunities for underutilized legumes to be promoted and widely adopted. Processing and value-adding underused beans into flour, protein powder, or other food products is another way to increase their marketability and appeal. Other factors that impact the marketability and consumer acceptance of underused legumes are food safety and quality.

Customers are more likely to accept underutilized legumes if they are grown and processed using sustainable and safe methods, then properly cleaned, inspected, and packaged. It is possible to finance and stimulate research on the breeding of underused legumes. To increase the genetic diversity of underused legumes and gather new germplasms, expeditions must be carried out all over the world. To comprehend the significance of underused legumes in human diets, a thorough investigation of their nutritional and anti-nutritional characteristics might be conducted. It is necessary to promote the use of data on the genomes, proteomics, transcriptomics, and metabolomics of underutilized legumes for breeding, pest, disease, and climate-resilient lines. The advanced breeding techniques discussed in this paper can boost underutilized legumes in a number of ways.

9. Modernizing the Traditional Breeding Method

Conventional methods of plant breeding are frequently labor-intensive, time-consuming, and resource-intensive, especially when it comes to crops with complicated trait inheritance. Crop improvement initiatives have been transformed in recent years by sophisticated molecular breeding techniques like Marker-Assisted Selection (MAS) and Genomic Selection (GS), which allow for the quick and accurate identification of desired genetic features. By selecting superior genotypes at early embryonic stages and predicting phenotypic performance using molecular markers and genome-wide data, these methods drastically shorten the breeding cycle.

When GS and MAS are incorporated into legume breeding projects, selection efficiency is improved, high-yielding and stress-resistant cultivars are developed more quickly, and field evaluation expenses are reduced. Moreover, these modern breeding tools play a pivotal role in ensuring sustainable agricultural productivity and meeting the growing global demand for nutritious legume crops.

10. Improvement of Grain Yield and Quality

Due in large part to limited genetic improvement and selection pressure, underutilized legumes frequently display inferior quality features and comparably lower yield potential when compared to regularly cultivated species. By making it possible to identify and choose genes linked to important agronomic characteristics like increased yield, improved nutritional quality, and resistance to biotic and abiotic stresses, advanced breeding methods, such as molecular-assisted and genomics-driven techniques, offer practical ways to get around these restrictions. Breeders can speed the creation of superior genotypes with increased resistance and production through focused introgression and accurate trait mapping. In addition to improving the agronomic performance of underutilized legumes, the use of these contemporary breeding strategies promotes their broader acceptance, cropping system diversification, and increased food and nutritional security.

11. Priority preference between Sustainable Agriculture Methods and Climate Resilience

To guarantee future food security, priority-based research on orphan legume pulses should carefully combine climate resilience with sustainable farming methods. These underutilized crops are important genetic resources under shifting climatic conditions because of their innate resistance to abiotic stresses like heat, drought, and low soil fertility. In addition to conserving and utilizing their genetic variety, research efforts must concentrate on identifying and characterizing climate-resilient genotypes using cutting-edge techniques like genomics, phenomics, and marker-assisted breeding. Promoting sustainable methods can improve soil health and lessen reliance on chemical fertilizers at the same time. These methods include low-input farming, biological nitrogen fixation, intercropping, and agroecological management. The development of resilience and high performance will be further supported by combining traditional knowledge with contemporary biotechnological interventions. Such a holistic, priority-driven approach can elevate the role of orphan legumes in climate-smart agriculture, contributing significantly to nutritional security, livelihood diversification, and the long-term sustainability of food systems.

12. Nutritional Enhancement Employing Cost-Cutting Omics-Based Technology

Although underutilized legumes are naturally high in proteins, dietary fiber, micronutrients, and bioactive compounds, they frequently contain anti-nutritional elements like phytates, tannins, and protease inhibitors that limit nutrient bioavailability and consumer acceptance, making nutritional enhancement of these crops a crucial research priority. Genes linked to both nutrient accumulation and anti-nutritional substances can be identified and altered thanks to sophisticated breeding methods like marker-assisted selection and genomic-assisted improvement.

Legume varieties with improved nutritional profiles and less unwanted components can be created through targeted breeding and genetic improvement. In addition to improving digestibility and nutrient absorption, these advancements raise the legumes' potential for use in a variety of food products. Consequently, the application of modern breeding strategies can significantly improve the functional and

nutritional quality of underutilized legumes, supporting their wider adoption and contribution to human health and food security.

13. Prioritization for Generating New Varieties

Setting priorities for the creation of new varieties: In order to improve the adaptability, productivity, and resilience of underused legumes in a variety of agroecological situations, new varieties must be created using contemporary breeding techniques. The precise identification and incorporation of desirable traits, such as high yield potential, pest and disease resistance, and tolerance to abiotic stresses like drought and salinity, is made possible by sophisticated techniques like marker-assisted selection, genomic selection, and biotechnological interventions.

When compared to traditional procedures, these approaches greatly speed up the breeding cycle and increase selection efficiency. The resulting improved cultivars increase crop performance and reliability because they are better adapted to particular agricultural systems and environmental circumstances. As a result, developing better legume varieties can increase their production, increase their accessibility in food systems, and support sustainable agriculture. Consequently, the generation of superior legume varieties can expand their cultivation, improve availability in food systems, and contribute to sustainable agriculture and nutritional security.

14. Global Perspective in Consumer Behaviour

What needs a greater emphasis today is towards availability, marketing, purchase and consumption of orphan legumes, which should be greatly encouraged. From a global perspective, consumer behaviour plays a pivotal role in determining the availability, marketing, purchase, and consumption of orphan legumes, which remain underutilized despite their high nutritional and ecological value. Encouraging their integration into mainstream food systems requires a multifaceted approach that combines increased consumer awareness, improved supply chains, and targeted marketing strategies. Public education on the health benefits, sustainability advantages, and culinary versatility of orphan legumes can stimulate demand, while policy support and market incentives can enhance their availability and affordability.

Additionally, value addition through product development, branding, and inclusion in modern dietary patterns can improve consumer acceptance across diverse populations. Understanding cultural preferences, purchasing patterns, and socio-economic factors is also essential for designing effective interventions. Collectively, fostering positive consumer behaviour towards orphan legumes can drive their commercialization, promote dietary diversification, and contribute to global food and nutritional security.

15. Conclusion

- In conclusion, in the future of molecular improvement in orphan legumes, integrated multi-omics and data-driven breeding will probably take the place of single-omics discovery. Transcriptomics, proteomics, metabolomics, phenomics, and machine learning approaches can be used with reference genomes and pangenomes to provide more system-level acquaintances of complex phenomena. Candidate genes and beneficial alleles may be found more quickly as a result. Machine learning-assisted genomic approaches are also gaining popularity for orphan crops, where limited datasets and resources remain major challenges to traditional genomics-assisted breeding [70]. The success of these technologies will eventually depend on the availability of well-characterized germplasm, susceptible and stress-tolerant genotypes, standardized phenotyping systems, repeatable multi-environment trials, and confirmed genetic resources. It will be necessary to integrate molecular signatures with physiological and agronomic performance in order to go beyond the cataloging of stress-responsive genes to the identification and functional validation of causal genes and regulatory networks that can be used through marker-assisted selection, genomic selection, and precision breeding.
- Legumes are unquestionably strategic crops for the future, providing a long-term solution to the interconnected problems of global food insecurity, agricultural instability, and climate change. Because of their innate ability to withstand a variety of biotic and abiotic challenges, including heat, drought, salinity, and pest pressure, they are positioned as climate-smart crops that can maintain productivity in the challenging circumstances brought on by human-caused climate change. Additionally, their capacity to fix atmospheric nitrogen greatly increases soil fertility, lessens the need for chemical fertilizers, and promotes environmentally friendly farming methods.
- Legumes are much more significant from a worldwide nutritional standpoint, especially when considering developing and third-world nations. Due to restricted availability to a variety of nutrient-rich foods, many individuals in these areas suffer from chronic protein-energy malnutrition and micronutrient deficiencies. Rich in critical amino acids, iron, zinc, folates, dietary fiber, and high-quality plant proteins, legumes offer economically disadvantaged areas an accessible and reasonably priced food option. Regularly including them into daily diets can greatly reduce undernutrition, enhance maternal health and child growth, and lower the incidence of hidden hunger. Legumes provide a balanced dietary solution across socioeconomic strata by simultaneously avoiding overnutrition-related ailments like obesity, diabetes, and cardiovascular diseases in more affluent populations.

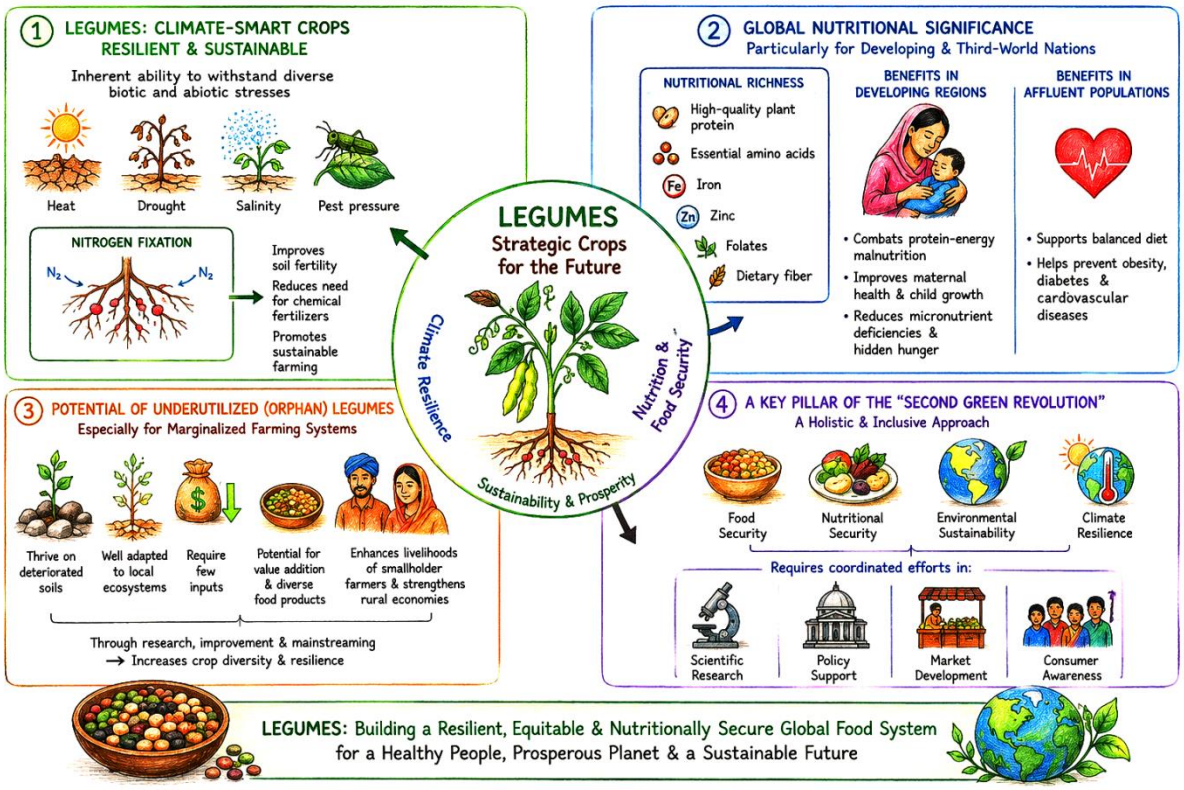


Fig. 24 Strategic role of legumes in promoting climate resilience, global nutrition, and sustainable agricultural ecosystems for a hunger-free world

Furthermore, underutilized or orphan legumes have enormous unrealized potential, especially for marginalized farming systems in underdeveloped nations. These crops can flourish on deteriorated soils where big crops fail, are frequently well-suited to local ecosystems, and require few inputs. These legumes can be included into mainstream food systems to increase crop diversity and resilience through targeted research on genetic improvement, agronomic techniques, and value addition. Additionally, by creating chances for smallholder farmers to make a living, their promotion can boost local economies and empower rural communities.

Underutilized legumes can be seen in this larger context as key elements of a "second green revolution," which eschews the previous yield-centric paradigm in favor of a more comprehensive strategy that takes into account food security, nutritional security, environmental sustainability, and climate resilience. Coordinated international efforts in market development, policy making, scientific research, and consumer awareness are required for such a shift. In the end, a resilient, equitable, and nutritionally secure global food system that can meet the demands of an expanding population—especially in the world's most vulnerable areas—may be built through the intentional promotion and use of legumes.

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