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REVIEW ARTICLE

Advancements in Genetic Enhancement Addressing Key Challenges in Pearl Millet (*Pennisetum glaucum* (L.) R. Br.)

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Abstract

Pearl millet (*Pennisetum glaucum* R. Br.), an important cereal crop widely grown in arid and semi-arid regions of Asia and Africa, plays a vital role in global food security. However, it faces significant challenges due to climate change, soil degradation, emerging pests, and diseases. Genetic enhancement is a promising approach to mitigate these challenges and to improve pearl millet resilience and yield. This review explores recent advancements in genetic enhancement to addresses key constraints in boosting pearl millet yields sustainably. The developments in genetic enhancement hold promise for addressing the complex challenges faced in pearl millet cultivation and offers the way forward for increased productivity, resilience, and food security in vulnerable regions. This review highlights modern strategies such as molecular breeding, genomic selection, systems biology tools, genetic engineering, and gene editing, which enable the precise identification and incorporation of desirable traits such as abiotic stress tolerance, resistance to biotic stress, improved nutritional content, etc. However, continued research, collaboration, and investment are essential to fully harness the potential of genetic resources and technologies to benefit smallholder farmers and global food and nutritional security.

Keywords Climate change · Food security · Gene editing · Genetic enhancement · Genetic transformation · Heterosis · Nutrition · Pearl millet

Abbreviations

DM	Downey mildew
Mag	Magainin
IPA1	Ideal plant architecture 1
CRISPR/CAS	Clustered regularly interspaced short palindromic repeats/CRISPR-associated proteins
QTL	Quantitative trait loci
LOX	Lipoxygenase
TAG	Triacylglycerol
DAG	Diacylglycerol
MAG	Monoacylglycerol
FFA	Free fatty acids
C-GF’s	C-glycosyl flavone
MiMe:	Mitosis instead of meiosis
MTL:	Matrilineal
GS	Genomic selection
ZFN	Zinc finger nuclease
TALEN	Transcription activator-like effector nucleases
ODM	Oligonucleotide-directed mutagenesis
NHEJ	Non-homologous end joining
HDR	Homology directed repair
SDN	Site directed nucleases

Introduction

Climate change threatens global food security by disrupting agricultural ecosystems and exacerbating existing challenges. Rising extreme temperatures, altered precipitation patterns, and increased frequency of extreme weather events such as droughts, floods, and storms adversely impact crop yields, water availability, and soil fertility (FAO 2015). Hence, there is an immediate demand for crops with high nutritional value, resilience to climate stresses, and widespread accessibility. Millets are an ideal solution to address these imperatives, showcasing excellence across multiple

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attributes. Pearl millet (*Pennisetum glaucum* (L.) R. Br) is the sixth most important cereal crop grown in 30 million ha in the arid and semi-arid regions of Asia and Africa, which accounts for more than half of the total worldwide production of all millets (Satyavathi et al. 2021). It is a highly cross-pollinated diploid cereal grass ($2n = 2x = 14$) with an estimated genome size of 1.79 Gb, having higher photosynthetic efficiency and dry matter production capacity (Varshney et al. 2017). Pearl millet is cultivated on more than 30 million hectares globally, with the most significant portion of cultivation in Africa and Asia (Raheem et al. 2021). Pearl millet can withstand adverse environmental conditions where other crops like wheat, rice, sorghum, and maize fail to produce good grain yields, making pearl millet an excellent model crop for stress tolerance studies (Merga 2020). It is an excellent cereal crop with a short duration and a high growth rate. It is widely grown primarily for grain production but is also valued for its fodder. The importance of pearl millet has risen in recent years due to its broad climatic adaptations and excellent nutritional qualities (Satyavathi et al. 2021; Patil et al. 2023). While it does possess several excellent qualities, there is always room for improvement to enhance its productivity, resilience, and nutritional enrichment.

Pearl millet is the principal energy source, nutrients, and minerals for the downtrodden and forage in the dry lands. Pearl millet grain boasts superior protein content at 14%, along with higher fat (5.7%) and fiber (2%) levels (Rani et al. 2018) compared to various other cultivated cereal crops such as wheat (Kavitha and Parimalavalli 2014), rice (Ahmed et al. 2014), and sorghum (Awadelkareem et al. 2015). Recently, millets have been increasingly spotlighted in combating diabetes as a dietary option (Muthamilarasan et al. 2016; Kavi Kishor et al. 2021). It is an exceptionally nutritious cereal with higher proteins and energy, rich in iron and zinc, and a balanced amino acid profile than other cereal crops such as maize or sorghum (Satyavathi et al. 2021). Additionally, it is an excellent forage crop, rich in protein, calcium, phosphorous and other minerals. It is also being utilized as a multipurpose crop ranging from poultry feed, dairy cattle feed, building material and alcohol extraction (Basavaraj et al. 2010).

However, despite being nutri-rich and climate resilience, its sustainable production is significantly challenged by several constraints such as uneven rainfall, low-soil fertility, and awful weather conditions, causing severe losses in the yields, thereby impacting the economy of the marginal farmers (Sharma and Ortiz 2000). Between 2007 and 2019, the global cultivation area for millets decreased from 35 million hectares to 32 million hectares, primarily due to reduced cultivation in Asia. Conversely, there was a notable increase in yields in Asia, rising from 1100 to 1292 kg/ha while experiencing a slight decline in Africa (Vetriventhan et al. 2024). The genetic improvement in pearl millet is critical to provide nutritional security with climate resilience. Abiotic factors, such as extreme drought, varying temperatures, salinity, poor soil condition and lodging, drastically affect almost all crops, and pearl millet is also vulnerable. These abiotic factors drastically affect not only pearl millet production, but also the nutritional quality (Zegada-Lizarazu and Iijima 2005).

Additionally, biotic factors include pests (white grub, stem borer, shoot fly, green bug, blister beetle, grasshopper, etc.) and diseases (downy mildew, blast, rust, ergot, smut, and *Striga*) also significantly affect the pearl millet crop yield (Shrama et al. 2020). Despite these agroclimatic factors, global utilization of pearl millet is also restricted due to rancidity trait (Bhargavi et al. 2024). The rapid development of off-flavours and taste in pearl millet flour is the major hindrance to wider consumer acceptability and increases users' drudgery, especially women in the household (Aher et al. 2022). This reduces the shelf life of millet products, poses low economic incentives to farmers, creates post-harvest waste at the consumer level, and limits the consumption of milled pearl millet.

Traditional breeding methods have dramatically culminated into new cultivars with enhanced crop improvement in the last few decades. However, the development of new cultivars are still less in pearl millet due to scant research attention and a lack of precise genomic and biotechnological tools in comparison with other major millets and cereals. Until now, there have been minimal studies on the identification and exploitation of potential candidate genes for abiotic tolerance or biotic stress resistance in pearl millet (Reddy et al. 2022). The availability of well-annotated pearl millet genome sequence information opens new avenues for advanced genetic research, enabling researchers to identify key genes and regulatory elements responsible for important traits (Varshney et al. 2017; Sun et al. 2023). The insights gained from extensive datasets can facilitate the identification of candidate genes, proteins, and metabolites for integration into crop improvement programs, ultimately leading to the development of superior cultivars with enhanced stress tolerance (Shivhare and Lata 2017; Palakolanu et al. 2022). Thus, translating the information obtained from omics data and other major cereals and adapting new breeding tools (NBTs) to explore and create genetic variability is vital in improving millet productivity and yield. In diverse crop species, new breeding/biotechnological tools such as molecular breeding, systems biology tools, transgenics, and gene editing are being used. They could be integrated to induce precise genetic variations to produce heritable mutations on the target genome without affecting any phenotypic or agronomic traits.

Pearl millet: a boon for alleviating food scarcity in parched areas

The distinctive outrageous environmental challenges like an unusually dry season, intense heat waves, increased CO_2 concentrations, and glacial run-off influence the yields significantly in staple food crops. Additionally, nutritional insecurity poses a significant global threat, affecting around 195 million people who suffer from various health issues, including malnutrition and hunger, especially among those living below the poverty line (FAO 2015). To address this challenge, crops that are resilient to climate change, nutritious, and affordable are unavoidable. Assessment of the effects of climate changes on crop growth and yield might help to suitably anticipate and adapt farming to exploit agricultural production in semi-arid and arid regions. Distorted weather patterns and depleting water resources encourage farmers to move from maize and sorghum to super-grain-like pearl millet. Pearl millet is a poor man's wheat, a staple food for more than 90 million people in Asia and Africa's arid and semi-arid tropical regions (Burgarella et al. 2018). It emerges as a promising solution for tackling future environmental challenges compared to other cereal crops. Its numerous advantages, including resilience to prolonged drought and extreme temperatures, low maintenance requirements, short growth duration, pest resistance, and ecological benefits, make it a “climate-smart” crop (Kheya et al. 2023).

Millets, prevalent in semi-arid and drought-prone regions like Asia and Sub-Saharan Africa (SSA), stand out for their nutritional superiority, offering proteins, essential amino acids, micronutrients, minerals, vitamins, and antioxidants with the added value of low glycemic index offer great potential as components of diets to combat diabetes, micronutrient deficiency, and obesity (Varsha and Narayana 2017; Kavi Kishor et al. 2021). Pearl millet is now seeking attention because of its similar or even higher nutrient profile as wheat, which also has a higher amount of zinc and iron (Ghatak et al. 2021). Pearl millet, recognized by the Government of India as a "Nutri-cereal" crop, is gaining attention for its exceptional nutrient profile, even surpassing wheat in certain aspects such as zinc and iron content. Moreover, pearl millet exhibits health benefits such as anti-cancer properties, neurodegenerative disorders, blood pressure regulation, and reduced risk of heart attacks, attributed to its phenolic components (Porwal et al. 2023). Pearl millet's resilience, nutritional value, adaptability, economic importance, and contribution to climate change mitigation make it a beacon of hope in the fight against food scarcity. Promoting its cultivation and utilization holds promise for ensuring food security, improving livelihoods, and building resilience in agriculture, especially in marginalized regions facing food insecurity.

Exploring challenges and initiatives in enhancing pearl millet crop performance

Pearl millet is a hardy, small-grain cereal crop cultivated in semi-arid regions in marginal environments of the world. Despite the great benefits of millet, productivity and use are confined due to various climatic and environmental changes. Among these, biotic, abiotic stresses, and combined stresses have a powerful impact on the quality and productivity of millet. In addition, a combination of drought and heat stress severely affects plants' physiological, cellular, and molecular functions, determining grain yield and crop productivity (Yadav et al. 2020). Exploring the molecular intricacies of individual and combined stresses and their impact on overall plant performance is of paramount significance. Figure 1 illustrates the schematic representation of the molecular mechanisms governing pearl millets' response to varying stress conditions and how plants respond to these challenges. However, to overcome these effects and further enhance millet productivity, it is critical to develop climate-resilient, highly nutritious, and high-yielding lines. Developing new millet cultivars through conventional breeding is much lower than in other cereals, even under optimum conditions. Thus, utilizing new breeding/biotechnological tools is of utmost importance to improve millet productivity and yield (Fig. 2).

Downey mildew (DM) disease

Downy mildew disease, caused by the pathogen *Sclerospora graminicola* (Sacc.), is one of the most devastating diseases afflicting pearl millet yield, particularly in the arid and semi-arid regions of India and Africa (Singh et al. 1993). Pearl millet is a vital food crop in these areas and DM results in significant economic losses by dramatically reducing crop yield and quality. The disease typically begins by manifesting at the base of the leaf lamina, spreading upward to cover newly emerging leaves. DM-affected plants exhibit stunted growth and fail to develop panicles, leading to substantial yield losses (Singh et al. 1993). Given the essential role of pearl millet in food security for millions, there has been a long-standing effort to develop DM-resistant varieties. Over the years, traditional breeding and advanced biotechnological approaches have been deployed to develop resistance against DM in pearl millet. Early efforts focused on screening germplasm collections and breeding lines to identify natural resistance sources, which were then used to develop resistant cultivars (Singh et al. 1997). Notably, India’s first DM-resistant pearl millet hybrid, HB3, was developed through conventional breeding practices and was widely cultivated (Singh 1995). Somatic embryogenesis techniques have also played a crucial role, leading to the development of DM-resistant cultivars such as ICTP 8203, ICMS 9555,

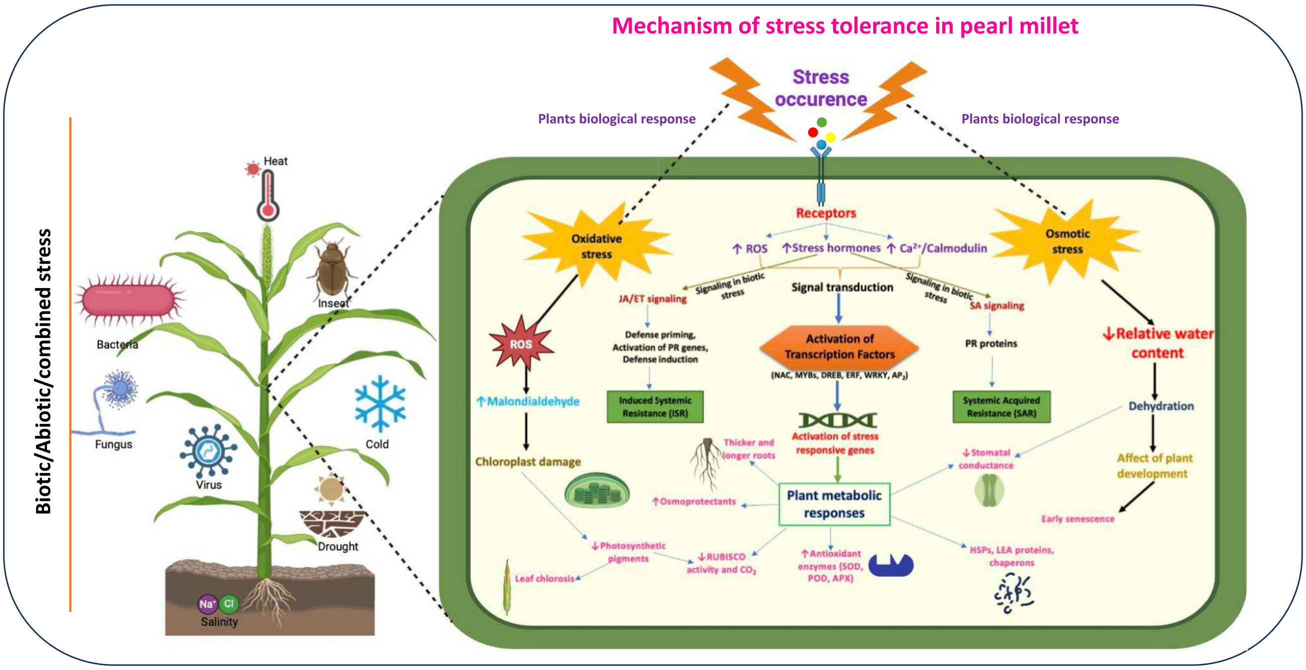

Fig. 1 Unveiling Stress Tolerance: Insights into Pearl Millet's Mechanisms and Plant Responses. Upon stress occurrence, plants exhibit oxidative and osmotic stress, resulting in chloroplast damage and premature senescence of plants. Plasma membrane receptors detect external stress signals, initiating intracellular signaling via reactive oxygen species (ROS), calcium ions (Ca^{2+}), and stress hormones (ABA, JA, SA). These secondary messengers activate kinases and phosphatases, facilitating the phosphorylation/dephosphorylation of transcription factors such as AP2/ERF, NAC, WRKY, MYB, and bZIP. Subsequently, these transcription factors regulate the expression of stress-responsive genes encoding late embryogenesis abundant (LEA) proteins, osmoprotectants, antioxidant enzymes, heat shock proteins (HSPs), chaperones, and transporters (ions and aquaporins). Notably, stress hormone signaling pathways such as JA/ET induce Induced Systemic Response (ISR), while SA signaling induces Systemic Acquired Resistance (SAR), enhancing biotic stress tolerance in pearl millet. Plants showed diverse metabolic responses that are crucial in enhancing stress tolerance

and HB3. These varieties demonstrated resistance throughout their growth period, offering a significant breakthrough in DM control (Kumar et al. 2012). The introduction of transgenics in pearl millet research marked another pivotal moment. Girgi et al. (2006) produced the first transgenic pearl millet with over 90% resistance to DM, a landmark achievement in the field. In parallel, Latha et al. (2006) developed transgenic plants carrying the *pin* gene, further enhancing DM resistance. Subsequent studies, such as those by Ramadevi et al. (2014), employed *Agrobacterium*-mediated transformation to express the synthetic *magainin* (*mag*) gene, resulting in stable transgenic plants with enhanced resistance to DM. Lavanya et al. (2022) have highlighted the critical role of elicitors in triggering resistance against DM in pearl millet. Such discoveries underscore the importance of further understanding plant-pathogen interactions at the molecular level to improve resistance mechanisms. Looking into the future, developing more effective and sustainable strategies to combat DM appears essential. Advancements in genome editing, mainly through CRISPR/Cas technology, offer promising new avenues for precisely targeting and modifying key genes associated with disease resistance. By identifying candidate genes involved in pearl millet's immune response and manipulating them to strengthen defense mechanisms, researchers can create pearl millet varieties that are not only resistant to DM but also adaptable to changing environmental conditions. While significant progress has been made in the fight against DM in pearl millet, continuous research and innovation are necessary to stay ahead of evolving pathogens, safeguarding the future of crops and ensuring food security for millions in vulnerable regions.

Blast disease

Blast disease, caused by *Pyricularia grisea* (also known as *Magnaporthe grisea*), is a severe fungal infection that adversely affects the growth and yield of millet crops, particularly pearl millet. Also known as brown leaf spot or *Pyricularia* leaf spot, the disease can cause early seedling death in high-moisture environments and forage discoloration, significantly reducing grain and forage production. The transmission of blast disease is mainly airborne, with windborne conidia being a primary vector, though seedborne transmission has also been reported (Singh and Pavgi 1977). Since pearl millet is an important food crop in arid

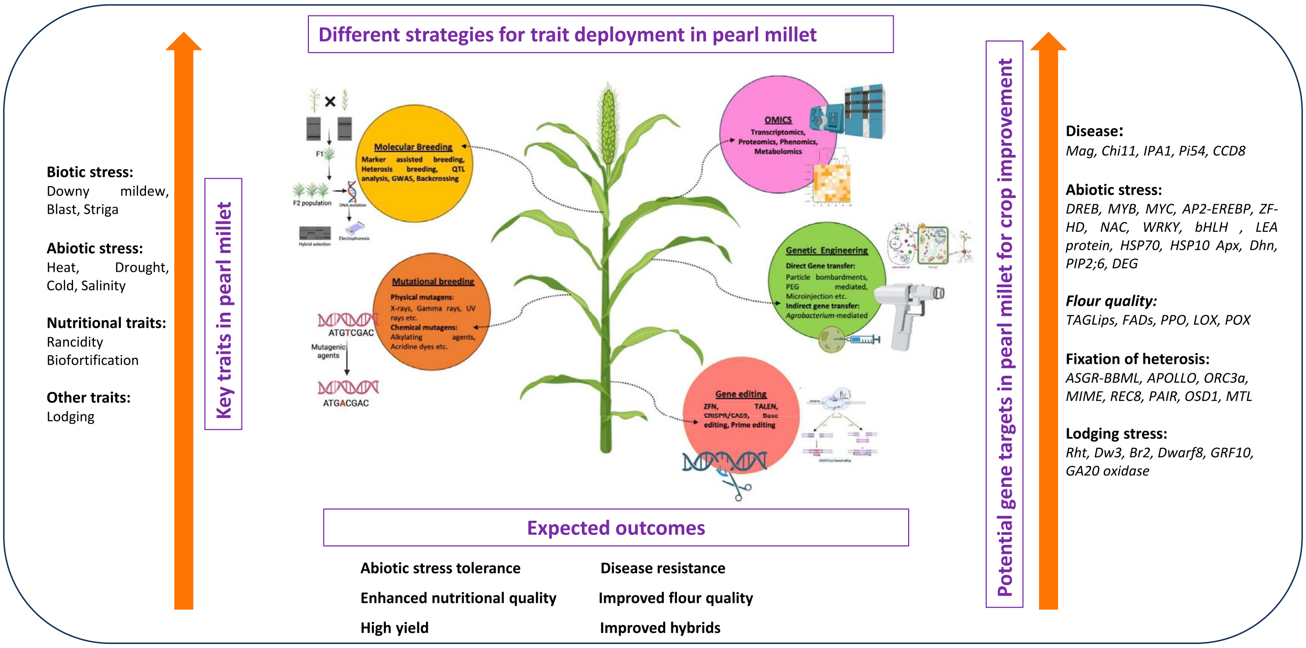

Fig. 2 Pearl Millet's Key Traits: Unlocking Potential Gene Targets and Deployment Strategies for Crop Improvement. Illustration depicting the multifaceted approach to unlocking the genetic potential of pearl millet for crop improvement. This comprehensive strategy involves identifying key traits such as abiotic stress tolerance, disease resistance, nutritional quality, and other traits. Targeted gene discovery and deployment strategies, including molecular breeding, mutational breeding, OMICS, genetic engineering and gene editing techniques, are employed to enhance desirable traits. The integration of cutting-edge genetic tools and breeding methodologies facilitates the development of improved pearl millet cultivars with enhanced agronomic performance and resilience to environmental stresses, ultimately contributing to sustainable crop production and food security

and semi-arid regions, developing resistance to blast disease is critical for maintaining sustainable yields. Efforts to develop blast-resistant pearl millet varieties have made significant strides. Blast resistance to Indian-origin isolates of *M. grisea* is controlled by a single dominant gene, making it easier to incorporate resistance into breeding programs (Singh et al. 2022). Early transgenic efforts have focused on transferring resistance genes from other crops into pearl millet. For instance, Ignacimuthu and Kanan (2013) successfully developed transgenic pearl millet lines by introducing the rice *chitinase* (*chi11*) gene, which conferred resistance to leaf blast disease. The *Ideal Plant Architecture 1* (*IPA1*)/*OsSPL14* gene has improved yield and blast resistance in rice. This gene functions through post-translational regulation, where phosphorylation alters the specificity of the IPA1 protein, enabling it to activate *WRKY45*, a defense regulatory transcription factor that provides quantitative resistance (Wang et al. 2018). If this regulatory mechanism is conserved in pearl millet, there is potential for leveraging *IPA1* expression to improve blast resistance in millet. Moreover, Singh et al. (2020) demonstrated that rice transgenic lines developed with the *Pi54* gene, originally intended for rice blast resistance, showed moderate resistance to other fungal and bacterial pathogens, such as blight and sheath blight. This cross-resistance highlights the potential for using similar approaches in pearl millet to confer broad-spectrum disease resistance. Advances in RNA sequencing have provided new insights into host–pathogen interactions in pearl millet. Further, Singh et al. (2022) identified key genes and transcription factors involved in pathogenesis-related proteins, reactive oxygen species regulation, cell wall defense, and metabolic signaling pathways, all of which play crucial roles in the plant's defense against blast. This work has opened up new avenues for identifying candidate genes that could be targeted to enhance blast resistance in pearl millet. Further research should focus on identifying and characterizing genes from other cereals and crops that confer resistance to blast disease. Candidate genes, such as those involved in post-translational regulation (e.g., *IPA1*) and resistance-related transcription factors like *WRKY45*, can be key targets for improving blast resistance in pearl millet. Gene-editing technologies like CRISPR/Cas9 also offer the potential for precise manipulation of these candidate genes, accelerating the development of blast-resistant varieties. Integrating molecular approaches with traditional breeding can provide more robust resistance.

Furthermore, exploring cross-resistance mechanisms, as demonstrated by the *Pi54* gene in rice, could help researchers develop pearl millet varieties resistant to blast and other major fungal and bacterial pathogens. This approach could provide a more comprehensive disease management strategy, reducing the need for chemical treatments and improving the sustainability of pearl millet cultivation and productivity. While significant progress has been made in developing blast-resistant pearl millet through transgenic/genetic engineering and traditional breeding, further research is needed to fully understand the molecular mechanisms of resistance. By identifying key resistance genes from other crops and leveraging biotechnological tools, it is possible to create pearl millet varieties that are more resilient to blast and other diseases, ultimately improving crop productivity and food security in regions where pearl millet is a staple food crop.

Combined stresses

Plants in their natural habitats constantly face a multitude of biotic and abiotic stresses that can significantly impact their growth, development, and overall health. Drought, heat, salinity, and various pathogen attacks represent some of the most pressing environmental challenges. Notably, the convergence of these stressors can create a more formidable threat to plant survival than individual stress factors alone (Pandey et al. 2015). While extensive research has focused on understanding single stress impacts, it is indispensible to recognize that plants often experience multiple stressors concurrently. This simultaneous exposure leads to complex interactions, unique physiological responses, and altered developmental pathways crucial for plant resilience. For instance, during summer, plants may face drought and heat stress, resulting in accelerated soil water evaporation and exacerbating the drought conditions. Similarly, plants attacked by bacterial and fungal pathogens must allocate resources to combat two different threats simultaneously, which can significantly hinder their overall growth and productivity. Understanding these combined effects is vital for enhancing plant resilience and developing sustainable agricultural practices, particularly in the face of climate change, which is expected to increase the frequency and severity of such stressors. Significant advancements have been made in understanding the impacts of combined stresses on plant health and productivity. Genetic manipulation has emerged as a powerful tool for enhancing plant resilience. Research has demonstrated that manipulating transcription factors, as well as proteins associated with late embryogenesis (LEA) and antioxidant defense mechanisms, can effectively confer resistance to various abiotic stresses across multiple crop systems (Bhatnagar-Mathur et al. 2008). For example, specific transcription factors can enhance stress signaling pathways, enabling plants to cope better with adverse environmental conditions. Research has also revealed that abiotic stress factors, particularly high temperatures, can negatively affect a plant's resistance capabilities to biotic stresses, leading to increased vulnerability (Sharma et al. 2007). The development of stress combinations and their Interactions in the Plants Database (SCIPDb) by Priya et al. (2023) represents a significant milestone in this field. This comprehensive database provides valuable insights into how plants respond morphologically, physiologically, biochemically, and at the molecular level to various combinations of stress factors. It is a vital resource for researchers seeking to understand the complexities of plant stress responses. In the specific case of pearl millet, extensive research has focused on elucidating its physiological and molecular responses to a range of abiotic stresses such as salinity, drought, and heat. Functional validation studies have identified key genes, including *Rab7, Hsc70*, and *Hsc90*, which are crucial in conferring tolerance to various abiotic stresses in pearl millet (Shivhare and Lata 2017; Reddy et al. 2010). Moreover, the aquaporin gene *PgPIP2;6* has been shown to be vital for the tolerance of plants to multiple abiotic stressors, including drought, heat, and cold, when expressed in transgenic tobacco (Reddy et al. 2022). Recent findings by Dhawi (2024) emphasized pearl millet's exceptional ability to endure simultaneous salt and heat stress, a feat attributed to the activation of shared KEGG pathways involved in abiotic stress responses. Furthermore, a meta-QTL analysis conducted by Gupta et al. (2024) has unveiled significant genomic regions associated with biotic stress tolerance, nutritional traits, and yield-related characteristics in pearl millet, thereby enhancing our understanding of the genetic basis for stress resilience. Looking ahead, it is crucial to enhance our understanding of how multiple stressors interact to develop resilient crop varieties capable of withstanding these challenges. Researchers should focus on identifying and characterizing candidate genes and pathways involved in multi-stress responses, utilizing advanced techniques like proteomics, metabolomics, RNA-seq, CRISPR/Cas9, and exploring stress tolerance mechanisms. Integrating this knowledge into breeding programs will be essential for producing varieties that can endure individual and combined stress effects. Additionally, field trials will be necessary to evaluate the performance of these varieties in diverse environmental conditions. Interdisciplinary collaboration among geneticists, molecular biologists, agronomists, and environmental scientists will foster comprehensive strategies to enhance crop stress tolerance. By uncovering the synergistic effects of stressors and elucidating associated molecular mechanisms, researchers can develop innovative approaches to mitigate the impacts of various stresses on agricultural productivity and food security, ultimately contributing to sustainable practices that adapt to the growing challenges of climate change.

Nutrition

Nutritional insecurity is an escalating global crisis, impacting millions of individuals and families, particularly in developing regions. Recent estimates indicate that approximately 195 million people worldwide suffer from undernutrition, with India disproportionately affected, accounting for over 25% of the global hunger burden. This situation is exacerbated by micronutrient deficiencies, often termed "hidden hunger," which significantly hinder health, growth, and cognitive development. Vulnerable groups, including school going children, pregnant women, and the elderly, are particularly susceptible to these deficiencies, resulting in severe health implications and reduced quality of life. To combat this pressing issue, innovative strategies must be employed, and biofortification of staple crops has emerged as a compelling solution. By enhancing these crops' essential vitamins and minerals, biofortified foods can contribute significantly to improving public health, reducing micronutrient deficiencies, and ultimately fostering food security. Millets are particularly well-suited for the arid and semi-arid regions due to their resilience to adverse environmental conditions, such as drought and poor soil quality. Studies including research by Teklu et al. (2023), have demonstrated the potential of soil amendments—such as the addition of zinc and iron to increase the micronutrient content of finger millet grains. This biofortification strategy holds promise for alleviating deficiencies in these critical nutrients, contributing to improved health outcomes in populations that depend on millet as a staple food. The ICRISAT has spearheaded efforts to develop biofortified pearl millet varieties designed to address micronutrient deficiencies prevalent in India. These biofortified varieties have been shown to provide substantial health benefits, with estimates suggesting that daily consumption of just 130 g of these millet-based foods can meet 100% of the daily iron and zinc requirements for children while providing around 70% of the daily needs for adults. The integration of advanced phenotyping, biotechnology, and traditional breeding techniques has facilitated the development of new millet cultivars with improved nutritional profiles, as highlighted by Govindraj et al. (2019). Certain pearl millet lines, such as ICTP 8203, exhibit high levels of iron and zinc. ICTP 8203 is widely cultivated on over 0.8 million hectares in India by 1995, contributing to improved micronutrient intake (Rai et al. 2013). Several high-iron hybrids and varieties have been developed, including Dhanashakti, HHB 299, AHB 1200Fe, AHB 1269Fe, HHB 311, RHB 233, and RHB 234 (Singhal et al. 2022), to enhance nutritional intake and combat micronutrient deficiencies. Singhal et al. (2021) identified 14 quantitative trait loci (QTLs) linked to Fe and 8 QTLs associated with Zn content in a recombinant inbred line (RIL) population derived from the cross between PPMI 683 and PPMI 627. Similarly, Kumar et al. (2016) mapped specific QTLs for Fe and Zn in another RIL population formed from ICMB 841-P3 and 863B-P2. In a different RIL population from the cross ICMS 8511-S1-17-2-1-1-B-P03 and AIMP 92901-S1-183-2-2-B-08, Kumar et al. (2018a, b) identified 11 QTLs for Fe and 8 for Zn. Additionally, Pujar et al. (2020) reported 18 marker-trait associations (MTAs) for Fe, 43 MTAs for Zn, and 17 MTAs for protein content in advanced inbred lines, while Anuradha et al. (2017) discovered 21 MTAs for Fe and 24 for Zn in an association mapping panel. These findings underscore the genetic variability present in pearl millet for enhancing essential micronutrients, providing valuable insights for breeding programs aimed at improving the nutritional quality of this important crop.

Despite these biofortification advancements, millets face significant challenges threatening their place in modern diets. Post-harvest issues, particularly rancidity in milled flour, have contributed to a decline in their consumption. Rancidity can negatively affect the taste, smell, and overall quality of millet products, deterring consumers. Researchers have identified several factors responsible for rancidity, including the presence of volatile compounds, lipid oxidation, and enzymatic changes in C-glycosyl flavones, as noted by Aher et al. (2022), Ali et al. (2022), and Pallavi et al. (2023). For instance, research conducted by Muzumdar et al. (2016) examined various pearl millet varieties, revealing variations in their susceptibility to rancidity, leading to the development of a rancidity matrix by Goswami et al. (2020) that assessed 93 diverse genotypes. This foundational work provides essential insights for breeding programs to develop varieties more resistant to rancidity. In-depth studies have shown that certain biochemical indicators, such as total lipid content, primary and secondary oxidation products, total phenolic content, and enzyme activities, are critical in determining a genotype's susceptibility to rancidity. Ali et al. (2022) characterized four diverse pearl millet genotypes and found a correlation between higher linoleic acid and C-glycosyl flavones content with increased rancidity, making the flour unsuitable for human consumption after only ten days of storage. As we look to the future, addressing the rancidity challenges faced by biofortified millets will be essential to enhance their market viability and nutritional impact. Recently, Aher et al. (2022) have identified key lipases, such as *PgTAGLip1* and *PgTAGLip2*, which play significant roles in hydrolytic rancidity. These findings pave the way for gene editing techniques, like CRISPR/Cas9, to develop low-rancidity genotypes that could significantly improve millet flour's shelf life and consumer acceptability. Additionally, low decortication treatments have proven effective in mitigating rancidity and extending the shelf life of pearl millet flour (Goswami et al. 2024). Future research efforts should prioritize understanding the intricate relationship between high levels of iron and zinc in millets and their implications for rancidity. By exploring the underlying mechanisms that link micronutrient content to shelf life, researchers can strategically incorporate high-iron lines into breeding programs, thereby enhancing the overall nutritional security of millet products globally.

Furthermore, an integrated approach combining biochemistry, genetics, and post-harvest technology will be critical for overcoming the challenges posed by rancidity. This multidisciplinary strategy will enhance the nutritional profile of biofortified millets and ensure that these grains remain viable options for improving the dietary health of populations facing malnutrition. Collaborative efforts among researchers, policymakers, agricultural stakeholders, and industry partners will be essential to promote the cultivation and consumption of biofortified millets. Education and awareness campaigns can help inform communities about the benefits of incorporating these nutrient-rich grains into their diets. By overcoming the barriers to accepting biofortified millets, we can unlock their potential to significantly improve nutritional outcomes, particularly in regions most affected by hunger and malnutrition. The path forward for biofortified millets is filled with potential. With concerted efforts to enhance their nutritional composition, improve their post-harvest handling, and raise awareness about their benefits, biofortified millets can play a transformative role in alleviating nutritional insecurity and contributing to global nutritional security. As climate change continues to pose challenges to food production systems, millets' resilience and dietary benefits make them essential to sustainable agricultural practices to ensure food security for future generations.

Capturing the heterosis

Heterosis, called hybrid vigor, is a key phenomenon in plant breeding that significantly enhances crop yield, particularly in increasing global food demands. This biological advantage arises from the genetic interaction between diverse parental lines, improving offspring growth, adaptability, and overall fitness. The mechanisms underlying heterosis involve genetic factors such as dominance, overdominance, and epistasis, making it an essential tool in crop improvement programs (Birchler et al. 2010). With next-generation sequencing, researchers can identify genome-wide variations contributing to phenotypic diversity. Current studies focus on examining transcriptomic, proteomic, and epigenomic factors to understand the mechanisms behind hybrid vigor. Researchers have discovered several heterotic genes, *ZAR1*, *CNR1*, *SFT*, *LaAP2L1*, *CCA1*, *LHY*, *TOC1* and *GI*, which together enhance hybrid vigor by regulating growth, flowering and stress response, offering significant advantages in crop improvement. Current cereal grain yields have nearly increased five-fold compared to levels observed during the pre-hybrid era. This significant rise can be attributed to advancements in breeding techniques, particularly the development and widespread adoption of hybrid varieties (Srivastava et al. 2020). Developing male sterile lines is crucial for producing hybrid seeds and effectively utilizing heterosis. Male sterility allows for controlled cross-pollination between inbred lines without the risk of self-fertilization, ensuring that hybrid seeds are produced exclusively from desired parent combinations.

While heterosis enhances traits such as yield and stress resistance, apomixis,an asexual reproduction method in flowering plants offers significant advantages, including clonal seed formation and the ability to fix heterosis. Apomixis is a natural reproductive process in plants that produces seeds asexually, resulting in clones of the parent. In contrast, synthetic apomixis is an engineered method that induces asexual seed production in sexually reproducing plants through genetic manipulation, aiming to harness the benefits of hybrid vigor. The development of synthetic apomixis for clonal seed production involves several critical processes: first, the formation of functional female gametophytes without meiosis, utilizing genes such as *SPO11, SPO11-1, PRD1, DFO, PAIR1, SDS, REC8, SGO1, BRK1, OSD1*, and *TAM*. Second, it requires developing embryos without fertilization, facilitated by genes like *CENH3, MTL, POD65, DMP, PLD3, BBML, PAR,* and *RWP*. Finally, viable endosperm must be produced through mechanisms involving genes such as *FIS1/MEA*, *FIS2*, *MSI1*, *EMF2a*, *YUC11*, and *NF-YB1*. By disrupting key chromosomal processes, researchers can bypass meiosis while employing strategies like parthenogenesis and genome elimination, allowing them to circumvent fertilization. This approach can produce haploid and recombined progeny, significantly advancing crop improvement (Xiong et al. 2023). This capability is particularly beneficial for commercial hybrid seed production (Fujimoto et al. 2018). The genetic basis of apomixis involves specific loci, such as the apospory specific genomic region (ASGR), underscoring its importance in crop improvement (Xu et al. 2022). For instance, Conner et al. (2015) demonstrated that the expression of the *PsASGR-BABY BOOM-like* (*PsASGR-BBML*) gene in transgenic sexual pearl millet could trigger parthenogenesis, resulting in the generation of haploid progeny via overexpression. Various crop species have been investigated for their apomictic traits, leading to the identification of crucial genes such as *APOLLO* (Boechera), *HpARI* (*Hypericum), PsORC3a* (*Paspalum*), and *ASGR-BBML* (*Pennisetum*) that play vital roles in inducing natural apomixis (Fiaz et al. 2021). Additionally, certain sexual plants have exhibited traits reminiscent of specific components of the apomictic pathway due to genetic mutations (Conner et al. 2017). In *Arabidopsis*, a mutation in the *dyad* gene led to a meiotic division transforming into a mitotic one, producing apomictic-like traits in the female gametophyte (Ravi et al. 2008). Similar phenomena have been noted in the *am1* gene of maize (Pawlowski et al. 2009). Different combinations of MiMe systems have been used to produce clonal gametes, but some may decrease fertility. The *pair1/rec8/osd1* combination in hybrid rice CY84 has achieved seed-setting rates similar to wild-type hybrids (Mieulet et al. 2016). A CENH3 variant (GEM) line was employed to induce genome elimination for generating clonal seeds through crosses with *mime* or *dyad* mutants, leading to the production of 34% diploid offspring from MiMe crosses and 13% from *dyad* mutants (Marimuthu et al. 2011). Genome editing using CRISPR/Cas9 technology was applied to four genes (*REC8*, *PAIR1*, *OSD1*, and *MTL*) in rice hybrids CY84, a technique referred to as Fix for Fixation of hybrids (Wang et al. 2019). Although limited information is currently available for pearl millet, the strategies employed in other cereals can be leveraged to fix heterosis in hybrid pearl millet through advanced breeding techniques, such as CRISPR/Cas9 technology.

Lodging stress

Historically, pearl millet has garnered significant interest from breeders and researchers due to its resilience to adverse stress conditions and high nutritional value. However, the productivity of pearl millet is not higher, primarily due to factors such as lodging stress. Lodging is a common problem in pearl millet; it leads to permanent bending of the stem from an upright position induced by various forces like rain, wind, or combinations. The main targets of lodging stress are the stem and root, as a heavy flow of wind in combination with the greasing of soil by rainwater can bend the plant towards the soil (Choudhary et al. 2023). In root lodging, the angle between the stem and soil changes due to strong wind force on the stem, crown bending, or root disanchoring, whereas stem lodging is associated with bending the stem towards the soil or breaking the stem (Van Delden et al. 2010). The yield potential of millets like tef, foxtail millet and pearl millet are reduced mainly due to lodging stress.

Pearl millet has less impact on lodging stress compared to other minor millets. Nevertheless, existing methods need to be improved, or novel strategies need to be developed to overcome lodging stress in millets and benefit from fertilizer application to enhance productivity. One of the efficient strategies to decrease the impact of lodging is to reduce plant height so that the plant can withstand it. During the green revolution, *Rht* dwarfing genes responsible for reducing plant height were introduced in wheat to boost its responsiveness to nitrogen fertilizer (Shivhare and Lata 2017). Changing the seed sowing date, tilling practices, and increasing the intra-row space or reducing the number of plants in a row are some other crop management practices used to reduce the effect of lodging stress (Cannarozzi et al. 2018). Inhibition of plant growth regulators like gibberellic acid (GA) can reduce plant height to improve lodging tolerance. The inhibitors of GA, like daminozide, mepiquat-Cl, chlormequat-Cl, and paclobutrazol (PBZ), play a pivotal role in developing dwarf semi-dwarf and sturdier plants that can tolerate lodging effects (Fallah 2012). Also, PBZ causes height reduction, increased seed yield, and enhanced nutrient uptake (Hedden and Graebe 1985). Bizuayehu and Getachew (2021) reported the application of PBZ in tef and finger millet, which showed reduced plant height and increased lodging tolerance.

Mutation in the Kodo millet CO3 variety through ethyl methanesulfonate (EMS) showed improved lodging tolerance due to photosynthesis efficiency (PhE) and culm thickness (Jency et al. 2020). Jost et al. (2015) created mutations in the *alpha-tubulin-1* gene in tef by EMS and developed a new cultivar, 'Kegne', which showed tolerance to lodging. Recently, Liu et al. (2018) identified several quantitative trait loci (QTLs) that contribute to lodging stress tolerance and are successfully integrated into developing improved varieties. RFLP marker-based lodging-resistance QTLs in wheat X spelt population was reported by Keller et al. (1999). In winter wheat, a 30% reduction in lodging risk was recorded with 15-days of delayed sowing (Spink et al. 2000). It has been reported that lodging-resistant wheat genotypes have higher culm wall thickness and culm diameter of the lower internodes (Xiang et al. 2019). According to Gupta et al. (2020), culm strength emerged as a weather-independent, cost-effective, and easily assessable trait, providing a valuable tool for screening lodging tolerance in pearl millet. More recently, the novel *Rht* locus QPH.caas-6A, designated as Rht24, located on chromosome 6A, was reported. It showed highly phenotypic variations. This QTL is attributed to reducing plant height (Tian et al. 2017). This knowledge is now available for these crops, and these traits can be exploited for pearl millet, where lodging leads to a considerable loss in their harvest. Identifying height-related and root architecture genes in crops such as rice, wheat, and sorghum provides a foundation for evolutionary and functional analysis of such proteins, defining a comprehensive view of *Rht*, *dw3,* or *Br2* family genes in pearl millet. Using available genome sequence information, the same family or group of genes may be considered and targeted for gene editing in pearl millet crops where genes for lodging tolerance have not yet been determined.

Integration of the tools for pearl millet improvement

Pearl millet crop improvement involves a multifaceted approach encompassing various strategies. These include traditional breeding methods and molecular techniques like marker-assisted selection (MAS), which facilitate the efficient identification and incorporation of beneficial genes into breeding programs. Additionally, genomic tools and technologies, such as next-generation sequencing should be exploited. OMICS, genetic engineering and genome editing further accelerate the breeding process by enabling precise manipulation of target genes for desired traits (Fig. 2), fostering the creation of cultivars with superior agronomic performance and resilience to environmental challenges. By integrating cutting-edge genetic methodologies, the development of improved pearl millet cultivars is facilitated, thus advancing sustainable crop production and bolstering food security efforts. Collaborative efforts between researchers, breeders, and farmers are essential for implementing these strategies effectively and ensuring sustainable pearl millet production in the face of evolving agricultural challenges.

Genomic selection (GS) has emerged as a promising approach in pearl millet breeding

Genomic selection (GS) represents a cutting-edge approach in plant breeding that harnesses genomic information to predict the performance of individuals for desired traits. By analyzing genome-wide marker data, GS enables breeders to identify and select plants with favourable genetic profiles, thereby accelerating the breeding process (Spindel et al. 2015). In recent years, GS has gained traction in various crops, including cereals like maize, wheat, and rice, revolutionizing breeding practices and enhancing genetic gains. This technology holds immense promise for pearl millet breeding. It is a better alternative for marker-assisted and phenotype selection, potentially expediting the development of improved varieties with enhanced traits (Ramadoss et al. 2024). Genomic selection enables selections during the off-season, facilitating annual improvements in genetic gains (Heffner et al. 2009). A group of ICRISAT scientists sequenced and assembled the genome for pearl millet (Varshney et al. 2017). Kanfany et al. (2020) examined 309 pearl millet genotypes to assess genetic diversity, revealing the smallest genetic distance of 0.09 between ICML197458 and ICML197279, derived from Nigerian and Indian landraces, while the largest distance of 0.33 was found between ICML197390 and ICML197314. GWAS is an effective approach for studying the genetic architecture of complex agronomic traits in crops through genome-wide SNP markers. The pearl millet draft genome has greatly enhanced the potential to identify QTLs, and the functions of associated genes related to diverse traits. However, high heterozygosity and heterogeneity in pearl millet germplasm accessions make association mapping challenging, resulting in limited strategies for effectively analyzing genetic diversity. A GWAS analysis with 34 SSR markers in 250 full-sib progenies identified associations between specific markers and traits in pearl millet, including grain yield, plant height, stover dry matter yield, and panicle length, each linked to distinct loci across *LG2*, *LG5*, *LG6,* and *LG7* (Kannan et al. 2014). Numerous major QTLs and genes have been identified in pearl millet for key input and output traits, including *Xibmsp27*, *Xibmsp9*, *Xibmsp12*, *Xibmsp60*, *Xibmsp14*, *Xibmsp24*, *Xibmsp31*, *Xibmsp15*, *SNPs*, *InDels, Ppb6981*, *head5* and *head7* for flowering time; *Xibmsp27*, *Xibmsp9*, *Xibmsp12*, *Xibmsp60*, *Xibmsp34*, *Xpsm592*, *Xpsm356*, *Xpsm464,* and *Xpsm716* for grain yield; *D1/d1*, *D2/d2*, *Pgpb6112*, *Pgpb9106*, *Pgpb9498*, *Xipes017*, and *Xipes203* for plant height; and *Xctm03*, *Xpsmp2072*, *Xibmsp53*, *Xibmsp55*, and *Xibmsp51* for drought tolerance, as well as *Rr1* and *Rust Res* for rust resistance (Singh and Nara 2023). Hence, genomic selection becomes feasible for predicting the overall combining ability (GCA) of recently inbred parents and the specific performance of individual pearl millet hybrids (Liang et al. 2018; Ramadoss et al. 2024). Liang et al. (2018) employed two different genotyping methods, RAD-seq and tGBS, to examine how well genomic selection and prediction worked to profile a set of inbred pearl millet lines developed by ICRISAT. Three different contrasted genomic selection (GS) models were implemented using data on grain yield and high-density molecular markers obtained from two different genotyping platforms for pearl millet. These platforms were labelled as C (conventional genotyping by sequencing, GBS RAD-seq) and T (tunable genotyping by sequencing, tGBS) (Jarquin et al. 2020). Genomic selection (GS) in pearl millet breeding is promising, as it offers a transformative approach for accelerating genetic gains and improving crop traits. Given that GS leverages genome-wide marker data, it facilitates the rapid selection of plants with favourable genetic profiles, allowing annual improvements even in the off-season. The successful application of GS in other cereals such as maize, wheat, and rice suggests its potential for a similar impact in pearl millet, especially with the recent pearl millet genome assembly.

Systems biology tools utilized in pearl millet

Systems biology tools encompass a range of high-throughput approaches that allow researchers to study biological systems comprehensively (Dai and Shen 2022). These technologies have revolutionized plant breeding and crop improvement by providing insights into the genetic and molecular mechanisms underlying traits of interest. Progress in next-generation sequencing (NGS) (Ashraf et al. 2022) has opened avenues for a new era of various systems biology disciplines, including genomics, transcriptomics, and proteomics. Nevertheless, crop science has also extensively documented metabolomics, ionomics, and phenomics. Several studies have implemented systems biology technologies in various cereal crops, including rice, wheat, barley, foxtail millet, and maize (Yang et al. 2021). Researchers have been paying attention to millets in recent years due to their excellent properties. The primary obstacle in improving millet lies in effectively understanding genetic and genomic reservoirs and utilizing variations specific to traits. The release of the pearl millet draft genome database (Varshney et al. 2017; Sun et al. 2023) has provided valuable genomic information and resources, which have the potential to enhance crop improvement efforts in pearl millet. Kumar et al. (2018a, b) identified 14 conserved miRNAs in pearl millet across 11 families by analyzing public datasets and validating five of these in different tissues of two genotypes. Target genes of these miRNAs are linked to stress response, transcriptional regulation, growth, and other critical functions, providing insights into miRNA roles in pearl millet. The study from Ramu et al. (2023) provided three high-quality, near-complete genome assemblies for pearl millet breeding lines, highlighting structural variations and gene enrichments related to seed protein quality and disease resistance. The assemblies and identified marker-trait associations offer a robust platform for advanced breeding techniques like marker-assisted selection and haplotype-based breeding. An improved assembly for the pearl millet cultivar Tift 23D2B1-P1-P5 was developed using Oxford Nanopore and Bionano Genomics technologies, adding 200 Mb to the genome and enhancing contig continuity, especially in centromeric regions. The assembly achieves 98.4% gene completeness, providing a valuable resource for studying structural variants and advancing pearl millet genomics and breeding (Salson et al. 2023). Transcriptomic studies have been successfully used in pearl millet improvement for biotic/abiotic stress tolerance and nutritional enhancement. Some of these studies include different sequencing strategies like Illumina Hiseq (drought tolerance), ION S5Next Generation Sequencer (iron and zinc content), PacBio Sequel and Illumina (Heat and drought stress) and Illumina HiSeq 2500 (salinity stress) (Banshidhar et al. 2023). Other systems biology studies like proteomics for identifying marker proteins for drought stress (Ghatak et al. 2021) and metabolomic approach identified 738 candidate genes for metabolite, starch, and vitamin synthesis (Yadav et al. 2021). Shinde et al. (2020) investigated the role of microRNAs (miRNAs) in salinity stress responses in pearl millet, revealing 81 conserved and 14 novel miRNAs associated with stress tolerance through small RNA sequencing. Target prediction identified 448 mRNAs, with approximately 25% encoding transcription factors, indicating that these miRNAs may regulate the auxin response pathway. Quantitative real-time PCR confirmed consistent expression patterns, highlighting the significance of miRNAs in enhancing salinity stress tolerance in pearl millet. Palakolanu et al. (2022) examined the physiological responses of pearl millet genotypes ICMR 1122 and ICMR 1152 to varying vapor pressure deficit (VPD) conditions. Under high VPD, ICMR 1152 showed lower transpiration rates and higher efficiency, while deep sequencing revealed 116 known and 61 novel Pg-miRNAs in ICMR 1152, and 26 known and six novel Pg-miRNAs in ICMR 1122, with specific miRNAs linked to root physiology and drought tolerance responses. Available sequence information facilitates the application of the CRISPR/Cas toolkit in improving pearl millet or other crop species. Pearl millet research will benefit significantly from the integration of systems biology tools, which can enhance our understanding of the complex biological processes governing this important crop. By leveraging high-throughput sequencing technologies, advanced bioinformatics, and data integration platforms, researchers can analyze the intricate interactions between genes, proteins, and metabolic pathways. This holistic approach will enable the identification of key regulatory networks that influence traits such as drought tolerance, disease resistance, and nutritional quality.

Genetic transformation in pearl millet

Various methods have been explored for transforming pearl millet, addressing challenges like recalcitrance and genotype dependence. Initially, electroporation with protoplasts was attempted (Hauptmann et al. 1987), but difficulties in plant regeneration persisted (Potrykus 1990). The introduction of the gene gun facilitated DNA transformation in intact cells and tissues, with successful attempts reported by Taylor et al. (1993) and Girgi et al. (2002). Devi and Sticklen (2002) developed a genotype-dependent transformation system using shoot apical meristem tissue. Goldman et al. (2003) increased transformation frequency using various explants and plasmids. Despite these advancements, the biolistic gene gun method remains predominant in pearl millet transformation (Ceasar and Ignacimuthu 2009), with attempts focusing on herbicide resistance (Tiecoura et al. 2015) (Table 1). Despite particle bombardment being the primary method, stable *Agrobacterium*-mediated transformation in millets still faces challenges (Sharma et al. 2022). Researchers seek an efficient and reproducible gene transformation system for pearl millet. Recently, an improved leaf segment-based *Agrobacterium*-mediated rapid transformation was utilized in maize and sorghum (Wang et al. 2023). This successful attempt in maize and sorghum will be used in other cereals, especially in pearl millet, to improve transformation efficiency. Bhatt et al. (2021) developed A reproducible protocol for *Agrobacterium* tumefaciens-mediated genetic transformation of kodo millet, achieving high transformation efficiency with specific *Agrobacterium* strains and optimized conditions. The successful expression of the *uidA* gene led to the regeneration of normal transgenic plants, providing a basis for developing improved kodo millet varieties. The increased activity of morphogenic genes, particularly those related to plant embryos or meristematic functions, regulates plants' overall growth and development (Yuan et al.

Table 1 Successful transformation attempts in Pearl millet

Sr. no	Explant	Transformation method	Gene	Reference
1	Protoplast	Electroporation	–	Hauptman et al. (1987)
2	Immature embryo	Gene gun method	*uidA*	Taylor et al. (1993)
3	Callus	Gene gun method	*uidA, hptII*	Lambe et al. (1995)
4	Scutellar tissue	Gene gun method	*GUS/uidA*	Girgi et al. (2002)
5	Shoot apical meristem tissue	Gene gun method	*uidA*	Devi and Sticklen (2002)
6	Embryogenic tissue derived from immature embryo, inflorescences, apical meristems	Gene gun method	*bar* *uidA*	Goldman et al. (2003)
7	Proliferating immature zygotic embryos	Gene gun method	*PMI*	O'Kennedy et al. 2004
8	Immature embryo	Gene gun method	*afp*	Grigi et al. (2006)
9	Shoot-tip-derived embryogenic calli	Gene gun method	*pin*	Latha et al. (2006)
10	Shoot apical medium	*Agrobacterium*-mediated transformation	*hpt, uidA*	Jha et al. (2011)
11	Compact nodular calli	*Agrobacterium*-mediated transformation	*mag*	Ramadevi et al. (2014)
12	Embryogenic cell suspensions	Gene gun method	*GUS*	Tiecoura et al. (2015)

The table comprises some successful transformation attempts in pearl millet with efficient transformation protocols. Gene transformation methods such as direct (electroporation, gene gun method) and indirect (*Agrobacterium*-mediated), have been utilized to integrate target genes in a variety of explants, including protoplasts, immature embryos, calluses, shoot tips, scutellar tissues, apical meristems, nodular calluses, and cell suspensions

2024). Numerous studies have investigated the genetic factors influencing somatic embryogenesis, meristem upkeep, and phytohormone metabolism (Snipes et al. 2018).

Genetic engineering

Traditional plant breeding techniques were widely employed for crop enhancement in the past. However, these methods proved to be laborious, expensive, and time-consuming. Furthermore, they often needed to improve in facilitating significant genetic crop advancements (Ahmar et al. 2020). In the last decade, transgenic technology has evolved to enhance agronomically important crops, including major cereals (Ceasar and Ignacimuthu 2009; Marone et al. 2023). Transgenic technology has been deployed to enhance the resilience of cereal crops against diverse stresses, including insect resistance in maize and rice, herbicide tolerance in rice, maize and wheat, well and drought tolerance in wheat, rice, maize, and barley (Bakshi and Dewan 2013) and so on. Additionally, it has been utilized for nutritional improvements in cereals like rice (Vit A, Fe, Zn, folic acid), wheat (Vit A, Fe), and maize (Vit A) (Bakshi and Dewan 2013; Kumar et al. 2019). A genetic engineering approach has also been implemented in millets, such as pearl millet, finger millet, foxtail millet, barnyard millet, elephant grass, guineas grass, and bahiagrass. Among all millets, pearl millet has been the highest priority in transformation studies (Ceasar and Ignacimuthu 2009). The details of the attempts made so far for genetic engineering have been included in Table 1. Genetic engineering often involves the integration of entire genes from unrelated organisms, which may raise ethical concerns and public skepticism about genetically modified organisms. This is where new breeding tools, such as CRISPR/Cas9, offer a promising alternative, allowing precise modifications in plants' genomes.

Gene editing tools—CRISPR/Cas9

The precise alteration of an organism's DNA within its genome is called "gene editing". It enables scientists to add, remove, or change nucleotide sequences, among other precise DNA modifications. This technique has wide applications in different research fields, including medicine and agriculture. There are several gene editing techniques, such as Meganucleases, Zinc finger nuclease (ZFN), Transcription activator-like effector nuclease (TALEN), Oligonucleotide-directed mutagenesis (ODM), and Clustered regularly interspaced short palindromic repeats (CRISPR) and most recent prime and base editing represent a significant tool for precise genetic modification (Sharma et al. 2022). CRISPR/Cas9 is one of the most well-known and often applied strategies. A double-strand break (DSB) repair system is used in genome editing and is based on a non-homologous end joining (NHEJ) and homology-directed repair (HDR) mechanism. The results are often classified into three categories: SDN-1 (NHEJ), SDN-2 (HDR), and SDN-3 (HDR), representing different levels or kinds of site-directed nucleases (Matres et al. 2021).

CRISPR/Cas9, a powerful gene editing tool, utilizes RNA-programmed DNA cleavage with sgRNA and Cas9 (Liu et al. 2022). PAM sequences are crucial for CRISPR targeting, enabling precise plant modifications, including stress tolerance and nutritional enhancement. This technology holds immense promise for crop improvement, allowing scientists to enhance traits such as yield, quality, nutritional content, and resistance to pests and diseases (Basu et al. 2023). Several recent successful examples of employing this tool for crop enhancement have been documented. Some examples of trait improvement in cereals using CRISPR/Cas9 are mildew resistance in wheat (Shan et al. 2014; Tripathi et al. 2020), bacterial-blight resistance in rice (Oliva et al. 2019), apomixis in rice (Biswas et al. 2020), fungal pathogen resistance in rice (Kim et al. 2022), improved plant immunity in barley (Galli et al. 2022) etc. Although extensively utilized in crops such as rice, wheat, and maize, its potential in pearl millet still needs to be explored, highlighting the necessity for efficient transformation and gene editing techniques (Liu et al. 2021). Traditional breeding is time-consuming, costly, and labour-intensive, necessitating efficient tools for crop improvement. Biotechnological methods offer economical, rapid, and error-free results, yet their utilization in millets, particularly pearl millet, remains limited (Kothari et al. 2005). The prerequisite resources required for utilizing these tools, such as a well-established transformation system, access to a genome database, comprehensive gene information, and an advanced vector system, are either limited or yet to be developed in pearl millet (Sapara et al. 2024). Enhancing millet transformation methods could facilitate significant gene targeting for improved stress resistance and trait enhancement. Adopting novel biotechnological methods like gene editing requires efficient transformation systems to validate stress tolerance candidate genes. Researchers can target potential genes identified in other crops to improve pearl millet resistance to different stress levels without any compromise on nutritional quality. These genes include *waxy* (starch quality), *PHYC* (flowering time and spike length), *CAD* (production of biofuel), LOX, lipases (improved flour quality and shelf life), *Dwarf 8* (small height), ACCase (high yield in drought condition), *OMTN3/4/6*, *ARR1/10/12*, *GhWRKY17*, *ADC* (drought), h*sp101*, *ZFP*, *FAD7*, *TamiR159* (heat), *KO2*, *GRF mRNA*, *GRF10*, *GA20-oxidase*, *BRI1*, *Rht1* (lodging) etc., (Numan et al. 2021; Panda et al. 2024).

Conclusion

Pearl millet can be considered a poor man’s crop, primarily grown by smallholder farmers from India and Africa. Pearl millet is considered a ‘powerhouse of nutrients’ and possesses various health benefits; it can also withstand harsh environmental conditions. In the face of leading climatic conditions, it is a crop with great potential for food security and nutrition to feed billions worldwide and combat adverse climatic conditions. These features make pearl millet an important crop for plant breeders/researchers and policymakers in the crop improvement program. Pearl millet is becoming highly important as a food or forage crop and will soon become a globally crucial alternative food grain. Still, there is a need to address several constraints and bottlenecks to improve pearl millet's economically important traits. Advancements in genetic enhancement have emerged as a promising solution to address key challenges in pearl millet cultivation. Researchers have targeted yield potential, drought tolerance, disease resistance, and nutritional quality through strategic breeding programs and biotechnological interventions. These efforts have resulted in improved varieties that exhibit higher productivity and resilience and possess traits aligned with nutritional requirements and market demands. Hence, appropriate research and development are crucial to tackling the significant constraints in pearl millet. By applying the power of genetic enhancement, pearl millet farmers can mitigate the impacts of climate change, combat pests and diseases and enhance food security for farming communities who are reliant on this staple crop. As these advancements continue to evolve, they hold the potential to revolutionize pearl millet agriculture, ensuring sustainable production and improved livelihoods for farmers worldwide.

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Author contributions RRA and PSR wrote the original manuscript. RRA and PSR edited, modified and finalized the manuscript.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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