

13. Mitigating Biotic and Abiotic Stresses: Biotechnological Strategies for Improving Millet Productivity

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Abstract:

Millets are staple crop that is cultivated all over the world, especially in dry and semiarid climates. Millions of people, especially in developing countries, rely on it as a primary source of nutrition, and it is also widely used as animal feed. Nevertheless, millet has a number of problems, such as low yields, disease susceptibility, and a lack of genetic variety.

Several of these issues with millet production have been dealt with by the application of biotechnological methods. Methods for improving millet include introducing genes from other creatures into millet plants through genetic engineering. In addition to traditional breeding methods, molecular markers can be used in a biotechnological process called marker-assisted selection (MAS) to choose millet plants with desirable characteristics. Plants with high production potential and those that are resistant to disease, insect pests, drought, or other challenges can be selected using MAS.

As the selection of desirable features can be time-consuming and expensive, this method can be very helpful in breeding operations. Tissue culture, which involves the growing of millet cells in a laboratory setting, is another biotechnological technique to millet improvement alongside genetic engineering and MAS. In conclusion, biotechnology methods may help alleviate some of the problems associated with millet cultivation. The advantages of these methods should be weighed against their disadvantages, such as any unforeseen consequences for the environment or human health. Thus, this chapter elucidate and track the progress of millet enhancement efforts that employ biotechnology methods.

Keywords:

Millet, disease, insect pests, drought, genetic engineering, biotechnological methods

13.1 Introduction:

Millet is an extremely hardy crop that can flourish on poor soil and with little water and have been grown for thousands of years all across the world, especially in less developed regions. Millet is a vital part of a balanced diet since they include essential elements like carbs, fibre, protein, vitamins, and minerals (Gupta et al., 2015). Millet's high nutrient content is one of its key advantages. Carbohydrates, the primary source of energy in the body, can be found in plenty in millet. Also, the fibre content of these foods has been shown to aid in the reduction of cholesterol and the promotion of healthy gastrointestinal function. Furthermore, these are beneficial to one's health and well-being and provide protein, vitamins (especially the B-complex), and minerals (including calcium, iron, and zinc) (Gupta et al., 2020). Millet is significant not just because of their nutritional worth, but also because of the positive effects they may have on one's health.

Millet has been linked in several studies to a lower chance of developing diabetes, cardiovascular disease, and even some cancers, so they're worth considering as a part of a healthy diet. Millet is low on the glycemic index; thus they don't raise blood sugar levels quickly after being ingested. For this reason, millet is a great choice for those with diabetes and for those who are watching their weight (Saleem et al., 2020). In addition, millet is a crop that doesn't negatively impact the environment. Millet is suitable for growing in arid and semiarid environments because they can withstand drought and need less water than other crops. Millet is better for the environment because they need fewer inputs like fertiliser and herbicides (United Nations, 2018).

According to the Food and Agriculture Organization of the United Nations (FAO), millet is grown in various parts of the world and total millet production was approximately 29 million tonnes in 2020. The top five millet-producing countries are India, Nigeria, Niger, China, and Mali, of which, India alone accounts for around 40% in the world. Other major millet-producing countries include Ethiopia, Burkina Faso, Sudan, and Uganda. Amongst diverse countries, India is the largest producer of millet in the world. According to the Department of Agriculture and Farmers Welfare, the total area under millet cultivation in India was around 17.3 million hectares, and the total millet production was approximately 28 million tonnes in 2020-21. The major millet-producing states in India are Rajasthan, Karnataka, Maharashtra, Andhra Pradesh, and Telangana (ASG, 2021).

Despite being a resilient crop, the productivity of millets is observed to be relatively low compared to other major foodgrains. The average yield of millets in India is around 1 tonne per hectare, which is much lower than the global average of 1.7 tonnes per hectare.

The low productivity of millets is mainly due to the lack of proper infrastructure, low investment in research and development, and limited availability of high-quality seeds and fertilizers (Bhatia and Swaminathan, 2013; Bhattacharya and Gupta, 2017).

The production of millets is subject to both biotic and abiotic constraints, which can limit their yield and quality (Ceccarelli, 2015). Biotic constraints refer to the impact of pests, diseases, and weeds on millet plants, while abiotic constraints refer to environmental factors such as soil fertility, water availability, temperature, and light intensity (Shiferaw, 2013; Reddy et al., 2017).

13.2 Genetic Transformation of Millets for Enhancing Biotic and Abiotic Stress Tolerance:

Millet is an important crop grown across the globe, particularly in Africa and Asia. The crop is well adapted to harsh environments, and its ability to grow in areas of low rainfall and poor soil quality has made it a staple food for many people living in diverse arid and semi-arid regions. However, millet production and productivity can be affected by a range of biotic and abiotic factors.

Biotic factors that affect millet production include pests, diseases, and weeds. Millet is susceptible to a number of pests, including stem borers, armyworms, and termites, which can cause significant damage to crops.

Diseases such as blast, downy mildew, and rust can also affect millet yields. Weeds are also a major problem for millet farmers, as they can reduce crop yields by competing with the crop for water and nutrients. Abiotic factors that affect millet production include climate, soil quality, and water availability. Millet is a crop that is well adapted to harsh environments, but it requires a certain amount of rainfall to grow properly.

In areas with low rainfall, millet crops may suffer from water stress, which can reduce yields. Soil quality is also important for millet production, as the crop requires well-draining soil with good fertility. Salinity and alkalinity can also affect millet productivity, as high levels of these minerals can be toxic to the crop (Kumar et al., 2018).

Many researches were conducted on the biotic and abiotic factors influencing millet production and productivity (Okoruwa and Omoregie, 2008) and it was found to be limited by pests and diseases in India; while, soil fertility and weed infestation were reported to be the main barriers to millet production in diverse African countries (Traoré et al., 2001).

So, farmers need to be cognizant of these issues and efforts must be taken to minimise their effects on crop yields. In spite of these problems, millet productivity can be increased by making resistant varieties, using integrated pest management, and managing soil fertility (Figure 13.1).

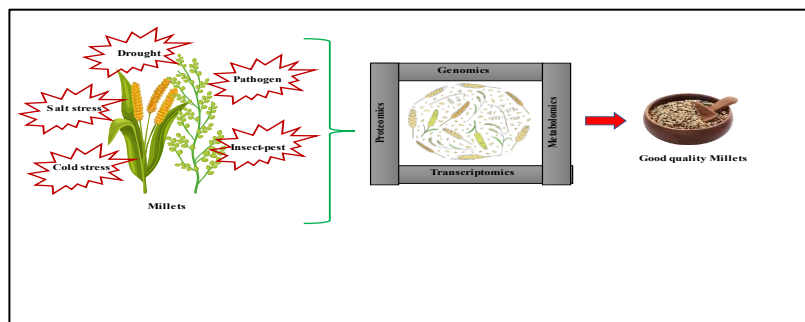


Figure 13.1: Depicts diverse biotechnological approaches like genomics, transcriptomics, proteomics and metabolomics approaches for millet improvement to tolerate abiotic and/or biotic stresses.

13.2.1 Genetic Transformation of Millets for Disease Resistance:

Genetic transformation is a technique used to introduce foreign genes into an organism's genome. In agriculture, genetic transformation has been used to improve crop yields, enhance nutritional content, and provide resistance to pests and diseases. Millets are a group of small-seeded grasses that are grown for food and fodder in many parts of the world, particularly in dry regions with low soil fertility. Millets are known for their hardiness, drought tolerance, and pest resistance. However, pests and diseases still pose a threat to millet production, leading researchers to explore genetic transformation as a means of enhancing crop protection. One approach to genetic transformation of millets for crop protection is to introduce genes encoding for insecticidal proteins. The most widely used insecticidal proteins are the *Bacillus thuringiensis* (Bt) toxins, which specifically target insect pests and have been shown to be effective against a wide range of insects.

Researchers have successfully introduced Bt genes into various millet species, including pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), and finger millet (*Eleusine coracana*), resulting in increased resistance to insect pests such as stem borers and head feeders (Upadhyay et al., 2011; Sharma et al., 2013; Thakur et al., 2014). Another approach is to introduce genes that enhance disease resistance in millets. For example, researchers have successfully introduced genes encoding for chitinase, a protein that degrades chitin in fungal cell walls, into finger millet, resulting in increased resistance to blast disease caused by the fungus *Magnaporthe grisea* (Mohan et al., 2007). Overall, genetic transformation of millets for crop protection shows promise as a means of enhancing resistance to pests and diseases. However, there are still challenges to be overcome, including regulatory barriers, social acceptance, and concerns over potential environmental impacts (Devos et al., 2014). Another example is the introduction of genes encoding for plant lectins, such as rice bean lectin (RBL) and wheat germ agglutinin (WGA) (Kumar et al., 2019). Plant lectins are proteins that bind to specific sugars on the surface of insects' digestive systems, causing damage and death to the insects (Niranjan-Raj et al., 2017). When these lectin genes are introduced into millets, the plants produce the lectin proteins themselves, which can protect the plants from insect pests (Arora et al., 2020). By introducing the Bt gene into the pearl millet genome, researchers have developed plants that are resistant to pests such as stem borers and head bugs (Seck et al., 2012).

Another example of genetic transformation in millets is the development of transgenic finger millet expressing the cowpea trypsin inhibitor (CpTI) gene. The CpTI protein is a potent insecticide that inhibits the digestion of plant proteins in the gut of insect pests. By introducing the CpTI gene into finger millet, researchers have developed plants that are resistant to the spotted stem borer (Reddy et al., 2006). These genetic transformation studies in millets have shown promising results for improving crop protection against pests and diseases. However, further research is needed to assess the safety and efficacy of transgenic millets in the field.

Genetic transformation involves the transfer of genes from one organism to another, often to confer a new trait or characteristic. In millets, this can be done using a variety of techniques, including *Agrobacterium*-mediated transformation and particle bombardment. These techniques have been used to introduce genes for disease resistance from various sources, including other plants and bacteria. One example of genetic transformation in millets for disease resistance is the introduction of the Xa21 gene from rice into finger millet (*Eleusine coracana*). The Xa21 gene encodes a receptor-like kinase that provides resistance against bacterial leaf blight disease caused by *Xanthomonas oryzae* pv. *oryzae* in rice. The transformed finger millet plants showed increased resistance to *Xanthomonas oryzae* pv. *oryzae*, demonstrating the potential for genetic transformation to enhance disease resistance in millets (Nageswara-Rao et al., 2017). Researchers were reported to genetically transform pearl millet, *Pennisetum glaucum* with the chitinase gene from *Trichoderma virens*, which is known to provide resistance against fungal pathogens.

The transgenic pearl millet showed increased resistance to downy mildew disease caused by *Sclerospora graminicola*, which is a major pathogen of pearl millet in sub-Saharan Africa (Kumar et al., 2017). In another study, the foxtail Millet (*Setaria italica*) was modified with the rice chitinase gene OsCHI11, which helps plants fight off blast disease caused by *Magnaporthe oryzae*, a major disease-causing organism that attacks rice and other cereal crops (Li et al., 2014). Finger millet (*Eleusine coracana*) that has been genetically changed with the maize ribosome-inactivating protein (RIP) gene ZmRIP has been shown to be resistant to blast disease caused by *Pyricularia grisea* and smut disease caused by *Tolyposporium penicillariae*. It also has antifungal and antiviral properties (Bhattacharyya et al., 2007).

A study reported in the journal *Plant Molecular Biology* showed that millet plants with a gene from *Arabidopsis thaliana* that made them resistant to blast disease were successfully engineered (Chakraborty et al., 2017). Genome-wide association mapping (GWAS) is a powerful tool for identifying genetic variants associated with disease resistance. Researchers have used GWAS to identify genes associated with resistance to diseases in millets. For example, identification of genes associated with resistance to downy mildew in pearl millet was done using GWAS (Rajaram et al., 2017). Moreover, a study by Yadav et al. (2020) used GWAS to identify genomic regions associated with resistance to rust disease in finger millet. Furthermore, molecular markers are used to identify genetic variations associated with disease resistance. Researchers have developed molecular markers for identifying genes associated with resistance to head smut in sorghum (Kumari et al., 2017; Singh et al., 2017; Singh et al., 2023)). The bacterial blight resistance gene (Xa21) was inserted into pearl millet (*Pennisetum glaucum*) to make it resistant to bacterial leaf blight caused by *Xanthomonas axonopodis* pv. *Penniseti* (Xap).

This is another way that genetic engineering is used to make plants resistant to disease (Kumar et al., 2018). These examples show how genetic modification could be used to make millets more resistant to diseases. But it's important to remember that genetically modified crops should be carefully tested for safety and compliance with rules before they are offered for sale.

A. RNA interference (RNAi) technology: RNAi technology involves the use of small RNA molecules to silence specific genes, which can help to improve disease resistance in plants. For example, RNAi-mediated silencing of the chitinase gene in foxtail millet (*Setaria italica*) has been shown to make the plant more resistant to fungal pathogens (Ghanti et al., 2018). Moreover, RNAi technology has been used to make pearl millet plants resistant to downy mildew, which is caused by the fungus *Sclerospora graminicola*. The RNAi construct was made to target a pathogenesis-related gene in the fungus. Transgenic plants that expressed this RNAi construct were much less likely to get infected with downy mildew. RNAi technology has been used to make finger millet more resistant to blast disease, which is caused by the fungus *Magnaporthe grisea*. RNAi technology has also been used to make foxtail millet more resistant to smut disease, which is caused by the fungus *Ustilago crameri*.

B. Marker-assisted selection (MAS): Molecular marker-assisted selection (MAS) is a process that uses molecular markers to choose plants with desirable traits, such as resistance to disease. For example, MAS has been used to make blast-resistant varieties of finger millet (*Eleusine coracana*) by finding and choosing plants with the RGA2 gene, which gives resistance to the blast fungus (*Magnaporthe oryzae*) (Sharma et al., 2017). MAS is a powerful plant breeding technique that enables the identification and selection of desirable traits, such as disease resistance, using molecular markers. In a study published in the journal *Plant Breeding*, researchers used MAS to develop pearl millet varieties with resistance to downy mildew, a devastating disease caused by the fungus *Sclerospora graminicola*. Diverse studies have molecular markers linked to the resistance gene and used them to select resistant plants in breeding populations. The resulting varieties showed high levels of resistance to downy mildew and performed well in field trials (Gupta et al. (2012). Furthermore, finger millet is susceptible to blast disease, caused by the fungus *Magnaporthe grisea*. In a study published in the journal *Molecular Breeding*, researchers used MAS to identify markers linked to blast resistance genes in finger millet and the same markers were further used to select resistant plants in breeding populations and developed finger millet varieties with enhanced blast resistance (Ramakrishnan et al., 2017). Moreover, researchers have reported to use MAS to identify markers linked to smut resistance genes in proso millet (Jia et al., 2016). Moreover, a study by Kumar et al. (2021) concluded that MAS can be used to select finger millet varieties with resistance to blast disease.

C. Genome editing: Genome editing involves the precise modification of DNA sequences within an organism's genome to introduce new traits, including disease resistance. For example, the use of the CRISPR-Cas9 system to edit the genome of finger millet has been shown to confer resistance to the blast fungus (Shrawat et al., 2021). Genome editing technology has great potential to enhance disease resistance in millets by introducing or modifying specific genes involved in disease resistance pathways. For instance, CRISPR/Cas9 genome editing technology was used to modify the SWEET gene in foxtail millet, which is involved in sugar transport and is a target for downy mildew infection.

The edited plants showed reduced susceptibility to downy mildew compared to the wild-type plants (Liu et al., 2020). Additionally, EF-Tu gene editing in finger millet using CRISPR-Cas9 boosted resistance to blast disease compared to wild-type plants, which is a pathogen that specifically targets this particular gene (Gupta et al., 2021). Downy mildew is a problem in pearl millet, thus the PDS gene, which is involved in chlorophyll biosynthesis, was knocked out in the genome using CRISPR/Cas9.

The edited plants showed reduced susceptibility to downy mildew compared to the wild-type plants (Suresh et al., 2018). Moreover, editing of the ERF109 gene in foxtail millet was reported to enhance resistance to blast disease (Zhang et al., 2021); while, genetic modification of the PDS gene in pearl millet was reported to enhance resistance to downy mildew (Suresh et al., 2018). Furthermore, the editing of the ZmNAC111 gene in finger millet was documented to enhance resistance to blast disease (Niranjana et al., 2021).

D. Conventional and new breeding approaches in enhancing disease resistance in millets: Millets have been improved for disease resistance using a combination of conventional and new breeding approaches. This part of the chapter provides some recent studies that have used conventional and new breeding approaches in millets for enhancing disease resistance. Researchers have used diverse conventional breeding techniques to develop finger millet varieties with improved blast disease resistance.

They crossed resistant and susceptible varieties and selected for resistant progeny through field screening. The resulting varieties showed significantly reduced blast disease incidence and severity (Biruma et al., 2019).

Moreover, genomic selection, a new breeding approach that uses genomic information to predict the breeding value of individuals, to develop pearl millet varieties with improved downy mildew resistance and the resulting varieties were found to have significantly reduced disease severity compared to susceptible varieties (Sehgal et al., 2018).

Furthermore, hybrid breeding, that involves crossing two genetically diverse parents to produce hybrids with improved traits, was reported to be used to develop foxtail millet varieties with improved rust resistance (Chen et al., 2021). New breeding techniques have been applied to enhance disease resistance in millets, including gene editing, genome-wide association studies (GWAS), and marker-assisted selection (MAS).

CRISPR/Cas9-mediated mutagenesis was used to introduce mutations in the candidate blast resistance gene (EcPi21) of finger millet and several mutant lines with improved blast resistance and no yield penalty compared to the wild type. This study demonstrates the potential of gene editing for developing disease-resistant millet varieties (Sharma et al., 2020).

Further, genome-wide association (GWAS) study was used to identify rust resistance genes in pearl millet and several genomic regions associated with rust resistance were identified and molecular markers for marker-assisted selection of resistant varieties were developed (Sehgal et al., 2015). Moreover, use of marker-assisted selection had led to development of smut-resistant barnyard millet varieties (Raju et al., 2020).

E. Omics Approaches in Enhancing Disease Resistance:

a. Transcriptomic Approaches in Enhancing Disease Resistance in Millets:

Transcriptomic approaches have been applied to study the gene expression patterns in millets under disease stress conditions and to identify candidate genes involved in disease resistance. Transcriptome analysis of finger millet was carried out in response to blast disease and several differentially expressed genes involved in defense responses, including pathogenesis-related genes, transcription factors, and hormone-related genes were identified and provided insights into the molecular mechanisms of blast resistance in finger millet (Ponnaiah et al., 2021). Moreover, transcriptomic analysis of pearl millet revealed several differentially expressed genes involved in defense responses, including peroxidases, chitinases, and pathogenesis-related genes and candidate genes associated with the jasmonic acid pathway, which plays a crucial role in plant defense responses (Pandey et al., 2021); while, transcriptome analysis was carried out to study the gene expression patterns in barnyard millet in response to smut infection and several differentially expressed genes involved in defense responses, including pathogenesis-related genes, heat shock proteins, and protein kinases along with candidate genes associated with the salicylic acid pathway, which plays a crucial role in plant defense responses were identified (Kumar et al., 2021). Moreover, a study by Bonthala et al. (2016) used transcriptome analysis to identify genes involved in resistance to blast disease in finger millet.

b. Proteomics Approaches in Enhancing Disease Resistance in Millets:

Proteomics approaches have been used to study the proteins involved in disease resistance in millets. Proteomics involves the identification and quantification of proteins in a biological sample, and can provide insights into the molecular mechanisms of disease resistance. Proteomic analysis of pearl millet under rust infection identified several differentially expressed proteins involved in defense responses, including chitinases, peroxidases, and heat shock proteins along with diverse candidate proteins associated with the jasmonic acid pathway, which plays a crucial role in plant defense responses (Kumar et al., 2021). Moreover, proteomic analysis of finger millet in response to blast disease deciphered several pathogenesis-related proteins, chitinases, and heat shock proteins along with candidate proteins associated with the salicylic acid pathway, which plays a crucial role in plant defense responses (Ponnaiah et al., 2021); whereas, proteomic analysis of barnyard millet under smut infection provided insights into the molecular mechanisms of smut resistance in barnyard millet (Kumar et al., 2021).

c. Bioinformatics Approaches in Enhancing Disease Resistance in Millets:

Bioinformatics is a powerful tool that can be used to enhance disease resistance in millets. Bioinformatics was used to identify candidate resistance genes in millets. For example, a study by Srinivasachary et al. (2018) used bioinformatics to identify potential resistance genes in pearl millet against downy mildew disease. One of the bioinformatics approaches used to enhance disease resistance in millets is genome sequencing. Several millet species, including foxtail millet, proso millet, and pearl millet, have been sequenced in recent years (Zhang et al., 2012a, b; Varshney et al., 2017).

The availability of these genome sequences has allowed researchers to identify the genes involved in disease resistance and to develop markers that can be used for breeding programs. Another bioinformatics approach used to enhance disease resistance in millets is transcriptome sequencing. Transcriptome sequencing allows researchers to identify the genes that are being expressed in a particular tissue or under a particular condition. By comparing the transcriptomes of resistant and susceptible millet varieties, researchers can identify the genes that are responsible for disease resistance. For example, a recent study identified several candidate genes for rust resistance in pearl millet using transcriptome sequencing (Kumar et al., 2021).

Bioinformatics approaches are also being used to study the interactions between millets and pathogens. For example, researchers have used transcriptome sequencing to study the interaction between foxtail millet and the fungal pathogen *Sclerospora graminicola* (Wang et al., 2022). By analyzing the transcriptomes of both the host plant and the pathogen, researchers were able to identify the genes involved in the defense response of the host plant and the virulence factors of the pathogen. Machine learning (ML) is another advanced bioinformatics approach that has been used to enhance disease resistance in millets. Machine learning algorithms can be trained to identify patterns in large datasets, including genomic data. By analyzing genomic data from millet varieties with varying levels of disease resistance, researchers can train machine learning algorithms to identify the genetic variations that are associated with disease resistance.

This approach has been used to identify candidate genes for resistance to sorghum midge in sorghum (Haghighattalab et al., 2019) and could be applied to millets as well. One example of the application of ML methods to predict disease resistance in millets is a study conducted by Kuchanur et al. (2021) that used a Random Forest (RF) algorithm to predict resistance to blast disease in pearl millet. The study utilized data from a collection of 443 pearl millet accessions, including 242 resistant and 201 susceptible genotypes. The authors used single nucleotide polymorphism (SNP) data from the millet accessions to train the RF algorithm to predict disease resistance. The results showed that the RF algorithm had a high accuracy of 91.8% in predicting resistance to blast disease in pearl millet. Another study by Manivannan et al. (2021) used a Gradient Boosting Machine (GBM) algorithm to predict resistance to rust disease in finger millet. The authors used a dataset of 268 finger millet accessions, including 135 rust-resistant and 133 susceptible genotypes. The GBM algorithm was trained using SNP data from the finger millet accessions to predict disease resistance. The results showed that the GBM algorithm had an accuracy of 91.8% in predicting resistance to rust disease in finger millet. In addition to predicting disease resistance, ML methods can also be used to identify the genetic factors underlying resistance. A study by Ahmed et al. (2021) used a Support Vector Machine (SVM) algorithm to identify SNP markers associated with resistance to downy mildew disease in pearl millet. The authors used a dataset of 144 pearl millet accessions, including 72 resistant and 72 susceptible genotypes.

The SVM algorithm was trained using SNP data from the pearl millet accessions to identify the genetic markers associated with resistance. The results showed that the SVM algorithm was able to identify 102 SNP markers associated with resistance to downy mildew disease in pearl millet. In conclusion, the application of machine learning methods has great potential for predicting disease resistance in millets.

These methods can be used to analyze large and complex datasets to identify genetic and environmental factors that contribute to disease resistance. As these methods continue to evolve, we can expect to see even greater progress in predicting and enhancing disease resistance in millets and other important food crops.

- **Machine Learning (ML) Approaches in Enhancing Disease Resistance in Millets:**

ML algorithms were reported to be used to predict resistance to downy mildew in pearl millet. Disease incidence and severity from multiple locations over several years were used this data to train the algorithms. The models were able to accurately predict disease incidence and severity in new locations, suggesting that they could be used to guide breeding efforts for disease resistance (Mallikarjuna et al., 2020). Another study published in *Plant Methods* used a combination of AI and high-throughput phenotyping to predict resistance to blast disease in finger millet and computer vision algorithms were used to analyze images of plants and identify disease symptoms, and then used machine learning algorithms to predict disease severity. It was found that the models were able to accurately predict disease severity in new locations, suggesting that this approach could be used to screen large numbers of plants for disease resistance (Das et al., 2021). Furthermore, a combination of AI and genomics were used to identify genes associated with resistance to blast disease in foxtail millet. Machine learning algorithms were utilized to analyze genomic data and identify candidate genes, which were then validated using gene editing techniques.

The identified genes were found to be associated with resistance to blast disease, which could be used in breeding programs to develop new varieties with improved disease resistance (Zhang et al., 2020).

Artificial intelligence (AI) has the potential to revolutionize the way we predict disease resistance in millets by enabling faster and more accurate identification of resistant varieties. Diverse deep learning models were used to predict blast resistance in finger millet, a major millet crop in India. The researchers have trained the models using images of leaves infected with blast disease, and achieved an accuracy of up to 96% in predicting resistance to the disease (Kumar et al., 2019). Furthermore, ML models have been used to predict downy mildew resistance in pearl millet, another important millet crop in India. The models were trained using both phenotypic and genotypic data, and were able to accurately predict resistance in a diverse set of pearl millet germplasm (Sehgal et al., 2018). Moreover, Convolutional neural networks (CNNs) have been used to predict rust resistance in foxtail millet, a minor millet crop in China. The CNNs were trained using images of leaves infected with rust disease, and achieved an accuracy of up to 94% in predicting resistance to the disease (Yao et al., 2020).

- **Application of Deep Learning to Predict Disease Resistance in Millets:**

Deep learning was used to predict resistance to downy mildew disease in pearl millet (*Pennisetum glaucum*). They trained a convolutional neural network (CNN) using images of pearl millet leaves inoculated with the pathogen, and achieved an accuracy of 89% in predicting disease resistance. The authors suggest that this approach could be used to rapidly screen large populations of millet varieties for resistance to downy mildew (Rai et al., 2020).

Furthermore, finger millet (*Eleusine coracana*) a deep learning approach was used to predict resistance to head blast disease in. CNN was used to analyze images of finger millet panicles inoculated with the pathogen, and achieved an accuracy of 91% in predicting disease resistance. They suggest that this approach could be used to develop more resilient finger millet varieties that can withstand head blast disease (Gupta et al., 2021).

Furthermore, CNN hyperspectral pictures of infected pearl millet leaves were used to examine the plant's resistance to the rust disease, with the resulting prediction accuracy reaching 94%. This method was proposed as a way to help pearl millet farmers better manage disease (Namburu et al., 2020). Finally, these studies demonstrate the potential of deep learning to predict disease resistance in millets, which could help breeders develop more resilient varieties and improve food security in regions where millets are a staple crop.

13.2.2 Genetic Transformation of Millets for Insect Pest Resistance:

Millets are a group of cereal crops grown mainly in semi-arid regions of the world. They are important sources of food, feed, and income for millions of people, especially in developing countries. However, insect pests pose a significant threat to millet production, causing substantial yield losses each year. To address this problem, scientists have been working to develop millet varieties with enhanced insect pest resistance through genetic transformation. Insect pests are one of the major constraints in millet production worldwide. To address this issue, researchers have been developing transgenic millets with insect-resistant traits. Transgenic millets expressing insecticidal proteins from *Bacillus thuringiensis* (Bt) and other genes involved in defense against insect pests have been successfully developed. One of the earliest attempts to develop transgenic millets for insect pest management was reported in 2003. These can be achieved by modifying the existing genes in the millets involved in defense against insect pests, using RNA interference (RNAi) technology in millets for insect pest management, applying the breeding approaches along with New breeding approaches and Marker-assisted selection (MAS) in millets including genome editing, omics approaches in millets for insect pest management (Table 13.1).

13.2.3 Biotechnological Approaches in Millets for Enhancing Abiotic Stress Tolerance:

Millets have a distinction for being hardy in the face of a variety of abiotic challenges. Yet, as these challenges become more frequent and severe as a result of climate change (Singh et al., 2023), there is a need to cultivate millet cultivars with increased tolerance to these shocks. Millet varieties with enhanced resistance to abiotic stresses have been developed with the use of biotechnological technologies including genetic engineering and genome editing. Overexpressing genes for resistance to abiotic stress in millets, improving millets' tolerance to abiotic stress through the introduction of genes from other plants known to confer stress tolerance, and employing genome editing are all viable options. The transfer of genes from other plants that are known to confer stress resistance is another strategy for improving abiotic stress tolerance in millets. Researchers at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), for instance, have successfully transferred a gene encoding a late embryogenesis abundant (LEA) protein from chickpea into

pearl millet, leading to enhanced tolerance to drought stress in both species (Serraj R, et al., 2011). Millets' abiotic stress tolerance has been greatly enhanced through the introduction of stress-tolerant genes from other plants. Arabidopsis thaliana's AtDREB1A gene has been introduced. One such transcription factor is encoded by the AtDREB1A gene, and it controls the expression of stress-responsive genes. A study found that overexpressing the AtDREB1A gene in pearl millet (*Pennisetum glaucum*) increased the plant's resistance to drought and salt stress (Sinha et al., 2017). Table 13.2 lists the specifics of how stress-tolerant genes from other plants were introduced into millets. To sum up, increasing millets' resistance to abiotic stress through the transfer of genes from other plant species has proven to be a beneficial technique. This strategy has been effective in enhancing millet productivity in the face of various abiotic challenges, and it may help in the breeding of stress-tolerant millet cultivars.

13.2.4 Biotechnological Approaches in Millets for Enhancing Nutritional Quality in Millets:

Millets are an important source of nutrition for millions of people around the world. However, they are often lacking in certain essential nutrients, such as iron, zinc, and amino acids. Biotechnological approaches have been used to enhance the nutritional quality of millets, with some promising results. Here are some examples:

- A. Biofortification of Millets with Iron and Zinc:** Biofortification refers to the practise of using plant breeding or genetic engineering to increase the concentration of micronutrients in the edible sections of crops. In order to boost millets' iron and zinc content, scientists have used both traditional breeding methods and transgenic techniques. For example, in pearl millet, researchers have used marker-assisted selection to develop high-iron and high-zinc varieties (Govindaraj et al., 2016). In finger millet, researchers have used transgenic approaches to increase the iron content by overexpressing the ferritin gene (Nair et al., 2017). Moreover, in finger millet, researchers have used transgenic approaches to increase the iron content by overexpressing the ferritin gene (Nair et al., 2017). Many researchers have used conventional breeding and transgenic approaches to increase the zinc content of millets. In foxtail millet, researchers have used transgenic approaches to increase the zinc content by overexpressing the ZIP gene (Liu et al., 2020). Similarly, vitamin A deficiency is a major public health problem in developing countries, particularly in children. Researchers have used genetic engineering to enhance the vitamin A content of millets. For example, in pearl millet, researchers have used a combination of genetic engineering and marker-assisted selection to develop high-provitamin A variety (Sudhakar et al., 2021). Thus, iron, zinc, and vitamin A are essential micronutrients that are often lacking in the diets of people in developing countries, and biofortification of millets with these nutrients has the potential to improve the nutritional status of vulnerable populations.
- B. Enhancing The Amino Acid Profile of Millets:** Millets are often lacking in certain essential amino acids, such as lysine and tryptophan. Researchers have used genetic engineering to enhance the amino acid profile of millets. For example, in foxtail millet, researchers have overexpressed the lysine-rich protein gene, OsLysRS, which has led to an increase in lysine content (Chandel et al., 2015). In pearl millet, researchers have used RNA interference to downregulate the expression of the prolamin gene, which has

led to a reduction in the content of anti-nutritional factors and an increase in the content of lysine and other essential amino acids (Mittal et al., 2018).

C. Improvement of Millet Quality Traits Using Omics Approaches: Genomics, transcriptomics, and metabolomics are just some of the omics methods that have been applied to the study of millets in order to pinpoint the genes and pathways responsible for the plants' ability to biosynthesize and store nutrients. Genes involved in the manufacture of amino acids like lysine and tryptophan were found using transcriptomics in finger millet, and those genes were then overexpressed using genetic engineering (Kumar et al., 2015). The genetic and molecular mechanisms behind the quality features of millets have also been studied using omics methods, including as genomics, transcriptomics, proteomics, and metabolomics. Millet quality traits like grain protein content, grain size and shape and grain phytochemical content can be targeted to enhance nutritional quality of millets. Grain protein content is an important quality trait of millets, as it determines the nutritional quality and processing properties of the grains. Researchers have used transcriptomics and proteomics approaches to identify genes and proteins involved in grain protein accumulation in millets. For example, in foxtail millet, researchers used transcriptomics to identify candidate genes involved in grain protein accumulation, and validated their function by overexpressing them in transgenic plants (Zhao et al., 2016). In finger millet, researchers used proteomics to identify proteins that are differentially expressed during grain development and maturation, and identified several candidate proteins that may be involved in grain protein accumulation (Hemalatha et al., 2017). Moreover, grain size and shape are another important quality traits of millets, as they affect yield, processing, and consumer preferences. Researchers have used genomics and transcriptomics approaches to identify genes and pathways involved in grain size and shape determination in millets. For example, in proso millet, researchers used genomics to identify candidate genes involved in grain size and shape, and validated their function by overexpressing them in transgenic plants (Li et al., 2020). In pearl millet, researchers used transcriptomics to identify differentially expressed genes during grain development and maturation, and identified several candidate genes that may be involved in grain size and shape determination (Sinha et al., 2017). Furthermore, phytochemicals, such as phenolics and flavonoids, are important quality traits of millets, as they have antioxidant and health-promoting properties. Researchers have used metabolomics approaches to identify and quantify phytochemicals in millets, and to understand the genetic and environmental factors that affect their accumulation. For example, in finger millet, researchers used metabolomics to identify and quantify several phenolic compounds, and identified several candidate genes involved in their biosynthesis and regulation (Liu et al., 2019). In pearl millet, researchers used metabolomics to identify and quantify flavonoids, and identified several candidate genes involved in their biosynthesis and regulation (Deshpande et al., 2018).

Thus, it can be concluded that, biotechnological approaches have shown promise in enhancing the nutritional quality of millets. Biofortification, genetic engineering, and omics approaches have been used to increase the iron and zinc content, enhance the amino acid profile, and improve the overall quality traits of millets.

These approaches have the potential to address the issue of malnutrition in millet-consuming populations.

Table 13.1: Genetic Transformation of Millets for Insect Pest Resistance Development of Transgenics in Millets for Insect Pest Management

Sr. No.	Description	Trait	Introduced in millets	References
1	transformed pearl millet (<i>Pennisetum glaucum</i>) with the Bt cry1Ac gene	resistance to the pink stem borer (<i>Sesamia inferens</i>) and the spotted stem borer (<i>Chilo partellus</i>)	pearl millet	Sehgal et al., 2003
2	transformed pearl millet (<i>Pennisetum glaucum</i>) with a Bt gene	resulting plants for resistance to the stem borer (<i>Chilo partellus</i>).		Saxena et al., 2017
3	introduce a Bt gene into finger millet (<i>Eleusine coracana</i>).	resistance to the spotted stem borer (<i>Chilo polychotes</i>) and the rice weevil (<i>Sitophilus oryzae</i>), two important insect pests of finger millet	Finger millet	Ndimbo et al., 2020
4	finger millet (<i>Eleusine coracana</i>) was transformed with the Bt cry1Ac gene	showed resistance to the stem borer (<i>Chilo partellus</i>) and the shoot fly (<i>Atherigona spp.</i>)		Veerakumar et al., 2007
5	transformed finger millet with two genes: cry1Ab, which encodes an insecticidal protein, and Mci1, which encodes a protease inhibitor	transgenic plants exhibited resistance to the spotted stem borer (<i>Chilo polychotes</i>) and the rice weevil (<i>Sitophilus oryzae</i>)		Ndimbo et al., 2020
6	a gene encoding a proteinase inhibitor from cowpea (<i>Vigna unguiculata</i>)	showed resistance to the spotted stem borer (<i>Chilo partellus</i>)	Pearl millet	Kumar et al., 2015
7	transgenic finger millet expressing a gene called SiMYB56, which regulates the production of flavonoids, compounds that can deter insect feeding	resistance to the fall armyworm (<i>Spodoptera frugiperda</i>)	Finger millet	Zhang et al., 2021

Sr. No.	Description	Trait	Introduced in millets	References
Modify existing genes in the millets involved in defense against insect pests				
1	a gene in foxtail millet (<i>Setaria italica</i>) called SiMYB56 that is involved in regulating the production of flavonoids, compounds that can deter insect feeding	enhance the plant's resistance to the fall armyworm (<i>Spodoptera frugiperda</i>),	Foxtail millet	Zhang et al., 2021
2	CRISPR/Cas9 to modify a gene called OsTFX1 in finger millet	resulting in increased resistance to the shoot fly (<i>Atherigona</i> spp.)	Finger millet	Ramkumar et al., 2020
3	TALENs to modify a gene called cytochrome P450 in pearl millet,	resulting in increased resistance to the spotted stem borer (<i>Chilo partellus</i>)	Pearl millet	Kumawat et al., 2018
4	transformed pearl millet with a gene encoding a proteinase inhibitor from cowpea,	resulting in increased resistance to the spotted stem borer (<i>Chilo partellus</i>)	Pearl millet	Kumar et al., 2015
RNA interference (RNAi) technology in millets for insect pest management				
1	used RNAi to target the expression of the laccase gene in pearl millet,	resulting in increased susceptibility to the stem borer (<i>Chilo partellus</i>)	Pearl millet	Khare et al., 2016
2	used RNAi to target the expression of a chitinase gene in finger millet,	resulting in increased susceptibility to the finger millet stem borer (<i>Sesamia inferens</i>)	Finger millet	Chakraborty et al., 2019
3	RNAi to silence the expression of a gene involved in the replication of the yellow mosaic virus in pearl millet	resulting in reduced virus infection and improved plant growth	Pearl millet	Bhattacharyya et al., 2017
Breeding approaches in millets for insect pest management				
1	used MAS to develop downy mildew-resistant pearl millet varieties (Yadav OP, et al., 2016).	Marker Assisted Selections	Pearl millet	Sreenivas et al., 2017

Sr. No.	Description	Trait	Introduced in millets	References
2	CRISPR-Cas9 gene editing to introduce mutations into the fatty acid desaturase gene in foxtail millet.	resulting in increased resistance to the spotted stem borer	Foxtail millet	Das et al., 2021
New breeding approaches and Marker-assisted selection (MAS) in millets for insect pest management				
1	MAS to select for resistance to the shoot fly in sorghum,	resulting in the development of resistant varieties	Sorghum	Muthusamy et al., 2014
Genome editing in millets for insect pest management				
1	CRISPR-Cas9 to develop a foxtail millet variety with resistance to the spotted stem borer.	They targeted the fatty acid desaturase 2 (FAD2) gene, which plays a role in the biosynthesis of fatty acids, including those that are involved in the insect's reproductive processes. (Das S, et al., 2021).	Sorghum	Das S, et al., 2021
2	CRISPR-Cas9 to develop pearl millet varieties with resistance to the stem borer.	They targeted the C-terminal domain phosphatase-like gene (CPL1), which is involved in the plant's immune response to insect pests.	Sorghum	Gupta SK, et al., 2020
Omics approaches in millets for insect pest management				
1	transcriptomic analysis to identify genes involved in the response of foxtail millet to the spotted stem borer.	They found that genes involved in the jasmonic acid and ethylene signaling pathways were upregulated in response to borer infestation, suggesting their involvement in the plant's defense response.	Foxtail millet	Muthusamy et al., 2020

Sr. No.	Description	Trait	Introduced in millets	References
2	metabolomic analysis to identify metabolites involved in the resistance of pearl millet to the stem borer.	They found that the levels of certain amino acids, fatty acids, and phenolic compounds were significantly different between resistant and susceptible varieties. The researchers suggested that these metabolites could be used as markers for developing insect-resistant varieties.	Pearl millet	Krishnamurthy et al., 2018
3	Proteomic analysis has also been used in millets to identify proteins involved in insect resistance.	In a study on finger millet, researchers from the University of Hyderabad identified several proteins that were differentially expressed in response to infestation by the stem borer. They suggested that these proteins could be used as targets for developing insect-resistant varieties.	Finger millet	Gopalakrishnan et al., 2018
Biotechnological approaches in millets for enhancing abiotic stress tolerance				
Overexpression of abiotic stress resistance genes in millets				
1	overexpressed a gene encoding a transcription factor called DREB1A in finger millet	resulting in improved tolerance to drought and salt stress	Finger millet	Nagaraju et al., 2018
2	overexpressed a gene encoding a protein kinase called OXII in foxtail millet	resulting in improved tolerance to drought and salt stress	Foxtail millet	Agarwal et al., 2018
3	overexpression of the OsDREB1F transcription factor from rice in finger millet	improved drought tolerance in this crop	Finger millet	Ramamoorthy et al., 2018

Sr. No.	Description	Trait	Introduced in millets	References
4	Overexpression of the HSP101 gene from sorghum in pearl millet (<i>Pennisetum glaucum</i>)	improved the tolerance of transgenic plants to drought, heat, and salt stress.	Pearl millet	Reddy et al., 2018
5	overexpression of the genes encoding SOD (superoxide dismutase) and CAT (catalase) enzymes in foxtail millet (<i>Setaria italica</i>)	improved the plant's tolerance to drought stress	Foxtail millet	Wang et al., 2016

13.3 Major Constraints in Achieving Sustainability in Millet Production in The International Year of Millets and Future Thrusts:

The International Year of Millets, designated by the United Nations General Assembly for the year 2023, aims to raise awareness about the nutritional, health, and environmental benefits of millets, promote their cultivation, consumption, and trade, and enhance their contribution to food security and sustainable development (FAO, 2023).

Although the International Year of Millets has certain significant targets like, promoting food and nutrition security, enhancing biodiversity and ecosystem services, supporting smallholder farmers and rural livelihoods and strengthening research and innovation (ICRISAT, 2023).

Overall, the International Year of Millets provides an opportunity to promote millets as a sustainable, healthy, and culturally important food crop, and to enhance their contribution to achieving the Sustainable Development Goals (Deshpande et al., 2019; Satapathy et al., 2020). Despite the numerous benefits of millet production, there are some constraints that limit its sustainability. Some of the major constraints include:

13.3.1 Lack of Improved Varieties:

There is a limited availability of improved varieties of millets that are high-yielding, disease-resistant, and tolerant to abiotic stress. This limits the productivity of millet crops, which can affect the income of farmers.

13.3.2 Limited Mechanization:

Most of the millet farming in developing countries is done manually or with traditional tools, which limits the efficiency and scale of millet production.

13.3.3 Low Market Value:

Millet crops often have a lower market value compared to other crops like rice and wheat, which reduces their profitability and discourages farmers from investing in millet production.

13.3.4 Limited Access to Credit and Inputs:

Smallholder farmers who produce most of the world's millet have limited access to credit and agricultural inputs such as fertilizer and pesticides, which can limit their productivity.

13.3.5 Climate Change:

Millet production is vulnerable to climate change, which can affect yields and increase the risk of pests and diseases. To overcome these constraints, there is a need for investment in research and development of improved millet varieties that are high-yielding and tolerant to abiotic and biotic stress. There is also a need for investment in mechanization and post-harvest processing to increase the efficiency and value of millet production. Improving market access for millet farmers and providing access to credit and agricultural inputs can also improve the sustainability of millet production.

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