Biotechnological Approaches toward Blast Disease Resistance in Finger Millet

Abstract

Plant disease globally causes significant losses in crop production that hindering the urgent need for a 60% increase in food demand. The main impact is reduced food quantity and quality leading to compromised food safety from pesticides and toxins. Biotechnology is essential for protecting crop yield and enhancing sustainability in agriculture. Agricultural biotechnology has advanced offering options for understanding plant molecular mechanisms and breeding.The devastating finger millet blast disease caused by the fungus *Magnaportheoryzae* affects crop growth and yield leading to instability due to breakdown of blast resistance. Novel strategies are essential for sustainable finger millet production focusing on breeding techniques such as molecular markers, expressed sequence tags (ESTs), gene expression profiling, genome-wide association studies, and genetic transformations to develop new disease-resistance varieties. This comprehensive review explores current breeding strategies and future directions that may offers insights for effective disease control and optimizing finger millet productivity.

Keywords: Finger millet, *Magnaportheoryzae*, QTLs, genotyping by sequencing (GBS)

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Introduction

Finger millet scientifically known as *Eleusinecoracana*L. Gaertn. belongs to the *poaceae* family as an allotetraploid (2n=4X=36) plant. It is a staple crop grown mainly in impoverished less watery regions of Asia and Africa [36]. This crop stands out for its highly beneficial nutraceutical properties including high calcium, fiber, essential amino acids, vitamins, and iron that surpassing other major cereals [13]. Finger millet is gluten-free also has long shelf life and is highly resilient to various climatic conditions making it the fourth most produced crop globally after sorghum, pearl millet and foxtail millet [38,44,45,46]. The United Nations General Assembly declared 2023 the International Year of Millets for recognizing their resilience and potential for sustainability as they can grow on arid lands with minimal inputs. World production of millets is 30.85 million metric tons from an area harvested of 29.55 million hectares during 2022 in which over 90 million people in Africa and Asia rely on millets with Africa accounting for over 55% of globe production and Asia nearly 40% and Europe around 3% [9].Efforts are needed to enhance finger millet production by 40% to feed the ballooning population and sustain food security [18]. The genetic resources of finger millet are crucial for its improvement and have been conserved in various gene banks worldwide with significant collections held by institutions. The finger millet germplasm contains valuable alleles for desired traits aiding researchers and breeders in their improvement programme. Despite limited information the systematic utilization of GenBank accessions in finger millet programs has been slow. Blast disease caused by *Magnaportheoryzae* poses a significant threat to finger millet production impacting various crops and necessitating ongoing research for developing resistant varieties. Small grey or brownish specks on the leaves are the initial sign of blast infections. After two to three days of infection the spots develop into wide diamond-shaped lesions with a greyish or whitish centre [23]. According to reports in wide range of rice cultivation areas with suitable conditions blast can cause output losses of up to 50% [31]. This review explores available information and cutting-edge techniques including markers, gene mapping, NGS-based genomics and modern breeding tools to study finger millet blast disease.

Biology and Pathogenicity of *Magnaportheoryzae*

M. oryzae is a plant-pathogenic filamentous ascomycete fungus that causes blast disease on over 50 monocot plant species [11]. It is heterothallic ad has two mating types such as MAT1 and MAT2 which occur in a single gene. However, isolates from single agroecological regions usually have only one mating type and strains cannot interbreed [35]. Sexual recombination may contribute to genetic variability. The finger blast fungus infects the host in two stages: Biotrophic (nutrients from live cells) and necrotrophic (nutrients from dead cells) [28]. During infection conidia attach to the host leaf surface allowing germination and germ tubes to form melanizedappressoria. Once inside the host tissues the fungus spreads to adjoining cells through the plasmodesmata without causing any perceptible alteration to the host cell walls. Under favourable conditions the fungus sporulates abundantly from disease lesions that allowing the disease to spread quickly [21, 47].

Breeding strategies to develop disease resistant varieties

The pursuit of new finger millet varieties with combined traits of increase grain yield, resistance to stresses and enhanced nutritional quality is a current breeding objective. Conventional breeding methods aim to transfer robust resistance to blast disease into adapted finger millet

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germplasm but the challenges in finger millet breeding are varied inheritance models for resistance sources to *M. oryzae*and drawbacks of conventional breeding methods. To address these challenges the recent fast-growing high-throughput sequencing techniques together with machine learning and artificial intelligence has led in widespread for finding of multiple genetic resources in plants. A variety of crops including rice, soybeans, maize and wheat have benefited from the use of novel breeding techniques such as genomic-assisted breeding, and gene pyramiding are being explored to develop resistance against blast disease and improve overall crop trait [10]. In response to concerns over genetically modified organisms' researchers are exploring genome editing tools to induce genome editing without creating transgenic plants [24]. Finger millet a lag behind compare to other cereals in genetic research and biotechnological improvements due to limited interest and investment [32]. However, advancements in technology are reducing costs, facilitating more research and leading to the exploration of crop vast germplasm of genetic studies. The forthcoming whole-genome sequencing of finger millet will enable in-depth research on important traits through advanced breeding approaches. Leveraging genomics similarities with rice and other important crops researchers aim to enhance finger millet resistance to diseases through genetic improvement and mutational breeding. Speed breeding technology enables achieving multiple generations per year serving as a valuable tool to shorten breeding cycles significantly by utilizing extended photoperiods and controlled temperatures for the main cereal crops. Although finger millet is a short-day tropical plant and its specific speed breeding protocols remains undeveloped that emphasizing the need to optimize cost-effective parameters to fully leverage the benefits of speed breeding technology for accelerating research, enhance resilience and boosting yield of finger millet to meet the food security challenges posed by population growth. Genome collinearity studies reveals high genomic synteny between finger millet and rice, foxtail millet and maize [27]. Blast resistance in finger millet is investigated using genomics to improve resistance. Several candidate genes in finger millet connected to blast pathogen have been identified and sequenced [26]. These genes and QTLs display collinearity in rice and barley that indicating a common evolutionary origin. Comparative studies with data from various plants can help identification of new alleles for blast resistance. Mutation developed breeding in finger millet to develop elite cultivar has not been reported but it can be explored based on successful rice mutation breeding. Traditional finger

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millet landraces are key genetic backgrounds for breeding approach [25]. The long breeding cycle and polygenic nature of traits like blast disease resistance pose challenges.

Genomic resources pave into blast disease improvement

Genomic resources like DNA-base markers are highly valued in plant breeding for various applications in finger millet breeding [39]. Isozyme markers revealed fixed heterozygosity in finger millet while DNA-based markers like RFLP, AFLP, SSR and EST have been utilized to construct genetic maps [2]. High-throughput sequencing techniques such as genotyping by sequencing (GBS) that enables rapid and cost-effective generation of large sequence datasets for SNP identification in numerous agriculturally important crops [8]. GBS has been successfully employed in assessing genetic diversity in *Vanilla planifolia* and olive germplasm. Accordingly, GBS technology has proven effective in identifying genetic traits and markers in finger millet against disease like blast.

QTLs and genes for blast disease resistance

Genomic resources like markers have been key regulator for locating blast resistance and designing architecture in traits improvements in crops including rice and foxtail millet [34]. Blast resistance is complex trait that are governed by multiple genes. Various strategies have been employed to clone nine blast R-genes in rice all belonging to the NBS-LRR family [1]. Studies suggest that orthologous of rice blast R-genes may play a key role in conferring resistance in finger millet. Genetic mapping and molecular characterization aid in improving finger millet through genome-assisted breeding with tools like genome wide association study (GWAS) analysis being commonly used. In finger millet QTL for finger blast and neck blast resistance were identified using genic-SSR markers associated with blast QTL with [3] detecting five significant QTL linked to FMBLEST32 and rice SSR RM262 derived from Pi5 blast gene for broader resistance to *M. oryzae*. [29] found two leaf blast resistance QTL connected to markers UGEP101 and UGEP95 through association study.

Blast resistance through gene pyramiding

The challenge posed by blast disease on finger millet highlight the importance of developing innovative tactics to boost resistance against the changing *M. oryzae* pathogen under fluctuating

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environments. Fungicides are costly and inaccessible to many smallholder farmers in Africa and Asia making resistant millet varieties a more practical option. Although sources of resistance have been identified, developing durable resistance is challenging due to the pathogen virulence diversity [3]. incorporating diverse R-genes in a single plant can prevent infection from multiple pathogen strains hinders fungal evolution by blocking recombination between fungal races [41]. Gene pyramiding is stacking multiple genes in a plant [4] that has shown promise in controlling fungal diseases in crops like rice, maize, and wheat [43] but its application in finger millet remains unexplored. Empirical evidence suggest that gene pyramiding is the most effective strategy for providing durable resistance to plant pathogens thereby despite many challenges such as compromised efficacy and independent mutations in virulence genes. The study [5] evaluated three methods to manage root-knot nematode in pepper and lettuce over a period exceeding three years: cultivar mixture, crop rotation and pyramiding of R-genes. The research findings indicated that pyramiding multiple genes in one plant genotype provided more lasting resistance to nematodes compared to cultivar mixtures and crop rotations. Despite the benefits of gene pyramiding, challenges such as long breeding periods have been addressed through innovative techniques like marker assisted selection and genetic engineering to streamline the process of combining multiple resistance genes into a single variety thus enhancing disease control strategies [30]. Nonetheless, the risk of breakdown of pyramided genes leading to the emergence of more aggressive pathogen strains like *M. oryzae* underscores the need to find a balance between economic feasibility and effective disease management approaches [30].

Allele mining for blast disease resistance

The enhancement of crop breeding has greatly benefited from gathering valuable alleles from various plant genetic resources globally includes wild relatives and landraces that leads to the development of resilient cultivars. Introducing novel alleles from wild races into cultivated crop varieties has resulted in significant trait improvements as seen in studies like stripe rust resistant wheat [19] and tomato varieties resistant to various diseases [17]. Highlighting the potential for discovering new alleles to boost crop genetic potential in fighting diseases like blast disease. An examination of finger millet and rice genomes showed that most chromosomes are closely related with 85% synteny [33] while association mapping revealed a link between rice and finger

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millet blast resistance genes. Consequently, the rice blast resistance genes could be explored for allele mining in finger millet [3].

Blast disease resistance through transgenic approach

Finger millet biotechnological advancement has been slower than other cereals but genetic modifications crucial for enhancing its nutritional value and resilience. Challenges like recalcitrant nature and polyploidy hinder in vitro regeneration [20] and genotype-specific optimization is needed [7]. Techniques like biolistic and Agrobacterium mediated transformation have been used [14] but genotype-independent regeneration system are needed. Whole genome sequencing (WGS) is expected to facilitate genome editing technologies like CRISPR (Cluster regularly interspaced palindromic region). Building a protoplast-based regeneration system is critical to create blast-resistant finger millet and advance research in the crop [6].

Biotechnological implemented programme to boost immunity against *M. oryzae*

Immunity can prevent reduced plant growth and yield due to active defense responses which are controlled by transcriptional regulators [37]. Overexpression of specific transcription factors can engineer resistance without affecting yield. A study on rice gen Ideal Plant Architecture 1(IPA1), OsSPL14 showed that a naturally occurring allelic variant increased yield and resistance to rice blast. Phosphorylation of IPA1 in response to blast infection altered its binding specificity leading to the protein binding to WRKY45 a (defence regulator transcription factor) ensuring quantitative resistance [40]. Plants resistant to rice blast disease were generated through CRIPR/Cas9-mediated disruption of OsERF922 and OsSEC3A genes (38). OsSEC3A a mutant plant showed improved resistance against *M. oryzae* and higher level of salicylic acid and Dwarf stature. CRISPR/Cas9 editing of OsSWEET13 in rice achieved resistance to bacterial blight disease caused by *XanthomonasOryzae* [42].

Conclusion

Effective management strategies for diseases in crops are important for maintaining high-quality production and reducing environmental impacts. Identifying the genetic makeup of *M. oryzae* is crucial for developing finger millet varieties with different candidate genes to combat the devastating blast disease with methods like gene pyramiding, genome editing, mutational

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breeding, speed breeding and genetic engineering offering promising avenues for achieving durable resistance in improved cultivars. The creation of resistant varieties with lasting resistance by incorporating new genes is beneficial and sustainable method to manage finger millet blast disease. Molecular tools aid in identified and sourcing genes like blast R-genes and QTLs that offering a chance for enhancing finger millet through diverse breeding methods.

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