

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 due to climate changes. 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. This knowledge accelerates the identification and transfer of key resistance traits for crop breeding programs. Finger millet is crucial in southern Asia and Eastern Africa facing the devastating finger millet blast disease caused by *Magnaporthe oryzae*. This review is discussing on strategies for developing blast-resistance finger millet varieties and sharing information of analyzed and highlighting advanced molecular tools for breeding programs aiding in the introgression of disease resistance genes. This information useful for developing disease controlled elite cultivar of finger millet that may aid in crop productivity optimization.

Comment [M1]: A confusing sentence. Is the 60% decrease due to the impact of diseases or climate?

Keywords: Finger millet, *Magnaporthe oryzae*, QTLs, Genotyping by sequencing (GBS)

Comment [M2]: The most important findings of the article should be added, and introductions about the topic should be reduced in the abstract

Introduction

Finger millet scientifically known as *Eleusine coracana* 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 [30]. 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 [12]. 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 [31]. World production of millets is 89.17 million metric tons from an area of 74.00 million hectare during 2020 in which Sorghum and Pearl millet are the major millet crops grown, constituting above 90% of the world millets production followed by Finger millet, Foxtail millet, Proso millet, Barnyard, Little millet and Kodo millet[9].

Comment [M3]: There are more recent statistics from the United Nations organization, please refer to them

Efforts are needed to enhance finger millet production by 40% to feed the ballooning population and sustain food security [17]. 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 programmes programme. Despite limited information the systematic utilization of GenBankgenebank accessions in finger millet programs has been slow. Blast disease caused by *Magnaporthe oryzae* 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 center. According to reports in wide range of rice cultivation areas with suitable conditions blast can cause output losses of up to 50%. Continuous research on blast disease is crucial to create resistance against severe spread of blast by applying various multi-omics tools for sustaining production and productivity of finger millet in the upcoming future.

Comment [M4]: This phrase needs a reference because it refers to a numerical value of losses

Comment [M5]: I suggest that there be an overview of the disease, its life cycle, details of the conditions that lead to its spread, and methods of prevention.

Comment [M6]: Clearly rephrase the aim of the study

Formatted: English (United States)

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 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[21]. Finger millet lag behind compare to other cereals in genetic research and biotechnological

improvements due to limited interest and investment [27]. 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 multi-omics 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 [24]. 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 [23]. These genes and QTLs display collinearity in rice and barley that indicating a common evolutionary origin. Synthetic synteny 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 millet landraces are key genetic backgrounds for breeding approach [22]. 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 [32]. 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 [29]. 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*. [25] 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 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 [33]. 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 [34] 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. 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 [26].

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 [18] and tomato varieties resistant to various diseases [16]. 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 [28] while association mapping revealed a link between rice and finger 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 [19] and genotype-specific optimization is needed [7]. Techniques like biolistic and Agrobacterium mediated transformation have been used [13] 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].

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~~ ~~devasting~~ blast disease with methods like gene pyramiding, genome editing, mutational 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.

References

1. Ameline-Torregrosa C, Wang BB, O'Bleness MS, Deshpande S, Zhu H, Roe B, et al. Identification and characterization of nucleotide-binding site-leucine-rich repeat genes in the model plant *Medicago truncatula*. *Plant Physiol.* 2008;146, 5–21.
2. Babu BK, Agrawal PK, Pandey D, Jaiswal JP, Kumar A. Association mapping of agromorphological characters among the global collection of finger millet genotypes using genomic SSR markers. *Mol. Biol. Rep.* 2014a;41:5287–5297.
3. Babu BK, Dinesh P, Agrawal PK, Sood S, Chandrashekara C, Bhatt JC, et al. Comparative genomics and association mapping approaches for blast resistant genes in finger millet using SSRs. *PLoS One.* 2014b;9:e99182.
4. Delmotte F, Bourguet D, Franck P, Guillemaud T, Reboud X, Vacher C, et al. Combining selective pressures to enhance the durability of disease resistance genes. *Front. Plant Sci.* 2016;7:1916.
5. Djian-Caporalino C, Palloix A, Fazari A, Marteu N, Barbary A, Abad P, et al. Pyramiding, alternating or mixing: comparative performances of deployment strategies of nematode resistance genes to promote plant resistance efficiency and durability. *BMC Plant Biol.* 2014;14:53.
6. Dong OX, Ronald PC. Genetic engineering for disease resistance in plants: recent progress and future perspectives. *Plant Physiol.* 2019;180:26–38.

7. Dosad S, Chawla HS. In vitro plant regeneration and transformation studies in millets: current status and future prospects. *Indian J. Plant Physiol.* 2016;21:239–254.
8. Elshire RJ, Glaubitz JC, Sun Q, Poland JA, Kawamoto K, Buckler ES, et al. A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One.* 2011;6:e19379.
9. FAO. *FAO STAT. Food and Agriculture Data.* 2019.
10. Ghatak A, Chaturvedi P, Weckwerth W. Cereal crop proteomics: systemic analysis of crop drought stress responses towards marker-assisted selection breeding. *Front. Plant Sci.* 2017;8:757.
11. González-Barrios P, Bhatta M, Halley M, Sandro P, Gutiérrez L. Speed breeding and early panicle harvest accelerates oat (*Avena sativa* L.) breeding cycles. *Crop Sci.* 2020.
12. Gupta SM, Arora S, Mirza N, Pande A, Lata C, Puranik S, et al. Finger millet: a “Certain” crop for an “uncertain” future and a solution to food insecurity and hidden hunger under stressful environments. *Front. Plant Sci.* 2017;8:643.
13. Hema R, Vemanna RS, Sreeramulu S, Reddy CP, Senthil-Kumar M, Udayakumar M. Stable expression of mtID gene imparts multiple stress tolerance in finger millet. *PLoS One.* 2014;9:e99110.
14. Hickey LT, Germán SE, Pereyra SA, Diaz JE, Ziemis LA, Fowler RA, et al. Speed breeding for multiple disease resistance in barley. *Euphytica* 2017;213:64.
15. Jamaluddin M, Durga-Rani CV, Swathi G, Anuradha C, Vanisri S, Rajan CPD, et al. Marker assisted gene pyramiding (MAGP) for bacterial blight and blast resistance into mega rice variety “Tellahamsa”. *PLoS One* 2020;15:e0234088.
16. Kumar A, Jindal SK, Dhaliwal MS, Sharma A, Kaur S, Jain S. Gene pyramiding for elite tomato genotypes against ToLCV (Begomovirus spp.), late blight (*Phytophthora infestans*) and RKN (*Meloidogyne* spp.) for northern India farmers. *Physiol. Mol. Biol. Plants.* 2019;25:1197–1209.
17. Kumar A, Tomer V, Kaur A, Kumar V, Gupta K. Millets: a solution to agrarian and nutritional challenges. *Agric. Food Security* 2018;7:31.
18. Liu R, Lu J, Zhou M, Zheng S, Liu Z, Zhang C, et al. Developing stripe rust resistant wheat (*Triticum aestivum* L.) lines with gene pyramiding strategy and marker-assisted selection. *Genet. Resour. Crop Evol.* 2020;67:381–391.

Comment [M7]: Adopt more recent statistics

19. Loyola-Vargas VM, Ochoa-Alejo N. "An introduction to plant tissue culture: advances and perspectives," in Plant Cell Culture Protocols, eds V. M. Loyola-Vargas and N. Ochoa-Alejo (New York, NY: Humana Press). 2018;3–13.
20. Mgonja MA, Lenné JM, Manyasa E, Sreenivasaprasad S. Finger Millet Blast Management in East Africa. Creating Opportunities for Improving Production and Utilization of Finger Millet. Patancheru: International Crops Research Institute for the Semi-Arid Tropics. 2007
21. Miroshnichenko DN, Shulga OA, Timerbaev VR, Dolgov SV. Generation of non-transgenic genome-edited plants: achievements, challenges and prospects. Biotekhnologiya 2019;35:3–26.
22. Mirza N, Marla SS. "Finger millet (*Eleusine coracana* L. Gaertn.) breeding," in Advances in Plant Breeding Strategies: Cereals, eds J. Al-Khayri, S. Jain, and D. Johnson (Cham: Springer). 2019;83–132.
23. Odeny DA, Niazi A, Tesfaye K, Lule D, Wanyonyi S, Kunguni JS. "Genomic designing for climate smart finger millet," in Genomic Designing of Climate-smart Cereal Crops, ed. C. Kole (Cham: Springer). 2020;287–307.
24. Pandian S, Ramesh M. Decoding of finger millet genome: a milestone of millet genomics. Signal Transduction Insights 2019;8:1–3.
25. Ramakrishnan M, Ceasa SA, Duraipandiyam V, Vinod KK, Kalpana K, Al-Dhabi NA, et al. Tracing QTLs for leaf blast resistance and agronomic performance of finger millet (*Eleusine coracana* (L.) Gaertn.) genotypes through association mapping and in silico comparative genomics analyses. PLoS One. 2016;11:e0159264.
26. Rana M, Sood A, Hussain W, Kaldate R, Sharma TR, Gill RK, et al. "Gene pyramiding and multiple character breeding," in Lentils, ed. M. Singh (Cambridge, MA: Academic Press). 2019;83–124.
27. Sood P, Singh RK, Prasad M. Millets genetic engineering: the progress made and prospects for the future. Plant Cell Tissue Organ Culture 2019;137:421–439.
28. Srinivasachary, Dida MM, Gale MD, Devos KM. Comparative analyses reveal high levels of conserved colinearity between the finger millet and rice genomes. Theor. Appl. Genet. 2007;115: 489–499.

29. Tabien R, Li Z, Paterson A, Marchetti M, Stansel J, Pinson S. Mapping QTLs for field resistance to the rice blast pathogen and evaluating their individual and combined utility in improved varieties. *Theor. Appl. Genet.* 2002;105:313–324.
30. Takan JP, Chipili J, Muthumeenakshi S, Talbot NJ, Manyasa EO, Bandyopadhyay R, et al. *Magnaporthe oryzae* populations adapted to finger millet and rice exhibit distinctive patterns of genetic diversity, sexuality and host interaction. *Mol. Biotechnol.* 2012;50:145–158.
31. Upadhyaya HD, Gowd CL, Reddy VG. Morphological diversity in finger millet germplasm introduced from Southern and Eastern Africa. *J. SAT Agric. Res.* 2007;3:1–3.
32. Veluru A, Bhat KV, Raju DVS, Prasad KV, Tolety J, Bharadwaj C, et al. Characterization of Indian bred rose cultivars using morphological and molecular markers for conservation and sustainable management. *Physiol. Mol. Biol. Plants* 2020;26:95–106.
33. Yasuda N, Mitsunaga T, Hayashi K, Koizumi S, Fujita Y. Effects of pyramiding quantitative resistance genes Pi21, Pi34, and Pi35 on rice leaf blast disease. *Plant Dis.* 2015;99:904–909.
34. Zhu X, Zhao J, Abbas HMK, Liu Y, Cheng M, Huang J, et al. Pyramiding of nine transgenes in maize generates high-level resistance against necrotrophic maize pathogens. *Theor. Appl. Genet.* 2018;131:2145–2156.