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Advances in Breeding Methodologies to Develop Resistance Genes against Blast: A Review

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Abstract

Finger millet is still neglected cereal crop mostly grown and consumed by resource-poor people mainly in arid and semi-arid tropics. The projected food demand of the global population will pressure plant breedersto secure the demand by improving the yield of major cereal crops. To meet this demand, thereis a consistent need to look beyond the conventional farming system to increase production and productivity in sustainable ways. However, finger millet production and productivities are limited by many factors namely biotic and abiotic. Of biotic factors, finger millet blast diseaseis the most limiting factor. The disease is caused by the filamentous ascomycetous fungus called *Magnaportheoryzae*. Under favorable conditions, the pathogen can devastate the entire field in all finger millet growing regions. Moreover, the frequent breakdown of blast resistance causes significant yield losses worldwide. The utilization of innovative and efficient research strategies guarantees future sustainable production of finger millet. Therefore, exploring an advanced breeding methodologies presently being used and discussing future directions for development of blast resistant varieties will help providing a broad understanding concept for finger millet breeding.

Keywords: Finger millet, Blast disease, Breeding methodologies, Control method

1. Introduction

1.1 Finger Millet Biology

Finger millet [*Eleusine coracana*(L.) Gaertn.] is an extremely self-pollinated and small seeded C4 plant. It is member of *Poaceae* family grouped under an allotetraploid with (2n =4x=36). This group of family is a unique type of millet which belongs to the tribe Chlorideae whereas all other millets such as Panicum, Setaria, Pennisetum, Paspalum, and Echinochloa are belong to the tribe Paniceae.In a reality, finger millet is understood to be one of the few special cereal crops which provides nutritionally secured food for the global people(Vietmeyer *et al.*,1996).

1.2 Suitable Agro Ecologies

Finger millet is naturally a resilient crop which has ability to grow in diverse range agro climates. The crop is habitually grown and consumed by resource scarce people especially in arid and semi-arid tropics of Asian and sub-Saharan African countries. As a member of the small millets, finger millet is a crop with the best and most respondent to hazards. This characteristics of finger millet has made it as a special crop to be cultivated under a diverse range of agro ecologies (Sood *et al*., 2016).

1.3 Importance of the Crop

Finger millet is well known cereal crop which is considered as basis for food and nutritional security. It supports human beings as a food source as well as feed for domestic animals. The grains of finger millets constitutes an excellent source of dietary properties, such as a high fiber contents, amino acids (Arginie, valine, histidine, isoleucine, leucine, lysine, cystine, methionine, threoninephenylalanine and tryptophan), vitamin B-complex, calcium, phosphorous, magnesium and Iron compared with maize, rice, wheat, and sorghum (Gupta *et al*., 2017). In global context, finger millet is gluten-free crop which serves as a rich source of nutrients functioning as health benefits with no age limit and people with chronic diseases as compared to other domestically consumed cereals, such as wheat and rice (Ramashia *et al*., 2019). However, the required labor-intensive chain of cultivation process from planting to productionmade the crop to be considered still as a neglected crop.

1.4 Its Special Merits

Finger millet is believed to bean excellent source of calcium and potassium with four times more than other cereals. These elements are crucial to strengthen bones and teeth. Finger millet also contains nutrients with antioxidant properties such as polyphenols, which has an ability to protect tbody cells against diseases by which tissues deteriorate over time and loose its ability to function properly (Sharma *et al*., 2015). Reports from(Art, 2022)demonstrated finger millet as a key cereal crop to manage micronutrient deficiency due to its rich source of zinc, iron and manganese. The extended storage life finger millet grains, without insect damage, makes it as vital crop for famine-prone areas of the world (Ramashia *et al*., 2019). On top of using as grains, finger millet normally provides a sufficient quality straw for animal fattening and feeding. It is also very useful for making contingency crop plan due to its drought tolerance. All mentioned special characteristics of finger millet, shifted the world not only to be considered it as a source of food and nutritional security crop of the future but also a crop of unexploited storehouse of valuable genetic resources for abiotic stresses (Michael and Jackson, 2013).

1.5 Area coverage

Many authors agreed that finger millet, with its diversity, is originated particularly from the highlands of Ethiopia and Uganda. The crop was first demonstrated and cultivated in the region for more than 5000 years. Afterwards, it was introduced and distributed to different parts of the globe particularly to the Asian countries. As it is well documented that finger millet was specially and quickly distributed to Indian districts(Hilu *et al*., 1979). Supporting evidences showed that finger millet ranks 4thin global production and followed by sorghum, pearl millet and foxtail millet. Even though its production is more labor intensive relative to other cereal crops grown worldwide, it is believed that planting area and production has been increasing. Currently, it is reaching an estimation of 4.5 million ha and 29 metric tons in 2020 respectively (FAOSTAT, 2020). This incensement is imbalanced with the required demand of finger millet products globally due to the speedily increasing human population and industrialization. Therefore, it is important to fill this gap through timely boosted production of finger millet at least by half percent as compared to other major cereal crops(Kumar *et al*., 2018). In terms of policy, millet research is grossly neglected both nationally and internationally, compared to cereal crops like maize and rice.

2. Production Constraints

Biotic and abiotic factors penalize producers by causing abnormal development, growth and significant reduction of yield in food crops. Diseases are amongst several biotic factors which affect crop production and productivity worldwide. Of finger millet diseases, blast is often an important disease and forecasted potential threat for global food and nutrition security. It is caused by a filamentous fungus called *Magnaporthe oryzae*. This fungus is grouped under the most devastating pathogens which are affecting the production and productivity of finger millet. This could be due to its ability to distribute widely and destruct the crop easily under favorable conditions in all finger millet growing districts (Aru *et al*., 2013). *Magnaporthe oryzae* an important fungal pathogen which infects finger millet nearly in all its growth stages and cause put to 100 percent yield loss (Aru *et al*., 2013). Considering its importance, effective control measures are needed to ensure the global food security. Several years ago, various efforts have been made to develop new cultivars that are more or less resistant this disease. Unfortunately, unavailability of whole-genome sequence and limited genomic resources of millet has importantly hindered studies of the genetics of resistance to the blast disease as compared to other cereals. Consequently, the understanding concept of resistance to finger millet blast remains a knowledge gap. Many literatures on the pathogenicity and the nature of the blast disease on finger millet, depends primarily on the phenotypic features and virulence test using various hosts (Rasool *et al*., 2020).The screening and selection of finger millet for this disease is mostly with limited success due to variability of phenotypic and genetic instability of the pathogen. On top of this, it takes also long time and unaffordable cost for breeders to develop highly resistant variety to blast. Many literature reviews pointed out the status of many studies as they are influenced by environmental pressures and likely exposed to human errors. Therefore, increasing precision and possibilities for improvement of crops to fight against individual treats and multiple stresses are indicated as major goals of crop breeding (Babu *et al*., 2014; Rasool *et al*., 2020).

The start of new biotechnological tools helps the current researches to focus on understanding the biotic stresses in finger millet. This scheme is particularly targeted in advancing molecular genetics of blast disease to develop integrated management systems. With the innovation of high quality sequencing platforms, there has been a remarkable increase in availability of modern genomic tools for better achievements in crop breeding. For example, molecular markers, gene expression profiling, genome-wide association studies, and genetic transformations have been used successfully in various crops. The applications of these tools can help explore the genetic basis of stress tolerance for guidance in the development of plants of superior quality. The development of DNA molecular markers have also been used for population genetics and evaluation of genetic variations that can occur between and within plant populations. Exploring the occurrence of polymorphic structure and level has a significant role in crop breeding. This review paper can help breeder to give hint for a set of recent molecular and genomic tools which is used to study the finger millet blast fungus.

2.1 The Characteristics of Blast Disease

The blast disease, caused by *Magnaporthe oryzae*, is the most important disease affecting the overall growth and yield of finger millet throughout its life span. The fungus has ability to infect many other economically important crops. Many literatures explained the details of the infecting ability of the pathogen on crops like rice, foxtail millet, barley and wheat due to their vulnerability (Cruz and Valent, 2017). (Han *et al*., 2018)also suggested that the pathogen can affect other grass plants within the *poaceae* family. When the pathogen gets favorable conditions, it infect leaves, stem, node, neck, roots, fingers and collar, by causing significant losses in all finger millet growing districts. Early infections of blast, which occur mostly on the leaves, show the disease appearing symptoms by marking small gray or brownish dots. Following two to three days of infection, the dots grow into diamond-shaped lesions of 1.5cm in length and 0.3 up to 0.5 cm in width. According to (Mgonja *et al.*, 2007), they reported that the head blast has ability to reduce finger length, seed weight, number of seeds per finger, quality of the seeds and the total grain yield. The yield losses from 30 to 50 percent due to blast under favorable conditions, have been reported in rice producing areas (Skamnioti and Gurr, 2009). This could be an indication of how the pathogen is affecting various kinds of crops. Currently, efforts are ongoing to develop finger millet varieties with blast resistance. Therefore, continuous studies on blast disease are essential to overcome the disease and thus it hopefully sustains worldwide finger millet production in the future.

2.2 The Biology and pathogenicity of *Magnaporthe oryzae*

Magnaporthe oryzae is plant pathogenic filamentous ascomycete fungi that belong to the family *Pyricularia*. Ascomycete fungi are host specific and cause blast disease on more than fifty cultivated crops and wild grass species (Gladieux *et al.*, 2018). The reproduction system of fungus is believed to be asexual. Reports from research findings suggested that sexual recombination of this pathogen may contribute to its genetic variability. The genetic variability of this fungus make the control options difficult and helps sustain it over years. So far,a strengthen research has not been carried out on finger millet blast strains but similar results are postulated. Regardless of this suggestion, sexual recombination, pathogenicity variation, habitat adaptation and fitness of the finger millet blast fungus need to be investigated.

Magnaporthe oryzae infects the host plant in two stages namely biotrophic and necrotrophic. The biotrophic stage is a stage where the pathogen obtains nutrients from live cells. In opposite to biotrophic, necrotrophic infection stage is a stage where it obtains nutrients from dead cells(Liang *et al.*, 2022). Through the time of infection, conidia attach to the host leaf surface by gum secretions released from the apical part of the spore tip during hydration. Successively, the spore anchors itself tightly to the hydrophobic finger millet surface to allow germination. Then after, the conidia produce germ tubes. According to their research findings, (Singh *et al.*, 2020)stated the ability of mature appressorium to break the leaf cuticle by creating cellular turgor pressure through the accumulation of compatible solutes and secretions of cell wall degrading enzymes. And hence, it is the beginning of gaining entry in to the epidermal cells. When it is once inside to the host tissues, *Magnaporthe oryzae* spreads to adjoining cells through the plasmodesmata without causing any visible alteration to the cell walls of the host. However, under optimum conditions of high humidity, the fungus sporulates abundantly from diseased lesions. This condition assists in permiting the disease to quickly spread to adjacent finger millet plant and its relatives by different agents like wind and water droplets (Gupta *et al.*, 2021).

3. Appropriate Management Options

The application of management options of blast disease is a challenging issue and its control depends on 3 general strategies namely: a number of farming practices, application of chemical and biological agents and development of blast resistant varieties. (Margaret *et al.*, 2020)listed some of the cultural and farming practices that have been applied to control blast disease. As per their explanation, optimum planting time, adequate recommended spacing, regular crop rotation and intercropping, nutrient and water management are included as the best options. These farming practices are commonly used by resource poor subsistence farmers in developing countries who unable to afford other methods of disease management practices. Some of preventive and curative chemicals available in the market are critical elements for effective control. (Patro *et al.*, 2020)suggested a fungicide namely organophosphorus to manage blast not only in finger millet but also other cereal crops. The application of foliar sprays of kitazin at the time of early emergencehas been suggested to be effective in controlling blast disease(Patro *et al.*, 2020). Many authors reviewed and suggested that agrochemicals pose potential risks to human health, food safety and the environment. According to (Chaudhary *et al.*, 2014)explanations, the advantages and effectiveness of these chemicals is directly proportional to their proper application procedures. The growing global concern on the environment together with a strong drive to sustainable agriculture, has led to the advancement of non-chemical alternative management option to control blast disease. Of these methods, biological control is refereeing the use of microbial enemies to suppress finger blast disease has been consider a viable and sustainable alternative method (Chaudhary *et al.*, 2014).

In the current biotechnology era, the breeding of blast resistance varieties offer the best cost effective and reliable approach for the management of finger millet blast disease. This is particularly very important in developing countries where subsistent farming is dominated year to year. The use of such resistant varieties in integrated management of blast disease is desirable because they require minimum fungicides and labors, subsequently lowering production cost. Farmers should therefore be made aware of the benefits planting resistant varieties as opposed to their preferred cultivars. The local finger millet landraces and their wild relatives are commonly used as genetic sources of variation of introgression and hybridization to incorporate the range of useful adaptation for disease resistance in to cultivated finger millet. The introgression of these quality characters should be assisted with modern biotechnological techniques because they are simpler, cost-effective, lesstime taking and more efficient that classical breeding method (Dida *et al.*, 2021).

4. Alternative Breeding Methodologies for Resistance

The current breeding approach is relatively combined with two or more objectives. Some of these are, increasing grain yield, improving resistance to various biotic and abiotic stresses and enhancing nutritional quality. Therefore, developing new finger millet varieties with all these important characters is very crucial. Introgression of a healthy and durable resistance *to Magnaporathe oryzae* in to adapted finger millet germplasm by modern breeding approach has been a goal of many breeding programs (Mbinda and Masaki, 2021). To date, no finger millet variety has been developed and released yet based on marker assisted selection (MAS) technique. Many authors reported that MAS is effectively practiced in other cereal crops improvement except finger millet. Findings from previous studies demonstrated that the modern breeding technique was successful in developing important traits of bacterial blight resistance in rice (Jamil *et al.*, 2020). If modern methodologies are appropriately and timely applied in the case of finger millet improvement program, it would highly advance finger millet breeding efforts against blast disease. However, a lot of knowledge is required before full application of molecular breeding. This is because most of the available data for the crop is currently emphasizing on diversity studies and limited QTLs. As a neglected crop, the status of finger millet genetics and genomics still lags behind due to limited research interests and investments. Few biotechnological approaches have been tested on finger millet crop improvement *(Sood et al.*, 2019).

Blast resistance in finger millet has been studied by using different approaches. Some of these breeding approaches have been used to improve finger millet for effective and durable resistance. Reports from (Dida *et al.*, 2021)explained that resistance genes as R-genes and several R-genes and quantitative trait loci (QTL) in finger millet are linked to the blast pathogen. These genes and QTLs show high sequence similarity in rice and Barley (Khahani *et al.*, 2020).Similar methods could be applied to identify novel alleles for blast resistance through studies with the data available from rice, barley, pear millet and other related crops. Currently, there is no literature reporting mutation breeding in finger millet to generate new lines which can help in the development of resistance varieties. As a result, the traditional finger millet landraces have been used as genetic resources of breeding programs (Gebreyohannes *et al.*, 2021). However, the required lengthy duration which will take ten to fifteen years breeding cycle from crossing to variety release slows the process. It is known that finger millet is a tropical crop, short day plant, but its speed breeding protocol is yet to be developed. In order to realize the actual and potential opportunities of speed breeding technology, it is essential to optimize the parameters with a minimum cost for finger millet crop. This is because, speed breeding technology will accelerate research, improve stability and increase production and productivity to meet food as well as nutritional securities demands for the increasing population.

4.1 Markers and Genotyping Systems for Blast Resistance

Molecular markers are highly valued in plant breeding and genetics. Long ago, molecular markers have played a vital role in finger millet breeding for variety improvement, taxonomy, population genetics, plant physiology, and genetic engineering (Veluru *et al.*, 2020). DNA-based markers such as simple sequence repeats (SSR), restricted fragment length polymorphism RFLP), amplified fragment length polymorphism (AFLP), and expressed-sequenced tag (EST) and other markers have been used to generate genetic maps of finger millet (Anumalla *et al.*, 2015). However, analysis results from these markers have indicated a low variation within cultivated finger millet. Highly variable markers therefore are required for their valuable applications in the crop breeding process. The key challenge posed by the use of molecular markers in plant breeding is the high cost of establishing, maintaining of molecular laboratories and inadequate qualified human resource. Additional, the huge capital requirement for development of markers is another major obstacle in plant breeding programs. Developing countries should put more efforts to overcome these challenges. To fill these gaps, collaboration with other partners through establishment of regional and continental molecular laboratories for breeding activities is very important.

4.2 Exploiting Wild Relative Allele in Blast Resistance Genes

The advancement made in breeding of plants with superior quality has been achieved by gathering valuable alleles from plant genetic resources in different agro-ecological regions of the world. The wild relatives and landraces of crops are believed to be important genetic sources which still have numerous untapped valuable alleles. These alleles could be sustainably exploited for development of crop plants with superior characters. The developed varieties through this process are able to withstand environmental variations and still be retained the preferred qualities. The introgression of new alleles from wild relatives into well adapted and cultivated crop varieties as explained by (Jamil *et al.*, 2020)could produce dramatic trait changes in a suitable genetic background. Recently, no reports exist on gene pyramiding on finger millet. Further efforts should therefore be done to exploit available essential alleles to continually enrich the genetic potential of crops. Together with other constraints, blast causes a significant yield loss as high as 100% in areas infested with the pathogen (Mgonja *et al.*, 2007). This escalates maintaining a need to understand the molecular mechanism of blast resistance and identify R-genes for the blast disease(Anumalla *et al.*, 2015).

4.3 Integrated Disease Management Options

The current major challenges ongoing climate changes must be met with a suitable and environmentally friendly production of health food. This could be attained through balancing environmental protection and agricultural production. To keep the sustainability of the ecosystem, integrated management options should be practiced in regular production systems as core drivers of food security. Although the technological advancements made over the past two decades, a real food crisis due to plant diseases has appeared as a significant threat to food security worldwide. Introgression of durable disease resistances genes in the advent of pathogen evolution caused by climate change distresses and other evolutionary pressures provides durable protection against important crop diseases. The results from empirical studies and theoretical models demonstrate that an effective combination of different selective pressures delays the emergence of virulence (Anderson *et al.*, 2019). The durability of introgressed resistant gen targeting a pathogenic fungus could be significantly elevated through the application of fungicides affecting that particular pathogen.

In general, all agricultural practices intended to control a given pathogen should ideally be integrated to boost their respective effectiveness and durability (Law *et al*., 2012; Hu *et al.*, 2020). In understanding of the smallholder farmers who mainly produce finger millet, such combinations may be inhibited by financial, technical knowledge, human and animal health, and environmental factors. To achieve the goal of all integrated management options of blast disease in finger millet, community engagement and extension services, a healthy partnership between all partners in the finger millet value chain and training programs should be emphasized to attain long term success.

Conclusion and Recommendations

Farmers will continue to grow finger millet to win their day to day lives. There is a need to look beyond the conventional farming system approach to increase production and productivity in sustainable ways. The finger millet production and productivities are limited by biotic and abiotic stresses. *Magnaporthe oryzae* is one of the major biotic factors affecting the finger millet production and causing massive destruction and yield losses. The development of resistance varieties with its sustainable durability through breeding methodologies is therefore very important. The resistance of a variety can be broken through time may be due to various agents. An appropriate and effective disease management options are likely to be keys to sustain production of high quality crops as well as reduce the environmental hazards from the ecosystem. It is also recommended to have an effective, economical and sustainable approach for controlling the finger millet blast disease. Finally, by combining different available options of controlling blast disease, it is expected to improve the durability of genetic resistance in improved finger millet cultivars.

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