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Comprehensive Breeding Approach for Resistance to Disease in Crop: Research Review

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Abstract

Plant disease is caused by fungi, bacteria, and viruses and it significantly reduces crop yield globally. The newly emerged Maize Lethal Necrosis viral disease in maize is devastating to the to the maize crop and cause yield loss in Africa. This review paper discussed on conventional breeding methods and molecular-assisted selection for breeding resistance to foliar disease in major crops. A wide array of germplasm, such as landraces, recombinant inbred lines, pure lines, double haploid lines, elite lines, multi-parent populations, mutant lines, introgressed lines, hybrids, open population varieties, and wild relatives can be used as source germplasm and screened under artificial inoculation or at hotspot areas to develop disease resistance varieties. Many maize inbred lines and hybrids showed resistance to Turbinicum leaf blight, grey leaf spot, and common rust diseases, indicating that these genotypes have carrying genes or favorable alleles for multiple disease resistance and that it is possible to develop variety resistance to fungi foliar disease in maize. Similarly, several advanced lines and some varieties showed resistance to strip and leaf rust in wheat. However, host plant resistance could be broken down due to new emerging race pathogens. Thus, conventional and molecular breeding should be integrated for resistant variety development. Indeed, marker techniques through backcrossing, combined genome-wide association, and transcriptome approaches are useful to identify candidate genes and resistant parents in crops. Moreover, genome editing (CRISPR/Cas9 is a recent powerful technology that can serve as a platform for genetic improvement of traits by knocking out specific DNA and/or insertion of targeted novel coding sequences. It has been shown that CRISPR/ Cas9 can be used as a great alternative tool that helps to develop resistance to disease in crops. Today, gene edition is applying in Africa with joined projects on different crops and traits. Further, stepwise building is required on national biosafety policy and regulation for gene-edited products.

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Introduction

Biotic stress is the stress that damages plants and is caused by other organisms such as fungi, bacteria, viruses, insects, nematodes and weeds. More than 40% of world crop yield is lost due to biotic stresses; out of these, 15% is attributable to insects, 13% to weeds and 13% to other pathogens [1]. On the other hand, climate projections in Eastern and Southern Africa showed fluctuations in rainfall and temperature. This climatic change could be attributed to the spread of disease, the evolution of pathogens, or new races or biotypes expected to affect crop yield. For example, the outbreak of a new strain of stripe rust in wheat [2], the emerging white scale insect affecting mango in Ethiopia [3], the newly emerged Maize Lethal Necrosis viral disease in maize, and the invasive fall armyworm insect are devastating crops and cause yield loss in Africa, including Ethiopia.

Economic importance of major foliar diseases in crop

Major maize foliar diseases such as turcicum leaf blight, common leaf rust, and grey leaf spot diseases are widely distributed and severe in Ethiopia. Turcicum Leaf Blight (TLB) is caused by the fungus *Exserohilum turcicum* (Pass.), which causes the leaf from seedling to the physiological maturity stages. TLB is severe, especially in the warm and humid areas of mid-altitude tropical regions, including Ethiopia. (Raymundo and Hooker 1981) reported that yield reduction due to TLB was about 63% for early-maturity susceptible hybrids. Yield losses may occur up to 60-70% if infection with blight occurs in the early developmental stage of the plant. Two high-yielding maize hybrid varieties (BH 541 and BH 543) were withdrawn from production in Ethiopia [4] due to susceptibility to turcicum leaf blight disease.

Grey Leaf Spot (GLS) is caused by *Cercospora zeae-maydis* and is a major economic concern disease in many maize-growing regions [5,6]. GLS is also a severe disease, particularly in the low to mid-land of the southern and western provinces of Ethiopia, wherever maize production belt areas. The disease shows necrotic lesions tend to be long, and individual lesions may merge, leading to leaf senescence, greatly reducing the photosynthetic areas and resulting in yield loss. A yield loss due to GLS was estimated at 37% in Ethiopia [7] and 60% in South Africa [8].

Several devastating diseases were also observed and reported in wheat, sorghum, faba bean, and other crops. Strip rust (yellow rust) disease is caused by *Puccinia striformis,* and stem rust caused by *Puccina graminis* is one of the most damaging diseases of wheat worldwide [9]. Due to a newly emerged strain of strip rust in Ethiopia, more than 600,000 ha of wheat were lost [2]. On the other hand, anthracnose, caused by the fungus *Colletotrichum sublineolum,* is one of the most damaging diseases of sorghum. The grain yield losses due to anthracnose disease are estimated at 50% and have been reported for susceptible cultivars [10], and a yield loss due to kernel smut is estimated at 2-30% in sorghum [11]. Weed parasites such as Striga hermonthica sp. are the main production constraints for sorghum, millet, rice, and maize production in Africa, and the yield loss ranges from 30 to 100% in the most devastated areas and is aggravated by low soil fertility [12].

To reduce yield losses, the genetic basis of crops should be exploited by plant breeders to develop resistance to disease in crops. Thus, host plant resistance (varietal resistance) is the most effective and cost-efficient means of managing foliar disease because chemicals are expensive, often ineffective, and inconsistent with the environment. Strategies for improving resistance to disease can depend on the availability of diverse germplasm (genetic variation) and the application of biotechnological tools for crop breeding. Integrated breeding strategies can be conventional or molecular marker methods. In this paper, breeding for resistance to major foliar diseases in crops is reviewed. Moreover, applications of molecular markers (MAS), Genomic-Wide Association Study (GWAS) and gene editing (CRISPR-Cas9) for breeding resistance to diseases were explained. In brief, conventional breeding methods and molecular-assisted selection for breeding resistance to disease in major crops were discussed.

Breeding Techniques for Resistance to Disease in Crop

Conventional Breeding Approach

Germplasm Screening for Resistance to Major Foliar Disease in Cereal

Before evaluating and selecting germplasm for resistance to disease in a crop, first the occurrence and severity of disease in a triangle should be visualized, meaning that the susceptible host, virulent pathogen, and environment should be conducive to disease development [13]. After considering the occurrence and severity of disease, breeding for resistance to disease is advocated as an affordable option to tackle the challenges of yield loss and crop failure due to disease caused by fungi, bacteria, viruses, or a combination of diseases. The aim of plant breeders is to develop varieties that have high yield potential in target environments, disease resistance, and desirable agronomic traits. To develop such varieties, a wide array of germplasm, such as landraces, recombinant inbred lines, pure lines, double haploid lines, elite lines, multi-parent populations, mutant lines, introgressed lines, hybrids, open population varieties and wild relatives can be used as sources of germplasm. These diverse sources were screened under artificial inoculation or in hotspot areas over years and across environments for resistance to disease in crops. Artificial inoculation can be prepared from the infected leaf or grain of a susceptible cultivar, and then fine powder, uredospores, or spore suspension can be applied to ensure disease pressure. Some breeders might think that crop breeding for resistance to disease is considered complete resistance. However, this is rarely a realistic objective. Even moderate (minor) levels of disease are often found to have effects. Several studies have reported on germplasm evaluation for resistance to TLB and GLS diseases in maize. Fifty-two maize inbred lines were screened under artificial inoculation against Turcicum leaf blight, gray leaf spot, and common leaf rust disease at Bako and Hawasa agricultural research centers in Ethiopia. Out of these, six inbred lines showed resistance to three diseases [14]. Similarly, twenty-five quality protein maize inbred lines were evaluated over two years against Turcicum leaf blight and gray leaf spot diseases at the Bako National Maize Research Center, Ethiopia. Out of these, three inbred lines are resistant to two diseases, indicating that they can carry genes for multiple traits, Figures 1 and 2. Likewise, [15] reported that seven elite maize lines showed resistance to Turcicum leaf blight and a lower value of AUPDC (Table 1). Similarly, found that three inbred lines and five hybrids showed resistance to gray leaf spot disease, suggesting that it is possible to develop varietal resistance to foliar disease in maize.

Figure 1: Screening for resistance to Grey Leaf spot disease at Bako maize pathology field trial: the inbred lines on the right and left are resistant to grey leaf spot, meanwhile inbred lines located in the middle are susceptible (Source: photo taken by Belay [4]).

A wide range of wheat, sorghum, barley, faba bean, and other crops of genetic diversity were studied for resistance to foliar disease. For instance, due to the outbreak of strain strip rust in wheat or the evolution of new pathogen races, especially Ug99, as a primary wheat production constraint in East Africa, including Ethiopia. Thus, the development of varietal resistance is essential against such outbreak of disease [17]. Evaluated 843 advanced wheat lines under the greenhouse and at spot area for resistance to strip rust over two years. Out of these genotypes, 52 advanced lines and two cultivars showed potential resistance to non-race specific and race-specific genes, which is more du-

Figure 2: Screening for resistance to TLB at Bako maize pathology field trial: the inbred lines on the left side resistant whereas on the right side was susceptible to TLB (Source: photo taken by Belay [4]).

rable than check cultivars (Table 2). Similarly, 64 (38 bread and 26 durum) Ethiopian wheat genotypes were screened in greenhouse against leaf and strip rust. Out of these, 12 bread wheat and three durum wheat of cultivar and advanced lines showed resistant to both diseases' disease [18] (Table 2). Over 200,000 wheat varieties, accessions, and advanced breeding materials were screened from 2005 to 2010 for resistance to Ug99 in Kenya and Ethiopia, and resistant genotypes were identified [2]. Furthermore, 235 durum wheat including landraces, advanced lines, and varieties were evaluated for resistance to leaf rust (Puccinia triticina) in Ethiopia showed that some varieties and lines had low AUDPC and slow rusting under field conditions [19].

Table 2: Some of wheat varieties and advanced elite lines that resistant to stripe, stem, and leaf rust diseases in Ethiopia.

Notice: TRS: terminal rust severity; ACI: average coefficient of infection; AUDPC: area under disease progress curve.

Viral disease also affects crop yield in many parts of the world. Maize Lethal Necrosis Disease (MLND) is caused by a combination of two viruses, the Maize Chlorotic Mottle Virus (MCMV) and Sugarcane mosaic virus (SCMV), and it is considered the newly emerged devastating viral disease of maize in Eastern Africa including Ethiopia. To reduce such yield losses, the development of virus-resistant varieties is important through maize germplasm screening under artificial inoculation. Several inbred lines were evaluated against MLN disease and some of the elite lines showed resistance [20]. Similarly, Bako National maize research in Ethiopia set the released maize hybrids and elite lines and screened for MLN tolerance at Naivasha, Kenya, under artificial MLN infestation. Unfortunately, almost all of the released maize varieties are susceptible to the disease, except very few materials that displayed moderate tolerance till 2018. The next step has been taken to introduce proven MLN-tolerant maize from CIMMYT-Kenya and evaluate them under local growing conditions at various quarantine testing sites in Ethiopia. The result showed that only one variety was resistant to MLN in a specific area. This indicates further research is required to develop MLN resistance through introgression and gene edition.

Backcrossing and Gene Pyramiding for Resistance to Disease in Crop

Backcross breeding is an effective method to transfer one or a few genes controlling specific traits from the donor parent to the adaptive elite line. For example, a parent with high yield and adaptability but susceptible to disease can be improved through backcrossing breeding methods. Introgressed [21] the stripe resistance genes from the resistant genotype into a widely adapted cultivar that was susceptible to yellow rust and followed by backcrossing. These derived lines were evaluated against the disease and found that the advanced wheat lines were resistant to stripe rust resistance and comparable to the check in yield and other traits. Likewise, the introgressed gene into the recurrent parent showed resistance to bacteria blight in beans [22]. Due to the breakdown of the race-specific resistance gene (R gene), gene pyramiding is an alternative method that aims at the stacking of desirable genes from multiple parents into a single plant for the target trait. Recently, five genes linked to DNA markers were found for resistance to rice leaf blast disease, and among these genes, a combination of some genes showed more effective resistance to the blast disease [23]. Moreover, composite crossed population and multi-lines mixture, consisting of several genes, are also powerful and resistant to biotic stress and have higher yields than pure lines [24,25].

Mutation Breeding for Resistance to Disease in Crop

Broadening the genetic basis of the crop through germplasm collection, introduction, and wild relatives is important for resistance to disease. However, the broadened germplasm may not be resistant to disease due to the evolution of pathogens or new races. To overcome such problems, mutation breeding, such as mutagenesis, can generate genetic variation for resistance to disease and other traits. Found [26] that recessive mutations in the Mlo gene confer resistance to powdery mildew in Ethiopian landrace barley. Likewise, mutant lines of wheat revealed resistance to multiple diseases [27].

Molecular Breeding for Resistance to Disease in Crop

Resistance genes to diseases and insect pests in crops can be either major or minor genes, or both. Molecular tools, such as markers linked to target genes, can identify major or minor gene effects on chromosome regions. It is also noted that marker-assisted breeding can improve the efficiency of transferring the specific gene of interest into an adaptive. Molecular marker techniques are also useful in reducing the time of backcross generations and breeding cycles to develop host-resistant varieties. Therefore, QTL mapping, marker-assisted selection and gene editing are important tools to identify resistant genes or candidate genes and subsequently gene transfer for resistant variety development.

QTL Mapping and Genomic Wide Association region for Resistance to Major Diseases in Crop

Quantitative trait locus (QTL) is linked to phenotypic traits with genotyped data at specific chromosome regions. In resistance breeding, QTL analysis is used to identify the regions of the genome linked to resistance to specific or multiple diseases. DNA markers such as Simple Sequence Repeats (SSR), Single Nucleotide Polymorphism (SNP), and Diversity Arrays Technology (DArT) markers have been effectively utilized to identify the crop genome for disease resistance [28]. Found [29] that the quantitative Htn1 gene, resistant to turcicum leaf blight disease in maize, is located on chromosome eight. Furthermore, the resistant parent containing Ht1 genes was introduced into the turcicum leaf blight disease susceptible maize line. Flanking markers on chromosome eight were used to select backcrossed segregants, revealing that three recurrent parents were more resistant than the original parent. Similarly, high putative QTLs located on chromosomal regions can have a significant effect on resistance to grey leaf spot disease and are useful for introgression into adaptively susceptible cultivars.

Plants are not affected only by a single disease but also by multiple diseases under field conditions. Mapping QTLs resistant to multiple diseases and detecting clustered QTLs is crucial. RILs of maize were evaluated and mapped for resistance to three diseases, revealing 9, 8, and 6 QTLs identified for resistance to multiple diseases, with five co-located QTLs detected for three diseases [30], indicating the same genes control multiple diseases. Similarly, 615 maize inbred lines were utilized to study genome selection for resistance to maize lethal necrosis disease. More than half of the inbred lines showed the detection of co-located QTLs on chromosome three and had resistance genes to multiple potyviruses, including maize lethal necrosis disease [31]. Moreover, two genomic regions were found to be resistant to other fungal diseases such as turcicum leaf blight and grey leaf spot. These elite lines carry clustered QTLs that are beneficial for multiple disease resistance

Wheat varieties' resistance to stripe rust is either race-nonspecific resistance, which is controlled by multiple additive loci of minor effects and inherited quantitatively, but single genes for race-nonspecific resistance have also been reported [32,33]. Thus, identification and mapping of rust resistance gene(s) in wheat (bread and durum) are crucial for the development of effective and host-resistant varieties. Several studies have been conducted to identify sources of useful rust resistance genes in wheat; over 68 leaf rust resistance genes, 80 Yr resistance genes to stripe rust, and 53 stem rust resistance genes/QTLs linked to traits were identified [34]. Similarly, a doubled haploid population derived from two parents phenotyped at seedling and adult reaction in the field and mapped for resistance to three diseases in durum wheat found that QTLs on chromosomes 1B and 7B were detected for leaf and stripe rust disease. In addition, a QTL was detected on chromosome 2B for leaf rust at the same time

as Yr genes conferred leaf rust resistance [35]. Similarly, more than 9 genes (Sr2, Sr9d, Sr9e, Sr9g, Sr11, Sr12, Sr13, Sr14, and Sr17 were identified for resistance to stem rust in durum wheat [36]. Moreover, 177 Recombinant Inbred Lines (RILs) were derived from resistant and susceptible bread wheat landraces and found that a major effect QTL was located on chromosome 2B (Figure 3), where it accounted for up to 47.2% of the phenotypic variation. In addition, two other minor QTL genes are located on chromosomes 3B and 4B for adult plant resistance (Yuan et al., 2018). The major QTL was zoomed in using the flanking marker to be used for further marker-assisted selection and to identify candidate genes. Moreover, three hundred eighteen landraces of Ethiopian durum wheat were phenotyped for Septoria Tritici Blotch (STB) disease and genotyped with 16,000 polymorphic markers, and found that five major putative QTLs for STB resistance and four co-located on each of one found on Chromosomes 3A, 5A, 4B, and 5B for resistance to STB [37]. Suggesting that the same gene for controlling different traits or locus has a pleiotropic effect that controls disease and other traits.

Figure 3: Major QTLs detected where LOD peak value greater than 2.5 for related to stripe rust resistance on chromosome 2B for populations A, B, and C in common wheat [38].

Today, genomic selection is an important tool to capture several minor genes and improve polygenetic inherited types that attempt to develop durable resistance variety. It also helps characterize the genetic base architecture of crops for disease resistance. This means that by using high-density markers that cover the whole genome [28]. Moreover, Genomic Wide Association Study (GWAS) can predict the allelic diversity among germplasm at the molecular level that helps for disease resistance. For instance, 190 Ethiopian wheat elite lines were phenotyped in the field and genotyped using 24,281 SNP markers for resistance to stripe rust and stem rust disease. GWAS results showed 15 loci associated with resistance to stripe rust and 9 genomic regions associated with stem rust at seedling and adult plant resistance in wheat [39]. In addition, resistance to stripe rust is strongly linked to markers on chromosomes 5A and 7B,

meanwhile resistance to stem rust is found on chromosomes 3B and 7B, and thus may be novel candidate genes due to hotspot QTLs detected. Similarly, 182 Ethiopian durum wheat landraces were used for the GWAS study for stripe rust resistance and found that 12 loci associated with resistance on chromosomes 1A, 1B, 2B, 3B, 4A, 4B, and 5A were detected, suggesting that Ethiopian durum wheat landraces are abundant in novel Pst genes resistant to stripe rust and that may be introgressed into adapted cultivars [40].

Combined Genome-Wide Association Study and transcriptome approach to identify candidate genes for resistance to disease in maize

Genome-Wide Association Study (GWAS) can dissect complex traits; provide higher resolution than QTL, and used to detect the genetic architecture bases for phenotypic variation [41]. GWAS has been successfully applied to identify genomic regions conferring resistance to maize gray leaf spot, northern corn leaf blight and maize lethal necrosis [42]. 615 maize inbred lines evaluated and underlying the resistance to Maize Lethal Necrosis disease by Genome-Wide Association Study (GWAS) showed that 24 SNPs are adjacent to 20 putative candidate genes associated with plant disease resistance as well as few inbred lines with resistance to Maize Lethal Necrosis disease [43]. However, GWAS does not provide an accurate target gene (resistance genes) at a given locus. Thus, transcriptome analyses can overcome this limitation by detecting and distinguishing the expression of candidate genes of different genotypes. Therefore, combined GWAS and transcriptome analysis can strengthen the gene-trait associations and identify the candidate genes' resistance. Recently, [44] identified a set of candidate genes associated with ear rot and [45] pinpointed the co-expression genes for resistance to wilt in maize using combining the result of GWAS with transcriptome analysis. Most of these studies focus on fungal and bacterial pathogens. However, limited studies conducted on combined results of GWAS with transcriptome approaches such as RNA-seq to identify candidate genes for resistance to Maize Lethal Necrosis disease in maize.

Genome Editing for Resistance to Disease in Crop

In the 20th century, mutations were accelerated through chemicals and radiation. Subsequently, genome editing began with Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs) and currently the discovery of CRISPR/Cas technology that targeted multiple loci through specific modification [45]. The development of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas9 systems consists of guide RNA (gRNA), Cas9 protein, genomic target, and PAM sequence. The CRISPR/Cas9 technology has the following steps and is summarized in Figure 4.

CRISPR/Cas9 has an advantage over classical breeding in terms of reducing the cycle. Additionally, it has an advantage over transgenic because it does not integrate foreign DNA into random sites of the host genome. Therefore, genome editing tools can serve as a platform for the genetic improvement of traits by insertion, replacement, or deletion of specific DNA and introducing novel coding sequences. For example, resistance to rice blast and bacterial blight were obtained by mutagenesis of transcription factor genes [46]. Similarly, [47] reported that CsLOB1 is a susceptibility gene in fruit and resistance obtained through disrupting cis-elements at the promoter and coding region showed enhanced resistance to canker in fruits. Likewise, the gene of eIF4E was disrupted, resulting in broad virus resistance in cucumber plants showed resistance to yellow mosaic virus and Papaya ring spot mosaic virus [48]. Additionally, used CRISPR/Cas9 technology to knock out the susceptibility encoding gene like MLO; resulted in resistance to powdery mildew in wheat and tomato. Currently, gene editing is being applied in Africa on different crops and traits of interest. For example, genome editing banana for resistance to Streak Virus (BSV) and improving provitamin A quality through targeting Phytoene Desaturase (PDS) in cassava at IITA, genome editing for resistance to Maize lethal necrosis in maize by CIMMYT and CORTEVA in Kenya, and lodging resistance and improving grain size in teff through the joint project in the USA. This shows that genome editing is a potential tool for sustainable agriculture. Furthermore, stepwise building on national policy for biosafety is important for the regulation of gene editing products.

Conclusion

Conventional breeding approaches for resistance to diseases are still dominant in developing countries. These approaches involve large-scale screening of germplasm against diseases using either artificial inoculation or hotspot areas. Resistant varieties are then released and commercialized in this manner. However, traditional breeding is time-consuming for successful disease resistance breeding. Additionally, due to the emergence of new races, resistance genes may break down. Therefore, fast tracking and introgression into adaptive cultivars is challenging for breeding disease resistance in Africa. Molecular tools should support conventional breeding in Africa. Furthermore, genome editing by CRISPR/Cas9 could be a powerful approach for targeting multiple genes for disease resistance in crops.

Prospect: There are significant bottlenecks to translate basic research resistance to disease in order to enhance crop production in Africa. Among these bottlenecks; limited basic laboratory, lack of skill for application of biotechnical tools and climatic change. To reduce such challenges, comprehensive breeding approach; conventional, molecular tools and CRISPR/Cas9such technology platform and capacity building enable to strength for breeding resistance to disease in crop.

Conflicts of Interest: The authors declare that no conflict of interest.

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