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Original Research Article

Breeding for Resistance to Biotic Stresses in Crops: Conventional to Genome Editing

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Abstract: Biotic stress is caused by fungi, bacteria, viruses, insects, nematodes as well weeds and it significantly reduces crop yield globally. For instance, the outbreak of a new strain of stripe rust in wheat worldwide, the emergence of white scale insect affecting mango in Ethiopia, the newly emerged Maize Lethal Necrosis viral disease in maize, and invasive fall armyworm insect are devastating maize crop and causing yield loss in Africa. To reduce the yield losses due to biotic stresses, the development of resistant variety and integrated insect pest approach is the way forward for managing disease and insects at respective agroecologies. Thus, this review paper discussed on conventional breeding methods and molecular-assisted selection for breeding resistance to foliar disease in major cereal crops. Wide array of germplasm such as landraces, recombinant inbred lines, pure lines, Double haploid lines, elite lines, multi-parent population, mutant lines, introgressed lines, hybrids, open population variety and wild relative can be used as source germplasm and should be screened under artificial inoculation and or at hotspot areas to develop disease resistance variety. Many maize inbred lines and hybrids showed resistance to turcicum leaf blight, grey leaf spot and common rust diseases and indicating that these genotypes have carrying genes/favorable alleles for multiple disease resistance and 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 breeding and molecular screening should be integrated for resistant variety development. Indeed, Marker-assisted selection through backcrossing, gene pyramiding, combined Genome-Wide Association, and transcriptome approach is 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 the genetic improvement of traits by knocking out the specific DNA and or insertion targeted novel coding sequences the result showed CRISPR/Cas9 can be used as great alternative tool that helps to develop resistance to disease in crops. Currently, the gene edition is applied in Africa with joined projects on different crops and traits. Further; the stepwise building is required on national biosafety policy and regulation for gene-edited products.

Keywords: Foliar Disease, Breeding for Resistance, Molecular Tools.

1. INTRODUCTION

Biotic stress is the stress that damages plants and caused by other organisms such as fungi, bacteria, viruses, insects, nematodes and weeds. These biotic stresses significantly reduce crop yield globally. 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 fluctuation in rainfall and temperature. This climatic change could be attributed to the spread of disease, the evolution of pathogens, or new outcomes of races /biotypes expected to increase the loss of 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

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Necrosis viral disease in maize [4] and invasive fall armyworm insect are devastating crop and causes yield loss in Africa including Ethiopia.

1.1. Economic importance of major foliar diseases and insect pests in crops

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 fungi *Exserohilum turcicum* (Pass.) 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 [5]. 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 of 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 [6] due to susceptibility to turcicum leaf blight disease.

Grey leaf spot (GLS) is caused by *Cercospora zeae-maydis* and it is a major economic concern disease in many maize-growing regions [7, 8]. GLS is also a severe disease particularly in the low to mid-land of southern and western provinces of Ethiopia wherever maize production 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 [9] and 60% in South Africa [10].

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 Striiformis* and stem rust caused by *puccina graminis* are two of the most damaging diseases of wheat worldwide [11]. Due to a newly emerged strain of strip rust in Ethiopia, more than 600,000 ha of wheat were lost [2].; http://wheatrust.org, [12]. 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% have been reported for susceptible cultivars [13] and a yield loss due to kernel smut is estimated at 2-30 % in sorghum [14]). Weed parasitic such as Striga hermonthica sp. are the main of the major production constraints to sorghum, millet, rice, and maize production in Africa and the yield loss ranges from 30 to 100 % in most devastated areas and is aggravated by low soil fertility [15].

A yield loss due to arthropod insects is varied. Maize yield losses due to stem bores are variable, for example, the range to 10 % where less infestation of maize stem borer, grain losses due to maize weevil are estimated to be 20 to 30 % [16;17]. Russian wheat aphid insect affects barley and wheat crops [18]. Currently, a newly emerging alien pest such as a white scale insect is the most distractive mango in western Ethiopia [3], whereas fall armyworm is an invasive insect that highly damages maize in Africa.

To reduce such yield losses, the genetic basis of crops should be exploited by plant breeders to develop resistance to major biotic stress 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 a crop's resistance to biotic stress depend on the availability of diverse germplasm (genetic variation) and the application of biotechnological tools for crop breeding. The breeding strategy can be conventional and molecular marker/DNA marker methods.

In this paper, breeding for resistance to major foliar diseases in crops was reviewed. Likewise, breeding for resistance to maize stalk borer, Russian Aphid wheat, and post-harvest insects (maize and bean weevil) is also outlined. In addition, recent applications of molecular markers (MAS), Genomic-Wide Association Study (GWAS) and gene editing (CRISPR-Cas9) for breeding resistance to diseases are also explained. In brief, conventional breeding methods and molecular-assisted selection for breeding resistance to biotic stress in major cereal crops were discussed.

2. Breeding Techniques for Resistance to Disease in Crops

2.1. Conventional Breeding Approach:

2.1.1. Germplasm Screening for Resistance to Major Foliar Disease in Cereal

A wide array of germplasm such as as landraces, recombinant inbred lines, pure lines, Double haploid lines, elite lines, multi-parent population, mutant lines, introgressed lines, hybrids, open population variety and wild relative can be used as source of germplasm. These diverse sources should be screened under artificial inoculation or at hot spot areas over years for resistance to biotic stress. Artificial inoculation can be prepared from infected leaf/grain of susceptible cultivar and then, fine powder, uredospores, or spore suspension should be applied to ensure disease pressure. Several studies reported on germplasm evaluation for resistance to major foliar disease 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 center in Ethiopia. Out of these, six inbred lines showed resistance to three diseases [19]. Similarly, twenty-five quality protein maize inbred lines were evaluated over two years against Turcicum leaf blight and gray leaf spot diseases at Bako National Maize Research Center, Ethiopia. Three inbred lines are resistant to two diseases, indicating that they can carry genes for multiple traits [6] Figure 1. Likewise [20], reported that seven elite maize lines showed resistance to turcicum leaf blight and a lower value of AUPDC (Table 1). Similarly [21], found that three inbred lines and five hybrids showed resistance to grey leaf spot disease suggesting that it is possible to develop varietal resistance to foliar disease in maize.

Table 1: Some maize inbred lines that resistant to turcicum leaf blight and grey leaf spot diseases in Ethiopia

Inbred lines	**turcicum leaf blight (scale 1-5)**	**Reaction type, R**	**sources**	**grey leaf spot (1-5 scale)**	**Reaction type**	**sources**
BQ00RC3- 356-1-1-2-1-1-1	1.5	R	Garoma *et al.,* 2016	1.9	R	Garoma *et al.,* 2016
CML144	1.8	R	Tilahun *et al*., 2012	2	R	Tilahun *et al*., 2012
BKL004	2	R	Abera *et al*., 2016	1.8	R	Abera *et al*., 2016
CML-176/Kulen(F2)-4-3-1-1-1	2	R	Garoma *et al*., 2016	1.75	R	Garoma *et al.,* 2016
Obtanpa 204-3-2-2-1	1.5	R	Garoma *et al*., 2016	2	R	Garoma *et al.,* 2016
CML-197 x 142-1-e(F2) 60-1-1-2-1-1	1.5	R	Deresa *et al*., 2018	2	R	Deresa *et al*., 2018
CML 383	1.75	R	Deresa *et al*., 2018	1.75	R	Deresa *et al*., 2018
DE-38-Z-126-3-2-2-1-1	1.75	R	Deresa *et al*., 2018	1.5	R	Deresa *et al*., 2018

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 Garoma, 2016)

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 disease [22]. 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 durable than check cultivars (Table 2). Similarly, 64 (38 bread and 26 durum) Ethiopian wheat genotypes 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 [23] (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 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 [24].

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

Wheat varieties or advanced elite lines	Infection type scored < 2 is resistance	ACI	AUPDC	TRC	Severity %	Sources	Remark
Shorima and Hulluka	Resistance to three races of stem rut	< 10	< 20	< 30	< 30	Bekele *et al.,* 2018	Bread wheat
52 advanced elite lines	Resistance to three races of stem rut	< 10	<20	< 30	< 30	Bekele *et al.,* 2018	Bread wheat, in addition, was evaluated in WANA region and Ethiopia
Selam, Mossobo, Bekelcha, and Utuba	Resistance to stem rut	< 10	< 20	< 30	< 30	Habtamu, 2019	Four commercial varieties of durum wheat
Twelve bread wheat varieties	Resistance to five races of leaf rust and two strip rust	< 10	< 20	< 30	< 30	Hussein and Pretorius, 2005	Bread and durum wheat types
Eight advanced elite lines	Resistance to five races of leaf rust and two strip rust	< 10	< 20	< 30	< 30	Hussein and Pretorius, 2005	Bread and durum wheat types

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

Genetic variation for resistance to Anthracnose disease is also reported in sorghum. Ninety-nine dwarf Ethiopian sorghum breeding lines evaluated over two years against Anthracnose disease under artificial inoculation showed that three of the breeding lines possessed gene-conferring resistance [25]. Parallel to this, twenty-three genotypes evaluated over two years for resistance to smut and grain mold showed four different lines of resistance to smut and grain mold indicating that controlled by different genes.

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 [4]. 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 [26]. Similarly, Bako National maize research in Ethiopia set the released maize hybris 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.

2.1.2. 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 adaptive but susceptible to disease can be improved through backcrossing breeding methods [27]. introgressed the strip resistance genes from the resistant genotype into a widely adapted cultivar that was susceptible to yellow rust and followed backcrossing as well as evaluated against disease and found that the advanced wheat lines resistant to strip rust resistance as well as comparable to check in yield and other traits. Likewise, the introgressed gene into recurrent parent showed resistance to bacteria blight in beans [28].

Due to the breakdown of the race-specific resistance gene (R gene), stacking Resistance-R genes into adaptive cultivars is quite essential, thus it provides durable disease resistance in crops. 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 blast disease [29]. Moreover, composite crossed population and multi-lines that consist of several genes are also powerful and resistant to biotic stress and have higher yields than pure lines [30, 31].

2.1.3. Mutation Breeding for Resistance to Disease in Crops

Broadening the genetic basis of the crop through germplasm collection, introduction, and wild relative is important for resistance to biotic stress. However, the broadened germplasm may be not 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 disease resistance and other traits. [32] found 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 [33].

2.1.4. Breeding for Resistance to Insect Pests in Crops

2.1.4.1. Germplasm Screening for Resistance to Field and Storage Insect Pests in Crops

Field insect pests such as stalk borer, Russian aphid, and storage pests (maize weevil, bean weevil, adzuki bean beetle, and others) cause losses. Thus, using resistant varieties or genetic resistance is essential as part of integrated insect control. Breeding for resistance to insect pests depends on the magnitude of genetic variability within the germplasm, heritability of the trait, and the level of selection intensity applied by the breeder.

Several numbers of maize germplasms screened against maize weevil and stem borer insects found that hybrids and elite maize resistant to post-harvest storage and stem borer [17], suggesting that the genetic variability for resistance to field and postharvest in crop. Likewise [34], screened 27 maize hybrids grain against maize weevil and large grain borer under field and in glass jars (laboratory) in Kenya and Ethiopia and found that two hybrids showed resistance to pre- and post-insects as well as moderately resistant to major foliar disease and comparable grain yield and subsequently recommended for variety release. Similarly, Check pea resistance to insect studied [35] indicated that one genotype exhibited resistance to the adzuki bean beetle out of 130 genotypes. Likewise, a large number of wheat and barley genotypes were evaluated in a greenhouse against Russian aphids, and some resistant genotypes were identified [18], resistant genotype consists of a high number of tillers provide yield and low damage by aphids than the susceptible variety.

2.1.4.2. Mechanisms of Grain Crop Resistance to Storage Insect Pests

Response of plant resistance to insects showed different mechanisms such as morphological or chemical factors that inhabit the insect pest. Physical barriers such as the tightness of the glumes in un-milled rice and the hardness of seed coat traits are attributed to resistance to storage pest insects [36] Maize genotypes which are characterized by full husk cover inhabit the entrance of adult weevil into cob under field conditions. Biochemicals found in grain such as phenolic compounds and antibiosis in the pericarp as well as aleurone layer are a means of mechanism for resistance to maize weevil [37] Enzymes like α-amylases inhibit adzuki bean beetles in peas and common beans. Studies also showed that the presence of phenolic compounds and a high density of trichomes in leaves has a great role in resistance to field insects. In cereal, metabolites include chlorogenic acid and benzoxazinoids provide resistance to insects and pathogens [38].

2.2. Molecular Breeding for Resistance to Biotic Stress in Crop

Resistance genes to disease and insect pests in crops can be either major and minor resistance genes or both. Molecular tools such as markers that are linked to target genes can identify either major or minor gene effects on chromosome regions. It also noted that marker-assisted breeding can improve the efficiency of transferring the specific gene of interest into adaptive [39]. Molecular marker techniques are also useful to reduce the time backcross generations and breeding cycles to develop host resistance variety. Thus, identification, QTL mapping, marker-assisted selection and gene edition are important tools to identify resistant genes/candidate genes and subsequently gene transfer/pyramiding for resistant variety development.

2.2.1. QTL Mapping and GWAS for Resistance to Major Diseases in Cereals

Quantitative traits locus (QTL) is linked to phenotypic traits and genotype data at specific chromosome regions. In resistance breeding, QTL analysis is to identify the regions of the genome linked to biotic stress resistance. DNA marker is defined as a particular segment of DNA sequence on a known chromosome location for particular genes or specific traits. DNA markers such as simple sequence repeats (SSR), single nucleotide polymorphism (SNP) and Diversity Arrays Technology (DArT) markers have been utilized effectively to identify the crop genome for disease resistance [40, 41] found that the quantitative Htn1 gene encodes a wall-associated receptor-like kinase (a gene that is resistant to turcicum leaf blight disease in maize) mapped and found on chromosome eight. Furthermore, the resistant parent that consists of Ht1 genes was introduced into the turcicum leaf blight disease susceptible maize line. On chromosome eight, flanking markers were used to select backcrossed segregants and found that three recurrent parents were more resistant than the original parent. Likewise, several QTLs were detected for grey leaf spot resistance in maize and almost found in each chromosome [42]. High putative QTLs located on chromosomal regions may contribute a large effect on resistance to grey leaf spot disease and are used for introgression into adaptive susceptible cultivars.

Plants are not only affected by a single disease but also by multiple diseases under field conditions. Mapping QTL that is resistant to multiple diseases and finding clustered QTL is essential. RILs of maize were evaluated and mapped for resistance to three diseases and found that 9, 8, and 6 QTLs were identified for resistance to multiple diseases, and out of these, five co-located QTLs were detected for three diseases [43] suggesting that the same genes controlling multiplies diseases. Similarly, 615 maize inbred lines were used to study genome selection for resistance to maize lethal necrosis disease. More than half of inbred lines showed the detection of co-located QTLs on chromosome three and had resistance genes to multiple potyviruses including maize lethal necrosis disease [44]. In addition, two genomic regions are also found resistant to other fungal diseases like turcicum leaf blight and gray leaf spot. Such elite lines carry clustered QTLs that are useful for multiple disease resistance.

QTL mapping for resistance to anthracnose disease was also studied in sorghum. 117 recombinant inbred lines (RILs) population was developed from a cross between resistance elite cultivar of sorghum and susceptible to anthracnose was phenotyped across environments and genotyped with high-density markers and found that one major quantitative trait locus (QTL) detected on chromosome 5 and other minor QTLs consistently identified across environments. The major QTLs showed that stable and a source of anthracnose resistance in all tested environments [45] Major anthracnose-resistance QTLs detected on chromosomes 5 and 9 were observed in all environment's genomes explained from 20 to 65% of the phenotypic variance [46] and based on annotation, many associated genes that disease resistance observed. Likewise, using nested association mapping, several QTLs for resistances to stripe rust and leaf rust disease in barely identified, and the resistance due to the accumulation of numerous small effect loci as well as wild donor QTL alleles present in cultivar barely [47]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 [21, 22]. Thus, the identification and mapping of rust resistance gene(s) in wheat (bread and durum) is 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 [48]. Similarly, a doubled haploid population derived from two parents was phenotype at seedling and adult reaction in the field and mapped for resistance to three diseases in durum wheat and found that QTLs on chromosome 1B and 7B were detected for leaf and strip ruts disease. In addition, QTL was detected on chromos 2B for leaf rust at the same of Yr genes conferred to leaf rust resistance [49]. 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 [50]. 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 2), 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 [51]. The major QTL should zoom in using the flanking marker to use further marker-assisted selection and to identify candidate genes.

Likewise, three hundred eighteen landraces of Ethiopian durum wheat were phenotype for septoria tritici blotch (STB) disease and genotyped with 16, 000 polymorphic markers and found that five major putative QTL for STB resistance and four co-located each of one found on Chromosome 3A, 5A, 4B and 5B for resistance to STB [52]. Co-located QTLs revealed that the same gene for controlling different traits or locus has the pleiotropic effect that controls disease and other traits.

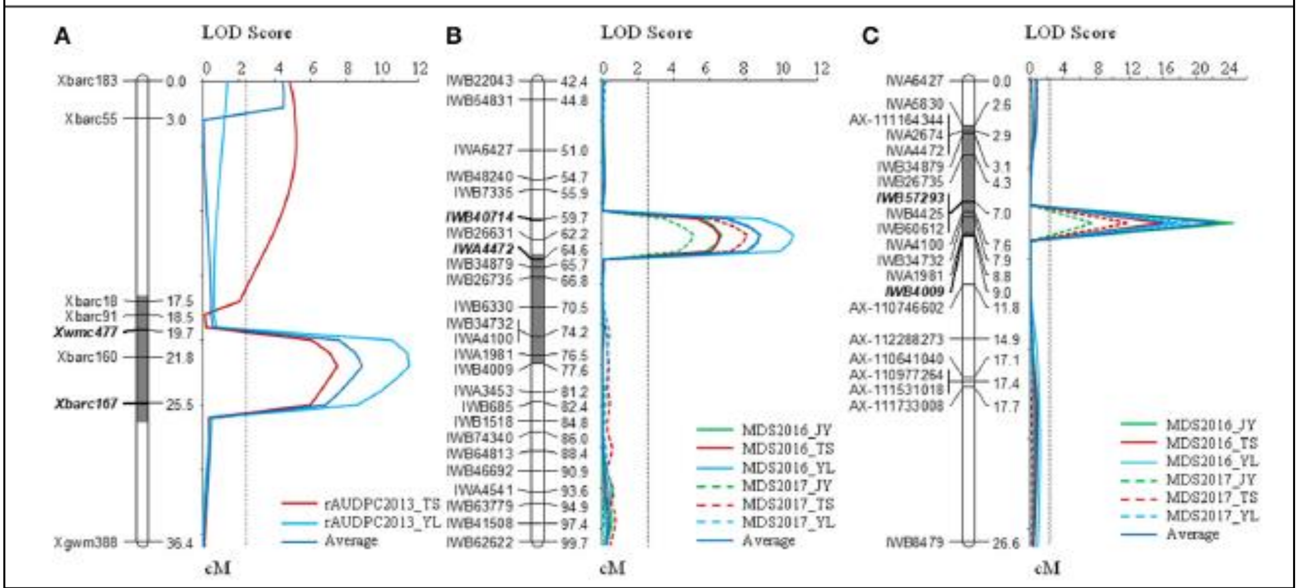

Figure 2: Major QTLs detected where LOD peak value greater than 2.5 for related to stripe rust resistance on chromosome 2B for populations A, B, and C in common wheat (Yuan *et al*., 2018)

Today, Genomic selection could be an important tool to capture several minor genes and to improve polygenetic inherited types that attempt to develop durable resistance variety. It also helps to characterize the genetic base architecture of crops for disease resistance. This means that by using high-density markers that cover the whole genome [40]. Moreover, Genomic Wide Association Study (GWAS) can predicate the allelic diversity among germplasm at the molecular level that helps for disease resistance. For instance, 190 Ethiopian wheat elite lines phenotype in the field and genotyped using 24, 281 SNP markers for resistance to stripe rust and stem rust disease and GWS results showed 15 loci associated with resistance to strip rust and 9 genomic regions associated with stem rust at seedling and adult plant resistance in wheat [53]. On top of this, resistance to strip 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 GWS 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 and, thus Ethiopian durum wheat landraces are abundant in novel Pst genes resistance to strip rust and that may be introgressed into adapted cultivars [54].

2.2.2. 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 be used to detect the genetic architecture bases for phenotypic variation [55]. GWAS has been successfully applied to identify genomic regions conferring resistance to maize gray leaf spot [56], northern corn leaf blight [57], and maize lethal necrosis [58]. 615 maize inbred lines evaluated and underlying the resistance to Maize Lethal Necrosis disease by genome-wide association study (GWAS) and 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 [59]. 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 [60], identified a set of candidate genes associated with ear rot and [61] 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.

2.2.3. 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 [11]. 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 summered in Figure 3.

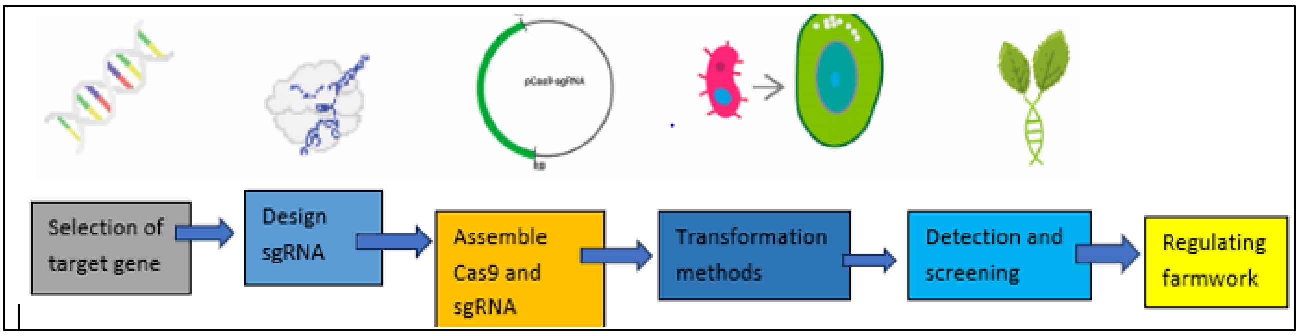

Figure 3: The basic steps of CRISPR/Cas9 technology used to edit target genes in plants (Erdogan *et al.*, 2023)

CRISPR/Cas9 is an advantage over classical breeding in terms of reducing the cycle. Also, it has an advantage over transgenic hence it has no integration of foreign DNA into random sites of host genome. Thus, 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, enhanced rice blast and bacterial blight resistance were obtained by mutagenesis of transcription factor genes [62]. Likewise [63], reported that the CsLOB1 is a susceptibility gene in fruit and resistance obtained through disrupting cis-elements at promoter and coding region showed enhanced resistance to canker in fruits. Similarly, the Gene of eIF4E was disrupted which broad virus resistance the cucumber plants showed resistance to yellow mosaic virus and Papaya ring spot mosaic virus [64]. Furthermore [65], used CRISPR/ Cas9 technology to knock out the susceptibility encoding gene like MLO; resulted in resistance to powdery mildew in wheat and tomato.

Currently, the gene edition is 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 USA. This showed that Genome editing is a potential for sustainable agriculture. furthermore, stepwise building on national policy for biosafety is important for regulation of gene editing products.

3. CONCLUSION

The conventional approach for breeding disease resistance is still the dominant one in developing countries. This approach applied large screening germplasm against disease using either artificial inoculation or a hotspot area. In such a way resistant varieties are released and commercialized, in the case of maize, wheat, barley, and other crops. However, traditional breeding is time-consuming for the success of breeding resistance to disease. Also, due to the new emergence of races the resistance genes might be broken down. Hence, fast tracking and introgression into adaptive cultivars is tough for breeding resistance to disease in Africa. Therefore, molecular tools should support conventional breeding in Africa. Moreover, Genome editing by CRISPR/Cas9 could be a powerful approach by targeting multiple genes for disease resistance in crops.

4. 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, application of biotechnical tools and projection of climate change. To reduce such challenges, integrated conventional breeding with molecular tools including CRISPR/Cas9such technology platform and capacity building should be strengthen for breeding resistance to disease and they're by contribute to sustainable agriculture in Africa.

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

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