Journal of Experimental Agriculture International

Volume 46, Issue 7, Page 448-466, 2024; Article no.JEAI.117613
ISSN: 2457-0591
(Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

Advancements in Plant Breeding for Biotic Stress Resistance in Cereal Crops: A Comprehensive Overview

Ajay Chandra [a], Chetariya C.P [a], Nilesh Suryakant Talekar [a] and Rahul Singh [a*]

[a] Department of Genetics and Plant Breeding, Lovely Professional University, Kapurthala, Punjab, India.

Authors' contributions

This work was carried out in collaboration among all authors. Author AC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors RS and CCP managed the analyses of the study. Author NST managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/jeai/2024/v46i72599

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/117613

Review Article

Received: 01/04/2024
Accepted: 03/06/2024
Published: 20/06/2024

ABSTRACT

Current developments in plant breeding techniques used to improve cereals resistance against biotic stress. To overcome the problems provided by biotic stress, the focus is on investigating cutting-edge strategies and techniques that have transformed the area of cereal breeding. The creation of biotic stress-resistant varieties has been greatly accelerated by the introduction of Marker-Assisted Backcrossing (MABC), which enables the controlled transfer of advantageous genes from wild germplasm into elite cultivars. To better understand the genetic basis of resistance traits and support marker-assisted selection in breeding programs, Genome-Wide Association Studies (GWAS) have been useful in identifying genetic markers linked to biotic stress resistance.

**Corresponding author: E-mail: rahul.29548@lpu.co.in;*

Cite as: Chandra , Ajay, Chetariya C.P, Nilesh Suryakant Talekar, and Rahul Singh. 2024. "Advancements in Plant Breeding for Biotic Stress Resistance in Cereal Crops: A Comprehensive Overview". Journal of Experimental Agriculture International 46 (7):448-66. https://doi.org/10.9734/jeai/2024/v46i72599.

Researchers have been able to decipher intricate gene regulatory networks involved in plant responses against biotic stress using microarray technique; which leads to identifying critical genes and pathways underlying resistance mechanisms. New possibilities for precise genetic alterations in genomes, including as the introduction of novel resistance alleles using allele mining and the targeted disruption of susceptibility genes, have been made possible by genome editing tools like CRISPR/Cas9. Apart from these modern-day techniques, conventional methods such as mutation breeding are still being improved and combined with genomic technologies to create genetic variation and find new alleles that confer resistance to biotic stress. A promising method such as targeted gene silencing and RNA interference (RNAi) technology enables the suppression of genes linked to biotic stress susceptibility and also increases resistance. The improvement of cereal crop varieties resistant against biotic stress has advanced significantly due to the integration of these many breeding tactics and technologies. These new developments have the potential to significantly improve agricultural sustainability and food security by reducing yield losses from biotic stressors and supporting international efforts to improve crops. This review article enlightens the different conventional and advanced breeding techniques used against biotic stress found in cereal crops.

Keywords: Cereal crops; biotic stress; MABC; CRISPR/Cas9; RNAi; microarray technique.

1. INTRODUCTION

A large proportion of the world's population feeds cereal grains like rice, wheat, and maize as their primary source of energy and nutrition for billions of people globally (Table 1). According to the FAO (Food and Agriculture Organisation) [1] of the United Nations, global cereal production reached approximately 2.8 billion tonnes in 2021. According to FAO wheat accounts for approximately 20% of the world's caloric intake. Rice contributes around 21% of the global dietary energy supply as per the estimation of IRRI (International Rice Research Institute) Philippines [2]. USDA (United States Department of Agriculture) [3] states that maize accounts for approximately 15% of world caloric intake. Rice, wheat and maize account for two-thirds of the total and also make up more than 90% of world cereal production, (FAO).

However, a variety of biotic stressors, such as infections raised by pathogens including fungus, bacteria, viruses and pests; pose a continual danger to these crops. The percentage losses caused by some major pathogens in Rice (*Xanthomonas O*; 30%, *Pyricularia O*.10-30%, *Rhizoctonia solani;* 20% etc [5], wheat (*Puccinia spp*.; 70%, *Fuasrium graminearum*; 10-50%, etc, [6], and Maize (Chilo pertellus; 80%, Sesamia inferans; 25.5-78.9%, Antherigona soccata; 21.28%, A. orientalis; 20%, fall army worm; 73%, etc [7]. To strengthen cereal crop's resistance to these risks and promote sustainable agriculture and global food security, breeding for biotic resistance has shown to be an essential approach. Considerable improvement has been achieved recently in decrypting the genetic keystones of biotic resistance and applying sophisticated breeding methods to create resistant cultivars. The molecular processes behind plant-pathogen interactions have been elucidated by recent research, offering important new information on the identification and description of resistance genes in cereal crops. For example, in rice, breeding efforts have been transformed by identifying and using important resistance genes, such as Xa21 and Pi-ta, against bacterial blight and blast disease, respectively [8].

Table 1. Global production and consumption of the major cereals

Crop	Production (Million Tons/Year)			Consumption (Million Tons/Year			Major Exporting Countries
	2021/22	22/23	23/24	2021/22	22/23	23/24	
Wheat	780	803	789	784	794	806	Argentina, Australia, Canada, EU, Russia, Ukraine, USA
Rice	514	514	512	520	159	516	India, Pakistan, Thailand, USA, Vietnam
Maize	1224	1163	1223	1213	1181	1212	Argentina, Brazil, Ukraine, USA

International Grains Council (IGC):(2024) Website - igc.int [4]

Phenotyping and high throughput genotyping technology have also sped up the process of finding resistant alleles and introducing them into elite germplasm. Breeders may now quickly identify genomic areas linked to resistance features and accelerate the creation of resistant cultivars using genome-wide association studies (GWAS) and genomic selection [9]. Genetic resources from landraces and wild relatives in wheat have been essential in widening the genetic diversity for disease resistance. Traditional breeding and marker-assisted selection techniques have successfully transferred genes that confer resistance to wheat diseases such as wheat rusts and powdery mildew into elite genotypes [10]. Similarly, efforts have been focused on maize to improve resistance against severe diseases like northern leaf blight and maize fatal necrosis using available genetic variety and natural variation. The discovery and implementation of novel resistance genes in maize improvement pipelines have been made easier by the integration of genomic technologies with traditional breeding techniques [11].

The introduction of novel pathogen strains, the stability of resistance genes, and the complicated genetic makeup of resistance features are some of the obstacles that remain in the way of biotic resistance breeding, despite notable advancements. To generate robust and sustainable cereal crop types, multidisciplinary techniques incorporating plant pathology, breeding, and genomics will be necessary to address these difficulties. Finally, new developments in the breeding of cereals for biotic resistance provide encouraging chances to reduce the negative effects of biotic stressors on the world's food supply. To provide food security and sustainable agriculture in the face of changing biotic challenges, research and development initiatives focused on improving cereal crops' genetic resilience must continue. Although there have been significant advancements in the last ten years in the creation and application of sustainable technologies to improve plant resistance against biotic stress but there is still a lacuna between controlled vs open field studies [12]. Over time, breeding programs have been changed since these programs utilize genomic tools instead of more traditional breeding methods. Many breeding programs have been benefited from the genetic information supplied through modern biotechnology such as allele mining and NGS platforms, that would not possible with only conventional breeding techniques [13]. Biotechnological "omics" technologies have greatly aided plant stress tolerance breeding by providing insight into genetic diversity, genotype variants, genetic maps, and other useful information related to the genetics of plant populations. This article offers a thorough summary of the most important tactics, difficulties, and potential future developments in the breeding of biotic resistance in cereal crops such as rice, wheat and maize etc.

a) Impact of biotic stressors on cereal crops

Many different types of organisms, including bacteria, fungus, viruses, and insects, can produce biotic stressors on plants. Numerous studies have demonstrated how biotic and abiotic stressors significantly lower cereal crop development and output. At several development phases (panicle formation, booting stage, and lactation stage), the bacterial leaf blight disease decreased rice production and grain quality [14]. Sorghum's total seed weight, 100-seed weight, and seeds per panicle were all decreased by fusarium stalk rot and charcoal rot disease [15]. The most destructive *Magnaporthe oryzae* disease in cereals is blast disease. The growth of the leaf, stem, collar, node, neck, and panicle in all cereals is impacted by blast disease. Additionally, it has decreased the growth and production of all crops that are significant to the economy, including barley [16], wheat [17], finger millet (*Eleusine coracana*) [18], foxtail millet (*Setaria italica*) [19], and rice [20]. Barley and wheat yields were decreased by more than 40% due to yellow dwarf virus [21].

Several historical occurrences, such as potato blight in Ireland, coffee rust in Brazil [22], and maize leaf bight in the USA [23], resulted in total crop failure and starvation in the affected areas. Another instance of crop failure brought on by illnesses is the Great Bengal Famine of 1943. Millions of people died because of these incidents, and many more moved to other areas. According to Christou and Twyman (2004) [24], biotic stressors such as insects and diseases significantly lower grain productivity. Specifically, just illnesses account for 10% of the world's food production, which results in 800 million people being hungry. In a similar vein, developing agricultural plants that are resistant to biotic stressors is challenging due to the lack of reliable resistance sources [25]. On the other hand, plants are resistant to biotic stressors due to one

or more genes. Therefore, plant breeders may use this genetic base to help agricultural plants become resistant to pest insects and illnesses. There are several breading approaches have been successfully utilized against biotic stress. These are categorized under conventional and advanced breeding approaches.

2. CONVENTIONAL BREEDING APPROACHES FOR BIOTIC STRESS RESISTANCE

The availability of resistance sources determines the techniques and tactics used to induce and improve agricultural plant resistance to biotic stressors. Breeding tactics may be classified into traditional and contemporary ways. Conventional plant breeding techniques played a major part in the creation of biotic stress-tolerant cultivars. The different approaches employed for this goal are explained below.

2.1 Introduction of Exotic Lines

In the 1970s, a devastating corn leaf blight epidemic caused by the *Cochliobolus heterostrophus* pathogen wiped out corn crops in the southern United States. This was due to the lack of genetic diversity in the corn varieties grown in that region [23]. The epidemic was triggered by the emergence of a new race of pathogens called Race T, which possessed the T-cms virulence gene. To address this issue, a new type of corn, known as Texas cytoplasm, was introduced. Similarly, Shah et al. [41] reported that potato germplasm imported from the USA, India, and the Netherlands showed promising resistance against potato leaf roll and blight diseases. This highlights the importance of introducing new genetic material to enhance the diversity and disease resistance of crop plants when local germplasm lacks these traits. Such introductions can be made through multinational companies or foreign gene banks. A recent and significant example is the introduction of Bt-cotton in Pakistan and India. As a result, a large area of the Pakistani cotton belt has been converted to transgenic Bt-cotton, replacing indigenous non-Bt cotton varieties [42]. The use of wild relatives also plays a major role in the cultivar development for biotic stress resistance (Table 2).

Table 2. Use of wild relatives in the development of biotic stress resilient cultivars

Crop	*Wild sp.*	Trait	References
Wheat	*Triticum timopheevii*	Stem rust resistance	[26]
	Triticum turgidum	Yellow rust resistance	[27]
	Aegilops ventricosa	Eye-spot resistance	[26]
	Aegilops geniculata	powdery mildew resistance genes	[28]
	T. turgidum var. dicoccoides	Powdery mildew resistance	[29]
	Aegilops tauschii	Hessian fly resistance	[30]
	Triticum monococcum	powdery mildew & leaf rust	[31]
	Aegilops variabilis.	nematode (*Heterodera avenae*) resistance	[32]
Rice	*Oryza longistaminata*	bacterial blight disease resistant	[33]
	Oryza nivara	Grassy stunt virus resistance	[34]
	O. brachyantha	Yellow stem borer resistance	[35]
	Oryza rupifogen	Brown plant hopper	[36]
	Oryza nivara	Bacterial blight	[37]
Maize	*Tripsacum dactyloides*	Common Rust	[38]
	Zea diploperennis	Maize Chlorotic Dwarf Virus	[38]
	Tripsacum dactyloides	Northern Corn Leaf Blight, Maize weevil	[38]
	Zea mexicana	Downy mildew, gray leaf spot	[38]
	Zea parviglumis	Gray leaf spot, banded leaf and sheath blight	[38]
Barley	*Hordeum bulbosum*	Stem rust resistance	[26]
	Hordeum vulgare	spot blotch, leaf blotch and leaf scald	[26]
Oat	*Avena strigosa*	multiple herbicide resistance	[39]
	A. barbata	leaf rust resistance	[40]

2.2 Hybridization and Cultivar Development

Hybridization is a technique to combine desirable genes from different sources, such as higher yield, disease resistance, and insect resistance, into a single plant variety. Plant breeders have successfully developed numerous disease-resistant hybrids and cultivars of various crop plants through conventional hybridization followed by selective breeding. One notable example is Lasani-2008, a wheat cultivar resistant to the Ug-99 stem rust pathotype. Pakistani plant breeders have developed Lasani-2008 variety, which exhibits high resistance to the Ug-99 race of stem rust [43].

The first step in this process is to identify the source of resistance for selecting the parental lines for crossing and developing resistant genotypes. The provided table lists various crop plant cultivars released world-wide for disease and insect resistance (Table 3).

2.3 Backcross Breeding

One of the most popular techniques for introducing a oligogene resistant to disease or insects into a high-yielding, vulnerable cultivar is backcross breeding. In this instance, the resistant cultivar is the donor, and the high-producing variety is the recipient. Researchers in India employed marker-assisted backcross breeding (MABC) to introduce blast resistance genes (xa13 and Xa21) from a donor variety into four popular Basmati rice cultivars (Pusa 3037, Pusa 3054, Pusa 3060, and Pusa 3066) [53]. This approach aimed to enhance blast resistance while retaining the desirable quality traits of Basmati rice.

2.4 Gene Pyramiding (Multi Line Breeding)

Crop plant resistance is vertical or horizontal. Vertical resistance is regulated by a single gene and is short-lived because pathogen races evolve [54]. However, horizontal resistance is regulated by multiple genes and may withstand many pathogen races, making it persistent [55]. Thus, gene pyramiding from numerous sources in single cultivars gives long-term resistance to biotic stressors (especially diseases) and has become a technique for plant breeders to generate disease-resistant cultivars against various pathogen races (Fig. 1). Composite crosses, synthetics, and multiline breeding are used [56]. Finckh et al. [57] reported different features using gene pyramiding methods, especially of composite cross, and found that composite crosses give improved and lasting resistance against diseases and insect pests. Steffan et al. [58] performed 218 crossings of 30 wheat types to integrate the bunt resistance genes in a single population and finally in a cultivar. Molecular markers were employed for analyzing the polymorphism in the F2 and F3 generations. After each crossing cycle, the populations were bulked and later investigations indicated transmission of resistance genes throughout this procedure. McDonald [59] suggested that with the availability of molecular tools, composite crosses may be utilized to accumulate the R genes in populations.

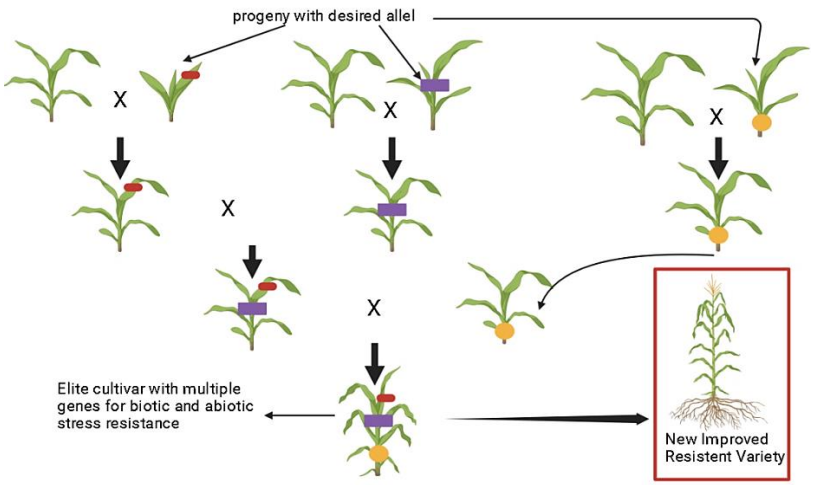

Fig. 1. Stepwise simultaneous transfer of genes into maize crop using gene pyramiding
(Source: Bio render)

Table 3. Different cultivars of cereals around the world and their disease resistance

Cereal Crops	Cultivar	Place	Resistance to Diesase	References
Rice	Koshihikari	Japan	Blast	[44]
Rice	Gangyuan8	China	Rice sheath blight	[45]
Rice	Karnal Local (indica rice)	India	Yellow stem borer	[46]
Rice	IR64	India	Blast	[46]
Rice	Swarna	India	Bacterial blight	[46]
Rice	Pusa Basmati-1	India	Blast, BLB	[46]
Rice	IR8	IRRI (Philippines)	BLB	[47]
Wheat	Pusa Yashasvi	India (ICAR)	Yellow, Brown rust	[48]
Wheat	Karan Vrinda	India (ICAR)	Leaf rust	[48]
Wheat	Karan varuna	India (ICAR)	Leaf rust	[48]
Wheat	Pusa Ojaswi	India (ICAR)	Stem & Leaf rust	[48]
Wheat	PBW 826	India (PAU)	Wheat blast	[48]
Maize	NC250P	United states	Southern corn leaf blight	[49]
Maize	Ganga 7	India	Maize Rust	[50]
Maize	Rajendra Hybrid makka -2	India	Maize rust	[50]
Maize	Nyamula	IRRI (Phillipines)	Fall army worm	[51]
Maize	ZD05	Africa	Striga resistance	[52]

Another viable method for developing horizontal resistance in agricultural plants is multiline breeding [60]. Since several iso-lines are created in multiline breeding to improve a high-yield cultivar, it is also known as the "dirty crop" strategy. Multiline combinations consist of plants with similar morphologies but distinct genetic profiles; alternatively, a single variety may be employed [56]. By using established methodologies, Marshall and Pryor (1978) investigated the effectiveness of multilines for long-term durable resistance and concluded that multilines provide steady yield because they include a large number of resistant genes [61]. Multiline cultivars are an effective strategy for managing insects and diseases. Multilines, which are combinations of iso-lines, are much more valuable in epidemiological settings since several resistance genes are at play. To generate powdery mildew-resistant wheat, Brunner et al. (2012) developed a novel method of imparting persistent resistance that blends traditional breeding with a cutting-edge transgenic technique [62]. In their study, near-isogenic lines (NILs) with various resistance genes were combined to create multilines. Except for the single R gene, these NILs were produced with the identical genetic background. Using distinct locus Pm3 alleles, they created NILs of the same origin called Pm3a, Pm3c, Pm3d, Pm3f, or Pm3g based on the allele expressed in a given line. While all of these transgenic lines demonstrated to enhanced resistance against powdery mildew the resistance increased to a greater extent than individual transgenic lines when a multiline including Pm3a, Pm3b, and Pm3d transgenic lines was created. Future studies will aim to generate agricultural plants with lasting resistance.

3. MODERN BREEDING METHODS FOR BIOTIC STRESS RESISTANCE

The problems associated with classical breeding methods are the longer time required to develop resistance cultivars, more effort and labor requirements, and the transfer of non-desirable genes along with resistance genes. Therefore, there was a need to develop new and efficient modern methods to overcome the above-mentioned problems. With the advancement of molecular genetics knowledge, many modern methods have been developed for this purpose. The modern breeding procedures to overcome the problems associated with traditional breeding strategies are given below.

3.1 Marker-Assisted Backcross Breeding for Resistance Traits

The MABC process includes molecular markers to introduce traits from a donor parent into a recurrent parent (often a popular variety). As a result, the product (variety/cultivar) keeps the

entire genome of the recurrent parent, together with the gene of interest from the donor parent. In MABC, the introduction of DNA markers dramatically improves selection efficiency while shortening varietal development time by many years. This method may also be used to generate near-isogenic lines (NILs) or chromosomal segment substitution lines (CSSLs), which are commonly utilized for gene/QTL analysis and foreign gene introgressions [63].

The steps of MABC are as follows (Fig. 2).

1. Generating F1 by crossing recurrent and donor parents.
2. Backcrossing F1 with the recurrent parent for foreground and background selections.
3. Selfing of backcross generation to obtain the gene of interest in homozygous form.

The MABC breeding program effectively improved stress tolerance in rice by introducing bacterial blight resistance genes Xa13 and Xa21 into the parental lines of the superfine grain aromatic rice hybrid Pusa RH 10 [64]. Similarly, a simultaneous but progressive transfer approach was used to transfer the blast resistance genes Pi-Kh and Piz5 from the donors Tetep and C101A51. However, the majority of breeders' desirable traits are regulated by several genes, such as resistance to multiple pathogen races. In such circumstances, the MABC technique is proven to be rather difficult in keeping the optimal gene combinations [65].

3.2 Mutation Breeding

When natural sources of resistance are not readily available within germplasm, one possible approach is to induce heritable changes or mutations in crop plants and then identify rare mutants that are resistant to biotic stress. The discovery of new germplasms can be attributed to the identification of advantageous mutations, which are genetic changes caused by the application of chemicals or radiation [66]. Additionally, novel compounds such as benzothiadiazole (BTH) have been used in wheat for this purpose. According to the International Atomic Energy Agency, a leading organization involved in mutant database development, 3250 mutant varieties from various plant species have been officially released worldwide.

According to a study by Taheri et al. [67], cereals make up 49.5% of the total, while ornamental plants and legumes account for 21.9% and 15%, respectively. Rp1-based Les Maize mutant provided protection against the *Puccinia sorghi* rust pathogen and *Cercosporazea maydis,* which causes gray leaf spot disease [68].

The development of the TILLING (targeting induced local lesions in genomes) method leads to the identification of beneficial mutations significantly, and these genome sequence data for cereal crops can be used to identify new alleles in the germplasms that could help to improve important traits like yield, disease resistance, and nutritional value [69]. It allows for the quick detection of mutations in genes of interest within a mutagenized population [70].

3.2.1 Tilling and eco-tilling

Claire McCallum and associates developed the TILLING technique in the late 1900s while working on Arabidopsis in their research [71]. Although TILLING has exposed a useful technique for detecting induced mutations, naturally existing variants may be assessed using the EcoTILLING approach. To find novel allelic variants for upcoming molecular breeding, EcoTILLING has been applied to a variety of crops, such as Arabidopsis, rice, wheat, and sorghum [72]. High-resolution melting (HRM), next-generation sequencing, and the LI-COR approach, which employs the enzyme CEL I for mutation identification, are the three main techniques for screening the mutant population utilizing TILLING [73] Out of all of these techniques, LI-COR [74] is the most recommended method for TILLING in plants. The following procedures are part of the standard approach for developing a TILLING platform in plants (Fig. 3).

3.2.2 Applications of tilling in cereal crops

For the past eight decades, mutation breeding has played a significant role in the agricultural industry. The application of TILLING to mutant breeding utilizing radiation or chemical treatment for starch synthesis, for Developing plant architecture has led to greater yielding of rice, Maize, and Chen et al [75].

3.2.3 Tilling to combat disease resistance

Multiple factors of living organisms' stress can impede wheat production, with one significant barrier being powdery mildew, stemming from *Blumeria graminis f. sp. Tritici*. Through TILLING, a technique identifying genetic variations, barley's Mlo gene equivalent in wheat, TaMlo,

was analyzed. Among the three TaMlo homoeologs in wheat, sixteen mutations altering amino acids were discovered. Four mutant lines exhibiting resistance to powdery mildew were consequently generated, marking a notable stride in creating commercially viable, non-genetically modified wheat strains resistant to this fungal disease, as reported by Acevedo-Garcia et al. [76]. Similarly, in maize TILLING populations, two genes (ZmWAK-RLK1 and ZmWAK-RLK2) associated with northern corn leaf blight resistance were identified.

3.3 Transgenic Approach

Resistance cannot be introduced via traditional hybridization when resistance genes are absent in a given species, even from its wild relatives and landraces. In this case, recombinant DNA technology is used to transfer resistant genes from another species to get past the genetic barriers. Different transformation technologies, such as gene cannon or particle bombardment, electroporation, floral dip (direct transformation methods), and Agrobacterium-mediated transformation (in direct transformation methods), are used to deliver foreign genes to agricultural plants. Even though appropriate biosafety procedures are performed while evaluating GMOs, their unnatural nature makes them risky. Between 1996 and 2003, the area planted with transgenic crops expanded 40 times globally, from 1.7 million hectares to 67.7 million hectares [77].

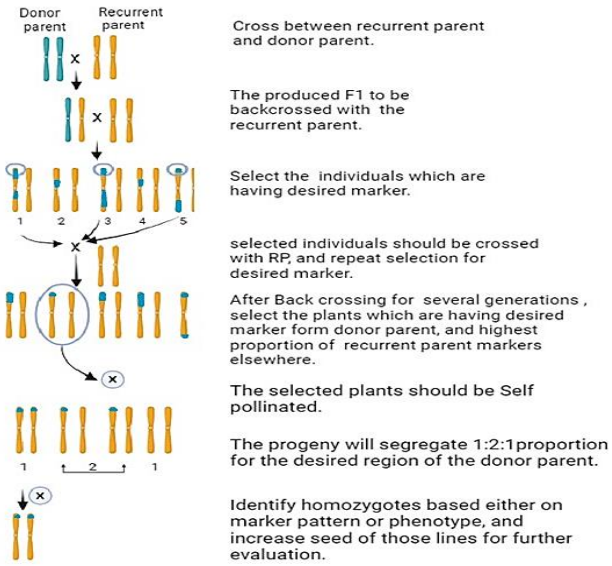

Fig. 2. Workflow of marker-assisted backcross selection
(Source: Bio render)

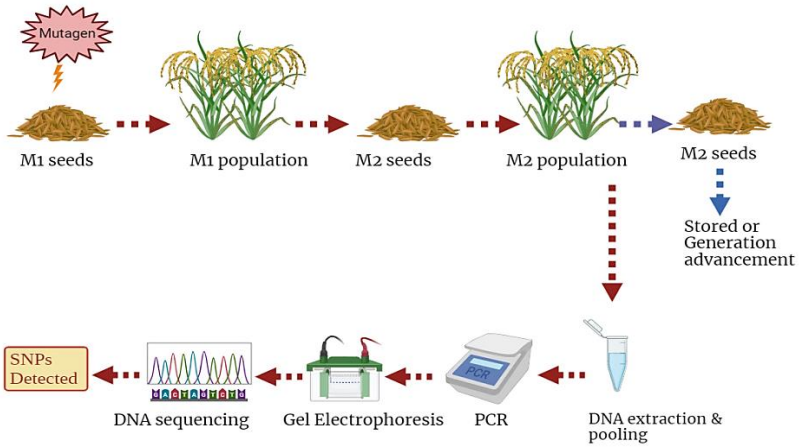

Fig. 3. Standard approach for developing a TILLING platform in plants
(Source: Bio render)

3.3.1 RNAi-mediated gene silencing

RNAi silencing has been applied against viruses, bacteria, fungi, and nematodes for the development of biotic stress-resistant plants. The RNAi phenomenon was initially recognized in Caenorhabditis Elegans, a free-living worm [78] wherein exogenously given sense and antisense RNAs effectively silenced gene expression in this model nematode. Double-stranded RNA (dsRNA) molecules produce RNA interference (RNAi), a biological process that stops the production of certain genes by causing post-transcriptional gene silencing (PTGS) [79]. It also plays a useful function in evaluating the alterations that take place in signaling networks [80].

There are essentially three steps involved in RNAi-mediated gene silencing [81]. The first step includes ribonuclease III breaking down long dsRNA into little dsRNA; the second stage involves unwinding these small RNAs to generate one guide strand, which is loaded into the RISC, while the other strand, referred to as the passenger strand, degrades. Ultimately, the RISC finds mRNAs with guide-complementary sequences, attaches to these sequences, and either destroys the mRNA or prevents its translation, all under the guidance of the guide strand [82].

3.3.2 RNAi for plant disease resistance

Pathogens pose a threat to extinction for entire plant species and can drastically reduce agricultural yields, which can have a severe negative economic impact. RNAi-induced gene silencing has been a useful tool for engineering plants resistant to pathogens in the past ten years [83]. According to Jiang CJ et al. [84], rice can employ RNAi to knockdown OsSSI2 (OsSSI2-kd), which is responsible for fatty acid desaturase activity and increased resistance against blast fungus (*Magnaporthe grisea*) and bacterial pathogens (*Xanthomonas oryzae* pv. Oryzae). Avra10, a host-induced gene that causes fungal disease in wheat (*Triticum aestivum*) and barley (*Hordeum vulgare*), can be used to silence the genes by causing temporary gene expression through RNA interference. This shows that RNAi-based plant protection against these infections results from the transfer of RNA from the host plant to the fungal pathogen *Blumeria graminis* [85]. Viral-induced gene silencing is another natural antiviral defense mechanism that RNA silencing uses to create resistance to viral infections [86]. By RNA interference (RNAi), plants may also regulate viral infections and exhibit resistance provided they possess appropriate hairpin or anti-sense RNAi constructions [87].

3.3.3 RNAi for resistance to pests and plant insects

A strong and more intensive method for combating a variety of harmful insects and pests that result in large financial losses is provided by RNAi. It is known that insects have two different types of RNAi pathways: non-cell-autonomous and cell-autonomous. Only the cells that the supplied or administered have cell-autonomous RNAi. Environmental and systemic RNAi are the two classes into which non-cell-autonomous RNAi may be divided, depending on how the cell obtains the dsRNA. Environmental RNAi refers to the uptake of dsRNA by a cell from its surrounding background. The reaction does not always propagate throughout the body as a result of environmental RNAi. Environmental RNAi is the term for dsRNA that has to be absorbed by gut cells from the gut lumen to effectively elicit RNAi. If the target gene transcripts are widely expressed in organs other than the intestine, systemic RNA interference (RNAi) is required for the silencing signal to propagate. Delivering dsRNA to the trypsin-like serine protease gene NItry of *Nilaparvata lugens*, the carboxypeptidase gene NIcar, and the hexose transporter gene NIHT1 allowed for the development of transgenic rice. The study found that the insects that consumed these transgenic rice plants had lower transcript levels of these three targeted genes. Insect lethality, however, was not mentioned [88].

3.4 GWAS (Genome-wide Association Mapping)

The substantial correlation between a marker locus and phenotypic trait is necessary for genome-wide association mapping (GWAS). This method takes thousands of polymorphisms into consideration when assessing the impact of QTLs, or quantitative trait loci. By using high resolution to identify single nucleotide polymorphisms (SNPs) inside genes that cause phenotypic change, the GWAS circumvents several limitations associated with traditional QTL/gene mapping techniques [89]. In maize, the idea of association analysis was used both on a genome-wide level [90] and as a candidate gene-based association research [91]. Since its

creation, GWAS has been enhanced to increase the validity of marker-trait connections by utilizing a variety of statistical procedures and target population configurations. These days, GWAS mapping is frequently used to analyze the genetic makeup of many complex plant characteristics, including cereals. Apart from the traditional/wide GWAS, various methods were created using association principles to identify the relationships between markers and traits. These methods include nested association mapping (NAM), multi-parent advanced generation intercross (MAGIC), and other techniques related to population structure like genomic control (GC) [89] and structured association (SA) [92].

To get around the drawbacks of biparental populations, geneticists have recently developed multi-parent populations (MPPs). The MPPs have been produced and used with success in a variety of crops, including cereals, for gene mapping and breeding [93]. With the establishment of the NAM population in maize, high-resolution mapping of the genomic areas linked to leaf architecture and quantitative resistance to leaf blight was achieved [94].

3.4.1 Nested Association Mapping (NAM)

The benefits of association mapping and linkage mapping are combined in NAM, an integrated technique involving several parents. Fixed lines (RILs, NILs, and DHs) that were created by a mixture of multiple unique and common founding parents make up NAM. The collective RIL/NIL/DH populations derived from every cross of the donor-common founder parents make up the NAM population. Using the first NAM population created in maize, at least a dozen research has been published [95].

The RILs produced by a sophisticated crossover operation incorporating multiple paternal lines make up the MAGIC population. These populations are essentially a continuation of highly developed inbred lines through intercrossing. The benefits of large germplasm collections and biparental populations are combined in the MAGIC populations. Using GWAS and MPP-based mapping techniques, important QTLs and genes for different biotic and abiotic stress tolerances in cereals have mainly been identified. In terms of disease resilience, GWAS searches revealed a novel allele Pikx at the Pik locus for blast resistance and two LRR-containing loci (Os01g0601625; Os01g0601675) linked to Bakanae resistance [96]. Furthermore, genomic regions associated with resistance to brown spot disease, submergence tolerance, and the brown planthopper (*Nilaparvata lugens*), a major rice insect pest, have been identified through the utilization of MAGIC panels (Satturu et al., 2020). Using multiple genome-wide association mapping in a 391-line MAGIC panel, thirteen putative genes linked to BPH resistance were found. According to Satturu et al. [97], these genes included WRKY70, NHL repeat-containing protein, NB-ARC domain-containing protein, and LRR-containing protein.

Because of wheat's complex genome and a higher percentage of repetitive sequences than other important cereal crops like rice, typical GWAS applications are extremely difficult to implement in this crop [98]. However, four pleiotropic QTLs for leaf and yellow rust diseases have been identified by recent GWAS using 90K SNP-chip genotyping: Lr46/Yr29, QLr-2AL.1/QYr-2AL.1, QLr-2AL.2/QYr-2AL.2, and QLr-5BL/QYr-5BL.1 [99]. Additionally, possible QTLs for much additional biotic stress tolerance in wheat, such as stem rust [100], tan spot [101], wheat blast [102], and spot blotch [103] have also been found by recent GWAS research.

Maize is an ideal crop for GWAS studies owing to quick LD decay, but barely any GWAS studies have been carried out on examining the genetic basis of stress tolerance-associated traits. Many general/classical GWAS investigations were reported for different stress tolerance phenotypes in maize, however only a small number of studies were able to uncover key genes, QTLs or alleles linked to stress tolerance. F-box protein (ZmFBL41) for banded leaf and sheath blight (BLSB) [104] and caffeoyl-CoA O-methyltransferase (ZmCCoAOMT2) for both southern leaf blight (SLB) and gray leaf spot (GLS) illnesses [105] were identified as the products of GWAS research related to biotic stress tolerance. In contrast to other cereals, MPPs were created and applied extensively for trait mapping in maize.

3.5 Genome Editing (GE)

It takes a lot of time, effort, and money to develop disease resistance into elite cultivars using the traditional strategy [106] But with the dawn of the genomics age, genome editing has become a viable way to deal with the problems associated with agricultural output. The enhancement of disease resistance in various

crops has been made possible by the ongoing advancements in genome-editing tools such as transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), site-specific mutagenesis (SSM), meganucleases (MNs), and the CRISPR/Cas system [107,108]. When compared to SSM, MNs, and ZFNs, TALENs and the CRISPR/Cas system stand out among these technologies due to their variety, speed, and relative efficiency.

a) CRISPR: The CRISPR gene family comprises repeated DNA sequences that are present in the genomes of bacteria (45%) and archaea (84%). It was initially discovered in Escherichia coli downstream of the alkaline phosphatase isozyme gene [109]. Previously referred to as short, regularly spaced repeats (SRSRs), it aids in the identification and elimination of the virus's DNA. Small guide RNAs (gRNAs) are used by the CRISPR system to interfere with invasive nucleic acids in a sequence-specific manner. A collection of brief repeating sequences, or repeats, spaced apart by distinct sequences, is known as CRISPR. The CRISPR/Cas mechanism can be broadly divided into three stages: (1) spacer acquisition or adaptation, which involves inserting unique sequences into the CRISPR locus; (2) transcription of the CRISPR locus and processing of gRNA, which involves expression or gRNA biogenesis; and (3) target interference, which involves the detection and degradation of nucleic acids by gRNA and Cas proteins [110]. Two classes (Classes I and II) comprise the CRISPR/Cas system [111]. Although the genomes of both classes of CRISPR systems might be edited, the class II system is preferred for genome editing because of its far more straightforward technique. Three hallmark proteins—Cas1, 2, and 9—which comprise three subtypes (II-A, II-B, and II-C)—classify the class II CRISPR/Cas systems. The most used CRISPR system for editing genomes in a variety of species among the three proteins is Cas9 (Fig. 4).

3.5.1 Improving biotic stress tolerance in cereals by CRISPR/Cas system

Various pesticides, herbicides, and fungicides are used to manage diseases of cereal crops, but they all contaminate the environment and lead to risks to the health of human and animal. Therefore, plants become resistant to diseases including bacteria, viruses, fungi, and insects. CRISPR/Cas variants that have been used to target certain biotic stress-responsive genes in cereal are presented below.

3.5.2 Bacterial disease tolerance

Numerous diseases in which bacteria infect grains such as Bacterial blight is a serious rice disease in West Africa and South Asia. According to some studies, rice plants afflicted with bacterial blight disease exhibit higher growth due to the presence of sucrose family (SWEET) transporters. Rice growth against bacterial blight disease was enhanced by OsSWEET13 gene knockout using CRISPR/Cas9 [112]. In another research, the CRISPR/Cas9 system altered three SWEET family genes (SWEET11, 13, and 14) to improve rice plant height and panicle length while lowering bacterial blight infection [113]. Only one family transporter has yet to be targeted by the CRISPR/Cas system. Numerous genes belonging to the resistance (R) and bacterial blights (BB) families were found and given functional descriptions in cereals. CRISPR/Cas variants that target the BB and R family genes improve grain development in the presence of bacterial illness.

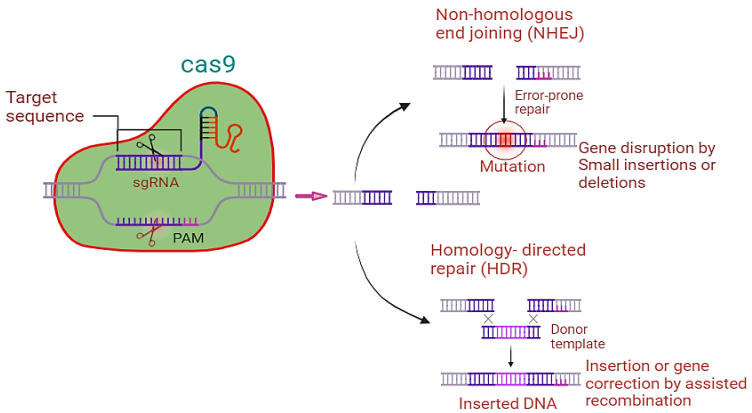

Fig. 4. CRISPR cas9 mediated genome editing
(*Source: Bio render)*

3.5.3 Fungal disease tolerance

Chemical fungicides are used in agriculture to inhibit fungal infections from causing losses. To create plants resistant against powdery mildew wheat, [114] used the CRISPR/Cas9 technology to modify the mildew resistance locus (MLO) genes (TaMLO-A1, B1, and D1). The ethylene-responsive factor 22 (OsERF922) gene in rice was knocked out using the CRISPR/Cas9 technology allowing the generation of a disease-resistant rice variety without compromising the rice's normal growth [115]. The CRISPR/Cas9 system's disruption of the rice component exocyst complex 3A (OsSEC3A) gene improved rice plant development resistance to blast disease [116]. With the use of the CRISPR/Cas9 system, Zhang et al, [117] produced powdery mildew-resistant wheat by utilizing the enhanced disease resistance1 (EDR1) gene. Therefore, using the CRISPR/Cas system to target the genes that confer resistance to fungal illnesses may reduce the virulence of infections, aiding in the management of fungal diseases throughout plant growth.

3.5.4 Insects or pest tolerance

Insects are the main carriers of viral infections in cereals, so a lot of pesticides are needed to stop the infection from spreading. The tango bacilliform virus and the tungro spherical virus work together to cause tungro disease, which is a serious obstacle to rice farming throughout tropical Asia. The function of the translation initiation factor 4 gamma (eIF4G) gene in controlling tungro spherical viruses in rice has been demonstrated in earlier research [118] Rice lines resistant to the tungro spherical virus were successfully created by researchers [119] by introducing mutations into the eIF4G gene using a CRISPR/Cas9 system. With the use of a base editor, plant genome can be modified or added which develop point mutations, assisting in the development of herbicide-resistant plants. When the acetolactate synthase (OsALS) gene was modified using a base editor, a herbicide-resistant rice plant was developed [120]. Additionally, rice growth was increased against five herbicides (nicosulfuron, imazapic, pyroxsulam, flucarbazone, and bispyribac) when the same OsALS gene was targeted by CBE (editing efficiency 37.5–61.5%) [121]. They have specifically targeted the OsALS and ideal plant architecture 1 (OsIPA1) genes in rice using prime editing. Among them, rice growth was boosted by the OsALS gene (editing efficiency: 26%) against the herbicide Bispyribac sodium. The function of several weeds and insect stress-responsive genes in wheat has been described using functional genomics techniques. Nevertheless, the existing CRISPR/Cas system has not yet been able to pinpoint the precise function of weed and insect genes in any cereals.

3.6 Microarray Technique in Biotic Stress Development

Microarray analysis is a cutting-edge method for determining how hundreds of genes express themselves in response to biotic stressors in cereal crops, like pathogenic infection. they activate a cascade of molecular responses to defend themselves. This technique involves attaching DNA fragments or probes derived from certain genes to a solid surface, like a glass slide or microchips. An RNA sample or a DNA sample labeled with fluorescent, or radiolabels makes up the label. Biological samples are subjected to gene expression pattern analysis using microarray technology. Breezing eight pathogenic connections has been transformed by this microarray research. Microarray study has yielded several insights, such as the expression patterns altered during the interaction, the way pathogen-synthesized enzymes corrupt the gene protector in cereals, and how changes are induced in plants as a counterattack [122].

3.6.1 Microarray studies in biotic stress in cereals

Plant transcriptomes have been extensively studied using DNA microarrays to address a range of biological concerns about tolerance against biotic illnesses or abiotic stressors, toxicity or shortage etc. In cereals, biotic stress resistance is a complex trait regulated by multiple genes and pathways. Microarray analysis is used to identify key genes that are upregulated and downregulated during a stressful environment. For example, genes involved in pathogen recognition, such as receptor-like kinases (RLKs) and pathogenesis-related (PR) proteins are often induced upon pathogen attack. Moreover, microarray analysis can allow the identification of candidate genes for breeding programs aimed at developing stress-resistant cereals. [122].

IET8585 (Ajaya) an indica rice cultivar exhibited resistance against both bacterial leaf blight (blb) and various strains of the X. oryzae disease. The microarray analysis showed variations in the expression of several genes resistant to the blb-

infected IET8585 cultivar compared to susceptible IR24. The resistant cultivar showed hypersensitive cell death in response to bacterial infection may be caused by increased expression of the EREBP TF gene and decreased expression of the genes that scavenge ROS and alcohol dehydrogenase. Additionally, it was proposed that upregulating defense genes during infection and inducing glutathione-mediated detoxification and flavonoid biosynthesis pathways would inhibit pathogen dissemination in host tissues [123]. Moreover, Wheat's Lr34/Yr18 gene contributes to resistance against yellow rust, leaf rust, and several other diseases. The flag leaves of two pairs of wheat near-isogenic lines for Lr34/Yr18 was subjected to microarray analysis after being mock- and rust-inoculated [124]. Mock-inoculated leaf tips of resistant plants showed upregulation of 57 genes linked to seed development, osmotic stress, cold stress, and/or ABA inducible stress. Plants having false infection did not exhibit up-regulation of PR proteins in their resistant flag leaves. On the other hand, they were up-regulated in the flag leaves of rust-inoculated plants, both resistant and vulnerable. However, increased PR gene expression in resistant plants indicated that Lr34/Yr18 might play an important role in defense reactions.

4. CONCLUSION AND FUTURE PROSPECTS

Traditional breeding methods have significantly contributed to cereal crops against biotic stress. These methods have less precession with more labor and time, thus instead of that method advanced approaches such as CRISPR-Cas9 gene editing, RNA interference, mutant breeding, Marker-Assisted Backcrossing, RNA interference, and genome-wide association studies have been intensively used in addressing biotic stress challenges. These methods allow accurate and rapid change to the plant genome enabling breeders to respond faster with agility to introduce the desired traits. MABC has greater importance in the development of resistance cultivars against oligogene disease resistance. This technique has an efficient ability to transfer desirable genes from donor cultivars or wild relatives to elite crop varieties. To combat a wide range of pests and diseases, RNA interference has arisen as a powerful tool for suppressing pathogen gene expression or bolstering plant defense systems. Advancements in breeding programs have been simplified with the identification of genomic regions associated with resistance to biotic stress through GWAS. The use of CRISPR-Cas9 technology has transformed precision breeding tool by permitting for precise modification of genes that deliver resistance to biotic stress through targeted mutations in plant genetics. Additionally, microarray technologies have aided in the creation of new breeding strategies by revealing the complex molecular pathways involved in plant-pathogen relationships.

In future breeding, the production of cereal crops that can withstand various challenges by using genomics, biotechnology, and bioinformatics can speed up. These approaches not only safeguard food security and sustainability in changing environmental conditions but also help address new issues caused by evolving pests and diseases. In the coming years, the full potential of plant breeding technologies will require collaboration across different fields and increased investment in research and development. Efforts to enhance the adoption of genetically modified crops must also focus on addressing regulatory concerns and gaining public trust. By leveraging advanced scientific and technological advancements, plant breeders can play a key role in addressing biotic stressors affecting cereal crop productivity and creating a resilient and sustainable agricultural environment.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. FAO: Food and Agriculture Organization of the United Nations; 2024.
(Accessed 2024 April 10).
2. IRRI: International Rice Research Institute; 2024.
(Accessed 2024 April 10).
3. USDA: United States Department of Agriculture; 2024.
(Accessed 2024 April 10)
4. International Grains Council. International Grains Council Grain Market Report Five-

Year Baseline Projections of Supply and Demand for Wheat, Maize (Corn), Rice and Soybeans; 2024.
Available:https://www.igc.int/
5. Savary S, Willocquet L, Elazegui FA, Castilla NP, Teng PS. Rice pest constraints in tropical Asia: quantification of yield losses due to rice pests in a range of production situations. Plant Disease. 2000;84(3):357-69.
6. Singh RP, Singh PK, Rutkoski J, Hodson DP, He X, Jørgensen LN, Hovmøller MS, Huerta-Espino J. Disease impact on wheat yield potential and prospects of genetic control. Annual Review of Phytopathology. 2016;54:303-22.
7. Kumar P, Singh R, Suby SB, Kaur J, Sekhar JC, Soujanya PL. An overview of crop loss assessment in maize. Maize Journal. 2018;7(2):56-63.
8. Khan MA. Molecular breeding of rice for improved disease resistance, a review. Australasian Plant Pathology. 2015;44:273-82.
9. Anilkumar C, Lokeshkumar BM, Sunitha NC, Basavaraj PS, Hanjagi PS. Genome-wide association mapping and genomic selection approaches for stress resilience in rice. In Next-generation plant breeding approaches for stress resilience in cereal crops. Singapore: Springer Nature Singapore. 2022;45-94
10. Kang Y, Zhou M, Merry A, Barry K. Mechanisms of powdery mildew resistance of wheat–a review of molecular breeding. Plant Pathology. 2020;69(4):601-17.
11. Ding J, Ali F, Chen G, Li H, Mahuku G, Yang N, Narro L, Magorokosho C, Makumbi D, Yan J. Genome-wide association mapping reveals novel sources of resistance to northern corn leaf blight in maize. BMC Plant Biology. 2015;15:1-1.
12. Aghnoum R, Marcel TC, Johrde A, Pecchioni N, Schweizer P, Niks RE. Basal host resistance of barley to powdery mildew: Connecting quantitative trait loci and candidate genes. Molecular Plant-Microbe Interactions. 2010;23(1):91-102.
13. Selvakumar R, Jat GS, Manjunathagowda DC. Allele mining through TILLING and EcoTILLING approaches in vegetable crops. Planta. 2023;258:15.
14. Noh TH, Lee DK, Park JC, Shim HK, Choi MY, Kang MH, Kim JD. Effect of bacterial leaf blight occurrence on rice yield and grain quality in different rice growth stage. Res Plant Dis. 2007;13:20–23.
15. Bandara YM, Weerasooriya DK, Tesso TT, Prasad PV, Little CR. Stalk rot fungi affect grain sorghum yield components in an inoculation stage-specific manner. Crop Protection. 2017;94:97-105.
16. Aghnoum R, Bvindi C, Menet G, D'hoop B, Maciel JL, Niks RE. Host/nonhost status and genetics of resistance in barley against three pathotypes of Magnaporthe blast fungi. Euphytica. 2019;215:1-9.
17. Cruz CD, Valent B. Wheat blast disease: Danger on the move. Tropical Plant Pathology. 2017; 42:210-22.
18. Mbinda W, Masaki H. Breeding strategies and challenges in the improvement of blast disease resistance in finger millet. A current review. Frontiers in Plant Science. 2021;11: 602882.
19. Sharma R, Girish AG, Upadhyaya HD, Humayun P, Babu TK, Rao VP, Thakur R. Identification of blast resistance in a core collection of foxtail millet germplasm. Plant Disease. 2014;98(4):519524.
20. Kihoro J, Bosco NJ, Murage H, Ateka E, Makihara D. Investigating the impact of rice blast disease on the livelihood of the local farmers in greater Mwea region of Kenya. Sprin Plus. 2013;2(1):308
21. Perry KL, Kolb FL, Sammons B, Lawson C, Cisar G, Ohm H. Yield effects of barley yellow dwarf virus in soft red winter wheat. Phytopathology. 2000;90:1043–1048.
22. Rogers DL. Genetic erosion: No longer just an agricultural issue. Native Plants. 2004;113–122.
23. Ullstrup AJ. The impact of the southern corn leaf blight epidemics of 1970–71. Annu Rev Phytopathol. 1972;10:37–50.
24. Christou P, Twyman RM. The potential of genetically enhanced plants to address food insecurity. Nut Res Rev. 2004;17:23–42.
25. Strange RN. Plant disease: A threat to global food security. Annu Rev Phytopathol. 2005;43:83–116.
26. Kashyap A, Garg P, Tanwar K, Sharma J, Gupta NC, Ha PT, Bhattacharya RC, Mason AS, Rao M. Strategies for utilization of crop wild relatives in plant breeding programs. Theoretical and Applied Genetics. 2022;135(12):4151-67.
27. Chhuneja P, Kaur S, Garg T, Ghai M, Kaur S, Prashar M, Bains NS, Goel RK, Keller B, Dhaliwal HS, Singh K. Mapping of adult plant stripe rust resistance genes in diploid

A genome wheat species and thseir transfer to bread wheat. Theor. Appl. Genet. 2008;116:313-324.
28. Stoilova T, Spetsov P. Chromosome 6U from Aegilops geniculata Roth carrying powdery mildew resistance in bread wheat. Breed. Sci. 2006;56:351-357.
29. Blanco A, Gadaleta A, Cenci A, Carluccio AV, Abdelbacki AMM, Simeone R. Molecular mapping of the novel powdery mildew resistance gene Pm36 introgressed from Triticum turgidum var. dicoccoides in durum wheat. Theor Appl Genetics. 2008;117(1):135–142.
30. Suszkiw J. Hessian fly-resistant wheat germplasm available Agricultural Research Service, News and Events, United States Department of Agriculture; 2005.
31. Yao G, Zhang J, Yang L, Xu H, Jiang Y, Xiong L, Zhang C, Zhang Z, Ma Z, Sorrells ME. Genetic mapping of two powdery mildew resistance genes in einkorn (*Triticum monococcum L.*) accessions. Theor. Appl. Genet. 2007;114:351-358
32. Barloy D, Lemoine J, Abelard P, Tanguy AM, Rivoal R, Jahier J. Marker-assisted pyramiding of 2 cereal cyst nematode resistance genes from *Aegilops variabilis* in wheat. Mol. Breed. 2007;20:31-40.
33. Khush GS, Bacalangco E, Ogawa T. A new gene for resistance to bacterial blight from Oryza longistaminata. Rice Genetic News. 1991;l7:121–122.
34. Brar DS, Khush GS. Alien introgression in rice. In: Sasaki T., Moore G. (Eds) Oryza: From Molecule to plant. Springer, Dordrecht; 1997.
35. Behura N, Sen P, Kar MA. Introgression of yellow stem borer (*Scirpophaga incertulus*) resistance genes into cultivated rice (*Oryza sp.*) from wild species. Indian J Agric Sci. 2011;81(4).
36. Yang M, Lin J, Cheng L, Zhou H, Chen S, Liu F, Li R, Qiu Y. Identification of a novel planthopper resistance gene from wild rice (*Oryza rufipogon Griff.*). The Crop Journal. 2020;8(6):1057-70.
37. Cheema KK, Grewal NK, Vikal Y, Sharma R, Lore JS, Das A, Bhatia D, Mahajan R, Gupta V, Bharaj TS, Singh K. A novel bacterial blight resistance gene from Oryza nivara mapped to 38 kb region on chromosome 4L and transferred to *Oryza sativa L*. Genetics Research. 2008;90(5): 397-407.
38. Wani SH, Samantara K, Razzaq A, Kakani G, Kumar P. Back to the wild: Mining maize (*Zea mays L.*) disease resistance using advanced breeding tools. Molecular Biology Reports. 2022;49(6):5787-803.
39. Lehnhoff EA, Keith BK, Dyer WE, Menalled FD. Impact of biotic and abiotic stresses on the competitive ability of multiple herbicide resistant wild oat (*Avena fatua*). Plos One. 2013;8:64478.
40. Adhikari KN, McIntosh RA, Oates JD. Distribution and temperature sensitivities of genes for stem rust resistance in Australian oat cultivars and selected germplasm. Aust. J. Agr. Res. 1999;51:75-83.
41. Shah MA, Hussain CA, Hussain KI. Selection of high yielding, disease resistance and frost tolerant varieties from exotic germplasm. Pak J Agric Res. 1982;3:479–482.
42. Sabir HM, Tahir SH, Khan MB. BT cotton and its impact on cropping pattern in Punjab. Pak J Soc Sci. 2011;31:127–134.
43. Singh RP, Hodson DP, Huerta-Espino J, Jin Y, Bhavani S, Njau P, Herrera-Foessel S, Singh PK Singh S, Govindan V. The emergence of Ug99 races of the stem rust fungus is a threat to world wheat production. Annu Rev Phytopathol. 2011;49:465 –481.
44. Ishizaki K, Hoshi T, Abe SI, Sasaki Y, Kobayashi K, Kasaneyama H, Matsui TA, Azuma S. Breeding of blast resistant isogenic lines in rice variety “Koshihikari” and evaluation of their characters. Breeding Science. 2005;55(3):371-7.
45. Wang, Y., Luo, H., Wang, H. et al. Comparative transcriptome analysis of rice cultivars resistant and susceptible to *Rhizoctonia solani* AG1-IA. BMC Genomics. 2022;23:606.
46. Ali J, Wani SH. Rice improvement: Physiological, molecular breeding and genetic perspectives. Springer Nature; 2021.
47. Khush GS. What it will take to feed 5.0 billion rice consumers in 2030. Plant Molecular Biology. 2005;59:1-6.
48. Arun Gupta, Vineet Kumar, Pradeep Kumar, Rajinder Pal, Gurudyal, Charan Singh, BS Tyagi, and Gyanendra Singh. Compendium of Wheat Varieties Notified in India during 2018-2023. Research Bulletin No. 51, ICAR- Indian Institute of Wheat and Barley Research, Karnal- 132001, India. 2023;32.
49. Belcher AR, Zwonitzer JC, Cruz JS, Krakowsky MD, Chung CL, Nelson R,

Arellano C, Balint-Kurti PJ. Analysis of quantitative disease resistance to southern leaf blight and of multiple disease resistance in maize, using near-isogenic lines. Theoretical and Applied Genetics. 2012;124:433-45.
50. Naser M, Ahamad S, Sheikh SA, Pilla A, Tandon P. Screening of maize germplasm against maize rust under natural field conditions in mid hill region of Jammu.
51. Xavier Chiriboga M, Amanuel T, Islam S S, Zeyaur K. Evaluation of African Maize Cultivars for Resistance to FallArmyworm *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) Larvae.
52. Rich PJ, Ejeta G. Towards effective resistance to Striga in African maize. Plant Signaling and Behavior. 2008;3(9):618-21.
53. Singh G, Singh N, Ellur RK, Balamurugan A, Prakash G, Rathour R, Mondal KK, Bhowmick PK, Gopala Krishnan S, Nagarajan M, Seth R. Genetic enhancement for biotic stress resistance in basmati rice through marker-assisted backcross breeding. International Journal of Molecular Sciences. 2023;24(22):16081.
54. Van der Plank JE. Principles of Plant Infection. New York, San Francisco, and London: Academic Press. 1975;216.
55. Browning JA, Frey KJ. Multiline cultivars as a means of disease control. Annu Rev Phytopathol. 1969;7:355–382.
56. Keneni G, Bekele E, Imtiaz M, Dagne K. Genetic vulnerability of modern crop cultivars: Causes, mechanism and remedies. Int J Plant Res. 2012;2:69–79.
57. Finckh M, Gacek E, Goyeau H, Lannou C, Merz U. Cereal variety and species mixtures in practice, with emphasis on disease resistance. Agronomie EDP Sci. 2000;20:813–837.
58. Steffan P, Borgen A, Lazzaro M, Backes G, Torp AM, Rasmussen SK. Marker assisted breeding and mass selection of wheat composite cross populations. In: Organic Plant Breeding: What makes the difference? 10 Year's Anniversary Conference, 3–4 Nov 2011 Frankfurt, Germany; 2011.
59. McDonald BA. Using dynamic diversity to achieve durable disease resistance in agricultural ecosystems. Trop Plant Pathol. 2014;39:191–196.
60. Mundt CC. Durable resistance: A key to sustainable management of pathogens and pests. Infect Genet Evol. 2014;27:446–455.
61. Marshall DR, Pryor AJ. Multiline varieties and disease control. Theor Appl Genet. 1978;51:177–184.
62. Brunner S, Stirnweis D, Quijano CD, Buesing G, Herren G, Parlange F, Barret P, Tassy C, Sautter C, Winzeler M et al. Transgenic Pm3 multilines of wheat show increased powdery mildew resistance in the field. Plant Biotechnol J. 2012;10:398–409.
63. Varshney RK, Mohan SM, Gaur PM, Gangarao NV, Pandey MK, Bohra A, Sawargaonkar SL, Chitikineni A, Kimurto PK, Janila P, Saxena KB. Achievements and prospects of genomics-assisted breeding in three legume crops of the semi-arid tropics. Biotechnology Advances. 2013;31(8):1120-34.
64. Singh P, Verma RL, Singh RS, Singh RP, Singh HB, Arsode P, Kumar M, Singh PK. Biotic stress management in rice (*Oryza sativa L*.) through conventional and molecular approaches. New Frontiers in Stress Management for Durable Agriculture. 2020;609-44.
65. Peleman JD, Van der Voort JR. Breeding by design. Trends in Plant Science. 2003;8(7):330-4.
66. Suprasanna P, Mirajkar SJ, Bhagwat SG. Induced mutations and crop improvement. Plant. Biol. Biotechnol. 2015;593–617.
67. Taheri S, Abdullah TL, Jain SM, Sahebi M, Azizi P. TILLING, high-resolution melting (HRM), and next-generation sequencing (NGS) techniques in plant mutation breeding. Mole. Breed. 2017;37.
68. Johal GS. Disease lesion mimics mutants of maize. Online. APSnet Features; 2007. DOI: 10.1094/APSnetFeatures-2007-0707
69. Jankowicz-Cieslak J, Mba C, Till BJ. Mutagenesis for crop breeding and functional genomics.Biotechnol. Plant. Mutation Breed. 2017;3–18.
70. Kumar APK, Mckeown PC, Boualem A, Ryder P, Brychkova G, Bendahmane A, Sarkar A, Chatterjee M, Spillane C. TILLING by Sequencing (TbyS) for targeted genome mutagenesis in crops. Mol. Breed. 2017;37:14.
71. Borevitz JO, Liang D, Plou_e D, Chang H, Zhu T, Weigel D, Berry CC, Winzeler E, Chory J. Large-scale identification of single-feature polymorphisms in complex genomes. Genome Res. 2003;13:513–523.
72. Jawher M, Arabi MIE, MirAli N, Till BJ. E_cient discovery of single-nucleotide

variations in Cochliobolus sativus vegetative compatibility groups by EcoTILLING. J. Plant Biochem. Physiol. 2018;6:2.

73. Blomstedt CK, Gleadow RM, O'Donnell N, Naur P, Jensen K, Laursen T, Olsen CE, Stuart P, Hamill JD, Møller BL. A combined biochemical screen and TILLING approach identifies mutations in *Sorghum bicolor L*. Moench resulting in acyanogenic forage production. Plant. Biotechnol. J. 2012;10: 54–66.
74. Dahmani-Mardas F, Troadec C, Boualem A, Leveˆque S, Alsadon AA, Aldoss AA, Dogimont C, Bendahmane A. Engineering melon plants with improved fruit shelf life using the TILLING approach. Plos One. 2010;5:e15776.
75. Chen L, Hao L, Parry MA, Phillips AL, Hu YG. Progress in TILLING as a tool for functional genomics and improvement of crops. J. Integr. Plant. Biol. 2014;56:425–443.
76. Acevedo-Garcia J, Spencer D, Thieron H, Reinstädler A, Hammond-Kosack K, Phillips AL, Panstruga R. Mlo-based powdery mildew resistance in hexaploid bread wheat generated by a non-transgenic TILLING approach. Plant. Biotechnol. J. 2017;15:367.
77. Sathishkumar R, Kumar SR, Hema J, Baskar V, editors. Advances in plant transgenics: Methods and applications. Berlin/Heidelberg, Germany: Springer; 2019.
78. Fire A, Xu S, Montgomery MK et al. Potent and specific genetic interference by double-strandedRNA in C. elegans. Nature. 1998;391:806–811.
79. Bosher JM, Labouesse M. RNA interference: Genetic wand and genetic watchdog. Nat Cell Bio. 2000;2:31-36.
80. Tierney MB, Lamour KH. An introduction to reverse genetic tools for investigating gene function. The Plant Health Instructor; 2005.
DOI: 10.1094/PHI-A-2005-1025-01
81. Siomi H, Siomi MC. On the road to reading the RNA-interference code. Nature. 2009;457:396–404.
82. Winter J, Jung S, Keller S et al. Many roads to maturity: Microrna biogenesis pathways and their regulation. Nat Cell Biol. 2009;11:228–234.
83. Duan CG, Wang CH, Guo HS. Application of RNA silencing to plant disease resistance. Silence. 2012;3:5.
84. Jiang CJ, Shimono M, Maeda S, Inoue H, Mori M, Hasegawa M, Sugano S, Takatsuji H. Suppression of the rice fatty-acid desaturase gene OsSSI2 enhances resistance to blast and leaf blight diseases in rice. Mol Plant-Microbe In. 2009;22:820-829.
85. Escobar MA, Civerolo EL, Summerfelt KR, Dandekar AM. RNAi-mediated oncogene silencing confers resistance to crown gall tumorigenesis. Proc Nat Acad Sci. USA. 2001;98:13437-42.
86. Ding SW. RNA-based antiviral immunity. Nat Rev Immunol. 2010;10:632-644.
87. Sudarshana MR, Roy G, Falk BW. Methods for engineering resistance to plant viruses. Methods Mol Bio. 2007;354:183-195.
88. Zha W, Peng X, Chen R et al. Knockdown of midgut genes by dsRNA-transgenic plant-mediated RNA interference in the hemipteran insect *Nilaparvata lugens.* Plos One. 2011;6:e20504.
89. Soto-cerda BJ, Cloutier S. Association mapping in plant genomes: Genetic diversity in plants. 2012;14:29–54.
90. Beló A, Zheng P, Luck S, Shen B, Meyer DJ, Li B, Tingey S, Rafalski A. Whole genome scan detects an allelic variant of fad2 associated with increased oleic acid levels in maize. Molecular Genetics and Genomics. 2008;279:1-0.
91. Thornsberry JM, Goodman MM, Doebley J, Kresovich S, Nielsen D, Buckler ES. Dwarf8 polymorphisms associate with variation in flowering time. Nature Genetics. 2001;28(3):286-9.
92. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus genotype data. Genetics. 2000;155(2):945-59.
93. Scott MF, Ladejobi O, Amer S, Bentley AR, Biernaskie J, Boden SA, Clark M, Dell'Acqua M, Dixon LE, Filippi CV, Fradgley N. Multi-parent populations in crops: A toolbox integrating genomics and genetic mapping with breeding. Heredity. 2020;125(6):396-416.
94. Kump KL, Bradbury PJ, Wisser RJ, Buckler ES, Belcher AR, Oropeza-Rosas MA, Zwonitzer JC, Kresovich S, McMullen MD, Ware D, Balint-Kurti PJ. Genome-wide association study of quantitative resistance to southern leaf blight in the maize nested association mapping population. Nature Genetics. 2011;43(2): 163-168.

95. Gireesh C, Sundaram RM, Anantha SM, Pandey MK, Madhav MS, Rathod S, Yathish KR, Senguttuvel P, Kalyani BM, Ranjith E, *Venkata Subbarao L*. Nested association mapping (NAM) populations: Present status and future prospects in the genomics era. Critical Reviews in Plant Sciences. 2021;40(1):49-67.
96. Li C, Wang D, Peng S, Chen Y, Su P, Chen J, Zheng L, Tan X, Liu J, Xiao Y, Kang H. Genome-wide association mapping of resistance against rice blast strains in South China and identification of a new Pik allele. Rice. 2019a;12:1-9.
97. Satturu V, Vattikuti JL, Kumar A, Singh RK, Zaw H, Jubay ML, Satish L, Rathore A, Mulinti S, Lakshmi VG I, Fiyaz R A. Multiple genome wide association mapping models identify quantitative trait nucleotides for brown planthopper (*Nilaparvata lugens*) resistance in MAGIC indica population of rice. Vaccines. 2020;8(4):608.
98. Pang Y, Liu C, Wang D, Amand PS, Bernardo A, Li W, He F, Li L, Wang L, Yuan X, Dong L. High-resolution genome-wide association study identifies genomic regions and candidate genes for important agronomic traits in wheat. Molecular Plant. 2020;13(9):1311-27.
99. Zhang P, Yan X, Gebrewahid TW, Zhou Y, Yang E, Xia X, He Z, Li Z, Liu D. Genome-wide association mapping of leaf rust and stripe rust resistance in wheat accessions using the 90K SNP array. Theoretical and Applied Genetics. 2021;134:1233-51.
100. Megerssa SH, Ammar K, Acevedo M, Brown-Guedira G, Ward B, Degete AG, Randhawa MS, Sorrells ME. Multiple-race stem rust resistance loci identified in durum wheat using genome-wide association mapping. Frontiers in Plant Science. 2020;11:598509.
101. Galagedara N, Liu Y, Fiedler J, Shi G, Chiao S, Xu SS, Faris JD, Li X, Liu Z. Genome-wide association mapping of tan spot resistance in a worldwide collection of durum wheat. Theoretical and Applied Genetics. 2020;133:2227-37.
102. Juliana P, He X, Kabir MR, Roy KK, Anwar MB, Marza F, Poland J, Shrestha S, Singh RP, Singh PK. Genome-wide association mapping for wheat blast resistance in CIMMYT's international screening nurseries evaluated in Bolivia and Bangladesh. Scientific Reports. 2020;10(1):15972.
103. Tomar V, Singh D, Dhillon GS, Singh RP, Poland J, Joshi AK, Singh PK, Bhati PK, Kumar S, Rahman M, Tiwari BS. New QTLs for spot blotch disease resistance in wheat (*Triticum aestivum* L.) using genome-wide association mapping. Frontiers in Genetics. 2021;11:613217.
104. Li N, Lin B, Wang H, Li X, Yang F, Ding X, Yan J, Chu Z. Natural variation in Zm FBL41 confers banded leaf and sheath blight resistance in maize. Nature Genetics. 2019b;51(10):1540-8.
105. Yang Q, He Y, Kabahuma M, Chaya T, Kelly A, Borrego E, Bian Y, El Kasmi F, Yang L, Teixeira P, Kolkman J. A gene encoding maize caffeoyl-CoA O-methyltransferase confers quantitative resistance to multiple pathogens. Nature Genetics. 2017;49(9):1364-72.
106. Romero FM, Gatica-Arias A. CRISPR/Cas9: Development and application in rice breeding. Rice Science. 2019;26(5):265-281.
107. Mishra R, Joshi RK, Zhao K. Genome editing in rice: Recent advances, challenges, and future implications. Frontiers in Plant Science. 2018;9:409924.
108. Zafar K, Khan MZ, Amin I, Mukhtar Z, Yasmin S, Arif M, Ejaz K, Mansoor S. Precise CRISPR-Cas9 mediated genome editing in super basmati rice for resistance against bacterial blight by targeting the major susceptibility gene. Frontiers in Plant Science. 2020;11:532184.
109. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isozyme conversion in Escherichia coli, and identification of the gene product. Journal of Bacteriology. 1987;169(12):5429-33.
110. Liu C, Zhang L, Liu H, Cheng K. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications. Journal of Controlled Release. 2017;266:17-26.
111. Makarova KS, Wolf YI, Iranzo J, Shmakov SA, Alkhnbashi OS, Brouns SJ, Charpentier E, Cheng D, Haft DH, Horvath P, Moineau S. Evolutionary classification of CRISPR–Cas systems: A burst of class 2 and derived variants. Nature Reviews Microbiology. 2020;18(2):67-83.
112. Zhou J, Peng Z, Long J, Sosso D, Liu BO, Eom JS, Yang B. Gene targeting by the TAL effector PthXo2 reveals cryptic

resistance gene for bacterial blight of rice. Plant J. 2015;82:632–643.
113. Oliva R, Ji C, Atienza-Grande G, Huguet-Tapia JC, Perez-Quintero A, Li T, Eom JS, Li C, Nguyen H, Liu B, Auguy F. Broad-spectrum resistance to bacterial blight in rice using genome editing. Nature Biotechnology. 2019;37(11):1344-50.
114. Wang Y, Cheng X, Shan Q, Zhang Y, Liu J, Gao C, Qiu JL. Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. Nature Biotechnology. 2014;32(9):947-51.
115. Wang F, Wang C, Liu P, Lei C, Hao W, Gao Y, Liu YG, Zhao K. Enhanced rice blast resistance by CRISPR/Cas9-targeted mutagenesis of the ERF transcription factor gene OsERF922. Plos One. 2016;11(4):e0154027.
116. Ma J, Chen J, Wang M, Ren Y, Wang S, Lei C, Cheng Z, Sodmergen. Disruption of OsSEC3A increases the content of salicylic acid and induces plant defense responses in rice. Journal of Experimental Botany. 2018;69(5):1051-64.
117. Zhang Y, Bai Y, Wu G, Zou S, Chen Y, Gao C, Tang D. Simultaneous modification of three homoeologs of Ta EDR 1 by genome editing enhances powdery mildew resistance in wheat. The Plant Journal. 2017;91(4):714-24.
118. Lee JH, Muhsin M, Atienza GA, Kwak DY, Kim SM, De Leon TB, Angeles ER, Coloquio E, Kondoh H, Satoh K, Cabunagan RC. Single nucleotide polymorphisms in a gene for translation initiation factor (eIF4G) of rice *(Oryza sativa)* associated with resistance to Rice tungro spherical virus. Molecular Plant-Microbe Interactions. 2010;23(1):29-38.
119. Macovei A, Sevilla NR, Cantos C, Jonson GB, Slamet-Loedin I, Čermák T, Voytas DF, Choi IR, Chadha-Mohanty P. Novel alleles of rice eIF4G generated by CRISPR/Cas9-targeted mutagenesis confer resistance to Rice tungro spherical virus. Plant Biotechnology Journal. 2018;16(11):1918-27.
120. Kuang Y, Li S, Ren B, Yan F, Spetz C, Li X, Zhou X, Zhou H. Base-editing-mediated artificial evolution of OsALS1 in planta to develop novel herbicide-tolerant rice germplasms. Molecular Plant. 2020;13(4):565-72.
121. Zhang R, Chen S, Meng X, Chai Z, Wang D, Yuan Y, Chen K, Jiang L, Li J, Gao C. Generating broad-spectrum tolerance to ALS-inhibiting herbicides in rice by base editing. Science China Life Sciences. 2021;64:1624-33.
122. Da Silva JA, Yücel M. Revealing response of plants to biotic and abiotic stresses with microarray technology. Genes Genomes Genomics. 2008;2(1):14-48.
123. Kottapalli KR, Rakwal R, Satoh K, Shibato J, Kottapalli P, Iwahashi H, Kikuchi S. Transcriptional profiling of indica rice cultivar IET8585 (Ajaya) infected with bacterial leaf blight pathogen Xanthomonas oryzae pv oryzae. Plant Physiology and Biochemistry. 2007;45(10-11):834-50.
124. Hulbert SH, Bai J, Fellers JP, Pacheco MG, Bowden RL. Gene expression patterns in near isogenic lines for wheat rust resistance gene Lr34/Yr18. Phytopathology. 2007;97 (9):1083-93.

© Copyright (2024): Author(s). The licensee is the journal publisher. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/117613