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Review Article

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A Study on Disease Resistance on Rice: Strategies and Challenges

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Abstract: Though Rice is cultivated in huge quantities, various disease causing agents will reduce the yield leading to not only losing the economy but also a food crisis. Production of rice is constrained by fungal, bacterial and viral diseases. In the current review, we focused on various pathological symptoms in *Oryza* species that cause high yield losses. In this context, plant breeders are attempting progressive research activity to achieve more yield and disease-resistant varieties that balance the world's rice demand and increase the farmers' income. Rice was recognized as a genetic model for research in genetics and molecular biology, for understanding growth, development, tolerance to stress and disease resistance because of its small genome. The present review focuses on the various causative agents of diminishing rice yield along with the strategies to eradicate the pathogen and thereby increasing the yield. Recent research advances at genetic level have paved a way for novel approach to understand the significance between the pheno-genotypic variations with the crop yield of rice. Further, the review also includes the advanced methodologies at molecular level so as to save the rice cultivators from economic crisis. Disease resistant genes are identified and screened using molecular markers like SSR (simple sequence repeats), RAPD (Randomly Amplified Polymorphic DNA), and RFLP (restriction fragment length polymorphisms) analysis. There exist few reports in the literature about rice cultivation, but to the best of our knowledge in a single review both cause and remedy were not discussed in detail. In this context, our review provides an insight into the aspects attributing the crop loss followed by suggesting the suitable alternative method for enhancing crop yield.

Keywords: Molecular Marker, *Oryza*, Random Amplified Polymorphic Deoxy Ribonucleic Acid, Simple Sequence Reports, Restriction Fragment Length Polymorphism

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1. INTRODUCTION

Rice is considered as one of the most important staple foods in Asian countries which diminish the hunger of huge number of people. The economy of India depends mainly on agricultural products and also it is a major producer as well as the exporter of rice [1]. Rice is primarily cultivated by farmers as a cash crop and it also provides an opportunity for agricultural workers. White rice has a lower food value than brown rice; outer brown coatings contain proteins and essential minerals. Brown rice/golden rice that contains sufficient amounts of β-carotene saves 40 million children lives from Vitamin A deficiency syndrome [2]. Rice is rich in vitamins (B&E), proteins, minerals and also has a better assortment of amino acids such as lysine and methionine. Rice is low in fat, cholesterol but has high nutritional content. Rice is composed of 77% of carbohydrates. For high yield and benefit, farmers use pesticides which are environmentally undesirable. Thus, novel techniques need to be developed that provide durable resistance, and give protection for a longer time. Transforming rice genetically offers many important opportunities for the development of contemporary varieties and development of new cultivars [3]. Breeders can rapidly develop new varieties by introducing genes that are cloned into commercial varieties. For controlling the important agronomic traits in all the major crop varieties molecular markers are used for tagging and mapping of genes. Considerable progress has been made in rice towards cloning and identification of disease-resistant genes, characterization of defense responses, and elucidation of signal transduction, which leads to the activation of defense responses resulting in the development of new rice varieties with extensive productivity. The main aim of this review is to bring at one place not only the responsible agents for a steep decrease in rice crop, but also the methods to detect and overcome the crop loss, thereby enhancing the standards of the farmers in terms of their wealth.

1.1 Factors Influencing Production Efficiency

Various cereals, pseudocereals, pulses, oil-yielding plants, fiber-yielding plants, spices, and medicinal plants are consumed by humans across the globe. All these varieties of plants are cultivated either in large scale or small scale in the fields. This leads to exposure of the plants to the environmental stresses and cause negative impact on the crop production worldwide and further can be classifies into two groups : (i) biotic factors and (ii) abiotic factors. The Biotic factors like insects, rodents, pests which have either predatory or symbiotic relationships with the host plant spread the disease and reduce crop production. The phytopathogens among the biotic stresses cause a variety of diseases in non-resistant plants by misbalancing the plant's metabolism at cellular, molecular, hormonal, and physiological levels [4].

1.2 Identification Of Genes For Nutrient Use Efficiency

The reduction of fertilizer application in both fertile as well as production poor soils requires improvement of nutrient-use efficiency that includes uptake as well as utilizing efficiency.Nutrient-efficient cultivars cannot be developed directly due to lack of many appropriate genes. Thus most of the work is still at the stage of discovering genes and QTL for nutrient efficiency. Several genes have been engineered to enhance N utilization efficiency in plants[5].

1.3 Identification Of Genes For Drought Resistance

Drought escape via a short life cycle or developmental plasticity, drought avoidance (DA) via enhanced water uptake and reduced water loss, drought tolerance (DT) via osmotic adjustment, antioxidant capacity, and desiccation tolerance all come under the mechanisms of drought resistance [6]. Thus the genetic basis of the individual components must be understood to formulate strategies for developing drought-resistant cultivars.

1.4 Identification Of Genes For Quality Improvement

Grain quality of rice includes many components such as cooking quality, eating quality, appearance quality, milling quality, and nutritional quality. The major problem of rice production in many rice-producing areas across the world begins with appearance quality of the rice grain, cooking and eating. Currently, there is a strong emphasis in China on improving eating, cooking, and appearance qualities of hybrid rice, especially the quality of indica hybrids. Amylose content, gelatinization temperature (GT), and gel consistency (GC) of the grain starch determine the cooking and eating quality. Grain shape as defined by grain length, grain width, the length–width ratio, and the translucency or chalkiness of the endosperm , all contribute to the appearance quality of the grain [7].

1.5 Identification Of Genes For Yield Trait

In rice, grain yield is multiplicatively determined by three component traits: grain weight, number of panicles per unit surface and number of grains per panicle. Large number of QTL for yield and yield component traits have been recognized during the last decade, whose credit to high-density molecular marker linkage maps. For many years, yield was generally regarded as a complex trait that is controlled by multiple genes of small effects. Several QTL have major effects in homogeneous genetic backgrounds, which has enabled QTL cloning followed by the map-based cloning approach [8].

1.6 Improving Host Recognition Of Pathogens

In order for a host to launch an appropriate defense response and mechanism against attacking pathogens, the host must first be able to identify a non-self entity. Plants have various receptors and sensors that recognize the non-self entities and depending on the nature of the receptor, they directly or indirectly interact with a variety of microbial components [9]. Based on the recognition of specific microbial components plant immunity is divided into 2 categories. The first category of defense is triggered by recognizing the host of conserved microbial components, known as pathogen-associated molecular patterns (PAMPs), or microbial-associated molecular patterns (MAMPs). The host proteins that recognize and bind a PAMP/MAMP are known as pattern recognition receptors (PRR). Binding of a PAMP to a PRR triggers an immune response, which is classified as PAMP-triggered immunity (PTI).The second category of defense is activated by the R gene-mediated recognition of pathogen effectors, and is termed as effector-triggered immunity (ETI).

This is different from PAMPs , which generally do not play a major role in pathogenicity, microbial effectors often serve as virulence factors and enter host cell to facilitate the establishment of disease. Effectors often work by suppressing PTI [10].

1.7 Phytoalexins

Low molecular weight antimicrobial compounds that are produced as a response to biotic and abiotic stresses are defined as phytoalexins. They are induced upon pathogen infection. The majority of phytoalexins in rice are diterpenoids including momilactones . A cluster of five rice genes involved in momilactone biosynthesis, which include two diterpene cylcase genes, OsKS4 and OsCyc1, along with two putative microsomal cytochrome P450 monooxygenases and one dehydrogenase [11]. In a follow-up study, authors have shown that the expression of these five genes is controlled by a chitin oligosaccharide elicitor-inducible basic leucine zipper (bZIP) transcription factor OsTGAP1 [12].

1.8 Modification of Transgenes

Genes and their products are subject to regulation at various levels in response to environmental cues in any organism. Current technology allows for modification of transgenes both at the posttranscriptional level through alteration of protein stability and at the expression level through the use of different promoters [13]. Key trends working against an increase in rice production:

1. Decline in arable land more of the reduction in arable land occurs in areas with fertile soils [14].
2. This reduction in arable land is largely caused by the construction of new roads and buildings and by reforestation of marginal arable land. Rice is often in a disadvantageous position when it comes in line along with cash crops for planting area. Therefore, most of the future increase in rice production must come from greater yields on existing crop land to avoid environmental degradation, the destruction of natural ecosystems, and loss of biodiversity [15].

1.9 Drought And Heat Tolerance

Drought and heat have occurred more frequently than ever before, causing high yield losses in the major rice-growing areas of China [16]. Scientists in China have dissected the genetic basis and mapped the genes (QTLs) in crosses between drought-tolerant germ plasm and elite cultivars [17]. A molecular breeding approach has been used to develop new varieties with drought tolerance. Candidate genes have been identified for engineering drought tolerance in rice [18]. Studies demonstrated significant genotypic variation in high temperature-induced spikelet sterility and tolerance [19].

1.10 Mutants and advanced lines exhibiting BSR

We define broad-spectrum resistance (BSR) to diseases as genetic mechanisms conferring resistance to multiple races of a pathogen and/or multiple diseases caused by different pathogens [20]. Mutational analysis indicates that many single gene mutations can confer a BSR phenotype but a majority of them have pleiotropic effects on development. Nonetheless, these mutants provide a suitable platform to understand the genetic pathways involved in resistance to multiple pathogens [21]. We have identified a number of lesion mimic mutants exhibiting BSR to blast and bacterial blight [22]. One of these lesion mimics mutations, spl11, has been recently isolated by map-based cloning. The Spl11 locus encodes a U-Box E3 ubiquitin ligase that presumably controls ubiquination of target proteins involved in programmed cell death and defense response [23]. Identification of a pivotal point in defense response is particularly useful; we can now place a number of candidate defense genes in relation to the position of Spl11 in the defense pathways. These mutants show quantitative resistance to blast and bacterial blight without any apparent lesion mimic phenotypes [24]. Expression analysis of these mutants show distinct patterns, therefore suggesting different mechanisms involved for the observed resistance phenotypes [25].

1.11 Various Causative Agents

1.11.1 Bacteria

Xanthomonas oryzae causes the Bacterial leaf blight (BLB) of rice crops. The rice yield loss of up to 10–20% was recorded under moderate infection while losses were as high as 50% in the fields severely affected by bacterial blight. Bacterial blight was first noticed by the farmers of Japan in 1884, and subsequently its existence was reported from various parts of Asia, Africa, northern Australia, and the USA [26]. Based on the crop stage, this disease reduces grain productivity in different levels based on the environmental conduciveness. The pathogen is a yellow-colored motile gram-negative rod with a polar flagellum that enters the host through wounds or natural openings. It reaches particularly the xylem. At xylem, the pathogen multiplies and infects the whole plant. *Burkholderia glumae* and *B. gladioli* are affiliated with the hot and dry weather, causes bacterial panicle blight which is considered as an important disease of rice that is seed-borne in the South India [27]. Due to the growth of secondary fungi, diseased grains change colour from gray to black or pink over time. Disease resistant varieties should be chosen because no chemical control measures are recommended.

1.11.2 Transcription Factors

Transcription factor is a protein that controls the rate of transcription of genetic information from DNA to messenger RNA, by binding to a specific DNA Sequence. They also activate transcription. Transcription factors are classified into different families based on the presence of DNA binding domains and conserved sequences. The WRKY family of transcription factors is a major group of plant-specific transcription factors involved in plant defense response and disease resistance. However, other transcription factors have also been shown to play a role in defense signaling and resistance response. In rice, several transcription factors from the WRKY superfamily have been implicated in resistance against both M. oryzae and X. oryzae pv . oryzae. Overexpression studies have shown that three WRKY-type transcription factors, OsWRKY13, OsWRKY45, and OsWRKY71 are involved in resistance to M. oryzae and X. oryzae pv [28,29].

1.12 Fungi

Magnaporthe grisea is a rice blast fungus, which is one of the most serious diseases of rice worldwide that causes a dangerous threat to the world's food production[5]. Various studies have been engrossed with resistance mechanisms of rice to the blast fungus, resulting in the isolation of several antifungal substances from rice leaves [30]. The plants have established defense strategies to resist plant pathogens. The rice blast defense response at the molecular level remains poorly understood. It is hoped that the gene manipulation may result in the generation of broad-spectrum resistant rice plants to rice pathogens. Along with the genetic approach, a direct assessment of the physiological and biochemical changes during disease infection has been used to identify genes involved in defense pathways in many plants. *Ustilaginoidea virens* a false smut fungus infects the rice at flowering stage [31]. The disease is characterized by large orange or olive green spore balls that replace one/more grains on a head. The middle of the spore masses is called sclerotia (survival structure). These sclerotia spread with the seeds and infect the next crop [32]. So, removal of the sclerotia structures in seed-cleaning reduces spread of disease. The false smut spore causes the discoloration of milled rice, but no yield loss is associated with this disease [33]. The fungus, *Cercospora janseana,* causes narrow brown coloured spots on the leaves. Spots appears to be linear and reddish-brown. This grain infection appeared as brown discoloration. They tend to be narrower, shorter and darker on resistant cultivars. The sheaths and glumes can be infected, causing significant discoloration and necrosis. On sheaths, the disease is known as sheath net blotch because of the brown cell walls and the tan-to-yellow intracellular areas that form a Netlike pattern. *Sclerotium oryzae*, the fungus causes stem rot. Damage appears as severe lodging, which makes harvesting difficult. A resistance of high level to stem rot is not available. High nitrogen and low levels of potassium favour the disease. Sclerotium comes in contact with the plant during early floods, then germinates and infects tissues near the water surface [34]. The outer sheath may die due to the development of lesions and the fungus penetrates inner sheaths and then finally to the culm. But new races of the pathogen develop very quickly. Low nitrogen content appears to favor the development of the disease. A limited number of fungicide trials were performed for the determination of the best timing against all stages. Rice spikelet rot disease is another fungal disease caused by *Fusarium proliferatum*. This disease leads to a reduction of yield by 30%. Pathogen first invades rice plants at the heading stage and results in grain discolouration, further leading to accumulation of toxic compounds [35].

1.13 Virus

The major hindrances limiting the production of rice in Africa and Asia are Insect pests and diseases associated. The (RYMV) Rice yellow mottle virus was first reported in Kenya in 1966 [36.]. The RYMV has by far been reported in many countries in Eastern and Western Africa including Cameroon, wherein some cases the whole field has been devastated[13]. There is a great necessity to study RMYV as the studies on it are very limited. Sampling methods were combined to evaluate the population of insect's vectors of rice yellow mottle virus (RYMV) in the three major ecosystems of irrigated rice in northern Cameroon, and in the low land rice fields. Another disease caused by viruses is rstripe disease caused by Rice Stripe Virus [37].

1.14 Strategies for Inducing Disease Resistance

Plant breeders are now using marker-assisted selection for the identification of specific genes; markers are small sequences of nucleic acids that make a segment of DNA [38]. With the availability of genetic linkage maps and the development of molecular markers, important progress has been achieved in the molecular marker location [39]. Marker assisted selection is also used in producing a variety of rice crops especially CSR-30 [40]. The genes combined markers are closed together on the same chromosome, they combine to stay together for a lot of generations. In recent years, there is considerable progress in the development of molecular markers, starting from protein-based isoenzymes to the RFLPs (restriction fragment length polymorphisms) and other DNA-based markers. Among DNA-based markers, simple sequence repeat (SSR) markers are comprehensively used in plants due to their suitability, abundance, hyper-variability, and for highly specific analysis. Microsatellites / Simple Sequence Repeats (SSR) have become major molecular markers for a wide range of research in plants and animals following its emergence as a polymerase chain reaction (PCR)-based genetic marker. The SSR has been widely used in various plants and in rice alone it has been used for fingerprint accessions, analyze diversity, and to identify introgressions in interspecific crosses, trace pedigrees, locate genes and quantitative trait loci on rice chromosomes and in marker-assisted selection. The SSR Markers are isolated either from DNA libraries or from data banks [41]. Association mapping will detect correlation between genetic markers[20.]. Molecular markers related closely with desirable traits are being utilized to improve the efficiency and effectiveness of breeding in conventional manner by selection of the desirable plants in segregating a gene pool, indirectly. High density molecular markers will be used for fine mapping of blast R genes [22]. The development of molecular markers that are gene-based with the sequencing of the rice genome, and the molecular markers that are microarray-based, increased the density of markers and identification of expression QTL[29]. The advancement in sequencing technologies led to large repertoire of SNP Markers to allow accurate QTL[42]. It was also emphasized that a revolutionary technology of mass spectrometry has raised the possibility that proteins may once again become important markers for breeding and genetics. Numbers of DNA markers are now available due to the evolution of DNA marker technology and these DNA markers are available to plant breeders and geneticists, helping them to overcome the difficulties faced during conventional breeding. Using both cDNA differential screening and SSH methods, 56 defense genes were identified responsive to blast infection. Among the 56 defense genes, some genes differentially expressed their function in susceptible plants after pathogenic infection. The variations which occur rapidly in the virulence characteristics of the population raise a continuous threat to the effectiveness of existing blast-resistant varieties. Although major genes have often been cited as the underlying cause of resistance instability, mapping blast resistance genes and locating closely linked markers made it possible to identify and confirm the existence of a gene in a variety with multiple genes[19]. Pathogen related proteins were studied well as a major defense response in numerous dicot plants, both in R gene-mediated resistance and also in SAR. The R genes are the foundation for disease resistance breeding. Development of genome sequence technology promoted rapid identification and cloning of rice blast R genes [43]. Classic SAR Models were developed in Rice which demonstrated the ability of systemic resistance which was considered in various plant families offered a broad spectrum of proteins against viral, bacterial and fungal pathogens. Several defense-related PR-like genes have been cloned from rice. The defense response in rice against the bacterial leaf blight was characterized by an increase in peroxidase activity. The peroxidase genes, pox 8.1 and pox 22.3 were expressed during incompatible interactions. Increases in activities of specific extracellular peroxidases were temporally and spatially associated with a decrease in the rate of pathogen multiplication and spread, suggesting an active role for peroxidase in resistance against bacterial leaf blight [44].

2. CONCLUSION

Various infections of rice are monitored and regulated by the application of molecular markers. The disease resistance genes for the different infections are identified and tagged with the markers, and insertion to rice species finally observed disease resistance to the specific pathogen. Molecular markers help in the effective identification of pathological symptoms expressed in different parts of the plant body, which in turn provide control measures by causing the inactivation of disease-inducing characteristics. However, targeting a single gene specific for overcoming tolerance to various stress conditions or to a biological causative agent is very difficult and requires huge expertise.

3. AUTHORS CONTRIBUTION STATEMENT

Nadeem Siddiqui Idea assisted in gathering the data. Nannapaneni Sreeja g gave inputs for designing the manuscript. Chadalavada Supriya Involved collecting information about causative agents of the manuscript. Potluri Bhavana Contributed for collection of references . Vemuri Sarvani and Siva Reddy Contributed for revising the entire manuscript. All authors discussed the topics to be covered in the manuscript and contributed equally and approved the final manuscript.

4. CONFLICT OF INTEREST

Conflict of interest declared none.

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