Recent Advances in Rice Varietal Development for Durable Resistance to Biotic and Abiotic Stresses through Marker-Assisted Gene Pyramiding

Md Azadul Haque 1,2, Mohd Y. Rafii 1,3,*, Martini Mohammad Yusoff 3, Nusaibah Syd Ali 4, Oladosu Yusuff 1, Debi Rani Datta 1, Mohammad Anisuzzaman 1,5 and Mohammad Ferdous Ikbal 1,2

Abstract: Abiotic and biotic stresses adversely affect rice growth, development and grain yield. Traditional rice breeding techniques are insufficient in modern agriculture to meet the growing population’s food needs on a long-term basis. The development of DNA markers closely linked to target genes or QTLs on rice chromosomes, and advanced molecular techniques, such as marker-assisted selection (MAS), have encouraged the evolution of contemporary techniques in rice genetics and breeding, such as gene pyramiding. Gene pyramiding refers to the act of combining two or more genes from multiple parents into a single genotype, which allows the overexpression of more than one gene for broad-spectrum abiotic and biotic stress resistance. Marker-assisted pedigree, backcrossing and pseudo-backcrossing methods can increase the conventional breeding speed by reducing the number of breeding generations in order to enhance the pyramiding process. Pyramiding is affected by several factors: the number of transferred genes; the range within gene and flanking markers; the number of chosen populations in every breeding generation; the features of genes and germplasms; and the potentiality of breeders to identify the target genes. Modern breeding methods, such as the marker-assisted backcrossing approach, have made gene pyramiding more precise and reliable for the development of stress-tolerant rice varieties in the coming decades. This review presents up-to-date knowledge on gene pyramiding schemes, marker-assisted gene pyramiding techniques, the efficiency of marker-assisted gene pyramiding and the advantages and limitations of gene pyramiding methods. This review also reports on the potential application of marker-assisted selection breeding to develop stress-tolerant rice varieties that stabilize abiotic and biotic stresses. This review will help rice breeders to improve yields by increasing rice productivity under abiotic and biotic stress conditions.

Keywords: gene pyramiding; rice; abiotic and biotic stresses; durable resistance; marker-assisted selection (MAS)

1. Introduction

Rice (Oryza sativa L.) is the world’s largest staple food crop, especially in Asia and Africa, and is consumed by more than 3.5 billion people. By the year 2050, the world’s population is expected to reach 9.6 billion people [1,2]. Hence, there is an urgent need to enhance rice production to meet the rising global food demand. This aim must be met in the face of rising abiotic and biotic stresses brought on by climate change, as well as growing competition for scarce resources, including land and water [3]. Rice is subjected to
an increasing number of abiotic and biotic stress combinations as a result of global warming and possible climatic change. This has a negative impact on rice growth and grain yield production [4–11]. Biotic stresses (pathogens, pests, weeds) and abiotic stresses (drought, submergence, salinity, heat, cold) negatively impact rice production worldwide. These abiotic factors affect rice growth, survival, grain filling and yield, depending on these factors’ time of occurrence [12]. Pathogens, drought, cold, submergence, high salinity and other stress factors all have a significant influence on global rice production, reducing average rice yields by more than 50% [13]. Drought, extremes of temperature and salinity have all been shown to impact the incidence and spread of diseases, insects and weeds [11,14].

The development of rice varieties resistant to abiotic and biotic stresses is the most effective and promising solution to tackle this problem. Some methods such as pedigree selection, backcrossing, recurrent selection and mutation breeding are used in conventional breeding techniques. During crossing programs that use these conventional breeding methods, unwanted genes are transferred along with target genes to the next generation and cannot be eliminated—even with several backcrosses. Marker-assisted introgression of new resistance genes/QTLs [15] can improve a rice genotype’s broad-spectrum resistance/tolerance capacities with accuracy; as it has been mentioned, conventional procedures, even with many generations of backcrosses, cannot achieve this [16]. The deployment of a single disease-resistant gene into elite rice genotypes through MAS frequently leads to a breakdown in resistance within a short time, as the relevant pathogen develops and becomes resistant to gene activity. Previously, the transferral of a single disease-resistant gene was declared to have imbued resistance into elite rice varieties [17,18]; however, within 2 or 3 years, breakdowns of resistance occurred. This was due to changes in patho-type frequency, the development of new pathogens via mutation or other processes. As a result, combining many genes that provide resistance to several stresses into a single genetic background is known to be required for long-term resistance [19]. When compared to one-, two- and three-gene combinations, multiple resistance genes confer broad-spectrum resistance to a diverse variety of races through synergistic and complementary gene activity [20,21]. The improvement in resistant varieties through gene pyramiding can be regarded as the most successful approach for stabilizing biotic and abiotic stresses in rice.

Gene pyramiding is the stacking of two or more genes from multiple parents into a single genotype for long-lasting tolerance in rice. The gene pyramiding process was first developed in wheat crops by Watson and Singh [22]. MAS-based gene pyramiding is the most promising method to strengthen durable, resistant varieties against stresses. Several researchers have pyramided multiple genes/QTLs into a single rice variety for resistance to abiotic and biotic stresses. Das et al. pyramided six QTLs/genes into the elite Tapaswini rice variety through marker-assisted selection for biotic and abiotic stress resistance [23]. Das et al. also improved the rice variety CRMAS2621-7-1, denoted Improved Lalat, that had already been pyramided with three BB-resistant genes (Xa5, Xa13 and Xa21). Here, resistance and tolerance to biotic and abiotic stresses were increased by pyramiding with ten genes/QTLs [24]. In this review, we highlight the successful applications of MAS-based gene pyramiding for improving rice varieties against biotic and abiotic stresses. This up-to-date guidance will help breeders to develop rice varieties with durable resistance against abiotic and biotic stresses.

2. A Definite Gene Pyramiding Diagram

In the below gene pyramiding diagram, a plan to combine all the desired genes from multiple parents into a single genetic background for durable resistance is depicted. DNA marker applications allow the identification of genes/QTLs at each breeding generation and quicken the gene pyramiding process. Gene pyramiding implies the derivation of a perfect genotype where favorable alleles are homozygous states at all loci. There are two parts to gene pyramiding; the pedigree step and the fixation step [25]. First, the pedigree step desires to accumulate all of the targeted genes from multiple parents into a single root
genotype. The fixation phase is the second stage, and it entails fixing the target genes into a homozygous condition, i.e., to create the ideal genotype from a single genotype. Each of the tree’s nodes is referred to as an intermediate genotype, and it has two parents. This intermediate genotype variation has the ability to resist. The intermediate genotypes are not just any offspring of a given cross; they are a specific genotype chosen from among the offspring that has all of the parental target genes. The pedigree breeding approach offers innovation through enhanced performance via crossing, recombinant parents and the selection of segregating progeny. Here, the genotypes enter a gametes population and multiply their genetic material. This leads to a completely homozygous population that can contain the ideotype. Applying this technique, breeders can produce the ideal genotypes after achieving the root genotype within just one extra generation (Figure 1). This method is effective for hereditary traits such as seed size, shape, disease and insect resistance, height and ripeness. The pedigree breeding method is widely applied when a trait is regulated by major genes when developing disease-resistant varieties [26]. However, in certain plant species, generating a large population of doubled haploids is difficult and time-consuming [27].

Figure 1. A definite gene pyramiding diagram of accumulating six desired/targeted genes [27].

Selfing the root genotype directly to achieve the ideal genotype is an alternative to this technique. On the other hand, selfing the root genotype will result in breaking the linkage between the desired alleles, which will be difficult to detect because the linkage phase is seldom apparent in selfed populations. As a result, it may cover too many generations, causing the gene pyramiding scheme to be stretched. The other alternative method is to acquire a genotype carrying all positive alleles in pairing next to crossing the parent without a favorable allele with the root genotype. This confirms that the offspring linkage phase is known and that the genotype may be determined without mixing. Within two
generations of the root genotype, the ideal genotype will be found. This technique is easy when one of the founding parents crosses the root genotype, instead of crossing it with a blank parent. In this type of program, the genetic linkage will be known, and genotypic selections will be based on a homozygous state with the desired gene except for other regions transferring by founding parents. There is no need to fix the target gene later, thereby increasing the possibility of obtaining an ideal genotype. The procedure is termed marker-assisted backcrossing (MABC) gene pyramiding [25,28–30].

3. Gene Pyramiding Methods

3.1. Gene Pyramiding through Conventional Backcrossing

Backcrossing is a conventional breeding strategy for transferring alleles from a donor parent to a well-known elite variety at one or more loci [31,32]. This elite rice variety (recurrent parent) has many desirable traits but lacks only a few traits [33]. The objective of backcrossing is to transfer the target traits into an elite genetic background and recover the recurrent parent genome (RPG). In the process of backcrossing with the targeted traits, some unwanted traits from donor parents can be introgressed into the elite genetic background. Therefore, the backcrossed progenies are selected based on phenotypic traits. Normally, the recovery of a recurrent parent genome (RPG) requires about six to eight backcrossings [34]. Selected individual plant populations are self-pollinated after the last backcross generation, and these selected lines are homozygous for the desired trait.

The backcrossing technique may also be used to achieve target characteristics; however, due to the phenotypic-based plant selection, it is difficult to acquire the target results precisely. The rice varieties developed through traditional backcrossing contain unwanted deoxyribonucleic acid (DNA) from donor parents. Such varieties fail to become a well-known exclusive cultivar. As a result, the traditional backcrossing method of gene pyramiding for rice varietal development has been criticized as slow, laborious and ineffective. Thus, any technique that can overcome the limitations of traditional backcrossing and promote rice development is acceptable [35,36].

3.2. Gene Pyramiding through Marker-Assisted Selection

Conventional breeding methods such as backcrossing, recurrent selection and pedigree selection techniques have been applied through MAS to develop stress-tolerant rice varieties (Figure 2). The selection of progeny with targeted genes during rice improvement programs using the PCR-based molecular marker application is termed marker-assisted selection (MAS) [37]. The application of molecular markers has multiple benefits over the conventional breeding approaches [38]. Marker-assisted breeding can solve problems by permitting the breeder to select immature plants with desired genes by providing the means to discard unwanted DNA parts/unwanted genes from in-between backcrosses. As a result, the development of a well-known rice variety within two to three years is possible without unwanted genes from in-between backcrosses. The use of genetic markers can save time by reducing breeding generations as the method allows breeders to select plants at the early stage of their growth. In rice breeding, genetic mapping of QTLs and development of markers have accelerated the use of molecular techniques. Genetic markers are useful tools for selection in backcrossing and can be classified into four categories [39]. Genetic markers may help to choose desired genes whose reactions are complex to observe based on plant phenotype. Multiple disease-resistant and recessive genes are assembled into a single plant (epistematically, the gene can disguise every interaction). Additionally, molecular markers can be used for selecting rare offspring where the chromosomes containing the target allele and surrounding DNA from the donor parent have been generated by recombination near the target gene.
Figure 2. Some common breeding and selection breeding methods in rice [40].

Furthermore, DNA markers could be applied to choose an exceptional genotype. Recombination adjacent to the desired gene-formed chromosome consists of available neighboring DNA and desired genes from donor parents. Lastly, DNA markers or SNPs can be used for background selection to choose the lines having the highest recovery rate of the recurrent parent genome among the backcross progenies in MABC.

3.3. Marker-Assisted Gene Pyramiding Techniques

Simply, MAS-based gene pyramiding could be accomplished using three approaches/techniques, namely, stepwise transfer, simultaneous transfer and simultaneous and stepwise transfer combined (Figure 3).
Figure 3. Schematic presentation of different backcrossing techniques for marker-assisted gene pyramiding. RP, recurrent parent; DP, donor parent; BC, backcross; IRP, improved recurrent parent; BCd, double backcross. (A) Stepwise transfer. (B) Simultaneous transfer. (C) Simultaneous and stepwise transfer combined. In order to develop a population of BC$_3$F$_1$, the F$_1$ populations are crossed back to the recurrent parents (RP) $^{[36,41]}$. 

In the first approach, the F$_1$ plants are produced from the cross between the recurrent parent (RP1) and the donor parent (DP1). Thereafter, the F$_1$ plant populations are backcrossed with recurrent parents until the third backcross generation acquires the improved recurrent parent (IRP1). Pyramiding/stacking of multiple genes is performed by crossing the improved recurrent parent with an additional donor parent (DP2). This approach is less acceptable because it is time-consuming; however, pyramiding is extremely precise. In the second approach, the F$_1$ hybrid plants are produced from crossing between the recurrent parents and different donor parents (DP1 and DP2). The improved F$_1$ (IF$_1$) hybrids are produced by intercrossing the two F$_1$ generations. Subsequently, backcrossing will be performed between recurrent parents (RP1) and the improved F$_1$ to produce improved recurrent parents (IRP). Such pyramiding takes place at the pedigree stage when the donor parents are not the same. However, this approach is less likely to be utilized because the pyramided gene may be lost in the process. The third approach is the combination of the first and second approaches by crossing the recurrent parent (RP1) with multiple donor parents at the same time and backcrossing them up to the BC$_3$ generation. Then, the backcrossed progenies are intercrossed to acquire the pyramid lines with the target genes $^{[41]}$. This design is very acceptable because it is time-efficient and ensures the presence of the desired genes in a single genetic background. The effectiveness of marker-assisted backcrossing relies upon factors such as the distance between markers and the desired genes, the number of genes transferred, the genetic nature of traits, the markers applied, lack of technical facilities and the genetic background. When all the selection criteria are fulfilled, a good and efficient gene pyramiding design will aid in the development of a durable genotype that can withstand biotic and abiotic stresses.

4. Popularly Used Marker System in MAS-Based Gene Pyramiding in Rice

Several markers have been developed and applied in a wide range for abiotic and biotic stress resistance improvement in rice. These marker systems have been applied in rice breeding programs with remarkable success rates. Some of the marker systems include: restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), simple sequence repeat
(SSR), single-nucleotide polymorphism (SNP) and competitive allele specific PCR (KASP) markers. Meanwhile, microsatellite markers are the most frequently used markers in rice breeding programs. The most commonly applied marker system for rice improvement activities is single-nucleotide polymorphisms (SNPs) [42,43]. Several high-throughput and flexible SNP genotyping methods have been developed in order to compensate for the benefits of SNP arrays in rice breeding programs. These include fluorescent PCR-based SNP assays, such as TaqMan and KASP (competitive allele specific PCR) markers, which are especially helpful since individual indicators may be evaluated and findings can be acquired using PCR equipment or fluorescence plate readers in real-time PCR [44,45].

KASP tests are more cost-effective in genotyping compared to the TaqMan systems and have been developed in order to reduce costs and improve genotyping efficiency [44] as an alternative to TaqMan. KASP is a one-step genotyping method that employs a co-dominant pre-identified allele for SNP and InDel variants [45] and is appropriate for various experimental designs with widely varied target loci and sample counts [46]. For Korean temperate japonica rice varieties, 771 polymorphic KASP markers have been developed. KASP markers were effectively employed in rice cultivars for genetic map building and QTL analyses of disease resistance and pre-harvest sprouting resistance. For example, genetic maps were built using the KASP 205, 158 and 175 markers with three populations of F$_2$ generations from crosses between Junam and Nampyeong [47,48]. Among these markers, SSR markers are highly prioritized in breeding programs due to their abundance, co-dominance and highly polymorphic nature [26].

5. Efficiency of MAS-Based Gene Pyramiding in Rice

Pyramiding can be performed using traditional breeding methods; however, it is difficult to find plants with multiple desired genes [42,49]. Compared to traditional breeding, marker-assisted backcrossing is the most accurate and speedy technique to transfer multiple genes into popular elite rice varieties for broad-spectrum resistance. Tanksley et al. reported that 99.2% of the recovery parent genome (RPG) can be recovered by six backcrossings using traditional/conventional backcrossing [15]. By applying MABC, it is possible to achieve the same ratio of the recovery parent genome (RPG) within two to four backcrosses [33]. Jain et al. noted that up to six genes can be incorporated into an elite background using marker-assisted selection; however, in the case of conventional techniques, it is necessary to conduct independent trials to confirm the selection of a single trait. Another study reported that marker-assisted selection has enhanced the gene pyramiding procedure by permitting rice breeders to choose and select the target plants at the very early growth stage, thereby helping them to conserve their resources, e.g., field space/greenhouse space, water and nutrients [50]. Pyramiding in plant breeding is most commonly used to combine several disease-resistant genes or QTLs in rice to produce long-term disease resistance [25,51]. MAS-based gene pyramiding has been proven as a swift, efficient and advanced strategy in rice breeding programs to boost durability and resistance against biotic and abiotic stresses [52,53].

6. Advantages of Marker-Assisted Gene Pyramiding/Stacking

6.1. Speedy Recovery of Recurrent Genome Parent (RPG)

Molecular breeding by MAS is one of the most accurate techniques in which multiple resistant genes are simultaneously transferred into an elite background to solve the constraints of conventional breeding. Rice breeders have succeeded in transferring broad-spectrum multiple R-genes into elite varieties with the aid of MAS to improve stress-resistant rice cultivars. Conventional breeding requires six backcross generations to recover the recovery parent genome. However, in the case of marker-assisted selection, only three to four backcross generations are required to recover the whole RPG, thus reducing three to four backcross generations [54–56]. A combination of foreground and background selection can recover 99% of the recurrent parent genome through the utilization of marker-assisted backcross breeding (MABB) [15]. In the foreground selection, the tightly linked primers
are used to identify the introgressed R-genes into the target breeding populations at any growth phase of rice plants. On the contrary, background selection applies polymorphic primers between the donor and recurrent parents to recover the RPG in each backcross generation at any growth phase of rice plants [57]. For example, Samuels et al. in their research, used 79 verified polymorphic microsatellites for the background selection to calculate the proportion of the RPG in the chosen lines. The result of their study indicated that the RPG was 80.11% for BC$_1$F$_1$, 95.30% for BC$_2$F$_1$ and 95.9% for BC$_3$F$_2$ [58]. Recently, Kim et al. [59] applied KASP markers in foreground and background selection to select rice lines with a high cooking and eating quality in each backcross generation. The results indicated that KASP markers were efficient in identifying BC$_1$F$_1$ and BC$_2$F$_1$ plants with a high cooking and eating quality and in quickly recovering the RPG. Seven BC$_2$F$_1$ plants with targeted traits were selected and the recovery of the RPG ranged from 97.4 to 99.1%. Meanwhile, in another study, 73 KASP markers were used to recover the RPG in the BC$_2$F$_1$ and BC$_2$F$_2$ generations, and the result indicated that the RPG was 84.5% and 96.2% in the BC$_2$F$_1$ and BC$_2$F$_2$ generations, respectively [60].

6.2. Solidity or Firmness

Environmental variables are of major significance and may hinder plant character expression. However, molecular markers are consistent with any important environmental impact, which offers tremendous potential for the selection of molecular markers for MABC [34]. Since environmental factors cannot affect the application of marker-assisted breeding, rather, DNA markers can select the plant carrying the target genes at any growth stage of the plant to improve stress-tolerant plants [61].

6.3. Minimization of Linkage Drag

A minimum of six backcrossed generations are required to reduce DNA parts/unwanted genes from in-between backcrosses, while MAS may require two to three backcrossed generations. Linkage drag requires many more generations of backcrosses which might be difficult to remove using a traditional backcross if DNA parts/unwanted genes are closely linked with the target locus [62,63].

6.4. Efficiency and Cost-Effectiveness

The application of molecular markers for the selection and screening of biotic and abiotic stress-resistant rice cultivars is diversified. For this reason, MAS has been proven as an effective and promising method for rice breeding [54,64]. Traits such as salt stress or blast disease are complex because they are genetically and physiologically controlled by multiple genes/QTLs. Therefore, they are problematic and more complex to select phenotypically; however, these traits may be selected directly using molecular markers [65]. Multiple genes can be pyramided for stress tolerance through MAS. A single plant can be selected. Despite traits having poor heritability, there is no problem during selection. MAS provides a quick advance for selecting stress-tolerant plants with high-precision selection. Furthermore, minimization of linkage drags takes care of recessive genes without progeny tests. Lastly, progeny with target genes could be chosen for crossing programs through molecular markers [66].

6.5. Availability of Markers and Molecular Techniques

The continuous development of DNA markers, understanding the genetic dissection of complex traits controlled by multiple genes/QTLs, fine mapping and identification of genes/QTLs for complex traits and the interrelationship between target genes and the environment positioned MAS as one of the best, most efficient and cost-effective techniques [67].
6.6. Reduce Breeding Generations

Different techniques such as conventional backcrossing, recurrent selection, pedigree selection and induced mutation are applied in conventional breeding to create genetic variation. However, through the application of molecular techniques, DNA markers can flank a target gene, thus reducing the number of backcross generations [34].

6.7. Accuracy of Selection

It is very difficult to identify polygenic traits through the use of traditional breeding procedures. In the case of MAS, however, traits based on gene expression may be selected with markers [62].

7. Limitations of Marker-Assisted Gene Pyramiding

The high cost associated with the application of marker-assisted gene stacking has posed a major limitation to the use of this technique. Equipment and consumables are two further constraints that restrict the use of marker-assisted selection in rice breeding. These two constraints are necessary for the establishment and operation of a molecular marker laboratory; therefore, their expenses have been a major challenge in adopting marker-assisted gene pyramiding [61,68]. In developing countries, electricity poses a serious threat to preserving molecular markers in the freezer. Meanwhile, there is a dearth of literature on the economic use of marker-assisted selection compared to traditional breeding, and the cost-effectiveness of this method is different in various studies. The initial cost of using markers may appear to be higher in the short term in marker-aided backcross breeding (MABB). However, in the long term, the rapid release of newly improved rice varieties due to the use of marker-assisted selection may result in larger revenues than the cost of production [34,69]. The accuracy and reliability of QTL mapping depend on the success of MAS, and this is essential when performing QTL mapping for complex traits such as yield controlled by many QTLs with small effects compared to simple traits. Many factors affect the reliability and accuracy of QTL mapping, such as replications to create phenotypic data and the sample size [70]. Experimental study and simulations indicated that the QTL detection ability lowers with the typical populations (<200) used in the study [70]. In addition, the sampling bias affects the estimations of QTL effects in small-size populations [71].

In some cases, recombination and crossing over occur between the markers and QTLs/gens due to a loose linkage [72]. During DNA copying, recombination or crossing-over can occur, which is a fundamental problem in marker-assisted selection technology since recombination makes it impossible to know which marker variation or allele is connected with which gene variant or allele. As a result, molecular markers are classified as either direct or indirect. A marker located within a major gene is called a direct marker, whereas when a marker is located near a major gene, it is called a linked or indirect marker [73]. The functional distance between a gene or QTL and the marker associated with it is termed recombination. The greater the distance between a maker and a major gene, the larger the problem of recombination [74]. Rice breeders and other plant scientists have not been able to fully understand the concepts and ideas of molecular biologists [75]. There is also a knowledge gap among rice breeders, plant breeders and other crop scientists, which restricts the application of marker-assisted gene pyramiding.

8. Marker-Assisted Gene Pyramiding for Abiotic and Biotic Stresses in Rice—Some True Success Stories

The successful application of MAS-based gene pyramiding for improving abiotic and biotic stress-tolerant rice varieties is summarized in Tables 1 and 2.
| Improved Rice Genotype | Resistance Gene/QTLs | Traits/ Diseases/ Resistance | Country/ Regions | Markers | Donor Parents | Reference |
|------------------------|----------------------|------------------------------|------------------|---------|---------------|-----------|
| Tainung82              | Xa4, xa5, Xa7, xa13 and Xa21 | BLB                           | Taiwan           | Xa4F/4R, RM604F/604R, Xa7F/7-1R/7-2R, Xa13F/13R, and Xa21F/21R | IRBB66       | [76]       |
| Jin 23B                | Bph3+Bph14+ Bph15+Bph18+Bph20+Bph21 | BPH                           | China            | RM58, RM19324, RM3331, RM28427, RM28561, RM16553, HJ34, RR28561, B212 | PTB33, IR65482-7-216-1-2, IR71033-121-15, B5 | [77]       |
| Jalmagna               | Xa5, Xa13, a21        | BLB                           | India            | Xa5S (Multiplex), Xa5SR/R (Multiplex), RG136, pTA248 | CRMAS 2232-8S | [28]       |
| Pyramided lines        | Xa13, Xa21, Xa5, Xa4  | BLB                           | China            | Xa4F/4R, RM604F/604R, Xa7F/7-1R/7-2R, Xa13F/13R, and Xa21F/21R | IRBB66       | [21]       |
| CO39                   | Pi1, Pita, Piz5       | Blast                         | Philippines      | RZ536, RZ64, RZ612, RG456, RG869, RZ397 | C101LAC, C101A51, C101FKT | [78]       |
| Jin 23B                | P1, P2, D12           | Blast                         | China            | RM144, RM224, P12-4, HC28, RM277, M309 | BL6, Wuyuqing 2 | [79]       |
| Pusa RH10              | Xa13, Xa21            | BLB                           | India            | RG136, pTA248 | Pusa1460       | [80]       |
| –                      | Gm-2, Gm-6t           | Gall midge                    | –                | –         | Duokang1, Phalguna | [81]       |
| R2381                  | Bph3, Bph27 (t)       | BPH                           | China            | RH1078, RH7, Q31, Q52, Q56, RM471 | Balamawee, Ningjing3, CV 93–11 | [82]       |
| Swarna-Sub1            | Pi1, Pi2, Pi54        | Blast                         | India            | RM224, RM527, RM206, PI54 MAS | Swarna-LT, Swarna-A51 | [83]       |
| PRR78                  | Piz5+Piz4             | Rice blast                    | India            | AP5930, RM206, RM6100 | C101A51, Tetep | [84]       |
| Improved PR106         | Xa5+Xa13+Xa21         | BLB                           | India            | RG 556, RG 136 and pTA248 | IRBB66       | [21]       |
| Tapaswini              | Xa4+Xa5+Xa13+Xa21     | BLB                           | India            | RG 556, RG 136 and pTA248 | MABC         | [20]       |
| Improved Lalat         | Xa4+Xa5+Xa13+Xa21     | BLB                           | India            | RG 556, RG 136 and pTA248 | IRBB 60      | [21]       |
| Improved TNG82         | Xa4, xa5, Xa7, xa13, Xa21 | BLB                           | Taiwan           | Xa4F/4R, RM604F/604R, Xa7F/7-1R/7-2R, Xa13F/13R, and Xa21F/21R | IRBB66       | [21]       |
| Mangeumbyeo            | Xa4+Xa5+Xa21          | BLB                           | India            | 10603, TI10Dw, MP1 + MP2, U1/11 | IRBB57       | [85]       |
| MR219                  | qDTY2.2, qDTY3.1, qDTY12.1 | Drought                       | Malaysia         | RM236, RM511, RM520 | IR77298-14-1-2-10, IR81896-B-B-195, IR84984-83-15-18-B | [86]       |
| MRQ74                  | qDTY2.2, qDTY3.1, qDTY12.1 | Drought                       | Malaysia         | RM12460, RM511, RM520 | IR77298-14-1-2-10, IR81896-B-B-195, IR84984-83-15-18-B | [86]       |
| ADT 43                 | Pi1+Pi2+Pi33+Pi54     | Blast                         | India            | RM206, RM72, RM527, RM1233 | CT 13432-3R | [87]       |
| LuoYang69              | Bph6, Bph9            | BPH                           | China            | InD2, RM28466 | 93–11, Pokkali | [88]       |
| Improved Rice Genotype          | Pyramided Genes/QTLs | Traits/ Diseases/ Resistance | Country/ Regions | Markers                                      | Donor Parents | Reference |
|--------------------------------|----------------------|------------------------------|------------------|---------------------------------------------|---------------|-----------|
| Pyramided rice lines           | Pi2, Pi46, Pita      | Rice blast                   | China            | RM224, Ind306, Pita-Ext, Pita-Int           | H4, Huazhan   | [89]      |
| PL-(S5-n + f5-n + pf12-j)      | S5-n, f5-n, pf12-j   | Fertility improvement        | China            | SNP markers                                 | Dular, 9311   | [90]      |
| Rice                           | qCTF7, qCTF8, qCTF12  | Cold stress                  | Japan            | RM5711, RM22674                            | Eikei88223, Suisei | [91]      |
| Hua-jing-xian                  | qCTBB-5, qCTBB-6, qCTS-6, qCTS12 | Cold tolerance            | China            | RM170, RM589, RM17, RM31                   | Nan-yangzhan   | [92]      |

Table 2. List of improved rice genotypes through marker-assisted gene pyramiding with their resistance genes, donor parents, recurrent parents and available linked markers for multiple traits.

| Improved Rice Genotype          | Pyramided Genes/QTLs | Traits/ Diseases/ Resistance | Country | Linked Markers                                      | Donor Parents | Reference |
|--------------------------------|----------------------|------------------------------|---------|-----------------------------------------------------|---------------|-----------|
| ASD 16 and ADT 43              | xa5, xa13, and Xa21  | BLB, blast, ShB              | India   | pTA248, Xa13-prom, Xa5, Pi54-MAS, RM224, RM536, RM209 | IRBB60, Tetep | [93]      |
| Pyramided line                 | Pi2, Pi9, and Xa4, Xa5, Xa13, Xa21 | BLB, blast                | Malaysia | RM6836, RM8225, RM13, RM21, pTA248                 | Putra 1, IRBB60 | [94]      |
| Swarna +drought                | Pi9+, xa5+, xa21+ Bphi7+Bph3+Gm4+ Gm3+ qDTY1.1+ qDTY3.1 | Blast, BLB, BPH, drought and gall midge resistance | India   | PIRTSZ2, Xa4, XasDR, Xa13prom, pTA248, RM586, RM213, Gm4LRR, Gm SPPR, RM 431, RM 136 | IRBB60, IRBL9, Rathu Heenati, Abhaya and Aganni | [95]      |
| Tellahamsa                     | Xa21, Xa13, Pi54 and Pii | BLB, blast              | India   | pTA248, Xa13 Prom, r Pi54MAS, RM 224               | Improved Samba Mahsuri, Swarnamukhi | [96]      |
| JGL1798                        | Xa13, Xa21, Pi54     | BLB, blast                   | India   | Xa13-prom, pTA248, Pi54-MAS                       | Improved Samba Mahsuri, NLR145 | [97]      |
| RPHR-1005                      | Xa21, Gm4, Gm8, R3, R4 | BLB, gald midge, fertility restorer genes | India   | pTA248, LRR-del, PRP, DRRM-Rf3–10, DRCG-RF4–14   | SM1, SM2       | [98]      |
| Improved Lalat (Recurrent Parent) | Pi2, P9, Gm1, Gm4, Sub1 | Blast, gald midge, submergence, salt tolerance | India   | RM444, RM547, RG64, SUB1BC2, RM10745                | CIO1A51, WHD-1S-75-1-127, Kavya, Abhaya, FR13A, FL478 | [20,24,66,78, 99–105] |
| MH725                          | Xa21, Xa4, Xa27, Sub1A, Pii9, Bas2.1, Bas2 | Blast, BLB, submergence Aromatic fragrance | China   | M265, M355, NB5-2-1, RM23887, RM224, RM21, M124    | KDML105, IRBB27, 75-1-127, IR64 | [106,107] |
| Pink3                          | Genes (Sub1A-C, SSLa, Xa5, Xa21, TPS1, QTLs (qBph3, qBbl1, qB11)) | Submergence, BLB, blast, BPH | Thailand | SNP and SSR markers                               | CholSub1, Xa497, BphPQ, Bph162 | [108]      |
| Wuyujin3                       | S1w/ Wx-mq             | Low amylase content, rice strip disease | China   | ST-10, Wx-mq-OF, Wx-mq-IR                           | Kanto 194     | [109]      |
| Junam                          | PH18+Xa40+Xa3 +Pib+Pik+qSTV11GC | BPH, BB, blast, SSV       | India   | 7312.TA4+HinfI, ID35.WA3, RM1233, 10571.T7+HinfI, NSB, K6415, Indel7 | IR65482-7–216-1-2 | [110]      |
### Table 2. Cont.

| Improved Rice Genotype | Pyramided Genes /QTLs | Traits/ Diseases/ Resistance | Country | Linked Markers | Donor Parents | Reference |
|------------------------|------------------------|------------------------------|---------|----------------|---------------|-----------|
| Cultivar 9311          | BXa21, Sub1A, Pi9      | LB, blast and submergence    | China   | RM224,5198     | WH21, IR64, 1892S, Guangzhou 63S, IR31917 | [107,111,112] |
| Jinbubyeo              | Xa4, Xa5, Xa21, Pi40, Bph18 | BLB, rice blast, brown planthopper | China   | MP1+MP2, 10603, T10Dw, U1+11, 9871.77E2b and 7312.74A | IRBBS7, IR65482-4-136-2-2, IR65482-7-216-1-2 | [85,113–115] |
| Pyramided lines tms2, t gums, tms5 | Thermosensitive genetic male sterility (TGMS) | Philippines | RM257, RM174, RM11 | DQ200047-21, Norin PL 12, SA2 | [116] |
| Pyramided lines Xa21, The Bt fusion gene, The RC chitinase gene | BLB, yellow stem borer, sheath blight | Philippines | B14, B15, S1 | B5 | [117] |
| Shengdao15, Shengdao16, Xudao3 | Bph14, Bph15 Stv-b | Brown planthopper, rice stripe disease | China | B14, B15, S1 | B5 | [118] |
| Hua1015S               | Pi2, Xa23              | Blast, BLB                   | China   | RM527, Ind M-Xa23 | GZ63-4S, VE6219, HBQ810 | [119] |
| Hom Mali 821          | Sub1, Qph12            | Submergence and BPH          | Thailand | R1073indel, RM277, RM260 | IR49830, ABHAYA RGD9903 RGD9905 | [120] |
| Pyramided lines Sub1, badh2, qBl1 and qBl11 | Submergence, blast Strong fragrance | Thailand | R1073indel, RM212-RM519, RM224-RM144, Aromarker | TDK303 IR85264GID0729 | [121] |
| Junam                  | Xa3, Xa4 and QTL (qCT11) | BLB and cold tolerance       | Korea   | RM1233, RM3577, RM4112, RM5766, RM224 | IR72 | [122] |

#### 8.1. Marker-Assisted Gene Pyramiding for Blast Pathogens in Rice

Blast disease is prevalent in most of the world’s rice cultivation areas [112,123]. Blast is the devastating disease of rice associated with the fungal pathogen *Magnaporthe grisea*. About 50% of the rice yield is reduced due to blast when it occurs on a pandemic scale [124]. There are nearly 100 resistance (R) genes/alleles and 500 quantitative trait loci (QTLs) linked to blast resistance in rice [125]. Twenty major R-genes associated with rice blast resistance (Pi1, Pi2, Pi5, Pi9, Pi3, Pi25, Pi36, Pi37, Pi54, Pia/Pi-Co39, Pib, Pi2, Pikm, Pikp, Pik, Pish, Pit, Pita and Piz1) [126–135] have been cloned and described, as well as two partial resistance genes (Pi21 and Pb1) [136,137]. All cloned R-genes, with the exception of *Pd2* and *P21*, belong to the nucleotide-binding domain and R-gene class leucine-rich (NBS/LRR) [138], and some of these R genes such as *Pi5*, *Pia/Pi-Co39*, *Pikm*, *Pik* and *Pi-Co39* are required to have two neighboring NBS/LRR classes [133,134] which are not cloned in the nucleotide binding site. The *Pd2* gene is a B-lectin receptor kinase [139], whereas the *P21* gene is a proline-rich protein with a potential heavy metal-binding domain and probable protein–protein interaction motifs [137]. Identification of these R-genes has furthered scientists’ understanding of the molecular basis of blast resistance in rice [53,140]. This enabled the introgression of known functional resistance genes from wild relatives or landraces into commercial rice cultivars through MAS, enhancing host plant resistance to blast disease [93]. Marker-assisted introgression of multiple blast resistance genes into commercial rice cultivars enhanced broad-spectrum resistance against blast disease [94]. Hence, the use of resistant rice cultivars is the most viable breeding technique to combat blast pathogens. The development of resistant cultivars is a precise, innovative and environmentally friendly method of managing rice blast [54,141]. A researcher from China incorporated three blast-resistant genes (*Pi1*, *Pi2*, *D12*) in the *Jin 23B* rice variety using marker-assisted breeding. The improved *Jin 23B* carrying single, double and triple...
genes was screened using blast inoculations. The results established that the higher the number of pyramided genes in Jin 23B, the greater the blast disease tolerance [79]. Similarly, a rice breeder from the Central Rice Research Institute in India pyramided six genes (Pi2, Pi9, Gm1, Gm4, Sub1, Saltol) in an elite rice variety named Improved Lalat (Xa4, xa5, xa13 and Xa21) through MAS [24]. The pyramided lines showed a sustainable resistance against blast disease, gall midge, submergence and salt stress [24]. Similarly, Huang et al. [82] successfully pyramided three dominant genes (Pi(2)t, Pi(t)a, Piz5) for blast resistance in rice through marker-assisted selection. The two genes Pi(2)t and Pi(t)a are flanked by RFLP markers on chromosomes 11 and 6, and the Piz5 gene is flanked by SNP markers on chromosome 12 [112]. Pyramiding of multiple genes can lead to broad-spectrum disease resistance. For example, the Pi21 + Pi34 + qBR4-2 + qBR12-1 genes together [141], the Pi5 + Pi54 + Pid3 + Pigm genes together [140] and the Pik + Pita genes [142] were stacked to achieve broad-spectrum resistance to blast pathogen M. oryzae.

8.2. Marker-Assisted Gene Pyramiding for Bacterial Blight Resistance in Rice

Bacterial blight (BB) is the most destructive disease of rice caused by Xanthomonas Orzyae pv Orzyae. About 60% yield losses occurred in rice-growing areas of Asia due to bacterial blight disease. There are no useful chemicals or pesticides to prevent bacterial blight infestation [143]. About thirty-eight (Xa1, Xa3, Xa26, xa5, xa13, Xa10, Xa21, Xa23, xa25, Xa40, Xa27, Xa4, Xa7, Xa22, Xa30, Xa31, Xa33 and Xa34) genes were identified, fine mapped and cloned from various sources that are resistant to BB pathogens [21,144–146]. Pradhan et al. pyramided three BB resistance genes (Xa21, xa13 and xa5) into a BB-susceptible elite popular variety named Jalmagna, which is vulnerable to bacterial blight but tolerant to submergence through marker-assisted backcrossing, and the pyramided lines established resistance against BLB disease in the incorporated rice [28]. The Rice Science Center, Kasetsart University, Thailand, also performed gene pyramiding through marker-assisted pseudo-backcrossing by pyramiding five genes (Sub1A-C, xa5, Xa21, TPS and SSIIa) and three QTLs (qBph3, qBII and Bl11) into a popular rice variety for bacterial leaf blight, blast, BPH resistance and submergence tolerance [108]. Suh et al. also pyramided three BB-resistant genes (Xa4, xa5, Xa21), blast resistance gene (Pi40) and a brown planthopper tolerance gene (Bph18) in Jinbubyeo, a japonica rice variety, using PCR-based SSR markers through backcrossing and intercrossing for durable resistance [115]. Similar research was also conducted at the International Rice Research Institute using marker-assisted gene pyramiding for introgressing four bacterial blight resistance genes (Xa21, xa5, xa4 and xa13) into a popular variety of rice for broad-spectrum resistance [51]. The pyramided lines containing multiple genes also demonstrated a higher level of resistance compared to lines with a single gene (Table 1). Pyramiding of multiple BLB-resistant genes into an elite rice variety through MAS can enhance durability and broad-spectrum resistance [25,90,91].

8.3. Marker-Assisted Gene Pyramiding for Rice Sheath Blight Resistance in Rice

Sheath blight disease (ShB) is considered as one of the most destructive rice diseases in the world [147]. Rice sheath blight disease is caused by Rhizoctonia solani Kühn, which has a major influence on production and quality [148]. Sheath blight disease has increased dramatically as a result of the introduction of high-yielding cultivars and the administration of high dosages of nitrogen fertilizers [149]. R. solani Kühn is a soil-borne facultative parasite that occurs in the form of sclerotia, mycelium or basidiospores. Meanwhile, no sheath blight-resistant cultivars have been identified thus far [150], and chemical fungicides and cultivation techniques are currently the most common methods for avoiding and controlling the disease [148]. Due to the inability to find effective tolerance sources from germplasms, the pathogen’s ability to survive from season to season in dormant form as sclerotia, greater ranging host compatibility and high genetic variability, breeding of rice for tolerance to sheath blight has been quite unsuccessful [151]. Although no qualitative resistance to ShB has been found, quantitative resistance has been reported in some rice landraces, including Tetep, Teqing and Jasmine 85 [152,153]. From various rice sources, 50 QTLs conferring
modest resistance to rice sheath blight have been found [154]. The \( qSBR7-1 \), \( qSBR11-1 \) and \( qSBR11-2 \) QTLs were found in the Tetep [150] background and pyramided in Pusa 6B [155]. The effective strategy to develop ShB-resistant rice germplasms, viz., pyramiding of key R-genes/QTLs, for sheath blight in rice can greatly enhance host plant resistance [156]. Ramalingam et al. pyramided three QTLs (\( qSBR7-1 \), \( qSBR11-1 \) and \( qSBR11-2 \)) into two elite rice cultivars, viz., the ASD 16 and ADT 43 recurrent parents, to increase sheath blight resistance through MABC [93]. Zuo et al. yramided two QTLs, \( qSB-9^TQ \) and \( TAC1^TQ \), into two commercial varieties to develop a series of NILs. The NIL lines with both \( TAC1^TQ \) and \( qSB-9^TQ \) showed higher resistance against RhB compared to those containing one of them [157].

8.4. Marker-Assisted Gene Pyramiding for Brown Planthopper Resistance in Rice

Destructive yield reductions in rice have been majorly attributed to insects and pathogen aggression [61,158]. The rice brown planthopper (BPH) is a catastrophic insect that leads to severe yield reductions during rice cultivation [159,160]. Only a few researchers have pyramided genes resistant to brown planthopper. Using marker-assisted selection, \( Bph27(t) \) (a dominant BPH resistance gene) was introgressed into Ningjing3 (N3), a susceptible commercial japonica variety, and an indica variety, 93–11. \( Bph27(t) \) and \( Bph3 \), a long-lasting BPH resistance gene, were also pyramided by intercrossing single-gene introgressed lines using MAS. This study’s improvement in single- and double-gene pyramided lines offers novel tools for molecular breeding of long-lasting BPH-resistant rice cultivars and BPH control using resistant cultivars [82]. Wang et al. applied marker-assisted selection to produce near-isogenic \( Bph9 \) lines (NIL-Bph9) by backcrossing elite cultivar 93–11 with Pokkali (harboring \( Bph9 \)). In addition, the researchers used MAS to pyramid \( Bph6 \) and \( Bph9 \) in a 93–11 genetic background. LuoYang69, a \( Bph6 \) and \( Bph9 \) pyramided line, showed greater antixenotic and antibiosis effects on BPH, and considerably increased resistance to BPH compared to the near-isogenic lines NIL-Bph6 and NIL-Bph9 [161]. Similarly, Xu et al. also pyramided two brown planthopper-resistant genes (\( Bph14, Bph15 \)) and a rice stripe disease-resistant gene (\( Stv-b^i \)) through marker-assisted backcrossing into japonica rice [118]. The pyramided lines showed a better tolerance against BPH and rice stripe disease in terms of broader resistance (Table 1).

8.5. Marker-Assisted Gene Pyramiding for Drought Stress in Rice

Rice, as a tropical crop, is highly vulnerable to abiotic stresses such as drought, salt and submergence [162]. Drought is a major abiotic stress that affects about 42 million hectares of rice planted in rainfed lowlands and uplands annually, resulting in production losses of 13–35% [86]. The majority of common rice varieties are sensitive to drought, which widens the yield gap between potential and realized yields under marginal environments. Traditional attempts at genetic improvement in rice for drought stress tolerance have had little success due to the complex nature of the mechanisms controlling drought. Due to advanced molecular genetics and genotyping, drought tolerance QTLs such as \( qDTY1.1, qDTY2.1, qDTY3.1 \) and \( qDTY6.1 \) have been identified and fine mapped in Apo [163–165] and \( qDTY12.1 \) in Way Rarem [166] as a major effect (QTLs) linked to drought. The use of MAS-based pyramiding to deploy the above-mentioned drought QTLs led to the improvement in drought-tolerant versions of IR64, such as DRR Dhan 42, drought-tolerant MR219 [86,167] and drought-tolerant Savitri [168].

8.6. Marker-Assisted Gene Pyramiding for Submergence Tolerance in Rice

Abiotic factors such as submergence stress adversely affect the poor farmers of South and Southeast Asia living on 15 million hectares of rice-growing area. Farmers grow local rice landraces that are moderately tolerant to submergence and characterized by low yield. However, some farmers grow high-yielding rice varieties susceptible to submergence but suffer crop losses due to periodic flash floods and monsoon rain. The lasting solution to overcome this problem is developing a submergence tolerance rice variety that
is acceptable to farmers in the area through marker-aided selection [105,169]. A single major quantitative trait locus (QTL), \( \text{Sub1} \) on chromosome 9, controlling submergence tolerance, along with several minor QTLs, was fine mapped and identified [109,110]. Scientists have majorly employed the FR13A landrace as the ideal donor for submergence tolerance. The major submergence tolerance QTL named \( \text{Sub1} \) provides complete submergence tolerance for two weeks with an LOD score of 36, and an \( R^2 \) value of 69% [102], and \( \text{Sub1} \) indicates the position of approximately 0.06 cM in the genomic region [104]. The \( \text{Sub1} \) QTL region on an FR13A-derived line shows three genes (\( \text{Sub1A} \), \( \text{Sub1B} \) and \( \text{Sub1C} \)) encoding the putative ethylene-responsive factor, and these three genes have been identified as the major indicators of submergence tolerance [170]. The IR64-\( \text{Sub1} \), Samba Mahsuri-\( \text{Sub1} \), Thadokkamq-\( \text{Sub1} \), BR11-\( \text{Sub1} \) and Swarna-\( \text{Sub1} \) rice varieties were developed after analysis and identification of \( \text{Sub1} \) QTLs through marker-assisted backcross breeding [170–176]. Scientists introgressed the submergence tolerance gene (\( \text{Sub1} \)) into different varieties through marker-assisted backcrossing for submergence tolerance (Table 1). Some scientists pyramided the \( \text{Sub1} \) gene with other traits such as blast, BLB and BPH in rice [23] (Table 2). To the best of our knowledge, no one has pyramided submergence-tolerant genes/QTLs into an elite rice variety to manage flooding stress in rice.

8.7. Marker-Assisted Gene Pyramiding for Salt Tolerance Rice

Salt tolerance is a major constraint in rice-growing areas of the world. The changing climate is posed to worsen the salinity problem, and this, alongside other abiotic stresses such as submergence, high temperature and drought, will have a detrimental effect on rice production and food sustainability [177–180]. Therefore, it is necessary to increase the current rice production by about 70% to feed the world’s population estimated at 9.6 \( \times \) 10^9 by the year 2050 [181]. Based on the present scenario, improvement in salinity-tolerant rice cultivars is a promising approach through marker-assisted breeding to meet the global food demand [182]. Rice is the most salt-susceptible crop among the cereals [183]; it can tolerate about 3 dS m\(^{-1}\) ECe (electrical conductivity of saturated extract), and the yield declines above the 3 dS m\(^{-1}\) ECe level [184–186]. The osmotic effect, ion toxicity, nutritional content and rice growth are substantially affected by salinity [183,187]. Ion homeostasis, ion compartmentalization, ion transport, ion uptake, biosynthesis and accumulation of osmo-protectants, osmolytes and compatible solutes activate antioxidant enzymes for ROS detoxification, and hormone modulation is related to salt tolerance mechanisms [188–192]. The salt-tolerant landraces Pokkali and Nona Bokhra are the donors of the \( \text{Saltol} \) QTLs [193] and \( \text{SKC1} \) genes [194,195]. Popular salt-susceptible high-yielding rice varieties have been improved by introgression of \( \text{Saltol} \) QTLs/genes through the utilization of SSR and SNP markers [196–201], and the resultant improved lines can tolerate salinity stress. Many researchers have also improved rice genotypes by pyramiding salt-tolerant QTLs/genes with other traits such as rice blast, brown planthopper and bacterial leaf blight (Table 2).

8.8. Marker-Assisted Gene Pyramiding for Multiple Traits against Biotic and Abiotic Stresses in Rice

As a result of global warming and changing climatic conditions, various abiotic and biotic stresses occur individually or in combination [5,10], thereby negatively impacting rice growth, development and grain yield production [201]. In most regions of the world, particularly Asia and Africa, abiotic and biotic stresses have been shown to have profound adverse effects on rice crop survival, growth, development and production [202]. Rice crops are subjected to a variety of stresses at various phases of growth and development, and a 70% yield decrease has been observed as a result of the occurrence of abiotic stresses [202]. Similarly, significant biotic stresses (bacterial leaf blight, blast, brown planthopper and gall midge) have been shown to result in substantial rice yield losses or even rice crop failure during infestation [34]. Rice yield growth has slowed from 2.3% per year in the 1970s–1980s to approximately 1.5% in the 1990s and less than 1% in the early decades of this century [203]. Although rice production has improved significantly over time, it is still insufficient to meet the world demand [204]. The improvement in high-yielding multiple
stress-tolerant/resistant rice cultivars with improved grain quality is an effort that is long overdue [205]. To improve the current scenario, marker-assisted breeding has made an effort to introgress desired genes/QTLs conferring resistance to key abiotic and biotic stresses, as well as enhancing production and quality [23,161,206–208]. Marker-assisted gene pyramiding is an effective breeding method for transferring more than one tolerance/resistance gene into a single rice line in order to create a long-lasting and wider resistance level that prevents tolerance/resistance breakdown against certain races/pathogens [62]. For example, Luo et al. reported that an introgressed rice line established strong resistance against blast and blight diseases [106]. It can also tolerate the 14-day periodic cycle of submergence, and its rice grains have a strong aroma with 95% genetic background recovery. In rice, genes (Pi2, Pi9, Gm1, Gm4, Sub1, Saltol) have been pyramided by [24] application of marker-assisted backcrossing for multiple resistance against biotic and abiotic stresses. Datta et al. reported, from the International Rice Research Institute, the stacking of three genes (the Xa21 gene, Bt fusion gene and chitinase gene) for multiple resistance to pathogens and insects through a molecular technique using the reciprocal crossing of two transgenic rice lines previously developed by genetic engineering [117]. Furthermore, MAS has been used to successfully incorporate different genes that provide higher resistance to various biotic and abiotic stresses, e.g., pyramiding of QTLs of submergence tolerance (Sub1A), leaf/neck blast (qBL1 and qBL11), brown planthopper (Bph3) and BLB (Xa5 and Xa21) in the high-yielding and aromatic rice variety ‘Pink30’ (Table 2).

9. Conclusions

Yield reductions due to water shortage, drought, submergence, increased salinity and the emergence of pests and diseases are the major limitations facing rice production worldwide. Rice breeders must consider gene pyramiding as a promising approach to improve on these challenges through the stacking of multiple genes into a single rice genotype. The application of resistant rice varieties has been heralded as a promising approach that is economical, long-lasting and environmentally friendly to combat abiotic and biotic stresses. Deployment of single disease-resistant genes into rice through MAS frequently leads to a breakdown in the resistance within a short time. The development of more stress-resistant rice varieties for long-term tolerance/resistance is possible through combining/stacking multiple stress-resistant genes/QTLs into a single genetic background. The marker-assisted gene pyramiding technique has been successfully applied in rice improvement programs for broad-spectrum resistance to biotic stresses compared to abiotic stresses. This article reviewed gene pyramiding, methods of gene pyramiding, strategies of gene pyramiding, durable resistance to abiotic and biotic stresses, success stories and the past and present prospects of gene pyramiding. The conventional techniques of gene pyramiding are slower, time-consuming and ineffective. Therefore, molecular marker-assisted gene pyramiding techniques are the most proven methods due to their accuracy, speed and reliability. Therefore, for the minimization of biotic and abiotic stress effects, developing countries such as India, Bangladesh, Sri Lanka, Nepal and Bhutan can use gene pyramiding techniques to improve durable resistant rice varieties against biotic and abiotic stresses and initiate the application of advanced molecular instruments to enhance the breeding programs.

Author Contributions: M.A.H., M.Y.R. and M.M.Y. drafted the manuscript, while the proofreading, editing and finishing were carried out by N.S.A., O.Y., D.R.D., M.A. and M.F.I. All authors offered suggestions on various drafts of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The Higher Institution Centres of Excellence (HICoE), Research Grant (Vot number 6369105), Ministry of Education, Malaysia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.
Data Availability Statement: Not applicable.

Acknowledgments: We gratefully acknowledge the financial support from the Higher Institution Centres of Excellence (HICoE) Research Grant, the Ministry of Higher Education, Malaysia. The first author also acknowledges the Bangabandhu Science and Technology Fellowship Trust (BB-STFT) under the Ministry of Science and Technology, The People’s Republic of Bangladesh, for Ph.D. scholarship.

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

References
1. Bordey, F.H. The Impacts of Research on Philippine Rice Production. Ph.D. Thesis, University of Illinois at Urbana-Champaign, Wright St. Urbana, IL, USA, 2010.
2. Leridon, H. Population. *I. Popul. Societies* 2020, 573, 1–4.
3. Finatto, T.; de Oliveira, A.C.; Chaparro, C.; da Maia, L.C.; Farias, D.R.; Woyann, L.G.; Mistura, C.C.; Soares-Bresolin, A.P.; Llauro, C.; Panaud, O.; et al. Abiotic stress and genome dynamics: Specific genes and transposable elements response to iron excess in rice. *Rice* 2015, 8, 1–18. [CrossRef] [PubMed]
4. Ramegowda, V.; Senthil-Kumar, M. The Interactive Effects of Simultaneous Biotic and Abiotic Stresses on Plants: Mechanistic Understanding from Drought and Pathogen Combination; Elsevier GmbH.: München, Germany, 2015; Volume 176, ISBN 9126735229.
5. Prasad, P.V.V.; Pispipati, S.R.; Momčilović, I.; Ristic, Z. Independent and Combined Effects of High Temperature and Drought Stress During Grain Filling on Plant Yield and Chloroplast EF-Tu Expression in Spring Wheat. *J. Agron. Crop Sci.* 2011, 197, 430–441. [CrossRef]
6. Prasch, C.M.; Sonnewald, U. Simultaneous Application of Heat, Drought, and Virus to Arabidopsis Plants Reveals Significant Shifts in Signaling Networks. *Plant Physiol.* 2013, 162, 1849–1866. [CrossRef] [PubMed]
7. Fatimah, F.; Prasetyono, J.; Dadang, A.; Tasliah, T. Improvement of Early Maturity in Rice Variety BY Marker Assisted Backcross Breeding OF HId2 Gene. *Indones. J. Agric. Sci.* 2014, 15, 55. [CrossRef]
8. Dean, R.A.; Talbot, N.J.; Ebbole, D.J.; Farman, M.L.; Mitchell, T.K.; Orbach, M.J.; Thon, M.; Kulkarni, R.; Xu, J.-R.; Pan, H.; et al. The genome sequence of the rice blast fungus Magnaporthe grisea. *Nature* 2005, 434, 980–986. [CrossRef] [PubMed]
9. Atkinson, N.J.; Lilley, C.J.; Urwin, P.E. Identification of genes involved in the response of arabidopsis to simultaneous biotic and abiotic stresses. *Plant Physiol.* 2013, 162, 2028–2041. [CrossRef] [PubMed]
10. Pandey, P.; Ramegowda, V.; Senthil-Kumar, M. Shared and unique responses of plants to multiple individual stresses and stress combinations: Physiological and molecular mechanisms. *Front. Plant Sci.* 2015, 6, 723. [CrossRef]
11. Ziska, L.H.; Tomecek, M.B.; Gealy, D.R. Competitive Interactions between Cultivated and Red Rice as a Function of Recent and Projected Increases in Atmospheric Carbon Dioxide. *Agron. J.* 2010, 102, 118–123. [CrossRef]
12. Akram, R.; Fahad, S.; Masood, N.; Rasool, A.; Ijaz, M.; Zahid, M.; Ihsan, S.; Hussain, S.; Ahmad, S.; Ijaz, M.; et al. Rice Responses and Tolerance to Metal/Metalloid Toxicity; Woodhead Publishing: Cambridge, UK, 2018; ISBN 9780128053744.
13. Anami, B.S.; Malvade, N.N.; Palaih, S. Classification of yield affecting biotic and abiotic paddy crop stresses using field images. *Inf. Process. Agric.* 2020, 7, 272–285. [CrossRef]
14. Coakley, S.M.; Scherrm, H.; Chakraborty, S. Climate Change and Plant Disease Management. *Annu. Rev. Phytopathol.* 1999, 37, 399–426. [CrossRef] [PubMed]
15. Tanksley, S.D.; Young, N.D.; Paterson, A.H.; Bonierbale, M.W. RFLP mapping in piant breeding: New tools for an old science. *Bio/Technology* 1989, 7, 257–264. [CrossRef]
16. Young, N.D.; Tanksley, S.D. Restriction fragment length polymorphism maps and the concept of graphical genotypes. *Theor. Appl. Genet.* 1989, 77, 95–101. [CrossRef]
17. Mew, T.W. Changes in Race Frequency of Xanthomonas oryzae pv. oryzae in Response to Rice Cultivars Planted in the Philippines. *Plant Dis.* 1992, 76, 1029. [CrossRef]
18. George, M.L.C.; Bustamam, M.; Cruz, W.T.; Leach, J.E.; Nelson, R.J. Movement of Xanthomonas oryzae pv. oryzae in Southeast Asia Detected Using PCR-Based DNA Fingerprinting. *Phytopathology* 1997, 87, 302–309. [CrossRef]
19. Singh, A.K.; Singh, A.; Singh, V.K.; Gopala, K.S.; Ellur, R.K.; Singh, D.; Ravindran, G.; Bhowmick, P.K.; Nagarajan, M.; Vinod, K.K.; et al. Public private partnership for hybrid rice. In Proceedings of the 6th International Hybrid Rice Symposium, Hyderabad, India, 10–12 September 2012.
20. Dokku, P.; Das, K.M.; Rao, G.J.N. Pyramiding of four resistance genes of bacterial blight in Tapaswini, an elite rice cultivar, through marker-assisted selection. *Euphytica* 2013, 192, 87–96. [CrossRef]
21. Dokku, P.; Das, K.M.; Rao, G.J.N. Genetic enhancement of host plant-resistance of the Lalat cultivar of rice against bacterial blight employing marker-assisted selection. *Biotechnol. Lett.* 2013, 35, 1339–1348. [CrossRef] [PubMed]
22. Watson, I.A.; Singh, B.D. The future for rust resistant wheat in Australia. *J. Aust. Inst. Agric. Sci.* 1952, 18, 190–197.
23. Das, G.; Rao, G.J.N.; Varier, M.; Prakash, A.; Prasad, D. Improved Tapaswini having four BB resistance genes pyramided with six genes/QTLs, resistance/tolerance to biotic and abiotic stresses in rice. *Sci. Rep.* 2018, 8, 1–16. [CrossRef] [PubMed]
24. Das, G.; Rao, G.J.N. Molecular marker assisted gene stacking for biotic and abiotic stress resistance genes in an elite rice cultivar. *Front. Plant Sci.* 2015, 6, 1–18. [CrossRef] [PubMed]
25. Ji, Z.; Shi, J.; Zeng, Y.; Qian, Q.; Yang, C. Application of a simplified marker-assisted backcross technique for hybrid breeding in rice. *Biology* 2014, 69, 463–468. [CrossRef]

26. Miah, G.; Rafii, M.Y.; Ismail, M.R.; Puthe, A.B.; Rahim, H.A.; Islam, N.K.; Latif, M.A. A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *Int. J. Mol. Sci.* 2013, 14, 22499–22528. [CrossRef] [PubMed]

27. Servin, B.; Martin, O.C.; Mézard, M.; Hospital, F. Toward a theory of marker-assisted gene pyramiding. *Genetics* 2004, 168, 513–523. [CrossRef] [PubMed]

28. Pradhan, S.K.; Nayak, D.K.; Mohanty, S.; Behera, L.; Barik, S.R. Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. *Rice* 2015, 8, 1–14. [CrossRef] [PubMed]

29. Pinta, W.; Toojinda, T.; Thummabenjapone, P.; Sanitchon, J. Pyramiding of blast and bacterial leaf blight resistance genes into rice cultivar RD6 using marker assisted selection. *Afr. J. Biotechnol.* 2013, 12, 4432–4438. [CrossRef]

30. Rajpurohit, D.; Kumar, R.; Kumar, M.; Paul, P.; Awasthi, A.; Osman Basha, P.; Puri, A.; Jhang, T.; Singh, K.; Dhaliwal, H.S. Pyramiding of two bacterial blight resistance and a semi dwarfing gene in Type 3 Basmati using marker-assisted selection. *Euphytica* 2011, 178, 111–126. [CrossRef]

31. Hospital, F. Selection in backcross programmes. *Philos. Trans. R. Soc. B Biol. Sci.* 2005, 360, 1503–1511. [CrossRef]

32. Reyes-Valdés, M.H. A Model for Marker-Based Selection in Gene Introgression Breeding Programs. *Crop Sci.* 2000, 40, 91–98. [CrossRef]

33. Brumlop, S.; Finckh, M. *Applications and Potentials of Marker Assisted Selection (MAS) in Plant Breeding*; Bundesamt für Naturschutz (German Federal Agency for Nature Conservation): Bonn, Germany, 2011; ISBN 9783896240330.

34. Hasan, M.M.; Rafii, M.Y.; Ismail, M.R.; Alam, M.A.; Ashkani, S.; Malek, M.A.; Latif, M.A. Marker-assisted backcrossing: A useful method for rice improvement. *Biotechnol. Biotechnol. Equip.* 2015, 29, 237–254. [CrossRef]

35. Lema, M. Marker Assisted Selection in Comparison to Conventional Plant Breeding: Review Article. *Agric. Res. Technol. Open Access J.* 2018, 14, 555914. [CrossRef]

36. Suresh, S.; Malathi, D. Gene Pyramiding For Biotic Stress Tolerance In Crop Plants. *Wkly. Sci. Res. J.* 2013, 23, 1–14.

37. Yadav, S.; Anuradha, G.; Kumar, R.R.; Vemireddy, L.R.; Sudhakar, R.; Donempudi, K.; Venkata, D.; Jabeen, F.; Narasimhan, Y.K.; Marathi, B.; et al. Identification of QTLs and possible candidate genes conferring sheath blight resistance in rice (*Oryza sativa* L.). *Springerplus* 2015, 4, 1–12. [CrossRef] [PubMed]

38. Khan, M.A.; Ahmad, J. In vitro wheat haploid embryo production by wheat x maize cross system under different environmental conditions. *Pak. J. Agric. Sci.* 2011, 48, 49–53.

39. Chukwu, S.C.; Rafii, M.Y.; Ramlee, S.I.; Ismail, S.I.; Hasan, M.M.; Oladosu, Y.A.; Magaji, U.G.; Akos, I.; Olalekan, K.K. Marker-assisted selection for bacterial leaf blight resistance in rice: A review of conventional breeding to molecular approach. *Mol. Biol. Rep.* 2019, 46, 1519–1532. [CrossRef] [PubMed]

40. Moose, S.P.; Mumm, R.H. Molecular plant breeding as the foundation for 21st century crop improvement. *Plant Physiol.* 2008, 147, 969–977. [CrossRef]

41. Kumar Joshi, R.; Nayak, S. Gene pyramiding-A broad spectrum technique for developing durable stress resistance in crops. *Biotechnol. Mol. Biol. Rev.* 2010, 5, 50–61.

42. Choudhary, K.; Choudhary, K.; Choudhary, O.P.; Shekhwat, N.S. Marker Assisted Selection: A Novel Approach for Crop Improvement. *J. Agron.* 2008, 1, 26–30.

43. Hayashi, K.; Hashimoto, N.; Daigen, M.; Ashikawa, I. Development of PCR-based SNP markers for rice blast resistance genes at the Piz locus. *Theor. Appl. Genet.* 2004, 108, 1212–1220. [CrossRef]

44. Thomson, M.J. High-Throughput SNP Genotyping to Accelerate Crop Improvement. *Plant Breed. Biotechnol.* 2014, 2, 195–212. [CrossRef]

45. Semagn, K.; Babu, R.; Hearne, S.; Olsen, M. Single nucleotide polymorphism genotyping using Kompetitive Allele Specific PCR (KASP): Overview of the technology and its application in crop improvement. *Mol. Breed.* 2014, 33, 1–14. [CrossRef]

46. Steele, K.A.; Quinton-Tulloch, M.J.; Amgai, R.B.; Dhakal, R.; Khatiwada, S.P.; Vyas, D.; Heine, M.; Witcombe, J.R. Accelerating public sector rice breeding with high-density KASP markers derived from whole genome sequencing of indica rice. *Mol. Breed.* 2018, 38, 38. [CrossRef]

47. Cheon, K.-S.; Jeong, Y.-M.; Oh, H.; Oh, J.; Kang, D.-Y.; Kim, N.; Lee, E.; Baek, J.; Kim, S.L.; Choi, I.; et al. Development of 454 New Kompetitive Allele-Specific PCR (KASP) Markers for Temperate japonica Rice Varieties. *Plants* 2020, 9, 1531. [CrossRef] [PubMed]

48. Cheon, K.-S.; Jeong, Y.-M.; Lee, Y-Y.; Oh, J.; Kang, D.-Y.; Oh, H.; Kim, S.L.; Kim, N.; Lee, E.; Baek, J.; et al. Kompetitive Allele-Specific PCR Marker Development and Quantitative Trait Locus Mapping for Bakanae Disease Resistance in Korean Japonica Rice Varieties. *Plant Breed. Biotechnol.* 2019, 7, 208–219. [CrossRef]

49. Pradhan, S.K.; Nayak, D.K.; Pandit, E.; Behera, L.; Anandan, A.; Mukherjee, A.K.; Lenka, S.; Barik, D.P. Incorporation of Bacterial Blight Resistance Genes Into Lowland Rice Cultivar Through Marker-Assisted Backcross Breeding. *Phytopathology* 2016, 106, 710–718. [CrossRef] [PubMed]

50. Dreher, K.; Morris, M.; Khairelah, M.; Ribaut, J.M.; Shivaji, P.; Ganesan, S. Is marker-assisted selection cost-effective compared with conventional plant breeding methods? The case of quality protein Maize. *Econ. Soc. Issues Agric. Biotechnol.* 2002, 203–236. [CrossRef]
51. Huang, N.; Angeles, E.R.; Domingo, J.; Magpantay, G.; Singh, S.; Zhang, G.; Kumaravadivel, N.; Bennett, J.; Khush, G.S. Pyramiding of bacterial blast resistance genes in rice: Marker-assisted selection using RFLP and PCR. Theor. Appl. Genet. 1997, 95, 313–320. [CrossRef]

52. Sharma, T.R.; Rai, A.K.; Gupta, S.K.; Vijayan, J.; Devanna, B.N.; Ray, S. Rice Blast Management Through Host-Plant Resistance: Retrospect and Prospects. Agric. Res. 2012, 1, 37–52. [CrossRef]

53. Wu, Y.; Xiao, N.; Chen, Y.; Yu, L.; Pan, C.; Li, Y.; Zhang, X.; Huang, N.; Ji, H.; Dai, Z.; et al. Comprehensive evaluation of resistance effects of pyramiding lines with different broad-spectrum resistance genes against Magnaporthe oryzae in rice (Oryza sativa L.). Rice 2019, 12, 1–13. [CrossRef] [PubMed]

54. Hospital, F.; Charcosset, A. Marker-assisted introgression of quantitative trait loci. Genetics 1997, 147, 1469–1485. [CrossRef]

55. Frisch, M.; Bohn, M.; Melchinger, A.E. Minimum Sample Size and Optimal Positioning of. Design 1999, 975, 967–975.

56. Visscher, P.M.; Haley, C.S.; Thompson, R. Marker-assisted introgression in backcross breeding programs. Genetics 1996, 144, 1923–1932. [CrossRef] [PubMed]

57. Singh, S.; Sidhu, J.S.; Huang, N.; Vikal, Y.; Li, Z.; Brar, D.S.; Dhaliwal, H.S.; Khush, G.S. Molecular progress on the mapping and cloning of functional genes for blast disease in rice (Oryza sativa L.). Current status and future considerations. Theor. Appl. Genet. 2001, 102, 1011–1015. [CrossRef]

58. Chukwu, S.C.; Rafii, M.Y.; Ramlee, S.I.; Ismail, S.I.; Oladosu, Y.; Muhammad, I.; Musa, I.; Ahmed, M.; Jatto, M.I.; Yusuf, B.R. Recovery of recurrent parent genome in a marker-assisted backcrossing against rice blast and blast infections using functional markers and SSRs. Plants 2020, 9, 1411. [CrossRef]

59. Kim, M.-S.; Yang, J.-Y.; Yu, J.-K.; Lee, Y.; Park, Y.-J.; Kang, K.-K.; Cho, Y.-G. Breeding of High Cooking and Eating Quality in Rice by Marker-Assisted Backcrossing (MAeB) Using KASP Markers. Plants 2021, 10, 804. [CrossRef]

60. Kang, J.-W.; Shin, D.; Cho, J.-H.; Lee, J.-Y.; Kwon, Y.; Park, D.-S.; Ko, J.-M.; Lee, J.-H. Accelerated development of rice stripe virus-resistant, near-isogenic rice lines through marker-assisted backcrossing. PLoS ONE 2019, 14, e0225974. [CrossRef] [PubMed]

61. Akhtar, S.; Bhat, M.A.; Bhat, K.A.; Chalkoo, S.; Mir, M.R. Marker assisted selection in rice. J. Phytol. 2008, 985–993. [CrossRef] [PubMed]

62. Kearsey, M.J. The principles of QTL analysis (a minimal mathematics approach). Curr. Opin. Plant Biol. 2010, 13, 213–218. [CrossRef] [PubMed]

63. Hospital, F. Size of donor chromosome segments around introgressed loci and reduction of linkage drag in marker-assisted backcross programs. Genet.ica 2001, 158, 1363–1379. [CrossRef] [PubMed]

64. Frisch, M.; Bohn, M.; Melchinger, A.E. Minimum Sample Size and Optimal Positioning of. Design 1999, 975, 967–975.

65. Jena, K.K.; Mackill, D.J. Molecular markers and their use in marker-assisted selection in rice. Crop Sci. 2008, 48, 1266–1276. [CrossRef]

66. Singh, A.K.; Gopalakrishnan, S.; Singh, V.P.; Prabhu, K.V.; Mohapatra, T.; Singh, N.K.; Sharma, T.R.; Nagarajan, M.; Ellur, R.K.; Singh, A.; et al. Marker assisted selection: A paradigm shift in Basmati breeding. Indian J. Genet. Plant Breed. 2011, 71, 120.

67. He, J.; Zhao, X.; Laroche, A.; Lu, Z.X.; Liu, H.K.; Li, Z. Genotyping-by-sequencing (GBS), An ultimate marker-assisted selection

68. Das, G.; Patra, J.K.; Baek, K. Corrigendum: Insight into MAS: A molecular tool for development of stress resistant and quality of rice through gene stacking. Front. Plant Sci. 2017, 8, 3389. [CrossRef] [PubMed]

69. Amagai, Y.; Watanabe, N.; Kuboyama, T. Genetic mapping and development of near-isogenic lines with genes conferring mutant phenotypes in Aegilops tauschii and synthetic hexaploid wheat. Mol. Breed. 2011, 27, 313–320. [CrossRef] [PubMed]

70. Kearsey, M. The principles of QTL analysis (a minimal mathematics approach). J. Exp. Bot. 1998, 49, 1619–1623. [CrossRef]

71. Melchinger, A.E.; Friedrich Utz, H.; Schör, C.C. Quantitative trait locus (QTL) mapping using different testers and independent population samples in maize reveals low power of QTL detection and large bias in estimates of QTL effects. Genetics 1998, 149, 383–403. [CrossRef] [PubMed]

72. Miao, Z.H.; Fortune, J.A.; Gallagher, J. Anatomical structure and nutritive value of lupin seed coats. Aust. J. Agric. Res. 2001, 52, 985–993. [CrossRef]

73. Chukwu, S.C.; Rafii, M.Y.; Ramlee, S.I.; Ismail, S.I.; Oladosu, Y.; Okporie, E.; Onyishi, G.; Utobo, E.; Ekwu, L.; Swaray, S.; et al. Marker-assisted selection and gene pyramiding for resistance to bacterial leaf blight disease of rice (Oryza sativa L.). Biotechnol. Biotechnol. Equip. 2019, 33, 440–445. [CrossRef]

74. Ashkani, S.; Rafii, M.Y.; Rahim, H.A.; Latif, M.A. Genetic dissection of rice blast resistance by QTL mapping approach using an F3 population. Mol. Biol. Rep. 2013, 40, 2503–2515. [CrossRef] [PubMed]

75. Negrão, S.; Cecília Almadanim, M.; Pires, I.S.; Abreu, I.A.; Maroco, J.; Courtois, B.; Gregório, G.B.; McNally, K.L.; Margarida Oliveira, M. New allelic variants found in key rice salt-tolerance genes: An association study. J. Exp. Bot. 2001, 52, 66–81. [CrossRef] [PubMed]

76. Ashkani, S.; Rafii, M.Y.; Rahim, H.A.; Latif, M.A. Genetic dissection of rice blast resistance by QTL mapping approach using an F3 population. Mol. Biol. Rep. 2013, 40, 2503–2515. [CrossRef] [PubMed]

77. Jiang, H.; Hu, J.; Li, Z.; Liu, J.; Gao, G.; Zhang, Q.; Xiao, J.; He, Y. Evaluation and breeding application of six brown planthopper resistance genes in rice maintainer line Jin 23B. Rice 2018, 11, 1–11. [CrossRef] [PubMed]

78. Huang, N. Fine mapping and DNA marker-assisted pyramiding of the three major genes for blast resistance in rice. Theor. Appl. Genet. 2000, 100, 1121–1128. [CrossRef] [PubMed]

79. Jiang, H.; Hu, J.; Li, Z.; Liu, J.; Gao, G.; Zhang, Q.; Xiao, J.; He, Y. Evaluation and breeding application of six brown planthopper resistance genes in rice maintainer line Jin 23B. Rice 2018, 11, 1–11. [CrossRef] [PubMed]
79. Jiang, H.; Feng, Y.; Bao, L. Improving blast resistance of Jin 23B and its hybrid rice by marker-assisted gene pyramiding. *Mol. Breed.* 2012, 30, 1679–1688. [CrossRef]

80. Vikas, S.H.B.; Atul, K.S.; Ashutosh, S. Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. *Mol. Breed.* 2010, 26, 293–305. [CrossRef]

81. Katiyar, S.; Verulkar, S.; Chandel, G.; Zhang, Y.; Huang, B.; Bennett, J. Genetic analysis and pyramiding of two gall midge resistance genes (Gm-2 and Gm-6t) in rice (*Oryza sativa* L.). *Euphytica* 2001, 122, 327–334. [CrossRef]

82. Liu, Y.; Chen, L.; Liu, Y.; Dai, H.; He, J.; Kang, H.; Pan, G.; Huang, J.; Qiu, Z.; Wang, Q.; et al. Marker assisted pyramiding of two brown planthopper resistance genes, Bph3 and Bph27 (t), into elite rice Cultivars. *Rice* 2016, 9, 1–7. [CrossRef]

83. Patroli, P.; Vishalakshi, B.; Umakanth, B.; Suresh, J.; Senguttuvel, P.; Madhav, M.S. Marker-assisted pyramiding of major blast resistance genes in Swarna-Sub1, an elite rice variety (*Oryza sativa* L.). *Euphytica* 2019, 215, 1–11. [CrossRef]

84. Singh, M.K.; Singh, R.P.; Singh, P. Identification of good combiners in early maturing × high yielding cultivars of Indica rice (*Oryza sativa* L.). *Bangladesh J. Bot.* 2013, 42, 247–255. [CrossRef]

85. Suh, J.; Jeung, J.; Noh, T.; Cho, Y.; Park, S.; Park, H.; Shin, M.; Kim, C.; Jena, K.K. Development of breeding lines with three pyramided resistance genes that confer broad-spectrum bacterial blight resistance and their molecular analysis in rice. *Rice* 2013, 1, 1–11. [PubMed]

86. Abd, N.; Shamsudin, A.; Swamy, B.P.M.; Ratnam, W.; Teressa, M.; Cruz, S.; Raman, A.; Kumar, A. Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice. *BMC Genet.* 2016, 17, 1–14. [PubMed]

87. Pandian, B.A.; Joel, J.; Nachimuthu, V.V. Marker-aided selection and validation of various Pi gene combinations for rice blast resistance in elite rice variety ADT 43. *J. Genet.* 2018, 97, 945–952. [CrossRef] [PubMed]

88. Pan, Y.; Chen, K.; Wang, X.; Wang, W.; Xu, J.; Ali, J.; Li, Z.; Roy, S.J. Simultaneous Improvement and Genetic Dissection of Salt Tolerance of Rice (*Oryza sativa* L.) by Designed QTL Pyramiding. *Front. Plant Sci.* 2017, 8, 1275. [CrossRef] [PubMed]

89. Xiao, W.; Yang, Q.; Huang, M.; Guo, T.; Liu, Y.; Wang, J.; Yang, G.; Zhou, J.; Yang, J.; Zhu, X.; et al. Improvement of rice blast resistance by developing monogenic lines, two-gene pyramids and three-gene pyramid through MAS. *Rice* 2019, 12, 1–11. [CrossRef]

90. Mi, J.; Lei, Y.; Kim, S. An effective strategy for fertility improvement of indica-japonica hybrid rice by pyramiding SS-n, f5-n, and pf12-j. *Mol. Breed.* 2019, 39, 3–6. [CrossRef]

91. Shinada, H.; Iwata, N.; Sato, T.; Fujino, K. QTL pyramiding for improving of cold tolerance at fertilization stage in rice. *Breed. Sci.* 2014, 68, 483–488. [CrossRef] [PubMed]

92. Yang, T.; Zhang, S.; Zhao, J.; Liu, Q.; Huang, Z.; Mao, X.; Dong, J.; Wang, X.; Zhang, G.; Liu, B. Identification and pyramiding of QTLs for cold tolerance at the bud bursting and the seedling stages by use of single segment substitution lines in rice (*Oryza sativa* L.). *Mol. Breed.* 2016, 36, 1–10. [CrossRef]

93. Ramalingam, J.; Raveendra, C.; Savitha, P.; Vidya, V.; Chaitra, T.L.; Velprabakaran, S.; Saraswathi, R.; Ramanathan, A.; Arumugam Pillai, M.P.; Arumugachamy, S.; et al. Gene Pyramiding for Achieving Enhanced Resistance to Bacterial Blight, Blast, and Sheath Blight Diseases in Rice. *Front. Plant Sci.* 2020, 11, 1662. [CrossRef]

94. Chukwu, S.C.; Rafii, M.Y.; Ramlee, S.I.; Ismail, S.I.; Halidu, J.; Muhammad, I.; Ahmed, M. Marker-Assisted Introgression of Multiple Resistance Genes Confers Broad Spectrum Resistance against Bacterial Leaf Blight and Blast Diseases in PUTRA-1 Rice Variety. *Agronomy* 2019, 10, 42. [CrossRef]

95. Dixit, S.; Singh, U.M.; Singh, A.K.; Alam, S.; Venkateshwarlu, C.; Nachimuthu, V.V.; Yadav, S.; Abbai, R.; Selvaraj, R.; Devi, M.N.; et al. Marker Assisted Forward Breeding to Combine Multiple Biotic-Abiotic Stress Resistance/Tolerance in Rice. *Rice* 2020, 13, 1–15. [CrossRef]

96. Jamaloddin, M.; Durga Rani, C.V.; Swathi, G.; Anuradha, C.; Vanisri, S.; Rajan, C.P.D.; Krishnam Raju, S.; Bhuvaneswari, V.; Jagadeeswar, R.; Laha, G.S.; et al. Marker Assisted Gene Pyramiding (MAGP) for bacterial blight and blast resistance into mega rice variety “Tellamansa”. *PLoS ONE* 2020, 15, e0234088. [CrossRef]

97. Swathi, G.; Rani, C.V.D.; Madhav, M.S.; Vanisree, S. Marker-assisted introgression of the major bacterial blight resistance genes, Xa21 and xa13, into the popular rice variety, JGL1798. *BMC Genet.* 2019, 20, 97. [CrossRef]

98. Kumar, V.A.; Balachiranjievi, C.H.; Naik, S.B.; Rekha, G.; Ramababu, R. Marker-assisted pyramiding of bacterial blight and gall midge resistance genes into RPHR-1005, the restorer line of the popular rice hybrid DRRH-3. *Mol. Breed.* 2017, 37, 1–14. [CrossRef]

99. Septiningsih, E.M.; Pamplona, A.M.; Sanchez, D.L.; Neeraja, C.N.; Vergara, G.V.; Heuer, S.; Ismail, A.M.; Mackill, D.J. Development of submergence-tolerant rice cultivars: The Sub1 locus and beyond. *Ann. Bot.* 2009, 103, 151–160. [CrossRef] [PubMed]

100. Deng, Y.; Zhu, X.; Shen, Y.; He, Z. Genetic characterization and fine mapping of the blast resistance locus Pigm(t) tightly linked to Pi2 and P9 in a broad resistance spectrum Chinese variety. *Theor. Appl. Genet.* 2006, 113, 705–713. [CrossRef] [PubMed]

101. Biradar, S.K.; Sundaram, R.M.; Thirumurugan, T.; Bentur, J.S.; Amudahan, S.; Shanoy, V.V.; Mishra, B.; Bennett, J.; Sarma, N.P. Identification of flanking SSR markers for a major rice gall midge resistance gene Gm1 and their validation. *Theor. Appl. Genet.* 2004, 109, 1468–1473. [CrossRef]

102. Himabindu, K.; Suneetha, K.; Sama, V.S.A.K.; Bentur, J.S. A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. *Euphytica* 2010, 174, 179–187. [CrossRef]

103. Xu, K.; Mackill, D.J. A major locus for submergence tolerance mapped on rice chromosome 9. *Mol. Breed.* 1996, 2, 219–224. [CrossRef]
104. Nandi, S.; Subudhi, P.K.; Senadhiraja, D.; Manigbas, N.L.; Sen-Mandi, S.; Huang, N. Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. *Mol. Gen. Genet.* 1997, 255, 1–8. [CrossRef]

105. Xu, K.; Xu, X.; Ronald, P.C.; Mackill, D.J. A high-resolution linkage map of the vicinity of the rice submergence tolerance locus Sub1. *Mol. Gen Genet.* 2000, 263, 681–689. [CrossRef]

106. Luo, Y.; Ma, T.; Zhang, A.; Ong, K.H.; Li, Z.; Yang, J.; Yin, Z. Marker-assisted breeding of the rice restorer line Wanhui 6725 for disease resistance, submergence tolerance and aromatic fragrance. *Rice* 2016, 9, 1–13. [CrossRef]

107. Luo, Y.; Sangha, J.S. Marker-assisted breeding of Xa4, Xa21 and Xa27 in the restorer lines of hybrid rice for broad-spectrum and enhanced disease resistance to bacterial blight. *Mol. Breed.* 2012, 30, 1601–1610. [CrossRef]

108. Ruengphayak, S.; Chaichumpoo, E.; Phromphan, S.; Kamolsukyunyong, W.; Sukhaket, W.; Phuvanartnarubal, E.; Korinsak, S.; Korinsak, S.; Vanavichit, A. Pseudo-backcrossing design for rapidly pyramiding multiple traits into a preferential rice variety. *Rice* 2015, 8, 1–16. [CrossRef] [PubMed]

109. Tao, C.; Hao, W.; Ya-dong, Z.; Zhen, Z.; Qi-yong, Z.; Li-hui, Z.; Shu, Y.; Ling, Z.; Xin, Y.; Chun-fang, Z.; et al. ScienceDirect Genetic Improvement of Japonica Rice Variety Wuyujing 3 for Stripe Disease Resistance and Eating Quality by Pyramiding Stv-b 1 and Wx-mq. *Rice Sci.* 2016, 23, 69–79. [CrossRef]

110. Reinke, R.; Man, S.; Kim, B.K. Developing japonica rice introgression lines with multiple resistance genes for brown planthopper, bacterial blight, rice blast, and rice stripe virus using molecular breeding. *Mol. Genet. Genom.* 2018, 293, 1565–1575. [CrossRef] [PubMed]

111. Luo, Y.; Ma, T.; Zhang, A. Marker-assisted breeding of Chinese elite rice cultivar 9311 for disease resistance to rice blast and bacterial blight and tolerance to submergence. *Mol. Breed.* 2017, 37, 1–12. [CrossRef]

112. Liu, G.; Lu, G.; Zeng, L.; Wang, G.-L. Two broad-spectrum blast resistance genes, Pi9(t) and Pi2(t), are physically linked on rice chromosome 6. *Mol. Genet. Genom.* 2002, 267, 472–480. [CrossRef] [PubMed]

113. Suh, J.-P.; Noh, T.-H.; Kim, K.-Y.; Kim, J.-J.; Kim, Y.-G.; Jena, K.K. Expression levels of three bacterial blight resistance genes against K3a race of Korea by molecular and phenotype analysis in japonica rice (*O. sativa* L.). *J. Crop Sci. Biotechnol.* 2009, 12, 103–108. [CrossRef]

114. Jeung, J.U.; Kim, B.R.; Cho, Y.C.; Han, S.S.; Moon, H.P.; Lee, Y.T.; Jena, K.K. A novel gene, Pi40(t), linked to the DNA markers Xa23, into GZ63-4S, an elite thermo-sensitive genic male-sterile line in rice. *Mol. Plant Pathol.* 2010, 11, 248–257. [PubMed]

115. Suh, J.; Cho, Y.; Won, Y.; Ahn, E.; Baek, M.; Kim, M.; Kim, B.; Jena, K.K. Development of Resistant Gene-Pyramided Japonica Rice Variety 994002-216 for bacterial blight, rice blast and strong fragrance in glutinous rice. *Mol. Gen. Genet.* 2011, 284, 1601–1610. [CrossRef]

116. Nas, T.M.S.; Sanchez, D.L.; Diaz, M.G.Q.; Mendioro, M.S.; Virmani, S.S. Pyramiding of thermosensitive genetic male sterility (TGMS) genes and identification of a candidate tms5 gene in rice. *Euphytica* 2005, 145, 67–75. [CrossRef]

117. Datta, S.K. Pyramiding transgenes for multiple resistance in rice against bacterial blight, yellow stem borer and sheath blight. *Theor. Appl. Genet.* 2002, 106, 1–8. [CrossRef] [PubMed]

118. Xu, J. Pyramiding of two BPH resistance genes and Stv-b i gene using. *Crop Breed. Appl. Biotechnol.* 2013, 13, 99–106. [CrossRef]

119. Jiang, J.; Yang, D.; Ali, J.; Mou, T. Molecular marker-assisted pyramiding of broad-spectrum disease resistance genes, Pi2 and Pi3, into GZ63-4S, an elite thermo-sensitive genic male-sterile line in rice. *Mol. Breed.* 2015, 35, 1–8. [CrossRef]

120. Korinsak, S.; Siangliw, J.L.; Vanavichit, A.; Toojinda, T. Pseudo-backcrossing design for rapidly pyramiding multiple traits into a preferential rice variety. *Rice* 2015, 8, 1–16. [CrossRef] [PubMed]

121. Manivong, P.; Korinsak, S.; Korinsak, S.; Siangliw, J.L.; Vanavichit, A.; Toojinda, T. Marker-assisted selection to improve bacterial resistance and strong fragrance in glutinous rice. *Mol. Gen Genet.* 2000, 263, 681–689. [CrossRef]

122. Babujee, L.; Gnanamanickam, S.S. Molecular tools for characterization of rice blast pathogen (*Magnaporthe grisea*) population and molecular marker-assisted breeding for disease resistance. *Curr. Sci.* 2010, 99, 110–122. [CrossRef]

123. Chou, C.; Castilla, N.; Hadi, B.; Tanaka, T.; Chiba, S.; Sato, I. Rice blast management in Cambodian rice fields using Trichoderma harzianum and a resistant variety. *Crop Prot.* 2020, 135, 104864. [CrossRef]

124. Babujee, L.; Gnanamanickam, S.S. Molecular tools for characterization of rice blast pathogen (*Magnaporthe grisea*) population and molecular marker-assisted breeding for disease resistance. *Curr. Sci.* 2000, 78, 248–257.

125. Ashkani, S.; Yusop, M.R.; Shabanimofrad, M.; Azadi, A.; Ali Ghasemzadeh, P.A.; Mohammad, A.L. Allele Mining Strategies: Principles and Utilisation for Blast Resistance Genes in Rice (*Oryza sativa* L.). *Curr. Sci.* 2014, 105, 99–106. [CrossRef]

126. Liu, J.; Wang, X.; Mitchell, T.; Hu, Y.; Liu, X.; Dai, L.; Wang, G.-L. Recent progress and understanding of the molecular mechanisms of the rice-* Magnaporthe oryzae* interaction. *Mol. Plant Pathol.* 2010, 11, 419–427. [CrossRef]

127. Cesari, S.; Thilliez, G.; Ribot, C.; Chalvon, V.; Michel, C.; Jaunee, A.; Rivas, S.; Alaux, L.; Kanzaki, H.; Okuyama, Y.; et al. The Rice Resistance Protein Pair RGA4/RGA5 Recognizes the Magnaporthe oryzae Effectors AVR-Pia and AVR1-CO39 by Direct Binding. *Plant Cell* 2013, 25, 1463–1481. [CrossRef]

128. Okuyama, Y.; Kanzaki, H.; Abe, A.; Yoshida, K.; Tamiru, M.; Saitoh, H.; Fujibe, T.; Matsumura, H.; Shenton, M.; Galam, D.C.; et al. A multifaceted genomics approach allows the isolation of the rice Pi-blast resistance gene consisting of two adjacent NBS-LRR protein genes. *Plant J.* 2011, 66, 467–479. [CrossRef] [PubMed]
Sustainability 2021, 13, 10806

129. Rai, A.K.; Kumar, S.P.; Gupta, S.K.; Gautam, N.; Singh, N.K.; Sharma, T.R. Functional complementation of rice blast resistance gene Pi-k h (Pi54) conferring resistance to diverse strains of Magnaporthe oryzae. *J. Plant Biochem. Biotechnol.* 2011, 20, 55–65. [CrossRef]

130. Hua, L.; Wu, J.; Chen, C.; Wu, W.; He, X.; Lin, F.; Wang, L.; Ashikawa, I.; Matsumoto, T.; Wang, L.; et al. The isolation of Pi1, an allele at the Pik locus which confers broad spectrum resistance to rice blast. *Theor. Appl. Genet.* 2012, 125, 1047–1055. [CrossRef]

131. Hayashi, K.; Yasuda, N.; Fujita, Y.; Koizumi, S.; Yoshida, H. Identification of the blast resistance gene Pi in rice cultivars using functional markers. *Theor. Appl. Genet.* 2010, 121, 1357–1367. [CrossRef]

132. Huang, H.; Huang, L.; He, X.Y.; Wang, L.; Zhang, W.S.; Liu, W.; Liu, X.Q.; Lin, F. Development of a marker specific for the rice blast resistance gene Pi39 in the Chinese cultivar Q15 and its use in genetic improvement. *Biotechnol. Biotechnol. Equip.* 2015, 29, 448–456. [CrossRef]

133. Das, A.; Soubam, D.; Singh, P.K.; Thakur, S.; Singh, N.K.; Sharma, T.R. A novel blast resistance gene, Pi54rh cloned from wild species of rice, Oryza rhizomatis confers broad spectrum resistance to Magnaporthe oryzae. *Acta Integr. Genom.* 2012, 12, 215–228. [CrossRef]

134. Hua, L.X.; Liang, L.Q.; He, X.Y.; Wang, L.; Zhang, W.; Liu, W.; Liu, X.Q.; Lin, F. Development of a marker specific for the rice blast resistance gene Pi39 in the Chinese cultivar Q15 and its use in genetic improvement. *Biotechnol. Biotechnol. Equip.* 2015, 29, 448–456. [CrossRef]

135. Liu, Y.; Liu, B.; Zhu, X.; Yang, J.; Bordeos, A.; Wang, G.; Leach, J.E.; Leung, H. Fine-mapping and molecular marker development for Pi56(t), a NBS-LRR gene conferring broad-spectrum resistance to Magnaporthe oryzae in rice. *Theor. Appl. Genet.* 2013, 126, 985–998. [CrossRef]

136. Hayashi, N.; Inoue, H.; Kato, T.; Funao, T.; Shirota, M.; Shimizu, T.; Kanamori, H.; Yamane, H.; Hayano-Saito, Y.; Matsumoto, T.; et al. Durable panicle blast-resistance gene Pb1 encodes an atypical CC-NBS-LRR protein and was generated by acquiring a promoter through local genome duplication. *Plant J.* 2010, 64, 498–510. [CrossRef] [PubMed]

137. Fukuoka, S.; Saka, N.; Koga, H.; Ono, K.; Shimizu, T.; Ebana, K.; Hayashi, N.; Takahashi, A.; Hirochika, H.; Okuno, K.; et al. Loss of Function of a Proline-Containing Protein Confers Durable Disease Resistance in Rice. *Science* 2009, 325, 998–1001. [CrossRef]

138. Fahad, S.; Rehman, A.; Shahzad, B.; Tanveer, M.; Saud, S.; Kamran, M.; Ihtisham, M.; Khan, S.U.; Turan, V.; ur Rahman, M.H. Rice Responses and Tolerance to Metal/Metalloid Toxicity; In *Advances in Rice Research for Abiotic Stress Tolerance*; Woodhead Publishing: Cambridge, UK, 2019; pp. 299–312.

139. Chen, X.; Shang, J.; Chen, D.; Lei, C.; Zou, Y.; Zhai, W.; Liu, G.; Xu, J.; Ling, Z.; Cao, G.; et al. A B-lectin receptor kinase gene conferring rice blast resistance. *Plant J.* 2006, 46, 794–804. [CrossRef] [PubMed]

140. Wu, Y.; Xiao, N.; Yu, L.; Pan, C.; Li, Y.; Zhang, X.; Liu, G.; Dai, Z.; Pan, X.; Li, A. Combination Patterns of Major R Genes Determine the Level of Resistance to the M. oryzae in Rice (Oryza sativa L.). *PLoS ONE* 2015, 10, e0126130. [CrossRef]

141. Hua, L.; Wu, J.; Chen, C.; Wu, W.; He, X.; Lin, F.; Wang, L.; Ashikawa, I.; Matsumoto, T.; Wang, L.; et al. The isolation of Pi1, an allele at the Pik locus which confers broad spectrum resistance to rice blast. *Theor. Appl. Genet.* 2012, 125, 1047–1055. [CrossRef]

142. Xiao, W.; Luo, L.; Wang, H.; Guo, T.; Liu, Y.; Zhou, J.; Zhu, X.; Yang, Q.; Chen, Z. Pyramiding of Pi46 and Pita to improve blast resistance and to evaluate the resistance effect of the two R genes. *J. Integr. Agric.* 2016, 15, 2290–2298. [CrossRef]

143. Khush, G.S.; Mackill, D.J.; Sidhu, G.S. Breeding Rice for Resistance to Bacterial Blight; Int. Rice Res. Inst.: Los Baños, Philippines, 1989; Volume 16, ISBN 971104188X.

144. Bhasin, H.; Bhatia, D.; Raghuvanshi, S.; Lore, J.S.; Sahi, G.K.; Kaur, B.; Vikal, Y.; Singh, K. New PCR-based sequence-tagged site marker for bacterial blast resistance gene Xa38 of rice. *Mol. Breed.* 2012, 30, 607–611. [CrossRef]

145. Han, X.; Yang, Y.; Wang, X.; Zhou, J.; Zhang, W.; Yu, C.; Cheng, C.; Cheng, Y.; Yan, C.; Chen, J. Quantitative trait loci mapping for bacterial blast resistance in rice using bulked segregant analysis. *Int. J. Mol. Sci.* 2014, 15, 11847–11861. [CrossRef] [PubMed]

146. Tian, D.; Wang, J.; Zeng, X.; Gu, K.; Qiu, C.; Yang, S.; Zhou, Z.; Goh, M.; Luo, Y.; Murata-Hori, M.; et al. The Rice TAL effector-dependent resistance protein XA10 triggers cell death and calcium depletion in the endoplasmic reticulum. *Plant Cell* 2014, 26, 497–515. [CrossRef] [PubMed]

147. Bhaskar Rao, T.; Chopperla, R.; Prathi, N.B.; Balakrishnan, M.; Prakasam, V.; Laha, G.S.; Balachandran, S.M.; Mangruthia, S.K. A Comprehensive Gene Expression Profile of Pectin Degradation Enzymes Reveals the Molecular Events during Cell Wall Degradation and Pathogenesis of Rice Sheath Blight Pathogen Rhizoctonia solani AG1-IA. *J. Fungi* 2020, 6, 71. [CrossRef]

148. Singh, P.; Mazumdar, P.; Harikrishna, J.A.; Babu, S. Sheath blight of rice: A review and identification of priorities for future research. *Planta* 2019, 250, 1387–1407. [CrossRef]

149. Savary, S.; Willocquet, L.; Teng, P.S. Modelling sheath blight epidemics on rice tillers. *Agric. Syst.* 1997, 55, 359–384. [CrossRef]

150. Channamallikarjuna, V.; Sonah, H.; Prasad, M.; Rao, G.J.N.; Chand, S.; Upreti, H.C.; Singh, N.K.; Sharma, T.R. Identification of major quantitative trait loci qSBR11-1 for sheath blast resistance in rice. *Mol. Breed.* 2010, 25, 155–166. [CrossRef]

151. Molla, K.A.; Karmakar, S.; Molla, J.; Bajaj, P.; Varshney, R.K.; Datta, S.K.; Datta, K. Understanding sheath blast resistance in rice: The road behind and the road ahead. *Plant Biotechnol. J.* 2020, 18, 895–915. [CrossRef] [PubMed]

152. Wang, Y.; Pinson, S.R.M.; Fjellstrom, R.G.; Tabien, R.E. Phenotypic gain from introgression of two QTL, qSB9-2 and qSB12-1, for rice sheath blast resistance. *Mol. Breed.* 2012, 30, 293–303. [CrossRef]

153. Singh, R.; Singh, Y.; Xalaxo, S.; Verulkar, S.; Yadav, N.; Singh, S.; Singh, N.; Prasad, K.S.N.; Kondayya, K.; Rao, P.V.R.; et al. From QTL to variety-harnessing the benefits of QTLs for drought, flood and salt tolerance in mega rice varieties of India through a multi-institutional network. *Plant Sci.* 2016, 242, 278–287. [CrossRef]
154. Zhang, M.; Wang, S.; Yuan, M. An update on molecular mechanism of disease resistance genes and their application for genetic improvement of rice. *Mol. Breed.* **2019**, *39*, 154. [CrossRef]

155. Singh, A.K.; Singh, V.K.; Singh, A.; Ellur, R.K.; Pandian, R.T.P.; Gopala Krishnan, S.; Singh, U.D.; Nagarajan, M.; Vinod, K.K.; Prabhhu, K.V. Introggression of multiple disease resistance into a maintainer of Basmati rice CMS line by marker assisted backcross breeding. *Euphytica* **2015**, *203*, 97–107. [CrossRef]

156. Li, D.; Li, S.; Wei, S.; Sun, W. Strategies to Manage Rice Sheath Blight: Lessons from Interactions between Rice and Rhizoctonia solani. *Rice* 2021, **14**, 21. [CrossRef][PubMed]

157. Zuo, S.; Zhang, Y.; Chen, Z.; Jiang, W.; Feng, M.; Pan, X. Improvement of Rice Resistance to Sheath Blight by Pyramiding QTLs Conditioning Disease Resistance and Tiller Angle. *Rice Sci.* **2014**, *21*, 318–326. [CrossRef]

158. Hu, G.; Lu, F.; Zhai, B.P.; Lu, M.H.; Liu, W.C.; Zhu, F.; Wu, X.W.; Chen, G.H.; Zhang, X.X. Outbreaks of the brown planthopper Nilaparvata lugens (Stål) in the yangtze river delta: Immigration or local reproduction? *PLoS ONE* **2014**, *9*, 1–12. [CrossRef]

159. Way, M.J.; Heong, K.L. The role of biodiversity in the dynamics and management of insect pests of tropical irrigated rice—A review. *Bull. Entomol. Res.* **1994**, *84*, 567–587. [CrossRef]

160. Du, B.; Zhang, W.; Liu, B.; Hu, J.; Wei, Z.; Shi, Z.; He, R.; Zhu, L.; Chen, R.; Han, B.; et al. Identification and characterization of Bph14, a gene conferring resistance to brown planthopper in rice. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 22163–22168. [CrossRef][PubMed]

161. Wang, Y.; Jiang, W.; Liu, H.; Zeng, Y.; Du, B.; Zhu, L.; He, G.; Chen, R. Marker assisted pyramiding of Bph6 and Bph9 into elite restorer line 93–11 and development of functional marker for Bph9. *Rice* **2017**, *10*, 51. [CrossRef]

162. Dixit, S.; Singh, A.; Sandhu, N.; Bhandari, A.; Vikram, P.; Kumar, A. Combining drought and submergence tolerance in rice: Marker-assisted breeding and QTL combination effects. *Mol. Breed.* **2017**, *34*, 143. [CrossRef]

163. Kumar, R.; Venuprasad, R.; Atlin, G.N. Genetic analysis of rainfed lowland rice drought tolerance under naturally-occurring stress in eastern India: Heritability and QTL effects. *F. Crop. Res.* **2007**, *103*, 42–52. [CrossRef]

164. Venuprasad, R.; Dalid, C.O.; Del Valle, M.; Zhao, D.; Espiritu, M.; Sta Cruz, M.T.; Amante, M.; Kumar, A.; Atlin, G.N. Identification and characterization of large-effect quantitative trait loci for grain yield under lowland drought stress in rice using bulk-segregant analysis. *Theor. Appl. Genet.* **2009**, *84*, 167–170. [CrossRef]

165. Vikram, P.; Swamy, B.; Dixit, S.; Ahmed, H.; Teresa Sta Cruz, M.; Singh, A.; Kumar, A. qDTY1.1, a major QTL for rice grain yield under reproductive-stage drought stress with a consistent effect in multiple elite genetic backgrounds. *BMC Genet.* **2011**, *12*, 89. [CrossRef][PubMed]

166. Bernier, J.; Kumar, A.; Ramaiah, V.; Spaner, D.; Atlin, G. A Large-Effect QTL for Grain Yield under Reproductive-Stage Drought Stress in Upland Rice. *Crop Sci.* **2007**, *47*, 507–516. [CrossRef][PubMed]

167. Shamsudin, N.A.A.; Swamy, B.P.M.; Ratnam, W.; Cruz, M.T.S.; Sandhu, N.; Raman, A.K.; Kumar, A. Pyramiding of drought yield QTLs into a high quality Malaysian rice cultivar MRQ74 improves yield under reproductive stage drought. *Rice* **2016**, *9*, 1–13. [CrossRef][PubMed]

168. Dixit, S.; Yadaw, R.B.; Mishra, K.K.; Kumar, A. Marker-assisted breeding to develop the drought-tolerant version of Sabitri, a popular variety from Nepal. *Euphytica* **2017**, *213*, 184. [CrossRef]

169. Tiwari, D.N. A Critical Review of Submergence Tolerance Breeding Beyond Sub 1 Gene to Mega Varieties in the Context of Climate Change. *Int. J. Adv. Sci. Res. Eng.* **2018**, *4*, 140–148. [CrossRef]

170. Kuanar, S.R.; Ray, A.; Sethi, S.K.; Chattopadhyay, K.; Sarkar, R.K. Physiological Basis of Stagnant Flooding Tolerance in Rice. *Rice Sci.* **2017**, *24*, 73–84. [CrossRef][PubMed]

171. Sarkar, R.K.; Panda, D. Distinction and characterisation of submergence tolerant and sensitive rice cultivars, probed by the fluorescence OJIP rise kinetics. *Funct. Plant Biol.* **2009**, *36*, 222–233. [CrossRef][PubMed]

172. Singh, S.; Mackill, D.J.; Ismail, A.M. Responses of SUB1 rice introgression lines to submergence in the field: Yield and grain quality. *Field Crops Res.* **2009**, *113*, 12–23. [CrossRef][PubMed]

173. Neeraja, C.N.; Maghirang-Rodriguez, R.; Pamplona, A.; Heuer, S.; Collard, B.C.Y.; Septiningsih, E.M.; Vergara, G.; Sanchez, D.; Xu, K.; Ismail, A.M.; et al. A marker-assisted backcross approach for developing submergence-tolerant rice cultivars. *Theor. Appl. Genet.* **2007**, *115*, 767–776. [CrossRef]

174. Toojinda, T.; Tragoonrung, S.; Vanavichit, A.; Sianglw, J.L.; Pa-In, N.; Jantaboob, J.; Sianglw, M.; Fukai, S. Molecular breeding for rainfed lowland rice in the Mekong region. *Plant Prod. Sci.* **2005**, *8*, 330–333. [CrossRef][PubMed]

175. Sianglw, M.; Toojinda, T.; Tragoonrung, S.; Vanavichit, A. Thai jasmine rice carrying QTLch9 (SubQTL) is submergence tolerant. *Ann. Bot.* **2003**, *91*, 255–261. [CrossRef][PubMed]

176. Sarkar, R.K.; Reddy, J.N.; Sharma, S.G.; Ismail, A.M. Physiological basis of submergence tolerance in rice and implications for crop improvement. *Curr. Sci.* **2006**, *91*, 899–905.

177. Wassmann, R.; Jagadish, S.V.K.; Heuer, S.; Ismail, A.; Redona, E.; Serraj, R.; Singh, R.K.; Howell, G.; Pathak, H.; Sunmpleth, K. *Chapter 2 Climate Change Affecting Rice Production. The Physiological and Agronomic Basis for Possible Adaptation Strategies*, 1st ed.; Elsevier Inc.: Amsterdam, The Netherlands, 2009; Volume 101, ISBN 9780123748171.

178. Welch, J.R.; Vincent, J.R.; Auffhammer, M.; Moya, P.F.; Dobermann, A.; Dawe, D. Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 14562–14567. [CrossRef]
Sustainability 2021, 13, 10806

179. Sreenivasulu, N.; Butardo, V.M.; Misra, G.; Cuevas, R.P.; Anacleto, R.; Kishor, P.B.K. Designing climate-resilient rice with ideal grain quality suited for high-temperature stress. *J. Exp. Bot.* 2015, 66, 1737–1748. [CrossRef]

180. Calanca, P.P. Quantification of Climate Variability, Adaptation and Mitigation for Agricultural Sustainability. *Quantif. Clim. Var. Adapt. Mitig. Agric. Sustain.* 2017, 165–180. [CrossRef]

181. Godfray, H.C.J.; Beddington, J.R.; Crute, I.R.; Haddad, L.; Lawrence, D.; Muir, J.F.; Pretty, J.; Robinson, S.; Thomas, S.M.; Toulmin, C. Food security: The challenge of feeding 9 billion people. *Science* 2010, 327, 812–818. [CrossRef] [PubMed]

182. Rana, M.M.; Takamatsu, T.; Baslam, M.; Kaneo, K.; Itoh, K.; Harada, N.; Sugiyama, T.; Ohnishi, T.; Kinoshita, T.; Takagi, H.; et al. Salt tolerance improvement in rice through efficient SNP marker-assisted selection coupled with speed-breeding. *Int. J. Mol. Sci.* 2019, 20, 2585. [CrossRef]

183. Munns, R.; Tester, M. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 2008, 59, 651–681. [CrossRef] [PubMed]

184. Rao, P.S.; Mishra, B.; Gupta, S.R.; Rathore, A. Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. *Plant Breed.* 2008, 127, 256–261. [CrossRef]

185. Negrao, S.; Coutois, B.; Ahmadi, N.; Abreu, I.; Saibo, N.; Oliveira, M.M. Recent updates on salinity stress in rice: From physiological to molecular responses. *CRC. Crit. Rev. Plant Sci.* 2011, 30, 329–377. [CrossRef]

186. Marcos, M.; Sharifi, H.; Grattan, S.R.; Lingquist, B.A. Spatio-temporal salinity dynamics and yield response of rice in water-seeded rice fields. *Agric. Water Manag.* 2018, 195, 1837–1849. [CrossRef]

187. Tiwari, S.; SL, K.; Kumar, V.; Singh, B.; Rao, A.; Mithra SV, A.; Rai, V.; Singh, A.K.; Singh, N.K. Mapping QTLs for Salt Tolerance in Rice (*Oryza sativa* L.) by Bulked Segregant Analysis of Recombinant Inbred Lines Using 50K SNP Chip. *PLoS ONE* 2016, 11, e0153610. [CrossRef]

188. Horie, T.; Karahara, I.; Katsuhara, M. Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. *Rice* 2012, 5, 1–18. [CrossRef]

189. Roy, S.J.; Negrao, S.; Tester, M. Salt resistant crop plants. *Curr. Opin. Biotechnol.* 2014, 26, 115–124. [CrossRef] [PubMed]

190. Deinlein, U.; Stephan, A.B.; Horie, T.; Luo, W.; Xu, G.; Schroeder, J.I. Plant salt-tolerance mechanisms. *Trends Plant Sci.* 2014, 19, 371–379. [CrossRef]

191. Hanin, M.; Ebel, C.; Ngom, M.; Laplaze, L.; Masmoudi, K. New insights on plant salt tolerance mechanisms and their potential use for breeding. *Front. Plant Sci.* 2016, 7, 1–17. [CrossRef]

192. Chen, Z.C.; Yamaji, N.; Horie, T.; Che, J.; Li, J.; An, G.; Ma, J.F. A magnesium transporter *OsMGT1* plays a critical role in salt tolerance in rice. *Plant Physiol.* 2017, 174, 1837–1849. [CrossRef]

193. Ren, Z.-H.; Gao, J.-P.; Li, L.-G.; Cai, X.-L.; Huang, W.; Chao, D.-Y.; Zhu, M.-Z.; Wang, Z.-Y.; Luan, S.; Lin, H.-X. A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nat. Genet.* 2005, 37, 1141–1146. [CrossRef]

194. Thomson, M.J.; de Ocampo, M.; Egdane, J.; Rahman, M.A.; Sajise, A.G.; Adorada, D.L.; Tumimbang-Raiz, E.; Blumwald, E.; Seraj, Z.I.; Singh, R.K.; et al. Characterizing the Saltol quantitative trait locus for salt tolerance in rice. *Rice* 2010, 3, 148–160. [CrossRef]

195. Lin, H.X.; Zhu, M.Z.; Yano, M.; Gao, J.P.; Liang, Z.W.; Su, W.A.; Hu, X.H.; Ren, Z.H.; Chao, D.Y. QTLs for Na+ and K+ uptake of the shoots and roots controlling rice salt tolerance. *Theor. Appl. Genet.* 2004, 108, 253–260. [CrossRef]

196. Vu, H.T.T.; Le, D.D.; Ismail, A.M.; Le, H.H. Marker-assisted backcrossing (MABC) for improved salinity tolerance in rice (*Oryza sativa* L.) to cope with climate change in Vietnam. *Aust. J. Crop Sci.* 2012, 6, 1649–1654. [CrossRef]

197. Linh, L.H.; Linh, T.H.; Xuan, T.D.; Ham, L.H.; Ismail, A.M.; Khanh, T.D. Molecular breeding to improve salt tolerance of rice (*Oryza sativa* L.) in the Red River Delta of Vietnam. *Int. J. Plant Genom.* 2012, 2012, 1–9. [CrossRef] [PubMed]

198. Gregorio, G.B.; Islam, M.R.; Vergara, G.V.; Thirumeni, S. Recent advances in rice science to design salinity and other abiotic stress tolerant rice varieties. *Sabraw. J. Breed. Genet.* 2013, 45, 31–41.

199. Babu, N.N.; Krishnan, S.G.; Vinod, K.K.; Krishnamurthy, S.L.; Singh, V.K.; Singh, M.P.; Singh, R.; Ellur, R.K.; Rai, V.; Bolliniedi, H.; et al. Marker aided incorporation of salttol, a major QTL associated with seedling stage salt tolerance, into *Oryza sativa* ‘pusa basmati 1121’. *Front. Plant Sci.* 2017, 8, 1–14. [CrossRef]

200. Quan, R.; Wang, J.; Hui, J.; Bai, H.; Lyu, X.; Zhu, Y.; Zhang, H.; Zhang, Z.; Li, S.; Huang, R. Improvement of salt tolerance using wild rice genes. *Front. Plant Sci.* 2018, 8, 1–11. [CrossRef] [PubMed]

201. Narsai, R.; Wang, C.; Chen, J.; Wu, J.; Shou, H.; Whelan, J. Antagonistic, overlapping and distinct responses to biotic stress in rice (*Oryza sativa*) and interactions with abiotic stress. **BMC Genom.** 2013, 14, 93. [CrossRef]

202. Akram, R.; Fahad, S.; Masood, N.; Rasool, A.; Ijaz, M.; Ihsan, M.Z.; Masood, M.M.; Ahmad, S.; Hussain, S.; Ahmed, M.; et al. Plant Growth and Morphological Changes in Rice Under Abiotic Stress. In *Advances in Rice Research for Abiotic Stress Tolerance*; Woodhead publishing: Cambrige, UK, 2019; pp. 69–85.

203. Khush, G.S. Strategies for increasing the yield potential of cereals: Case of rice as an example. *Plant Breed.* 2013, 132, 433–436. [CrossRef] [PubMed]

204. Sasaki, T.; Burr, B. International Rice Genome Sequencing Project: The effort to completely sequence the rice genome. *Curr. Opin. Plant Biol.* 2000, 3, 138–142. [CrossRef]

205. Khush, G.S. What it will take to Feed 5.0 Billion Rice consumers in 2030. *Plant Mol. Biol.* 2005, 59, 1–6. [CrossRef] [PubMed]

206. Kumar, A.; Sandhu, N.; Dixit, S.; Yadav, S.; Swamy, B.P.M.; Shamsudin, N.A.A. Marker-assisted selection strategy to pyramid two or more QTLs for quantitative trait-grain yield under drought. *Rice* 2018, 11, 35. [CrossRef] [PubMed]
207. Sandhu, N.; Dixit, S.; Swamy, B.P.M.; Raman, A.; Kumar, S.; Singh, S.P.; Yadaw, R.B.; Singh, O.N.; Reddy, J.N.; Anandan, A.; et al. Marker Assisted Breeding to Develop Multiple Stress Tolerant Varieties for Flood and Drought Prone Areas. *Rice* 2019, 12, 8. [CrossRef]

208. Muthu, V.; Abbai, R.; Nallathambi, J.; Rahman, H.; Ramasamy, S.; Kambale, R.; Thulasinathan, T.; Ayyenar, B.; Muthurajan, R. Pyramiding QTLs controlling tolerance against drought, salinity, and submergence in rice through marker assisted breeding. *PLoS ONE* 2020, 15, e0227421. [CrossRef]