

Review

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

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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].

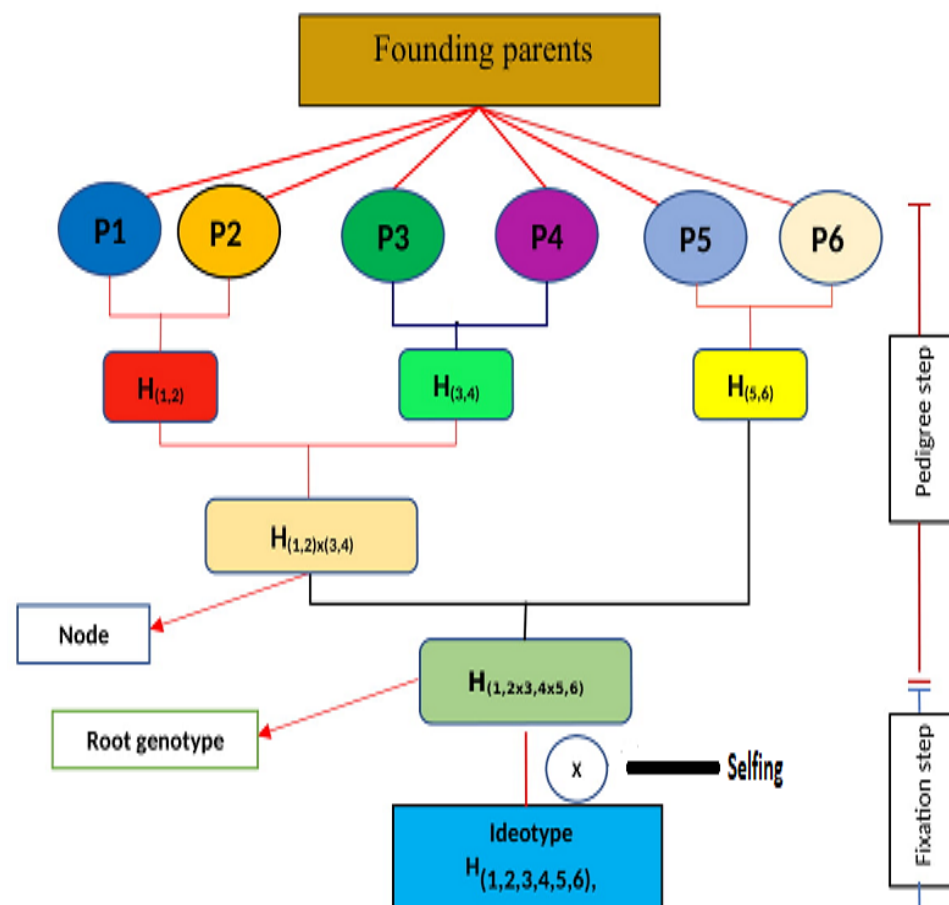


Figure 1. A definite gene pyramiding diagram of accumulating six desired/targeted genes [27].

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 (epistatically, 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.

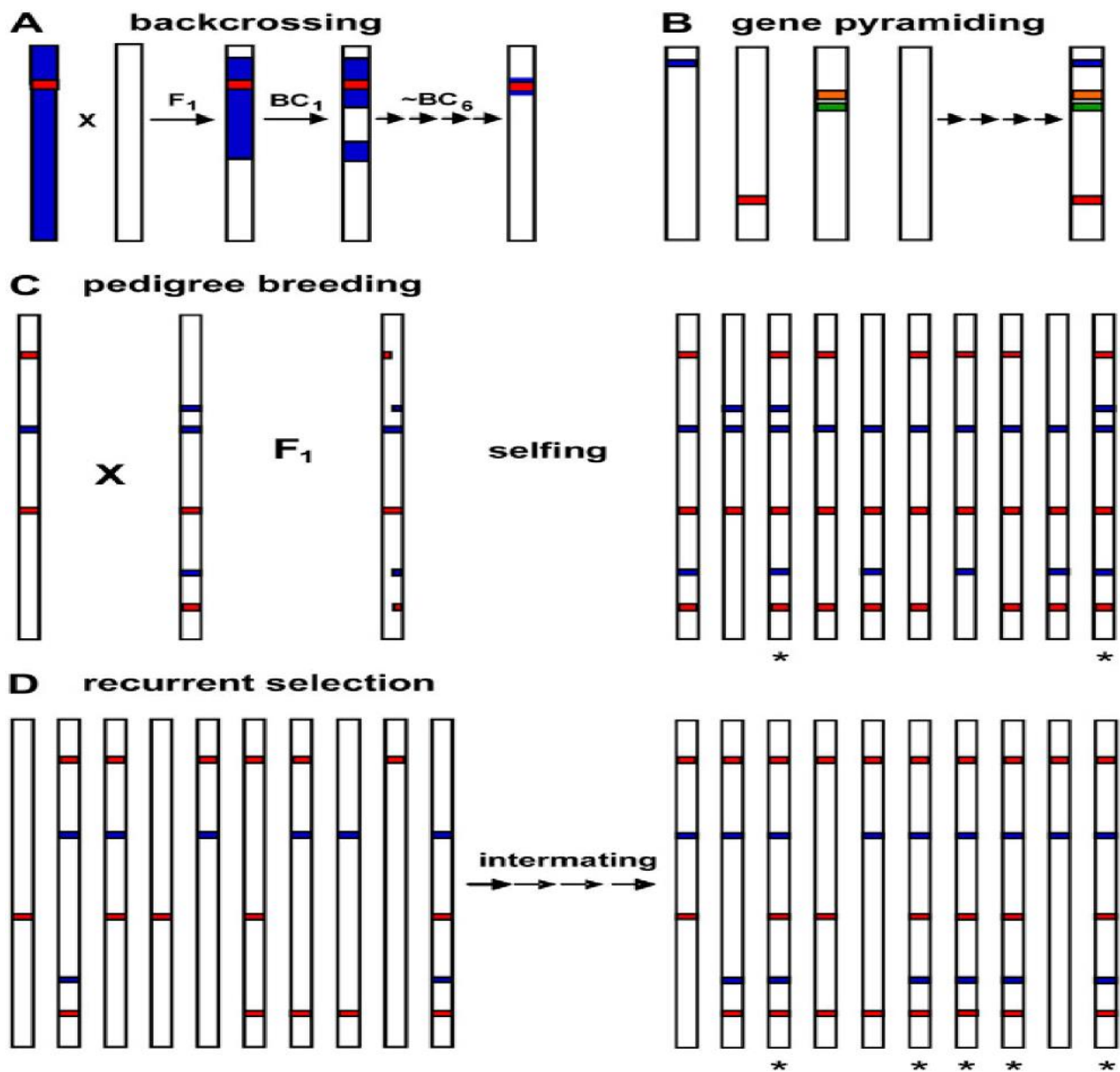


Figure 2. Some common breeding and selection breeding methods in rice [40].

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).

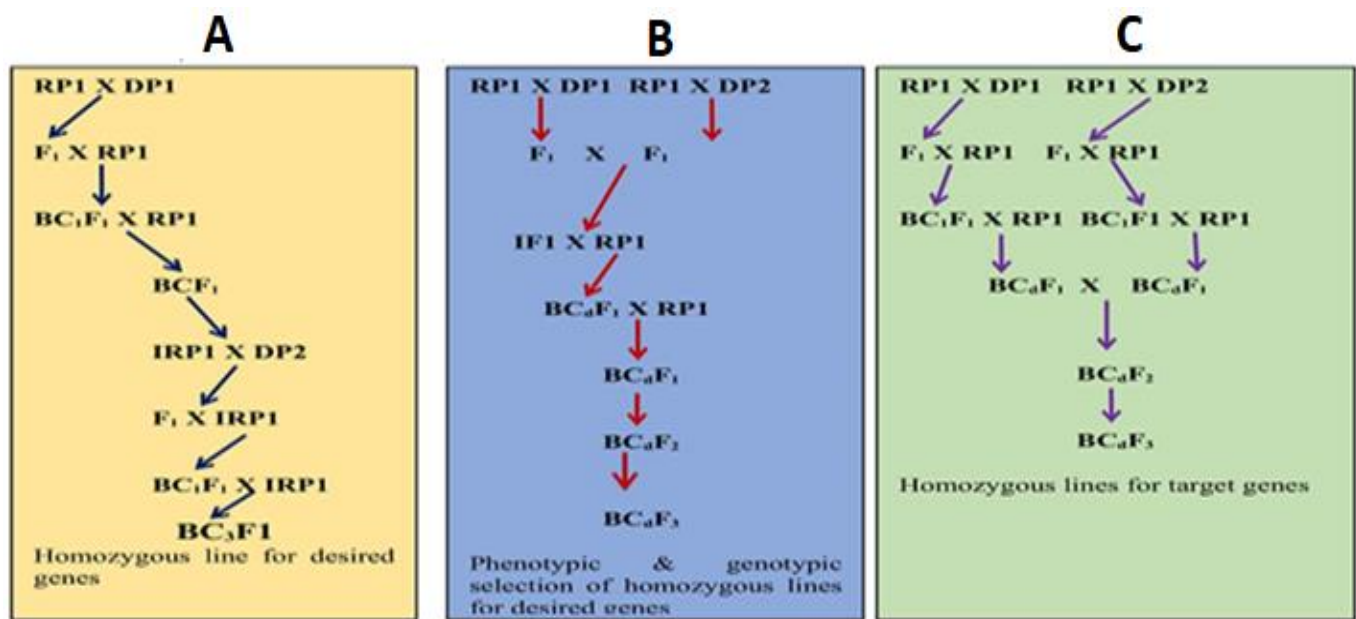


Figure 3. Schematic presentation of different backcrossing techniques for marker-assisted gene pyramiding. RP, recurrent parent; DP, donor parent; BC, backcross; IRP, improved recurrent parent; BCd, double backcross. (A) Stepwise transfer. (B) Simultaneous transfer. (C) Simultaneous and stepwise transfer combined. In order to develop a population of BC_1F_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 (RPI) 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 kompetitive 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 (kompetitive 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 F₂ 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 backcrosses 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 BC₁F₁, 95.30% for BC₂F₁ and 95.9% for BC₂F₂ [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 BC₁F₁ and BC₂F₁ plants with a high cooking and eating quality and in quickly recovering the RPG. Seven BC₂F₁ 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 BC₂F₁ and BC₂F₂ generations, and the result indicated that the RPG was 84.5% and 96.2% in the BC₂F₁ and BC₂F₂ 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/genes 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 marker 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.

Table 1. List of improved rice genotypes through marker-assisted gene pyramiding with their resistance genes, donor parents, recurrent parents and available linked markers for single traits.

Improved Rice Genotype	Resistance Gene/QTLs	Traits/ Diseases/ Resistance	Country/ Regions	Markers	Donor Parents	Reference
Tainung82	<i>Xa4, xa5, Xa7, xa13</i> and <i>Xa21</i>	BLB	Taiwan	<i>Xa4F/4R, RM604F/604R, Xa7F/7-1R/7-2R, Xa13F/13R, and Xa21F/21R</i>	IRBB66	[76]
Jin 23B	<i>Bph3+Bph14+Bph15+Bph18+Bph20+Bph21</i>	BPH	China	RM58, RM19324, RM3331, RM28427, RM28561, RM16553, HJ34, RR28561, B212	PTB33, IR65482-7-216-1-2, IR71033-121-15, B5	[77]
Jalmagna	<i>Xa5, Xa13, a21</i>	BLB	India	<i>Xa5S (Multiplex), Xa5SR/R (Multiplex), RG136, pTA248</i>	CRMAS 2232-85	[28]
Pyramided lines	<i>Xa13, Xa21, Xa5, Xa4</i>	BLB		<i>Npb18, RG556, RG136, pTA248</i>	IRBB4, IRBB5, IRBB13, IRBB21	[31,53,61]
CO39	<i>Pi1, Pita, Piz5</i>	Blast	Philippines	RZ536, RZ64, RZ612, RG456, RG869, RZ397	C101LAC, C101A51, C101PKT	[78]
Jin 23B	<i>Pi1, Pi2, D12</i>	Blast	China	RM144, RM224, PI2-4, HC28, RM277, M309	BL6, Wuyujing 2	[79]
Pusa RH10	<i>Xa13, Xa21</i>	BLB	India	RG136, pTA248	Pusa1460	[80]
–	<i>Gm-2, Gm-6t</i>	Gall midge	–	–	Duokang1, Phalguna	[81]
R2381	<i>Bph3, Bph27 (t)</i>	BPH	China	RH078, RH7, Q31, Q52, Q58, RM471	Balamawee, Ningjing3, CV 93-11	[82]
Swarna-Sub1	<i>Pi1, Pi2, Pi54</i>	Blast		RM224, RM527, RM206, PI54 MAS	Swarna-LT, Swarna-A51	[83]
PRR78	<i>Piz5+Pi54</i>	Rice blast		AP5930, RM206, RM6100	C101A51, Tetep	[84]
Improved PR106	<i>Xa5+Xa13+Xa21</i>	BLB	India	RG 556, RG 136 and pTA248	IRBB62	[21]
Tapaswini	<i>Xa4+Xa5+Xa13+Xa21</i>	BLB	India	RG 556, RG 136 and pTA248	MABC	[20]
Improved Lalat	<i>Xa4+Xa5+Xa13+Xa21</i>	BLB	India	RG 556, RG 136 and pTA248	IRBB 60	[21]
Improved TNG82	<i>Xa4, xa5, Xa7, xa13, Xa21</i>	BLB	Taiwan	<i>Xa4F/4R, RM604F/604R, Xa7F/7-1R/7-2R, Xa13F/13R, and Xa21F/21R</i>	IRBB66	[21]
Mangeumbyeo	<i>Xa4+Xa5+Xa21</i>	BLB		10603, T10Dw, MP1 + MP2,U1/I1	IRBB57	[85]
MR219,	<i>qDTY2.2, qDTY3.1, qDTY12.1</i>	Drought	Malaysia	RM236, RM511, RM520	IR77298-14-1-2-10, IR81896-B-B-195, IR84984-83-15-18-B	[86]
MRQ74	<i>qDTY2.2, qDTY3.1, qDTY12.1</i>	Drought	Malaysia	RM12460, RM511, RM520	IR77298-14-1-2-10, IR81896-B-B-195, IR84984-83-15-18-B	[86]
ADT 43	<i>Pi1+Pi2+Pi33+Pi54</i>	Blast	India	RM206, RM72, RM527, RM1233	CT 13432-3R	[87]
LuoYang69	<i>Bph6, Bph9</i>	BPH	China	InD2, RM28466	93-11, Pokkali	[88]

Table 1. Cont.

Improved Rice Genotype	Resistance Gene/QTLs	Traits/ Diseases/ Resistance	Country/ Regions	Markers	Donor Parents	Reference
Pyramided rice lines	<i>Pi2, Pi46, Pita</i>	Rice blast	China	RM224, Ind306, Pita-Ext, Pita-Int	H4, Huazhan	[89]
PL-(S5-n + f5-n + pf12-j)	<i>S5-n, f5-n, pf12-j</i>	Fertility improvement	China	SNP markers	Dular, 9311	[90]
Rice	<i>qCTF7, qCTF8, qCTF12</i>	Cold stress	Japan	RM5711, RM22674,	Eikei88223, Susei	[91]
Hua-jing-xian	<i>qCTBB-5, qCTBB-6, qCTS-6, qCTS12</i>	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	<i>xa5, xa13, and Xa21 Pi54, qSBR7-1, qSBR11-1, and qSBR11-2)</i>	BLB, blast, ShB	India	pTA248, Xa13-prom, Xa5, Pi54-MAS, RM224, RM336, RM209	IRBB60, Tetep	[93]
Pyramided line	<i>Piz, Pi2, Pi9 and Xa4, Xa5, Xa13, Xa21</i>	BLB, blast	Malaysia	RM6836, RM8225, RM13, RM21, pTA248	Putra 1, IRRBB60	[94]
Swarna +drought	<i>Pi9+ Xa4+ xa5+ Xa21+ Bph17+Bph3+Gm4+ Gm8+ qDTY1.1+ qDTY3.1</i>	Blast, BLB, BPH, drought and gall midge resistance	India	Pi9STS2, Xa4, Xa5DR, Xa13prom, pTA248, RM586, RM8213, Gm4LRR, Gm 8PRP, RM 431, RM 136	IRBB60, IRBL9, Rathu Heenati, Abhaya and Aganni	[95]
Tellahamsa	<i>Xa21, Xa13, Pi54 and Pi1</i>	BLB, blast	India	pTA248, Xa13 Prom, r Pi54MAS, RM 224	Improved Samba Mahsuri, Swarnamukhi	[96]
JGL1798	<i>Xa13, Xa21, Pi54</i>	BLB, blast	India	Xa13-prom, pTA248, Pi54-MAS	Improved Samba Mahsuri, NLR145	[97]
RPHR-1005	<i>Xa21, Gm4, Gm8, Rf3, Rf4</i>	BLB, gall midge, fertility restorer genes	India	pTA248, LRR-del, PRP, DRRM-Rf3-10, DRCG-RF4-14	SM1, SM2	[98]
Improved Lalat (Recurrent Parent)	<i>Pi2, Pi9, Gm1, Gm4, Sub1 Saltol</i>	Blast, gall midge, submergence, salt tolerance	India	RM444, RM547, RG64 SUB1BC2, RM10745	C101A51, WHD-1S-75-1-127 Kavya, Abhaya, FR13A, FL478	[20,24,66,78, 99-105]
MH725	<i>Xa21, Xa4, Xa27, Sub1A, Pi9, Badh2.1, Badh2</i>	Blast, BLB, submergence, Aromatic fragrance	China	M265, M355, NBS2-1, RM23887, RM224, RM21, M124	KDML105, IRBB27, 75-1-127, IR64	[106,107]
Pink3	<i>Genes (Sub1A-C, SSIIa, Xa5, Xa21, TPS), QTLs (qBph3, qBL1, qBL11)</i>	Submergence, BLB, blast, BPH	Thailand	SNP and SSR markers	CholSub1, Xa497, RBPiQ, Bph162	[108]
Wuyujin3	<i>Sto-bⁱ Wx-mq</i>	Low amylase content, rice strip disease	China	ST-10, Wx-mq-OF, Wx-mq-IR	Kanto 194	[109]
Junam	<i>PH18+Xa40+Xa3 +Pib+Pik+qSTV11^{SG}</i>	BPH, BB, blast, SSV		7312.T4A+HinfI, ID55.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	<i>BXa21, Sub1A, Pi9</i>	LB, blast and submergence	China	RM224,5198	WH21, IR64, 1892S, Guangzhan 63S, IR31917	[107,111,112]
Jinbubyeo	<i>Xa4, Xa5, Xa21, Pi40, Bph18</i>	BLB, rice blast, brown planthopper	China	MP1+MP2, 10603, T10Dw, U1+I1, 9871.T7E2b and 7312.T4A	IRBB57, IR65482-4-136-2-2, IR65482-7-216-1-2	[85,113–115]
Pyramided lines	<i>tms2, tgms, tms5</i>	Thermosensitive genetic male sterility (TGMS)	Philippines	RM257, RM174, RM11	DQ200047-21, Norin PL 12, SA2	[116]
Pyramided lines	<i>Xa21, The Bt fusion gene, The RC chitinase gene</i>	BLB, yellow stem borer, sheath blight	Philippines	SSR markers	TT103, TT9	[117]
Shengdao15, Shengdao16, Xudao3	<i>Bph14, Bph15, Stv-b</i>	Brown planthopper, rice stripe disease	China	B14, B15, S1	B5	[118]
Hua1015S	<i>Pi2, Xa23</i>	Blast, BLB	China	RM527, Ind M-Xa23	GZ63-4S, VE6219, HBQ810	[119]
Hom Mali 821	<i>Sub1, Qbph12</i>	Submergence and BPH	Thailand	R10783indel, RM277, RM260	IR49830, ABHAYA RGD9903 RGD9905)	[120]
Pyramided lines	<i>Sub1, badh2, qBl1 and qBl11</i>	Submergence, blast Strong fragrance	Thailand	R10783indel, RM212-RM319, RM224-RM144, Aromarker	TDK303 IR85264GD07529	[121]
Junam	<i>Xa3, Xa4 and QTL (qCT11)</i>	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, Pid3, Pi25, Pi36, Pi37, Pi54, Pia/Pi-Co39, Pib, Pid2, Pikm, Pikp, Pik, Pish, Pit, Pita* and *Pizt*) [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 *Pid2* and *Pi21*, belong to the nucleotide-binding area 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 *Pid2* gene is a B-lectin receptor kinase [139], whereas the *Pi21* 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 Oryzae pv Oryzae*. 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*, *qBl1* 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. pyramided 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 (NJ3), 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-bⁱ*) 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), *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 *Sub1* provides complete submergence tolerance for two weeks with an LOD score of 36, and an R^2 value of 69 % [102], and *Sub1* indicates the position of approximately 0.06 cM in the genomic region [104]. The *Sub1* QTL region on an FR13A-derived line shows three genes (*Sub1A*, *Sub1B* and *Sub1C*) encoding the putative ethylene-responsive factor, and these three genes have been identified as the major indicators of submergence tolerance [170]. The IR64-Sub1, Samba Mahsuri-Sub1, Thadokkamq-Sub1, BR11-Sub1 and Swarna-Sub1 rice varieties were developed after analysis and identification of *Sub1* QTLs through marker-assisted backcross breeding [170–176]. Scientists introgressed the submergence tolerance gene (*Sub1*) into different varieties through marker-assisted backcrossing for submergence tolerance (Table 1). Some scientists pyramided the *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 9.6×10^9 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 dSm^{-1} ECe (electrical conductivity of saturated extract), and the yield declines above the 3 dSm^{-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 Bokra are the donors of the *Saltol* QTLs [193] and *SKC1* genes [194,195]. Popular salt-susceptible high-yielding rice varieties have been improved by introgression of *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.

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