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REVIEW ARTICLE

Gene pyramiding an advanced approach for disease management in rice: A review

Sanju Thorat[1], Rakesh Kumar Gangwar[1], Dilipsinh Sisodiya[2]

1 *Main Rice Research Station, Anand Agricultural University, Nawagam, Kheda, Gujarat-387 540, India*

2 *Department of Agricultural Entomology, B. A. College of Agriculture, Anand Agricultural University, Anand, Gujarat-388 110, India*

Corresponding author: Sanju Thorat (sanjuthorat2@gmail.com)

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Abstract

Gene pyramiding is a vital strategy for varietal development in different crops. By using fewer generations than conventional breeding, molecular marker genotyping can streamline the gene pyramiding process. Enhancing the genetic foundation of resistance is crucial for reducing the burden of pesticide residues in the food chain, making it an effective strategy. The development of different pyramided lines carrying single or in combination of different diseases resistant genes in rice viz., Xa4, xa5, xa13, Xa21, and Xa38 for bacterial leaf blight; Pi1, Pi2, Pi9, Pi54, Pi46, and Pita for blast; qSBR7-1, qSBR11-1, and qSBR11-2 for sheath blight. The pyramided lines consist of either single or combinations of various resistance genes, which are instrumental in disease-resistant genetic improvement programs. These lines are utilized to develop profitable varieties that exhibit resistance to multiple diseases.

Keywords

Gene pyramiding, rice, varietal resistance

Introduction

Rice, scientifically classified as *Oryza sativa* L., carries substantial significance as a staple cereal crop, playing a crucial role in providing sustenance, and livelihood for millions of people worldwide, and it is cultivated extensively in various regions across the globe. Approximately 85% of the total rice produce is utilized for human consumption. India has the biggest rice crop area *i.e.*, 45.77 million ha with production 124.37 million tones, and productivity of 2717 kg per hectare during 2020–21 (Anonymous 2023a). During 2021–22 in Gujarat, the cultivation of rice spanned across 0.89 million hectares, producing 2.10 million tonnes of output, and 2355.05 kg of productivity per hectare (Anonymous 2023c). Various biotic, and abiotic limitations restrict rice productivity in India. There are several pests that can limit the yield of rice, among them weeds were responsible for the highest loss in seed yield, accounting for 37.02%, insect-pests ranked second, causing a loss of 27.9%, while diseases accounted for the remaining losses (15.6%) (Mondal et al. 2017). The diseases causing substantial yield losses to the rice crop, among them bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae* (*Xoo*)) has been reported to cause the losses ranging from 20 to 80% (Khush et al. 1989; Pradhan et al. 2015; Mohapatra et al. 2021; Pradhan et al. 2022), rice blast (*Pyricularia oryzae*) recorded more than 50% losses (Babujee and Gnanamanickam 2000), sheath rot (*Sarocladium oryzae*) causes 10 to 85% losses Liu et al. (2014); Bigirimana et al. (2015) and *Rhizoctonia solani*, sheath blight reported to causes 50% losses (Singh et al. 2019).

Various strategies, and techniques, including cultural, mechanical, physical, biological, and chemical methods, are utilized for managing diseases in rice crops. One of the most crucial, affordable, and environmentally beneficial elements for integrated disease management in rice is host plant resistance. The theory of gene pyramiding is outlined by Watson and Singh (1953). The technique

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of gene pyramiding involves deliberately incorporating a variety of desirable genes from different parent plants into one genotype. The simultaneous expression of multiple genes in a variety is achieved through the stacking of different genes, resulting in long-lasting resistance expression. It involves the use of several genes in a single cultivar to provide a wider bases of disease resistance. Gene pyramiding is a proven methodology that has been utilized in the past to improve the genetic composition of elite cultivars by selectively incorporating key genes from multiple sources. Ye and Smith 2010 revealed that the verification of the target genes necessitates the use of phenotyping, a technique that is solely effective for major genes. Nevertheless, the effort to identify the existence of targeted genes is challenging, thus limiting the range of selectable offspring (Malav and Chandrawat 2016). The number of genes intended for transfer, the distance among the target genes, and nearby markers, and the genotype selection made in each breeding generation are all critical variables that affect the success of gene pyramiding. To increase the frequency of better genotypes in the target population, marker-assisted selection (MAS) is a commonly used method to transfer genes between pyramided lines to the intended crop (Magar et al. 2014; Das and Rao 2015; Pradhan et al. 2015; Shamsudin et al. 2016). Various crops have specific genes incorporated into them to enhance their resistance against different pathogens. The utilization of the gene pyramiding strategy offers a proficient approach to augmenting the genetic basis of resistance, and its compatibility with diverse management strategies further enhances its efficacy (Tiruvaipati et al. 2022).

Why gene pyramiding is needed?

The most popular method of disease management in the farming community is application of chemical pesticides, but the drawback of using chemical pesticides is well known. For years, chemical pesticides have been employed to decrease biotic damage to crops; however, recent adverse effects have prompted a discouragement of their use. Most small-scale farmers cannot afford them due to much product costs, and the requirement for many sprays. There may be development of resistance, resurgence of pathogens, residues etc. Chemical pesticides represent a substantial hazard to human health, and are also harmful to the environment (Vincelli 2016; Sharifzadeh et al. 2018). In May 2011, the rice shipment from India, containing tricyclazole levels exceeding 0.1 ppm, was slated for return from the American shore. Throughout the past decade, 90% of shipments were declined because they contained tricyclazole (Anonymous 2013). Many biotic stressors, primarily caused by bacteria, and fungus, frequently pose a threat to the yield potential. To tackle these issues and enhance productivity, there's a need to cultivate varieties with enduring resistance. Optimal enhancement of host-plant resistance can be attained by amalgamating major resistant genes for diverse diseases, and biotic stress factors (Jegadeesan et al. 2020). Combining further dominant genes with the xa5 gene results in enhanced, and long-lasting resistance against *X. oryzae* compared to plants possessing only a single bacterial blight (BB) resistant gene (Huang et al. 1997). One possible strategy to reduce blast disease damage is to incorporate effective resistance genes into rice varieties. It has been suggested that using a resistance gene to create novel strains that are resistant to a extensive range of diseases is a useful, and environmentally friendly way to manage the disease. Hence, utilizing resistant varieties is required to overcome such issues, and it is an important tool of integrated disease management (Peng et al. 2023).

Strategies for gene pyramiding

Identification of resistant gene sources is one of the crucial features contributing to the success of gene pyramiding. Introduce the resistant gene into the elite genotype deficient through selective breeding techniques. "Gene pyramiding" is the term used to describe the act of incorporating multiple genes from distinct parents to create improved lines, and variations. The pyramiding process entails the stacking or combination of numerous genes, important to the simultaneous expression of numerous genes in various ways. Molecular markers play a crucial contribution to the selection of optimal plants for further advancement. A breeding strategy to attain disease control, and increased crop output is referred to as "gene pyramiding." The emergence of gene pyramiding as a novel field in plant breeding can be attributed to the progress made in molecular genetics, and its allied technologies, including marker assisted selection. The primary aim of gene pyramiding is to amalgamate all the desirable genes within a single genotype. When there are more than three parental lines possessing the desired genes (founding parents), the generation of the target genotype can be achieved through various crossing schemes. Henceforth, it is possible to categorize it into two sections (Ji et al. 2014). The initial section is known as pedigree, which aims to accumulate a single genotype called root genotype containing all target genes. Cross between two parent, and developed hybrid having two desirable genes. Finally in F_3 generation root genotype developed having all desirable genes. The subsequent stage is referred to as the fixation step, with the objective of establishing the target genes in a homozygous state, thereby attaining the desired genotype from the initial genotype (root genotype). Fixation steps aim to attaining the homozygosity. Double haploid techniques are implemented *i.e.* tissue culture method, artificial induction method, naturally haploid method etc to develop ideal plant type having targeted genes (Joshi and Nayak 2010). Breeders can speed up the gene pyramiding process by evaluating fewer generations to find the appropriate gene combination by molecular marker genotyping. Gene pyramiding plays a dynamic role in enhancing germplasm development (Rajpurohit et al. 2011; Miah et al.

2013; Pinta et al. 2013; Ji et al. 2014; Pradhan et al. 2015). Pedigree crossing, backcross breeding, cumulative hybridization, and repeated selection are a few instances of gene pyramiding techniques (Janila et al. 2016; Dormatey et al. 2020).

Advantages of gene pyramiding

Gene stacking, often referred to as pyramiding, is a technique that effectively transfers numerous desired genes from various parents into a single genotype. In contrast to conventional breeding, which usually needs a minimum of six generations to improved 99.2 per cent of the recurrent parent genome Suresh and Malathi (2013); Bai et al. (2018). Widely used for combining multiple disease resistance. Developing "durable" disease resistance against many different races is crucial. Its primary purpose is to enhance the current elite cultivar, do away with significant phenotyping, manage linkage drag, and shorten the breeding period (Zhang et al. 2006; Malav and Chandrawat 2016). It is recommended as a potential strategy to enhance both quantitative, and qualitative plant attributes (Richards 2006; Moose and Mumm 2008; Chukwu et al. 2019a).

Limitations of gene pyramiding

The initial cost implication of gene pyramiding is higher. Incorporating multiple major genes into a single cultivar necessitates a significant amount of effort, and this task is much more dedicated efforts to incorporate a gene to another one (Michelmore et al. 1991; Malav and Chandrawat 2016; Chukwu et al. 2019a; Dormatey et al. 2020).

Techniques of gene pyramiding

1. Conventional technique

It is a successive gene pyramiding involves the deployment of genes in the same plant one after another. The method utilizes sequential gene pyramiding within a single plant. It's a crop breeding technique that can be utilized to establish new lines in both conventional, and improved molecular breeding programmes. The conventional crop breeding technique, in the context of modern, and advanced technologies, entails using natural processes, and obsolete techniques to create new crop types (Floros et al. 2010; Zhang et al. 2018; Su et al. 2019). By adopting traditional breeding methods, during the previous thirty years, the International Rice Research Institute (IRRI), Philippines, has developed a diverse array of elite cultivars that exhibit resistance to a range of abiotic stresses, and diseases. In contemporary conventional breeding, backcrossing, recurrent selection, and pedigree selection have emerged as the main techniques (Khush 1984; Lafitte et al. 2006; Miah et al. 2013; Pang et al. 2017).

(i) Pedigree breeding

It is most common technique of breeding for the advancement of disease resistance genotypes. When the resistance character is regulated by polygene/minor genes, this approach is employed. Pedigree breeding is a technique employed to improve the genetic quality of self-pollinated species. It involves meticulously selecting superior genotypes from segregating generations, and keeping thorough records at each stage of the selection method of the chosen plants' lineage (Khush 1984; Yadira et al. 2011). For the majority of self-pollinating crops, such as rice, plant breeders have always found that recurrent selection is preferable than pedigree selection (Miah et al. 2013). The technique aims to produce F_1 offspring by crossing two distinct individuals with appropriate, and complementary traits. The initial segregating generations, typically ranging from F_2 to F_5, are combined together without any selection. In subsequent generations, while maximum plants are homozygous, plant are selected, and evaluated for resistance against diseases (Malav and Chandrawat 2016; Dormatey et al. 2020). Nonetheless, the primary drawback of pedigree selection is laborious in nature, necessitating the frequent assessment of numerous lines during planting seasons while meticulously recording the selection measures. Pedigree selection, as one of the breeding procedures, requires a comprehensive understanding of the breeding materials, as well as an awareness of how the environment influences the genotype in relation to desirable traits (Oladosu et al. 2019). A group of scientists recommended rice genotype IET-14726 exhibited multiple resistance against bacterial leaf blight (BLB), leaf, and neck blast diseases from MRRS, AAU, Nawagam, Kheda, Gujarat, India. This promising genotype was used to develop rice variety GAR 13 (GR 11 × IET-14726) (Fig. 1) by pedigree method of selection in the year 2009, which is resistant against major disease of rice (Anonymous 2002). GAR 13 is now more popular in Gujarat state, and covering 1.2 to 1.3 lakh ha area out of total 9.0 lakh ha area of rice in Gujarat (Anonymous 2023b).

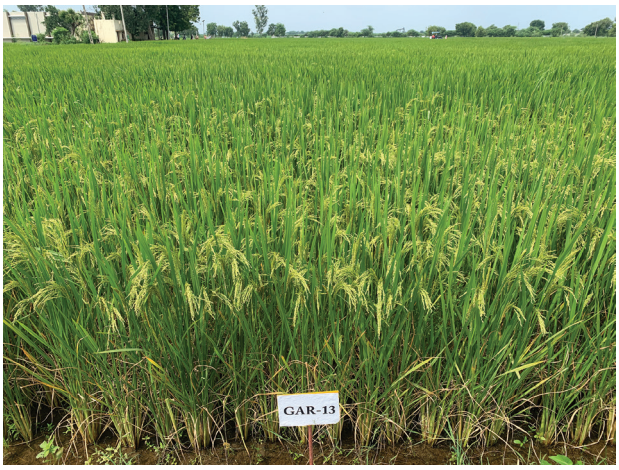

Figure 1. Rice variety GAR 13 exhibited multiple resistance.

(ii) Backcross breeding

In the traditional pyramiding method, backcross breeding is used. In order to select for the desired traits, a hybrid must be hybridized with a single of its parental lines. The process of backcrossing involves repetitively crossing a donor genotype with a popular cultivar in order to transfer a specific target gene. Rice breeding extensively utilizes the process of introgression to transfer desirable or target genes responsible for specific traits between a receiving parent, and a donor parent. The primary goal is to decrease the genetic makeup of the donor parent while achieving a notable enhancement in the successful incorporation of the desired traits into the recipient parent. The recurrent parent is routinely crossed with both the elite genotype, and a donor genotype that contains a specific desirable gene. Backcrossing procedures involve selecting for the desired gene, and recovering a larger percentage of the elite line's genome. This methodology offers a precise, and accurate means of producing a significant quantity of advanced breeding lines. The disease resistance trait of the progeny is screened through artificial inoculation with the pathogen (Lafitte et al. 2006; Malav and Chandrawat 2016; Assefa 2018; Dormatey et al. 2020).

(iii) Recurrent selection

The recurrent selection process entails selecting individuals, followed by intermating among the chosen ones, as a part of breeding procedure so that frequency of genes within a population, favorable conditions can lead to an increase, while unfavorable conditions can result in a decrease. Intermating between individual within or between populations. It is a productive, and modified kind of progeny selection in which certain qualities are selected for successive generations of segregating progeny based on phenotypic traits. The selection procedure is repeated in every succeeding generation, thus acquiring the term recurrent selection. After several cycles or generations, plants that are acquired closely resemble the recurrent parent, except for the amalgamation of resistance genes. In varietal improvement, recurrent selection is employed to acquire beneficial alleles through many crossings while preserving genetic variability. It enables accelerated, and well-defined cycles of reproduction, extra precise genetic advancements, and the development of enormously varied breeding lines. Extensive research has been conducted by this technique in rice (Pang et al. 2017). Recurrent selection proves to be ineffective due to its reliance on environment-dependent phenotypic selection, which in turn prolongs the selection process to approximately two to three crop seasons every cycle (Cui et al. 2009; Malav and Chandrawat 2016; Dormatey et al. 2020).

Advantages of conventional breeding methods

The conventional breeding required lower input cost as compared with molecular techniques. It requires minimum infrastructure, and easily conducted in the supervision of breeder/scientist. Conventional breeding has gradually moved its emphasis to selecting on physiological features because these qualities need less effort, and genetic variation. In general, the generation of novel genetic characteristics, hybridization between sexually different parents, and germplasm conservation all benefitted from traditional breeding techniques. The IRRI, Philippines has developed many more genotypes, different diseases, and abiotic stresses can be combated through the utilization of conventional techniques to develop resistant varieties (Khush 1984; Malav and Chandrawat 2016).

Limitations of conventional breeding methods

It needs large area, and resource, i.e., many generations, multilocation trials etc. Periodically evaluating lines takes significantly more time., i.e., several generations, phenotypic selections. The main drawback of the conventional method is due to its time-consuming character, involving frequent assessment of many lines throughout planting seasons while meticulously recording the selection criteria. In the traditional approach, a thorough understanding of the breeding materials, and the effect of environment on genotype on desirable traits is essential. This approach is inappropriate for traits where multiple genes are involved. The transmission of unwanted genes along with the target genes into the recipient line can lead to a decrease in the performance of supplementary characters, resulting from the phenomenon known as linkage drag. Phenotyping has predominantly confirmed the existence of target trait genes, mostly on an individual basis. The combination of allelic genes within the same genotypes are not permissible, for instance. Progeny testing is essential to assess the effect of a recessive gene as it cannot be determined in individuals with heterozygous traits (Khush 1984; Miah et al. 2013; Malav and Chandrawat 2016). Conventional breeding can still be utilized, although it becomes exceedingly challenging, if not almost impossible, in the early generations because each plant must undergo phenotypical screening for every attribute under consideration. It becomes extremely challenging to evaluate plants in specific populations, such as the F_2 generation or for traits that require destructive bio-assays (Pradhan 2015; Malav and Chandrawat 2016). Conventional breeding alone poses challenges in pyramiding genes due to the persistent impact of linkage drag, which proves difficult to overcome through repeated backcrossing. The effect of each individual gene cannot be clearly distinguished through phenotyping alone when two or more genes are introgressed (Chukwu et al. 2019a). Improved drought tolerance genotypes are still screened conventionally using a manual procedure. This procedure is prone to errors, requires a significant amount of manual labour, consumes a considerable amount of time, frequently lacks effectiveness, and necessitates the use of destructive sampling (Yang et al. 2013; Crossa et al. 2017).

2. Molecular technique

Simultaneous gene pyramiding involves the introducing simultaneously inserting several genes into a single plant. In recent times, crop breeding has undergone significant

advancements, and the introduction of modern molecular tools has made it possible to accomplish precision breeding in the shortest possible duration. Innovative molecular breeding technologies, particularly marker-assisted selection (MAS), and gene transformation, are utilized for transferring desirable genes (Hospital 2009). Molecular technology, and genetic engineering are the primary tools utilized in biotechnological methods, resulting in the advancement of biotic, and abiotic tolerance in rice. Two methods are used by biotechnology in rice molecular breeding. This process uses marker-assisted selection, whereas the other would necessitate the creation of genetically engineered crops (Miah et al. 2013; Oladosu et al. 2019). Molecular strategies have greatly enhanced conventional plant breeding in the current era. The use of molecular marker genotyping has streamlined the breeding selection process, leading to fewer generations needed for evaluation, and the integration of genes into desired genotype (Dormatey et al. 2020).

(i) Marker assisted selection

(a) Molecular marker assisted selection

Molecular marker assisted selection (MMAS) is well-organized technique for swiftly integrating desirable characteristics into novel cultivars. An additional option to support phenotypic screening is to use DNA markers that are closely related to the target locus. A specific site in a genome that regulates a certain phenotypic trait can be identified using a "genetic tag" called a molecular marker. The utilization of molecular markers for the indirect selection of various traits are widely employed approach. It is facilitating the improvement of traits. The screening of genotypes of interest through molecular markers. Gene pyramiding with marker assistance offers the potential to expedite breeding programs, and ensure that the resistance imposed in the host plant is durable. MAS enables the concurrent monitoring of multiple traits, while conventional breeding necessitates distinct field experiments to assess particular characteristics (Rai et al. 2018). By selecting appropriate plants during the initial growth phase, MAS facilitates the economical stacking of genes, resulting in reduced field area requirements, lower costs for maintaining germplasm, and decreased expenses for agronomic inputs during field trials. It is crucial to employ rice varieties that have resistant genes added in order to protect against the disease's threat. To provide persistent resistance to BLB in rice, it is crucial to take into account the pyramiding of several genes (Zhang et al. 2006). Marker assisted selection shows great potential in the expansion of rice cultivars resistant to the BLB disease (Das and Rao 2015; Oladosu et al. 2018). The transferred BLB resistant genes from a donor rice genotype IRBB59 through MAS. The 22 pyramided lines having BLB resistance genes, and exhibited a greater degree of resistance (Deshmukh et al. 2017). Using the MAS technique, blast resistant genes (Pi2, and Pi9) were added to the elite rice variety "Improved Tapaswini," which already has four BLB gene strains. The findings revealed that the Improved Tapaswini a recurrent parent displayed susceptible reactions with a score of 4, comparable to both the susceptible controls, while the donor parents exhibited maximum levels of resistance with a score of '0'. The ITGP2, and ITGP5 gene pyramids demonstrated a significant level of resistance against blast disease, as indicated by a score of 'zero' for the resistant reaction. The pyramided lines exhibited resistance with lesion lengths extending from 0.70 to 2.50 cm against BLB (Das et al. 2018). The blast resistant pyramided lines developed by using MAS strategy in rice. Pyramided line BL-40-21-86-28-208 demonstrated resistance against blast, with a disease scale range of 1 to 2, and a Recurrent Parent Genome (RPG) of 95.50%. Its grain yield was slightly higher than that of the recurrent parent (Kumar et al. 2019). Through the use of MAS, the resistance of the rice cultivar with pyramided features to bacterial leaf blight was evaluated in the field with the Taiwanese *X. oryzae* strain isolate, XF89-b. Lower lesion sizes varied from 0.37 to 0.46 cm among all five gene pyramided cultivars. The pyramided genotypes, comprising five genes, showed a spectrum of reduced lesion lengths, spanning from 0.37 to 0.46 cm. Additionally, they displayed greater production, and quality of grain along with increased levels of disease resistance (Hsu et al. 2020). The BLB, blast, and sheath blight resistance genes were combined in the genetic profiles of ASD 16, and ADT 43 using the marker assistant selection method. The IRBB60, served as the source of bacterial blight resistance genes. Conversely, Tetep, which carries the blast resistance gene (Pi54) as well as the sheath blight resistance genes, served as the source for blast, and sheath blight resistance. In the recurrent parents, the disease reaction score for bacterial blight, blast, and sheath blight was determined to be 9, indicating a high level of susceptibility. The pyramided lines exhibited highly resistant reaction against BLB. The chosen pyramided lines, which contain the Pi54 gene, displayed strong resistance to blast, as evidenced by disease scores ranging from 1 to 3. The lines with qSBR7-1, qSBR11-1, and qSBR11-2 pyramided exhibit a spectrum of relative lesion height (RLH) values spanning from 28–45%, alongside disease scores ranging from 3 to 5, signifying a moderate resistance to moderately susceptible levels (Ramalingam et al. 2020). Elite rice maintainers, and restorers were equipped with blast, and BLB resistance genes through a combination of conventional crossbreeding, and the MAS breeding technique. A total of Seventy-four improved lines (ILs) with a gene combination for blast, and BLB resistance were developed (Wang et al. 2021).

(b) Marker assisted backcrossing

The process of moving a required gene from a donor cultivar to a cultivar that is widely grown is accomplished through a recurring crossbreeding technique referred to as backcrossing. However, this method is unfortunately characterized by its slow, and uncertain nature. The utilization of marker assisted backcrossing (MAB) involves improving the desired trait in a recipient parent by transferring one or more desired genes from a donor parent

through multiple rounds of backcrossing (Hospital 2009). In contrast to conventional backcrossing, MAB presents several advantages viz., effective selection of target gene, minimize linkage drag, accelerated recovery of recurrent parent etc. By employing MABC, it is possible to achieve the desired outcomes in a shorter span of time, typically within approximately two years (Chukwu et al. 2019a). By employing marker-assisted backcross breeding (MABB) in rice, the blast-resistant genes Pi46, and Pita were effectively introduced from the donor parent into HH179, resulting in the development of three near isogenic lines (NILs): R1791, R1792, and R1793. NILs, and parents were screened against 34 different isolates of blast disease pathogen at seedling stage under green house condition. These NILs recorded 64.7 to 97.1% resistance spectra which was significantly higher than the recurrent parent, 23.5% (Xiao et al. 2016). The pyramided lines developed by using MABB strategy against blast in rice. The Samba Mahsuri (BPT 5204) variety, known for its high yield, and excellent grain quality, was chosen as the recurrent parent. It is a popular Indica variety that also exhibits a high resistance to blast disease, and Tetep (pi-54 gene) was used as donor parent. Pyramided line BT-8-47-22-6-203 showed resistant reactions, and given recovery of RPG 92.80 per cent, and grain yield marginally more than recurrent parent (Kumar et al. 2018b). The resistance gene Xa38, which is the major dominant gene against bacterial leaf blight, has been successfully incorporated into the genetic makeup of APMS 6B rice through MABB. The backcross derived lines demonstrated varying levels of resistance, ranging from moderate to high, as indicated by the mean lesion length of 1.63 to 4.27 cm against all Xoo strains (Yugander et al. 2019). The disease resistance attributes of the high yielding commercial rice variety MR219, specifically against BLB disease, have been effectively enhanced through the implementation of the backcross breeding approach, supported by the MAS tool. Two lines, PB-2-107, and PB-2-34, have been identified as having great potential due to their exceptional performances, and high resistance against BLB (Yazid et al. 2021). By using MABB, four BLB resistance genes were able to be pyramided into the well-known rice variety Ranidhan. Among the tested lines, CRBR203-32-82-1036, which had been genetically enhanced with multiple resistance genes, displayed the highest resistance reaction, as indicated by a lesion length of 2.51 cm (Pradhan et al. 2022). The renowned Japonica Italian rice variety has been enhanced against blast by integrating four Pi genes such as Piz, Pib, Pita, and Pik using MABB. Several lines exhibit broad patterns of resistance to blast diseases, featuring the presence of four Pi genes (Zampieri et al. 2023). The implementation of gene pyramiding, utilizing marker assisted backcrossing, was carried out through three distinct strategies (Joshi and Nayak 2010; Ye et al. 2016; Pathania et al. 2017).

(1) Stepwise transfer

The initial approach involves generating an F_1 hybrid through the crossbreeding of the recurrent parent (RP_1) with the donor parent (DP_1). Following this, an enhanced recurrent parent (IRP_1) is developed via successive backcrossing, extending up to the third backcross generation (BC_3). Subsequently, the refined recurrent parent is crossed with an alternative donor parent (DP_2) in order to facilitate multiple gene stacking. This method is distinguished by its focused targeting of individual genes, ensuring heightened accuracy, and simplicity in execution. As a result, there's a reduction in both population size, and the volume of genotyping required. However, this approach does have certain limitations as it requires a longer duration to complete the pyramiding process. Consequently, this strategy is considered less favorable (Pazhamala et al. 2015; Malav and Chandrawat 2016; Kushwah et al. 2020).

(2) Simultaneous transfer

The alternative method entails crossing the recurrent parent (RP) with multiple donor parents (DP_1, DP_2, DPn, etc) to produce F_1 hybrids. These F_1 hybrids are then interbred to yield the enhanced F_1 generation (IF_1). By backcrossing this IF_1 with the recurrent parent, the improved recurrent parent (IRP) is attained. The process of gene stacking is integrated within the pedigree phase itself. One benefit of backcrossing that is simultaneous or synchronized is that it is faster to execute. In cases where the donor parents differ, the utilization of this approach becomes less probable due to the potential risk of losing the pyramided gene during the process (Hu et al. 2016; Rana et al. 2019; Kushwah et al. 2020). The improvement of the esteemed rice variety Lalat utilized the marker-assisted forward breeding (MAFB) technique, employing a simultaneous crossing method. This tactic effectively integrated four BLB genes as well as the blast resistance gene Pi9. The selected ILs demonstrated a high degree of resistance to BLB by lesion lengths varying from 1.3–3.0 cm, and blast scale between 1, and 3 (Singh et al. 2021).

(3) Simultaneous and stepwise transfer

In the third approach, integrating elements from both the first, and second strategies includes backcrossing up to the BC_3 generation after concurrently crossing the recurrent parent (RP_1) through various donor parents. Pyramided lines are derived from intercrossing the backcross populations containing individual genes with each other. Employing this method not only streamlines the timeframe but also guarantees the full fixation of genes, rendering it the most widely accepted, and dependable approach (Joshi and Nayak 2010; Pazhamala et al. 2015; Hu et al. 2016; Malav and Chandrawat 2016; Ye et al. 2016; Pathania et al. 2017; Rana et al. 2019; Kushwah et al. 2020). The incorporation of two BLB resistance genes (Xa21, and xa13) and the blast resistance gene (Pi54) into the Indian rice variety MTU1010 was achieved through both simultaneous, and stepwise Marker Assisted Backcrossing (MABB) strategies. The donor parents utilized were Improved Samba Mahsuri, identified for its high produce, fine grain type, and BLB resistance carrying genes xa5, xa13, and Xa21, and NLR145 (Swarnamukhi), a widely known long slender grain type, and late variety harboring

the blast-resistant gene pi54. Resultantly, the pyramided lines ICF3-16-59, and ICF3-16-521 showed high resistance against both BLB, and blast diseases, with a disease scoring of One (Kumari et al. 2016). The simultaneous, and stepwise marker assisted backcrossing (MABB) was used in a simultaneous, and sequential manner to incorporate two BLB resistance genes, and blast resistant genes into the rice variety Tellahamsa. The selection of donor parents involved choosing Improved Samba Mahsuri, and NLR 145 (pi54, and pi1). The resulting two-gene pyramided lines, namely TH-625-159, and TH-625-491, displayed significant resistance to both diseases, achieving a disease score of One (Jamaloddi et al. 2020).

(c) Marker assisted recurrent selection

Utilizing recurrent selection proves to be an effective technique for improving polygenic traits. Recurrent selection is thought to be a productive method for pyramiding various plant attributes; however, the effectiveness of its selection is unsatisfactory as phenotypic selection relies on environmental factors, whereas genotypic selection requires a significant amount of time (2 to 3 cropping seasons for a selection cycle) (Jiang et al. 2013; Cai et al. 2019). The core MARS process involves several phases, such as the selection of parent lines from both homogeneous, and heterogeneous populations (Ali et al. 2020). To improve agronomic characteristics such as grain yield, and resistance to both abiotic, and biotic stresses, it is recommended to employ pyramiding to combine multiple QTLs in crops (Crosbie et al. 2006; Ribaut et al. 2010; Almeida et al. 2013; Gu et al. 2016). Marker assisted recurrent selection (MARS) includes utilizing molecular markers to perform recurrent selection, and identify multiple genomic regions linked to complex traits. The objective of this method is to find the most favorable genotype, either within a single population or across interconnected populations. For a single selection cycle, it permits intercrossing, and genotypic selection within the same crop season. By increasing the frequency of advantageous alleles, MARS improved the effectiveness of long-term selection. The process of choosing parents from either homogeneous or heterogeneous populations is the first step in the early phases of MARS (Gokidi et al. 2016; Dormatey et al. 2020).

(ii) Transgenic method

Transgenic procedures are used to modify the plants, where genes are genetically engineered from one plant to another. Natural or artificial methods are used in transgenic methods. In natural method, agrobacterium mediated gene transformation is used whereas in artificial method, gene gun, particle bombardment mediated transformation, electrophoresis, sonification approach, laser treatment etc are used. The successful cloning of the rice Xa21 gene, conferring resistance against the blight pathogen *X. oryzae*, has demonstrated robust resistance against various isolates (Rahangdale et al. 2020). Using the freeze-thaw technique, the genes Pib, Pi25, and Pi54 were inserted into the *Agrobacterium tumefaciens* strain LBA 4404 (An et al. 1988). Using an *Agrobacterium*-mediated method, ripe seeds of Kasalath, and Zhenghan 10 were used to extract *scutellar calli* for genetic transformation (Saika and Toki 2010; Sahoo et al. 2011). Utilizing transgenesis to stack the Pi genes offers a promising strategy to enhancing rice resistance against the pathogen *Magnaporthe oryzae* (Peng et al. 2021). While the MAS implanted comparable lines with the resistance gene were compared to transgenic plants expressing the Xa21 gene for bacterial blight resistance. It was also shown that both were almost identical at the molecular level, the disruption of utmost pathways caused by transgenesis was also observed in MAS breeding (Gao et al. 2013). The parental transgenic lines utilized in this investigation were near-isogenic lines (NILs) derived from IR72. These NILs exhibited a single locus variation from IR72, with TT103 for Xa21, and TT9 for Bt, and RC7. This method substantially minimized the time, and effort necessary for recurrent backcrossing, enabling nearly complete restoration of the recipient background. The transgenes additionally functioned as markers for molecular selection, and early detection, which led to the creation of stable homozygous lines. In the imminent future, these stable homozygous pyramided lines will be cultivated to multiply seeds, and undergo field-testing (Datta et al. 2002).

Advantages of molecular techniques of gene pyramiding

The molecular methods are simpler, rapid, competent, and cost-effective strategies compared to phenotypic evaluation (Collard et al. 2005; Moose and Mumm 2008; Kumar et al. 2018a; Angeles et al. 2020). Introducing some desired genes from various parents into a single genotype can be accomplished in merely 2 to 3 generations, a notable acceleration compared to the conventional breeding method, which typically demands at minimum six generations to improve 99.2 per cent of the RPG (Suresh and Malathi 2013; Hasan et al. 2015). Opting for individual plant selection during the seed-

Table 1. Successfully pyramided genes with their traits in rice.

Diseases	Pyramided genes	References
Bacterial leaf blight	Xa4, xa5, xa13, Xa21, and Xa38	Kumari et al. (2016); Das et al. (2018); Yugander et al. (2019); Ramalingam et al. (2020); Singh et al. (2021); Pradhan et al. (2022)
Rice blast	Pi1, Pi2, Pi9, Pi54, Pi46, Pita, Piz, Pib, Pita and Pik	Kumari et al. (2016); Das et al. (2018); Kumar et al. (2019); Ramalingam et al. (2020); Singh et al. (2021); Zampieri et al. (2023)
Sheath blight	qSBR7-1, qSBR11-1 and qSBR11-2	Ramalingam et al. (2020)

ling stages provides a notably reliable approach. Marker genotyping or DNA marker tests can substitute extensive field trials with large populations. The plants not carrying the targeted genes, which can be discard by use of foreground markers. Moreover, the selection of plants based on molecular markers is highly dependable as it takes into account the diverse environmental factors that can influence field trials (Collard and Mackill 2008). When adding genes or QTLs from landraces, introgression using molecular markers is particularly successful since it decreases the time required to creation a better cultivar, and the tricky of linkage drag (Dwivedi et al. 2007). Breeders can speed up the gene pyramiding process by evaluating fewer generations to find the appropriate gene combination by molecular marker genotyping. The utilization of gene pyramiding is a vital tactic in improving germplasm (Rajpurohit et al. 2011; Pinta et al. 2013; Ji et al. 2014; Pradhan et al. 2015). Decreasing the number of plants to be tested improves the efficiency of target trait selection within an effective breeding framework. By doing this, the breeder also saves costs by avoiding tedious work, and wastage of time. Moreover, employing molecular markers for plant selection is deemed more dependable as it accounts for the variations in environmental factors affecting field trials (Collard et al. 2005; Bishwas et al. 2016). Advancements in molecular technologies have transformed the breeding landscape, facilitating precise, sophisticated, and swift breeding via molecular markers, surpassing the constraints of traditional breeding methods (Hasan et al. 2015; Kage et al. 2016; Dormatey et al. 2020).

Limitations of molecular techniques of gene pyramiding

The initial cost is higher for establishment, and maintaining a molecular marker, equipments, consumables etc in laboratory. The development of markers involves considerable initial costs, and the preservation of these markers at a constant temperature is a major challenge due to its dependency on electricity supply. The reliability of markers in determining the phenotype is generally low. Sampling bias can still have an impact, especially in small populations. An extended gap among a marker, and a major gene compounds the challenge of recombination (Chukwu et al. 2019a). Additionally, constraints related to consumables, and equipment further hinder the utilization of MAS in plant breeding. The creation, and maintenance of a molecular marker laboratory necessitate the presence of both these elements, thereby warranting careful consideration of their associated expenses (Akhtar et al. 2010; Das et al. 2017). The initial investment required for incorporating markers in marker-assisted backcross breeding (MABB) might appear higher in the short term. However, over time, the rapid deployment of newly developed varieties through MAS may outcome in greater financial gains surpassing the production costs (Amagai et al. 2015; Hasan et al. 2015).

Key elements influencing the pyramiding of genes

(i) Features of the desired traits or genes

Successful gene pyramiding relies on well-defined functional characteristics of the genes to be pyramided, and the utilization of ideal markers for selection that are as effective as the genes themselves. The effectiveness of pyramiding may be reduced when dealing with target genes that have moderate or small effects (Chukwu et al. 2019b). One or two markers per gene can be used to determine if the gene of interest is present or absent. It is strongly recommended to employ the bulk segregant analysis (BSA) technique for identifying closely linked markers to a major gene (Michelmore et al. 1991; Chukwu et al. 2016).

(ii) Reproductive characteristics

The reproductive potential of a crop is ascertained by the quantity of seeds generated by an individual plant (Chukwu et al. 2019b). It's also crucial to remember that using wild relatives as donor parents to add desired genes to cultivated rice varieties can lead to additional reproduction related issues like incompatibility in crossing (Singh et al. 2001; Sharma and Dubey 2005).

(iii) Operating capital

Breeding programs are conducted exclusively within the financial constraints of the available operating capital. Thus, it is of utmost significance to consider the factor of reducing the overall cost when opting for a particular strategy. Increasing the length of generations can alleviate the burden on population size required in each generation, potentially resulting in a reduction of the total cost. Extending the duration, however, results in a postponement of the release of the new cultivar (Malav and Chandrawat 2016; Chukwu et al. 2019b).

(iv) The ability to recognize the appropriate genotypes by a breeder

Factors such as the nature of germplasm, and the quantity of genes to be shifted determine the calculation of the distance among the flanking markers, and the target genes. The total of promising lines selected during respectively breeding cycle (Malav and Chandrawat 2016; Chukwu et al. 2019b).

Conclusion

Pathogens pose significant threats to rice crops, necessitating the incorporation of specific genes to confer resistance against these disease causing agents. The process of stacking different genes, termed "pyramiding," leads to the simultaneous expression of multiple genes in a variety, thereby fostering the development of enduring resistance. The development of different pyramided lines carrying single or in combination of different resistance genes in rice (Xa4, xa5, xa13, Xa21, and Xa38 for bacterial leaf blight; Pi1, Pi2, Pi9, Pi54, Pi46, and Pita for blast; qSBR7-1, qSBR11-1, and qSBR11-2 for sheath blight). The gene pyramiding strategy stands out as a competent method for augmenting the genetic basis of resistance. Its successful integration with all other management strategies further enhances its effectiveness.

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