



## Literature Review

# Biotic Stress Tolerant Rice Variety with Enhanced Method of Gene Pyramiding

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## Abstract

Rice, as a staple food crop, faces various difficulties from biotic burdens like sicknesses, bugs, and weeds. Customary raising strategies have shown a halfway outcome in creating rice arrangements with a thorough battle against numerous biotic burdens. The superbiotic elements that lessen rice efficiency are bacterial curse (BB) and organism-impact diseases. The potential for yield of the rice varieties must be developed by including genes for biotic stress resistance if yield improvements in rice are to be sustained. This research paper aims to explore innovative methods of gene pyramiding to upgrade biotic stress, and resilience in rice varieties. We examine the standards and headways in quality pyramiding procedures, including sub-atomic markers, and determination on marker-helped and genome-altering apparatuses, to stack numerous opposition qualities proficiently. By utilizing these methods, we propose a clever methodology for raising rice assortments with upgraded biotic pressure patience. In this review, two principal BB (Xa21 and xa13) and two critical impact obstruction qualities (Pi54 and Pi1) were all the while brought into Tellahamsa with the use of the marker-helped backcross rearing (MABB) system. The paper underlines the expected advantages, challenges, and moral contemplations related to quality pyramiding, and talks about future exploration bearings to speed up the organization of versatile rice assortments.

## Introduction

Rice is one of the main staple harvests internationally, giving a huge piece of the total populace a significant wellspring of calories. Notwithstanding, rice creation faces various difficulties, including biotic pressure brought about by microbes like parasites, microscopic organisms, infections, and vermin. These anxieties can prompt significant yield misfortunes and diminish the nature of rice grains. Creating biotic pressure lenient rice assortments is critical to guarantee food security and further develop rural maintainability [1].

## Background

Ordinary imitating approaches have been used to empower rice assortments with further developed resistance near unambiguous biotic weights. Notwithstanding, these strategies are in many cases tedious and depend on the accessibility of

safe benefactor qualities inside the rice germplasm. With progressions in atomic science and hereditary designing, scientists have gone to present-day strategies, for example, quality pyramiding to further develop rice assortments with a wide range of protection from different biotic anxieties [1].

Quality pyramiding includes the concurrent fuse of various qualities or quality alleles presenting obstruction against various microorganisms into a solitary rice assortment. This interaction consolidates the advantages of individual opposition qualities, prompting upgraded and sturdy obstruction against different biotic anxieties. Traditional raising methods and genetic planning procedures like as marker-helped assurance (MAS) and innate change have been used to achieve quality pyramiding in rice [2].

## Significance

There are key implications for agribusiness and food

security with the advancement of biotic pressure touchy rice groupings with work on quality pyramiding. A few significant arguments in Favor of the importance of this strategy are as follows [1,2]:

- I. **Increased resistance:** Strong resistance to the irregularities of varied biological weights is provided everywhere by high-quality pyramiding, which permits the blending of diverse barrier features. This strategy revolves around the general flexibility of rice crops and lessens the bet of yield disasters achieved by separated life structure outflows.
- II. **Less use of pesticides:** Rice lays that are receptive to biotic strains can through and through diminish the necessity for designed pesticides. This reduction is significant because it restricts the adverse consequences of pesticide use, which benefits human thriving, the climate, and property reasonableness.
- III. **Improved efficiency and quality:** Quality pyramiding adds to longer rice yields and further upgraded grain quality by moderating the impacts of biotic anxieties. Ranchers can accomplish higher monetary gains with fewer misfortunes because of infection or bug invasions.
- IV. **Climate versatility:** Change in the environment is supposed to raise the predominance and power of stresses particularly the biotic under way of rice. Biotic pressure open-minded assortments with improved quality pyramiding can assist ranchers with adjusting to changing natural circumstances and guarantee stable rice creation under testing environments.
- V. **Overall food security:** Rice is the focal nourishment for a large piece of the overall people, particularly in Asia. Making biotic strain liberal rice groupings with refreshed quality pyramiding adds to general food security by lessening yield occurrences and keeping a reliable hold of this earnest gather.

### Objective and research questions

To create a rice variety that can withstand biotic stress using an improved gene pyramiding technique.

Research Questions:

- What major biotic stressors have an impact on rice production?
- What particular genes are linked to resistance to various biotic stresses?
- How can gene pyramiding be improved to increase rice's ability to withstand biotic stress?
- What morphological and genetic traits does the resulting rice variety possess?
- How do the yield and resistance of the biotic stress-tolerant rice variety compare to those of the current varieties?

## Literature review

### Rice under biotic stress

About different biotic issues, which are brought about by weeds, sicknesses, and aggravations, rice is powerless. A portion of the run-of-the-mill biotic stressors influencing rice are [1]:

- a. **Bug irritations:** By benefiting from the leaves, stems, and grains of rice plants, nuisances, for example, natural-hued planthoppers, rice leaf coordinators, and rice stem drills can genuinely harm rice plants.
- b. **Diseases:** By influencing different plant parts, like leaves, stems, panicles, and roots, rice sicknesses, for example, bacterial scourge, influence, sheath revile, and rice tungro contamination, can bring about ominous yield results.
- c. **Weeds:** Weeds compete with rice plants for nutrients, moisture, and light, which stunts their growth and productivity. Common rice weeds include *Scirpus* spp., *Cyperus* spp., and *Echinochloa* spp.

### Impact on food security and yield

Biotic loads have a major impact on food security and rice yield:

- a) **Yield misfortunes:** Depending on the severity of the pressure and the rice variety's helplessness, biotic burdens can result in significant yield losses, ranging from 20% to 80%.
- b) **Diminished quality:** The attributes of rice grains can likewise be impacted by biotic tension, which can prompt a decrease in their reasonable worth and client worth.
- c) **Food Security:** A huge part of the total populace, particularly in Asia, relies generally upon rice for nourishment. Rice's moderateness and availability might raise doubt about biotic issues, which could affect many individuals' admittance to food [2].

### Normal replicating hardships and cutoff points

Standard replicating ways of managing foster rice plans liberal to biotic tension face a couple of challenges and obstacles:

- a. **Confounding genetic characteristics:** Biotic tension strength in rice is compelled by different characteristics, habitually with complex coordinated efforts. Recognizing and introgression all of the ideal characteristics through customary raising methodologies can be monotonous and testing.
- b. **Confined genetic assortment:** Rice germplasm may have a limited inherited assortment for explicit biotic tension impediment characteristics, making it trying to find suitable wellsprings of resistance.



- c. **Time and cost:** Conventional raising techniques require a couple of periods of crossing, assurance, and field primers, which can require a surprisingly long time and cause huge costs.
- d. **Contradiction:** Presenting qualities from remotely related species into rice might bring about hereditary inconsistencies and block the effective exchange of wanted attributes.
- e. **Limited concentration:** Conventional reproducing approaches frequently center around working on each or a couple of qualities in turn, which may not give thorough and tough opposition against different biotic burdens.

These difficulties feature the requirement for upgraded approaches, for example, quality pyramiding, to proficiently and successfully foster biotic pressure open-minded rice assortments with further developed flexibility and better return potential [2].

### Quality pyramiding: Guidelines and advancements

A single plant line is created by connecting several qualities associated with beneficial traits in a process known as “quality pyramiding.” Regarding rice’s ability to withstand biotic pressure, quality pyramiding aims to strengthen the plant’s ability to continuously combat diverse biotic stresses by stacking distinct obstructive qualities against different vermin or illnesses. The idea of quality pyramiding depends on the rule of consolidating correlative or synergistic qualities to accomplish a more extensive and sturdy obstruction. By stacking numerous opposition qualities, the probability of vermin or sicknesses defeating the plant’s safeguard systems is diminished, as it requires synchronous transformations in different qualities for effective microbe variation [3].

#### Benefits of stacking numerous obstruction qualities:

- I. **Enhanced solidness:** Stacking different opposition qualities gives a stronger obstruction against vermin or sicknesses contrasted with depending on a solitary quality. This decreases the gamble of microbe variation and forward leap.
- II. **Wider range of obstruction:** Quality pyramiding expands the insurance by combining traits that target different pests or illnesses, strengthening the plant against diverse biotic concerns.
- III. **Lessened choice tension:** By stacking different opposition characteristics, the determination strain on particular qualities can be lessened, making it harder for annoyances or microbes to proliferate and create obstruction.
- IV. **Synergistic impacts:** The combination of different blockage characteristics may result in synergistic effects, which could lead to a more sensible and effective defense response against biotic loads.

**Quality pyramiding: Subatomic markers:** Subatomic markers play a crucial role in quality pyramiding by supporting the identification and maintenance of specific attributes throughout the raising system. These markers are DNA groupings related to the presence or nonappearance of a specific quality or characteristic [3].

**Marker-Assisted Selection (MAS) strategies:** MAS is a strategy that uses sub-atomic markers to choose plants conveying explicit qualities or characteristics of interest. MAS can fundamentally speed up the raising framework by allowing reproducers to perceive and pick plants with wanted quality mixes at the beginning phases, without the requirement for tedious and asset-concentrated phenotypic assessment [4].

#### MAS strategies regularly utilized for quality pyramiding in rice incorporate:

- I. **Polymerase Chain Response (PCR):** PCR-based markers, for example, straightforward grouping rehashes (SSRs) and succession labeled destinations (STS) can be utilized to enhance explicit DNA areas related to target qualities. The presence or nonattendance of the quality is focused on not set in stone by the size or presence of PCR amplicons.
- II. **Single Nucleotide Polymorphism (SNP) markers:** SNP markers are progressively utilized because of their overflow and high-throughput genotyping abilities. They can be utilized to identify hereditary varieties related to target qualities.

### Genome altering devices for quality stacking

Headways in quality-altering abilities, like CRISPR-Cas9, give extra apparatuses to quality stacking in rice rearing. Genome altering permits exact alterations of the rice genome, including the addition, cancellation, or substitution of explicit qualities. Through the targeted and controlled use of CRISPR-Cas9, raisers can exhibit or modify characteristics associated with biotic pressure resistance. This invention presents the possibility of more accurate and skilled quality stacking, particularly when concentrating on attributes that are known to be capable of biotic pressure blockage [5]. Utilizing genome-altering devices, MAS techniques, and subatomic markers, breeders can improve biotic pressure-lenient rice varieties with enhanced resistance to various pests and contaminations, streamline the selection process, and accelerate the quality pyramiding process.

## Methodology

### Plant material

The recurrent parent was chosen as Tellahamsa, a well-modified and well-liked rice variety tolerant to cold, which was released in 1968. The donor parents were NLR 145 (Swarnamukhi, Pi54, and Pi1) and Better-quality Samba Mahsuri (ISM, Xa21, and xa13) [4].

### Gene ID and determination

The bioinformatics apparatuses Impact and Phytozome and Rice Genome Explanation Venture Data set (RGAP) data sets are used to distinguish and choose qualities that have been displayed to present resistance to explicit biotic burdens in rice. Qualities were considered from both rice and other related species that show potential for upgrading biotic pressure resistance.

### Gene pyramiding technique

An improved quality pyramiding system was intended to join different qualities related to biotic pressure resilience. Factors thought about like quality connection, similarity, and articulation levels to expand the synergistic impacts of the chosen qualities, as displayed in Figure 1.

### Genetic alteration

The genetic alteration was performed to bring the chosen qualities into the objective rice assortment. Laid-out change conventions and procedures were utilized to guarantee fruitful quality exchange and joining. 3.5 MABB and a crossing strategy for the introduction of the Bacterial blight genes and blast genes into the Tellahamsa.

All four genes were introgressed into a single genetic background simultaneously using a step-wise back cross-transfer technique [5]. To create resistant lines, Tellahamsa was used by way of the female and 2 benefactor guardians (ISM and NLR145) as per the folks in two separate back-crossing suites called Tellahamsa ISM (Cross-I) and NLR145 (Cross-II). Figure 2 diagrams the marker backcrossed raising (MABB) framework used in the survey. With the help of front-facing region markers, the "substantial" F1 half and parts from the two crosses were recognized, and they were then backcrossed through Tellahamsa to convey BC1F1s [6]. These BC1F1s were then tried for the presence of opposition qualities, explicitly Cross I- Xa21 and Xa13 and Cross II- Pi54 and Pi1, the two of which were heterozygous. Yet again to make BC2F1s, which were then screened involving closer view atomic markers as recently referenced, +ve BC1F1 lines were backcrossed

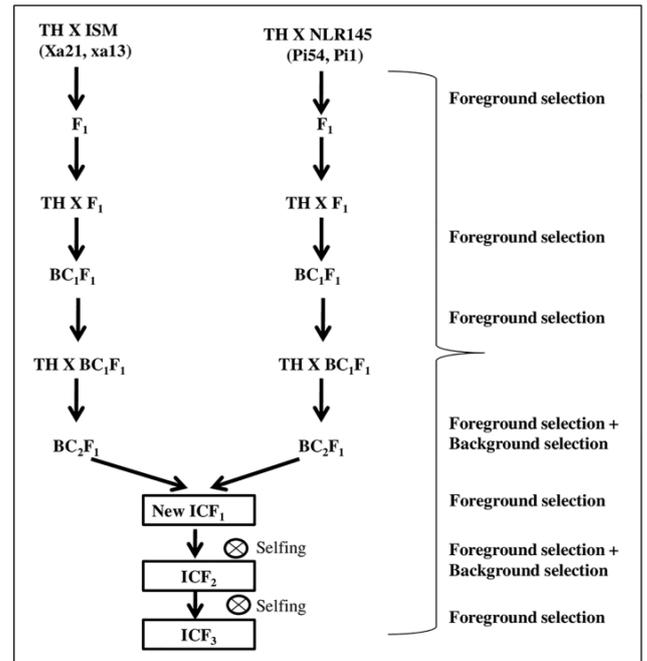


Figure 2: The study's marker-assisted backcrossing strategy [3].

by Tellahamsa. With the guide of a bunch of polymorphic markers-SSR, two separate BC2F1 lines from each cross were found to have Cross I- Xa21 and Xa13 and Cross II- Pi54 and Pi1 in heterozygous state with the most extreme genome of Tellahamsa recuperation. To incorporate the obstruction qualities Xa21, Xa13, Pi54, and Pi1 into a solitary plant, lines were crossed to make intercross ICF1 (F1s). The opposition qualities explicit/connected markers (closer view markers) were used to screen for "true" ICF1 hybrids, which were then selfed for 2 generations to produce ICF3 [7].

### Evaluating BB resistance

The chosen 9 ICF2 lines conveying BB opposition qualities were brought into the principal field with a separating of 15 x 20 cm and vaccinated with a bacterial suspension culture of 108-9cfu/ml containing two of the most harmful detaches of Xanthomonas oryzae pv. On the other hand, five youthful leaves from each plant were immunized utilizing the leaf-cutting technique for vaccination [8] and the sickness response was noted 22 days after immunization utilizing together visual slashing and the estimation of injury length under the IRR's 1996 Standard Evaluation System (SES) scale [6].

### Evaluating blast resistance

In Rabi, 3 highly virulent isolates of NLR-1 (Hyderabad), ID-14 (Nellore), and Magnaporthe oryzae (IB-16 (Maruteru) were used for artificial rice blast screening in 9 ICF2 lines along with their parents at three important Andhra Pradesh and Telangana state locations Regional Agricultural Research Station (RARS), N Each test entry was sewn in a single row that was 51 cm long and spaced 10 cm apart b/w rows. The susceptible checked (HR12) was propagated around the nursery and after every 10 test entries to ensure that blast disease propagated uniformly to the experimental material.

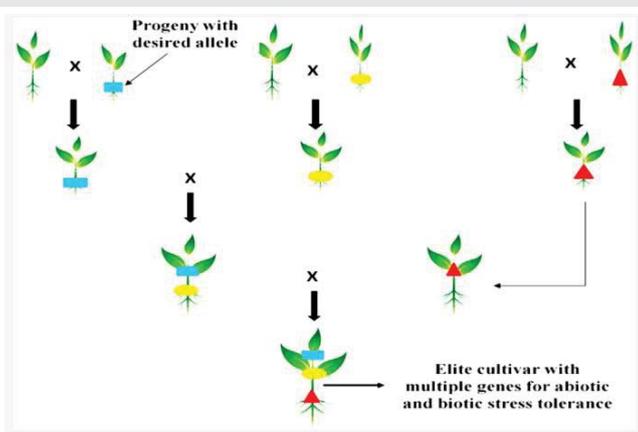


Figure 1: Framework of gene pyramiding for working on economical yields [10].

The normal protocol was followed for cultivating and storing the aforementioned pathogen strains [8]. To advance the improvement of the disease, the youthful seedlings at the 4-leaf phase were tainted by the parasitic conidial suspension at a centralization of  $1 \times 10^5$  spores/ml. Multi week after being vaccinated, immunized seedlings were noticed for the rise of impact sores, furthermore, the plants were evaluated on a 0-10 scale according to IRRI-SES [5].

### Data analysis

The Graphical Genotype (GGT) Version 2.0 software tool [9] was used to analyze the marker data and gauge the extent of repetitive parent chromosomal portion recuperation in the chosen backcrossed population segregants. The replicated agronomic information was exposed to an ANOVA using the statistics program GenStat (<http://www.biosci.global/software/genstat/>).

## Results and discussion

Marker-Helped Back Crossing (MABC) and thorough screening of aggregate were utilized to upgrade the protection from cold early developing rice collection Tellahamsa's protection from BB and impact illness [10]. The ongoing review analyzed the Tellahamsa ICF3 lines that have been introgressed with 2 qualities of protection from BB (Xa21 and Xa13) and 2 qualities of protection from impact (Pi54 and Pi1).

### Heterozygosity including the quality unequivocal front-facing region markers pTA248 for Xa21 and xa13 for Xa13

Tellahamsa x ISM (Cross-I) was used to make 60 F1 combinations. These half and parts were then pursued for heterozygosity including the quality express nearer view Xa21 and xa13 for markers pTA248 and Xa13 for prom. This method brought about the presentation of BB obstruction qualities Cross I- Xa21 and Xa13 into Tellahamsa. Yet again backcrossing the picked heterozygous F1 mixtures to repetitive guardians created 204 BC1F1 lines, which were established utilizing similar quality explicit frontal area markers. To make BC2F1 lines, a sum of 24 heterozygous lines for the Xa21 and Xa13 qualities were picked given their agro-morphological closeness to the repetitive parent. Six of the 102 BC2F1 lines that were made exhibited the +nce of the Xa21 and Xa13 qualities in heterozygous structure. Following the establishment decision using sixty polymorphic SSR markers, these plants showed the presence of an ordinary 90.2% discontinuous parent genome, going from 89% to 95%. Two BC2F1 lines (TH-BC2F1-16 and #TH-BC2F1-196) [11] with the most raised RPG recovery (92% and 95%, independently) were found and used for intercrossing out of the total of 6 lines.

### Blast-resistant genes (Pi54 and Pi1) were introduced into the Tellahamsa (Cross-II) cell line

Tellahamsa x NLR145 (Cross-II) created 125 F1 half and halves altogether, and 50 of those not set in stone to be "valid" F1s given the discoveries of PCR involving the quality explicit marker Pi54MAS for Pi54 and the quality connected marker RM 224 for Pi1 (Table 1) [8]. To make 200 BC1F1 lines, F1 half-breeds

**Table 1:** Pi54 and Pi1 are two important primers for identifying blast resistance [8].

Resistance gene	Chr.	Marker Name	Primer seq. for gene identification	Expected size (bp)
Pi-54	11	Pi-54 MAS	CAATCTCCAAAGTTTTCAGG-F GCTTCAATCACTGCTAGACC-R	200
Pi1	11	RM224	ATCGATCGATCTTCACGAGG-F TGCTATAAAAAGGCATTCGGG-R	130

were backcrossed to their intermittent guardians. To make 152 BC2F1 lines, a sum of 28 heterozygous lines for the Pi54 and Pi1 qualities were picked in light of their agro-morphological comparability to the repetitive parent. Thirteen BC2F1 lines — out of the 152 that were tried — presented the Pi54 and Pi1 in a heterozygous structure. These 13 BC2F1 lines were then submitted to foundation determination utilizing sixty polymorphic SSR markers. In the ongoing review, repetitive parent genome recuperation went from 89% to 95%, with a normal of 90.6%. Out of 15 lines, two lines (TH-BC2F1-6 and #THBC2F1-105) with the most noteworthy RPG recuperation and the best phenotypic resemblance to the recurrent parent Tellahamsa (93 and 94%, respectively) were found and used for intercrossing.

### BB and blast resistance genes pyramiding

The intermingling converges into a pyramid of the Xa21, xa13, Pi54, and Pi1 qualities, the line TH-BC2F1-196 (cross-I) was utilized as the female parent, and TH-BC2F1-105 (cross-II) as the male parent. Out of 154 ICF1 lines, 3 lines (Xa21+Xa13 + Pi54 + Pi1) were viewed as heterozygous for all of the objective qualities. Given DFF (days to half blooming), plant level, and grain type qualities like Tellahamsa, one intercross F1 cross breed (twentieth mixture) was browsed at this gathering. It was then selfed to create 1012 ICF2 lines. These 1012 ICF2 lines went through forefront determination to find the lines conveying a blend of two, three, or four qualities utilizing the proper closer view markers. Nine homozygous lines with different quality mixes were tracked down involving a closer view and phenotypic determination for agro-morphological attributes [12]. One plant had the Xa21+Pi54+Pi1 quality blend, one plant had the Xa21+xa13+Pi1 quality mix, 4 lines had Xa21 + xa13 + Pi54 + Pi1, three lines had Xa21 + xa13 + Pi54, and three lines had Xa21 + xa13 + Pi54. Foundation examination was finished on the 9 lines that were picked because they had polymorphic SSR markers that were heterozygous in the BC2F1 age. Given the greatest repetitive parent genome recuperation and adequate phenotypic characteristics, 2 lines — TH-625-159 (95.2%) and TH-625-491 (96.3%), were picked.

## Conclusion

The goal of the current work was to introduce the blast (Pi54 + Pi1) and BB (Xa21 + Xa13) resistance genes away from plain sight of the tolerance against cold, super rice assortment Tellahamsa. The results of the current study were two upgraded ICF3 lines with > 95% RPG, BB, and blast resistance. All in all, the improvement of stress opposition particularly biotic, rice combinations through upgraded quality pyramiding is a huge headway in farming biotechnology. This approach gives a useful asset to battle various biotic burdens all the while,



guaranteeing feasible rice creation, decreasing pesticide use, and improving worldwide food security.

### Hardships of value pyramiding for yield improvement

- **Innate multifaceted design:** Recognizing and joining various characteristics for pyramiding can be a result of the unpredictability of the inherited reason for needed credits. Understanding quality affiliations and likeness is critical for productive pyramiding.
- **Confined innate resources:** The openness of various genetic resources with accommodating characteristics may be limited, making it trying to find fitting characteristics for pyramiding. Getting to and introducing characteristics from related species or wild relatives can help with overcoming this obstruction.
- **Hereditary linkage drag:** Qualities of interest might be hereditarily connected to ominous or unfortunate attributes. During the pyramiding system, unwanted qualities may likewise be unintentionally moved, requiring extra adjustments of reproducing to kill them.
- **Time and asset escalated:** Quality pyramiding requires different rounds of crossing, determination, and assessment to accomplish the ideal quality blends. This cycle can be tedious, asset-concentrated, and work-escalated.
- **Administrative and acknowledgment difficulties:** Administrative endorsements for hereditarily adjusted harvests and public acknowledgment of hereditarily altered organic entities (GMOs) can introduce difficulties for quality pyramiding, especially when it includes hereditary designing strategies.
- Tending to these difficulties requires an extensive comprehension of the objective qualities, cautious choice of parental lines, productive reproducing systems, and powerful joining of sub-atomic devices and procedures, for example, marker-helped determination and genome altering. Defeating these difficulties can open the maximum capacity of quality pyramiding, which is used for improving harvests, and add to manageable agrarian practices [13,14].

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