

WRKY45 mediated immune priming confers durable *Magnaporthe oryzae* resistance without yield penalty in the rice mutant Pooya

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Research Article

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**WRKY45 mediated immune priming confers durable *Magnaporthe oryzae* resistance
without yield penalty in the rice mutant Pooya**

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Abstract

Rice blast, caused by the hemibiotrophic fungus *Magnaporthe oryzae*, remains a primary threat to global rice production and food security. While many resistance genes offer protection, their efficacy is often limited by race-specificity or detrimental growth-defense trade-offs that reduce grain yield. This study investigates the physiological and biochemical basis of enhanced resistance in Pooya, a blast-resistant mutant cultivar developed via mutation breeding from the susceptible Iranian cultivar Moosatarom. Following inoculation with various *M. oryzae* isolates, Pooya exhibited robust, broad-spectrum resistance without significant yield loss, suggesting an absence of fitness costs. Mechanistically, the mutant showed accelerated accumulation of hydrogen peroxide (H₂O₂) during early infection, signaling rapid oxidative-mediated defense activation. Furthermore, Pooya demonstrated sustained increases in peroxidase (POD) activity and consistently elevated phenylalanine ammonia-lyase (PAL) activity, facilitating enhanced lignification for cell wall reinforcement. Unlike the susceptible parent, Pooya maintained stable SPAD chlorophyll indices, indicating preserved photosynthetic capacity under pathogen pressure. Molecular analysis revealed that the mutant maintains constitutively higher expression levels of the transcription factor WRKY45, suggesting an immune-primed state compared to the delayed induction observed in Moosatarom. Collectively, our results indicate that the durable resistance in Pooya is driven by the coordinated integration of ROS signaling, phenylpropanoid metabolism, and *WRKY45*-mediated transcriptional regulation, all while preserving agronomic productivity. These findings provide essential molecular and physiological markers for breeding high-yielding rice varieties with durable blast resistance.

Keywords: *Magnaporthe oryzae*; broad-spectrum resistance; growth-defense trade-off; lignification; rice; *WRKY45*

Introduction

The development of broad-spectrum disease resistance has become a major objective in modern rice breeding because it provides protection against multiple pathogen species or a wide range of races and isolates within a single pathogen population [1, 2]. Unlike race-specific resistance, which is frequently rendered ineffective by the rapid evolution of virulent pathogen populations, broad-spectrum resistance generally exhibits greater durability and stability under field conditions. Consequently, it represents an essential component of sustainable crop production by reducing reliance on chemical pesticides, lowering production costs, and minimizing adverse environmental impacts. Considering that rice serves as the primary food source for more than half of the global population, the development of cultivars with durable resistance is not only critical for agricultural productivity but also for global food security [3]. Therefore, elucidating the genetic mechanisms responsible for broad-spectrum resistance has become a central focus in plant pathology and rice improvement programs.

Plants rely on a multilayered immune system to defend themselves against invading pathogens. The first layer, pattern-triggered immunity (PTI), also referred to as basal immunity, is activated through the recognition of conserved pathogen-associated molecular patterns. A second and generally stronger layer, effector-triggered immunity (ETI), is mediated primarily by disease resistance (R) genes. ETI is initiated when intracellular immune receptors, predominantly nucleotide-binding leucine-rich repeat (NLR) proteins, directly or indirectly recognize pathogen-derived effector molecules. Activation of these receptors triggers a cascade of defense responses, including calcium influx, rapid accumulation of reactive oxygen species (ROS), activation of mitogen-activated protein kinase (MAPK) signaling pathways, biosynthesis of defense-related

phytohormones such as salicylic acid, and transcriptional induction of pathogenesis-related genes [4, 5]. Together, these coordinated responses effectively suppress pathogen invasion and proliferation, ultimately determining disease outcome. Significant advances over the past two decades in the isolation and functional characterization of rice R genes have substantially improved our understanding of the molecular basis of disease resistance while providing valuable genetic resources for resistance breeding [6].

Rice blast, caused by the hemibiotrophic fungus *Magnaporthe oryzae*, remains the most devastating disease affecting rice production worldwide, with yield reductions commonly ranging from 10% to 35% during severe epidemics [7, 8]. The remarkable evolutionary capacity and genetic variability of this pathogen enable it to rapidly overcome resistance deployed in commercial cultivars, thereby limiting the durability of many resistance genes. To date, nearly 100 blast resistance genes or allelic variants have been identified in rice, of which more than 30 have been successfully cloned and functionally characterized. Several of these genes, including *Pi9*, *Pigm*, *Pi50*, *pi21*, *Pi7*, *Pi57*, and *Ptr*, have been demonstrated to confer broad-spectrum resistance against diverse *M. oryzae* isolates [9, 10]. These loci have become valuable genetic resources for breeding programs aimed at improving blast resistance in elite yet susceptible rice cultivars. Nevertheless, despite these achievements, many R genes continue to exhibit race-specific recognition and consequently lose effectiveness as new virulent pathogen populations emerge, restricting their long-term application in rice production.

In addition to classical resistance genes, increasing attention has recently been directed toward a distinct category of resistance-associated genes known as defense-regulator (DR) genes. Unlike conventional R genes, DR genes generally do not function through direct recognition of pathogen

effectors. Instead, they modulate essential immune signaling pathways and regulatory networks, thereby providing partial but quantitatively inherited resistance that is often effective against a broad range of pathogen races. Owing to their regulatory mode of action, these genes are considered promising targets for developing durable disease resistance in crop breeding programs. Numerous DR genes and resistance-associated regulators have been identified in rice, including both positive and negative modulators of blast resistance. Many of these genes encode transcription factors, protein kinases, or regulatory microRNAs. Among them, *bsr-d1*, *bsr-kl*, *spl11*, *spl33*, and *OsBB11* have been demonstrated to confer broad-spectrum resistance against rice blast [9–10]. Their importance arises from their ability to regulate immune signaling and transcriptional networks rather than relying on direct pathogen recognition, which contributes to more durable resistance across genetically diverse populations of *Magnaporthe oryzae*.

Despite the considerable benefits of enhanced disease resistance, constitutive activation of plant immune responses frequently imposes a substantial fitness cost. Excessive investment in defense can negatively affect plant growth, biomass accumulation, and grain production, resulting in the well-recognized growth–defense trade-off [11, 12]. Consequently, resistance genes that maintain effective immunity without adversely affecting agronomic performance are particularly valuable for crop improvement. Among the currently characterized genes, *bsr-d1*, *bsr-kl*, and *IPAI* represent notable examples because they provide broad-spectrum blast resistance while exerting little or no negative impact on yield-related traits [9].

The molecular mechanism underlying *bsr-kl* has been investigated in detail. This gene encodes a tetratricopeptide repeat (TPR)-containing protein that binds the messenger RNAs of *OsPAL1*–*OsPAL7* and promotes their degradation in the cytoplasm. Mutation or loss of *bsr-kl* function

results in the accumulation of *OsPAL* transcripts, leading to enhanced lignin biosynthesis and increased resistance to *M. oryzae*. Because lignin reinforces the structural integrity of plant cell walls, its increased deposition forms an effective physical barrier that restricts pathogen penetration and subsequent colonization [13]. A different regulatory mechanism has been described for *bsr-d1*. During pathogen infection, the transcriptional repressor MYBS1 binds to the *bsr-d1* promoter and suppresses its expression. Reduced activity of *bsr-d1* subsequently alters the transcription of peroxidase genes, decreases hydrogen peroxide scavenging, and promotes the accumulation of H₂O₂, thereby strengthening blast resistance. Thus, *bsr-d1* represents a transcriptional regulator that enhances broad-spectrum resistance through modulation of peroxidase-mediated reactive oxygen species metabolism rather than through direct pathogen recognition [14]. An additional breakthrough was reported by Wang et al. [15], who demonstrated that *Ideal Plant Architecture 1* (*IPAI*) simultaneously regulates grain yield and disease resistance. Under normal growth conditions, the non-phosphorylated form of *IPAI* promotes an ideal plant architecture associated with high productivity. Following infection by *M. oryzae*, however, *IPAI* becomes phosphorylated at Ser163 within its DNA-binding domain, resulting in a change in its DNA-binding specificity. The phosphorylated protein directly binds the promoter of the defense-related transcription factor *WRKY45*, activates its expression, and consequently enhances blast resistance. This mechanism illustrates how a single developmental regulator can be rapidly redirected toward immune activation in response to pathogen attack [15]. Collectively, the genes *bsr-k1*, *bsr-d1*, and *IPAI* establish robust, broad-spectrum immunity against rice blast by targeting essential defense pathways (*OsPALs*, *Peroxidase*, H₂O₂ and *WRKY45*), while simultaneously maintaining stable crop productivity.

Increasing evidence suggests that enhanced lignin deposition together with the maintenance of photosynthetic performance are two important characteristics associated with durable disease resistance that incurs little or no fitness penalty. As one of the principal constituents of the secondary cell wall, lignin reinforces cell wall structure, thereby creating a stronger physical barrier against pathogen penetration and restricting pathogen colonization within host tissues. Enhanced lignification is closely associated with activation of the phenylpropanoid biosynthetic pathway and has been widely implicated in broad-spectrum resistance to numerous plant pathogens [16-18]. Besides structural reinforcement, the capacity to sustain photosynthetic activity during pathogen challenge is increasingly recognized as an essential indicator of an optimal balance between plant growth and immune responses. Excessive activation of defense pathways often accelerates chlorophyll degradation and suppresses photosynthetic efficiency, ultimately leading to reduced biomass accumulation and grain yield. The Soil Plant Analysis Development (SPAD) index, a rapid and non-destructive estimate of leaf chlorophyll concentration, is therefore widely employed to evaluate photosynthetic capacity and overall plant physiological status. Resistant genotypes capable of maintaining relatively high SPAD values under disease pressure are considered to preserve carbon assimilation while simultaneously activating effective defense responses [19, 20]. Accordingly, lignin accumulation and SPAD values may serve as complementary physiological indicators linking durable broad-spectrum resistance with the absence of significant yield penalties and may provide valuable insight into the mechanisms coordinating defense responses with agronomic performance.

Rice mutants that exhibit broad-spectrum blast resistance without compromising yield represent particularly valuable genetic materials for both functional studies and breeding programs. In the present study, the broad-spectrum blast-resistant rice mutant 'Pooya' was compared with its

parental cultivar 'Moosatarom' to elucidate the mechanisms responsible for resistance without an associated yield penalty. To achieve this objective, several physiological and molecular parameters closely related to plant defense were evaluated, including the activities of phenylalanine ammonia-lyase (PAL) and peroxidase (POD), lignin accumulation, hydrogen peroxide (H₂O₂) content, SPAD values as an indicator of chlorophyll content and photosynthetic performance, and the expression of the defense-related transcription factor WRKY45. By integrating these biochemical and molecular analyses, this study aims to provide a more comprehensive understanding of the mechanisms underlying durable broad-spectrum blast resistance and to clarify how effective immune activation can be achieved while preserving agronomic performance.

Materials and Methods

Plant Materials

Two rice (*Oryza sativa* L.) genotypes were used in this study: the mutant cultivar Pooya and its parental cultivar Moosatarom. The mutant line was developed through gamma-ray mutagenesis with the aim of improving agronomic traits including early maturity, lodging resistance, and resistance to rice blast disease. The mutant was generated in 1997 and was subsequently registered by the Joint FAO/IAEA Programme as a mutant rice variety in 2004 [21]. Seeds of both cultivars were obtained from the Rice Research Institute of Iran (RRII), Amol, Mazandaran Province, Iran.

Assessment of Agronomic Performance

Field experiments were conducted during two consecutive growing seasons in paddy fields located in Babol and Ghaemshahr, Mazandaran Province, Iran. Standard agronomic practices

recommended for rice cultivation were followed throughout the growing period. Grain yield was recorded at maturity, and the average yield across locations and years was calculated for each genotype.

Evaluation of Rice Blast Resistance

Resistance to rice blast disease caused by *Magnaporthe oryzae* was evaluated under controlled greenhouse conditions. Three virulent Iranian isolates of *M. oryzae* representing blast pathogen populations from northern rice-growing regions of Iran were used for inoculation, including IA-81, IC-25, and IF-1. These isolates have been reported among pathogenic populations associated with rice fields in the Caspian rice-growing areas of Iran, including Guilan and Mazandaran provinces [22]. The fungal isolates were maintained on oatmeal agar (OMA) medium at 25°C under continuous illumination for 10-14 days to promote sporulation. Conidial suspensions were prepared by gently washing sporulating cultures with sterile distilled water containing 0.02% Tween-20. The suspensions were filtered through sterile gauze, and the conidial concentration was adjusted to 1×10^5 conidia/mL as determined using a hemocytometer. Rice seeds were surface sterilized with 1% sodium hypochlorite, rinsed thoroughly with sterile distilled water, and sown in plastic pots containing a mixture of sterilized soil and compost. Seedlings were grown under greenhouse conditions (25-28° C, 70-80% relative humidity) until the four-leaf stage. For inoculation, conidial suspensions were uniformly sprayed onto the foliage until complete wetting of the leaves was achieved. After inoculation, plants were maintained in a humid chamber (>90% relative humidity) at 25 °C for 24 h to facilitate fungal penetration and infection, and were subsequently transferred to greenhouse conditions. Disease severity was assessed 7 days after inoculation based on lesion development and sporulation according to the International Rice Research Institute (IRRI) Standard Evaluation System for Rice [23].

Experimental Design and Sampling

To investigate physiological and molecular responses associated with blast resistance, a time-course experiment was conducted. Seedlings of both cultivars were divided into two groups: control (mock-inoculated) and *M. oryzae*-inoculated plants at the four-leaf stage. Leaf samples were collected from both control and inoculated plants at 1, 2, 3, 4, and 5 days post inoculation (dpi). At each sampling time, the uppermost fully expanded leaves were harvested for determination of hydrogen peroxide (H₂O₂) content, peroxidase (POD) activity, phenylalanine ammonia-lyase (PAL) activity, lignin content, and expression analysis of the *WRKY45* gene. Chlorophyll status was assessed using SPAD measurements immediately before leaf sampling.

Determination of Hydrogen Peroxide Content

Hydrogen peroxide content was measured according to the potassium iodide (KI) method described by Velikova et al. [24]. Briefly, 0.1 g of fresh leaf tissue was homogenized in 5 mL of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at $10,000 \times g$ for 15 min. Subsequently, 0.5 mL of the supernatant was mixed with 0.5 mL of 10 mM potassium phosphate buffer (pH 7.0) and 1 mL of 1 M KI solution. Absorbance was recorded at 390 nm using a UV-visible spectrophotometer. H₂O₂ concentration was calculated from a standard curve and expressed as $\mu\text{mol g}^{-1}$ fresh weight (FW).

Plant Extraction and Enzyme Assay

Fresh plant leaf samples were collected, immediately transferred into liquid nitrogen, and kept at -80 °C. Frozen leaf samples were homogenized in 100 mM KH₂PO₄/Na₂HPO₄ buffer solution containing 2 mM EDTA, 1% (m/V) PVP, and 1 mM phenylmethylsulfonyl fluoride (PMSF) with pH 7.0 (2 g fresh weight per 3 mL) at 0 °C and filtered through one layer of Miracloth. The filtered

extract was centrifuged at $12,000 \times g$ at $4\text{ }^{\circ}\text{C}$ for 15 min. The soluble protein content was determined by the method of Bradford [25] using bovine serum albumin as a standard.

Peroxidase (POD) Activity Assay

The activity of peroxidase (POD, EC 1.11.1.7) was evaluated based on the protocol described by Lin and Kao [26]. For this purpose, a reaction mixture was prepared consisting of 50 μL of 20 mM guaiacol, 2.8 mL of 10 mM phosphate buffer (at pH 7.0), and 0.1 mL of the prepared plant extract. The enzymatic reaction was triggered by introducing 20 μL of 40 mM H_2O_2 . Following this, the absorbance was monitored at 470 nm for a duration of 1 minute using a UV-visible spectrophotometer. To determine POD activity, the absorption coefficient of tetraguaiacol ($26.6\text{ mM}^{-1}\text{ cm}^{-1}$ at 470 nm) was utilized. One unit of POD activity was defined as the enzyme quantity necessary to produce 1 μmol of tetraguaiacol per minute at ambient temperature.

Phenylalanine Ammonia-Lyase (PAL) Activity Assay

Following the methodology of Dickerson et al. [27], the activity of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) was determined. The assay was conducted by incubating a mixture of 100 μL plant extract, 500 μL of 50 mM Tris-HCl (pH 8.8), and 600 μL of 1 mM L-phenylalanine for 60 minutes at room temperature. To halt the reaction, 2 N HCl was subsequently added. The reaction mixture was then extracted using 1.5 mL of toluene via 30 seconds of vortexing, followed by centrifugation at 1,000 rpm for 5 minutes to recover the toluene phase. The absorbance of the resulting toluene layer, which contained the trans-cinnamic acid, was measured at 290 nm against a toluene-only blank. Enzyme activity was quantified as the amount of trans-cinnamic acid produced (nmol) per minute per mg of protein.

Chlorophyll Content (SPAD) Measurement

Relative chlorophyll levels in the rice leaves were assessed using a portable SPAD-502 chlorophyll meter (Konica Minolta Co., Tokyo, Japan). This handheld device provides non-destructive measurements by analyzing light transmittance at 650 nm and 940 nm wavelengths. To ensure accuracy and consistency, measurements were taken from the upper, middle, and lower sections of the leaves, specifically targeting areas away from the leaf veins. The final SPAD value for each leaf was determined by averaging these readings.

Quantification of Total Lignin Content

To determine the total lignin concentration, 0.3 g of fresh leaf tissue was pulverized in liquid N₂, followed by five successive washes with 80% ethanol to eliminate soluble metabolites. The samples were then washed with acetone and dried in an oven. The acetyl bromide analysis was performed according to the method of Peng et al. [28]. Briefly, samples were incubated in a 4:1 (v/v) acetic acid to acetyl bromide mixture at 70 °C for 2 hours. After reaching room temperature, the reaction was terminated by transferring the samples into 50-mL volumetric flasks containing 2 M NaOH, acetic acid, and 7.5 M hydroxylamine hydrochloride. The final volume was adjusted with acetic acid, and the absorbance was recorded at 280 nm. Lignin levels were expressed as OD₂₈₀ mL⁻¹ g⁻¹ of fresh weight (FW).

RNA Extraction and Quantitative Real-Time PCR (qRT-PCR)

Total RNA was extracted from leaf samples using pBIOZOL reagent (BioFlux, Japan) according to the manufacturer's instructions. RNA integrity was evaluated by agarose gel electrophoresis, and RNA concentration and purity were determined using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). To eliminate genomic DNA contamination, extracted RNA was treated with RNase-free DNase I (Thermo Fisher Scientific, USA). First-strand cDNA synthesis was performed using 3 µg of DNase-treated RNA with the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) using oligo(dT)18 and random hexamer primers. Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green Master Mix (Applied Biosystems, USA) on an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, USA). Each reaction was performed in a final volume of 20 µL containing SYBR Green Master Mix, gene-specific primers, and diluted cDNA template. The amplification conditions consisted of an initial denaturation at 95 °C for 5 min, followed by 40 cycles of 95 °C for 10 s, 62 °C for 30 s, and 72 °C for 10 s. A melting curve analysis was conducted to confirm the specificity of the amplified products. The *Actin* gene was used as the endogenous reference gene for normalization of transcript levels based on its validated suitability for qRT-PCR analysis in rice under biotic stress conditions, and the expression level of the defense-related transcription factor OsWRKY45 was determined using gene-specific primers reported previously [29, 30]. The primer sequences used in this study are listed in Table 1. Relative transcript abundance was calculated using the comparative threshold cycle method ($2^{-\Delta\Delta C_t}$) according to Livak and Schmittgen [31]. Expression levels were normalized against Actin and expressed as fold changes relative to the non-inoculated control samples. Three biological replicates with three technical replicates were analyzed for each treatment.

Statistical Analysis

Each value was the mean of three independent biological experiments that contained eight leaves in each experiment, and standard deviations were given. The results were analyzed using the Student's *t-test*.

Results

Agronomic Performance and Yield Stability

The evaluation of grain yield showed that the mutant cultivar Pooya achieved a mean yield of $3,950 \pm 257 \text{ kg ha}^{-1}$, while the parental cultivar Moosatarom produced $4,217 \pm 184 \text{ kg ha}^{-1}$. Although the mutant line exhibited a slightly lower average grain yield, the difference between the two cultivars was not statistically significant ($P > 0.05$).

Disease Reaction and Broad-Spectrum Blast Resistance

Phenotypic assessment revealed that the parental cultivar Moosatarom was highly susceptible to *Magnaporthe oryzae*, whereas the mutant cultivar Pooya consistently displayed incompatible reactions against all pathogen isolates evaluated (Figure 1).

Reactive Oxygen Species and Peroxidase-Mediated Defense

Marked differences in the dynamics of reactive oxygen species (ROS) were detected between the two cultivars following infection. In the susceptible cultivar Moosatarom, hydrogen peroxide (H_2O_2) levels decreased during the initial stages of infection and increased only at later sampling times, reflecting a delayed oxidative response. By contrast, the mutant cultivar Pooya maintained significantly higher H_2O_2 concentrations throughout the infection period, with the greatest accumulation occurring during the first three days after inoculation (Figure 2).

The pattern of peroxidase (POD) activity further supported these findings. Although Moosatarom exhibited only a temporary increase in POD activity after inoculation, Pooya showed a continuous

rise in enzyme activity, reaching its highest levels between the fourth and fifth days following inoculation (Figure 3).

Activation of the Phenylpropanoid Pathway

A clear difference in phenylalanine ammonia-lyase (PAL) activity was observed between the two cultivars following inoculation. The susceptible cultivar Moosatarom showed little variation in PAL activity over the course of infection, whereas Pooya exhibited a continuous and pronounced increase in enzyme activity throughout the experimental period (Figure 4).

***WRKY45* Expression Indicates Immune Priming**

Analysis of gene expression revealed that *WRKY45* was among the most prominent molecular features distinguishing the resistant mutant Pooya from its susceptible parental cultivar, Moosatarom. In Moosatarom, a significant increase in *WRKY45* transcript levels was detected only after three days of inoculation, whereas Pooya maintained consistently high expression throughout the infection period (Figure 5).

Lignin Accumulation and Structural Defense Reinforcement

Following inoculation, Pooya exhibited a pronounced increase in lignin accumulation, with lignin content rising significantly during the early stages of infection and remaining high throughout the experimental period. In contrast, the susceptible cultivar Moosatarom showed only slight changes in lignin levels, indicating a comparatively weak induction of structural defense responses (Figure 6).

Maintenance of Photosynthetic Performance under Pathogen Stress

Measurements of chlorophyll content using SPAD indicated clear differences in the photosynthetic responses of the two cultivars following pathogen infection. In the susceptible cultivar Moosatarom, blast infection resulted in a significant decline in SPAD values, reflecting chlorophyll loss and a reduction in photosynthetic efficiency. In contrast, Pooya maintained relatively constant SPAD values throughout the experimental period, indicating that its photosynthetic apparatus remained largely unaffected by disease stress (Figure 7).

Discussion

The lack of a significant reduction in grain yield suggests that the induced mutation did not negatively affect the balance between carbon allocation for defense mechanisms and the carbon required for biomass accumulation and grain development. This finding is consistent with previous studies demonstrating that resistant crop genotypes can maintain high productivity while enhancing disease resistance through efficient metabolic regulation and reduced losses caused by pathogen infection [32]. The combination of stable grain yield and improved blast resistance observed in Pooya indicates that this mutant successfully maintains an effective balance between growth and defense. These results imply that the resistance mechanism in Pooya enables the plant to mount an efficient defense response while avoiding excessive metabolic costs, thereby reducing the conventional trade-off between disease resistance and crop productivity.

The consistent resistance observed in Pooya indicates the involvement of broad-spectrum defense mechanisms rather than isolate-specific resistance mediated by single resistance (R) genes. Such broad-spectrum resistance is generally regarded as more durable because it exerts lower selection

pressure on pathogen populations, thereby reducing the likelihood of resistance breakdown under field conditions [33]. The enhanced resistance of Pooya likely played a key role in preserving grain yield by restricting pathogen establishment and minimizing damage to photosynthetically active leaf tissues. Since severe blast infection can substantially reduce yield through accelerated leaf senescence, impaired photosynthetic carbon assimilation, and disruption of source–sink relationships, limiting pathogen growth is critical for sustaining crop productivity under disease pressure [34, 35]. Notably, the improved blast resistance in Pooya was achieved without a significant decline in grain yield, suggesting that its resistance is mediated by a tightly regulated defense system rather than constitutive defense activation. This regulated response likely provides effective protection against infection while minimizing metabolic costs and maintaining normal physiological functions.

Rapid ROS generation is a hallmark of pattern-triggered immunity (PTI), where these molecules function not only as antimicrobial agents but also as important signaling components that initiate downstream defense responses [36]. The early and sustained accumulation of H_2O_2 in Pooya likely promoted faster activation of defense pathways, enabling the plant to restrict fungal invasion before extensive colonization and tissue damage occurred. Such an early defense response is essential for maintaining grain yield because prolonged pathogen infection can accelerate chlorophyll degradation, increase cellular injury, impair carbon assimilation, and ultimately reduce the availability of assimilates for grain filling [34, 36]. Consequently, the elevated H_2O_2 levels observed during the early stages of infection in Pooya were likely instrumental in limiting pathogen establishment through rapid induction of defense signaling. Peroxidases play a central role in regulating ROS homeostasis while contributing to lignin biosynthesis, phenolic compound cross-linking, and reinforcement of the secondary cell wall [37, 38]. The prolonged elevation of

POD activity in Pooya suggests efficient regulation of oxidative metabolism, allowing ROS to act effectively as signaling molecules without causing excessive oxidative damage to host tissues. Moreover, enhanced peroxidase activity likely strengthened cell-wall integrity, thereby reducing fungal penetration and slowing disease development. This protection helped preserve the physiological functions necessary for normal plant growth and grain production. Collectively, the coordinated induction of early ROS accumulation and sustained POD activity appears to constitute an effective defense strategy in Pooya, providing strong resistance to pathogen invasion while minimizing the metabolic costs that are often associated with prolonged defense activation.

As the key enzyme catalyzing the first committed reaction of the phenylpropanoid pathway, PAL diverts carbon flux toward the production of lignin, flavonoids, diverse phenolic metabolites, and phytoalexins, all of which play important roles in plant defense [39]. The sustained induction of PAL in Pooya therefore suggests a greater capacity to rapidly activate phenylpropanoid metabolism in response to pathogen invasion. Despite the increased investment in defense-related metabolism, Pooya did not experience a significant reduction in grain yield. This finding indicates that carbon partitioning between defensive compound synthesis and primary growth processes remained well balanced, allowing sufficient resources to support both resistance and grain development. Such efficient metabolic regulation likely prevented the defense response from imposing a substantial fitness penalty. The phenylpropanoid pathway enhances disease resistance through multiple complementary mechanisms. In addition to strengthening cell walls via lignin accumulation, it promotes the synthesis of antimicrobial phenolic compounds that suppress pathogen growth and restrict infection [40]. In resistant genotypes, rapid activation of PAL-mediated defense can limit pathogen establishment during the initial stages of infection, thereby reducing both the severity and duration of disease. By minimizing pathogen-induced damage, this

response helps preserve photosynthetically active tissues and decreases the energetic demands associated with prolonged defense activation. These coordinated effects may explain why Pooya was able to maintain stable grain yield while exhibiting enhanced resistance to blast disease. The sustained elevation of PAL activity in Pooya reflects a substantial shift in metabolic activity toward the production of defense-related secondary metabolites. Nevertheless, this enhanced metabolic response was not accompanied by a decline in grain yield, indicating that the reallocation of resources was carefully regulated rather than resulting in excessive diversion of carbon from growth and reproduction. Such coordinated regulation of the phenylpropanoid pathway allows plants to accumulate structural and antimicrobial compounds essential for disease resistance while preserving adequate assimilate supply for reproductive growth and grain filling [40, 41].

In rice, WRKY45 functions as a key transcriptional regulator of salicylic acid-dependent immunity and controls the expression of numerous genes involved in ROS signaling, phenylpropanoid biosynthesis, and antimicrobial defense [42]. The sustained abundance of *WRKY45* transcripts in Pooya provides a plausible molecular basis for the coordinated activation of several defense mechanisms observed in this study, including enhanced H₂O₂ accumulation, increased PAL activity, and greater lignification. Immune priming enables plants to respond rapidly after pathogen recognition without maintaining defense mechanisms in a permanently activated state, thereby minimizing the energetic costs associated with constitutive immunity. Consistent with this concept, previous studies have identified WRKY transcription factors as central regulatory nodes that connect upstream immune signaling with the activation of downstream defense genes in rice [43, 44]. Notably, although Pooya exhibited higher *WRKY45* expression, this enhancement was not associated with a significant decrease in grain yield. This finding indicates that disease resistance in the mutant is more likely attributable to enhanced immune readiness than to

continuous activation of defense pathways. Such a regulatory strategy allows plants to preserve normal physiological functions and sustain resource allocation for growth and reproductive development when pathogen pressure is absent [45, 46]. Consequently, the elevated expression of *WRKY45* in Pooya likely facilitated faster activation of defense responses and more effective restriction of *Magnaporthe oryzae* during the early stages of infection.

Lignin deposition constitutes an important physical defense mechanism against pathogen invasion by reinforcing secondary cell walls, reducing fungal penetration, and restricting the spread of pathogens within host tissues [47, 48]. The greater lignin accumulation observed in Pooya likely enhanced its resistance to rice blast while simultaneously protecting the integrity of leaf tissues during infection. By strengthening cell walls and limiting lesion expansion, lignification helps preserve photosynthetically active tissues, thereby sustaining carbon assimilation and providing sufficient assimilates for grain filling. Consistent with previous reports, increased lignin deposition has frequently been associated with improved resistance to fungal pathogens through enhanced structural reinforcement of host tissues. The higher lignin content detected in Pooya was closely associated with the increased activities of PAL and POD observed in the same genotype. PAL supplies the phenylpropanoid intermediates required for lignin biosynthesis, whereas POD catalyzes the oxidative polymerization of lignin precursors into the cell wall matrix [49]. The concurrent enhancement of PAL activity, POD activity, and lignin accumulation indicates that metabolic and structural defense mechanisms were activated in a coordinated manner. Such coordination is particularly important because excessive carbon investment in cell-wall fortification may compromise plant growth and productivity. In Pooya, however, lignification appeared to be tightly regulated, enabling effective reinforcement of structural barriers without impairing biomass production or reproductive development [41, 50]. By restricting pathogen

invasion and reducing infection-induced tissue damage, enhanced lignin deposition likely preserved photosynthetic capacity and contributed to the maintenance of grain yield under blast disease pressure. Therefore, lignification in Pooya not only functioned as a key component of disease resistance but also helped explain how improved protection against *Magnaporthe oryzae* was achieved without a measurable penalty in agronomic performance.

Preserving photosynthetic activity during infection is essential for sustaining plant productivity because defense responses depend on a continuous supply of energy and assimilated carbon. When immune responses are excessively activated, they can divert resources away from primary metabolism, leading to reduced photosynthetic performance, lower biomass accumulation, and ultimately diminished grain yield. Since successful grain filling relies on uninterrupted carbon assimilation and effective source activity, maintaining chlorophyll content enables resistant plants to continue supplying photoassimilates to developing grains despite pathogen challenge. Previous studies have shown that prolonged or excessive activation of defense mechanisms may impair photosynthesis by increasing competition between defense-related metabolism and primary metabolic processes for carbon and energy resources [34, 35]. However, the stable SPAD values recorded in Pooya suggest that this mutant effectively coordinated immune responses with photosynthetic function. Instead of compromising carbon assimilation to sustain defense, Pooya appears to limit pathogen development rapidly, thereby shortening the duration of stress and minimizing its negative effects on physiological performance. This efficient integration of defense and photosynthetic maintenance likely contributed to the enhanced blast resistance and stable grain yield observed in the mutant cultivar.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest

The author declares that there is no conflict of interest

Ethical approval

All plant experiments, field studies and plant material collection were conducted in accordance with relevant institutional, national and international guidelines and legislation. This study did not involve any endangered or protected species.

Consent to publish

Not applicable

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Table 1. Primers used for quantitative real-time PCR (qRT-PCR).

Gene	GenBank accession No.	Primer sequence (5'–3')	Reference
<i>OsWRKY45</i>	AK066255	F: CGGGTAAAACGATCGAAAGA R: TTTCGAAAGCGGAAGAACAG	Shimono et al. (2007)
<i>Actin</i>	AB047313.1	F: CAGCCACACTGTCCCCATCTA R: AGCAAGGTCGAGACGAAGGA	Chen et al. (2020)

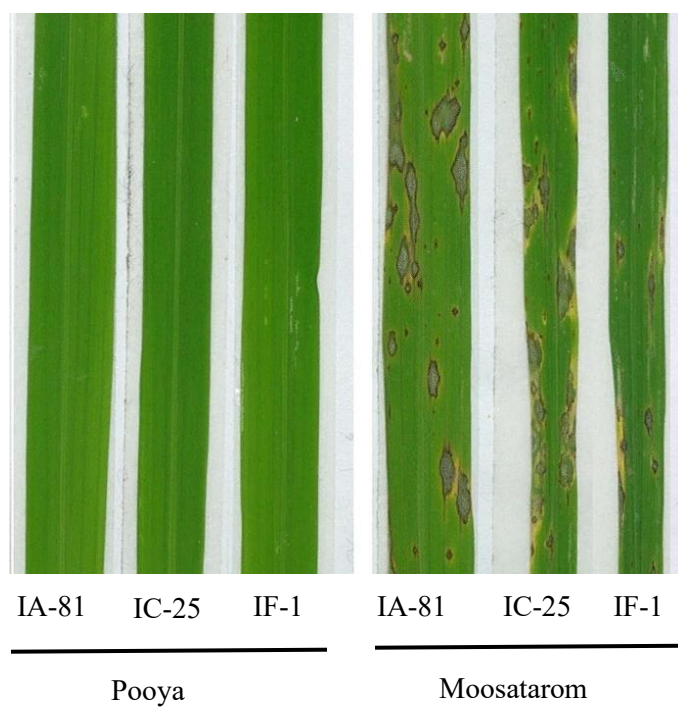


Fig 1. Blast disease severity seven days after inoculation with three isolates of *Magnaporthe oryzae* (IA-81, IC-25, and IF-1) in the rice cultivars Pooya and Moosatarom.

1

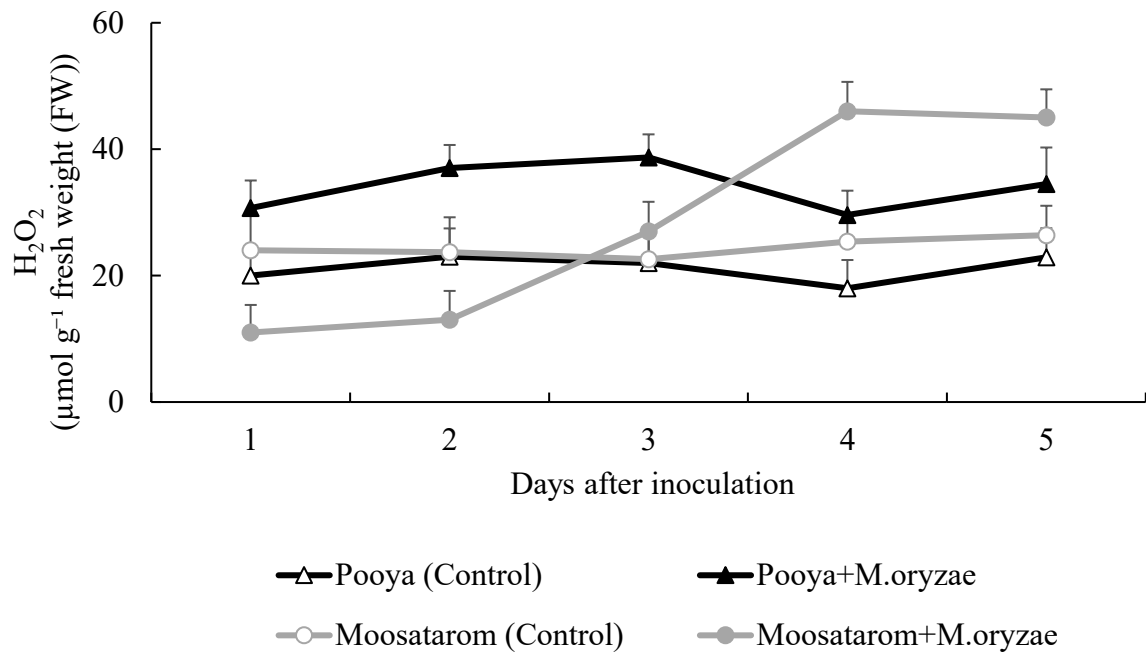


Fig 2. Hydrogen peroxide (H₂O₂) content in leaves of rice cultivars Pooya and Moosatarom under control and *Magnaporthe oryzae* infection conditions during 1-5 days after inoculation (DAI). The vertical lines represent $\pm$ SE of the mean of three replicates

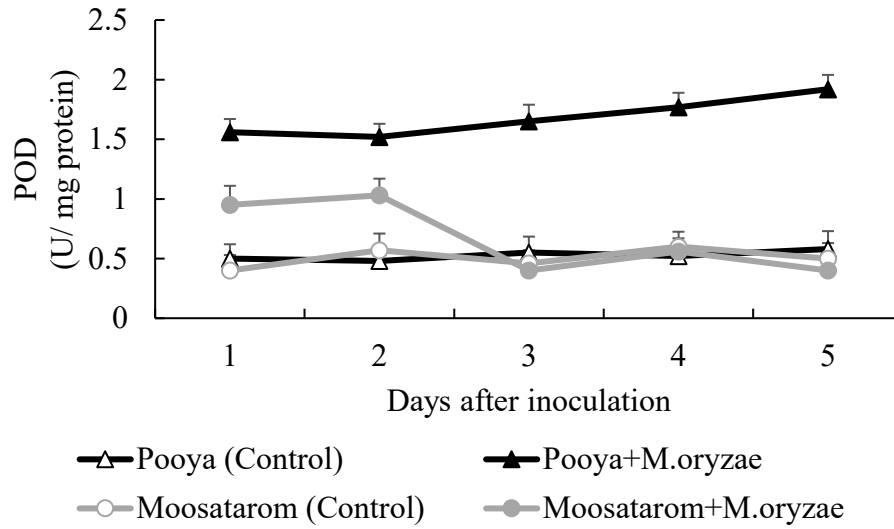


Fig 3. Peroxidase (POD) activity in leaves of rice cultivars Pooya and Moosatarom under control and *Magnaporthe oryzae* infection conditions during 1-5 days after inoculation (DAI). The vertical lines represent $\pm$ SE of the mean of three replicates

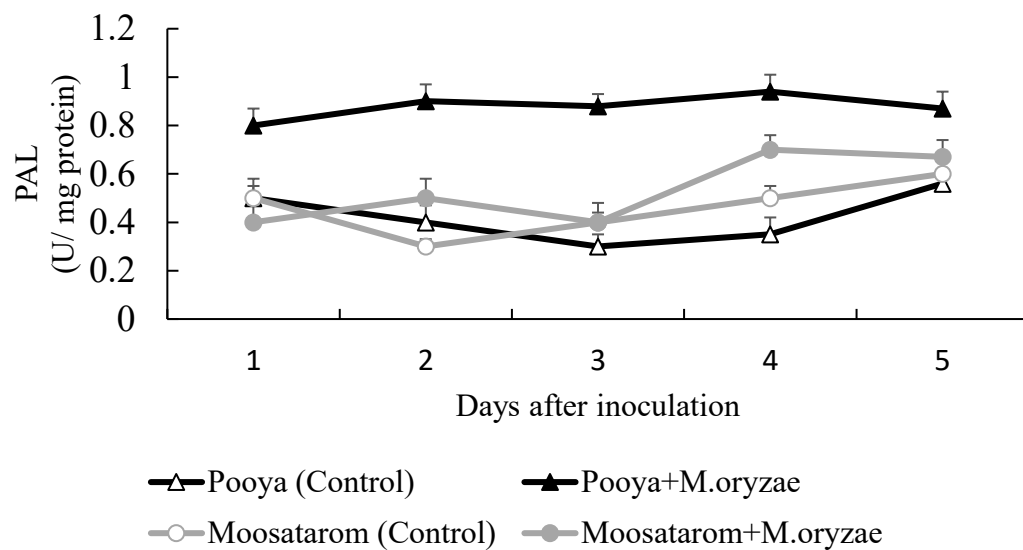


Fig 4. Phenylalanine ammonia-lyase (PAL) activity in leaves of rice cultivars Pooya and Moosatarom under control and *Magnaporthe oryzae* infection conditions during 1-5 days after inoculation (DAI). The vertical lines represent $\pm$ SE of the mean of three replicates

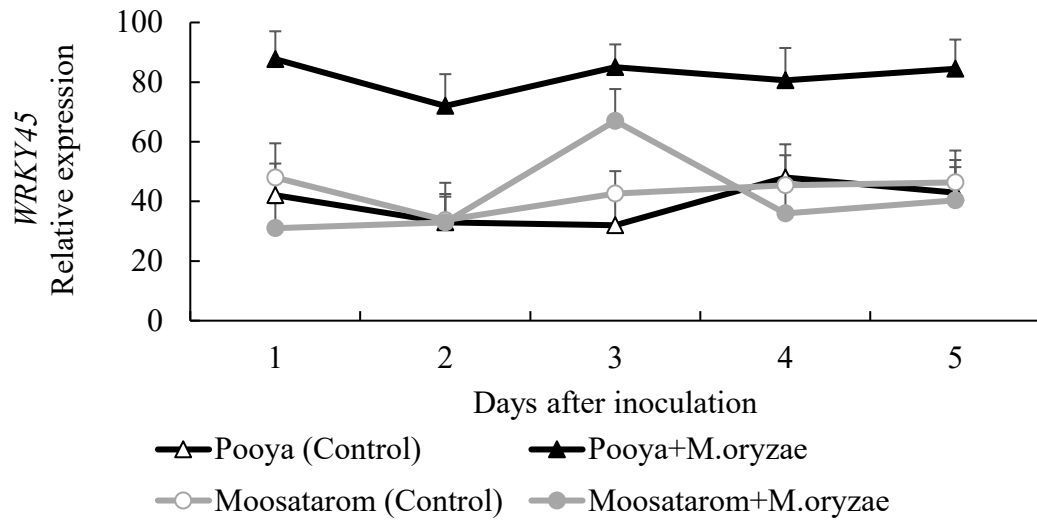


Fig 5. *WRKY45* transcript abundance in leaves of rice cultivars Pooya and Moosatarom under control and *Magnaporthe oryzae* infection conditions during 1-5 days after inoculation (DAI). The vertical lines represent $\pm$ SE of the mean of three replicates

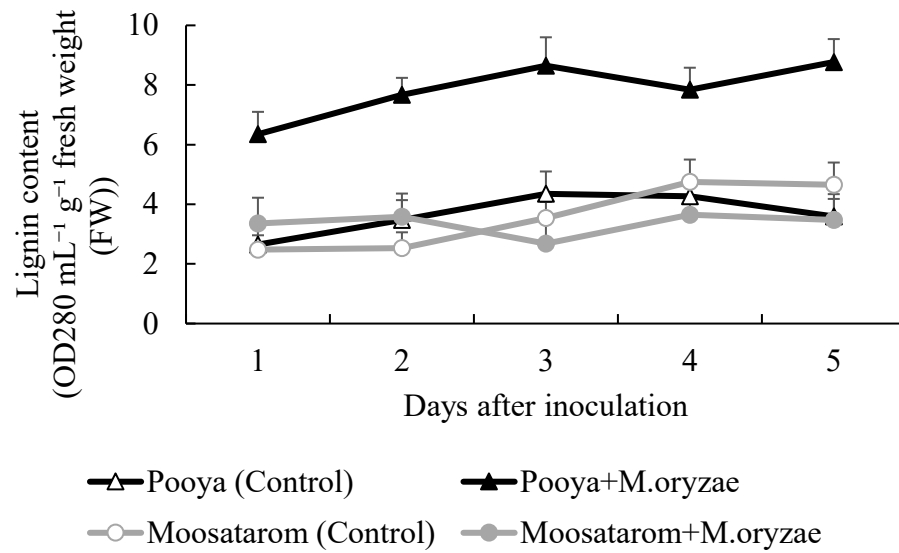


Fig 6. Lignin content in leaves of rice cultivars Pooya and Moosatarom under control and *Magnaporthe oryzae* infection conditions during 1-5 days after inoculation (DAI). The vertical lines represent $\pm$ SE of the mean of three replicates

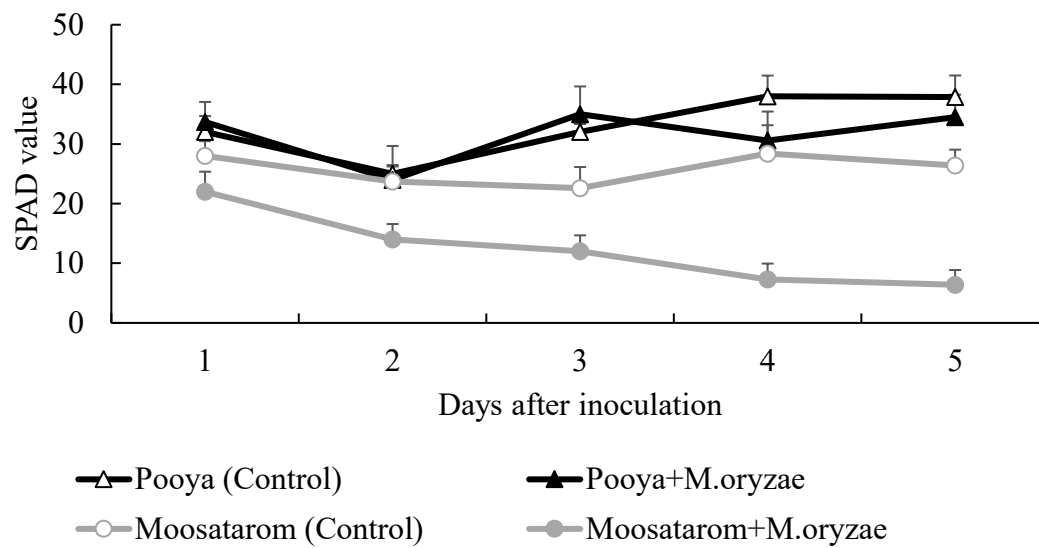


Fig 7. SPAD values in leaves of rice cultivars Pooya and Moosatarom under control and *Magnaporthe oryzae* infection conditions during 1-5 days after inoculation (DAI). The vertical lines represent $\pm$ SE of the mean of three replicates