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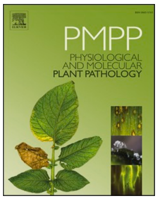

Assessment of morphological and genomic variation in *Attakkari* rice for selection of blast-resistant lines

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ABSTRACT

Traditional rice landraces remain vital for food security and cultural identity, yet their use in breeding is constrained by insufficient characterization. This study comprehensively evaluated *Attakkari,* a prominent local cultivar in Northern Sri Lanka, focusing on its genetic diversity, agronomic traits, and blast resistance potential. Farmer surveys (n = 119) revealed that *Attakkari* is highly valued for yield (2500–3150 kg/acre), favorable taste, grain quality, and strong local market demand, with 95.79 % reporting consistently high preference. Despite concerns of blast susceptibility noted by 65 % of farmers, cultivation persists due to adaptability and cultural significance. Morphological analysis of seed samples identified ten clusters based on grain traits, from which nine morphotypes were selected for further study. Image sensing using RGB and grayscale intensities differentiated subtle pericarp variations into four sub-groups. ITS sequencing confirmed high genetic similarity (98.5–100 %) among morphotypes, with some sequences showing 100 % similarity to *Oryza nivara* from South Korea, indicating possible contributions from wild progenitors. Screening for ten major blast resistance genes revealed considerable variation; notably, Att 44, Att 06, and Att 16 carried up to nine resistance genes (*Pita/Pita-2*, *Piz*, *Pizt*, *Pik*, *Pikh*, *Pib*, *Pikp*, *Pi1*, *Pi5*, *Pikm*). Field evaluations highlighted significant agronomic diversity, with Att 94, Att 44, and Att 116 exhibiting superior vigor, yield components, and grain weight compared to reference varieties (RY, RM, Co 10). Overall, this integrated morphological, molecular, and agronomic assessment demonstrates *Attakkari*'s strong potential for breeding programs aimed at enhancing yield, blast resistance, and sustainable rice production in Northern Sri Lanka.

1. Introduction

Rice (*Oryza sativa* L.) is the primary staple food for the majority of the Sri Lankan population, sustaining the dietary needs of over 18 million people. The rice sector plays a pivotal role in the economy of the country, involving approximately 1.8 million farming families and employing over 30 % of the agricultural labor force. Rice production occupying roughly one-third of the nation's cultivated land. In recent years, paddy is grown on about 1.10–1.15 million hectares (combined the major; *Maha* and minor; *Yala* seasons), producing on the order of 3.4–3.5 million metric tons of milled rice annually [1]. Government statistics indicate that modern semi-dwarf varieties dominate nearly all rice growing area; for example, white-pericarp, long-grained improved varieties cover over 75 % of the paddy area, while traditional landraces occupy only about 0.4 %–0.5 % [2]. The most widely cultivated varieties in 2023 were Bg 300 (~16 % of area) and At 362 (~16 %). Despite the use of improved cultivars, Sri Lankan rice farmers face major challenges including weather variability, periodic droughts, irrigation constraints, and recent agricultural policy shocks [3]. For instance, a government ban on chemical fertilizers (2021–22) drastically cut national output, and a severe drought in 2023 destroyed over 25,000 ha of paddy, affecting an estimated 60,000 farmers. Labor shortages, high input costs, and erratic rainfall also contribute to yield gaps under smallholder conditions, which occupying 70 %. Despite achieving near self-sufficiency in rice production, stagnating yields and biotic stress factors such as disease outbreaks pose ongoing challenges to sustainable

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productivity [4].

Northern Province of the country, comprising five districts, Jaffna, Kilinochchi, Mullaitheeevu, Mannar and Vavuniya, long affected by civil conflict, has been recovering its agricultural capacity. The Northern dry-zone has about 100,000 ha of land suitable for paddy, of which roughly 95–96 % is typically used for cultivation [5]. Rice cultivation in the province holds significant importance due to its contribution to food security, rural livelihoods, and the preservation of traditional agricultural practices. The region's agro-ecological conditions, characterized by a dry climate and reliance on both monsoonal rains and irrigation systems, have shaped distinct cultivation patterns and farmer preferences. Farmers in the Northern Province select rice varieties based on factors such as yield potential, disease resistance, maturity period, and market demand. The selection of rice varieties is influenced by factors including water availability, soil conditions, market demand, and cultural preferences. Seed sourcing is a critical aspect of rice cultivation. Farmers obtain seeds from various sources, including the Department of Agriculture (DoA), Agrarian Development Centers (ADCs), private vendors, and through farmer-to-farmer exchanges. While ADCs provide seeds at subsidized rates, the quantity is often limited, leading many farmers to rely on saved seeds or informal networks. The quality and availability of seeds from these sources can vary, influencing farmers' choices and cultivation outcomes.

Attakkari is a traditional rice cultivar extensively cultivated in the Northern Province of Sri Lanka. It is favored by farmers and consumers alike due to its bold, round seed type, red pericarp, superior taste, and resilience to flooding. Despite not being officially recommended by the DoA, largely due to its reported susceptibility to rice blast disease and unknown genetic parentage, *Attakkari* remains widely grown, especially for household consumption and niche markets. Its continued cultivation highlights its cultural significance and local economic value. Although believed to have been introduced to Mannar district around 2009, the exact origin of *Attakkari* rice remains unconfirmed due to a lack of reliable sources. Nevertheless, the cultivar has demonstrated strong adaptation to local conditions. Farmers particularly value its ability to withstand submergence, as the grains remain intact without shattering. The name "*Attakkari*" is derived from its characteristic appearance at maturity, when the plants sway in the wind, evoking a "dance" in the fields. Importantly, *Attakkari* rice commands a high market price compared to other rice varieties. For instance, seed paddy of *Attakkari* was sold at Rs. 10,500 (approximately USD 33) for 72 kg as of February 2025, making it one of the highest-priced varieties in the local market in Kilinochchi district. This price premium reflects strong consumer demand, perceived quality, and limited seed availability, despite the risks associated with blast susceptibility and lack of crop insurance eligibility due to its unofficial status.

Despite its cultural and economic relevance, the cultivation of *Attakkari* rice remains largely unregulated and lacks formal monitoring or improvement programs. As the variety is not officially recognized or recommended by the DoA, it has been propagated primarily through farmer-led selection and informal seed exchange systems. This unstructured approach, combined with environmental variability and continuous cultivation across seasons, has likely led to the emergence of multiple variants within the *Attakkari* rice in different parts of the Northern Province. The absence of standardized cultivation and seed certification practices raises concerns about genetic erosion, inconsistent agronomic performance, and heightened susceptibility to diseases such as rice blast. Without systematic conservation or breeding efforts, these unrecognized variants risk being lost or further compromised, threatening the sustainability and resilience of *Attakkari* rice in the region.

Rice blast, caused by the fungal pathogen *Magnaporthe oryzae*, is one of the most destructive diseases affecting rice production worldwide. Reported in over 85 rice-growing countries, it has historically caused yield losses ranging from 6 % to as high as 90 %, depending on varietal susceptibility and environmental conditions [6,7]. The pathogen attacks nearly all aerial parts of the rice plant including leaves, nodes, collars, panicles, and necks, causing lesions that lead to plant death, poor grain filling, and significant yield reductions. Outbreaks are particularly severe under conditions of high humidity, intermittent rainfall, and moderate temperatures (20–27 °C), which favor spore germination and rapid spread [8]. In Sri Lanka, rice blast remains a recurrent challenge, especially during the *Maha* season. The disease disproportionately affects traditional cultivars, which are grown under low-input systems with limited disease management [9]. The DoA identifies blast as a leading biotic constraint in rain-fed paddy fields, where severe outbreaks can undermine regional food security and rural incomes. The pathogen's seed and airborne nature further increase its spread risk in informal seed systems, commonly found in the Northern Province [10]. Managing rice blast is particularly complex due to the pathogen's high genetic variability and adaptability. While resistant varieties, fungicides, and cultural practices can reduce disease pressure, their effectiveness is often inconsistent across agro-ecological zones. Moreover, climate change projections suggest that increased humidity and temperature may further intensify disease prevalence in vulnerable zones [11].

The most sustainable strategy for managing rice blast is the development and deployment of resistant cultivars. Over 100 blast resistance (R) genes have been identified, including *Pi9*, *Pi54*, and *Pi-ta*, many of which function through pathogen-associated molecular pattern (PAMP)-triggered immunity or effector-triggered immunity mediated by nucleotide-binding leucine-rich repeat (NLR) proteins [12–15]. However, due to the high genetic variability of *M. oryzae*, single-gene resistance is often short-lived, typically being overcome within a few years of cultivar release [16]. To achieve durable resistance, current breeding programs focus on pyramiding multiple R genes and utilizing marker-assisted selection, supported by advances in molecular markers and bioinformatics tools [17–20]. These strategies provide a promising path toward long-term, environmentally friendly management of rice blast.

Locally preferred rice cultivars, often developed and maintained through farmer selection, play a vital role in regional food security, agrobiodiversity, and cultural identity. The continued preference for such cultivars by farming communities emphasizes their socioeconomic value and the need for targeted research. Despite the scale of the threat of blast, Sri Lanka lacks comprehensive studies on local cultivars, impeding resistance breeding and surveillance efforts. Therefore, it is necessary to study this disease and improve farmer access to blast-resistant cultivars. Despite its susceptibility to blast disease and lack of official recommendation, *Attakkari* rice continues to be cultivated in Northern Sri Lanka due to its favorable traits and strong farmer preference. However, its genetic potential remains underexplored. A systematic assessment of morphotype and genetic diversity is essential to identify promising variants. This will support the selection of superior lines for future improvement, aiming to enhance disease resistance while preserving the cultivar's agronomic and cultural value.

2. Materials and methods

2.1. Study site

The study was conducted using the laboratory facilities of the Department of Agricultural Biology, Faculty of Agriculture, and the Department of Zoology, Faculty of Science, University of Jaffna. Field studies were carried out at the Rice Research Station (RRS) in Paranthan, Sri Lanka.

2.2. Collection of background information

Background information on the *Attakkari* rice was gathered using two approaches. A structured questionnaire (Supplementary file 1) survey was conducted with farmers currently cultivating *Attakkari* rice across the five districts to collect primary data on cultivation practices, perceptions, and varietal traits. In addition, secondary data were obtained through discussions with Subject Matter Officers (SMOs) for Paddy, Agricultural Instructors, and officers from the DoA and RRS, Paranthan. Data analysis was carried out using SPSS. Key variables were summarized using descriptive statistics such as means, standard deviations, and frequency distributions. To explore regional variations, inferential statistical tests including cross-tabulations and ANOVA were applied to identify significant differences in farmer-reported traits, management practices, and varietal preferences across the study districts.

2.3. Evaluation of morpho-variants through seeds

2.3.1. Sample collection

A total of 117 seed samples were collected from farmers currently cultivating the cultivars across five districts: Kilinochchi (51 samples), Jaffna (35), Vavuniya (1), Mullaitheevu (14), and Mannar (16). A convenient sampling method was used, and each sample, weighing approximately 300 g, was labeled with the farmer's name, location, and contact information for reference.

2.3.2. Morphological characterization of seed parameters

From each seed sample, 100 seeds were randomly selected for evaluation. Seeds were visually classified as either healthy or diseased. Key parameters such as seed length and width were measured using a vernier caliper, while seed weight was determined using an electronic balance. Additionally, seed color and pericarp color variations were recorded using a standardized color chart [21,22].

2.3.3. Image sensing for color variation analysis

Representative seeds from each *Attakkari* rice sample were imaged using a stereo microscope under uniform lighting conditions. The RGB (Red, Green, Blue) values and grayscale intensity were recorded to quantify visual differences in grain color. These parameters were used to classify seed quality and identify variation among *Attakkari* morphotypes.

2.4. Field trials to confirm morpho variants

2.4.1. Selection of samples

Based on the results obtained from seed morphological evaluations and image sensing analysis, a cluster analysis was conducted to identify distinct morphotypes within the collected *Attakkari* rice samples. From this analysis, nine representative variants showing clear morphological differentiation were selected for field-level confirmation. In addition to these farmer-sourced variants, both reference cultivars and improved breeding lines were obtained from the RRS in Paranthan. The reference set included *Attakkari* rice collections from the *Maha* (2022/2023) and *Yala* (2023) seasons, together with breeding lines developed through conventional crosses involving *Attakkari* and other known lines such as AR-9-3/At 362. The Co 10 variety was included as an outgroup for comparative purposes, while the traditional cultivar *Pachchaperumal* which was already identified as a susceptible variety used as a control. Details of all selected samples are presented in Table 1.

Table 1
Details of selected *Attakkari* rice variants, reference cultivars, and breeding lines used for field evaluation.

Sample Code	Collected place	Remarks
Att 06	Paranthan, Kilinochchi	*Attakkari* variant
Att 14	Alaveddy, Jaffna	*Attakkari* variant
Att 16	Uruththirapuram, Kilinochchi	*Attakkari* variant
Att 29	Varany, Jaffna	*Attakkari* variant
Att 32	Mannar	*Attakkari* variant
Att 34	Vaddakkachi, Kilinochchi	*Attakkari* variant
Att 44	Vaddukkoddai, Jaffna	*Attakkari* variant
Att 94	Mannar	*Attakkari* variant
Att 116	Udayarkaddu, Mullaitheevu	*Attakkari* variant
CO 10	RRS, Paranthan	It was used for the comparison
Improved Line (ILa)	RRS, Paranthan	Improved Line using AR-9-3 and *Attakkari*
Improved Line (ILb)	RRS, Paranthan	Improved Line using *Attakkari* and AR-9-3
Improved Line (ILc)	RRS, Paranthan	Improved Line using *Attakkari* and At 362
Improved Line (ILd)	RRS, Paranthan	Improved Line using At 362 and *Attakkari*
Pachchaperumal	RRS, Paranthan	Blast susceptible variety
Reference *Maha* (RM)	RRS, Paranthan	*Maha* 2022/2023 Season collection
Reference *Yala* (RY)	RRS, Paranthan	*Yala* 2023 season collection

2.4.2. Germination and nursery establishment

Prior to nursery establishment, a seed viability test was conducted to ensure optimal germination. Approximately 90 % of the selected *Attakkari* seed samples were found to be viable. To promote uniform germination, seeds were pre-treated using a standard three-step protocol. First, seeds were soaked in water for 24 h to activate metabolic processes Subsequently, the soaked seeds were wrapped in moist cloth and incubated under warm conditions for 48 h to maintain humidity and encourage sprouting.

Germinated seeds were then transferred to nursery trays (parachute trays) filled with a prepared substrate composed of soil, a small quantity of inorganic fertilizer, 500 g of cow dung, and 2 kg of vermicompost. The trays were regularly watered and maintained under controlled conditions for 14 days until seedlings reached the appropriate stage for transplanting.

2.4.3. Field preparation and maintenance

Field preparation was carried out by puddling the soil using a tractor, followed by manual removal of weeds. Seedlings were transplanted into the field with an inter-row spacing of 30 cm and an intra-row spacing of 15 cm, with each cultivar line comprising 100 plants. Fertilizer applications were made in accordance with DoA recommendations: 50 kg of urea per acre at two weeks after transplanting; 75 kg of urea and 25 kg of muriate of potash (MOP) at four weeks; 65 kg of urea and 35 kg of MOP at six weeks; and 35 kg of urea at seven weeks. The field was irrigated manually every 4–5 days during the early growth stages, ensuring consistent soil moisture, and irrigation intervals were adjusted based on rainfall and plant growth stage.

2.4.4. Assessment of growth and yield parameters

To evaluate the morphological variance among *Attakkari* rice variants and reference varieties, the following parameters (Table 2) were recorded at biweekly intervals, beginning two weeks after transplanting.

To assess variability in yield performance, key post-harvest traits were evaluated after grain formation. Prior to harvesting, plants were

Table 2
Growth parameters used to assess morphological variation among *Attakkari* cultivars and reference varieties.

Parameter	Measurement Details
Plant Height	Measured from soil surface to the tip of the tallest leaf using a standard ruler.
Leaf Length	Measured from the base to the tip of the fully expanded uppermost leaf.
Number of Leaves	Counted manually per tiller to assess vegetative growth.
Flag Leaf Length	Measured at the reproductive stage for each plant using a measuring tape.
Leaf Color	Assessed using the IRRI Leaf Color Chart to estimate relative chlorophyll content.

individually labeled to ensure accurate tracking. The mature plants were harvested, placed in labeled bags, and dried for two days. Five representative plants from each line were then selected for analysis. Grain processing involved traditional winnowing to separate husks from the grains. Measurements were taken using a Vernier caliper and precision balance, focusing on the number of grains per spike, panicle count, spikelets per spike, 100-grain weight, and total grain yield per 100 plants.

2.5. Molecular characterization

Samples listed in Table 1 were used for molecular characterization to confirm the genetic variation among *Attakkari* cultivars identified through morphological and image-based analyses.

2.5.1. DNA extraction

To ensure high-quality DNA for downstream applications, seeds were first germinated and 5-day-old fresh seedlings were used as the tissue source. Genomic DNA was extracted using a modified CTAB method [23]. The extracted DNA was quantified using Nanodrop (Nanodrop 1000, Thermo Scientific, USA) and stored at −20 °C until further analysis.

2.5.2. PCR amplification of the ITS region

The Internal Transcribed Spacer (ITS) region was amplified using the universal primers ITS 5-F (5′-GGA AGT AAA AGT CGT AAC AAG G-3′) and ITS 4-R (5′-TCC TCC GCT TAT TGA GC-3′). Each 25 μL PCR reaction contained 12.5 μL of 2X Master Mix, 2.5 mM of $MgCl_2$, 1 Unit of *Taq* polymerase, 0.5 MM each of the forward and reverse primers, 2 μL of template DNA, and 7.3 μL of nuclease-free water. The thermal cycling conditions included an initial denaturation at 95 °C for 5 min, followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for 1 min, and extension at 72 °C for 1 min. A final extension was carried out at 72 °C for 10 min. Amplification success was confirmed by electrophoresis on 1.5 % (w/v) agarose gels stained with ethidium bromide. Bands were visualized and documented under UV light.

2.5.3. DNA sequencing and GenBank submission

PCR amplicons showing clear and distinct bands were sent to Macrogen Inc., South Korea for Sanger sequencing. The resulting sequences were edited using FinchTV to remove ambiguous base calls and aligned using Clustal Omega in MEGA 11 [24]. Sequences were compared against known entries in the NCBI database using the BLAST tool. Based on the results, sequences were submitted to GenBank and accession numbers were obtained.

2.5.4. Phylogenetic analysis

To infer evolutionary relationships and confirm genetic variability among the *Attakkari* variants, aligned sequences were analyzed using Clustal Omega. Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method with 1000 bootstrap replicates in MEGA 11 software [24]. Comparative sequence analysis included selected reference sequences from GenBank.

2.6. Detection of blast resistance genes

For the detection of blast resistance genes in *Attakkari* variants and other samples (Table 1), ten genes were selected based on their frequent usage and validation in existing literature (Supplementary file 2). DNA was extracted as mentioned in 3.4.1. PCR reactions were carried out in a total volume of 11.5 μL, comprising 6.25 μL of 2X Master Mix, 1.5 mM of $MgCl_2$, 1.5 Unit of *Taq* polymerase, 1 mM each of forward and reverse primers, 1 μL of template DNA, and 3.4 μL of nuclease-free water. A total of 30 cycles were performed, beginning with a pre-denaturation at 94 °C for 5 min. This was followed by denaturation at 94 °C for 30 s, primer-specific annealing for 45 s (as outlined in Table 3), and an extension step at 72 °C for 2 min. A final extension was conducted at 72 °C for 8 min, followed by a hold at 4 °C. Amplified products were electrophoresed on 1 % agarose gel prepared in 1X TAE buffer and run at 80 V for 2 h. Gels were stained with 0.5 μg/mL ethidium bromide, and a 100 bp DNA ladder was used for fragment size determination. Amplification results were visualized and documented using a UV gel documentation system.

Table 3
GenBank accession numbers and sequence similarity of *Attakkari* rice variants and reference samples.

Sample Code	Accession Number	Similarity Percentage (%)	Accession No. of closest match	Country
Att 34	PQ386307	98.98	KM036288.1	South Korea
Att 94	PQ386306	99.23	KM036284.1	South Korea
Att 29	PQ386303	99.38	KM036284.1	South Korea
Att 44	PQ386300	97.60	KM036288.1	South Korea
Att 14	PQ386298	99.24	KM036284.1	South Korea
Att 116	PQ386297	99.24	FJ949066.1	Bangladesh
Att 06	PQ386295	99.09	KM036284.1	South Korea
Att 16	PQ386294	99.85	KM036288.1	South Korea
Att 32	PQ386293	91.51	KM036283.1	South Korea
Improved Cultivar	PQ386304	99.09	KM036284.1	South Korea
Reference *Maha* (RM)	PQ386299	99.80	KM036285.1	South Korea
Reference *Yala* (RY)	PQ386302	99.38	KM036288.1	South Korea
Co 10	PQ386301	95.38	KM036284.1	South Korea

3. Results and discussion

3.1. Farmer survey and background information on Attakkari rice cultivation

A total of 119 structured questionnaires were administered across five districts in Northern Sri Lanka; Kilinochchi (43.69 %), Jaffna (29.41 %), Mannar (13.45 %), Mullaitheevu (9.24 %), and Vavuniya (4.2 %) to document farmers' experiences and perceptions of the *Attakkari* rice (Fig. 1A; Supplementary file 3). Farmers cultivated land areas ranging from 0.5 to 35 acres, with 2 acres the most frequently reported size (10.92 %, n = 13). Irrigation practices varied, with 44.54 % relying solely on rain-fed systems and the remainder using supplementary irrigation. Notably, 51.23 % of respondents cultivated *Attakkari* rice during both the *Maha* and *Yala* seasons, indicating dual-season adaptability that fits the production calendars and water availability patterns of low-input systems. Most respondents (98.32 %, n = 117) continued to grow *Attakkari* rice, citing high-yield and favorable taste as principal drivers (96.64 %, n = 115 for each), while the culturally preferred reddish grain further supported adoption. Despite its non-recommended status, none reported discontinuing cultivation; instead, traditional methods predominated (90.76 %), with only a small fraction (n = 6) practicing organic or mixed approaches. These preferences and practices are consistent with evidence that smallholders often prioritize cultivars offering sensory quality, cultural value, and reliable performance under modest inputs [25–27].

Farmer characterizations of *Attakkari* rice emphasized its agronomic profile: a medium-height, vigorous plant with robust tillering, good grain filling, and desirable grain traits. Within these ratings, 41.17 % (n

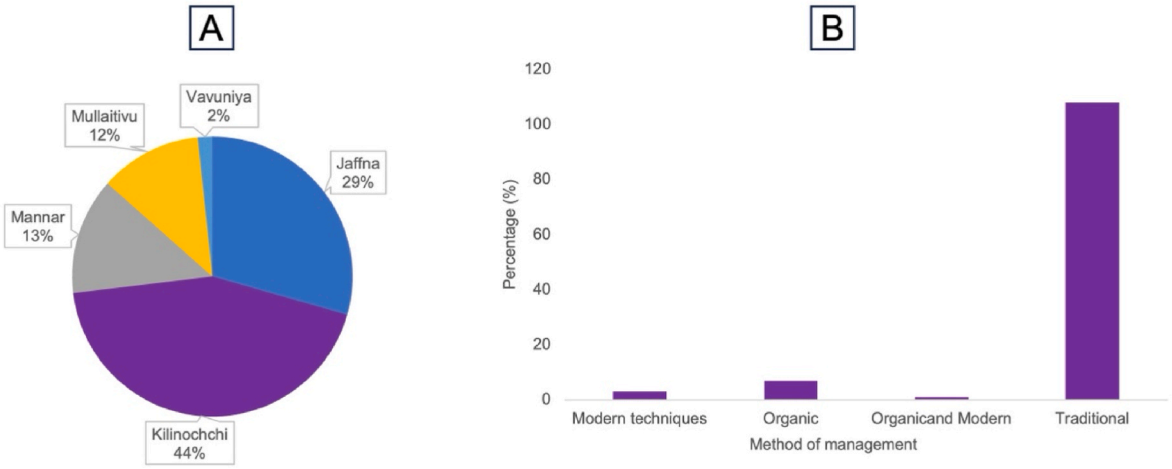

Fig. 1. ***Attakkari*** **rice cultivation practices and regional adoption patterns;** (A) District-wise distribution of farmers cultivating *Attakkari* rice based on questionnaire responses; (B) Common management method followed by farmers cultivating *Attakkari* rice.

= 49) highlighted sturdy plant growth and 34.45 % (n = 41) noted large, well-filled grains. Seed characteristics were most often described as golden yellow and medium-sized (53.78 %, n = 64), followed by golden yellow and oval-shaped (19.32 %, n = 23). Such within-cultivar heterogeneity is typical of landraces maintained through farmer selection and informal seed systems and likely contributes to functional stability across diverse management conditions; it also signals genetic variation of potential breeding value [26]. Market indicators were concordant: demand was reported as high by 95.79 % (n = 114), with 94.11 % noting increasing demand in recent years, largely attributed to limited production (91.6 %, n = 109).

In the survey, 97.5 % of farmers reported strong demand for *Attakkari* rice when evaluated in terms of market performance and varietal traits. Reported average yields ranged from 2500 to 3150 kg/acre, and 88 % rated yields as satisfactory to high under current management. Short crop duration (3.5–4 months) and high grain recovery rates were additional advantages in low-resource settings. Seed was commonly saved from previous harvests or sourced from local networks and extension offices, and a 72 kg bag of parboiled *Attakkari* rice sold for LKR 9000–10,500 (USD 30–35). Together, these findings suggest that informal seed channels sustain adoption but also constrain supply and uniformity, patterns observed in other landrace systems where farmer preferences outweigh access to certified seed [25–27].

Attakkari rice was reportedly introduced around 2009, primarily in Kilinochchi and Mannar, with its name derived from the characteristic swaying of the plants in the wind, likened to a dancer's movement. The cultivar remains in a non-recommended status due to its exclusion from National Coordinated Varietal Trials (NCVT) and Village Adaptive Trials (VAT), along with continuing concerns over its susceptibility to diseases, particularly blast. Disease and pest observations matched regional patterns: 65 % reported blast disease, while sheath blight and brown spot were reported by 25 % and 15 %, respectively, consistent with multi-season surveys from the Northern Province. This study's findings resonate with the survey by Terensan et al. [28], which demonstrated that cultivar choice is a primary factor influencing disease prevalence in Northern Sri Lanka, and *Attakkari* rice's blast susceptibility aligns with previous reports of high incidence during both *Maha* and *Yala* seasons [28]. Key pests included stem borer (35 %), leaf folder (22 %), and thrips (18 %), with outbreaks often linked to poor drainage or repeated monocropping, field conditions that can intensify inoculum carryover and stress. These environment–management interactions, combined with informal seed use and low external inputs, likely elevate disease pressure relative to recommended varieties grown under more managed systems.

Management practices were heterogeneous (Fig. 1B). Half of the farmers (50 %) reported no chemical treatments, reflecting reliance on traditional resilience; this aligns with studies showing that landraces often express moderate disease resistance yet remain vulnerable under humid, low-input conditions [29]. Selective fungicide use (28 %), primarily during blast outbreaks, and adoption of cultural measures (22 %), timely planting, crop rotation, and wider spacing indicate partial uptake of integrated disease management but also suggest constraints in access to inputs, information, and drainage improvements. Farm-ratings for agronomic traits (medium height, vigorous tillers, large grain, 41–34 %) and grain descriptions (golden yellow, medium or oval: 53–19 %) emphasize phenotypic diversity. Global and local diversity studies support significant landrace variation in Sri Lanka, often under-characterized due to limited molecular analysis [26]. The lack of formal cultivar improvement efforts, despite evident intra-cultivar diversity, points to a research and breeding opportunity: integrate blast resistance while preserving taste, grain type, and dual-season adaptability. Genetic analyses of Sri Lankan landraces have revealed previously uncharacterized diversity groups within indica rice [30], suggesting that *Attakkari* rice could similarly harbor useful alleles for resistance and adaptation.

3.2. Evaluation of morpho-variants through seeds characters

Hierarchical clustering of 117 *Attakkari* rice seed samples, based on seed characters, revealed ten distinct morphotype clusters (A–J) (Fig. 2; Supplementary file 4).

Among these, nine representative accessions; Att 34, Att 94, Att 29, Att 44, Att 14, Att 116, Att 06, Att 54, and Att 16 were selected for further field evaluation and molecular analysis as they captured the observed morphological diversity. Cluster I contained a single accession (Att 32), which was excluded from subsequent analyses due to poor germination, illustrating the practical importance of seed viability in cultivar selection. The clustering patterns showed no strict association with geographic origin, suggesting that phenotypic diversity is distributed across locations and is likely shaped by farmer selection practices and local seed exchange. Such findings are consistent with previous work on seed purity in other rice systems, such as IR841, where farmer-managed seed systems generated substantial phenotypic heterogeneity [31].

Complementing the clustering analysis, seed color variation was assessed using image sensing to quantify average RGB and grayscale intensities (Supplementary file 4). This approach enabled the detection of subtle differences in grain coloration, which were classified into two major groups within the yellow spectrum, further subdivided into four distinct sub-variations (A–D) (Fig. 3). These nuanced differences align with farmers' own grain characterizations, where seeds are often

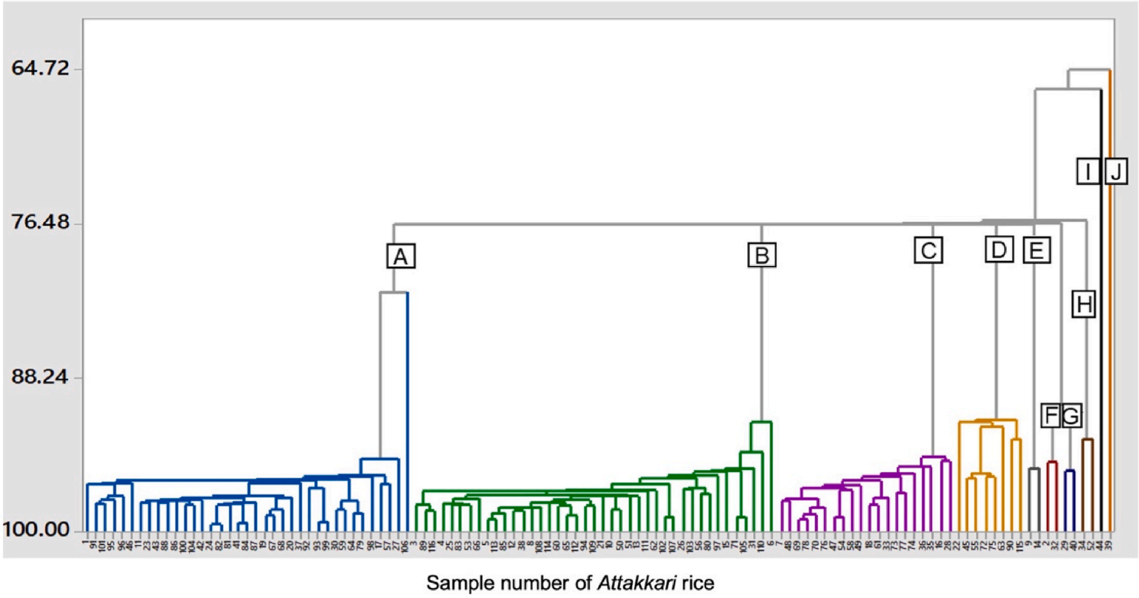

Fig. 2. Dendrogram of *Attakkari* seed morphotypes based on morphological parameters.

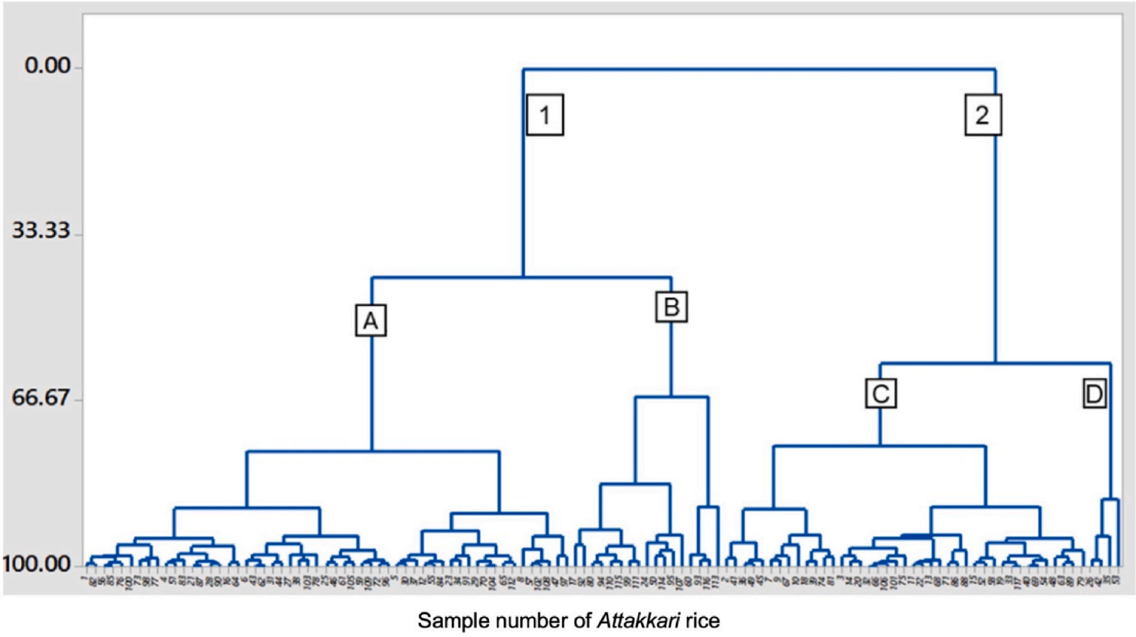

Fig. 3. Dendrogram of *Attakkari* rice seed morphotypes based on image sensing data.

distinguished into short or long types, indicating that visual phenotypic variation resonates with both genetic diversity and agronomic preference.

Together, the morphological and image-based analyses emphasize the high degree of intra-cultivar variation within *Attakkari* rice, despite the absence of formal breeding or varietal purification. Comparable studies, such as those on Italian rice germplasm, have similarly reported extensive variability across multiple traits, including seed size and shape, with clustering linked to different yield strategies. The patterns observed here reinforce the role of farmer-driven selection and community seed networks in maintaining and amplifying phenotypic diversity, independent of district boundaries. This diversity not only reflects the adaptability and resilience of *Attakkari* rice under farmer management but also provides a valuable foundation for targeted breeding and molecular studies aimed at combining preferred agronomic traits with improved stress resistance.

3.3. Field trials to confirm morpho variants

Comparative evaluation of growth parameters among *Attakkari* rice variants and reference cultivars (RY, RM, and CO 10) revealed clear phenotypic differences. Variant Att 116 exhibited the tallest stature (89.6 cm) and the highest tiller number (11.24 tillers/plant), outperforming RY (86.28 cm; 7.24 tillers/plant), RM (85.92 cm; 7.12 tillers/plant), and CO 10, which displayed the shortest height (62.28 cm) and lowest tillering capacity (5.56 tillers/plant). Superior plant height and tiller production, as observed in Att 116, are key indicators of vegetative vigor that directly contribute to resource capture and biomass accumulation, both critical to grain yield potential [32]. Leaf number also differed significantly, with Att 44 producing the highest count (41.84 leaves/plant), considerably more than RY (27.76), RM (30.0), and CO 10 (22.76). Increased leaf production is associated with enhanced photosynthetic capacity, which improves assimilate supply and stress resilience [34]. In terms of flag leaf length, Att 94 exhibited the longest flag leaves (25.8 cm), marginally exceeding RY (23.8 cm) and RM (23.2 cm), while CO 10 had significantly shorter flag leaves (18.4 cm). Since the flag leaf is a principal contributor of photosynthates during grain filling, the extended flag leaf length in Att 94 is agronomically advantageous. The consistently lower growth performance of CO 10 across these parameters highlights its limited potential compared to superior *Attakkari* rice variants.

Variation was also evident in yield-related traits. Att 34 exhibited the highest productive tiller number (7.8 tillers/plant) and a high seed number per panicle (148), surpassing reference cultivars RY (5.2 tillers/plant; 82 seeds/panicle), RM (6.2 tillers/plant; 82 seeds/panicle), and CO 10 (5 tillers/plant; 135.4 seeds/panicle). Att 94 demonstrated the highest seed number per panicle (179.6) and the greatest 100-grain weight (3.436 g), significantly exceeding all reference cultivars. High seed number and grain weight in this variant suggest strong yield potential when supported by favorable agronomic practices [33–35]. However, despite their large seed counts, Att 34 and Att 94 recorded lower-than-expected yields (0.779 kg and 1.761 kg per 100 plants, respectively), indicating possible inefficiencies in grain filling or resource allocation. Such discrepancies are commonly reported in traditional cultivars, where high tiller or seed numbers do not always translate into proportional yield gains [36–38]. Att 29 showed a notably high seeds-per-spikelet value (19.4) but only moderate yield (0.859 kg/100 plants), again suggesting limitations in assimilate partitioning.

By contrast, the reference cultivar RY produced a balanced yield of 1.473 kg per 100 plants, reflecting optimized grain filling and tiller productivity. RM yielded lower (1.2 kg/100 plants), while Co 10 performed poorly across yield parameters, recording only 0.801 kg per 100 plants. These results reinforce the relatively weak performance of Co 10, attributed to fewer productive tillers and reduced grain weight, in contrast to *Attakkari* rice variants that exhibited more robust agronomic attributes.

Overall, the evaluation revealed that Att 116, Att 44, and Att 94 exhibited superior vegetative traits, while Att 94 stood out for yield potential through a combination of high seed number and grain weight. These findings underscore the significant phenotypic diversity among *Attakkari* rice variants compared to reference cultivars, highlighting opportunities for targeted breeding. The superior traits of selected variants could be exploited to enhance yield stability and productivity under diverse agro-ecological conditions, while addressing limitations such as incomplete grain filling observed in some lines.

3.4. Molecular confirmation of Attakkari rice morpho-variants

PCR amplification targeting the ITS region successfully produced fragments of approximately 680 bp across 16 samples, including nine *Attakkari* rice morphotypes, four breeding lines, and the reference cultivars (RM and RY) and Co 10 (Fig. 4).

ITS amplicons from nine *Attakkari* rice morphotypes and four other samples (Co 10, RM, RY, and ILb) were sequenced using Sanger sequencing, generating high-quality chromatograms (Phred scores >30). The sequences have been deposited in GenBank under accession numbers PQ386293–PQ386307 (Table 3). Sequence similarity comparisons with international *Oryza sativa* accessions ranged from 91.5 % to 99.85 %.

Pairwise comparisons among the nine *Attakkari* rice variants, RM and RY indicated very high similarity (98.5–100 %), demonstrating minimal genetic divergence within the group. This genetic uniformity suggests that the observed phenotypic diversity in seed morphology and grain color (as revealed by clustering and image sensing analyses) may reflect phenotypic plasticity rather than deep genetic differentiation. Such findings align with previous studies of rice landraces, where high

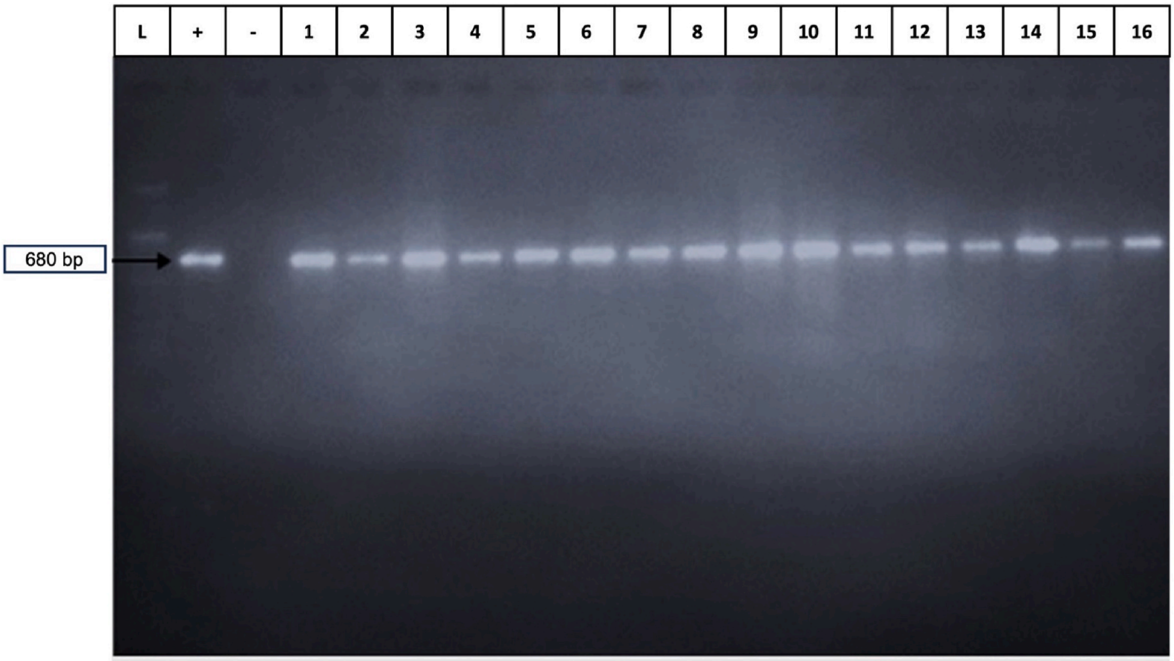

Fig. 4. The gel picture shows the results of agarose gel electrophoresis (1.5 %) used to confirm PCR amplification of the ITS region from DNA extracted from 13 *Attakkari* rice samples, 4 *Attakkari* breeding lines, Co 10 and *Pachchaperumal*. The lanes are organized as follows: Lane L, 100 bp DNA ladder; Lane + is the positive control using *Pachchaperumal*; Lane –, negative. Lanes 1 to 16 correspond to the tested samples as follows; Lane 1, Att 34; Lane 2, Att 94; Lane 3; AR 93/Att (ILa); Lane 4, Att 29; Lane 5, Co 10; Lane 6, RY (Reference *Yala*); Lane 7, Att 44; Lane 8, Att 14; Lane 9, RM (Reference *Maha*); Lane 10, Att 116; Lane 11, Att 06; Lane 12, Att 16; Lane 13, Att 32; Lane 14, Att/AR 93 (ILb); Lane 15, Att/At 362 (ILc); and Lane 16, At 362/Att (ILd). The presence of a clear band at approximately 680 bp in all positive lanes confirms successful amplification of the ITS region.

morphological variability often coexists with narrow molecular diversity due to farmer seed saving and localized exchange practices [36–38].

Despite their close relatedness, *Attakkari* rice variants also showed high similarity to several international accessions, particularly from South Korea and Bangladesh. For instance, PQ386307 exhibited 100 % similarity with KM036288.1 (*Oryza nivara*, South Korea), suggesting possible historical gene flow or shared ancestry. Similarly, PQ386298 demonstrated 99 % similarity with FJ949066.1 (*Oryza sativa* indica, Bangladesh). These findings imply that *Attakkari* rice may contain introgressed alleles from wild or regionally cultivated rice types, potentially contributing to its adaptability. *O. nivara* in particular has been reported as a source of resistance genes against major biotic stresses, including sheath blight and brown planthopper, raising the possibility that *Attakkari rice* carries valuable resistance traits [39].

The phylogenetic tree constructed using Clustal Omega (Fig. 5) grouped all nine *Attakkari* rice morphotypes, the ILb, and the local reference lines (RM and RY) into Clade 1. Within this clade, internal branch lengths were short (ranging from ~0.043 to 0.068), reinforcing the close genetic relationships observed in pairwise comparisons. The ILb (PQ386304) formed a distinct sub-branch (branch length ≈ 0.046), consistent with its known hybrid background involving AR-9-3, while RM and RY clustered closely with minimal divergence (branch lengths ≈ 0.043 and 0.059, respectively). Clade 2 comprised international accessions from South Korea and Bangladesh, which were more divergent (branch lengths ranging from ~0.08 to 0.15) but still showed notable similarity to certain *Attakkari* rice variants, such as PQ386307 and PQ386298.

These results support the conclusion that *Attakkari* rice represents a genetically coherent group of phenotypic variants maintained by farmers, with minimal internal divergence yet traceable genetic links to international Oryza lineages. Although the parental lines of the original cultivar remain unknown, the observed affinities to *O. nivara* and South Asian indica accessions suggest possible ancestral connections or ancient introgression events. This would explain how *Attakkari* rice combines distinctive morphological and sensory traits with adaptive potential. Previous work on Sri Lankan landraces has documented similar patterns, where close ties to both South and East Asian gene pools highlight complex domestication and diversification histories [39].

3.5. Detection of blast resistance genes in Attakkari variants and reference samples

PCR-based screening for ten major blast resistance genes revealed distinct resistance profiles among the tested *Attakkari* rice morphotypes and reference cultivars (Table 4), with gel images provided in Supplementary file 5.

Among the nine *Attakkari* rice variants, Att 44, Att 06, and Att 16 carried nine out of ten resistance genes, including *Pita/Pita-2*, *Piz*, *Pizt*, *Pik*, *Pikh*, *Pib*, *Pi1*, *Pi5*, and *Pikm*, suggesting strong resistance potential. Att 14 and RM also showed high resistance, each with eight genes, while Att 29 and RY possessed seven genes, indicating moderate resistance. By contrast, Att 94 contained only three resistance genes, making it comparatively vulnerable to blast. Other lines such as Att 34, ILb, and breeding derivatives carried between four and six genes, representing intermediate resistance levels. Notably, *Pikp* was absent in all tested samples, suggesting that this gene may be uncommon or absent in Sri Lankan germplasm. The most frequently detected genes across samples were *Pita-2*, *Pik*, *Pikh*, *Pi1*, *Pi5*, and *Pikm*, each present in more than 75 % of the accessions, indicating their prominence in shaping resistance within the *Attakkari* rice population.

The variation observed highlights the substantial genetic diversity in blast resistance within *Attakkari* rice morphotypes. Variants with high gene counts (e.g., Att 44, Att 06, Att 16) represent particularly valuable genetic resources for breeding programs, as they combine multiple resistance genes that can broaden and stabilize resistance against diverse blast pathotypes. Conversely, the limited gene presence in Att 94 (three genes) suggests greater vulnerability to disease outbreaks and

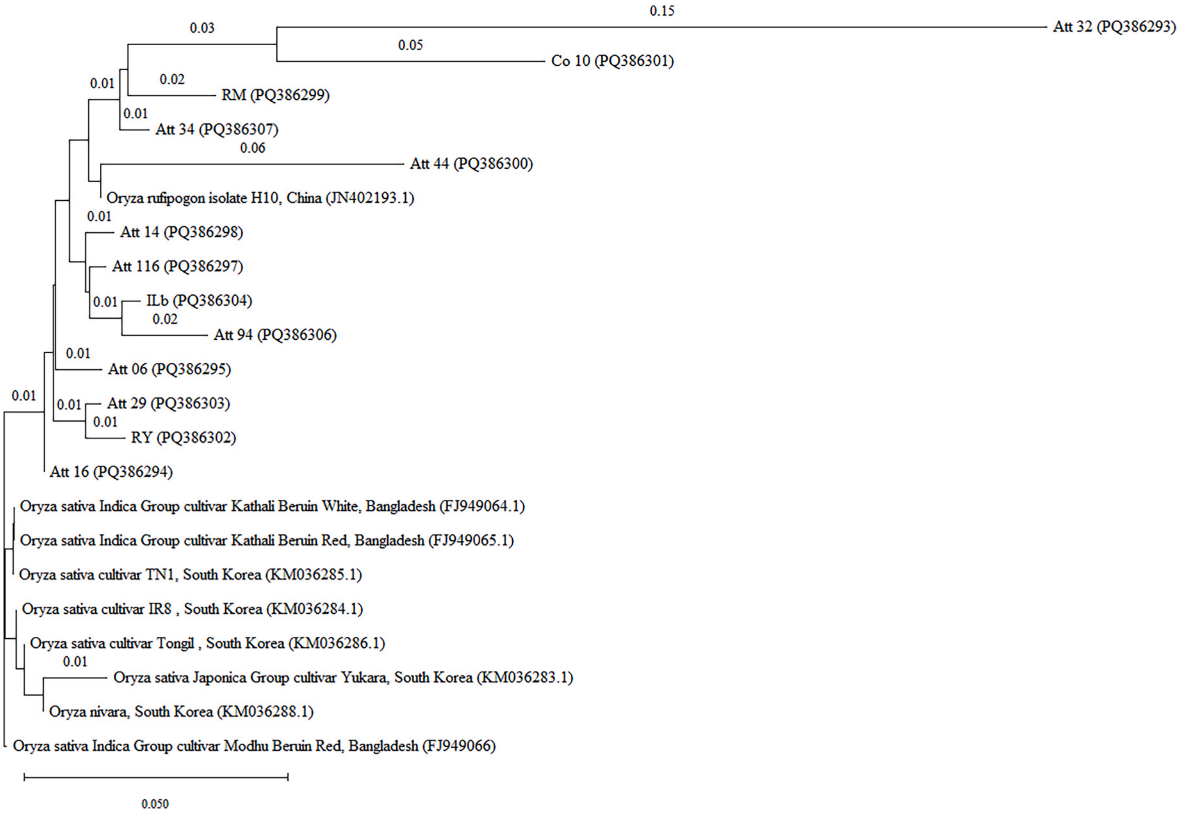

Fig. 5. Phylogenetic Tree of Attakkari Rice used in this study along with the other varieties with similar sequence.

Table 4

Presence and combination of blast resistant genes detected in *Attakkari* rice variants and other rice lines
Presence and absence are indicated by + and – signs, respectively.

Sample code	*Pita/Pita-2*	*Piz*	*Pizt*	*Pik*	*Pikh*	*Pib*	*Pikp*	*Pi1*	*Pi5*	*Pikm*	Total
Att 34	–	+	–	+	+	+	–	+	+	+	7
Att 94	+	–	–	–	–	–	–	+	+	–	3
ILa	+	+	–	+	+	+	–	+	+	+	8
Att 29	+	–	–	–	–	–	–	+	+	+	4
CO 10	+	–	–	+	+	–	–	+	+	+	6
RY	+	+	–	–	+	+	–	+	+	+	7
Att 44	+	+	+	+	+	+	–	+	+	+	9
Att 14	+	+	–	+	+	+	–	+	+	+	8
RM	+	+	–	+	+	+	–	+	+	+	8
Att 116	+	+	–	+	+	+	–	+	+	+	8
Att 06	+	+	+	+	+	+	–	+	+	+	9
Att 16	+	+	+	+	+	+	–	+	+	+	9
Att 32	–	+	–	+	+	+	–	+	+	+	7
ILb	+	–	–	–	+	–	–	+	+	+	5
ILc	+	–	–	–	+	–	–	+	+	+	5
ILd	+	–	–	+	+	–	–	+	+	+	6

underlines the need for careful selection before using this variant in breeding or cultivation. The absence of *Pikp* across all samples is notable, as this gene has been associated with durable resistance in other rice populations, indicating a possible gap in local germplasm that could be addressed by targeted gene introgression.

Reference cultivars RM and RY, derived from RRS at Paranthan, exhibited resistance profiles comparable to the higher-performing *Attakkari* rice variants, supporting their continued use as reliable benchmarks in breeding efforts. The overall pattern, presence of multiple resistance genes in most variants but variability in gene combinations suggests that *Attakkari* rice germplasm could serve as an effective reservoir of genetic diversity for developing improved blast-resistant cultivars suited to the Northern Province. These findings align with global evidence that landraces often harbor diverse resistance gene pools, which can be strategically mobilized through marker-assisted selection or genomic approaches to enhance disease resilience in cultivated rice.

However, closer analysis revealed that some of the detected resistance genes are likely allelic variants located at the same genetic loci, particularly at the Pik and Pi2/Pi9 regions. As a result, the apparent co-occurrence of these alleles in a homozygous state within the same genotype may reflect a methodological limitation stemming from the use of linked markers, which cannot reliably distinguish between functionally similar alleles. The molecular markers employed in this study target sequences adjacent to resistance loci, but may lack the resolution to identify specific allelic forms. To address this ambiguity and improve the precision of gene pyramiding assessments, future studies should employ gene-specific or functional markers such as those based on NBS-LRR domains or functional SNPs with higher discriminatory power.

The comprehensive evaluation of *Attakkari* rice variants revealed significant genetic and phenotypic diversity, highlighting promising traits suitable for future breeding and conservation programs. Variants such as Att 94, Att 34, Att 44, Att 06, and Att 16 demonstrated superior agronomic characteristics, including higher yield components, vigorous growth, and desirable grain quality traits. Notably, molecular analyses identified multiple blast resistance genes (up to nine genes in Att 44, Att 06, and Att 16), suggesting robust disease resistance potential. Genetic sequencing further indicated close relationships of certain *Attakkari* rice variants with international rice accessions, notably with wild progenitor *O. nivara*, emphasizing potential beneficial genetic introgressions. The combination of traditional farmer knowledge, phenotypic assessments, and molecular characterization underscores *Attakkari* rice's potential as a valuable genetic resource for sustainable rice production and varietal improvement, particularly in blast-prone environments.

CRediT authorship contribution statement

Kishanthan Anantharasa: Writing – review & editing, Methodology, Investigation, Formal analysis. **Suvanthini Terensan:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization. **Gajapathy Kanapathy:** Writing – review & editing, Supervision, Funding acquisition. **Ponnegipprenthiraraja Antonyrajah:** Writing – review & editing, Investigation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pmpp.2025.102969.

Data availability

Data will be made available on request.

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