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Assessment of rice (*Oryza Sativa l.*) Genotypes against different diseases under field conditions

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Abstract — **Present study aims to evaluate thirteen rice genotypes, comprising seven imported and six local varieties, along with the check cultivar IR24 (originating from China, RRI Kala-Shah-Kaku, and ICI, Pakistan), for resistance to bacterial leaf blight (BLB), brown spot (BS), and grain discoloration (GD) under artificial field inoculation conditions. The evaluation revealed significant differences between the hybrids and local rice types across various parameters. None of the varieties exhibited complete resistance to BLB, BS, or GD diseases. However, three varieties (CH3, CH9, and CH11) demonstrated moderate resistance (MR) to BLB. CH11 showed moderate resistance to BS, whereas CH3 and CH9 were moderately susceptible (MS). For GD, three genotypes (CH5, CH6, and CH7) were found to be susceptible (S) compared to the other genotypes. In terms of paddy yield, CH12 and CH13 recorded higher yields (10447 kg ha^{-1} and 10064 kg ha^{-1}, respectively) than the other hybrids and varieties. While conventional rice types had moderate disease incidence and severity, exhibited the lowest yield and yield-contributing characteristics compared to hybrid varieties.**

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Keywords: **rice, field evaluation, genotypes, resistance, disease screening**

Resumen — **El presente estudio tiene como objetivo evaluar trece genotipos de arroz, que comprenden siete variedades importadas y seis locales, junto con el cultivo de control IR24 (originario de China, RRI Kala-Shah-Kaku e ICI, Pakistán), para la resistencia al tizón bacteriano de la hoja (BLB), mancha marrón (BS) y decoloración del grano (GD), bajo condiciones de inoculación artificial en campo. La evaluación reveló diferencias significativas entre los híbridos y los tipos de arroz locales en varios parámetros. Ninguna de las variedades presentó resistencia completa a las enfermedades BLB, BS o GD. Sin embargo, tres variedades (CH3, CH9 y CH11) demostraron resistencia moderada (MR) a BLB. CH11 mostró resistencia moderada a BS, mientras que CH3 y CH9 fueron moderadamente susceptibles (MS). Para GD, tres genotipos (CH5, CH6 y CH7) resultaron ser susceptibles (S) en comparación con los otros genotipos. En cuanto al rendimiento del arroz, los cultivos CH12 y CH13 registraron rendimientos superiores (10 447 kg ha-1 y 10 064 kg ha-1, respectivamente) a los demás híbridos y variedades. Si bien los tipos de arroz convencionales presentaron incidencia y severidad moderadas de enfermedades, mostraron menor rendimiento y características que contribuyen a ese mismo rendimiento, en comparación con las variedades híbridas.**

Palabras Clave: **arroz, evaluación de campo, genotipos, resistencia, detección de enfermedades**

I. INTRODUCTION

RICE is a source of income for millions of households around the globe and uplift foreign exchequer to generate government revenue [1]. Rice serves as a staple food for half of the world's population. Due to the continuous increase in rice demand, it is projected that global rice production must increase by 25 % by 2025 to meet the needs of the growing population [2, 3]. However, to ensure food security for the rising global population consistent rice production is crucial [4]. The worldwide rice consumption has experienced a marginal upswing in recent years. As per the study, global rice consumption increased from 437.18 million metric tons in the years 2008 and 2009 to approximately 509.87 million metric tons in the 2021-2022 (Total Global Rice Consumption 2021/22). On a global scale, rice is positioned second only to wheat when it comes to production acreage. The two primary regions responsible for basmati rice production on a global scale are South and East Asia. Asia provides over 90% of the global rice production contribution and consumes it as its primary food source. Indonesia, Bangladesh, Vietnam, Thailand, Myanmar, Philippines, Brazil, Japan, India, and China are the major rice producer. Albeit, Pakistan does not stand in top rice producing countries, but it is the 4th largest exporter of rice in the world [5]. Furthermore, when compared to neighboring nations, Pakistan's average rice production is quite poor [6]. Despite advancements in developing disease-resistant varieties and other management techniques, many diseases continue to significantly contribute to agricultural yield loss. The rice crop is susceptible to a number of diseases among which bacterial leaf blight (BLB), brown spot and grain discoloration are the most destructive diseases of rice all over the world and sternly menace the unceasing cultivation of rice crop [7]. These are widespread in Asia, particularly in, Philippines, Japan, Indonesia, India and Pakistan [8]. *Xanthomonas oryzae* pv. *Oryzae (Xoo)* caused BLB befalls mostly during the wet season and in some Asian countries thin out crop yield up to 50%. BLB's increasing pervasiveness was also investigated, notably in the Kallar area of Punjab, Pakistan [9] where yield losses ranged from 35.8% to 50% [10, 11]. Septicity is essential during the tillering stage and can result in a yield loss of up to 100% [12]. Besides, rice blast, BLB, leaf streak, Brown spot (BS) is an important fungal disease caused *Bipolaris oryzae* that can cause up to 90% yield loss [13]. Brown spot disease is a globally prevalent threat to rice crops, significantly harming both nursery and field plants, and reducing the quality and yield of the grain. In nurseries, losses often occur due to the planting of infected seeds, leading to uneven germination. However, the most severe damage typically happens when the leaves and stems of seedlings become infected [14]. The fungus responsible for this disease can persist for at least two years in infected plant parts, particularly in the seeds. Brown spot disease was a major contributor to the Great Bengal Famine and remains a common problem in southern China, causing severe damage during cool summers (24-30°C) with high humidity (>92.5 %). This disease continues to impact rice cultivation worldwide, affecting crop yield and grain quality [15]. To minimize production losses, timely control of diseases is essential. Biological control, chemical protection, and the development of resistant host plants are all viable disease management methods [16]. Chemical controls can vary in effectiveness and may harm rice plants. Additionally, these chemicals are costly and not environmentally sustainable. Therefore, an integrated strategy is necessary to ensure sustained productivity. The use of bio-control agents against infections requires further investigation. An environmentally friendly, cost-effective, and efficient management strategy for bacterial blight and other diseases is crucial for sustainable rice production, not only in Pakistan but globally. Developing resistant varieties is the most effective approach to managing crop diseases and reducing yield loss. To develop a cultivar with specific traits such as disease resistance, its ability to endure environmental conditions and different genotypes must be validated [17, 18]. This study aims to assess several rice genotypes against various rice diseases through in vivo experimentation.

II. MATERIALS AND METHODS

An experiment was conducted at The Islamia University of Bahawalpur, Pakistan, in 2024 to screen rice germplasm against various rice diseases (Table I).

TABLE I
DETAILED DESCRIPTION OF RICE GENOTYPE WITH ORIGIN

Code	Genotype	Origin
CH-1	Chinese Coarse hybrid	China
CH-2	Chinese Coarse hybrid	China
CH-3	Chinese Coarse hybrid	China
CH-4	Chinese Coarse hybrid	China
CH-5	Chinese Basmati hybrid	China
CH-6	Chinese Basmati hybrid	China
CH-7	Chinese Basmati hybrid	China
CH-8	Super Basmati	KSK-Pakistan
CH-9	Basmati-515	KSK-Pakistan
CH-10	KSK-133	KSK-Pakistan
CH-11	KSK-434	KSK-Pakistan
CH-12	GRI-2	ICI-Pakistan
CH-13	GRI-3	ICI-Pakistan
CH-14	IR24	IRRI-Philippines

Seeds of each genotype were sown in single rows, 50 cm long, on raised beds. A row of the susceptible Basmati Super was planted after every two test rows to serve as a spreader. The experiment was arranged in a randomized complete block design (RCBD) with three replications. Standard agronomic practices were followed throughout the experiment. Environmental data (temperature, relative humidity, and rainfall) were recorded daily from the university weather station to document conditions influencing disease development.

A. Brown Spot Disease

At the three-week seedling stage, a spore suspension of *Bipolaris oryzae* (10^6 spores/mL) was prepared from laboratory-cultured isolates and applied uniformly to all test entries using a hand sprayer. To promote disease establishment, the nursery was misted frequently with tap water to maintain high humidity. Disease severity was recorded three weeks after inoculation using the Standard Evaluation System (SES) 0–9 scale.

B. Bacterial Leaf Blight (BLB), Pathogen Culture and Inoculum Preparation

BLB inoculum was prepared by culturing bacterial strain PSA medium and incubating them at 28°C for 48 hours. A bacterial suspension was adjusted spectrophotometrically to approximately 1×10^8 CFU/mL [19]. Plants were inoculated at the booting stage using the clipping method [20], in which the tips of the leaves were clipped with scissors dipped in the prepared suspension. This method ensures uniform infection. Disease incidence and severity were assessed 14 days after inoculation by measuring lesion length and scoring the reaction on the SES 0–9 scale.

C. Grain Discoloration (GD); Pathogen Culture and Inoculum Preparation

Fungal pathogens associated with grain discoloration were isolated from naturally infected panicles and cultured on appropriate media. A mixed inoculum (1×10^6 CFU/mL) was prepared and sprayed onto panicles at the flowering stage using a hand sprayer. High humidity was maintained after inoculation to facilitate infection, similar to the protocol followed for brown spot. At harvest, panicles were collected, visually inspected, and scored for grain discoloration severity using a scale comparable to the 0–9 SES rating. The GD score reflected the combined effect of the pathogen complex rather than individual species.

D. Disease Incidence

The disease incidence for all the diseases was recorded by using a 0-9 scale (Table II) according to [21].

TABLE II
STANDARD SCALE FOR BACTERIAL LEAF BLIGHT, BROWN SPOT DISEASE AND GRAIN DISCOLORATION

	Infection %	Score	Host Behavior
Bacterial leaf blight disease *(Xanthomonas oryzae* pv. *oryzae)*	0%	0	HR
	>1-10%	1	HR
	>10-30%	3	MR
	>30-50%	5	MS
	>50-75%	7	S
	>75-100%	9	HS
Brown spot disease (*Bipolaris oryzae*)	0	0	I
	>1%	1	HR
	1-3%	2	R
	4-10%	3-4	MR
	11-25%	5-6	MS
	26-50%	7	S
	51-100	8-9	HS
Grain Discoloration	No Incidence	0	I
	Less than 1%	1	HR
	1-5%	3	R
	6-25%	5	MR
	26-50%	7	MS
	51-100%	9	S

Standard Evaluation System for Rice (Annon, 1996). I=Immune, HR=Highly resistant, R=Resistant, MR=Moderately resistant, MS=Moderately susceptible, S=Susceptible, HS=Highly susceptible (HS)

The diseases incidence was calculated by using Eq. 1.

$$\text{Disease incidence}\,\% = \frac{\text{Number of Diseased Plants}}{\text{Total number of plant}} \times 100 \qquad (1)$$

E. Statistical Analysis

To determine the variation, the data acquired for various characteristics were subjected to statistical analysis using analysis of variance [22]. Recorded data were subjected to Pearson correlation using Statistical 8.1 software. Mean differences among the treatments were compared by Duncan's Multiple Range Test.

III. RESULTS

A. Pathogen

Isolates of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) were successfully cultured in PSA medium, producing the characteristic yellow, smooth, and viscous bacterial colonies were used for inoculation (Fig. 1A). Following inoculation, lesions in all genotypes progressed uniformly downward from the clipping point (Fig. 1B). However, lesion length showed clear genotypic variation, confirming differential resistance to BLB.

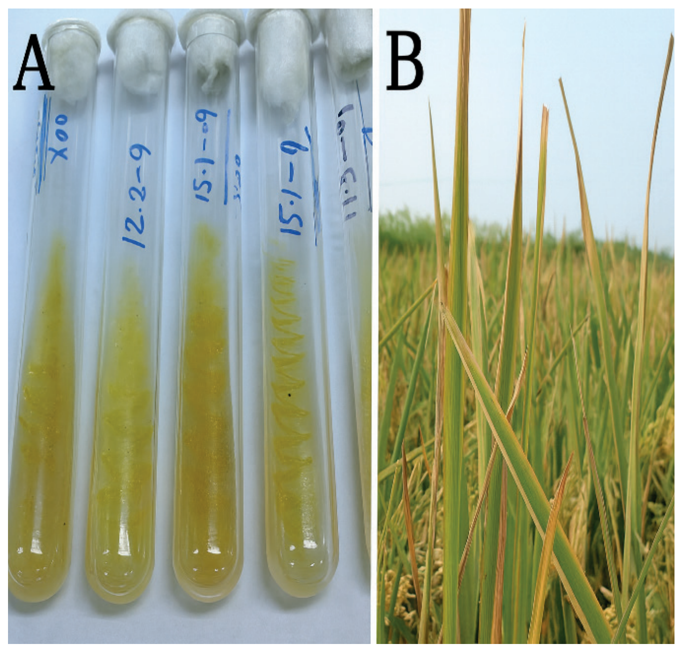

Fig. 1. Pure culture of *Xanthomonas oryzae* pv. *oryzae* (A). Inoculation of *Xanthomonas oryzae* pv. *oryzae* on plants (B).

B. Resistance Evaluation

Among all the 13 tested rice genotypes, including inbred lines and hybrids, CH3, CH9, and CH11 exhibited a moderately resistant (MR) response against bacterial leaf blight (BLB), brown spot (BS), and grain discoloration (GD) (Fig. 2).

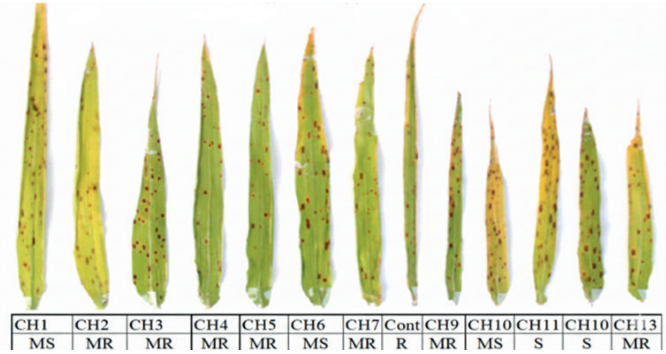

Fig. 2. Disease symptoms of Bacterial blight (A), Brown spot (B) and Grain Discoloration (C) on plant leaves after two weeks inoculation.

These genotypes showed reduced lesion expansion, lower disease incidence, and overall better maintenance of physiological integrity compared with moderately susceptible (MS) entries. Among the 13 genotypes, CH3, CH9, and CH11 showed ≥70% MR, CH7 and CH12 showed >50% moderately susceptible (MS), six genotypes (CH1, CH2, CH4, CH6, CH10, and CH13) showed >25% MS, and CH5, CH8, and the control IR-24 showed >100% highly susceptible (HS) response. CH3, CH9, and CH11 showed 29.9%, 28.9%, and 27.2% MR, respectively (Table III; Fig. 3).

C. Disease Infection Evaluation

Diseases Pressure, combined with Prevailing environmental conditions, significantly influenced plant health and yield outcomes. Notably CH11 exhibited the lowest overall disease incidence for brown spot (BS), aligning with its MR classification. Other genotypes showed an MS response. BS incidence increased progressively from the flowering to milking stages, particularly on the flag leaf, resulting in measurable yield reduction across susceptible entries. Overall BS incidence ranged from 10.5% to 19.5%, with CH13 showing the highest incidence (19.5%) and CH4 the lowest (10.5%). CH11 maintained an MR response, whereas CH3 and CH9 were classified as MS (Fig. 4).

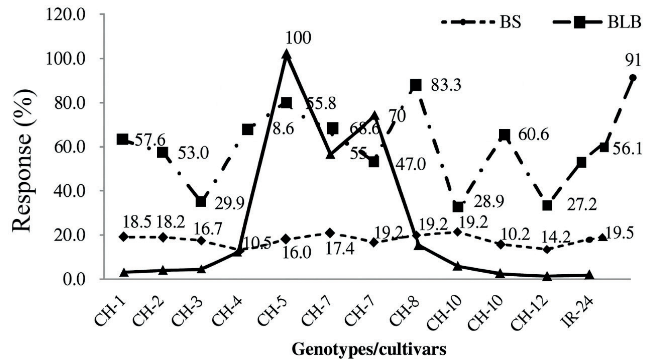

Fig. 3. Evaluating rice genotypes for resistance: Brown Spot, Bacterial Leaf Blight, and Grain Discoloration

TABLE III
MEAN PERFORMANCE OF YIELD AND ITS RELATED TRAITS OF RICE GENOTYPES. MEANS FOLLOWED BY THE SAME LETTER WITHIN A COLUMN ARE NOT SIGNIFICANTLY DIFFERENT AT P ≤ 0.05

Genotypes	Plant Height (cm)	Productive tiller/Plant	Panicle length(cm)	Primary Branches	Total spikelet's / panicle	Fertility %	1000-grain weight (gm)	Yield/plant (gm.)	Yield/ ha.(Kg/ha^1)
CH-1	99.7^{f}	4.0^{h}	25.3^{gh}	12.3^{ab}	198.0^{a}	96.0^{a}	27.0^{bc}	26.7^{ef}	5160^{c-f}
CH-2	107.7^{b-f}	6.3^{gh}	24.7^{hi}	10.7^{bc}	144.0^{b-e}	89.0^{ab}	25.0^{cde}	23.3^{fg}	4483^{ef}
CH-3	102.3^{def}	6.7^{fgh}	24.7^{hi}	9.0^{c}	132.7^{c-f}	83.3^{ab}	26.6^{bcd}	26.3^{ef}	5070^{c-f}
CH-4	101.7^{ef}	8.0^{fg}	24.3^{hi}	12.3^{ab}	149.0^{bcd}	84.3^{ab}	26.6^{bcd}	28.3^{de}	5390^{cde}
CH-5	110.0^{bcd}	13.0^{abc}	29.2^{bc}	11.0^{abc}	150.0^{bc}	57.3^{c}	24.6^{de}	26.0^{ef}	4879^{def}
CH-6	112.7^{b}	13.3^{abc}	32.2^{a}	11.0^{abc}	148.0^{bcd}	55.0^{c}	26.3^{bcd}	34.7^{b}	6641^{b}
CH-7	121.7^{a}	11.0^{cde}	30.7^{ab}	10.7^{bc}	150.3^{bc}	70.3^{bc}	28.3^{b}	30.7^{cd}	5888^{bcd}
CH-8	111.0^{bc}	15.0^{a}	26.7^{efg}	10.0^{bc}	120.3^{ef}	87.0^{ab}	27.0^{bc}	28.3^{de}	5440^{cde}
CH-9	121.3^{a}	8.7^{efg}	28.7^{cd}	10.0^{bc}	125.3^{c-f}	90.0^{ab}	25.0^{cde}	29.0^{c-f}	5568^{bcd}
CH-10	102.0^{def}	9.3^{def}	25.5^{fgh}	12.3^{ab}	111.0^{f}	90.7^{ab}	26.0^{cde}	26.7^{ef}	5185^{c-f}
CH-11	90.7^{g}	11.7^{bcd}	27.2^{def}	11.3^{abc}	122.0^{def}	88.3^{ab}	24.0^{e}	32.0^{bc}	6092^{bc}
CH-12	103.0^{c-f}	14.3^{ab}	28.0^{cde}	13.7^{a}	169.0^{b}	89.0^{ab}	31.06^{a}	54.3^{a}	10447^{a}
CH-13	109.0^{b-e}	13.0^{abc}	27.2^{def}	11.0^{abc}	124.7^{c-f}	88.7^{ab}	33.3^{a}	52.7^{a}	10064^{a}
Control	69.0^{h}	12.7^{abc}	23.0I	10.0^{bc}	109.3^{f}	87.0^{ab}	20.6^{f}	21.7^{g}	4164f
S.E.M	3.51	0.90	0.70	0.33	6.45	3.35	0.81	2.65	506.79

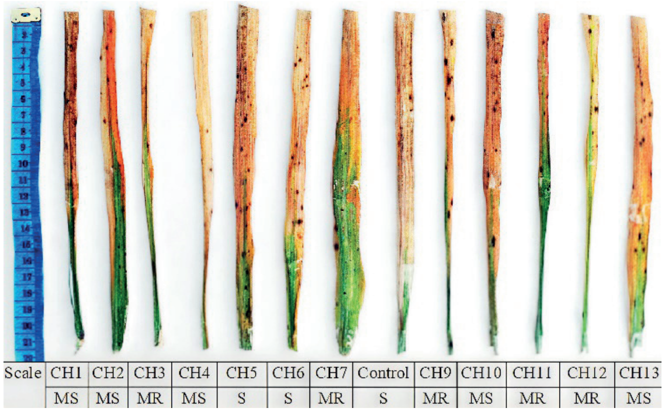

Fig. 4. Diseases infection on leaves (CH1, 2,3,4,5,6,7, Control, 9,10,11,12,13).

D. Yield and Yield Components of Rice

A comprehensive assessment of yield and yield-contributing traits revealed substantial genotypic variability (table 3): Plant Height: Ranged from 69 cm (IR-24) to 121.7 cm (CH7). Productive Tillers: Highest in CH8 (14.3), statistically similar to CH5, CH6, CH12, and CH13. Panicle Length: Varied from 23 cm (IR-24) to 32.2 cm (CH6). Panicle Density (Primary Branches): Ranged from 9 (CH3) to 13.7 (CH12), the latter significantly different from CH4 and CH10. Total Spikelet per Panicle: Ranged from 109 (IR-24) to 198 (CH1). Although CH1 had high spikelet numbers, its low tiller count contributed to its reduced final yield. Fertility Percentage: High across genotypes except CH5 (57%) and CH6 (55%). 1000-Grain Weight: Highest in CH12 (54.3 g) and CH13 (52.7 g), followed by CH6, CH11, and CH7. Hybrid seeds were generally thicker and heavier than those of indigenous varieties. Yield performance mirrored the superiority of the hybrids. CH12 and CH13, both hybrids developed by ICI Pakistan, recorded the highest yields (10,447 kg ha^{-1} and 10,064 kg ha^{-1}, respectively), each statistically superior to locally bred and Chinese cultivars. Consistent with prior findings, yield showed a positive association with productive tillers, panicle length, and number of primary branches, grains per panicle, fertility percentage, and 1000-grain weight (Fig. 5). Key environmental variable during for the year 2024 were Temperature 22-39ºC, Relative humidity 33-92% and Rainfall 0.0065-4.9 mm.

Fig. 5. Genotypes behavior on panicle regarding BLB, BS and GD.

IV. DISCUSSION

Rice-growing regions of Punjab frequently experience epidemic outbreaks of BLB, BS, and GD, with reported yield losses of 15–35% and, under severe BLB conditions, up to 90% [23, 24]. The primary factor contributing to these epidemics is the widespread cultivation of susceptible cultivars and the continuous introduction of imported hybrids that may harbor seed-borne pathogens [12; 25]. The environmental and cultural conditions used in the present study (25–35°C field temperatures and 28°C pathogen incubation) align well with optimal pathogen activity [26]. The bacterial strains were grown on PSA medium, with inoculum prepared on slants incubated at 28°C for 48 hours. Previous studies identified an optimal temperature of 28°C for the growth of *Xoo* on culture medium, with bacteria incubated at 37°C for 48 hours to screen rice varieties against bacterial blight [19, 27]. After 24 hours of incubation, the bacterial colonies appeared yellow, smooth, convex, and circular; however, they became somewhat irregular after 48 hours due to the viscous fluid secreted by the bacteria. Similar culture characteristics and viscous colonies have been reported on potato semi-synthetic agar medium [28]. The clipping method provided reliable and uniform BLB infection, consistent with previous studies showing strong correlation between clipping scores and natural BLB incidence [29]. To assess the rice plants, percent disease incidence was calculated based on lesion length, which also served to evaluate the pathogenicity of the bacterial strains on different rice varieties. Severity scores were assigned according to the standard evaluation system for rice (0-9 scale) [30]. Our findings confirm substantial genetic variability in pathogenic responses. CH3, CH9, and CH11 demonstrated strong multi-disease resistance, exhibiting minimal lesion expansion and lower disease incidence for BLB, BS, and GD. Conversely, CH5, CH8, and IR-24 showed high susceptibility, reinforcing their vulnerability in disease-prone environments. Brown spot and grain discoloration responses mirrored BLB trends, with CH11 uniquely maintaining MR status across all disease categories. The high susceptibility of CH5, CH6, and CH7 to GD suggests that these genotypes may carry seed traits or husk characteristics that favor pathogen establishment [31]. It spreads widely in dry environments with nutritional imbalances [32]. BLB causes yield losses depending on the stage of infection and the genetics of the rice cultivars [32].

Despite this, the best-performing hybrids under disease pressure, CH12 and CH13, excelled primarily in yield-related traits rather than disease resistance while other moderately susceptible genotypes showed incidences ranging from 13.3% to 19.5% [21]. Their large panicles, high fertility percentage, and heavier seeds contributed to superior yields but without the disease resilience observed in CH3, CH9, and CH11 [25]. This contrast highlights an important breeding insight: disease resistance and high yield potential do not necessarily co-occur naturally. Resistance genes may impose metabolic costs, while high productivity may leave plants physiologically vulnerable under pathogen stress. Therefore, the integration of resistance and high-yield traits through targeted hybridization is essential for long-term cultivar improvement. Average grain yield losses ranging from 8.2% to 23.0% were reported in northwestern Sierra Leone from 1983-1985 [33]. Earlier studies have reported up to 52%

yield loss due to brown spot disease at various rice growth stages [33]. The most severe impact on the next crop's germination is observed when glume blotch occurs at flowering [34]. Our results revealed that among the 13 rice genotypes, including inbred lines and hybrids, CH3, CH9, and CH11 showed the least severity (MR) for bacterial leaf blight (BLB), brown spot (BS), and grain discoloration (GD). The lowest disease incidence and severity in CH3, CH9, and CH11 align with previous findings of minimal pathogenic incidence for BLB, BS, and GD.

V. CONCLUSIONS

Under natural ecosystem of Punjab, Pakistan, the current study provide comprehensive framework of field evolution of rice genotypes against diseases by integrating standard disease rating scale, artificial inoculation and quantitative measurement of lesions. Evaluation of BLB, BS and GD in single experimental setup improve methodological rigor. It enables research to multi disease assessment along with agronomic performance

This study demonstrated substantial genotypic variation in response to bacterial leaf blight, brown spot, and grain discoloration under Pakistan's agro-climatic conditions. Three genotypes CH3, CH9, and CH11 exhibited the most reliable and consistent resistance across all diseases evaluated. CH11 further showed the lowest brown spot incidence among all entries. In contrast, the hybrid genotypes CH12 and CH13 demonstrated superior yield potential, producing the highest grain yields under disease pressure due to their robust panicle architecture, high fertility, and large grain size.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest. All authors contributed substantially to this work and approved the final version of the manuscript.

ARTIFICIAL INTELLIGENCE STATEMENT

The authors declare that generative artificial intelligence tools were used for the following purposes: Translation of abstract. The tool(s) used include: Google translator. The authors take full responsibility for the content of the manuscript.

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