Biological and Clinical Sciences Research Journal
eISSN: 2708-2261; pISSN: 2958-4728
www.bcsrj.com
DOI: https://doi.org/10.54112/bcsrj.v2024i1.1511
*Biol. Clin. Sci. Res. J., Volume, **2024**: 1511*
Original Research Article

GENETIC EVALUATION OF AROMATIC AND SUBMERGENCE TOLERANCE IN RICE (*ORYZA SATIVA* L.)

SHAHZAD A[1], SABAR M[2], ALI Q[1], ABBAS A[1,3], USAMA M[1], MUBASHAR U[4] , ASHFAQ M*[1]

[1]*Department of Plant Breeding and Genetics, University of the Punjab Lahore, Pakistan*
[2]*Rice Research Institue Kala Shah Kaku, Pakistan*
[3]*National Nanfan Research Institute (Sanya), Chinese Academy of Agricultural Sciences, Sanya 572024, China*
[4]*Government Girls High School No2 Ghakkhar, Gujranwala, Pakistan*
***Corresponding author email address:** ashfaq.iags@pu.edu.pk*

(Received, 19th September 2024, Revised 9th December 2024, Published 18th December 2024)

Abstract *This study investigates the morphological and genetic characterization of Oryza sativa germplasm for submergence tolerance and aroma. Conducted at the Rice Research Institute, Kala Shah Kaku, the research aimed to assess the adaptability of diverse accessions for breeding high-yielding, fast-growing, flood-tolerant, and aromatic rice varieties. Key agronomic traits, including plant height, tiller number, panicle length and weight, flag leaf dimensions, and grain morphology, were measured using a Vernier caliper. Analysis of variance (ANOVA) revealed significant genetic variation among accessions. Molecular characterization using SSR markers identified RM-7481 as associated with submergence tolerance, while Aroma and Badex loci were linked to aromatic traits. The SUB1 gene, a key regulator of submergence tolerance, was characterized by its role in suppressing premature growth and conserving energy under waterlogging conditions. Yield-contributing traits were analyzed through correlation analysis, simple regression, and path coefficient analysis, highlighting panicle weight and 1000-grain weight as key determinants of yield. Spearman's correlation analysis showed that there was a positive correlation between grain quality and submergence tolerance in some accessions. Some of these accessions have both high yield potential and flood resilience. The study establishes a precise relationship between genetic composition and key agronomic traits, thus providing critical information for breeding programs. The results contribute to the development of submergence-tolerant and high-quality aromatic rice aligned with consumer preferences and market demands. Further, this study gives insights into the genetic and phenotypic diversity in rice germplasm, therefore providing good direction toward rice production improvements under flood conditions.*

Keywords: *Submergence Tolerance; Aromatic Rice; Genetic Diversity; SSR Markers; SUB1 Gene; Yield Traits*

Introduction

Rice (*Oryza sativa* L.), the world's most important self-pollinated crop of the Poaceae family, provides one-fifth of the world's total caloric intake (J. Buelah et al., 2020; S. Velprabakaran et al., 2020). Domesticated about 10,000 years ago in China, rice is now cultivated globally, with the two major domesticated species, *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice), being originated from their wild progenitors *Oryza rufipogon* and *Oryza barthii*, respectively (Deng et al., 2021; Bianco et al., 2021; Junaid and Gokce, 2024). *O. sativa* is further classified into some groups like indica, temperate japonica, tropical japonica, and aromatic groups based on their ecological and genomic characteristics (Lu, 2023).

Rice is the second crop worldwide, providing more than half of the world's population with its staple, primarily in Asia, Sub-Saharan Africa, and South America (Saito et al., 2023). Rice needs some specific environmental conditions, such as high temperatures in the daytime, temperate nights, flat terrain, and an ample supply of water (Shi et al., 2016). In 2022, global rice production was valued at over three billion dollars, with the United States, India, Thailand, and Vietnam being major exporters (Statista, 2024). India led global rice exports in 2023/2024, shipping 16.5 million metric tonnes, followed by Thailand with 8.2 million metric tonnes (Top Rice Exporting Countries Worldwide, 2023/2024).

Despite its economic importance, rice production faces challenges from abiotic stresses, particularly submergence, which significantly impacts yield. Submergence tolerance, the ability of rice plants to survive and recover after prolonged flooding, is a critical trait for sustainable rice cultivation in flood-prone regions (Oladosu et al., 2020). Submergence causes a reduction in photosynthesis, reduces

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carbohydrate reserves, and elevates the content of ethylene, which further leads to growth inhibition or death. However, East Indian rice cultivars have the feature of submergence tolerance that minimizes shoot elongation with general conservation of energy, thus recovering after flooding has receded (Pradhan et al., 2015). Genetic diversity is the key factor for improving rice productivity and stress tolerance. However, only about 15% of rice's genetic potential has been exploited so far, which makes a strong case for further exploration of its genetic resources (Ying et al., 2019). Molecular markers, including SSRs, have become essential tools in identifying and utilizing genetic variation in rice breeding programs (Li et al., 2017). SSRs have been widely applied for genotyping and genetic mapping to identify QTLs linked to submergence tolerance, characterized by very high polymorphism and repeatability (Singh et al., 2009; Daware et al., 2016).

This research focuses on the genotypic and morphological analysis of the submergence tolerance of *Oryza sativa*. In this SSR markers are used to identify genetic variations that are responsible for the trait. Understanding the genetic basis of submergence tolerance, this research should contribute toward the development of resilient rice varieties for food security in flood-prone regions. These will also support efforts to improve the productivity and sustainability of rice production globally, in the context of climate change and increasing environmental stresses.

Material and methods

Experimental Site

This experiment was carried out at the Rice Research Institute, Kala Shah Kaku, Punjab, Pakistan. The Rice Research Institute, founded in 1926, is a prime rice breeding and research center working towards high-yielding, disease-resistant varieties of Basmati and non-Basmati types of rice. It is involved in state-of-the-art molecular breeding and biotechnology techniques for yield enhancement, disease resistance, and quality of grain in rice.

Soil and Climate Conditions

The experimental site contained clay-loamy soil with an average temperature of 36°C. The rainfall of 220 mm was recorded during the study period.

Experimental Design

The experiment, with 25 accessions, was laid out in a Randomized Complete Block Design (RCBD) with three replications. Each plot had rows spaced 9 inches apart, with plants spaced 9 inches within rows. Thirty plants were maintained per row.

Data Collection

Morphological data were collected following the Standard Evaluation System for Rice by the International Rice Research Institute (IRRI). The following parameters were measured using standard instruments such as a meter rod, measuring tape, weighing balance, and Vernier caliper.

Plant height was measured from the base of the tiller to the tip of the tallest panicle using a meter rod. Three plants per genotype were measured, and the average was recorded. Tillers, or secondary shoots, were manually counted at the maturity stage for each plant. Panicle length was measured from the base to the tip of the terminal spikelet using a measuring scale. Three panicles per replication were measured, and the average was calculated.

Panicles were cut and weighed using an electronic balance. Five panicles per replication were measured, and the average weight was recorded. Flag leaf length was measured from the base to the apex, while width was measured at three points along the leaf. Data were collected from three plants per replication, and averages were used for analysis. Grain length and width were measured using a Vernier caliper. Five grains per replication were analyzed, and average values were recorded. One thousand fully developed grains were dried to 13% moisture content and weighed using a balance. The process was repeated three times per genotype, and the average weight was calculated.

Statistical Analysis

Data were analyzed using Statistica 8.1 software. Analysis of Variance (ANOVA) was performed to compare means, with significance levels set at $*p < 0.05$ and $**p < 0.01$. Tukey's HSD test was used for post-hoc analysis to compare group means. Pearson's correlation coefficient (r) was calculated to assess linear relationships between variables.

Molecular Evaluation

DNA Extraction and Quantification

DNA was extracted from young leaf tissue using a modified CTAB method. DNA concentration was measured using a Nanodrop spectrophotometer. Samples were diluted to 15 ng/µL for further analysis.

Polymerase Chain Reaction (PCR)

PCR amplification was performed using a 20 µL reaction mixture containing DNA template, dNTPs, PCR buffer, $MgCl_2$, primers, Taq DNA polymerase, and distilled water. The PCR profile included initial denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation (95°C, 15 seconds), annealing (55.6°C–60.6°C, 15 seconds), extension (72°C, 30 seconds), and a final extension at 72°C for 5 minutes.

Polyacrylamide Gel Electrophoresis (PAGE)

PCR products were resolved on an 8% polyacrylamide gel prepared with TAE buffer, acrylamide, APS, and TEMED. Gels were stained and visualized to confirm marker amplification.

Results

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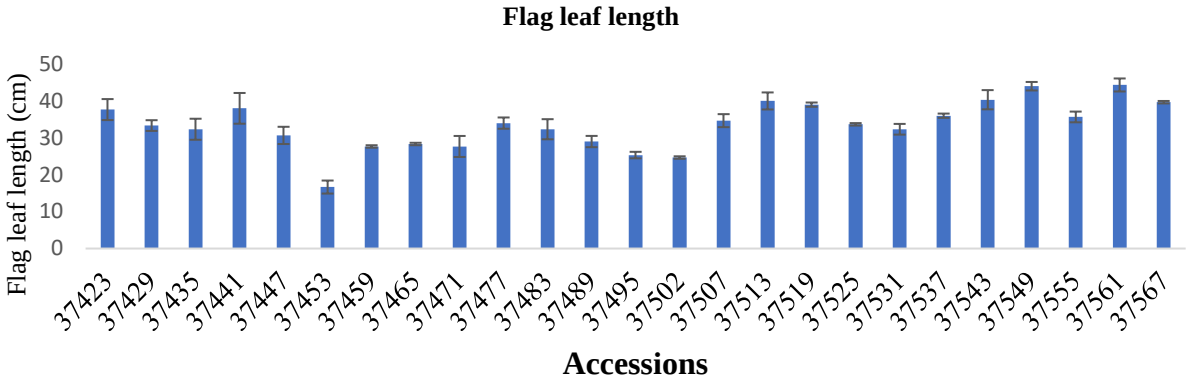
Phenotypic Evaluation of Ten Traits Under Submergence Condition

The flag leaf length of the accessions ranged from 16.67 cm to 44.33 cm, (Shown in Graph 1), with significant variation observed among genotypes (F = 11.00, p < 0.01). Accession 37561 exhibited the longest flag leaf (44.33 cm), while 37453 had the shortest (16.67 cm). Tukey's HSD test confirmed significant differences, with accessions grouped into distinct homogeneous categories (Table 1 and 2).

Graph 1: Graph for Flag Leaf Length



Source	DF	SS	MS	F
Rep.	2	1.79	0.893	
Accession	24	2970.75	123.781	11**
Error	48	540.21	11.254	
Total	74	3512.75		

Table 1: ANOVA table for Flag Leaf Length

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

Accessions	Mean	Homogeneous Groups
37561	44.333	A
37549	44	AB
37543	40.333	ABC
37513	40	ABC
37567	39.667	ABC
37519	39	ABCD
37441	38	ABCDE
37423	37.667	ABCDE
37537	36	ABCDE
37555	35.667	ABCDEF
37507	34.667	ABCDEFG
37477	34	ABCDEFG
37525	33.667	BCDEFG
37429	33.333	CDEFG
37435	32.333	CDEFG
37483	32.333	CDEFG
37531	32.333	CDEFG
37447	30.667	CDEFG
37489	29	DEFG
37465	28.333	EFG
37459	27.667	EFG
37471	27.667	EFG
37495	25.333	FGH
37502	24.667	GH
37453	16.667	H

Table 2:Tukey test table for Flag Leaf length

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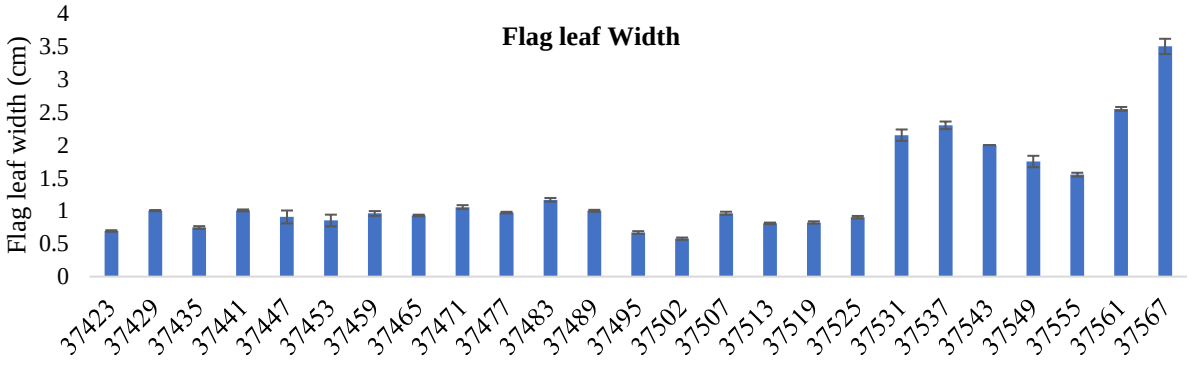
Flag leaf width varied from 0.5767 cm to 3.5 cm, with accession 37567 showing (Graph 2) the widest leaves and 37502 the narrowest. ANOVA revealed highly significant differences among genotypes ($F = 239.54$, $p < 0.01$). Tukey's test grouped accessions into 12 homogeneous categories, highlighting significant genetic variation (Table 3 and 4).

Graph 2: Graph for Flag Leaf Width



Source	DF	SS	MS	F
rep	2	0.0374	0.01872	
Accessions	24	36.7487	1.5312	239.54**
Error	48	0.3068	0.00639	
Total	74	37.093		

Table 3: ANOVA Table for Flag Leaf Width

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

Accessions	Mean	Homogeneous Groups
37567	3.5	A
37561	2.55	B
37537	2.3	BC
37531	2.15	CD
37543	2	DE
37549	1.75	EF
37555	1.55	F
37483	1.1667	G
37471	1.0567	GH
37441	1.0067	GH
37429	1.0033	GH
37489	1	GH
37477	0.9733	GHI
37507	0.9633	GHI
37459	0.96	GHI
37465	0.93	GHIJ
37447	0.9067	HIJK
37525	0.9033	HIJK
37453	0.8533	HIJK
37519	0.8233	HIJKL
37513	0.81	HIJKL
37435	0.7467	IJKL
37423	0.6933	JKL
37495	0.67	KL
37502	0.5767	L

Table 4:Tukey Test Table for Flag Leaf Width

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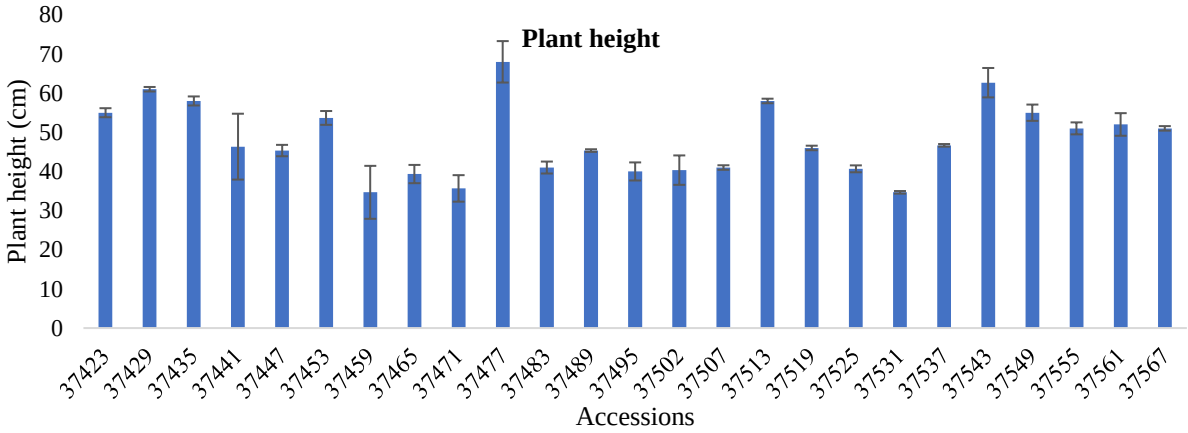
Plant height ranged from 34.67 cm to 68 cm, with accession 37477 being the tallest and 37531 the shortest (Graph 3). ANOVA indicated significant genotypic differences ($F = 9.21$, $p < 0.01$). Tukey's test grouped accessions into six homogeneous categories, with 37477 and 37531 representing the extremes (Table 5, 6).

Graph 3:Graph for Plant Height



Source	DF	SS	MS	F
Rep.	2	16.75	8.373	
Accessions	24	6084.35	253.514	9.21**
Error	48	1321.25	27.526	
Total	74	7422.35		

Table 5: ANOVA Table for Plant Height.

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

Accessions	Mean	Homogeneous Groups
37477	68	A
37543	62.667	AB
37429	61	ABC
37435	58	ABC
37513	58	ABC
37423	55	ABCD
37549	55	ABCD
37453	53.667	ABCD
37561	52	ABCDE
37567	51	BCDEF
37555	51	BCDEF
37537	46.667	BCDEF
37441	46.333	BCDEF
37519	46	CDEF
37447	45.333	CDEF
37489	45.333	CDEF
37483	41	DEF
37507	41	DEF
37525	40.667	DEF
37502	40.333	DEF
37495	40	DEF
37465	39.333	DEF
37471	35.667	EF
37459	34.667	F
37531	34.667	F

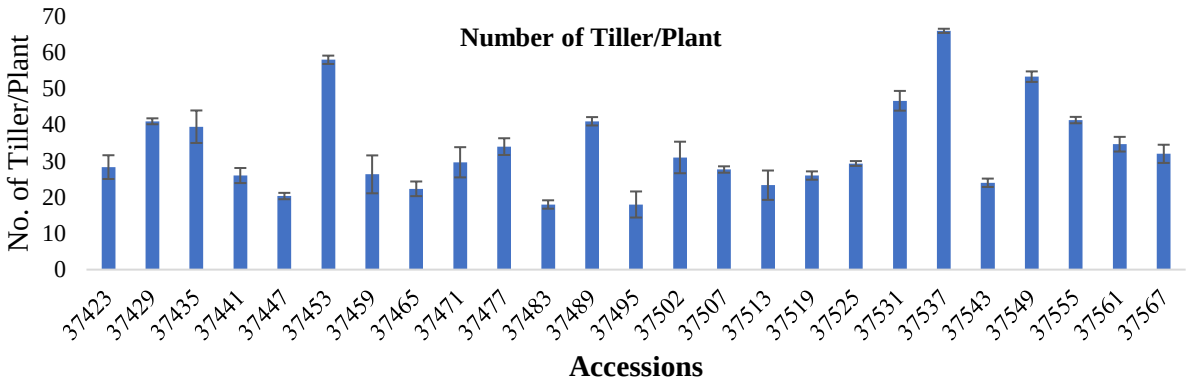
Table 6:Tukey Test Table for Plant Height

The number of tillers per plant varied from 18 to 66, with accession 37537 producing the highest number and 37495 the lowest (Graph 4). ANOVA confirmed significant genotypic differences ($F = 20.22$, $p <$

[Citation: Shahzad, A., Sabar, M., Ali, Q., Abbas, A., Usama, M., Mubashar, U., Ashfaq, M. (2024). Genetic evaluation of aromatic and Submergence Tolerance in Rice (*Oryza Sativa* L.). *Biol. Clin. Sci. Res. J.*, **2024***: 1511*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1511]

0.01). Tukey's test grouped accessions into nine homogeneous categories, with 37537 and 37495 representing the extremes (Table 7, 8).

Graph 4:Graph for Number of Tillers per Plant



Source	DF	SS	MS	F
rep	2	9	4.493	
Accessions	24	11225.3	467.722	20.22**
Error	48	1110.3	23.132	
Total	74	12344.7		

Table 7:ANOVA Table for Number of Tillers per Plant

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

Accessions	Mean	Homogeneous Groups
37537	66	A
37453	58	AB
37549	53.333	ABC
37531	46.667	BCD
37435	44.667	BCDE
37555	41.333	CDEF
37489	41	CDEFG
37429	39.667	CDEFG
37561	34.667	DEFGH
37477	34	DEFGH
37567	32	DEFGHI
37502	31	EFGHI
37471	29.667	EFGHI
37525	29.333	FGHI
37423	28.333	FGHI
37507	27.667	FGHI
37459	26.333	FGHI
37519	26	GHI
37441	26	GHI
37543	24	HI
37513	23.333	HI
37465	22.333	HI
37447	20.333	HI
37483	18	I
37495	18	I

Table 8: Tukey Test Table for Number of Tillers per Plant

Grain length ranged from 6.78 mm to 10.923 mm, with accession 37513 having the longest grains and 37459 the shortest (Graph 5). ANOVA revealed highly significant differences ($F = 94.86$, $p < 0.01$). Tukey's test grouped accessions into 13 homogeneous categories, with 37513 and 37459 representing the extremes (Table 9, 10).

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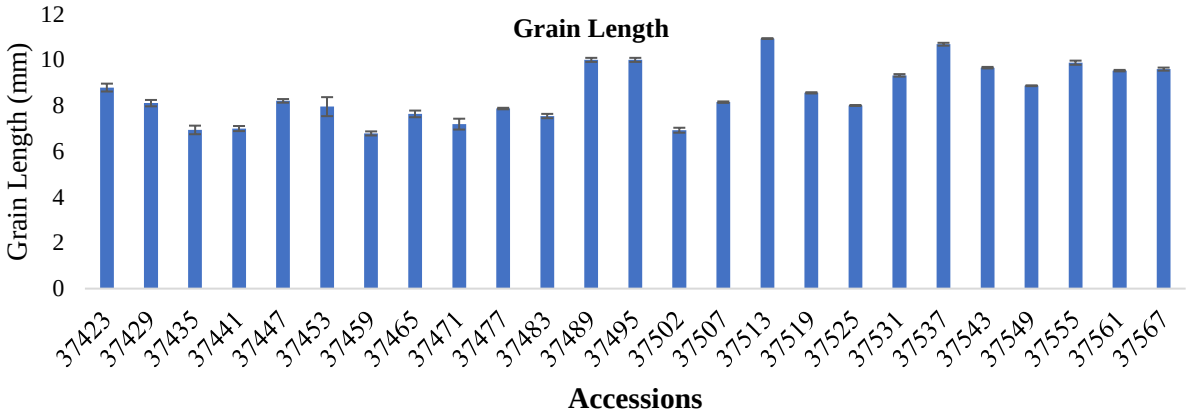
Graph 5: Graph for Grain Length



Source	DF	SS	MS	F
rep	2	0.238	0.11878	
Accessions	24	108.618	4.52575	94.86**
Error	48	2.29	0.04771	
Total	74	111.146		

Table 9:ANOVA Table for Grain Length

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

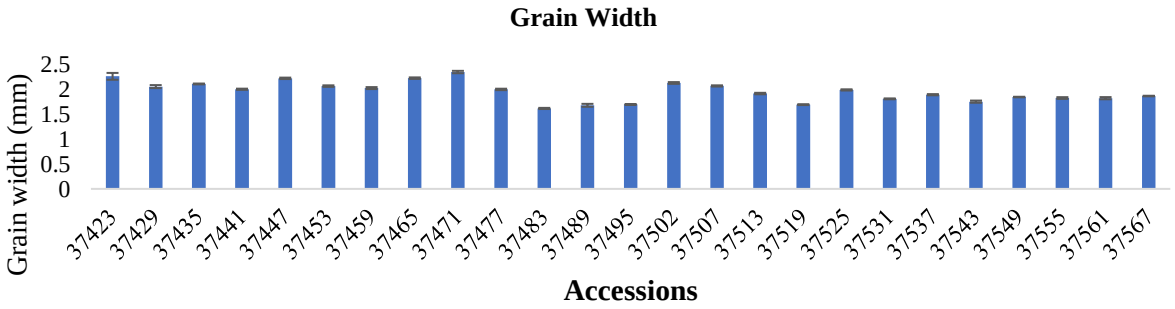
Accessions	Mean	Homogeneous Groups
37513	10.923	A
37537	10.677	AB
37489	9.993	BC
37495	9.993	BC
37555	9.87	C
37543	9.653	C
37567	9.59	C
37561	9.523	CD
37531	9.317	CDE
37549	8.863	DEF
37423	8.783	EFG
37519	8.553	FGH
37447	8.203	FGHI
37507	8.147	GHI
37429	8.103	GHI
37525	8	HI
37453	7.95	HI
37477	7.867	HIJ
37465	7.633	IJK
37483	7.54	IJKL
37471	7.187	JKLM
37441	6.993	KLM
37435	6.933	LM
37502	6.92	LM
37459	6.78	M

[Citation: Shahzad, A., Sabar, M., Ali, Q., Abbas, A., Usama, M., Mubashar, U., Ashfaq, M. (2024). Genetic evaluation of aromatic and Submergence Tolerance in Rice (*Oryza Sativa* L.). *Biol. Clin. Sci. Res. J.*, **2024***: 1511*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1511]

Table 10: Tukey Test Table for Grain Length

Grain width varied from 1.62 mm to 2.353 mm, with accession 37471 having the widest grains and 37483 the narrowest (Graph 6). ANOVA confirmed significant genotypic differences ($F = 87.95$, $p < 0.01$). Tukey's test grouped accessions into 14 homogeneous categories, with 37471 and 37483 representing the extremes (Table 11, 12).

Graph 6: Graph for Grain Width



Source	DF	SS	MS	F
rep	2	0.00282	0.00141	
Accession	24	2.83123	0.11797	87.95**
Error	48	0.06438	0.00134	
Total	74	2.89843		

Table 11: ANOVA Table for Grain Width

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

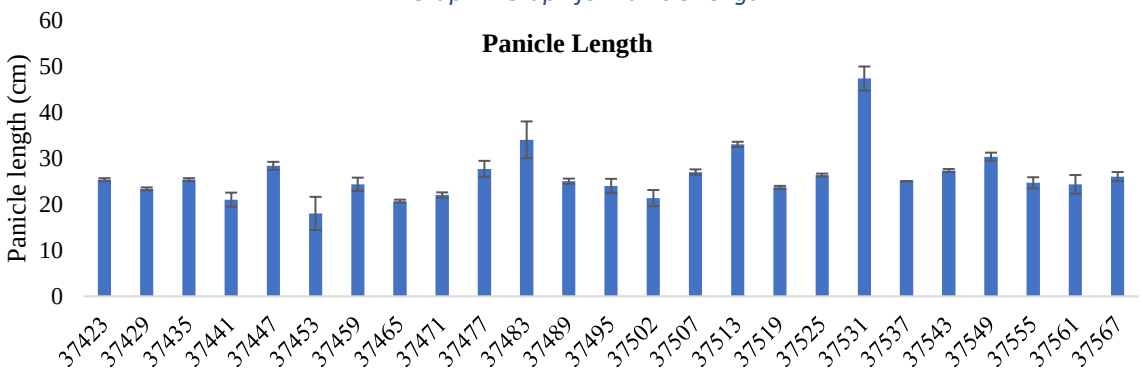
Accessions	Mean	Homogeneous Groups
37471	2.3533	A
37423	2.2667	AB
37465	2.23	BC
37447	2.2267	BCD
37502	2.1333	CDE
37435	2.1133	DEF
37507	2.0733	EFG
37453	2.07	EFG
37429	2.06	EFG
37459	2.03	EFGH
37441	2.0067	FGHI
37477	2.0033	FGHI
37525	1.9933	GHI
37513	1.92	HIJ
37537	1.8967	IJ
37567	1.87	JK
37549	1.85	JK
37555	1.83	JK
37561	1.8267	JK
37531	1.8133	JKL
37543	1.7567	KLM
37495	1.7	LMN
37519	1.6967	MN
37489	1.6833	MN
37483	1.62	N

Table 12: Tukey Test Table for Grain Width

Panicle length ranged from 18 cm to 47.333 cm, with accession 37531 having the longest panicles and 37453 the shortest (Graph 7). ANOVA revealed significant genotypic differences ($F = 15.52$, $p <$

0.01). Tukey's test grouped accessions into six homogeneous categories, with 37531 and 37453 representing the extremes (Table 13, 14).

Graph 7:Graph for Panicle Length



Source	DF	SS	MS	F
rep	2	52.83	26.4133	
Accessions	24	2336.59	97.3578	15.52**
Error	48	301.17	6.2744	
Total	74	2690.59		

Table 13: ANOVA Table for Panicle Length

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

Accessions	Mean	Homogeneous Groups
37531	47.333	A
37483	34	B
37513	33	BC
37549	30.333	BCD
37447	28.333	BCDE
37477	27.667	BCDE
37543	27.333	BCDE
37507	27	BCDE
37525	26.333	BCDE
37567	26	CDE
37423	25.333	CDEF
37435	25.333	CDEF
37489	25	DEF
37537	25	DEF
37555	24.667	DEF
37459	24.333	DEF
37561	24.333	DEF
37495	24	DEF
37519	23.667	DEF
37429	23.333	DEF
37471	22	EF
37502	21.333	EF
37441	21	EF
37465	20.667	EF
37453	18	F

Table 14: Tukey Test Table for Panicle Length

Panicle weight varied from 1.067 g to 4.91 g, with accession 37519 having the heaviest panicles and 37441 the lightest (Graph 8). ANOVA confirmed highly significant differences ($F = 128.08$, $p < 0.01$).

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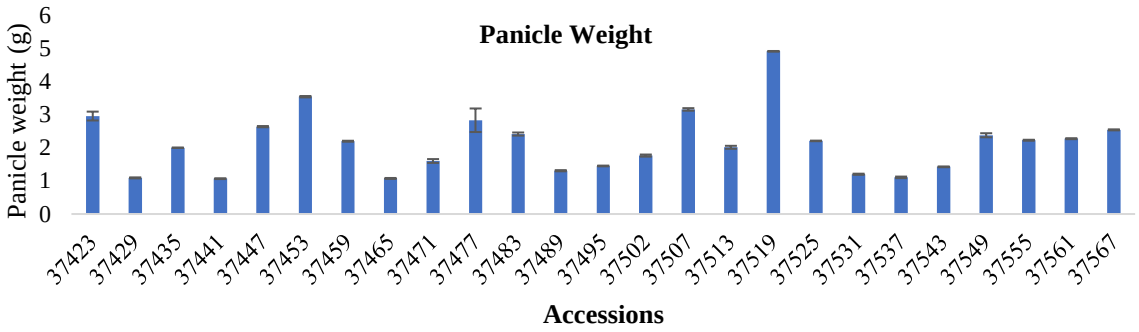
Tukey's test grouped accessions into 12 homogeneous categories, with 37519 and 37441 representing the extremes (Table 15, 16).

Graph 8:Graph for Panicle Weight



Source	DF	SS	MS	F
rep	2	0.0394	0.01969	
Accessions	24	59.6282	2.48451	128.08**
Error	48	0.9311	0.0194	
Total	74	60.5986		

Table 15: ANOVA Table for Panicle Weight

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

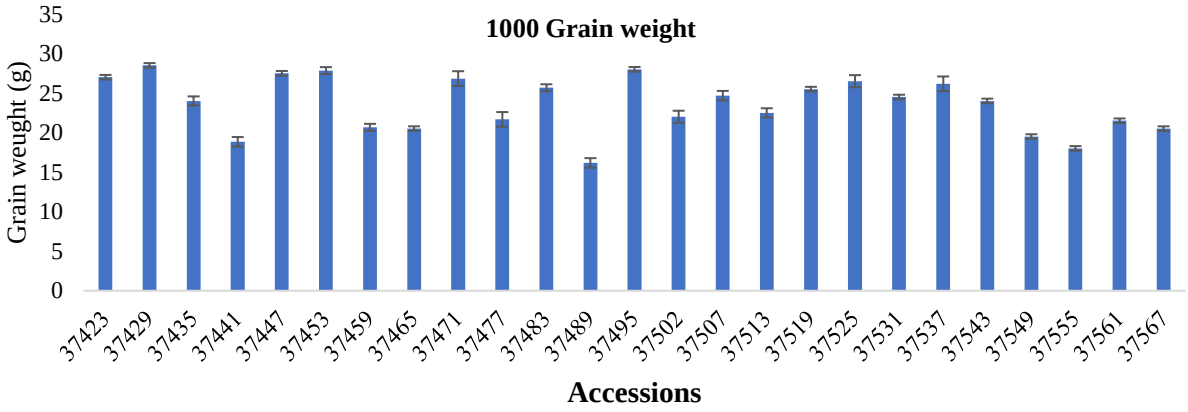
Accessions	Mean	Homogeneous Groups
37519	4.91	A
37453	3.54	B
37507	3.1567	BC
37423	2.9567	CD
37477	2.83	CDE
37447	2.6367	DEF
37567	2.54	DEF
37483	2.4133	EFG
37549	2.3767	FG
37561	2.2733	FG
37555	2.2267	FG
37525	2.2067	FG
37459	2.1967	FGH
37513	2.0133	GHI
37435	2	GHI
37502	1.7633	HIJ
37471	1.6	IJK
37495	1.45	JKL
37543	1.4233	JKL
37489	1.3067	KL
37531	1.2	KL
37537	1.1067	L
37429	1.09	L
37465	1.0733	L
37441	1.0667	L

Table 16: Tukey Test for Panicle Weight

The 1000-grain weight ranged from 16.167 g to 28.5 g, with accession 37429 having the heaviest grains and 37489 the lightest (Graph 9). ANOVA revealed significant genotypic differences ($F = 44.72$, $p < 0.01$). Tukey's test grouped accessions into 13 homogeneous categories, with 37429 and 37489 representing the extremes (Table 17, 18).

[Citation: Shahzad, A., Sabar, M., Ali, Q., Abbas, A., Usama, M., Mubashar, U., Ashfaq, M. (2024). Genetic evaluation of aromatic and Submergence Tolerance in Rice (*Oryza Sativa* L.). *Biol. Clin. Sci. Res. J.*, **2024***: 1511*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1511]

Graph 9:Graph For 1000-Grain Weight



Source	DF	SS	MS	F
rep	2	3.66	1.83	
Accessions	24	868.38	36.1825	44.72
Error	48	38.84	0.8092	
Total	74	910.88		

Table 17: ANOVA Table For 1000-Grain Weight

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

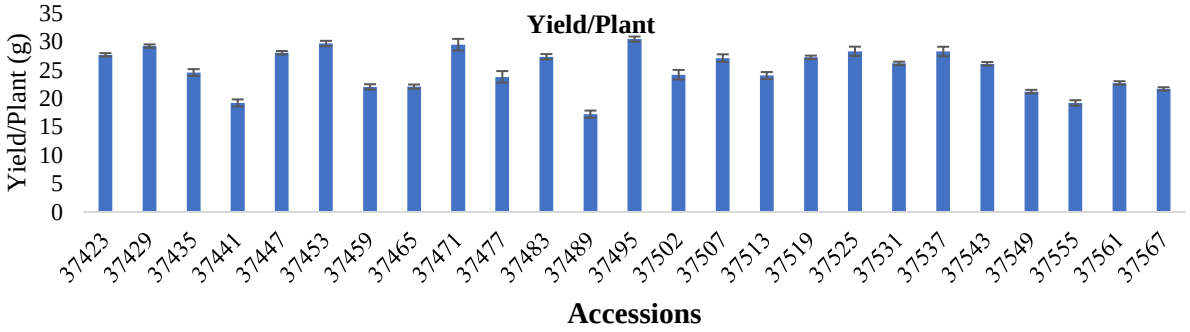
Accessions	Mean	Homogeneous Groups
37429	28.5	A
37495	28	AB
37453	27.833	AB
37447	27.5	ABC
37423	27	ABCD
37471	26.833	ABCDE
37525	26.5	ABCDE
37537	26.167	ABCDE
37483	25.667	ABCDE
37519	25.5	BCDE
37507	24.667	CDEF
37531	24.5	DEFG
37435	24	EFGH
37543	24	EFGH
37513	22.5	FGHI
37502	22	FGHIJ
37477	21.667	GHIJK
37561	21.5	HIJK
37459	20.667	IJKL
37465	20.5	IJKL
37567	20.5	IJKL
37549	19.5	JKL
37441	18.833	KLM
37555	18	LM
37489	16.167	M

Table 18:Tukey Test Table For 1000-Grain Weight

Grain yield per plant ranged from 17.128 g to 30.449 g, with accession 37495 yielding the highest and 37489 the lowest (Graph 10). ANOVA confirmed significant genotypic differences (F = 42.93, p <

0.01). Tukey's test grouped accessions into 11 homogeneous categories, with 37495 and 37489 representing the extremes (Table 19, 20).

Graph 10:Graph for Plant Yield



Source	DF	SS	MS	F
rep	2	3.73	1.8663	
Accessions	24	970.55	40.4397	42.93**
Error	48	45.21	0.9419	
Total	74	1019.5		

Table 19: ANOVA Table for Plant Yield

*= significant level is ≤0.05, **= significant level is ≤0.01, SOV: Source of variance, DF; Degree of freedom, SS: sum of the square, MS: mean square

Accessions	Mean	Homogeneous Groups
37495	30.449	A
37453	29.67	AB
37471	29.463	AB
37429	29.213	ABC
37525	28.297	ABCD
37537	28.264	ABCD
37447	28.05	ABCD
37423	27.675	ABCD
37483	27.335	BCDE
37519	27.229	BCDEF
37507	27.101	BCDEF
37531	26.161	CDEFG
37543	26.088	DEFG
37435	24.56	EFGH
37502	24.171	FGHI
37513	24.025	GHI
37477	23.79	GHI
37561	22.719	HI
37465	22.072	HIJ
37459	22.031	HIJ
37567	21.662	HIJ
37549	21.197	IJ
37441	19.21	JK
37555	19.207	JK
37489	17.218	K

Table 20: Tukey Test Table for Plant Yield

Correlation Analysis

Correlation analysis revealed a strong positive correlation between yield and 1000-grain weight (r = 0.986). Grain length and grain width showed a strong negative correlation (r = -0.528). Flag leaf length was positively correlated with plant height (r = 0.296) and flag leaf width (r = 0.467). These results highlight the importance of 1000-grain weight in yield improvement (Table 21).

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	Plant height	*Flag leaf length*	*No. of Tillers*	*Panicle Length*	*Flag leaf width*	*Grain length*	*Grain width*	*Panicle weight*	*1000-Grain weight*
Flag leaf length	0.2950								
No. of Tillers	0.2260	-0.0265							
Panicle Length	-0.0340	0.2465	0.0377						
Flag leaf width	0.0959	0.4660	0.3079	0.2651					
Grain length	0.2170	0.3593	0.2005	0.2682	0.4624				
Grain width	-0.0480	-0.2796	-0.0239	-0.3388	-0.3512	-0.5277			
Panicle weight	0.1209	0.0770	-0.0703	-0.0989	-0.1394	-0.1051	-0.0438		
1000-Grain weight	-0.0360	-0.2633	-0.0537	0.0309	-0.2337	-0.0818	0.2388	0.1869	
Yield	-0.0726	-0.2871	-0.0480	0.0378	-0.2224	-0.0606	0.2058	0.1884	0.9857

Table 21:Correlation analysis table

Regression Analysis

Regression analysis identified 1000-grain weight as the most significant predictor of yield (coefficient = 0.9812, $p < 0.01$). Other traits, such as plant height, flag leaf length, and grain width, had negligible effects on yield. This underscores the importance of 1000-grain weight in breeding programs (Table 22).

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	8.0749E-17	0.01937364	4.16798E-15	1	-0.038691831	0.038691831	-0.038691831	0.038691831
Plant height	-0.035434819	0.021820856	-1.623896817	0.109237946	-0.079014077	0.00814444	-0.079014077	0.00814444
Flag leaf length	-0.039636553	0.025492012	-1.554861707	0.124834907	-0.090547617	0.01127451	-0.090547617	0.01127451
No. of Tillers	0.004110476	0.022046069	0.186449397	0.852673052	-0.039918564	0.048139516	-0.039918564	0.048139516
Panicle Length	3.95449E-05	0.021882898	0.001807113	0.998563668	-0.043663621	0.043742711	-0.043663621	0.043742711
Flag leaf width	0.009585685	0.025885469	0.370311427	0.712354439	-0.042111167	0.061282536	-0.042111167	0.061282536
Grain length	0.025507494	0.025994347	0.981270796	0.330097583	-0.026406803	0.077421791	-0.026406803	0.077421791
Grain width	-0.023677052	0.025289678	-0.936233798	0.352618347	-0.074184027	0.026829924	-0.074184027	0.026829924
Panicle weight	0.015687734	0.021132451	0.742352818	0.460547901	-0.026516684	0.057892153	-0.026516684	0.057892153
1000-Grain weight	0.981185802	0.021932337	44.73694662	1.49899E-50	0.9373839	1.024987704	0.9373839	1.024987704

Table 22:Regression analysis table

Path Coefficient Analysis

Path coefficient analysis confirmed the dominant role of 1000-grain weight in yield (direct effect = 0.7728). Other traits, such as panicle weight and grain width, had smaller direct effects. The analysis highlighted the complex interactions between traits and their indirect contributions to yield (Table 23).

	Plant height	*Flag leaf length*	*No. of Tillers*	*Panicle Length*	*Flag leaf width*	*Grain length*	*Grain width*	*Panicle weight*	*1000-Grain weight*	*Yield*
Plant height	-0.0354	-0.0105	-0.0080	0.0012	-0.0034	-0.0015	0.0017	-0.0043	0.0013	-0.0589
Flag leaf length	-0.0117	-0.0396	0.0011	-0.0098	-0.0185	-0.0142	0.0111	-0.0031	0.0104	-0.0744
No. of Tillers	0.0009	-0.0001	0.0041	0.0002	0.0013	0.0008	-0.0001	-0.0003	-0.0002	0.0066
Panicle Length	0.00001	0.00002	0.00003	0.00002	0.00001	0.00003	0.00004	0.00002	0.00003	0.0001
Flag leaf width	0.0009	0.0045	0.0030	0.0025	0.0096	0.0044	-0.0034	-0.0013	-0.0022	0.0180
Grain length	0.0055	0.0092	0.0051	0.0068	0.0118	0.0255	-0.0135	-0.0027	-0.0021	0.0457
Grain width	0.0011	0.0066	0.0006	0.0080	0.0083	0.0125	-0.0237	0.0010	-0.0057	0.0089
Panicle weight	0.0019	0.0012	-0.0011	-0.0016	-0.0022	-0.0016	-0.0007	0.0157	0.0029	0.0145
1000-Grain weight	-0.0360	-0.2583	-0.0527	0.0303	-0.2293	-0.0802	0.2343	0.1834	0.9812	0.7727

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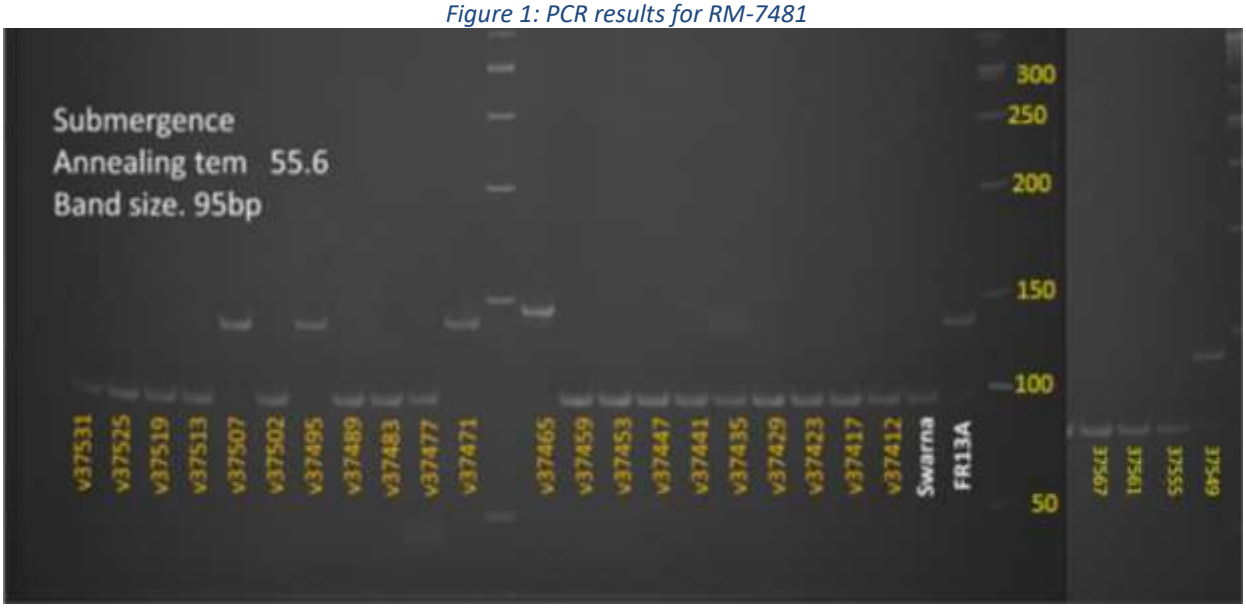
Table 23: Path Coefficient Analysis table

Molecular Analysis

RM-7481 (Submergence Tolerance)

The SSR marker RM-7481, linked to the SUB1A gene, was used to identify submergence-tolerant accessions. Accessions showing a 95 bp band (similar to the positive check, Swarna) were identified as tolerant, while those with a 300 bp band (similar to the negative check, FR13A) were susceptible. Accessions such as 37412, 37423, and 37531 exhibited submergence tolerance, while 37456, 37471, and 37507 were susceptible (Figure 1).

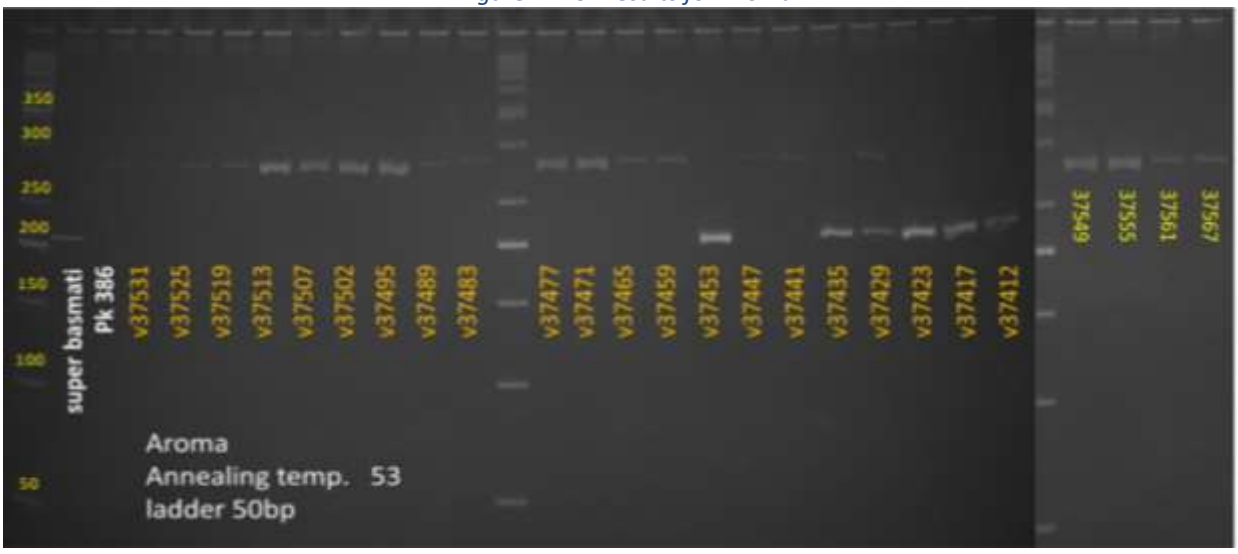
Figure 1: PCR results for RM-7481



Aroma Marker

The aroma marker identified aromatic accessions based on band size. Accessions showing bands similar to the positive check (Super Basmati) were aromatic, while those matching the negative check (PK-386) were non-aromatic. Accessions such as 37463 and 37495 lacked aromatic properties, while others, including 37412 and 37423, were aromatic (Figure 2).

Figure 2: PCR results for Aroma

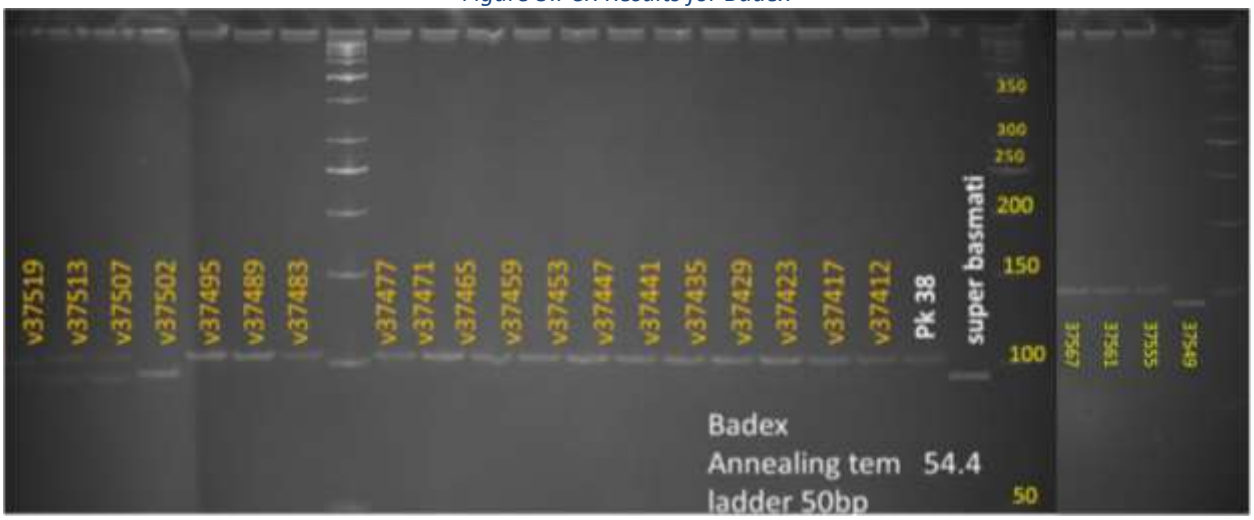


Badex Marker

The Badex marker further confirmed aromatic properties. Accessions with bands matching the positive check (Super Basmati) were aromatic, while those matching the negative check (PK-386) were non-aromatic. Accessions such as 37412 and 37423 were aromatic, while 37495 and 37507 were non-aromatic. These molecular analyses provide valuable understandings into the genetic basis of submergence tolerance and aromatic properties. Which assist in the selection of desirable traits for breeding programs (Figure 3).

[Citation: Shahzad, A., Sabar, M., Ali, Q., Abbas, A., Usama, M., Mubashar, U., Ashfaq, M. (2024). Genetic evaluation of aromatic and Submergence Tolerance in Rice (*Oryza Sativa* L.). *Biol. Clin. Sci. Res. J.*, **2024***: 1511*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1511]

Figure 3:PCR Results for Badex



Discussion

The study recognized notable genetic diversity of key morphological and yield-related traits in the 25 accessions, pointing to the suitability of the material for breeding programs targeting improvement of yield, tolerance to stresses, and grain quality. The critical flag leaf size for photosynthesis and general health of the plant showed substantial variability, and in this respect, accessions such as 37561 and 37567 proved to be the longest and widest flag leaves. These results agree with other previous studies, indicating that ideal flag leaf dimensions would improve photosynthesis efficiency and grain yield but increase lodging risk in adverse conditions (Alamin et al., 2018; Javed et al., 2024). The diversity observed in flag leaf traits underlines their importance in breeding for better yield and environmental adaptability (Fatima et al., 2023; Kumar et al., 2021).

Plant height was another important attribute that showed extensive variation, and accession 37477 was the tallest and accession 37531 shortest. The fact that taller plants absorb more light can lead to lodging in these plants, which can be higher due to higher wind or more significant rain loads (Ali et al., 2024ab; Shah et al., 2019). Genetic variability for plant height justifies the selection of appropriate architectures that maximize the yield potential at the same time resisting lodging.

Tiller number is a great determinant of grain yield and varied from 18 to 66 per plant, while accession 37537 produced the highest number. However, not all tillers contribute equally to yield since productivity depends much on the environment and resources. Genetic variation in tillering capacity gives the potential as a target trait in breeding programs optimized toward yield. Grain length and width, important in market preference and culinary use, also varied highly. Accession 37513 had the longest grains, whereas 37471 had the widest. These are traits controlled by many genes as well as by environmental factors; hence, these are essential for breeding programs aimed at targeting specific market demands (Hour et al., 2020).

Panicle length and weight, directly related to grain yield, showed considerable variation, with accession 37531 having the longest panicles and 37519 the heaviest. Though longer and heavier panicles can improve yield, they might increase lodging risk and tend to over qualify breeding in terms of balanced trait selection. The 1000-grain weight, an important yield determinant, ranged from 16.167 g to 28.5 g, with accession 37429 having the heaviest grains. This trait exhibited a strong positive correlation with yield, as seen in earlier reports that have highlighted its significance in breeding for high-yielding varieties.

Submergence tolerance and aromatic properties were analyzed through molecular analysis using SSR markers RM-7481, Aroma, and Badex. Accessions with the 95 bp band for RM-7481, such as 37412 and 37531, were submergence-tolerant and were found to be consistent with the role of the Sub1A gene in flood tolerance. Similarly, aromatic accessions were identified using the Aroma and Badex markers, confirming the presence of alleles associated with fragrance, which was consistent with the studies on the BADH2 gene. The correlations and regressions clearly showed that the 1000-grain weight had the highest influence on yield and is certainly a very important factor in breeding programs. Several other traits, like grain width and panicle weight, indicate significant yet context-dependent effects, indicating how yield in rice is complexly determined. Overall, this study shall be a good example of their ability to be used in breeding programs aimed at better yield, stress resistance, and grain quality. The strong genetic diversity obtained in key traits, coupled with molecular marker analysis, forms the basis for building high-performing rice varieties customized

[Citation: Shahzad, A., Sabar, M., Ali, Q., Abbas, A., Usama, M., Mubashar, U., Ashfaq, M. (2024). Genetic evaluation of aromatic and Submergence Tolerance in Rice (*Oryza Sativa* L.). *Biol. Clin. Sci. Res. J.*, **2024***: 1511*. doi: https://doi.org/10.54112/bcsrj.v2024i1.1511]

to specific environments and market needs. Future work should be more focused on building these findings into breeding strategies so that rice can be improved concerning both productivity and climate resilience.

Conclusion

In this study, 25 rice accessions were studied for key morphological, yield-related, and molecular traits, which showed considerable genetic variation in plant height, tiller number, flag leaf dimensions, panicle characteristics, grain size, and yield. 37412, 37423, 37453, and 37531 were shown to be accessions possessing submergence tolerance, which was confirmed to be associated with the SUB1A gene through the RM-7481 marker, while aromatic traits were identified in accessions such as 37412, 37423, and 37531 through the Aroma and Badex markers. Such accessions offer an ability to resist flood with a high monetary value for their aromatic flavor, thus emerging as prime candidates for breeding programs. These factors suggest that potential high-yielding, stress-tolerant varieties of rice may be developed by using these diverse accessions with high 1000-grain weight and diversity in key traits. MAS would further improve the accessions with better adaptability to flood-prone areas as well as satisfy the market for aromatic rice varieties. This work provides a robust basis for future breeding for improved rice productivity, resilience, and quality in changing climatic conditions.

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Declaration

Acknowledgement
Not applicable

Ethics Approval and Consent to Participate
Not applicable.

Consent for Publication
The study was approved by authors.

Funding Statement
Not applicable

Authors' Contribution
MK conducted research under the supervisions of MS and MA. QA, AA, UM, and MA provided technical support. All authors approved final version.

Conflict of interest
There is no conflict of interest among the authors of the manuscript.