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Estimation of Genetic Parameters and Identification of Leaf Blast-Resistant Rice RILs Using Cluster Analysis and MGIDI

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Abstract: Rice blast disease, caused by the fungus *Magnaporthe oryzae*, poses a significant threat to rice cultivation. One effective way to deal with this disease is to identify and introduce resistant varieties using different breeding methods. This study utilized a population of 153 recombinant inbred lines (RILs) derived from the crossing of the Shahpasand (SH) and IR28 varieties, characterized by susceptibility and resistance to leaf blast, respectively. In combination with 12 control varieties, these genotypes were subjected to an extensive evaluation of disease severity (5 stages), the area under the disease progress curve (AUDPC), type, and the infection rate in 2021 and 2022. Analysis of variance revealed significant genetic variation, highlighting the potential of the RIL population for identifying and selecting resistant lines. Employing cluster analysis and the multi-trait genotype-ideotype distance index (MGIDI), 17 lines were identified as the most resistant over a two-year evaluation period. The average AUDPC for these resistant lines was estimated at 2.435 ± 0.114, and lines 17 and 111 had the lowest AUDPC (1.526 and 1.630, respectively) and showed the least infection in two years. Conversely, lines 42 and 43 showed the highest AUDPC values (255.312 and 248.209) along with heightened sensitivity. The use of MGIDI yielded a substantial selection differential (SD) of −59.12% for traits related to leaf blast disease resistance, demonstrating the effectiveness of this method. Furthermore, new recombinant populations are expected to be developed in future plant breeding projects by crossing the most susceptible and resistant lines, which will be new sources of resistance to this disease.

Keywords: area under the disease progress curve (AUDPC); cluster analysis; disease severity; infection type

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1. Introduction

Rice (*Oryza sativa* L.) is one of the three largest grains in the world, providing nourishment for over half of the global population [1]. It is cultivated in 114 countries, with annual production exceeding 100,000 tons in more than 50 countries [2]. In Iran, rice cultivation has been an integral part of the Iranian people's diet and a vital pillar of the agricultural economy, particularly in the northern provinces. It is the second most extensively grown crop in terms of land area, following wheat, with a cultivated area of approximately 622,000 hectares in 2018–2019. Over 70% of this area is concentrated in the provinces of Guilan and Mazandaran [3].

Rice growth, development, and yield are continually confronted with diverse biotic and abiotic stresses. Abiotic stresses encompass drought, salinity, and nutrient deficiencies, whereas biotic stresses manifest as insect infestations and viral, bacterial, and fungal diseases, posing challenges to rice production [4]. Rice breeding programs primarily focus on understanding plant responses to both biotic and abiotic stresses and achieving high

yields [5]. Among these, diseases and pests are crucial limiting factors that impact rice production. More than 70 diseases caused by fungi, bacteria, viruses, or nematodes have been reported in rice [6]. Diseases resulting from living pathogens are contagious and can spread from infected plants to healthy ones, with fungal diseases being the most significant. Fungi exhibit a wide range of variations in terms of their form, mode of action, life cycle, and history [7].

Rice blast, sheath blight (SHB), and bacterial leaf blight (BLB) are major diseases reported in rice. Blast, recognized as the primary devastating disease [8], is caused by the fungus *Magnaporthe oryzae* B.C. Couch, formerly known as *Magnaporthe grisea* or *Pyricularia grisea* [9]. It is currently widespread in over 85 countries [4]. The fungus *Pyricularia oryzae* can cause damage to rice leaves and panicles, resulting in detrimental effects on both the vegetative and reproductive stages of the plant [10]. This can lead to an annual yield reduction of up to 30% [8]. Also, if favorable conditions for the fungus exist, such as cultivar susceptibility, high infection severity, fungicide usage timings, high humidity, drought, intense dew, high average temperature, high plant density, and excessive nitrogen fertilizer [11], the damage can escalate to 100% [8]. While the precise level of damage caused by blast disease in Iran is not known, it is substantial enough that every year, farmers only in Iran use tens of tons of fungicides, including tricyclazole, edifenphos, and carpropamid, for chemical management against the disease [12]. Leaf blast disease usually reaches its peak intensity around the maximum tillering stage and gradually diminishes thereafter, which is attributed to the resistance of adult plants. Leaf blasts primarily occur before flowering. This period is characterized by the formation of a source and a reservoir, while grain filling takes place after flowering. Thus, leaf blast indirectly causes a reduction in grain yield by affecting the source and a reservoir [13].

Although the utilization of fungicides and the adoption of resistant varieties are crucial strategies in managing rice blast disease due to the adverse environmental effects and application difficulties associated with fungicides, the identification and introduction of resistant varieties by breeders can be an effective approach to decreasing pesticide usage and minimizing rice losses resulting from this disease [6,14]. Numerous research studies have been conducted to assess the resistance of various rice genotypes against blast disease. These studies have successfully identified and introduced resistant genotypes [15–19]. Among the populations providing a suitable genetic background for selecting resistant genotypes, Recombinant Inbred Lines (RILs) stand out. The RIL population is created through the initial cross between two parents, followed by multiple generations of self-pollination. This crossing mechanism enables the exchange of genes between the parents, leading to a wide spectrum of diverse progeny in subsequent generations. From a breeding perspective, this diversity creates an opportunity for selection, enabling the choice of individuals who possess the best characteristics from both parents after purification. When forming RIL populations, selecting parents with noticeable genetic differences in the desired trait will result in increased effectiveness in selecting superior lines. It is even possible to identify offspring that surpass their parents [20,21].

Accordingly, a population of RILs was generated by crossing the IR28 and Shahpasand (SH) varieties. In the current study, a two-year evaluation of RILs (F11) was conducted under blast disease conditions to identify resistant RILs by cluster analysis and the MGIDI method.

2. Materials and Methods

2.1. Plant Materials

The plant materials of the current study include 153 RILs of the F11 generation, derived from crossing IR28 and SH, along with 12 control varieties, including parental varieties (SH and IR28), four aerobic rice genotypes (15A, 18A, 19A, and 26A), and the varieties Nona Bokra, Hashemi, Domsiah, Dorfak, Neda, and Sadri. The information on plant materials is presented in Table 1. Parental varieties IR28 and SH are blast-resistant and susceptible varieties, respectively [18].

Table 1. Information on rice genotypes in this experiment includes 153 RILs and 12 control varieties.

Code or Name	Designation	Parentage or Origin
1-154 [1]	F11 Recombinant inbred lines (RILs)	Iran
15A	IR 82635-B-B-82-2	IR 78875-176-B-2/IR 78875-207-B-3
18A	IR 82639-B-B-140-1	IR 78875-176-B-2/IR 78908-143-B-4
19A	IR 83749-B-B-46-1	IR 71524-44-1-1/2*IR 74371-54-1-1
26A	IR 82635-B-B-32-4	IR 78875-176-B-2/IR 78875-207-B-3
31A	Nona Bokra	India
Domsiah	Domsiah	Iran (native)
Dorfak	Dorfak	Iran (improved)
Neda	Neda	Iran (improved)
Sadri	Sadri	Iran (native)
Hashemi	Hashemi	Iran (native)
SH	Shaahpasand	Iran (native)
IR28	IR28	IRRI

[1] One of the lines (line 120) did not germinate and was removed from the list.

2.2. Experimental Design

This study was carried out in the research field of the Faculty of Agricultural Sciences, University of Guilan, Iran, during the 2021 and 2022 rice growth seasons. The experiment was conducted as a randomized complete block design with three replications in both years. The meteorological data for the two growing seasons of 2021 and 2022 in Rasht are provided in Table 2. The genotype seeds were sown in a nursery in late spring, corresponding to the period of leaf blast occurrence in this study region. Four days before seed sowing, approximately 50 kg·ha^{-1} pure nitrogen from a urea source and 50 kg·ha^{-1} phosphorus from a P_2O_5 source, along with organic fertilizer, were incorporated into the soil in a linear pattern with 10 cm intervals. To help with the better development of the leaf blast disease in the nursery during this study, the Hashemi susceptible variety was planted in two rows as vertical lines on both sides of the studied varieties. Furthermore, in addition to natural infection, suspensions of the causal agent fungus virulent strain spores were provided three times at a concentration of 105 spores/mL and sprayed weekly on the genotypes leaves after 2–3 leaf growth stages of the seedlings [18].

Table 2. Meteorological data in Rasht for the cropping seasons of 2021 and 2022.

Year	Month	Rainfall (mm)	Absolute Temperature (°C)		Mean Temperature (°C)	Relative Humidity (%)	Evaporation (mm)
			Minimum	Maximum			
2021	April	11.9	11.94	24.07	18	73.17	92.1
	May	38	16.42	26.12	21.27	79.73	104.6
	June	5.1	20.96	31.13	26.05	75.77	162.1
	July	108.1	22.35	30.93	26.64	78.87	117.2
	August	1.6	21.55	34.74	28.14	68.31	161.6
	September	80.9	18.86	27.9	23.38	79.32	79.1
2022	April	18.9	10.82	23	16.92	71.65	91
	May	46.7	13.47	22.54	18	80.66	83
	June	1.2	20.2	30.75	25.47	71.42	166.5
	July	35.7	20.67	30.82	25.74	74.4	135.7
	August	15.7	20.56	33.06	26.81	71.53	152.8
	September	129.3	18.45	29.67	24.05	78.98	90.7

2.3. Measurement of Phenotypic Traits Related to the Disease

The disease-related phenotypic traits were evaluated upon the appearance of blast symptoms on the leaves of the seedlings. Disease severity and the infection type of the genotypes were evaluated using the international scale provided by IRRI [22] (http://www.knowledgebank.irri.org/images/docs/rice-standard-evaluation-system.pdf) (accessed on

22 September 2023), which is shown in the text as severity 1–5, respectively (Figure 1, Table 3). The first recording of disease severity was carried out 24 and 29 days after sowing in 2020 and 2021, respectively. The disease severity of genotypes was recorded at seven-day intervals across five stages in the vegetative phase.

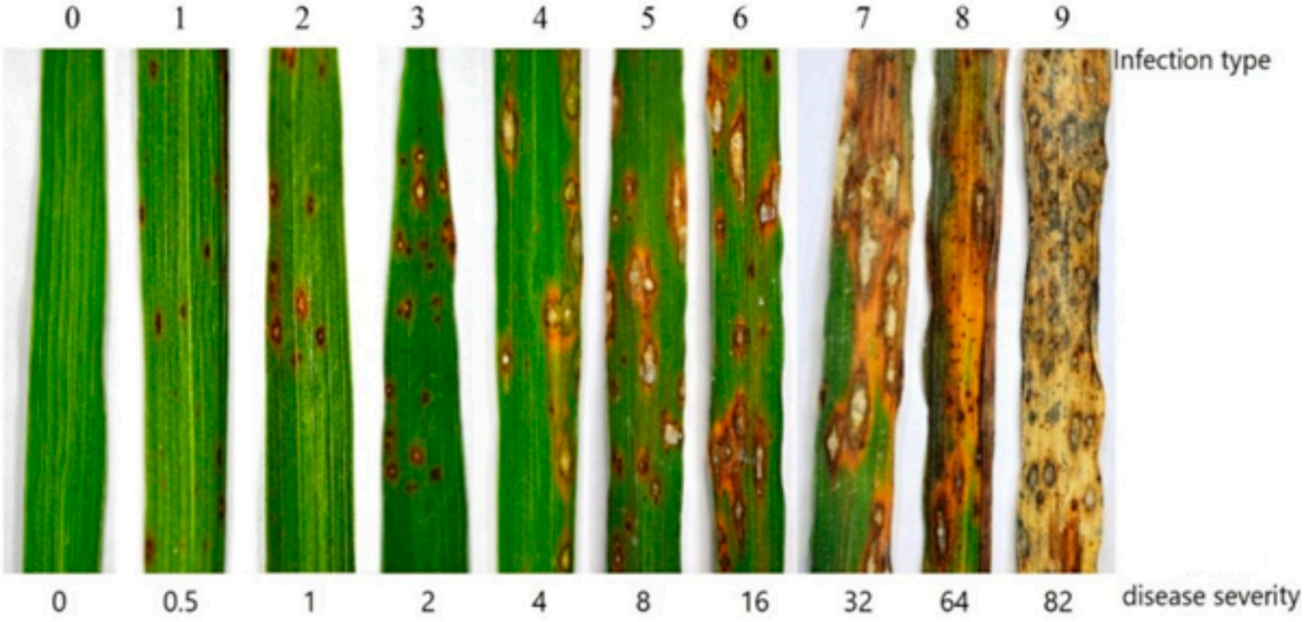

Figure 1. Infection type and disease severity of rice blast disease based on the scale of IRRI [22].

Table 3. Scale of IRRI [22] for scoring rice leaf blast disease.

0	No lesions were observed
1	Small brown specks of pin-point size or larger brown specks without a sporulating center
2	Small, roundish to slightly elongated, necrotic gray spots, about 1–2 mm in diameter, with a distinct brown margin
3	The lesion type is the same as in scale 2, but a significant number of lesions are on the upper leaves
4	Typical susceptible blast lesions are 3 mm or longer, infecting less than 4% of the leaf area
5	Typical blast lesions infect 4–10% of the leaf area
6	Typical blast lesions infect 11–25% of the leaf area
7	Typical blast lesions infect 26–50% of the leaf area
8	Typical blast lesions infect 51–75% of the leaf area, and many leaves are dead
9	More than 75% of the leaf area affected

The Area Under the Disease Progress Curve (AUDPC) was calculated using Equation (1) [23]:

$$\text{AUDPC} = \sum_{i=1}^{k} \frac{X_{i+1} + X_i}{2} (t_{i+1} - t_i), \quad (1)$$

where AUDPC is the area under the disease progress curve, X_i and X_{i+1} are the disease severity recorded on the first and second evaluations, and "$t_{i+1} - t_i$" is the number of days between the first and second evaluations.

The infection rate was calculated according to Equation (2) [24]:

$$r = [\ln \{\frac{X2}{(1 - X2)}\} - \ln \{\frac{X1}{(1 - X1)}\}]/(t_2 - t_1), \quad (2)$$

where r is the infection rate, *X*1 and *X*2 are the disease severity recorded on the first and final evaluation, and t1 and t2 are the first and final severity of the disease recorded on the genotypes.

2.4. Statistical Analysis

Prior to conducting the analysis of variance, a logarithmic transformation was applied to disease severity and AUDPC data, while an Arcsine transformation was used for infection type (IT) data. Once the assumptions were validated, a combined analysis of variance was performed using SAS Version 9.0 software [25].

Cluster analysis was employed with the objective of grouping and identifying disease-resistant and disease-susceptible genotypes, utilizing the Euclidean distance measure and Ward's minimum variance method in SPSS v.24 software [26]. Following the clustering, the Z-Score was calculated for various traits within each group using Equation (3).

$$Z\_Score = \frac{X - \mu}{\sigma} \tag{3}$$

In this Equation, μ and σ represent the mean and overall standard deviation, respectively [27]. After standardization, the group means are converted into relative values, enabling easy comparisons. It is important to note that a higher Z-Score for a group indicates a greater susceptibility of that group compared to the overall mean. This means that the group has assigned higher values to traits related to blast disease in comparison to the overall mean. Conversely, a more negative Z-Score for a group indicates a higher level of resistance.

Finally, after performing cluster analysis and identifying susceptible and resistant groups for each year, a Venn diagram was constructed using an online tool (https://bioinformatics.psb.ugent.be/webtools/Venn/ (accessed on 18 June 2023). This tool facilitated the identification of genotypes that were consistently among the most resistant and susceptible across both years.

Calculations of genetic parameters and the multi-trait genotype-ideotype distance index (MGIDI) with the best linear unbiased prediction (BLUP) for genotypes were performed as proposed in R using the 'metan' package [28] (https://github.com/TiagoOlivoto/metan, 22 September 2023). The polar plot was created using Polar Plot add-ins in Excel for MGIDI indices (https://andypope.info/charts/polarplot3.htm) (accessed on 22 September 2023).

The heritability on the mean basis (h^2_{mg}) was calculated according to Equation (4).

$$h^2_{mg} = \frac{\sigma^2_g}{\sigma^2_g + \frac{\sigma^2_{g\times e}}{e} + \frac{\sigma^2_e}{re}} \tag{4}$$

where σ^2_g, $\sigma^2_{g\times e}$, σ^2_e, r and e were the genotypic variance, the genotype × environment variance, the residual variance (error), the number of replicates, and the number of environments, respectively.

The selection accuracy was calculated according to Equation (5).

$$\text{Accuracy} = \sqrt{h^2_{mg}} \tag{5}$$

The selection differential for all traits was performed considering a selection intensity of 12%. Selection differential (SD) was computed as the difference between the mean of the selected genotypes ($\overline{X}_s$) and the original population ($\overline{X}_0$).

The predicted selection gain (SG) was computed using the MGIDI index for each trait using Equation (6).

$$SG(\%) = \frac{(\overline{X}_s - \overline{X}_0)h^2}{\overline{X}_0} \quad (6)$$

where $\overline{X}_s$, and $\overline{X}_0$ are the means of the selected genotypes and the original population, respectively, and h^2 is the heritability.

3. Results

3.1. Combined Analysis of Variance, Heritability, and Selection Accuracy

The results of the combined analysis of variance (Table 4) revealed a significant genotype effect for all assessed traits ($p < 0.01$). This indicates the existence of genetic diversity among the examined genotypes in terms of their response to blast disease on leaves. Hence, considering these observed variations, it is possible to select desirable genotypes based on the studied traits.

Table 4. Combined variance analysis and genetic parameters of evaluated traits of rice genotypes under blast treatment as a randomized complete block design across 2021 and 2022.

		Mean Squares							
S.O.V	**df**	**Severity 1** [2]	**Severity 2**	**Severity 3**	**Severity 4**	**Severity 5**	**AUDPC**	**r**	**IT**
Year	1	5.90×10^{-6} ns [1]	7.67×10^{-4} ns	2.27×10^{-3} ns	3.38×10^{-3} ns	1.70×10^{-1} **	6.14×10^{-1} ns	4.70×10^{-4} **	2.40×10^{-1} **
Block(Year)	4	3.58×10^{-6}	1.81×10^{-4}	1.37×10^{-3}	3.56×10^{-3}	1.28×10^{-3}	8.26×10^{-1}	1.72×10^{-5}	1.97×10^{-3}
Genotype	164	1.55×10^{-6} **	1.75×10^{-4} **	1.39×10^{-3} **	4.37×10^{-3} **	1.33×10^{-2} **	1.22 **	8.1×10^{-5} **	1.46×10^{-2} **
Genotype×Year	164	8.30×10^{-7} ns	7.86×10^{-5} **	1.68×10^{-4} ns	6.91×10^{-4} *	4.74×10^{-3} **	1.08×10^{-1} **	2.35×10^{-5} **	1.87×10^{-3} *
Erorr	656	7.30×10^{-7}	4.92×10^{-5}	2.45×10^{-4}	5.46×10^{-4}	8.47×10^{-4}	4.57×10^{-2}	6.41×10^{-6}	1.49×10^{-3}
CV%		0.26	2.65	4.74	13.64	5.047	14.27	5.77	17.65
h^2_{mg}		0.46	0.54	0.81	0.81	0.59	0.84	0.71	0.84
Accuracy		0.68	0.73	0.90	0.90	0.77	0.92	0.84	0.92

[1] ns, * and ** indicate no significant difference or significance at the five and one percent level, respectively. [2] Severity 1–5: Disease Severity in the first-fifth recording, AUDPC: area under the disease progress curve, r, IT: rate and type of infection, CV: coefficient of variation, h^2_{mg}: heritability on the mean basis, and Accuracy: selection accuracy.

Identifying and selecting superior genotypes should be based on screening for specific traits with high heritability. In the present study, the majority of variables manifested high heritability. The robustness of high heritability was verified through accuracy. AUDPC and IT achieved the highest accuracy.

3.2. Grouping of Rice Genotypes

3.2.1. Cluster Analysis of the 2021 Dataset

The dendrogram in Figure 2 represents the result of cluster analysis of rice genotypes in the first year of the experiment (2021). To effectively classify the genotypes and assess their disease response, they were divided into four groups based on the cluster analysis results. Genotypes that exhibited similar responses within a group (similar intra-group characteristics) were placed in the same group, while genotypes that displayed distinct reactions were assigned to different groups, depending on the extent of their dissimilarity. To evaluate and compare the groups based on the studied traits, the mean values and Z-Scores have been presented (Table 5). A higher positive Z-Score indicates that the genotypes in that particular group have higher values for disease-related traits compared to the overall average. This suggests that they are more susceptible to blast disease. Conversely, a more negative Z-Score indicates resistance within that group.

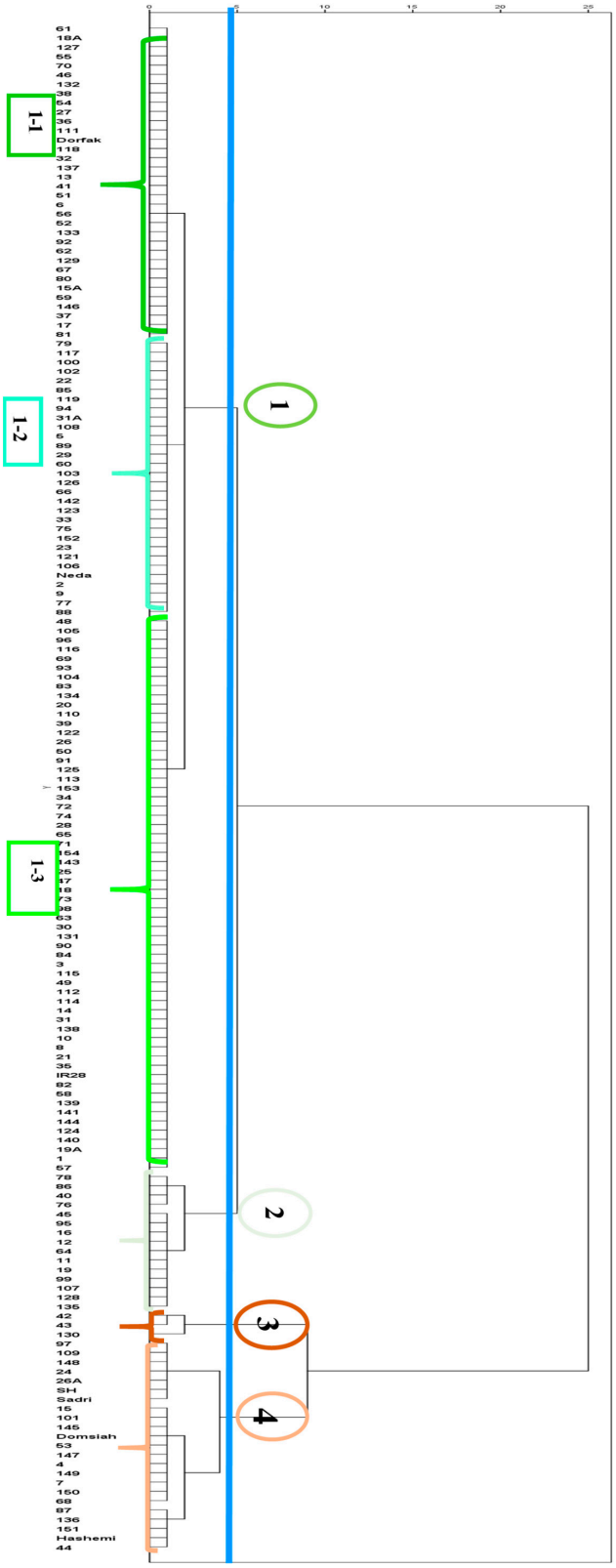

Figure 2. Dendrogram of cluster analysis of the recombinant inbred rice line population along with control varieties based on traits related to rice leaf blast disease using Ward's method in 2021. Dark green and dark brown colors have been used to show the most resistant and susceptible groups to blasts, respectively. Lighter colors have less sensitivity and resistance. (Information on rice genotypes was presented in Table 1).

Table 5. The information on separate groups from cluster analysis includes the mean, the number of group members, and the Z-score of the groups in response to blast disease compared to the total average in 2021.

Group Information	Trait	Group Mean	Z-Score
Group name: 1.1 Memberships: 34	Severity 1 [1]	0.0000	−0.1793
	Severity 2	0.0000	−0.2144
	Severity 3	0.0705	−0.3105
	Severity 4	0.1121	−0.3400
	Severity 5	0.3324	−0.3962
	AUDPC	2.4419	−0.3704
	r	0.0016	−0.4856
	IT	0.5882	−0.7183
Group name: 1.2 Memberships: 30	Severity 1	0.0013	−0.1333
	Severity 2	0.0114	−0.1815
	Severity 3	0.1877	−0.2092
	Severity 4	0.2592	−0.2870
	Severity 5	0.5702	−0.3569
	AUDPC	5.2087	−0.3136
	r	0.0026	−0.4121
	IT	1.4444	−0.2004
Group name: 1.3 Memberships 60	Severity 1	0.0005	−0.1615
	Severity 2	0.0024	−0.2076
	Severity 3	0.1316	−0.2577
	Severity 4	0.1790	−0.3159
	Severity 5	0.5321	−0.3632
	AUDPC	4.0550	−0.3373
	r	0.0026	−0.4131
	IT	0.9944	−0.4726
Group name: 2 Memberships: 15	Severity 1	0.0012	−0.1381
	Severity 2	0.0070	−0.1944
	Severity 3	0.3195	−0.0954
	Severity 4	1.0673	0.0042
	Severity 5	2.9782	0.0416
	AUDPC	20.1839	−0.0062
	r	0.0129	0.3477
	IT	2.5111	0.4448
Group name: 3 Memberships: 3	Severity 1	0.1385	4.7052
	Severity 2	1.7693	4.8747
	Severity 3	7.6764	6.2596
	Severity 4	18.6184	6.3278
	Severity 5	36.0052	5.5067
	AUDPC	322.9518	6.2097
	r	0.0428	2.5525
	IT	7.5556	3.4960
Group name: 4 Memberships: 23	Severity 1	0.0146	0.3367
	Severity 2	0.2784	0.5864
	Severity 3	1.1824	0.6499
	Severity 4	3.4782	0.8728
	Severity 5	10.3013	1.2533
	AUDPC	70.6786	1.0305
	r	0.0322	1.7733
	IT	4.7681	1.8100

[1] Severity 1–5: Disease severity in the first-fifth recording (%), AUDPC: area under the disease progress curve, r and IT: rate and type of infection. Dark green and dark brown colors have been used to show the most resistant and susceptible groups to blasts, respectively. Lighter colors have less sensitivity and resistance.

Based on the cluster analysis results, all individuals were classified into four groups. Group 1, being the largest group, consisted of genotypes that showed greater resistance compared to others in the entire population. To identify the most resistant genotypes, Group 1 was further subdivided into three subgroups (1-1, 1-2, and 1-3). Among these subgroups, 1-1 comprised the most resistant genotypes, consisting of 34 members. As depicted in

Table 5, the Z-Scores for all traits in this group were the most negative, indicating the lowest susceptibility and the greatest deviation from the overall average in terms of all the traits. The average AUDPC for subgroup 1-1 was estimated to be 2.442, with infection rates and types of 0.003 and 1.444, respectively. Subgroups 1-3, consisting of 60 members, ranked next, followed by subgroups 1-2 with 30 members. All three subgroups displayed negative Z-Scores for all traits. Group 3 and, subsequently, Group 4 were identified as the most susceptible genotypes. These groups had 3 and 23 members, respectively, and had the highest Z-Score values for all traits. The average AUDPC for these two groups was estimated to be 322.952 and 70.679, respectively. Additionally, these two groups exhibited the highest infection rate and type.

3.2.2. Cluster Analysis of the 2022 Dataset

Derived from the cluster analysis results from 2022, all individuals were categorized into seven groups (Figure 3, Table 6). Group 1, consisting of 32 genotypes, was identified as the most resistant group to blast disease that year. This group exhibited the lowest and most negative Z-Scores for all infection-related traits, and the average AUDPC for this group was estimated to be 3.068. The resistant control varieties, including Dorfak as a resistant verity and IR28 (a resistant parent), along with two aerobic rice varieties (18A and 19A), were included in this group. Following in terms of resistance were groups 3, 4, and 2 in the subsequent stages. The most susceptible genotypes were found in groups 5 and 7, with group 6 also showing susceptibility. Lines 42 and 43 were identified as the most susceptible lines in 2022. The aerobic rice 26A, along with these two lines, was placed in the most susceptible group, which had the highest average AUDPC of 172.627. Additionally, this group exhibited the highest Z-Score values for all traits. The next susceptible group was group 7, which included the Hashemi variety and the SH (susceptible parent), along with lines 12, 68, 101, 109, 130, and 151.

Table 6. The information on separate groups from cluster analysis includes the mean, the number of group members, and the Z-score of the groups in response to blast disease compared to the total average in 2022.

Group Information	Trait	Group Mean	Z-Score
Group name: 1 Memberships: 32	Severity 1 [1]	0.0009	−0.2941
	Severity 2	0.0113	−0.3505
	Severity 3	0.1534	−0.4072
	Severity 4	0.1934	−0.3963
	Severity 5	0.1595	−0.3528
	AUDPC	3.0683	−0.3944
	r	0.0014	−0.4591
	IT	0.2188	−0.9526
Group name: 2 Memberships: 20	Severity 1	0.0314	0.3594
	Severity 2	0.2408	0.1190
	Severity 3	0.6237	0.0106
	Severity 4	0.7702	0.0187
	Severity 5	0.5891	−0.0854
	AUDPC	13.6149	0.0096
	r	0.0052	0.0766
	IT	1.3500	0.3214
Group name: 3 Memberships: 41	Severity 1	0.0022	−0.2673
	Severity 2	0.0248	−0.3229
	Severity 3	0.2391	−0.3310
	Severity 4	0.2934	−0.3243
	Severity 5	0.2080	−0.3226
	AUDPC	4.6365	−0.3343
	r	0.0021	−0.3585
	IT	0.6667	−0.4482

Table 6. *Cont.*

Group Information	Trait	Group Mean	Z-Score
Group name: 4 Memberships: 51	Severity 1	0.0033	−0.2426
	Severity 2	0.0454	−0.2806
	Severity 3	0.2968	−0.2798
	Severity 4	0.3557	−0.2795
	Severity 5	0.2551	−0.2933
	AUDPC	5.7907	−0.2901
	r	0.0026	−0.2959
	IT	1.0000	−0.0728
Group name: 5 Memberships: 3	Severity 1	0.2359	4.7472
	Severity 2	3.1416	6.0511
	Severity 3	7.1521	5.8101
	Severity 4	9.0822	5.9984
	Severity 5	10.3343	5.9812
	AUDPC	172.6270	6.1013
	r	0.0446	5.6402
	IT	4.5556	3.9314
Group name: 6 Memberships: 8	Severity 1	0.0651	1.0821
	Severity 2	0.7228	1.1045
	Severity 3	2.7194	1.8723
	Severity 4	3.4860	1.9725
	Severity 5	4.0725	2.0831
	AUDPC	62.9790	1.9008
	r	0.0203	2.2066
	IT	3.2500	2.4611
Group name: 7 Memberships: 10	Severity 1	0.0270	0.2657
	Severity 2	0.6422	0.9398
	Severity 3	1.5403	0.8248
	Severity 4	1.5898	0.6083
	Severity 5	1.7816	0.6570
	AUDPC	32.7360	0.7422
	r	0.0106	0.8377
	IT	2.3667	1.4663

[1] Severity 1–5: Disease severity in the first-fifth recording (%), AUDPC: area under the disease progress curve, r and IT: rate and type of infection. Dark green and dark brown colors have been used to show the most resistant and susceptible groups to blasts, respectively. Lighter colors have less sensitivity and resistance.

3.2.3. Identification of the Most Resistant and Susceptible Common Rice Genotypes over Two Years

In order to identify the rice genotypes that were most resistant and susceptible in both years, a Venn diagram was utilized. Figure 4 represents the Venn diagram of the most resistant genotypes, while Figure 5 shows the Venn diagram of the most susceptible genotypes.

A total of 34 and 32 genotypes were considered from the most resistant group in 2021 and 2022, respectively. The genotypes that exhibited overlap in both years were identified. Among two groups, 20 genotypes were found to be common across the two years, including lines 13, 17, 27, 32, 37, 38, 46, 51, 54, 59, 61, 80, 81, 111, 127, 129, 132, 146, aerobic rice 18A, and the Dorfak variety. The average trait values for these 20 genotypes are presented in Table 7. The average AUDPC for these genotypes was estimated to be 2.611 $\pm$ 0.144, indicating their high level of resistance.

To identify the genotypes that were both most susceptible and common between the two years, a total of 26 and 21 genotypes from the most susceptible group in 2021 and 2022 were considered, respectively (Figure 5). Among them, 19 genotypes showed overlap in both years, including lines 4, 7, 24, 42, 43, 68, 97, 101, 109, 130, 147, 148, 149, 150, 151, 26A, Domsiah, Hashemi, and the SH parent. The average trait values for these 19 genotypes are presented in Table 7. The average AUDPC for these genotypes was estimated to be 90.633$\pm$15.686, indicating their high resistance.

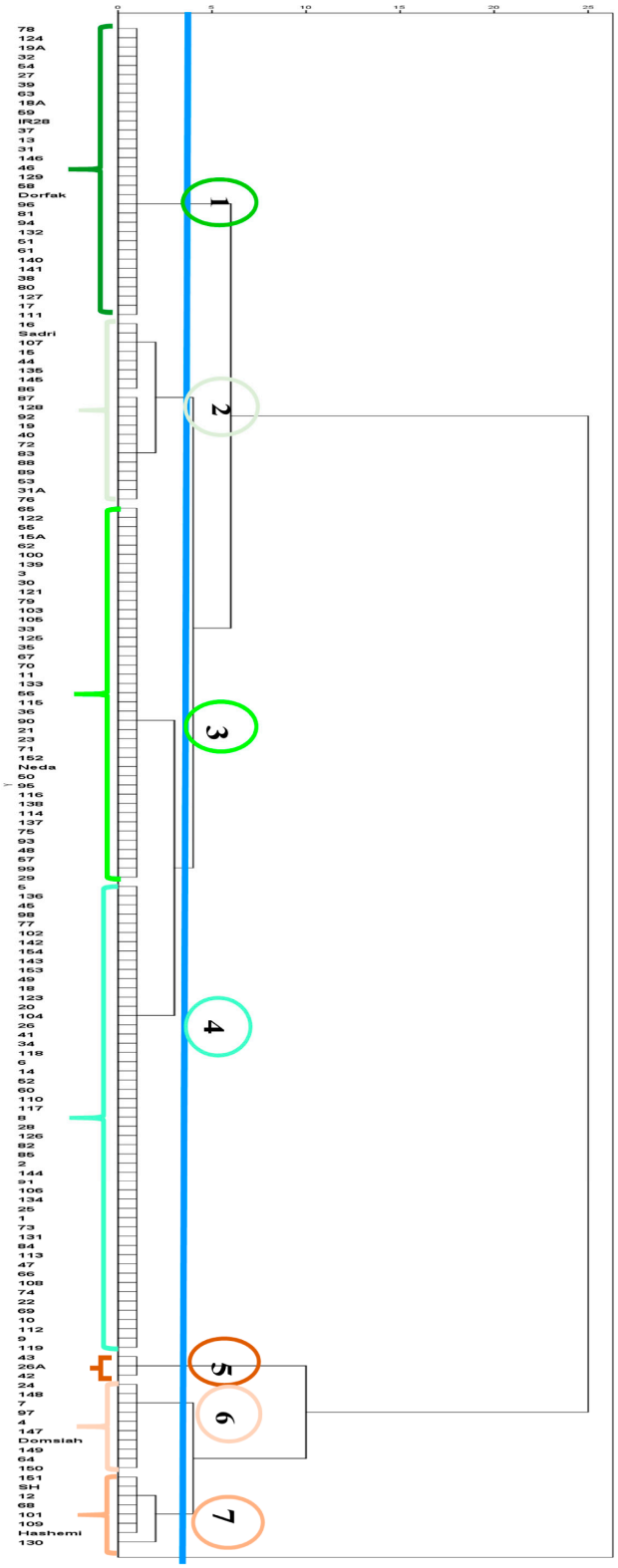

Figure 3. Dendrogram of cluster analysis of the recombinant inbred rice line population along with control varieties based on traits related to rice leaf blast disease using Ward's method in 2022. Dark green and dark brown colors have been used to show the most resistant and susceptible groups to blasts, respectively. Lighter colors have less sensitivity and resistance. (Information on rice genotypes was presented in Table 1).

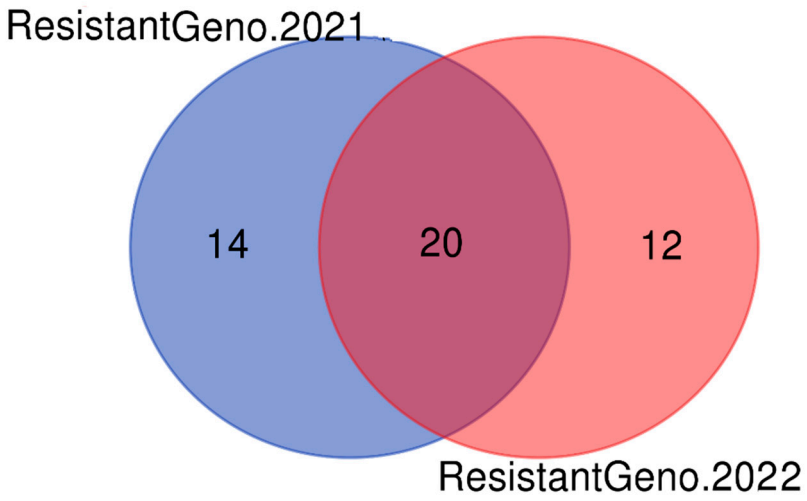

Figure 4. Venn diagram to identify the most resistant rice genotypes to leaf blast disease that were common in the two assessment years of 2021 and 2022. Thirty-four and 32 genotypes of the most resistant genotypes were selected in 2021 and 2022, respectively. Among these genotypes, 20 genotypes were common in two years.

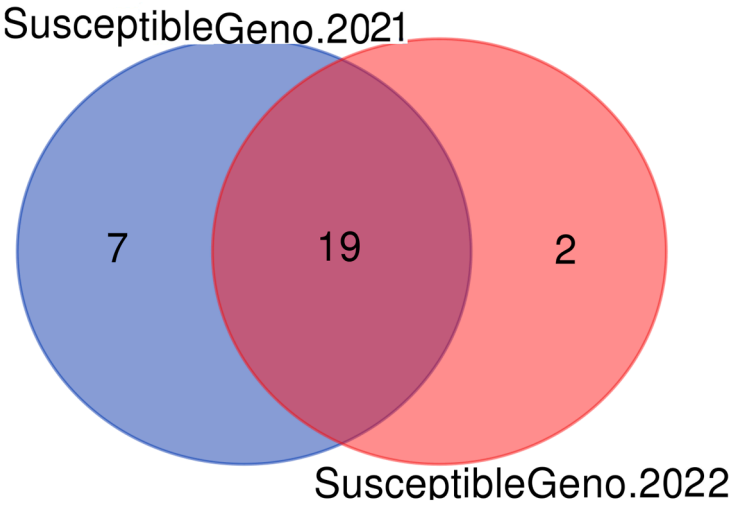

Figure 5. Venn diagram to identify the most susceptible rice genotypes to leaf blast disease that were common in the two assessment years of 2021 and 2022. Twenty-six and 21 genotypes of the most susceptible genotypes were selected in 2021 and 2022, respectively. Among these genotypes, 19 genotypes were common in two years.

Table 7. The mean of traits related to resistance to leaf blast disease in the most susceptible and resistant rice genotypes in two years.

	Traits Related to Resistance to Leaf Blast Disease							
Genotypes	**Severity 1** [1]	**Severity 2**	**Severity 3**	**Severity 4**	**Severity 5**	**AUDPC**	**r**	**IT**
Most resistants	0.001 ± 0.001	0.006 ± 0.002	0.097 ± 0.008	0.149 ± 0.009	0.241 ± 0.011	2.611 ± 0.144	0.001 ± 0.000	0.383 ± 0.024
Most susceptibles	0.050 ± 0.016	0.789 ± 0.164	2.541 ± 0.506	4.863 ± 0.912	9.458 ± 1.446	90.633 ± 15.686	0.028 ± 0.002	4.272 ± 0.217

[1] Severity 1–5: Disease Severity in the first-fifth recording (%), AUDPC: area under the disease progress curve, and r and IT: rate and type of infection.

In each series of susceptible and resistant genotypes, the fifth sampling stage exhibited the highest levels of infection severity, 0.241% and 9.458%, respectively. Initially, during the first and second recording stages, no significant differences were observed as disease symptoms had not yet emerged. However, as the evaluation progressed, the variations between genotypes became increasingly noticeable. Upon comparing the average infection severity in the fourth and fifth stages, along with the AUDPC, infection rate, and infection type indices, it became evident that significant disparities existed between the most resistant and most susceptible genotypes that were identified.

3.3. MGIDI Index Selections

The heritability (h2) ranged from 0.461 (for Severity 1) to 0.844 (for AUDPC). The result of PCA showed that the first component with eigenvalues ≥ 1 (7.08) accounted for 88.45% of the total variation among the traits. The selection differential and gain ranged from −46.11 to −72.44 and −0.002 to −10.35 for Severity 1 and AUDPC, respectively (Table 8). AUDPC showed the highest selection differential and genetic gain percent (−72.44% and −61.13%, respectively). In general, the MGIDI index provided a total SD of −59.12% for traits that all tended to decrease.

Table 8. The predicted selection differentials and selection gain for traits related to resistance to leaf blast disease in the MGIDI (multi-trait genotype-ideotype distance) analysis.

Genotypes	Xo	Xs	SD	SD%	h2	SG	SG%	Sense
Severity 1 [1]	0.010	0.005	−0.005	−46.113	0.461	−0.002	−21.264	decrease
Severity 2	0.129	0.062	−0.067	−51.948	0.539	−0.036	−28.005	decrease
Severity 3	0.521	0.169	−0.352	−67.580	0.810	−0.285	−54.767	decrease
Severity 4	0.900	0.283	−0.617	−68.605	0.808	−0.499	−55.456	decrease
Severity 5	1.727	0.846	−0.880	−50.995	0.588	−0.518	−30.007	decrease
AUDPC	16.924	4.665	−12.259	−72.437	0.844	−10.346	−61.131	decrease
r	0.006	0.003	−0.004	−54.688	0.706	−0.002	−38.629	decrease
IT	1.420	0.560	−0.861	−60.591	0.843	−0.726	−51.106	decrease
mean				−59.120		−1.552	−42.546	

[1] Severity 1–5: Disease Severity in the first-fifth recording (%), AUDPC: area under the disease progress curve, r, and IT: rate and type of infection. Xo: The original population mean. Xs: The mean of selected genotypes, SD, and SD%: The selection differential and selection differential in percentage, respectively, h2: The broad-sense heritability after selection, SG and SG%: The selection gains and selection gains in percentage, respectively, sense: The desired selection sense.

Figure 6 shows the ranking of the 165 rice genotypes according to the MGIDI index. In total, 20 genotypes—17, 80, 111, 127, 81, 38, 37, 61, 132, 59, Dorfak, 58, 46, 141, 18A, 54, 27, 140, 51, and 32—were selected.

3.4. Comparison of RILs with Control Varieties and Population Parents

Table 9 presents the mean traits associated with blast resistance for control varieties, population parents, and RILs over two years.

The RILs demonstrated intermediate trait values, as they were derived from crossing the two parents (SH and IR28). Despite the genetic diversity within the population and the presence of resistant, susceptible, semi-resistant, and semi-susceptible lines, the number of lines exhibiting resistance outnumbered the susceptible ones overall. In comparison to the control varieties, Dorfak displayed the highest level of resistance, whereas genotype 26A proved to be the most susceptible control variety in both years.

Table 9. The mean of traits related to the rice leaf blast RILs population, resistant and susceptible control varieties, and parents of the population in 2021 and 2022.

			Resistant Control Varieties								Susceptible Control Varieties			
Year	Trait	RILsMean	15A	18A	19A	Nona Bokra	Dorfak	Neda	IR28 [2]	SH [3]	Hashemi	Domsiah	26A	Sadri
2021	Severity 1 [1]	0.005	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
	Severity 2	0.067	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.519	1.499	0.000	0.000	0.000
	Severity 3	0.417	0.154	0.035	0.033	0.120	0.000	0.011	0.018	1.106	2.776	0.297	1.720	0.867
	Severity 4	0.982	0.043	0.069	0.154	0.180	0.058	0.342	0.020	8.002	6.834	1.175	4.436	2.604
	Severity 5	2.461	0.280	0.192	0.491	1.021	0.296	0.640	0.245	17.043	9.098	4.384	16.445	23.262
	AUDPC	18.898	2.363	1.399	3.029	5.678	1.444	4.710	1.116	127.037	109.603	25.646	100.653	105.709
	r	0.007	0.002	0.001	0.002	0.006	0.002	0.002	0.002	0.058	0.014	0.019	0.046	0.076
	IT	1.697	0.333	0.667	1.000	1.333	0.667	1.333	1.000	5.000	5.333	4.667	6.333	5.667
2022	Severity 1	0.012	0.000	0.000	0.000	0.014	0.000	0.000	0.000	0.083	0.000	0.167	0.200	0.177
	Severity 2	0.166	0.000	0.028	0.008	0.110	0.033	0.000	0.000	0.855	0.484	0.697	2.149	0.369
	Severity 3	0.557	0.189	0.144	0.155	0.525	0.100	0.233	0.175	2.855	1.789	1.389	7.236	0.933
	Severity 4	0.665	0.239	0.236	0.205	0.968	0.147	0.276	0.265	3.897	3.110	1.366	9.423	0.887
	Severity 5	0.634	0.139	0.182	0.135	0.429	0.118	0.273	0.180	3.874	4.761	2.140	10.064	0.544
	AUDPC	11.977	3.483	3.494	3.057	12.775	2.370	4.517	3.709	67.101	54.342	32.237	167.581	17.850
	r	0.004	0.002	0.002	0.002	0.006	0.001	0.002	0.002	0.022	0.020	0.008	0.046	0.005
	IT	1.031	0.667	0.333	0.333	0.667	0.333	0.667	0.333	2.667	3.333	2.333	4.333	2.000

[1] Severity 1–5: Disease Severity in the first-fifth recording (%), AUDPC: area under the disease progress curve, r and IT: rate and type of infection, [2, 3] IR28, and SH (Shahpasand) are resistant and susceptible parents of the RIL population, respectively.

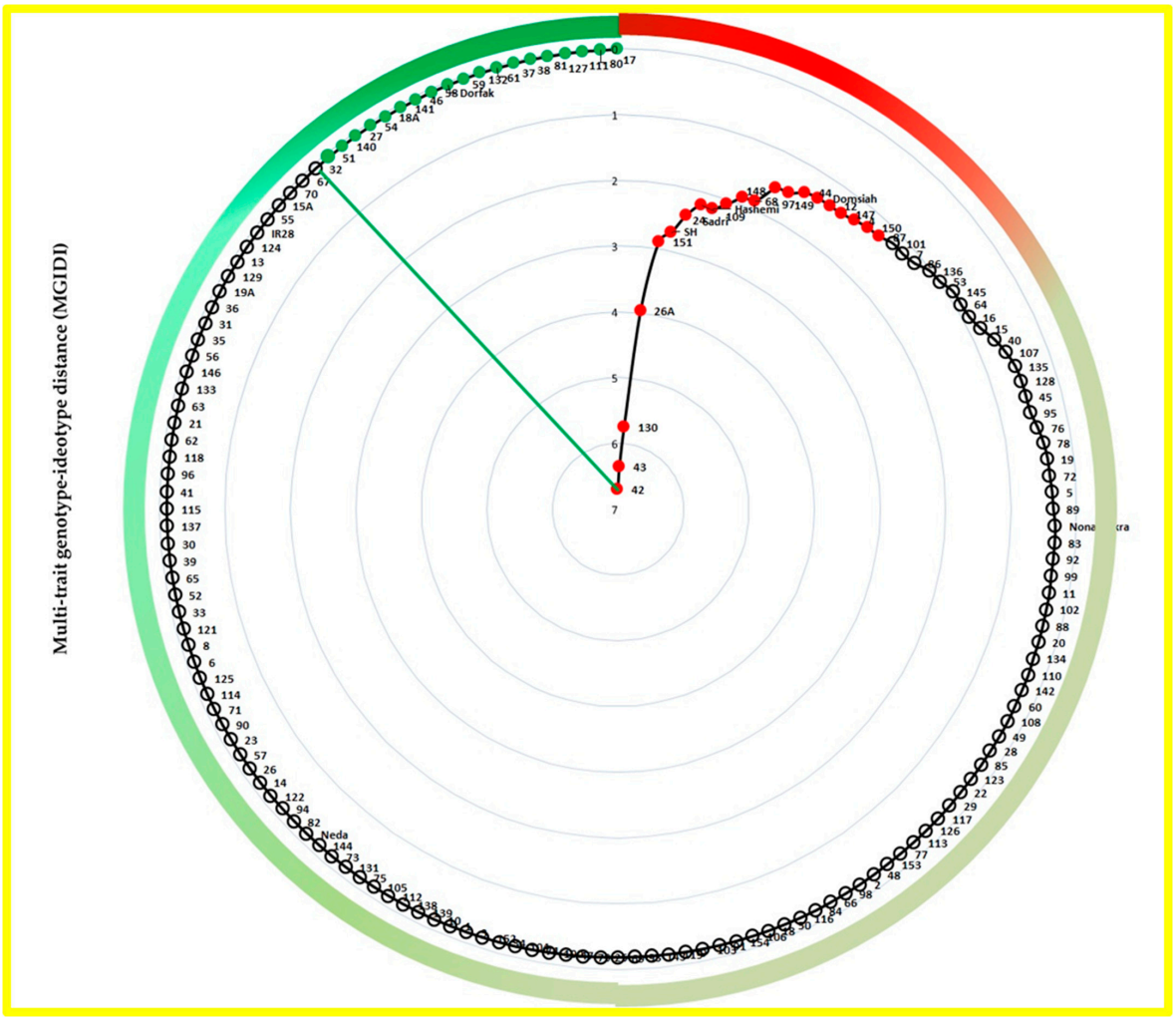

Figure 6. Polar plot based on the MGIDIs (multi-trait genotype-ideotype distance) indices. The selected superior genotypes as the most resistant genotypes are shown in green. The 43° cut angle represents the cut point according to the selection intensity of 12% (out of 165 genotypes). The selected resistant genotypes include 17, 80, 111, 127, 81, 38, 37, 61, 132, 59, Dorfak, 58, 46, 141, 18A, 54, 27, 140, 51, and 32. The dark green and dark red colors in the circle's circumference have been used to show the most resistant and susceptible. Lighter colors have less sensitivity and resistance.

4. Discussion

Studying rice blast disease is essential due to its primary impact on carbohydrate production. The main effect of blast disease is a reduction in carbohydrate synthesis caused by the damage inflicted on leaf photosynthesis and respiration. This decrease in carbohydrate production leads to a slowdown in crop growth and a decrease in leaf area. Consequently, there is a reduction in the amount of green leaf present during grain filling, resulting in decreased carbohydrate levels. This significant mechanism can negatively affect grain yield even before flowering occurs [13]. However, from a breeding perspective, using blast-resistant rice varieties has been proven to be an effective strategy to prevent damage caused by this disease [18]. Various research studies have been conducted to identify and develop resistant genotypes. A research study aimed at assessing the relative resistance of certain rice varieties to blast disease (*Magnaporthe grisea* (Hebert) Barr) and determining the AUDPC was conducted on 58 local rice varieties and promising lines under greenhouse and field conditions. The findings demonstrated that the IR24 variety, along with promising lines F125 and F120-2, exhibited the lowest levels of disease progression, as indicated by the smallest AUDPC ranging from 20 to 30. Conversely, the Domsiah and Hasani varieties, as well as promising lines F35-1 and F63-3, displayed the highest AUDPC values, ranging from 50 to 60. The genotypes were categorized into three groups following cluster analysis: resistant, tolerant, and susceptible to blast disease [16]. In another study that evaluated some of the resistance components to blast, it was reported that Iranian local varieties and lines, including C104-PKT and CO-39, were classified as susceptible based on the AUDPC and infection type. On the other hand, Iranian improved varieties, lines obtained from the International Rice Research Institute, and near-isogenic lines, except for the two lines mentioned above, were categorized as resistant. Varieties like Fuji-Minori, Onda, and Hassan-Saraei were placed in the moderate group (semi-susceptible) [15]. Results from another field experiment aimed at identifying and screening different rice genotypes against blast disease demonstrated that the genotype Shankharika exhibited high disease severity and AUDPC, indicating its increased susceptibility to blast disease. Conversely, the genotype Sabitri showed lower disease severity and AUDPC, indicating a higher resistance level to blast disease [17]. In a study conducted to identify resistant and susceptible rice genotypes by screening them against blast disease under field conditions, 72 genotypes were evaluated over two consecutive cropping seasons (2017 and 2018). The results revealed varying responses of the genotypes to blast disease between the two years. Eleven genotypes out of the 72 exhibited a wide range of AUDPC values from 1800 to 2550, indicating high susceptibility. Eight genotypes displayed AUDPC values ranging from 115 to 250, indicating susceptibility, while nine genotypes demonstrated AUDPC values between 55 and 115, indicating relative resistance [19]. The results of previous studies aimed at assessing the genetic diversity of blast resistance in different rice varieties have indicated that varieties like Domsiah, Sadri, and Hashemi are susceptible to blast disease, while Neda and Dorfak varieties are classified as genotypes showing relative resistance to blast disease [18,29,30], which aligns with the findings of this current study. However, the selection of resistant lines from the F11 recombinant inbred lines sets this study apart from previous research.

In this study, the accuracy values of the majority of variables, especially AUDPC and IT, were very high, as were the accuracy values of the rest of the variables; in general, estimates above 0.70 are sufficient to explicitly conclude the genetic value of genotypes [31,32]. In addition, AUDPC showed the highest selection genetic gain percent (−61.13%), and in general, the MGIDI provided a total SD of −59.12% for traits with decreased sense. According to the results, we found that the selection based on MGIDI provided satisfactory gain. The measured MGIDI, based on the BLUP data from multiple traits in two years, selected 20 resistant genotypes (17, 80, 111, 127, 81, 38, 37, 61, 132, 59, Dorfak, 58, 46, 141, 18A, 54, 27, 140, 51, and 32) that highly overlapped with selected genotypes using cluster analysis.

Since the selection of suitable genotypes has always been a challenge for plant breeders, the use of MGIDI has grown widely among breeders, helping to identify superior genotypes that combine desired different traits [33–36]. Amrate et al. [37] used the MGIDI method to identify high-yielding charcoal rot-resistant soybean genotypes.

The MGIDI is calculated based on BLUPs that have the ability to eliminate environmental variance and provide accurate estimations of individual breeding values. As a result, they are becoming more prevalent among plant breeders for precise genotypic value estimation [32]. Also, this method is free from the multi-collinearity problem [38].

Some lines, such as 17 and 111, demonstrated greater resistance with the lowest AUDPC values (1.526 and 1.630, respectively) compared to all the tested control varieties and the SH (resistant parent). The RILs are an ideal population for conducting breeding studies and can be generated using the single-seed descent (SSD) method. SSD enables the production of diverse genetic variations, enhances the chances of transgressive segregation, and facilitates the development of homozygous lines. According to previous research, resistance can be effectively achieved through transgressive segregation, in which a segregating hybrid exhibits novel phenotypes due to the epistatic interactions of genes ([39,40]). Transgressive segregation has also been observed for Verticillium wilt resistance in an F2 (resistant Pima S-7 $\times$ susceptible Acala 44) [41].

In addition, one notable advantage of RILs compared to F2 populations is their ability to generate an ample supply of seeds for experimental designs with replicates [42]. In the present study, the RILs population was developed through a cross between two parents, SH (susceptible) and IR28 (resistant). The selection of these parents was based on their distinct genetic and phenotypic responses to blast disease, enabling the creation of a population with maximal genetic diversity for targeted selection purposes. Through the implementation of cluster analysis and the MGIDI method, 17 genotypes, including 17, 111, Dorfak, 38, 127, 54, 81, 80, 18A, 27, 61, 46, 37, 59, 32, 132, and 51, were identified as the most resistant genotypes over two years. The mean value of the AUDPC for these resistant genotypes was determined to be 2.435 ± 0.114.

Furthermore, the identification of susceptible and resistant lines holds promising implications for the development of new populations and future breeding programs. By crossing highly susceptible and highly resistant lines, gene exchange between the parental lines can be facilitated, leading to the generation of a broad spectrum of diverse outcomes in subsequent generations. This approach provides a valuable genetic background for selecting new resistant lines possessing desirable phenotypic traits [20,21].

5. Conclusions

The research findings show significant diversity among the rice-inbred lines in their response to leaf blast. Specifically, certain genotypes displayed higher levels of resistance compared to others across both years of the study. Notably, 17 genotypes exhibited superior resistance. In this study, the selected genotypes, identified using cluster analysis and MGIDI, hold immense potential for utilization in breeding programs to develop recombinant populations by crossing highly susceptible and resistant lines. This approach will maximize the genetic diversity for selecting new blast-resistant lines.

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