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Genetic Variation in Tolerance to Iron Deficiency among Species of *Oryza*

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Abstract: Transplanted rice cultivation has caused groundwater depletion in several regions globally. Direct-seeded rice under aerobic conditions is a water-saving alternative. However, under aerobic conditions, iron in the soil is oxidized from the ferrous to ferric forms, which are not easily available to rice crops, resulting in iron-deficiency-induced chlorosis (IDIC) and causing significant reductions in yield. Cultivated rice accessions have limited variations in IDIC tolerance, while the wild Oryza germplasm could be a potential source of IDIC tolerance. In this study, 313 Oryza accessions were evaluated for IDIC tolerance at the tillering stage under aerobic conditions and 20 IDIC-tolerant lines were identified. The twenty lines showed no signs of chlorosis and had high levels of iron content and SPAD values, while the eight cultivated controls exhibited varying degrees of chlorosis symptoms and low levels of SPAD and iron content. To confirm their tolerance, the selected lines were evaluated again in a subsequent year, and they showed comparable levels of tolerance, indicating that these lines were efficient in iron uptake and utilization, resulting in maintained high chlorophyll and leaf area index. These accessions may be useful for developing IDIC-tolerant cultivars for aerobic rice cultivation and future studies of the molecular basis of IDIC tolerance.

Keywords: aerobic rice; chlorosis; iron deficiency; *Oryza*; wild germplasm; IDIC; SPAD

Citation: Kumar, R.; Yer, H. Genetic Variation in Tolerance to Iron Deficiency among Species of *Oryza*. *Crops* **2023**, *3*, 184–194. https://doi.org/10.3390/crops3030018

Received: 3 April 2023
Revised: 23 May 2023
Accepted: 19 June 2023
Published: 21 June 2023

1. Introduction

Rice (*Oryza sativa* L., 2n = 24) is an important cereal and the primary food source for over one-third of the world's population [1]. It is the second-largest crop grown globally, with 160 million hectares of rice cultivation worldwide [2]. Rice is a staple crop in many countries, and over 90% of its production is consumed in Asia [1]. Despite its significance as a food source, rice cultivation poses significant challenges, including high water usage. Irrigated agriculture accounts for 70–85% of global water usage, with rice being one of the most water-consuming crops [3]. This excessive water usage in rice cultivation has resulted in the depletion of groundwater levels in many rice-growing regions worldwide, which could have catastrophic consequences for future global rice production. Hence, the development of a new ideotype of rice that demands less water than the existing cultivation system is very important.

The high water requirement of rice poses a threat to future rice production, as the groundwater levels in many rice-growing regions are depleting [4,5]. Rice production in many high-yielding regions is at risk due to groundwater depletion, and there is a need to produce more rice with less water to sustain productivity [4,5]. Furthermore, the labor-intensive nature of transplanted rice has resulted in many farmers moving away from rice cultivation, necessitating a shift towards more efficient rice cultivation practices [5]. Aerobic rice cultivation offers several advantages over traditional rice cultivation methods, such as increased water-use efficiency, reduced greenhouse gas emissions, lower cultivation costs, and decreased labor requirements [6,7]. Bouman [8] suggested that aerobic rice could be grown under irrigated conditions, much like upland crops such as wheat and maize, by cultivating high-yielding rice varieties in direct-sown, non-puddled, aerobic soils with irrigation. This method of cultivation is known as direct-seeded aerobic rice and could potentially serve as an alternative to cope with depleting underground water and labor shortages in rice production [9,10].

Iron-deficiency-induced chlorosis (IDIC) is a major constraint in aerobic rice cultivation that significantly reduces crop yield and quality [11–14]. Although some studies have been conducted on IDIC in rice, there is still a lack of information on screening accessions for IDIC under aerobic conditions in rice germplasms [15]. Additionally, cultivated species have limited variation in these traits, making it difficult to develop iron-efficient cultivars. IDIC is a physiological disorder that affects the growth and yield of rice under aerobic conditions. In rice cultivated under aerobic conditions, the solubility of iron in the soil is limited due to its oxidation to the insoluble ferric form [11–14]. IDIC is characterized by the interveinal chlorosis of young leaves, reduced plant growth, and decreased yield [5]. The mechanism of IDIC in rice under aerobic conditions involves the regulation of iron uptake, translocation, and utilization [15,16].

Wild rice species possess a vast array of desirable alleles for abiotic stress tolerance, including IDIC tolerance, as they have evolved under diverse environmental conditions [17]. The *Oryza* genus includes 21 wild and 2 cultivated species of rice, and over 70% of the genetic variation in this genus is attributed to wild species [18]. *O. sativa* (L.), which originated from *O. nivara* and *O. rufipogon*, is grown worldwide, while *O. glaberrima* (Steud.), which originated in West Africa from *O. barthii* (A. Chev.), is grown on a limited scale [19].

IDIC is a common problem in rice cultivation, particularly under aerobic conditions. However, limited research has been conducted to date on screening rice accessions for IDIC tolerance under such conditions, and cultivated species exhibit limited variation in these traits [11–14]. Wild rice species have evolved under diverse environmental conditions and harbor desirable alleles for various biotic and abiotic stresses, making them a promising genetic resource for improving IDIC tolerance in cultivated rice. Punjab Agricultural University in Ludhiana, India, has a vast collection of wild rice species procured from the International Rice Research Institute in the Philippines. The purpose of this study was to screen wild *Oryza* species for IDIC tolerance at the tillering stage under direct-seeded aerobic conditions.

2. Materials and Methods

2.1. Climate

The experiment was conducted at Punjab Agricultural University, Ludhiana, utilizing both field and laboratory facilities. The geographical coordinates of the study site are 30°56′ N latitude and 75°52′ E longitude, and its mean altitude is 247 m above sea level. The area has a semi-arid sub-tropical climate with distinct seasonal variations. The summer season, which lasts from April to June, is hot and dry, while the monsoon season, from July to September, is hot and humid. The winter season can be divided into two parts, mild winter from October to November and cold winter from December to February. Soil samples were randomly obtained from six different locations within the experimental field prior to crop sowing. These field samples were initially air dried under shaded conditions and subsequently passed through a 2.0 mm sieve for chemical analysis. The analysis revealed that the soil contained 4.86 parts per million (ppm) of iron (Fe). Furthermore, the soil was classified as sandy loam and possessed a pH value of 6.2.

2.2. Plant Materials

In this study, a total of 313 rice accessions were used as plant material, consisting of 6 accessions of *O. glaberrima*, 105 accessions of *O. rufipogon*, 193 accessions of *O. nivara*, 1 accession of *O. barthii*, and 8 cultivated *O.* sativa accessions (Figure 1). Most of these species originated from India (271), followed by Myanmar (9) and Thailand (9). The *O. sativa*, *O. nivara*, and *O. rufipogon* accessions originated from Asia, while the *O. glaberrima* and *O. barthii* accessions originated from Africa. The experiment was conducted in a randomized block design (RBD) with three replications. Paired rows of 1.5 m length were sown for each entry, with a row spacing of 30 cm under dry direct-seeded conditions.

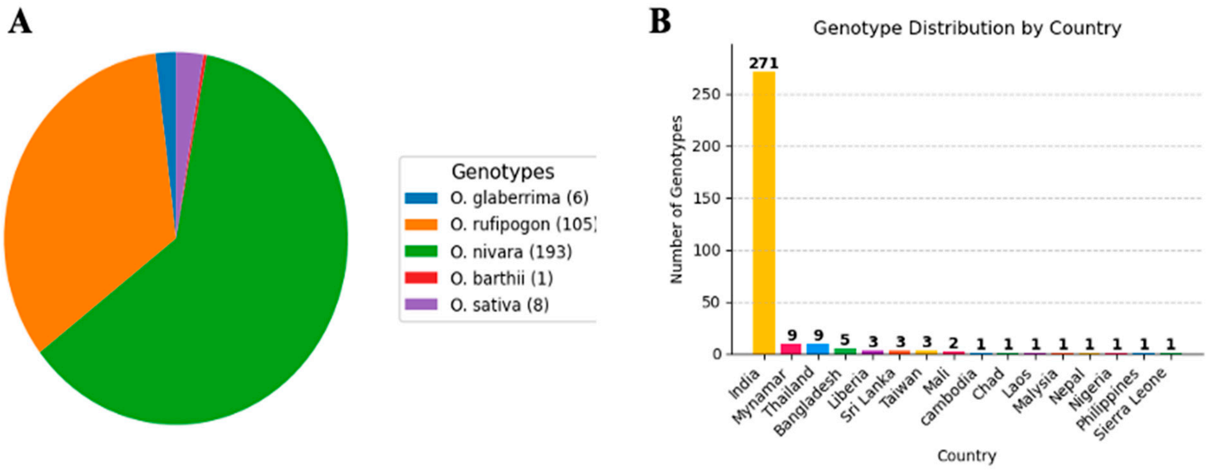

Figure 1. Information about the accessions used for the experiment. (**A**) Species-wise distribution of the germplasm into five groups. Majority of the accessions were *O. nivara* and *O. rufipogon.* (**B**) County-wise distribution of wild germplasm. Majority of wild germplasm belonged to India.

2.3. Irrigation Applied during Experiment

During the rice season, the field was irrigated immediately after sowing and thereafter on a weekly basis, depending on the amount of rainfall, to maintain the required soil moisture levels. In instances where there was rainfall, the scheduled irrigation was skipped, and additional irrigations were applied at 7-day intervals after the water drained from the field.

2.4. Screening Method for IDIC Tolerance of Wild Germplasm

To evaluate IDIC tolerance, the rice accessions were evaluated on a 1–5 scale after 28 days of sowing. The ratings were based on the degree of chlorosis observed in the plants [20]. A score of 1 indicated normal, green plants without any chlorosis, while a score of 2 indicated slight yellowing of the upper leaves. A score of 3 indicated interveinal chlorosis in the upper leaves without any stunting of growth or necrosis, and a score of 4 indicated interveinal chlorosis of the upper leaves with some stunting of growth or necrosis of plant tissue. A score of 5 indicated severe chlorosis with stunted growth and necrosis of the youngest leaves and growing point. The ratings were recorded in each plot to determine the IDIC tolerance of the rice accessions.

2.5. Chlorophyll Measurement and Leaf Area Index

The SPAD meter was used to measure chlorophyll content in the leaf. The SPAD value of each entry was determined after 28 days of sowing using a SPAD meter to measure the middle portion of the index leaf. Five randomly selected plants from each entry were measured to ensure accuracy, while wet leaves and plants that were widely spaced, tall, or short were avoided during the measurement process. The leaf area index (LAI) was recorded after four weeks at tillering using a digital plant canopy imager (Model CI-110/CI-120, CID Inc., Camas, WA, USA).

2.6. Iron Content in the Leaves

After four weeks of sowing, leaves were collected, thoroughly washed twice with deionized water, and then dried in a hot air oven at 65 °C for 3 days. The dried leaves were finely ground to analyze the Fe concentration. To analyze the total Fe, the dried samples were digested in a diacid mixture of HNO_3 and $HClO_4$ (3:1). The concentrations of Fe were determined using the atomic absorption spectrophotometry method described by Isaac and Kerber [21].

2.7. Statistical Analysis

The experiments were conducted in a randomized block design (RBD) with three replications. ANOVA was performed for all the recorded data using Python. A correlation analysis was carried out to investigate the correlation between IDIC rating, iron content, LAI, and SPAD reading using Python. Spearman's correlation coefficient was used to observed the correlation of IDIC (non-parametric data) with SPAD reading, Fe content, and leaf area index (parametric data). Pearson's correlation coefficient was used to observe the correlation for SPAD reading, Fe content, and the leaf area index. Ratings for IDIC were subjected to Kruskal–Wallis nonparametric tests using Python.

3. Results

3.1. Wild Oryza Germplasm Exhibited Significant Variation in IDIC Tolerance under Aerobic Field Conditions

IDIC is a major limiting factor for the adaptation of aerobic rice, affecting plant growth and yield. IDIC was scored on a 1–5 scale at the tillering stage, as described in the Materials and Methods section. The Kruskal–Wallis nonparametric test showed a significant difference ($p < 0.01$) between 313 genotypes in terms of IDIC rating. While 20 accessions remained green throughout the growing season (Rating 1), the remaining wild germplasm lines and conventional cultivars showed mild to severe chlorosis and stunting symptoms (Rating 2–5) (Table 1, and Figures 2 and 3). In contrast, all cultivars had a rating of 2 or higher, with Feng-ai-zai showing a rating of 5. Among the IDIC-tolerant accessions, 11 belonged to the *O. nivara* group and 9 belonged to the *O. rufipogon* group (Table 1). During the subsequent year, 20 selected IDIC-tolerant lines and 8 cultivars were evaluated for their tolerance to IDIC under aerobic conditions. Similar IDIC responses were observed compared with those from the previous year (Table 1). Of the 20 wild germplasm lines, 17 showed a rating of 1, while 3 showed a rating between 1 and 2. The correlation analysis showed that IDIC rating was negatively related to SPAD value (Spearman correlation, r = −0.69), iron content (Spearman correlation, r = −0.56), and LAI (Spearman correlation, r = −0.28) (Figure 4). These findings suggest that there is considerable variation in IDIC tolerance among rice accessions, with the twenty wild germplasm lines showing more tolerance compared with the conventional cultivars.

Table 1. List of the 20 selected tolerant and cultivated accessions with iron-deficiency-induced chlorosis (IDIC), SPAD value, leaf area index (LAI), and iron content. * A statistical difference was observed among the accessions.

Species	First Year				Second Year	
	IDIC Rating *	SPAD Reading *	Fe Content (ppm) *	LAI *	IDIC Rating *	SPAD Reading *
O. rufipogon (IR105491)	1 ± 0	32.5 ± 1.8	1294 ± 131	1.4 ± 0.13	1 ± 0	33.1 ± 1.3
O. rufipogon (CR100334A)	1 ± 0	31.6 ± 0.8	1119 ± 79	1.5 ± 0.21	1 ± 0	30.9 ± 0.9
O. rufipogon (CR100334B)	1 ± 0	31.2 ± 1.1	1261 ± 113	2.1 ± 0.19	1 ± 0	33.9 ± 1.3
O. rufipogon (CR100436)	1 ± 0	32.7 ± 1.9	1664 ± 143	1.4 ± 0.11	1.3 ± 0.47	30.2 ± 1.6
O. rufipogon (CR100462)	1 ± 0	32.8 ± 2.1	1042 ± 92	1.4 ± 0.16	1 ± 0	32.4 ± 1.7
O. rufipogon (IR80762A)	1 ± 0	32.9 ± 0.4	1229 ± 75	1.8 ± 0.09	1 ± 0	34.3 ± 0.9
O. rufipogon (CR100030)	1 ± 0	33.3 ± 1.4	2127 ± 193	1.2 ± 0.13	1 ± 0	32.8 ± 1.6
O. rufipogon (CR100001)	1 ± 0	35.3 ± 1.3	1056 ± 69	2.2 ± 0.10	1.3 ± 0.47	34.6 ± 1.6
O. rufipogon (CR100484)	1 ± 0	32.5 ± 1	1085 ± 82	1.4 ± 0.09	1 ± 0	33.1 ± 1.7
O. nivara (CR100454)	1 ± 0	31 ± 0.6	1005 ± 63	1.1 ± 0.14	1 ± 0	31.8 ± 1.6
O. nivara (CR100363)	1 ± 0	32.3 ± 2.2	2101 ± 173	1.4 ± 0.12	1 ± 0	31.2 ± 1.7
O. nivara (CR100371)	1 ± 0	33.8 ± 1.3	1055 ± 103	1.1 ± 0.12	1 ± 0	33.2 ± 1.1
O. nivara (CR100003)	1 ± 0	36 ± 1.6	1802 ± 144	1.5 ± 0.16	1 ± 0	34.7 ± 1.9
O. nivara (IR82018)	1 ± 0	29.7 ± 2.5	1287 ± 103	1.6 ± 0.17	1.66 ± 0.47	31.9 ± 1.9

Table 1. *Cont.*

Species	First Year				Second Year	
	IDIC Rating *	SPAD Reading *	Fe Content (ppm) *	LAI *	IDIC Rating *	SPAD Reading *
O. nivara (CR100124A)	1 ± 0	29.8 ± 0.8	1081 ± 89	1.5 ± 0.10	1 ± 0	31.5 ± 0.9
O. nivara (CR100127)	1 ± 0	30.3 ± 1.4	1662 ± 123	1.6 ± 0.12	1 ± 0	29.8 ± 1.1
O. nivara (CR100337)	1 ± 0	33.3 ± 1.1	1299 ± 91	1.3 ± 0.12	1 ± 0	33.9 ± 0.8
O. nivara (CR100426)	1 ± 0	31.6 ± 1.9	1079 ± 59	1.2 ± 0.08	1 ± 0	32.3 ± 1.7
O. nivara (CR100360)	1 ± 0	33.5 ± 0.9	1508 ± 179	1 ± 0.10	1 ± 0	31.5 ± 1.1
O. nivara (CR100429)	1 ± 0	29.4 ± 1.6	1318 ± 132	1.3 ± 0.07	1 ± 0	30.1 ± 1.5
PAU 201	2.7 ± 0.27	27.9 ± 1.2	565 ± 51	1.7 ± 0.11	3 ± 0	31.7 ± 0.9
Rasi	4 ± 0	29.2 ± 1.7	560 ± 60	1.2 ± 0.09	3.3 ± 0.27	29.7 ± 1.3
Basmati 370	1.67 ± 0.47	23.9 ± 0.9	580 ± 44	1.2 ± 0.07	2 ± 0.0	27.5 ± 2.1
Cheng-Hui 448	4 ± 0	26.9 ± 2.1	682 ± 77	1.2 ± 0.10	4 ± 0	26.9 ± 1.1
Feng-ai-zai	5 ± 0	28.2 ± 2.8	847 ± 92	1.1 ± 0.11	5 ± 0	29.1 ± 1.9
IR 64	3.3 ± 0.27	28.3 ± 1.7	734 ± 55	1.2 ± 0.10	4 ± 0	26.8 ± 1.9
Lemont	1.67 ± 0.47	31.1 ± 0.8	851 ± 73	1.6 ± 0.08	2 ± 0.0	32.1 ± 1.8
Nagina 22	3 ± 0	23.6 ± 1.9	765 ± 61	0.9 ± 0.08	3.3 ± 0.27	26.1 ± 1.4

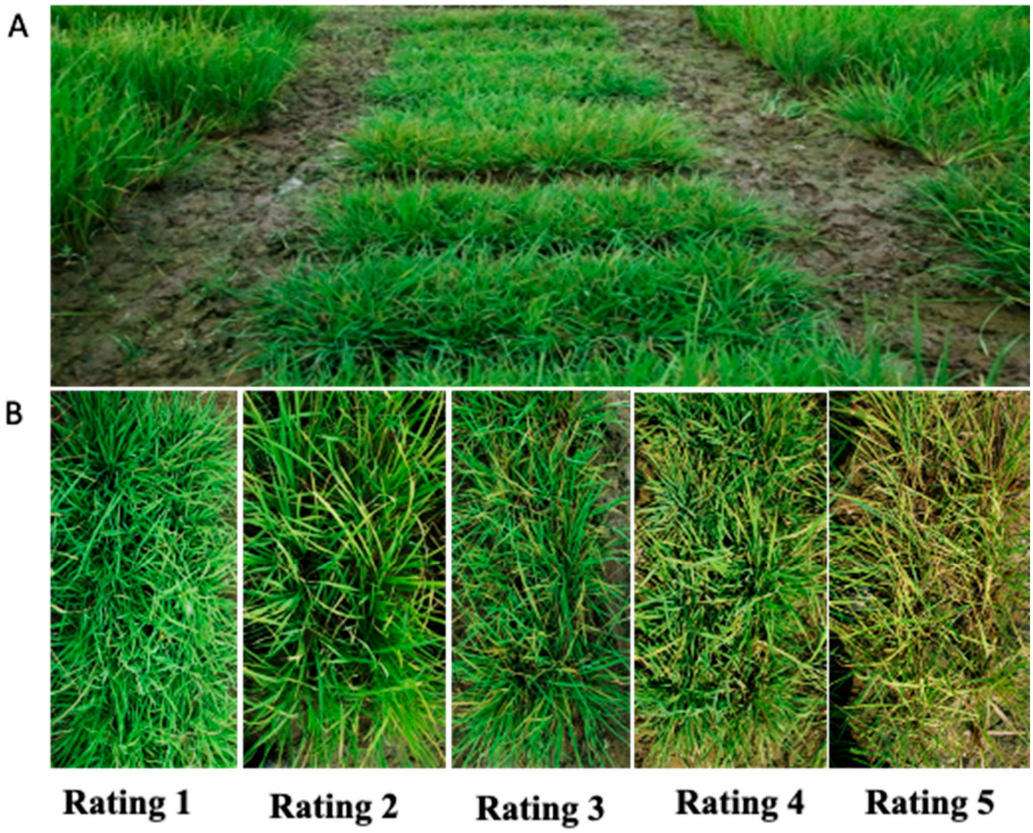

Figure 2. Evaluation of wild *Oryza* germplasm for iron-deficiency-induced chlorosis tolerance under aerobic conditions. (**A**) Wild *Oryza* wild germplasm comprising 313 accessions and cultivars were evaluated for iron-deficiency-induced chlorosis tolerance under aerobic conditions. IDIC rating was taken 28 days after sowing. (**B**) A 1–5 rating was given to score iron-deficiency-induced chlorosis tolerance. A score of 1 indicated no chlorosis and normal, green plants, while a score of 2 indicated slight yellowing of the upper leaves. A score of 3 indicated interveinal chlorosis in the upper leaves, but no obvious stunting of growth or death of leaf tissue (necrosis), and a score of 4 indicated interveinal chlorosis of the upper leaves with some stunting of growth or necrosis of plant tissue. A score of 5 indicated severe chlorosis with stunted growth and necrosis of the youngest leaves and growing point.

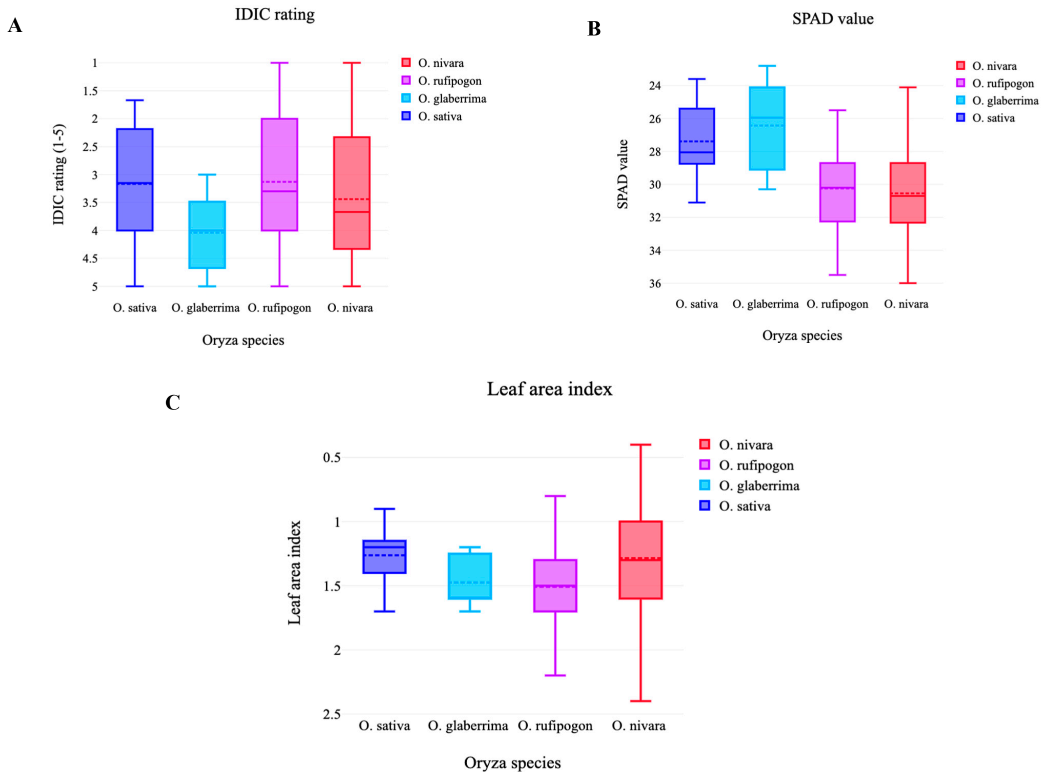

Figure 3. Characterization of wild germplasm for IDIC tolerance at the tillering stage. (**A**) A 1–5 rating was given to score iron-deficiency-induced chlorosis tolerance. A score of 1 indicated no chlorosis and normal, green plants, while a score of more than 1 indicated susceptibility to IDIC. (**B**) SPAD value (chlorophyll content) of the germplasm at the tillering stage. (**C**) LAI of the germplasm at the tillering stage.

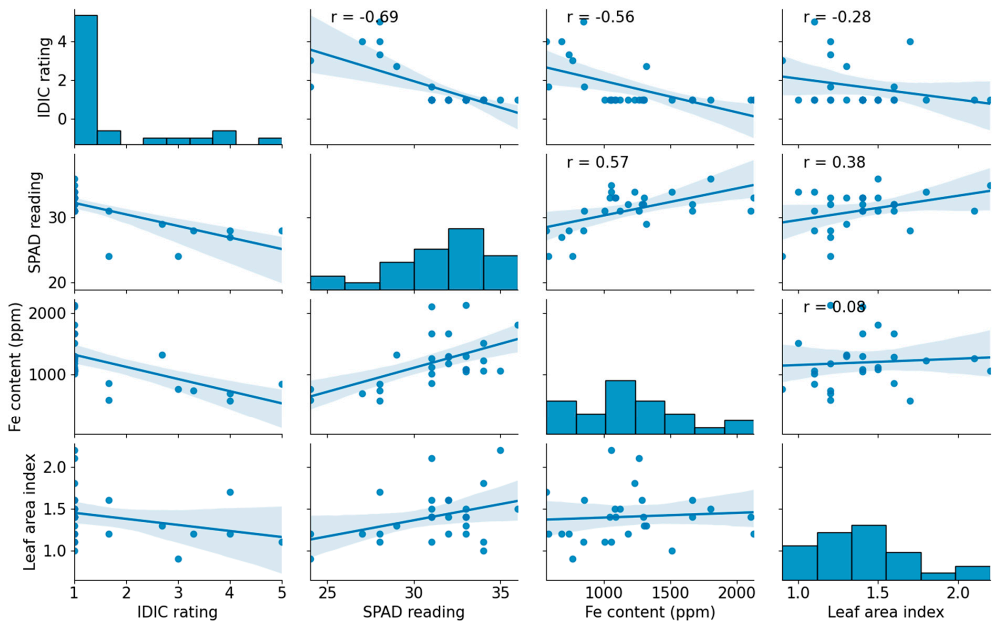

Figure 4. Correlation analysis between iron-deficiency-induced chlorosis (IDIC), SPAD value, leaf area index (LAI), and iron content. A strong correlation was observed between IDIC rating and iron content, while moderate correlation was observed between IDIC, SPAD value, and LAI. Spearman's correlation coefficient was used to observed correlation of IDIC (non-parametric data) with SPAD reading, Fe content, and leaf area index (parametric data), while Pearson's correlation coefficient was used to observe correlation for SPAD reading, Fe content, and leaf area index.

3.2. Wild Oryza Germplasm Exhibited Significant Variation in SPAD Value

A SPAD meter was used to measure chlorophyll content and relative greenness of leaves in all accessions. The results showed significant variations (F value, 0.33; $p < 0.01$) in the SPAD values across the accessions, ranging from 23.6 to 36, with the *O. nivara* and *O. rufipogon* species having the highest average SPAD values of 30.8 and 30.6, respectively, followed by *O. sativa* at 28.3 (Table 2). Among the wild accessions, *O. nivara* (CR 100003) had the highest SPAD value of 36, followed by *O. rufipogon* (CR 100001), *O. nivara* (CR 100371), and *O. nivara* (CR 100360), with values of 35.3, 33.8, and 33.5, respectively (Table 2 and Figure 3). Among the cultivars, Lemont had the highest SPAD value of 31.1, followed by Rasi, IR 64, and Feng-ai-zai, with values of 29.2, 28.3, and 28.2, respectively. Among the wild accessions, *O. nivara* (CR 100429) had the lowest SPAD value of 29.4, while Nagina 22 had the lowest value of 23.6 in the conventional cultivar group (Table 1). During the subsequent year, expectedly, IDIC-tolerant wild *Oryza* accessions had higher SPAD values than susceptible cultivated varieties. The correlation analysis showed that SPAD value was negatively related to IDIC rating (Spearman correlation, $r = -0.69$) and positively related to iron content (correlation coefficient = 0.57) and LAI (correlation coefficient = 0.38) (Figure 4). These results suggest that the 20 selected IDIC-tolerant accessions of *O. nivara* and *O. rufipogon* may have better ability to maintain chlorophyll content and plant health under iron-deficient conditions.

Table 2. Mean and range for SPAD value and leaf area index (LAI) of 313 accessions taken after 28 days of sowing.

	SPAD Value			LAI		
Species	**Mean**	**Maximum**	**Minimum**	**Mean**	**Maximum**	**Minimum**
O. nivara	30.6	36.0 (CR100003)	22.2 (CR100123)	1.3	2.6 (CR100007)	0.4 (CR100399)
O. rufipogon	30.8	35.3 (CR100001)	25.5 (CR100461)	1.5	2.2 (CR100001)	0.8 (CR100465B)
O. glaberrima	26.4	30.3 (IR102980)	22.8 (IR102489)	1.5	1.7 (IR102489)	1.2 (IR 102980)
O. barthii	24.9	24.9 (IR103580)	24.9 (IR103580)	1.4	1.4 (IR103580)	1.4 (IR103580)
O. sativa	28.3	31.1 (Lemont)	23.6 (Nagina 22)	1.2	1.7 (PAU 201)	0.9 (Nagina 22)

3.3. IDIC-Tolerant Lines Had More Iron Content in the Leaves than Susceptible Cultivars

The iron content in the leaves of 20 IDIC-tolerant lines and 8 conventional cultivars was determined four weeks after sowing, and the results showed significant differences (F value, 114.16; $p < 0.01$) between accessions. The tolerant lines had higher iron content compared with the conventional cultivars, with the wild *Oryza* germplasm exhibiting a wider range of iron content (1005 to 2121 ppm) compared with the conventional cultivars (560 to 847 ppm) (Table 1 and Figure 3). *O. rufipogon* (CR100030) had the highest iron content of 2127 ppm, followed by *O. nivara* (CR100363), *O. nivara* (CR100003), and *O. rufipogon* (CR100436) with iron contents of 2101, 1802, and 1664 ppm, respectively (Table 1). Conversely, the highest iron content in the conventional cultivars was observed in the Loment cultivar, with 851 ppm, followed by Feng-ai-zai, Nagina 22, and IR 64, with iron contents of 847, 765, and 734 ppm, respectively. The correlation analysis showed that iron content was negatively related to IDIC rating (Spearman correlation, $r = -0.56$) and positively related to SPAD value (correlation coefficient = 0.57) and LAI (correlation coefficient = 0.08) (Figure 4). These findings demonstrate that the IDIC-tolerant lines have significantly higher iron contents compared with the conventional cultivars and that the wild *Oryza* germplasm may serve as a valuable genetic resource for aerobic rice.

3.4. Wild Germplasm Exhibited Significant Variation for LAI

Significant differences (F value, 0.273; $p < 0.01$) were observed between the accessions for LAI under aerobic conditions, with average values ranging from 0.9 to 2.6 (Table 2). Among the wild accessions, *O. rufipogon* (CR100001) had the highest LAI 2.2, followed by *O. rufipogon* (CR100334B), *O. rufipogon* (IR80762A), and *O. nivara* (IR82018), with values of 2.1, 1.8, and 1.8, respectively (Table 1). In the cultivar accessions, PAU 201 had the highest LAI, with a value of 1.7, followed by Lemont, with a value of 1.6. The wild accession *O. nivara* (CR100360) had the lowest LAI of 1, while Nagina 22 had the lowest LAI of 0.9 among the conventional cultivar group (Table 1). The correlation analysis showed that LAI was negatively related to IDIC rating (Spearman correlation, $r = -0.28$) and positively related to SPAD value (correlation coefficient = 0.38) and iron content (correlation coefficient = 0.08) (Figure 4). These results suggest that there are significant differences in the LAI between the accessions, with some wild germplasm lines showing higher LAI values compared with the conventional cultivars.

4. Discussion

Iron deficiency is one of the major constraints in the adaptation of aerobic rice, affecting plant growth and yield. In the present study, significant genetic variation was observed for IDIC tolerance across the *Oryza* wild germplasm. Our results indicate that the wild rice germplasms, particularly of *O. nivara* and *O. rufipogon*, have a higher IDIC tolerance than the cultivated rice varieties. A moderate to high correlation was observed between IDIC, SPAD value, and iron content. The use of wild rice germplasms in breeding programs may lead to the development of IDIC-tolerant rice varieties with higher yield potentials and nutritional quality.

In our study, all *Oryza sativa* cultivars showed IDIC under aerobic conditions, suggesting an absence of efficient alleles that can efficiently uptake the Fe^{3+} form of iron. The domestication of lowland rice cultivars involved selecting plants that were well-suited for the lowland waterlogged environment, resulting in the loss of many alleles that were important for aerobic rice cultivation [22,23]. As a result of distinct adaptations to their respective environments, lowland and aerobic rice have differences in nutrient availability and uptake [11–14]. The availability of Fe (iron) and Zn (zinc) can become limited under aerobic conditions due to their oxidation [11,24]. Under anaerobic conditions, iron is available in its ferrous form, which is easily taken up by high-yielding cultivars developed for lowland cultivation [25]. However, under aerobic conditions, iron is insoluble (Fe^{3+} form) and not available for uptake by rice. In response to iron deficiency stress, plants release iron chelating substances called phytosiderophores [26]. These phytosiderophores solubilize inorganic Fe^{3+} compounds via chelation, and Fe^{3+}-phytosiderophore complexes are taken up through a specific transport system in the root plasma membrane [27]. Variations in the amount of phytosiderophores synthesized and iron transporter genes may play a role in IDIC tolerance in resistance lines. Wild species that have evolved under varying environmental conditions may possess desirable alleles for aerobic cultivation.

In plant breeding, various methods such as intervarietal selection, wide hybridization, transgene incorporation, and genome editing have been widely employed to enhance stress tolerance and other desirable traits [28–32]. Wild rice germplasms have been extensively demonstrated to be a source of stress resistance genes for rice breeding programs [17]. Zhang [33] demonstrated that the introduction of wild rice alleles into cultivated rice significantly improved yield under drought conditions. Similarly, Huang [34] found that some wild rice species possess genes that confer resistance to bacterial blight. Yuan [35] also showed that wild rice germplasms also contain genes related to cold tolerance. Furthermore, several studies have also shown that wild rice germplasms possess a high level of genetic diversity, which is essential for the development of new rice cultivars with enhanced stress tolerance [36–38]. The successful development of a rice cultivar, NERICA-10, through wide crossing between *O. sativa* and *O. glaberrima*, highlighted the promising potential of utilizing wide crosses in plant breeding [38]. Similar to these studies, significant variation in IDIC tolerance was observed in the wild germplasm in our study. The identification of twenty IDIC-tolerant lines in our study suggests the presence of allelic variations in phytosiderophore synthesis and iron transporter genes that can potentially be transferred to high-yielding lowland cultivars for aerobic cultivation.

The iron content in the IDIC-tolerant lines and cultivated susceptible rice varieties was estimated, and the results showed significant differences in the iron content among the accessions. The IDIC-tolerant wild accessions had higher iron contents compared with the cultivated susceptible rice varieties, with *O. rufipogon* and *O. nivara* having the highest iron contents among the wild rice germplasms. The moderately IDIC-tolerant Lemont cultivar had the highest iron content among the cultivated varieties, which is congruent with its SPAD value. A high level of iron has been reported in rice with low IDIC [39]. A significant variation in iron content was evident among the twenty IDIC-tolerant accessions. It is possible that IDIC tolerance in the selected lines may be controlled by different mechanisms, as the wild germplasm is known to contain a rich diversity of genetic variations. This could be due to the fact that different lines have adapted to iron-deficient conditions through various strategies, such as changes in root architecture, the production of iron-chelating compounds, or an increased expression of genes involved in iron metabolism. Therefore, the mechanisms underlying IDIC tolerance in different lines may vary depending on the specific genetic variations that have been selected for in each line. In addition to IDIC, we also measured SPAD values as an indicator of chlorophyll content and plant health. Our results showed significant differences in SPAD values among accessions, with *O. nivara* and *O. rufipogon* having higher SPAD values than *O. glaberrima*, *O. barthii*, and *O. sativa*. The higher chlorophyll content in the wild rice germplasms may be attributed to their ability to adapt to harsh environmental conditions, including iron-deficient soils. It is important to take into account that cultivated varieties typically have higher levels of chlorophyll than wild accessions under water submergence conditions [40–42]. Ishikawa [42] conducted a study in which they observed that *O. coarctata* (wild rice) displayed a higher level of salinity tolerance compared with the cultivated *O. sativa* control. Interestingly, the SPAD value was lower in both the control and salinity conditions for *O. coarctata,* indicating the potential for stress tolerance even without a higher SPAD value. Therefore, any differences in SPAD values between wild and cultivated accessions may not entirely indicate their tolerance to IDIC. The LAI of a plant is an important factor that determines its photosynthetic capacity and yield potential. Our results showed significant differences in LAI among accessions, with *O. rufipogon* having the highest LAI among the wild rice germplasm and PAU 201 having the highest LAI among the cultivated varieties. IDIC tolerance might contribute to high LAI, but there were genotypic differences for LAI even under the water submergence condition, suggesting that LAI might not be a good indicator of IDIC tolerance.

Our study provides important insights into the genetic variation of IDIC tolerance, chlorophyll content, LAI, and iron content among rice accessions. Our results indicate that wild rice germplasms, particularly of *O. nivara* and *O. rufipogon*, have higher tolerance to IDIC, chlorophyll content, LAI, and iron content than cultivated rice varieties. The use of wild rice germplasms in breeding programs may lead to the development of IDIC-tolerant rice varieties with higher yields.

5. Conclusions

This study identified twenty IDIC-tolerant rice lines from a collection of 313 *Oryza* accessions evaluated under aerobic conditions at the tillering stage. These tolerant lines exhibited no chlorosis symptoms, and high iron content and SPAD values, indicating efficient iron uptake and utilization. In contrast, the cultivated controls showed varying degrees of chlorosis, and lower SPAD and iron content. The selected lines maintained their tolerance in a subsequent year, confirming their reliability. These findings highlight the potential of the wild *Oryza* germplasm as a valuable resource for developing IDIC-tolerant cultivars for aerobic rice cultivation. The identified lines can contribute to addressing groundwater depletion issues and promote sustainable water-saving practices. Additionally, they are promising candidates for future research on the molecular basis of IDIC tolerance, facilitating improved breeding strategies and genetic engineering techniques for enhancing IDIC tolerance in rice crops.

Author Contributions: R.K. performed the experiments and data analysis. R.K. and H.Y. were involved in manuscript writing and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data is contained within the article.

Conflicts of Interest: The authors declare no conflict of interest.

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