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Bridging Biodiversity and Breeding: Characterisation of Wild Rice (*Oryza* spp.) Accessions and Development of Novel Interspecific Germplasm to Broaden the Genetic Base

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Simple Summary

Wild *Oryza* species constitute a critical reservoir of genetic diversity for rice improvement, offering adaptive traits for resistance to major biotic and abiotic stresses. Key gaps in the utilisation of these genotypes are mainly the lack of systematic characterisation of non-AA genome species, underscoring the need for uniform descriptors and genomics-enabled evaluation. This study synthesises detailed morphological characterisation of *Oryza* accessions with a species coverage of 15 wild species, along with two cultivated species, highlighting their trait diversity and the importance of this unexplored genetic resource for pre-breeding.

Abstract

Enormous genetic diversity exists in rice germplasm, including wild and weedy relatives, though they remain unexplored within in situ or ex situ collections. Characterisation and utilisation of the available biodiversity in plant breeding is essential for the detection of novel traits or genes for climate resilience. In this study, 97 rice genotypes, including 90 rice accessions belonging to various *Oryza* species and 7 check cultivars with an *O. sativa* background, were characterised for quantitative morphological characters following the guidelines based on distinctiveness, uniformity and stability (DUS) test by the Protection of Plant Varieties and Farmers' Rights Authority (PPVFRA), India. Characterisation of the genotypes based on 39 important DUS morphological descriptors revealed polymorphism in 35 traits, confirming high morphological diversity among wild rice accessions and distinguishing and unique traits from other wild accessions for the utilisation in pre-breeding programmes. Genotypes such as WD5_6, WD10_4, and WD3_3 consistently expressed a favourable combination of broad and long leaves, extended panicle length, and well-branched panicles with higher panicle number. In addition, these genotypes showed purple pigmentation across multiple vegetative and reproductive organs, indicating stable and enhanced anthocyanin accumulation. Accessions WD10_4 and WD3_3 also represent valuable donors for panicle architecture and yield component enhancement, while genotypes such as WD17_15 and WD12_8 may serve as specific donors for panicle length and branching traits. Characterisation studies and detection of unique traits provide the empirical foundation for conservation decisions, taxonomic clarity, and pre-breeding applications. Interspecific crosses in the genetic background of elite cultivars with donor species viz., *O.*

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barthii, *O. glaberrima* and *O. rufipogon* were developed as pre-breeding materials for further crop improvement as well as for the identification of novel genes of agronomic importance.

Keywords: wild species; DUS; *Oryza*; interspecific crosses; anthocyanin

1. Introduction

Rice is an annual, short-day and C3 plant and a member of the genus *Oryza* and belongs to the family Poaceae. The *Oryza* genus comprises 27 species, including two of the most extensively cultivated species of Asian rice (*Oryza sativa* L.) and African rice (*Oryza glaberrima* Steud.) with the AA ($2n = 24$) genome, along with 25 distinct wild species with various genome combinations. These species were divided into six diploid types (AA, BB, CC, EE, FF, and GG) and five allotetraploid types (BBCC, CCDD, HHKK, HHJJ, and KKLL) [1]. Rice is one of the most important cereal crops produced worldwide and feeds more than half of the world's population. More than 90% of the world's rice is grown and consumed in Asia [2]. During 2024–2025, India ranked first globally in terms of area (51 million hectares) and production (149.1 million tons), followed by China [3]. In India, rice sustains food production by contributing 20–25% of agricultural output and ensures food security for more than half of the population. Global agricultural resources are greatly impacted by population increase, unbalanced environmental conditions due to climate change, and diminishing agricultural resources. Based on updated estimates and demographic projections, it is necessary to raise rice output by developing cultivars with higher yield potential and improved yield stability [4]. To create new rice varieties with higher yield and quality than existing ones, it is essential to have a broad spectrum of genetic variation in the population. Ex situ and in situ preserved diversity in rice germplasm is enormous and can be effectively utilised to meet present demands for food and nutritional security.

However, the genetic diversity of cultivated rice species has been inevitably decreased due to a long history of domestication, intercrossing a few elite genotypes via artificial selection, ultimately resulting in potential yield stagnation and a narrow genetic base in the existing germplasm [5]. There was a significant reduction in genetic diversity caused by the domestication of wild relatives into cultivated forms of rice [6]. Repeated use of a few select rice cultivars in the breeding programmes not only limits the genetic base but also develops susceptibility to various abiotic and biotic stresses [7]. Crop wild relatives (CWRs) retain genetic diversity and, thus, represent a valuable genetic resource for modern agriculture, contributing novel genes and alleles for climate resilience [8,9]. The genus *Oryza* comprises species distributed across tropical and subtropical regions, spanning multiple genome groups with variable crossability to cultivated rice. Wild relatives have long been recognised for their breeding value, particularly for traits linked to stress tolerance and ecological adaptation. Millions of years of genetic adaptation and interaction with the environment resulted in evolution and the accumulation of several agronomically important traits in these wild rice species, which are desirable for crop improvement [10]. One of the major requirements in modern crop breeding is the recovery of abundant genetic diversity present in the gene pool and introgressing the favourable alleles into elite cultivars to develop climate-resilient varieties [11]. Identifying potential donors from wild genotypes for yield traits, input use efficiency, tolerance to biotic and abiotic stresses, and utilising them for gene discovery and crop improvement is a promising approach [12,13].

The successful outcome of any breeding programme mostly depends on the exploitation of existing variability and, therefore it is always advisable to collect, evaluate and utilise

the available diverse germplasm for crop improvement to suit specific needs concerning a definite ecosystem. The conservation and characterisation of these genetic resources is a necessity not only for posterity, but also for utilisation in different improvement programs [14]. Arunachalam (1981) stated that obtaining high variability in segregating population and superior heterotic hybrids mostly depends on the genetic divergence of the parents utilised in the hybridisation programme [15]. The novel characters and existing variability in the germplasm should be exploited to develop the varieties and hybrids based on needs, using crop improvement programmes. The available germplasm should be characterised, and genetic diversity should be studied efficiently prior to its use in any breeding programme.

There are several ways to estimate diversity in germplasm, viz., evaluation of phenotypic variation, biochemical and DNA polymorphisms. Being a signatory to the General Agreement on Trade and Tariffs (GATT), the Government of India has legislated a sui-generis system, the Protection of Plant Varieties and Farmers' Rights Act (PPV & FRA), for providing protection to plant varieties based on the Distinctiveness, Uniformity and Stability (DUS) test. The present study aimed to investigate wild rice accessions for key DUS traits, along with selected popular cultivars, and to develop crosses between wild accessions and cultivars.

2. Materials and Methods

2.1. Plant Materials

The present research programme was carried out during *Kharif* 2024 and *Rabi* 2024–2025 at the ICAR-Indian Institute of Rice Research (ICAR-IIRR), Rajendranagar, Hyderabad, Telangana, located at 17.32° N latitude, 78.39° E longitude, and 536 m above mean sea level. The experimental materials comprised 90 wild rice accessions and 7 cultivars obtained from ICAR-IIRR (Tables 1 and S1). The wild rice accessions were selected based on criteria to represent all available 17 species. Only those accessions that survived and completed various stages of crop growth for DUS testing are included in the study. Among the 7 cultivars used in the study, MTU1010 is a popular mega rice variety of *indica* origin; Nipponbare is a popular *japonica* variety which is commonly used as a reference genome; N22 or Nagina 22 belongs to another subspecies of *aus* type; and Dhanrasi, DRRDhan40, DRRDhan74, and DRRDhan65 are belong to interspecific varieties with wild introgression in *O. sativa* background, ensuring representation of all rice subspecies.

During *Kharif* 2024, the experimental materials were raised under net-house pot culture conditions at IIRR, Rajendranagar, to ensure uniform growth, following a completely randomised design (CRD). Healthy seeds were selected, pre-soaked for 24 h, and germinated in petri plates, initially sown in trays. Then, ten-day-old seedlings were transplanted to a plastic pot (25 cm diameter, 20 cm depth) filled with loamy soil and vermicompost mixture transferred to pots filled with loam soil, maintaining three plants per pot. Basal fertilisation with DAP was applied to the soil before transplanting, and urea was applied at active tillering as per the requirement. Pots were maintained under saturated water conditions, except during germination, when only moist conditions were maintained. Plants were grown in an open net house facility during *Kharif* 2024 at normal temperatures and were exposed to all ongoing weather changes during the season, similar to an open-field condition. Pots were arranged in a completely randomised design with three biological replicates per treatment, repositioned periodically to minimise positional effects, and standard plant protection measures were followed throughout the experiment. The variability observed in the wild rice accessions used in the study is depicted in Figure 1.

Table 1. Details of wild species and accessions used in the study.

Sl. No.	Species	Chromosome No.	Genome	Accessions (97)	Code
I. Sativa complex					
1	*O. sativa* L.	24	AA	7	-
2	*O. nivara* Sharma et Shastry	24	AA	5	WD12
3	*O. rufipogon* Griff.	24	AA	53	WD17
4	*O. meridionalis*	24	AA	2	WD10
5	*O. glumaepetula* Steud	24	AA	3	WD6
6	*O. glaberrima*	24	AA	5	WD5
7	*O. barthii* A. Chev. et Roehr	24	AA	1	WD3
II. Officinalis Complex/Latifolia complex					
8	*O. punctata* Kotschy ex Steud.	24	BB	5	WD14
9	*O. rhizomatis* Vaughan	24	CC	3	WD15
10	*O. officinalis* Wall. ex Watt	24	CC	1	WD13
11	*O. australiensis* Domin.	24	EE	2	WD2
12	*O. minuta* J.S.Pesl. ex C.B.Presl.	48	BBCC	1	WD11
13	*O. latifolia* Desv.	48	CCDD	4	WD8
14	*O. alta* Swallen	48	CCDD	2	WD1
15	*O. grandiglumis* (Doell) Prod.	48	CCDD	1	WD7
III. Ridleyi Complex					
16	*O. ridleyi* Hook. f.	48	HHJJ	1	WD16
17	*O. longiglumis* Jansen	48	HHJJ	1	WD18

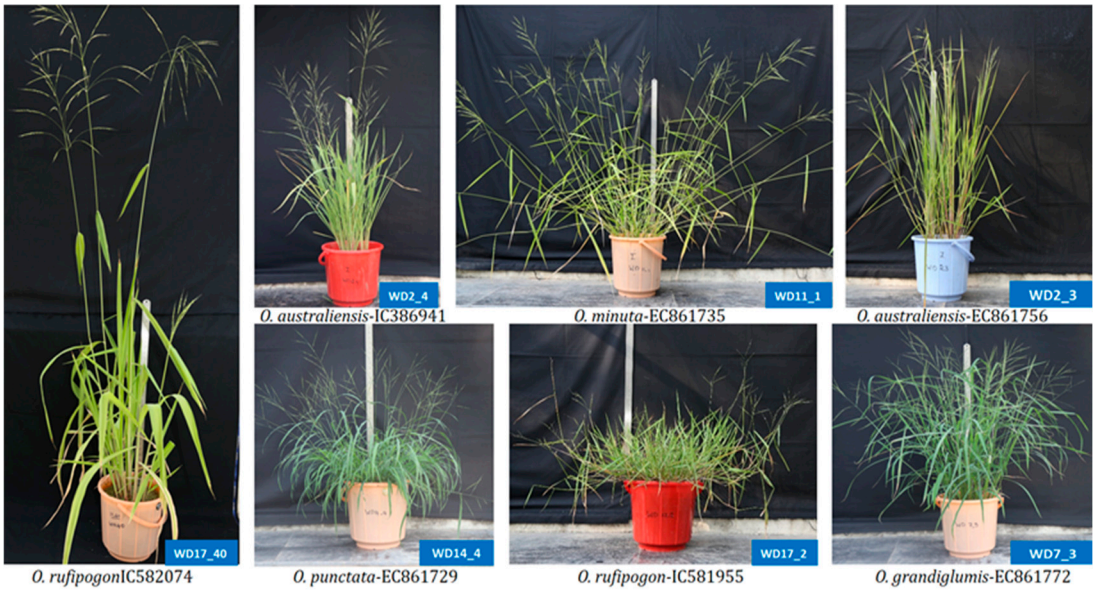

Figure 1. Variability in the plant architecture observed in wild rice accessions used in the study. WD2_4 (*O. australiensis*-IC386941), WD11_1 (*O. minuta*-EC861735), WD2_3 (*O. australiensis*-EC861756), WD17_40 (*O. rufipogon*-IC582074), WD14_4 (*O. punctata*-EC861729), WD17_2 (*O. rufipogon*-IC581955), WD7_3 (*O. grandiglumis*-EC861772).

2.2. Characterisation of Genotypes for DUS

The genotypes were characterised for Distinctness, Uniformity and Stability (DUS) traits involving 39 important morphological descriptors. Visual observations were recorded on a single-plant basis on three randomly selected plants in each genotype at appropriate growth stages according to the guidelines of PPVFRA on different morphological characters. Traits like time of heading, basal leaf sheath colour; culm attitude, decorticated grain, colour,

length, width; flag leaf attitude of blade (early and late stage); grain length, width; leaf ligule, leaf sheath, anthocyanin colouration, leaf anthocyanin colouration, anthocyanin colouration of auricles, anthocyanin colouration of collar, leaf auricles, leaf collar, colour of ligule, intensity of green colour leaf, leaf length of blade, leaf pubescence of blade surface, leaf shape of ligule, leaf width of blade, lemma anthocyanin colouration of apex, lemma anthocyanin colouration of the area below apex, lemma anthocyanin colouration of keel; panicle attitude of branches, panicle awns, panicle curvature length of main axis, panicle distribution of awns, panicle exsertion, panicle length of main axis, panicle number per plant, panicle presence of secondary branching, panicle secondary branching, spikelet colour of stigma, stem length (excluding panicle excluding floating), stem anthocyanin colouration of internodes and stem anthocyanin colouration of nodes were studied. Details on DUS characters, states, stage of observation and type of assessment of the morphological traits are presented in Tables S2–S4.

2.3. Development of Interspecific Crosses

Crosses between elite cultivars and wild species were attempted during *Kharif* 2024. Interspecific hybridisation involved six cultivars, such as DRR Dhan 65, DRR Dhan 50, ADT38, Vikram TCR, RNR15948, and WGL697, and nine wild accessions, viz., WD5_1, WD5_7, WD3_1, WD12_5, WD16_1, WD17_23, WD17_25, WD17_61, and WD17_64. Emasculation of selected female plants was carried out in the early morning between 6 and 8 o'clock by the clipping method, and pollination was carried out on the same day in the glasshouse. Then, the panicle was covered with a butter paper bag, labelled and pinned. The crossed seeds were collected from the recipient panicles three to four weeks later and placed in a small envelope with proper labelling.

2.4. DNA Extraction and Genotyping

Leaf samples from four-week-old hybrid (F_1) plants and their parents were collected, and genomic DNA was extracted using the Cetyl Tris Methyl Ammonium Bromide (CTAB) method [16]. The isolated DNA was quantified using a NanoDrop spectrophotometer (Thermo Fischer Scientific, Mumbai, India) and diluted to a concentration of 50 ng/mL in TE buffer (Gentrox, Worcester, MA, USA) for subsequent PCR analysis. Parental polymorphism was performed using nine SSR/InDel markers, (oligos synthesised at BioSquare Biotechnologies India Pvt Ltd., Hyderabad, India) spanning five rice chromosomes, namely RM1, RM9, A01P02097, A01P01132, A11P04101, A07P22185, A01019295, A03P09039, and A08P11468. The PCR reaction mixture of 10 µL contained 3 µL of DNA, 10 × Buffer of 1 µL, 0.2 µL of Taq polymerase, 0.5 µL of dNTP mix, 1 µL of forward primer, 1 µL of reverse primer, and 3.3 µL water. Amplification was performed using a Bio-Rad thermal cycler (Bio-Rad, Hercules, CA, USA) with the following program: initial denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and a final extension at 72 °C for 7 min. The PCR products were separated on 3% agarose gel and electrophoresed at 120 V for one hour. The banding pattern was visualised by ethidium bromide staining, and gel images were documented using a gel documentation system. After developing interspecific crosses between elite popular varieties with wild species, the F_1s obtained were raised in the field during *Rabi* 2024–2025. Parental polymorphism was assessed using SSR/InDel markers, and the true F_1s were determined using detected polymorphic markers.

3. Results

3.1. Characterisation of Rice Genotypes for Important DUS Characters

Characterisation of germplasm plays a vital role in confirming the identity of genetic resources and minimizing duplication. It serves as a foundation for developing a systematic approach to record and store valuable information, allowing easy access for plant breeders and making it readily available for use in future breeding programs. In the present study, characterisation of 97 rice genotypes—including 90 wild rice accessions and 7 cultivars—was carried out for 39 DUS characters viz., basal leaf sheath colour, leaf intensity of green colour, leaf anthocyanin colouration, pubescence of leaf blade, leaf auricle, collar and its anthocyanin colouration, shape and anthocyanin colouration of leaf ligule, length of leaf blade, width of leaf blade, time of heading, attitude of flag leaf (late and early), culm attitude, anthocyanin colouration of keel, apex and below apex of spikelet, colour of stigma, anthocyanin coloration of nodes and internodes, length of main axis (panicle), curvature of main axis, stem length, number of panicles plant, presence of awns, distribution of awns, presence of secondary branching, panicle exertion, panicle attitude of branches, length of grain, width of grain, decorticated grain length, and width and colour of decorticated grain. The variation observed for various DUS characters is depicted in Figures 2 and S1. The frequency distribution of 39 DUS characters among 97 rice genotypes is presented in Table S3.

Basal leaf sheath colour was observed at the booting stage. The trait exhibited polymorphism with four different types among the genotypes. Of 97 genotypes, 65 exhibited green basal leaf sheath colour, 10 light purple, 13 purple lines, and 9 uniform purple sheath colour. The genotypes with uniform purple sheath colour included an accession of *Oryza barthii* (WD3_3), *O. meridionalis* (WD10_4), and seven *O. rufipogon* accessions (WD17_15, WD17_18, WD17_19, WD17_51, WD17_52, WD17_65 and WD17_66). The intensity of leaf green colour was polymorphic: 9 genotypes had light green colouration, 60 had medium green, and 28 had dark green.. The intensity of leaf green colour was mostly medium (61.9%) followed by dark (28.9%) and light colouration (9.3%). Leaf auricles were present in 92.8% of the genotypes, and most of the genotypes had colourless auricles (77.8%), followed by purple (15.6%) and light purple auricles (6.7%).

The presence of anthocyanin in the leaf blade was observed in 6 genotypes, including an accession of *O. australiensis* (WD2_3), *O. barthii* (WD3_3), *O. meridionalis* (WD10_4) and three *O. rufipogon* accessions (WD17_65, WD17_73 and WD17_79), whereas it was absent in 91 genotypes. Among the 97 genotypes studied, only 9 showed anthocyanin colouration in the leaf sheath. These included an accession of *O. australiensis* (WD2_3), *O. barthii* (WD3_3), *O. meridionalis* (WD10_4), two *O. glaberrima* (WD5_1 and 6), one and four *O. rufipogon* genotypes (WD17_57, WD17_65, WD17_73 and WD17_82). The remaining 88 genotypes lacked anthocyanin in the leaf sheath. Leaf collar and ligule were present in all genotypes, and anthocyanin colouration of the collar was observed in only 15.5% of the genotypes. The colour of the ligule was green in most of the genotypes (77.3%), followed by light purple (13.4%) and purple (9.3%).

Leaf blade pubescence was found to be absent in 71 genotypes, weak in 6 genotypes, medium in 11 genotypes, strong in 5 genotypes, and very strong in 4 genotypes. The genotypes exhibiting very strong pubescence included one accession of *O. australiensis* (WD2_3), *O. glaberrima* (WD5_6), *O. meridionalis* (WD10_2) and Dhanrasi, which is derived from *O. rufipogon*.

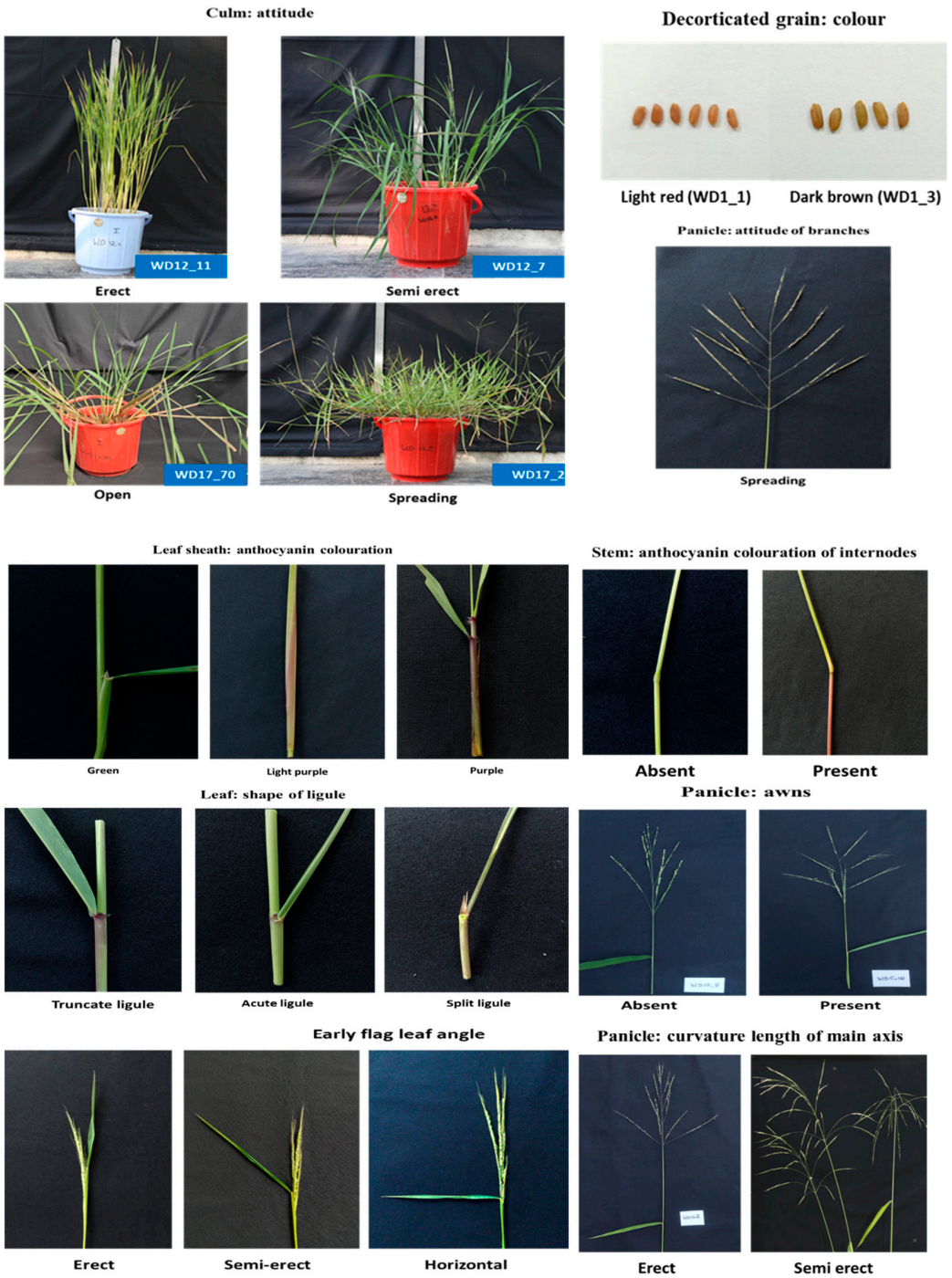

Figure 2. Variations observed among wild accessions for different DUS traits.

Leaf auricles wereobserved in 90 genotypes and were absent in 7 genotypes. The genotypes without auricles were accessions of *O. glumaepetula* (WD6_3), *O. latifolia* (WD8_4), two *O. punctata* (WD14_2 and WD14_4), *O. rhizomatis* (WD15_2), *O. ridleyi* (WD16_2), and *O. rufipogon* (WD17_12). Out of 90 genotypes with auricles, 70 genotypes were found to be colourless, 6 were light purple, and 14 had purple auricles. The genotypes with purple auricles included one accession of *O. glaberrima* (WD5_6), two *O. latifolia* (WD8_1 and 3), *O. meridionalis* (WD10_2), three *O. nivara* (WD12_3, WD12_5, and WD12_8) and seven *O. rufipogon* accessions (WD17_8, WD17_15, WD17_24, WD17_40, WD17_42, WD17_66 and WD17_77). Leaf collar and ligule were found to be present in all the 97 genotypes. Out of 97 genotypes, anthocyanin colouration of the collar was present in 15 genotypes, comprising one accession of *O. glaberrima* (WD5_6), two *O. latifolia* (WD8_1 and WD8_3),

one *O. meridionalis* (WD10_2), three *O. nivara* (WD12_3, WD12_5 and WD12_8), and eight *O. rufipogon* (WD17_8, WD17_15, WD17_20, WD17_24, WD17_40, WD17_42, WD17_66 and WD17_77). The remaining 82 genotypes exhibited colourless leaf collars. There are three categories in ligule shape, namely, truncate, acute and split. This character was found to be trimorphic among the genotypes studied. A total of 20 genotypes possessed truncate type, 52 genotypes had acute ligules, and 25 genotypes had split ligules in which all the seven cultivars exhibited split ligules. A total of 75 genotypes had green ligules, while light purple ligules were found in 13 genotypes, and purple ligules were found in 9 genotypes. The genotypes with purple ligules included one accession of *O. glaberrima* (WD5_6), *O. meridionalis* (WD10_2), two *O. nivara* (WD12_5 and WD12_8) and five *O. rufipogon* (WD17_8, WD17_15, WD17_42, WD17_66 and WD17_77) accessions.

Length of leaf blade was long in 18 genotypes, medium in 67 genotypes, and the remaining 12 genotypes had a short leaf blade. Based on width of the leaf blade, 97 genotypes were grouped into three classes: 8 genotypes possessed narrow width, 81 genotypes exhibited medium leaf width, and 8 genotypes exhibited broad leaf width. The broad-leaved genotypes included one accession of *O. glaberrima* (WD5_1), one *O. latifolia* (WD8_3), one *O. nivara* (WD12_3) and five *O. rufipogon* (WD17_8, WD17_15, WD17_20, WD17_24 and WD17_40) accessions. The first observation of the angle of flag leaf was taken at the beginning of anthesis. The genotypes were categorised based on flag leaf attitude as erect, semi-erect, horizontal, and drooping. The majority of the genotypes (65) possessed semi-erect flag leaf at early observation, followed by horizontal in 20 genotypes, and erect in 10 accessions. A deflexed flag leaf was observed in two genotypes, namely one accession of *O. minuta* (WD11_1) and one *O. rufipogon* (WD17_24) accession. The flag leaves were observed a second time during the ripening stage to document the change in angle resulting from bearing the panicle weight. Upon late observation, only two genotypes exhibited an erect flag leaf: one accession of *O. nivara* (WD12_11) and the cultivar Nagina 22. In contrast, 24 genotypes showed a semi-erect flag leaf, 11 genotypes had a horizontal angle, and the majority of the genotypes (60) had a deflexed flag leaf angle.

Based on the culm attitude, the genotypes were categorised as erect, semi-erect, spreading, and open plant types. Among the 97 genotypes studied, 14 had an erect plant type, 36 had a semi-erect plant type, 39 had an open plant type, and 8 had a spreading plant type. The spreading type included one accession of *O. glaberrima* (WD5_8), one *O. latifolia* (WD8_4), one *O. meridionalis* (WD10_4), one *O. nivara* (WD12_3), one *O. punctata* (WD14_2), one *O. rhizomatis* (WD15_2) and three *O. rufipogon* (WD17_2, WD17_9 and WD17_73) accessions. The genotypes were categorised into five classes based on days to heading.

Out of all the 97 genotypes, 17 genotypes were categorised as very early heading (<71 days) including an *O. glaberrima* (WD5_10), *O. rhizomatis* (WD15_1), *O. longiglumis* (WD18_1), and 14 *O. rufipogon* genotypes (WD17_1, 4, 7, 12, 16, 23, 26, 32, 35, 38, 49, 66, 75 and 82). The remaining were classified as very late heading (>130 days), which included one accession of *O. australiensis* (WD2_3), one *O. glaberrima* (WD5_1), one *O. meridionalis* (WD10_4), one *O. nivara* (WD12_11), and three *O. rufipogon* (WD17_65, WD17_79 and WD17_83) accessions.

All the lemma characteristics were observed during anthesis. None of the genotypes exhibited colouration at the keel or the area below the apex. The apical region of the lemma was observed for the presence of anthocyanin colouration. Among the 97 genotypes studied, 84 were devoid of anthocyanin coloration at the lemma apex, 4 had weak colouration, 5 had medium, and 4 genotypes had strong colouration , all of which were *O. rufipogon* (WD17_2, WD17_19, WD17_51 and WD17_82) accessions. The colour of stigma was observed halfway through the anthesis, and it showed trimorphic variation among the genotypes studied. The majority of genotypes (76) exhibited purple stigma, followed by white stigma in

12 genotypes, and light purple stigma in 9 genotypes. The anthocyanin colouration of the lemma apex was absent in most genotypes (86.6%). The colour of stigma in the spikelet was trimorphic, and 78.4% of the genotypes had purple stigma followed by white (12.4%) and light purple stigma (9.3%), while all seven cultivars had white stigma.

Stem length and anthocyanin colouration of nodes and internodes were observed during milk development stage. Some 34 genotypes had very short (<91 cm) stem length, 42 had short (91–110 cm), 15 had medium (111–130 cm), 2 had long (131–150 cm), and 4 genotypes had very long (>150 cm) stem length. Out of 97 genotypes, only 13 genotypes had nodal anthocyanin colouration, which included one accession of *O. glaberrima* (WD5_1), *O. glumaepatula* (WD6_3), *O. latifolia* (WD8_4), two *O. meridionalis* (WD10_2 and 4), *O. nivara* (WD12_3), *O. rhizomatis* (WD15_2) and six *O. rufipogon* (WD17_8, WD17_18, WD17_24, WD17_40, WD17_65 and WD17_77) accessions. In contrast, the remaining 84 genotypes were devoid of anthocyanin at the nodes. For internodal colouration, only 12 genotypes had internodal anthocyanin colouration, which included three accession of *O. glaberrima* (WD5_1, WD5_6 and WD5_11), *O. glumaepatula* (WD6_3), *O. latifolia* (WD8_4), *O. meridionalis* (WD10_4), *O. ridleyi* (WD16_2), and five *O. rufipogon* (WD17_2, WD17_54, WD17_57, WD17_77 and WD17_82) accessions; meanwhile, the remaining 85 genotypes were devoid of anthocyanin at the internodes.

Panicle length was observed during the milk and dough development stage. Some 7 genotypes had very short (<16 cm) panicles; 42 had short (16–20 cm); 37 had medium (21–25 cm); 7 had long (26 to 30 cm); and 4 genotypes had very long (>30 cm) panicles. The genotypes with very long panicles included one accession of *O. meridionalis* (WD10_2), one of *O. nivara* (WD12_3), and two of *O. rufipogon* (WD17_20 and WD17_40). The peduncle which was the main axis of the panicle and panicle curvature of main axis was observed during the ripening stage. Among 97 rice genotypes, a straight peduncle was found in 79 genotypes, a semi-straight peduncle in 10 genotypes, and a deflexed peduncle in 8 genotypes. Notably, the deflexed panicle curvature was observed in all seven cultivars and one accession of *O. rufipogon* (WD17_83). All panicle-related characteristics were observed during the ripening stage.

Of 97 genotypes, 29 had fewer panicles per plant (<11), while the majority (66) had a medium number (11–20). Only two genotypes produced a high number of panicles per plant (>20), namely one accession of *O. latifolia* (WD8_3) and one of *O. rufipogon* (WD17_82).

Panicle awns were absent in 15 genotypes, and 82 genotypes had awns. Although 82 genotypes were awned, they differed in the distribution of awns within the panicle. Some 72 genotypes had awns throughout the length of the panicle, and 9 genotypes had awns only at the tips, which included one accession of *O. glaberrima* (WD5_6), one *O. latifolia* (WD8_1), two *O. nivara* (WD12_5 and WD12_8), four *O. rufipogon* (WD17_40, WD17_42 WD17_66 and WD17_83), and the cultivar N22. Only one accession of *O. rufipogon* (WD17_77) had awns only at the upper half of the panicle. Secondary branching was present in 90 genotypes and absent in the remaining 7 genotypes. Among the 90 genotypes with secondary branching, 78 genotypes had weak branching, and 11 genotypes had strong secondary branching. The genotypes with strong secondary branching included all the cultivated varieties, one accession of *O. punctata* (WD14_3), and three *O. rufipogon* (WD17_54, WD17_55 and WD17_78) accessions. Only one accession of *O. rufipogon* (WD17_20) exhibited clustered secondary branching.

Based on the distance between the flag leaf cushion and the panicle neck, the germplasm was sorted based on panicle exertion properties. Well-exerted panicles were observed in 89 genotypes, mostly exerted panicles were spotted in 7 genotypes, and only 1 accession of *O. rufipogon* (WD17_19) exhibited partly exerted panicles. The attitude of branches of the main axis was classified into five classes: erect, erect to semi-erect,

semi-erect, semi-erect to spreading, and spreading. Erect branches were observed in only 1 accession of *O. nivara* (WD12_7); the erect to semi-erect type was seen in 7 genotypes, semi-erect in 18 genotypes, semi-erect to spreading in 15 genotypes, and spreading in 56 genotypes.

All grain characteristics were recorded at harvest. Some 60 genotypes showed very short grain length (<6.0 mm), 35 genotypes had short grain length (6.1–8.5 mm), and 2 genotypes (MTU1010 and N22) recorded medium grain length (8.6–10.5 mm). No genotypes in this study were found to exhibit long or very long grain lengths. The majority of genotypes (85) exhibited narrow grain width (2.1–2.5 mm). A total of 29 genotypes showed medium width (2.6–3.0 mm), and 2 genotypes had broad grain width (3.1–3.5 mm) (WD17_19 and N22), while only one genotype (Nipponbare) was very broad (>3.5 mm). Decorticated grain length and width were observed after milling. For decorticated grain length, 85 genotypes had short grain length (<5.6 mm), and 12 genotypes had medium grain length (5.6–6.5 mm). For decorticated grain width, 66 genotypes showed narrow decorticated grain width (<2.0 mm), while 29 genotypes were categorised as medium (2.0–2.5 mm), and 2 genotypes were identified as having broad width (>2.5 mm). N22 and Nipponbare were the genotypes with broad grain width. The colour of decorticated grains was observed at the post-harvest stage. Among the 97 genotypes evaluated, a majority of the wild accessions (83) exhibited dark brown grain colour. Light red grain colour was observed in six genotypes, comprising one accession of *O. alta* (WD1_1), *O. australiensis* (WD2_4), three *O. glaberrima* (WD5_6, 8 and 11), and one *O. rufipogon* (WD17_2) accession. A light brown grain colour was recorded in eight genotypes, which included all the cultivated varieties along with all the cultivated varieties and one *O. rufipogon* accession (WD17_83). Therefore, out of all the 39 DUS morphological descriptors, only 4 characteristics—i.e., presence of leaf collar, presence of leaf ligule, lemma and anthocyanin colouration of keel, and anthocyanin colouration area below apex—were monomorphic. All other 35 DUS characteristics were polymorphic among wild accessions and check cultivars.

3.2. Interspecific Crosses Developed in the Study

Interspecific crossing between elite cultivars with wild species was carried out to develop introgression lines (ILs) by backcrossing cultivated rice with diverse wild species, enabling the transfer of yield-enhancing traits and resistances to biotic and abiotic stresses. The list of crosses made and F_1s generated and confirmed is shown in Table S5. Obtained F_1s were raised in net house conditions during *Rabi* 2024–2025. Parental polymorphism analysis was conducted using nine SSR/INDEL markers: RM1, RM9, A01P02097, A01P01132, A11P04101, A07P22185, A01019295, A03P09039 and A08P11468 (Figure S2). Of these, seven markers exhibited polymorphism across different cross combinations, as detailed in Table 2. The confirmed F_1s and their parents are depicted in the Figures 3, S3 and S4. The F_2 seeds were collected from the confirmed F_1 plants and raised during *Kharif* 2025 for further advancement through backcross breeding.

Table 2. The list of successful interspecific crosses developed and polymorphic markers used in confirming the F_1s.

Sl. No.	Interspecific Crosses	Polymorphic Markers	Marker Used for F_1 Confirmation
1	ADT38 x WD5_7 (*O.glaberrima*)	A11P04101, A07P22185	A11P04101
2	ADT38 x WD3_1 (*O. barthii*)	A01P01132	A01P01132
3	DRR Dhan 65 x WD5_7(*O.glaberrima*)	A01P02097	A01P02097

Table 2. *Cont.*

Sl. No.	Interspecific Crosses	Polymorphic Markers	Marker Used for F_1 Confirmation
4	DRR Dhan 65 x WD17_23 (*O. rufipogon)*	RM 9, A01P02097, A01P19295	RM 9
5	DRR Dhan 65 x WD5_1 (*O.glaberrima*)	RM 1	RM 1
6	DRR Dhan 50 x WD5_1 (*O.glaberrima*)	RM 1, A01P01132	RM 1
7	Vikram TCR x WD17_23 *(O. rufipogon)*	RM 9, A01P19295	RM 9

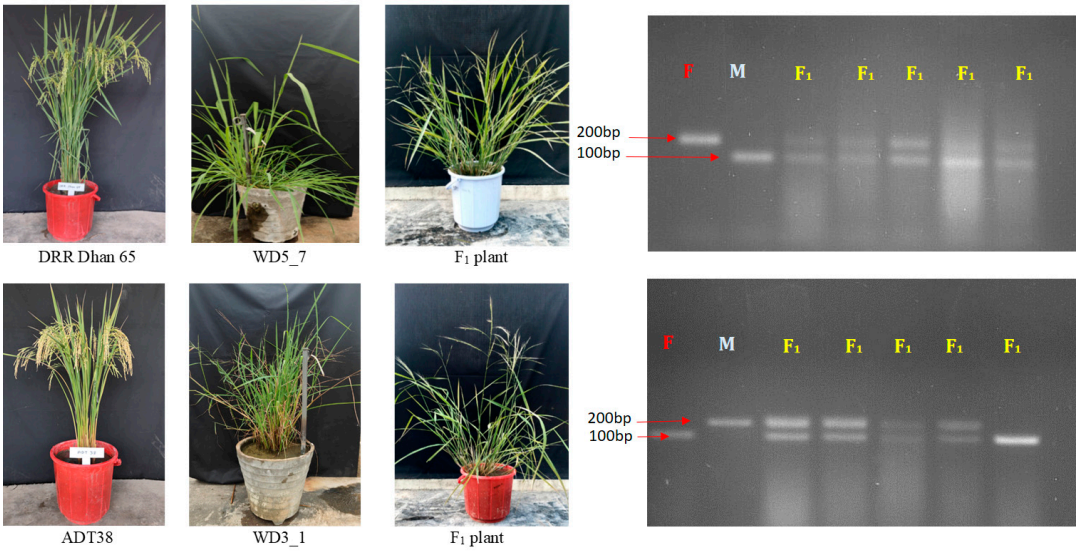

Figure 3. Interspecific hybrids developed and their parents along with molecular marker confirmation of true F_1s. F—female parent, M—male parent, F1—derived hybrids.

4. Discussion

The genetic improvement of rice (*Oryza sativa* L.) is essential to meet the growing global demand under increasingly challenging environmental conditions. Wild *Oryza* species represent a rich reservoir of untapped genetic diversity, offering novel alleles for agronomic traits such as yield, disease resistance and abiotic stress tolerance. They possess evolutionary adaptations not found in cultivated rice and provide valuable alleles for resilience to biotic and abiotic stresses, yield stability, and grain quality enhancement. Agromorphological characterisation of wild accessions is therefore essential for locating, documenting, and utilising this diversity effectively. Vaughan et al. (2003) emphasised that wild rice accessions contain unique phenotypes shaped by natural selection and ecological pressures, making them indispensable resources for long-term crop improvement [17].

Recent research studies has focused on AA-genome species such as *O. rufipogon* and *O. nivara* due to their close relationship and direct breeding utility as immediate progenitor species of cultivated rice. Characterisation has also extended to species with BB, CC, EE, FF, and GG genomes, as well as allopolyploids (BBCC, CCDD), particularly for identifying novel stress tolerance traits. However, the comparative depth of phenotyping remains uneven across genome groups. A systematic morphological evaluation enables precise discrimination among wild accessions, which often span broad geographic and ecological ranges. These species exhibit considerable variation in traits such as flowering behaviour, plant stature, tillering capacity, panicle architecture, grain morphology, and seed shattering. Detailed phenotypic documentation is necessary to capture trait variations that may relate to specific adaptive advantages [18]. Such characterisation also supports taxonomic clarification, particularly in cases where morphological overlap exists between closely related wild taxa. Agromorphological assessment is fundamental to unlocking

the breeding potential of wild *Oryza* germplasm. Phenotypic screening forms the first step in selecting promising accessions for pre-breeding and introgression pipelines [19]. Atwell et al. (2014) highlighted that phenotypic plasticity observed in wild accessions reflects functional genomic diversity that cannot be detected through DNA sequence analysis alone [20].

Beyond breeding applications, characterisation of wild accessions plays a vital role in the conservation and management of genetic resources. According to FAO (2010), comprehensive phenotypic data are required to guide germplasm curation, maintain representative diversity, and avoid redundancy in ex situ collections [21]. Agromorphological characterisation strengthens strategies to integrate wild genetic diversity into cultivated backgrounds. As noted by McCouch et al. (2016), phenotypic data provide the biological context required to interpret genomic information, validate allelic effects, and prioritise accessions for further molecular analysis [22]. As rice breeding increasingly adopts genomics-assisted selection, high-quality morphological characterisation remains indispensable for linking genes to function and improving trait introgression outcomes. A further dimension of agromorphological characterisation is its contribution to understanding the structure and magnitude of phenotypic diversity within and among *Oryza* species. Characterisation of traits such as flowering time, plant architecture, panicle morphology, plant stature, and leaf parameter types allows researchers to distinguish patterns of variation shaped by domestication, hybridisation, and environmental selection pressures [17]. This information supports cluster analysis, diversity assessment, and genome–phenotype associations, providing insight into evolutionary pathways.

Previous studies established the genus-level context and highlighted the large phenotypic diversity and complex gene pool relationships of *Oryza*. Field and population-level studies have since quantified that diversity; for example, ecogeographic surveys of *O. rufipogon* and *O. nivara* showed clear morphological clustering associated with geographic origins and ecological niche [23], while characterisation of weedy/red rice ecotypes documented consistent differences in plant stature, tillering, shattering and dormancy relevant to both taxonomy and management [18]. Many empirical screening studies have used standard descriptor sets (IRRI/IBPGR descriptors and their revisions) and combined field scoring with controlled-stress assays to identify trait donors [24,25]. Bierschenk et al. in 2020 evaluated 58 wild accessions across 21 species for iron toxicity responses and identified species- and accession-level differences that are useful for introgression studies [26].

Over the last decade, morphological characterisation has been strongly complemented by genomic resources and targeted trait screening, improving the capacity to prioritise wild donors for breeding. Large scale comparative genome projects (genomes of 13 wild and domesticated *Oryza* relatives) and recent reviews have emphasised that high-quality phenotypes remain essential for interpreting sequence diversity and translating genomic discoveries into breeding gains [27]. Nevertheless, there are recurring limitations: most phenotyping is concentrated on AA-genome relatives, and non-AA genomes remain undercharacterised phenotypically. Scoring systems and trial environments are often inconsistent across studies.

Considering the unique accessions identified in this study, regarding the time of heading, 44.5% of the genotypes were early, followed by very early (17.5%), late (17.5%), medium (1.4%), and very late (7.2%). All seven cultivars were categorised under medium (91–110 days) and late heading (111–130 days). Early wild accessions are suitable donors in pre-breeding programmes, given the number of large breeding cycles required to develop an advanced stabilised interspecific progeny.

Anthocyanins are flavonoid pigments present in several rice organs, including the leaf blade, sheath, nodes, glumes, awns, coleoptile, and pericarp. Functionally, they provide

photoprotection by absorbing excess light and UV radiation, thereby protecting chloroplasts from photo-oxidative damage [28]. They act as strong antioxidants, scavenging reactive oxygen species generated under abiotic stresses such as drought, salinity, cold, and flooding, contributing to improved stress tolerance [29–31]. Anthocyanins also play a defensive role, reducing palatability to insects and increasing resistance to pathogens, especially in exposed tissues like leaf sheaths and glumes [32]. Among the traits related to colouration of various plant organs, anthocyanin in leaf and leaf sheath was absent in most of the genotypes; however, other parts, which can be used as morphological markers, showed the presence of anthocyanin; basal leaf sheath colour was tetramorphic, and most of the genotypes exhibited green basal leaf sheath colour (67%) followed by purple lines (13.4%), light purple (10.3%), and uniform purple (9.3%). Anthocyanin colouration was present in all three of the basal leaf sheath, leaf blade and leaf sheath of the following genotypes: WD2_3, WD3_3, WD10_4, WD17_65 and 73. Anthocyanin in the leaf and leaf sheath was absent in most of the genotypes. Anthocyanin colouration was present in all three of the leaf auricle, collar, and ligule of the following genotypes: WD5_6, WD8_1 and 3, WD10_2, WD12_5 and 8 and WD17_8, 15, 24, 42, 66 and 77. Notably, all seven cultivated varieties possessed white stigma.

Anthocyanin colouration of nodes and internodes was present in the least genotypes, with rates of 13.4% and 12.4%, respectively. The decorticated grain colour was dark brown in most of the wild accessions (85.2%), followed by light brown (8.2%) in most of the cultivars and light red in 6.2% of the genotypes, including an *O. alta* (WD1_1), one *O. australiensis* (WD2_4), three *O. glaberrima* (WD5_6, 8 and 11) and one *O. rufipogon* (WD17_2). In rice improvement, anthocyanin pigmentation is widely used as a morphological and genetic marker for varietal identification, DUS testing, hybrid purity assessment, and taxonomic differentiation [33]. Additionally, anthocyanin-rich rice grains (black and purple rice) have high nutraceutical value, providing dietary antioxidants that are beneficial for human health. The qualitative descriptor analysis revealed substantial variation in pigmentation and agronomic traits among the evaluated accessions. Among the studied genotypes, WD5_6 emerged as the most promising donor, displaying multi-organ pigmentation coupled with robust morphological features, making it highly suitable for pre-breeding programmes targeting nutritional quality and stress resilience. Accessions such as WD10_4 and WD3_3 exhibited unique pigmentation combinations, suggesting their potential utility for genetic dissection of organ-specific anthocyanin regulation. Conversely, genotypes predominantly expressing green phenotypes represent valuable elite backgrounds for introgression breeding to balance yield and pigmentation traits. Overall, the identified accessions constitute a valuable genetic resource for yield improvement, stress tolerance, and anthocyanin-enriched rice breeding.

In rice (*Oryza* spp.), the leaf base ligule is a small, membranous outgrowth located at the junction of the leaf blade and leaf sheath. The ligule prevents the entry of water and pathogens at the blade-sheath junction; provides mechanical protection through its structural integrity, especially during wind or heavy rain; and plays a significant role in leaf formation, reflecting proper leaf polarity and morphogenesis. The shape of ligules showed trimorphic variation in which acute-shaped ligules were dominant among the genotypes studied, with 53.6%, followed by split (25.8%) and truncated (20.6%). All seven cultivars exhibited split-shaped ligules.

Leaf pubescence acts as a mechanical barrier, interfering with insect feeding, oviposition, and movement. Pubescence of the leaf blade surface was mostly absent in wild accessions (73.2%), followed by medium (11.3%), weak (6.2%), strong (5.2%) and very strong in 4.1% of genotypes including one *O. australiensis* (WD2_3), one *O. glaberrima* (WD5_6), one *O. meridionalis* (WD10_2), and the Dhanrasi cultivar. Wild-derived nutrient

use efficient varieties from *O. rufipogon* DRR Dhan65 and DRR Dhan 74 showed medium pubescence, and the *O.nivara*-derived high-yielding variety DRR Dhan 40 showed strong pubescence. Sandhu and Sarao (2021) evaluated several wild rice accessions for antixenosis against the brown planthopper (BPH), *Nilaparvata lugens* (Stål), and found that longer and denser leaf trichomes/pubescence were associated with lower planthopper populations [34]. Punithavalli et al. (2013) investigated the role of rice trichomes in conferring resistance to the rice leaf folder, *Cnaphalocrocis medinalis*, and demonstrated that trichomes significantly hinder larval movement and make the formation of leaf folds for feeding more difficult [35]. Awns were present in 84.5% of the genotypes, with their distribution being the whole length in 87.8% of the respective genotypes, followed by tip-only (11%) and the upper half of the panicle in only one genotype (WD17_77).

The length and width of the leaf blade were medium among the genotypes, at 69.1% and 83.5%, respectively. The attitude of the flag leaf upon early observation was semi-erect in most cases (67%), followed by horizontal (20.6%), erect (10.3%), and deflexed (2.1%). At the later stage of observation, the attitude of the flag leaf was deflexed in 61.9% of the genotypes, followed by semi-erect (24.5%), horizontal (11.3%), and erect (2.1%). Flag leaf angle significantly influences rice grain yield. Erect flag leaf angles improve light interception and canopy photosynthesis, and the larger flag leaf angles (more horizontal leaves) are associated with reduced grain yield under dense planting conditions [36]. Vertical flag leaf angles resulted in 13% higher photosynthetic rates, reduced photoinhibition, delayed leaf senescence, and 15% higher yields [37]. The culm attitude was open type in most genotypes (40.2%), followed by semi-erect (37.1%), erect (14.2%), and spreading type (8.2%). Open or spreading types have lower yield potential under intensive cultivation due to inefficient land use and a higher risk of lodging. Semi-erect and erect culm types orient leaves more vertically, reducing shading of lower leaves and enhancing light use efficiency, particularly in dense plantings. The attitude of branches was mostly spreading among the genotypes (57.7%), followed by semi-erect (18.6%), semi-erect to spreading (15.5%), erect to semi-erect (7.2%), and erect in only one genotype (WD12_7). The predominance of spreading panicles in wild rice accessions reflects adaptation to natural ecological conditions, where such architecture may facilitate seed dispersal and promote outcrossing, whereas domesticated rice exhibits reduced diversity and more compact panicle architecture due to strong artificial selection during domestication [17].

The panicle length was short in most genotypes (43.3%), followed by medium (38.1%), very short (7.2%), long (7.2%), and very long (4.1%). The curvature of the panicle was straight in most wild accessions (81.4%), followed by semi-straight (10.3%) and deflexed (8.2%). Straight or upright panicle types improve canopy architecture and light interception and enhance lodging resistance by reducing mechanical stress on the panicle neck and stem, thereby conferring greater structural stability than curved or deflexed panicles [38,39]. In the present study, wild rice accessions predominantly exhibited straight panicles, whereas modern cultivars largely possessed drooping or deflexed panicles. This difference may be attributed to the relatively lower grain number and lighter panicles in wild accessions, while modern cultivars bear a higher grain number, resulting in increased panicle weight and consequent deflexion. The secondary branching of panicles was present in 92.8% of the genotypes, among which 86.7% of the respective genotypes had weak secondary branching followed by strong (12.2%) and clustered branching in only one genotype (WD17_20). The panicle was well exserted in 91.8% of the genotypes followed by mostly exserted in 7.2% and partially exserted in only one genotype. The length of decorticated grain was short in most of the genotypes (87.6%) and medium in 12.4%. The width of decorticated grain was narrow in 68% of the genotypes followed by medium (29.9%) and broad (2.1%). Nevertheless, selecting desirable panicle types in combination with suitable plant architecture could

be an appropriate breeding strategy through which to improve yield potential without compromising lodging resistance. Out of 39 DUS morphological descriptors studied, only 4 were monomorphic. The other 35 DUS characters were polymorphic among wild accessions and check cultivars. This shows the broader genetic base of wild rice species by nature.

Evaluation of qualitative agronomic descriptors revealed that accessions WD5_6, WD10_4, and WD3_3 consistently expressed a favourable combination of broad and long leaves, extended panicle length, and well-branched panicles with higher panicle number. Among these, WD5_6 emerged as the most promising genotype, integrating enhanced source capacity (large leaf area) with strong sink traits (long, highly branched panicles), in addition to its anthocyanin pigmentation across multiple organs. Such trait convergence highlights WD5_6 as a superior pre-breeding line for yield improvement and functional trait introgression.

Interspecific crosses between elite cultivars and wild rice species were made to develop introgression lines (ILs), enabling the transfer of yield-enhancing traits and resistance to biotic and abiotic stresses. This crossing program comprised 17 cross combinations involving six elite *Oryza sativa* cultivars, namely ADT38, DRR Dhan 65, DRR Dhan 50, Vikram TCR, RNR 15948 and WGL697, and nine wild rice accessions including an *O. barthii* (WD3_1), *O. nivara* (WD12_5), *O. ridleyi* (WD16_1), two *O. glaberrima* (WD5_1, WD5_7), and four *O. rufipogon accessions* (WD17_23, WD17_25, WD17_61 and WD17_64). Of the 17 crosses attempted, 13 resulted in an F_1 seed set, while 7 crosses produced surviving and confirmed F_1 plants, indicating successful interspecific hybridisation (Table S5). All successful crosses involved wild species possessing the AA genome—namely *O. barthii*, *O. glaberrima* and *O. rufipogon*—indicating that genome homology facilitated fertilisation and early hybrid development. Although the crosses DRR Dhan 65 × WD12_5 (*O. nivara*), DRR Dhan 65 × WD17_25 (*O. rufipogon*), DRR Dhan 65 × WD17_64 (*O. rufipogon*), Vikram TCR × WD17_64 (*O. rufipogon*), and RNR 15948 × WD17_23 (*O. rufipogon*) produced F_1 seeds and showed initial germination, the seedlings failed to survive due to post-zygotic incompatibilities, which could be hybrid weakness, partial sterility, endosperm imbalance, cytoplasmic–nuclear incompatibility, or linkage drag from wild alleles. Rao et al. (2018) also reported reductions in F_1 vigor and survival in *O. sativa* × AA-genome wild species crosses [40]. In their study, seven popular high-yielding Indian rice varieties were used as recurrent parents, and three wild accessions (*O. nivara*—IC 336681, IC 283150 and *O. rufipogon*—IC 309814) served as donor parents. F_1 hybrid germination varied from 60 to 100%; however, only a small proportion of the F_1 plants (7–21%) were confirmed as true hybrids, highlighting the presence of post-zygotic reproductive barriers even among AA-genome species [41]. In contrast, the cross involving *Oryza ridleyi* (WD16_1), which possesses the HHJJ genome, failed to produce viable F_1 plants due to strong genomic incompatibility between AA and non-AA genomes, resulting in embryo abortion or hybrid lethality. This study was conducted at a single location and during a single season due to the material's wild nature, which is not amenable to field evaluation. Pots with 15 L capacity filled with clay soil were used to grow plants with a recommended dose of fertilisers, per irrigated cultivation practices under open net house conditions, and they were exposed to existing weather parameters; no specific temperature or light regime was fixed for growing the materials. Therefore, we acknowledge this limitation, as most of the DUS and agronomic traits are environment-sensitive.

Despite the poor agronomic performance of wild rice species, they are emphasised as a valuable source of novel alleles and important donors for stress tolerance, disease resistance, and adaptive traits. Incorporating this diversity into modern breeding programs has become more efficient with the advent of marker-assisted selection (MAS),

which enables precise identification and selection of progeny carrying desirable alleles. MAS has significantly enhanced the development of introgression lines (ILs) by utilizing molecular markers distributed across the genome [41]. Furthermore, modern genomic technologies have accelerated the discovery and transfer of stress-tolerance genes from wild rice into cultivated varieties, facilitating the development of resilient rice strains that can withstand environmental stresses [42]. Harnessing the genetic potential of wild relatives and landraces through genomics-assisted breeding strategies presents a promising pathway toward sustainable rice improvement. Ram et al. (2010) found an introgression line B90-15 (IET15420) derived from an *Oryza rufipogon* accession (WR107) in the genetic background of the high-yielding line B32-Sel-4, which was susceptible to salinity, blast, BLB, and plant hoppers [43]. However, the introgression line exhibited broad-spectrum resistance to pests and diseases, improved yield under saline stress, and better cooking quality compared to other released varieties. It was later named 'Jarava' and released in 2006 by the Directorate of Rice Research (DRR), Rajendranagar, Hyderabad, India [44]. An introgression line 'IL248S' derived from Swarna × *Oryza nivara* was found to have a yield advantage of 25% over the national check variety Jaya and was released as the variety 'DRR Dhan 40' for commercial cultivation in Maharashtra, Tamil Nadu, and West Bengal [45]. A high-yielding and salinity-tolerant rice introgression line 'IL50-13' originated from the KMR3 × *O. rufipogon* cross developed at ICAR-IIRR, Hyderabad, in collaboration with Rice Research Station, Chinsurah, West Bengal. It was later released in the name of 'Chinsura Nona 2' (S.O. 3220 (E) dt 9 July 2019) [46]. Dhanrasi—a lowland variety with genes for grain yield, blast resistance, bacterial blight and tungro disease—introgressed from *O. rufipogon*. DRR Dhan 65, DRR Dhan 74 and DRR Dhan 88 are high-yielding, nutrient-use-efficient varieties with multiple stress tolerance derived from *O. rufipogon* [47]. Thus, previous reports and released varieties indicate the very high potential of introgression lines derived from interspecific crosses for crop improvement, as well as for the identification of novel genes for biotic and abiotic stress tolerance and grain yield. Recent advances in whole-genome sequencing and comparative genomics have strengthened the capacity to link phenotypes to underlying genetic variation. Reference genomes and resequencing data for multiple wild species now support allele mining, association mapping, and introgression strategies. Nevertheless, high-resolution phenotypic datasets remain essential to validate functional hypotheses derived from genomic analysis. Agromorphological characterisation ensures that the existing diversity is not only preserved but also rendered accessible, interpretable, and transferable to modern crop improvement programmes.

5. Conclusions

Extensive trait variation among *Oryza* wild species within and among taxa demonstrates the practical value of wild accessions for conservation, taxonomy and pre-breeding. Among the studied traits, only four—presence of leaf collar, presence of leaf ligule, lemma–anthocyanin colouration of keel, and anthocyanin colouration area below apex—were found to be monomorphic in the tested genotypes, demonstrating remarkable phenotypic diversity within each species with various trait combinations as valuable donors for agronomic enhancement. None of the traits showed any species-specific exclusivity, and conclusions can be drawn only from studies with a large number of wild accessions per species. Anthocyanin pigmentation was found in the majority of wild species for grain and consistently across plant organs in *O. meridionalis* and *O. barthii* accessions. Important agronomic traits like very early heading were observed in *O. glaberrima*, *O. rhizomatis*, *O. longiglumis* and *O. rufipogon* accessions, while long panicles were present in *O. meridionalis*, *O. nivara* and *O. rufipogon*, and strong panicle branching was observed in *O. Punctata* and *O. rufipogon*. Interspecific crosses using *O. glaberrima*, *O. barthii*, and *O. rufipogon* were also

developed against various cultivar backgrounds for the generation of advanced backcross populations. However, further progress requires deeper and more standardised phenotyping across the genus, especially for non-AA species and the teritiary gene pool, combined with genomic approaches to enable targeted selection and introgression to detect novel traits from wild species, validate their breeding potential, and broaden the genetic base of cultivated rice for future agricultural resilience.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/wild3020023/s1, Figure S1: Variations observed among rice genotypes for various DUS traits; Figure S2: Parental polymorphism between male and female parents using SSR/INDEL markers; Figure S3: F_1 Interspecific hybrids and their parents; Figure S4: Confirmation of interspecific F1 hybrids using molecular markers. Table S1: Detailed list of wild rice accessions and cultivars from ICAR—the Indian Institute of Rice Research—used in this study; Table S2: Essential characters along with descriptors and stage of observation for DUS characterisation; Table S3: Frequency distribution of 39 DUS traits among the rice accessions and varieties. Table S4: Details of genotypes under each state of essential characteristics along with descriptors and stage of observation for DUS characterisation; Table S5: Interspecific crosses developed against the background of *O. sativa* varieties.

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