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RESEARCH ARTICLE

Phenotypic Characterization of Crop Genetic Resources of AA-genome Species in Rice (*Oryza spp.*)

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Abstract

Rice (*Oryza sativa* L.) is vital for food security of global population especially in Asian countries where more than 90% of the worlds rice is produced and consumed. For exploring variability in morpho-qualitative traits in crop wild relatives (CWRs) for genetic improvement of rice cultivars, we characterized 88 accessions of eight *Oryza* species of diploid AA-genome utilizing 20 morpho-qualitative traits. High extent of within and between species phenotypic variation was observed for all the morpho-qualitative traits except anther color. Shannon-Weiner's diversity (H' = 0.96) and Pielou's measure of evenness (J = 0.67) also confirmed the presence of a high level of variability in 19 morpho-qualitative traits for both within and between the *Oryza* species. The greater diversity (H' >1.00 to 1.39) was recorded for 11 qualitative traits, namely, flag-leaf angle, basal-leaf sheath color, culm angle, panicle type, panicle exsertion, awn color, apiculus color, lemma and palea color, seed shattering, threshability and pericarp color. A moderate level of diversity (H' = 0.50 to 0.99) was observed for leaf-blade pubescence, ligule color, ligule shape, auricle color, stigma color, and lemma and palea pubescence. However, growth habit and awning displayed low level of diversity (H' < 0.50). Within species variability assessed in 41 accessions of *O. nivara* and 29 accessions of *O. rufipogon* revealed existence of higher variability, in *O. nivara* accessions (H' = 0.87) as compared to *O. rufipogon* (H' = 0.69) for most of the traits. Ward's distance dendrogram grouped the accessions of CWRs into five major clusters, each containing accessions of three to seven species of *Oryza* AA-genome.

Keywords: AA-genome, *Oryza* species, Phenotypic characterization, Qualitative traits, Wild rice.

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Introduction

Rice (*Oryza sativa* L.) is one of the oldest domesticated cereals in the world. Rice is known as "wonder grain" because it is staple food for more than half of the world's population. For the ever-increasing human population, genetic improvement plays an important role in matching the enhancement of the production and productivity of rice crop to fulfil growing food demand (Khush, 2013). The current global rice (paddy) production is about 756.3 million tonnes, where India ranks second next to China, producing 178.3 million tonnes (FAO, 2022). To meet the challenge of increased production, the novel allelic variation needs to be explored in wild rice germplasm (Brar and Khush, 2018). The genus *Oryza* includes two cultivated ($2n = 24$) and 22 wild species ($2n = 24, 48$) consisting of six diploid (AA, BB, CC, EE, FF and GG) and five tetraploid (BBCC, CCDD, KKLL, HHKK and HHJJ) genome types (Vaughan, 1989; Stein *et al*., 2018). As the genetic variability is limited within the cultivated species of rice, the improvement of rice cultivars depends mainly on exploiting the diversity available in the gene pool of crop wild relatives (CWRs) (McCouch *et al*., 2012, 2013). The *Oryza* species are further divided into three gene pools based on crossability and phylogenetic relationships (Harlan and de Wet, 1971; Vaughan, 1989). The primary genepool consists of two cultivated (*O. sativa* and *O. glaberrima*) and six wild species namely, *O. nivara*, *O. rufipogon*, *O. barthii*, *O.*

longistaminata, *O. glumaepatula* and *O. meridionalis*), all belonging to *Oryza* AA-genome (Vaughan *et al.,* 2005). All eight AA-genome species are diploid, cross compatible and showed homologous pairing in the chromosomes (Vaughan *et al.,* 2008; Alsantely *et al*., 2023).

The wild species of *Oryza* are weedy grass like plants, which are inferior in morphological traits such as poor grain quality, inferior plant type, grain shattering and low grain yield (Xu and Sun, 2021; Fornaseiro *et al*., 2022). Though the wild rice species are inferior in morphological traits, they possess a repertoire of useful genes for resistance to biotic, abiotic stress and yield-related traits (Sanchez *et al*., 2013). The morphological traits of wild species differ markedly from cultivated species such as growth habit, tillering, plant height, leaf size, culm, flowering patterns, panicle exsertion, panicle size, panicle branching, awning, seed traits, and adaptation to different habitats and agronomic traits (Brar and Khush 2018; Stein *et al*., 2018; Gaikwad *et al*., 2020). Understanding the extent and type of genetic variation in wild rice populations may help to identify valuable accessions possessing desirable traits for germplasm conservation and utilization in crop improvement. The qualitative trait-based phenotypic characterization has been utilized in rice (Joseph *et al*., 2007; Kharel *et al*., 2022) and CWRs (Khan *et al*., 2021; Sandamal *et al*., 2021), especially to assess the genetic variation in AA-genome species of genus *Oryza* (Samal *et al*., 2018; Singh *et al*., 2018; Tiwari *et al*., 2020). Shannon-Weiner index has been used frequently to measure the diversity within and between the species utilizing both qualitative and quantitative traits in cultivated and wild rice germplasm (Hein *et al*., 2007; Sow *et al*., 2013; Rabara *et al*., 2014; Lei *et al*., 2018; Rao *et al*., 2021). For the effective utilization of rice germplasm consisting of CWRs in plant breeding programmes, firstly they need to be characterized and evaluated. Therefore, the present study was aimed to characterize wild rice accessions of AA-genome species utilizing morpho-qualitative traits.

Material and Methods

Experimental Materials

The experimental materials consisted of 90 *Oryza* accessions belonging to seven wild, one intermediate and two cultivated species of rice acquired from the Indian National Gene Bank, ICAR-National Bureau of Plant Genetic Resources (NBPGR), New Delhi. The wild rice species mainly consisted of AA genome of genus *Oryza* (88 accessions) and one species of CC genome (*O. Officinalis*; two accessions). Of these AA genome species, 41 accessions were of *O. nivara*, 29 accessions of *O. rufipogon*, five accessions of *O. glaberrima,* three accession of *O. meridionalis,* two accessions each of *O. glumaepatula, O. sativa* f. *spontanea,* and *O. longistaminata,* one accession of *O. barthii* and three accessions are of *O. sativa* (checks). The details of rice germplasm accessions studied are provided in Table 1. Geo-referencing of the wild rice accessions was performed using passport data information using 'DIVA-GIS' software version 7.5 (Hijmans *et al*., 2001). The collection sites of 90 *Oryza* spp. accessions were geo-referenced on world map and also depicted on enlarged India map (Figure 1). The geographic distribution of collection sites and number of collections from different countries, states and districts are provided in Table 2.

Morpho-qualitative Trait-based Characterization

Field experiment was conducted at Experimental Farm, Division of Genetics, ICAR-Indian Agricultural Research Institute, New Delhi during June to December 2021. The experiment was conducted in Augmented Block Design (ABD), and a 40 × 40 cm distance was maintained between line to line and plant to plant. Five blocks with, each containing 18 test accessions and three checks maintained in ABD. The cultural practices were followed as per the standard recommendations for raising a healthy cultivated rice crop. We utilized morphological descriptors developed by International Rice Research Institute (IRRI's) Standard Evaluation System (2013) and Bioversity International (2007) for the phenotypic characterization of wild rice germplasm. Observations were recorded on 20 qualitative traits *viz.,* growth habit, culm angle, basal-leaf sheath color, flag-leaf angle, leaf-blade pubescence, ligule color, ligule shape, auricle color, panicle type, panicle exsertion, awning, awn color, apiculus color, stigma color, anther color, lemma and palea color, seed shattering, panicle threshability, pericarp color, and lemma and palea pubescence. The data on five randomly chosen plants from each accession were recorded at an appropriate plant growth and development stage.

Statistical Analysis

The dendrogram was constructed based on Ward's distance method using R-software version 3.6.3 (Le *et al*., 2008). The bar diagrams depicting the frequency distributions of morpho-qualitative traits were prepared using MS Excel. The diversity estimates based on morpho-qualitative traits were derived utilizing Shannon-Weiner index (Shannon and Weaver, 1949). The formula used for calculating Shannon-Weiner index is as follows.

$$\text{Shannon-Weiner index (H')} = -\sum_{i=1}^{n} \text{Pi} \ln \text{Pi}$$

Where n is the number of phenotypic classes for a character, P_i is the relative frequency in the i^{th} class of the j^{th} trait and ln is the natural logarithm of P_i. The extent of diversity was interpreted as < 0.50 value of H': low diversity, 0.50 to 1.00 value of H' : moderate and >1.00 value of H': high diversity in the germplasm. The traits, which showed higher value of Shannon-Weiner index, revealed the existence of greater diversity among the accessions. The distribution of diversity was calculated based on Pielou's measure of evenness

Table 1: Passport data of accessions of *Oryza* species germplasm used in morphological analysis

Sl. No.	*Acc. code*	*Accession number*	*Species name*	*Local name*	*District of collection*	*Latitude*	*Longitude*	*State/Country*
1	WR727	IC283158	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
2	WR728	IC283160	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
3	WR729	IC283166	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
4	WR730	IC283172	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
5	WR731	IC283176	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
6	WR732	IC283180	*O. nivara*	Jhara	Malkangiri	18.257	81.953	Odisha
7	WR733	IC283149	*O. nivara*	Jhara	Koraput	18.856	82.734	Odisha
8	WR734	IC283152	*O. nivara*	Jhara	Koraput	18.856	82.734	Odisha
9	WR735	IC283155	*O. nivara*	Jhara	Koraput	18.856	82.734	Odisha
10	WR736	IC283181	*O. nivara*	Jhara	Koraput	18.856	82.734	Odisha
11	WR737	IC282978	*O. nivara*	Balunga	Jharsuguda	21.805	83.952	Odisha
12	WR738	IC282985	*O. nivara*	Jhara	Jharsuguda	21.805	83.952	Odisha
13	WR739	IC309827	*O. nivara*	Jhara	Jharsuguda	21.805	83.952	Odisha
14	WR740	IC256850	*O. nivara*	Jhara	Sambalpur	21.445	84.270	Odisha
15	WR741	IC282989	*O. nivara*	Balunga	Sambalpur	21.445	84.270	Odisha
16	WR743	IC336689	*O. nivara*	Paserbalunga	Balangir	20.866	83.216	Odisha
17	WR744	IC336693	*O. nivara*	Paserbalunga	Balangir	20.298	82.829	Odisha
18	WR745	IC283198	*O. nivara*	Jhara	Kalahandi	19.866	83.109	Odisha
19	WR746	IC336695	*O. nivara*	Paserbalnga	Kalahandi	19.866	83.109	Odisha
20	WR747	IC336699	*O. nivara*	Paser	Kalahandi	19.866	83.109	Odisha
21	WR748	IC283189	*O. nivara*	Jhara	Nabarangapur	19.575	82.336	Odisha
22	WR750	IC283195	*O. nivara*	Jhara	Nabarangapur	19.575	82.336	Odisha
23	WR751	IC282993	*O. nivara*	Balunga	Dhenkanal	20.826	85.526	Odisha
24	WR752	IC257161	*O. nivara*	Jhara	Ganjam	19.374	85.083	Odisha
25	WR753	IC330654	*O. nivara*	Jalidhan	Dakshin Dinajpur	25.365	88.580	West Bengal
26	WR754	IC330657	*O. nivara*	Kawa thekra	Dakshin Dinajpur	25.365	88.580	West Bengal
27	WR755	IC330612	*O. nivara*	Jhara	Malda	25.131	88.087	West Bengal
28	WR756	IC330653	*O. nivara*	Jalidhan	Malda	25.131	88.087	West Bengal
29	WR757	IC330610	*O. nivara*	Jhara	Murshidabad	24.161	88.232	West Bengal
30	WR758	IC330611	*O. nivara*	Palua	Murshidabad	24.161	88.232	West Bengal
31	WR759	IC330595	*O. nivara*	Jhara	Birbhum	23.953	87.665	West Bengal
32	WR760	IC330616	*O. nivara*	Jhara	Birbhum	23.953	87.665	West Bengal
33	WR761	IC330621	*O. nivara*	Pasarhi	Faizabad	23.953	87.665	U.P.
34	WR762	IC272981	*O. nivara*	Pasarhi	Faizabad	26.656	82.001	U.P.
35	WR763	IC272984	*O. nivara*	Pasarhi	Faizabad	26.656	82.001	U.P.
36	WR766	IC332599	*O. nivara*	Dhan	Lucknow	26.687	80.984	U.P.
37	WR767	IC598071	*O. nivara*	Tini	Varanasi	25.316	82.973	U.P.
38	WR768	IC598072	*O. nivara*	Tini	Sonbhadra	24.457	82.993	U.P.
39	WR769	IC598110	*O. nivara*	Beda Dhan	Garhwa	24.154	83.799	Jharkhand
40	WR770	IC598108	*O. nivara*	Beda Dhan	Garhwa	24.040	83.540	Jharkhand
41	WR771	IC598106	*O. nivara*	Beda Dhan	Palamau	24.128	84.185	Jharkhand
42	WR773	IC598078	*O. rufipogon*	Baunadhan	Dakshin Dinajpur	25.371	88.556	West Bengal
43	WR774	IC330666	*O. rufipogon*	Jhadadhan	Uttar Dinajpur	25.480	88.240	West Bengal
44	WR775	IC596548	*O. rufipogon*	-	Nagaon	26.460	92.580	Assam
45	WR776	IC381929	*O. rufipogon*	Uridol	Lakhimpur	27.233	94.116	Assam
46	WR777	IC596545	*O. rufipogon*	-	Lakhimpur	27.370	94.250	Assam
47	WR778	IC596547	*O. rufipogon*	-	Lakhimpur	27.608	94.769	Assam

48	WR779	IC336675	*O. rufipogon*	-	Dhemaji	20.212	84.999	Assam
49	WR780	IC256748	*O. rufipogon*	Balunga	Nayagarh	21.895	83.395	Odisha
50	WR781	IC309826	*O. rufipogon*	Jhara	Jharsuguda	21.805	83.952	Odisha
51	WR782	IC309829	*O. rufipogon*	Jhara	Jharsuguda	21.805	83.952	Odisha
52	WR783	IC256759	*O. rufipogon*	Jhara	Sambalpur	21.445	84.270	Odisha
53	WR784	IC598102	*O. rufipogon*	Boridhan	Lohardaga	23.433	84.647	Jharkhand
54	WR785	IC598103	*O. rufipogon*	Beda dhan	Lohardaga	23.433	84.647	Jharkhand
55	WR787	IC330594	*O. rufipogon*	Jhara	Birbhum	23.953	87.665	West Bengal
56	WR789	IC330627	*O. rufipogon*	Udi	Nadia	23.478	88.520	West Bengal
57	WR790	IC330607	*O. rufipogon*	Jhara	Murshidabad	24.161	88.232	West Bengal
58	WR791	IC330609	*O. rufipogon*	Jhara	Murshidabad	24.161	88.232	West Bengal
59	WR792	IC330668	*O. rufipogon*	Dal	Malda	25.131	88.087	West Bengal
60	WR793	IC283165	*O. rufipogon*	Jhara	Malkangiri	18.257	81.953	Odisha
61	WR794	IC283191	*O. rufipogon*	Jhara	Nabarangapur	19.575	82.336	Odisha
62	WR796	IC336687	*O. rufipogon*	Posari	Balangir	20.298	82.829	Odisha
63	WR798	IC283199	*O. rufipogon*	Jhara	Kalahandi	19.866	83.109	Odisha
64	WR799	IC336703	*O. rufipogon*	Paser	Kalahandi	19.866	83.109	Odisha
65	WR800	IC330672	*O. rufipogon*	Balunga	Puri	19.663	85.510	Odisha
66	WR801	IC330676	*O. rufipogon*	Balunga	Puri	19.663	85.510	Odisha
67	WR802	IC336710	*O. rufipogon*	Pansari	Gajapati	19.184	84.160	Odisha
68	WR803	IC257084	*O. rufipogon*	Danda	Cuttack	20.452	85.700	Odisha
69	WR804	IC362061	*O. rufipogon*	Valunki	Jagatsinghpur	20.223	86.181	Odisha
70	WR805	IC362066	*O. rufipogon*	Utennenditeh	Jagatsinghpur	20.223	86.181	Odisha
71	WR806	IC556030	*O. glaberrima*	-	Bamako	12.639	-8.003	Mali
72	WR807	EC132649	*O. glaberrima*	-	Timbuktu	16.766	-3.002	Mali
73	WR808	EC133117	*O. glaberrima*	-	Kwara	8.966	4.387	Nigeria
74	WR809	EC132675	*O. glaberrima*	-	Sikasso	11.322	-5.698	Mali
75	WR810	EC133087	*O. glaberrima*	-	Kaduna	10.501	7.440	Nigeria
76	WR811	IC556056	*O. meridionalis*	-	katherine	-14.452	132.269	Australia
77	WR812	EC384083	*O. meridionalis*	-	katherine	-14.087	132.487	Australia
78	WR813	EC384124	*O. meridionalis*	-	Tenant creek	-19.645	134.191	Australia
79	WR814	IC301735	*O. glumaepatula*	-	Costa Rica	9.748	-83.753	C. America
80	WR815	IC301738	*O. glumaepatula*	-	Panama	8.538	-80.782	C. America
81	WR816	IC380147	*O. barthii*	-	Ondo	6.914	5.147	Nigeria
82	WR818	IC301734	*O. officinalis*	-	Raipur	21.251	81.629	Chhattisgarh
83	WR819	EC384063	*O. officinalis*	-	Pattaya	12.923	100.882	Thailand
84	WR820	IC627921	*O. sativa* f. *spontanea*	Uri Dholl Ghas	Morigaon	26.110	92.220	Assam
85	WR821	IC627922	*O. sativa* f. *spontanea*	Uri Dholl Ghas	Morigaon	26.060	92.060	Assam
86	WR822	EC133275	*O. longistaminata*	-	Borno	11.884	13.152	Nigeria
87	WR823	EC784830	*O. longistaminata*	-	Jigawa	12.228	9.561	Nigeria
88	Nagina22	IC123319	*O. sativa*	-	Nagina	29.443	78.432	U.P.
89	Pusa44	IC590849	*O. sativa*	-	IARI Delhi	28.633	77.152	Delhi
90	Pusa 1850	IC627102	*O. sativa*	-	IARI Delhi	28.633	77.152	Delhi

Abbreviations: Acc. = Accession; O. = *Oryza*; C. America = Central America; U.P. = Uttar Pradesh

Table 2: Geographic distribution of 90 accessions of *Oryza* species germplasm

Sl. No.	*Species*	*State/Country*	*Site/District of collection*	*Total*
1.	*O. nivara*	Odisha	Malkangiri (6), Koraput (4), Jharsuguda (3), Sambalpur (2), Balangir (2), Kalahandi (3), Nabarangapur (2), Dhenkanal (1) and Ganjam (1)	24
		West Bengal	Dakshin Dinajpur (2), Malda (2), Murshidabad (2) and Birbhum (2)	8
		Uttar Pradesh	Faizabad (3), Lucknow (1), Varanasi (1) and Sonbhadra (1)	6
		Jharkhand	Garhwa (2) and Palamau (1)	3
	Total accessions	4 states	19 districts	41
2.	*O. rufipogon*	West Bengal,	Dakshin Dinajpur (1), Uttar Dinajpur (1), Birbhum (1), Nadia (1), Murshidabad (2), Malda (1)	7
		Assam	Nagaon (1), Lakhimpur (3), Dhemaji (1)	5
		Odisha	Malkangiri (1), Nabarangapur (1), Balangir (1), Kalahandi (2), Puri (2), Gajapati (1), Cuttack (1), Jagatsinghpur (2), Nayagarh (1), Jharsuguda (2), Sambalpur (1)	15
		Jharkhand	Lohardaga (2)	2
	Total accessions	4 states	17 districts	29
3.	*O. glaberrima*	Mali	Bamako (1), Timbuktu (1), Sikasso (1)	3
		Nigeria	Kwara (1), Kaduna (1)	2
4.	*O. meridionalis*	Australia	Katherine (2), Tenant creek (1)	3
5.	*O. glumaepatula*	C. America	Costa Rica (1), Panama (1)	2
6.	*O. barthii*	Nigeria	Ondo (1)	1
7.	*O. officinalis*	Chhattisgarh	Raipur	1
		Thailand	Pattaya	1
8.	*O. sativa* f. *spontanea*	Assam	Morigaon (2)	2
9.	*O. longistaminata*	Nigeria	Borno (1), Jigawa (1)	2
10.	*O. sativa* (Checks)	Delhi	New Delhi (3)	3
	Total accessions	7 countries	-	20

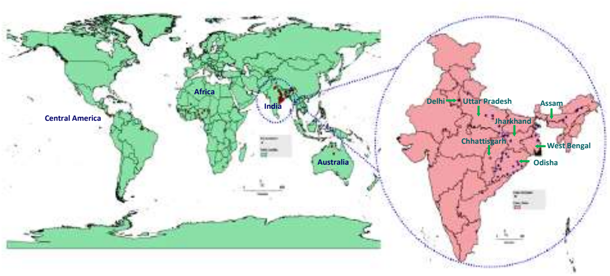

Figure 1: World map showing geo-referencing of 96 accessions of nine *Oryza* species. Enlarged India map depicting distribution of various accessions of wild rice species in seven states of Indian Union.

(Pielou, 1966). The value of evenness gives equality in the distribution of traits among the accessions.

Results

Morphological Variation for Qualitative Traits

The data of 20 morpho-qualitative traits was recorded in 90 accessions of 10 *Oryza* species using IRRI and Bioversity International descriptors. Bar diagrams depicting frequency distribution and morphological variability for 15 qualitative traits are presented vide Figure 2. A wide range of variability was observed for all the morpho-qualitative traits between different accessions within species and between accessions of different species (Figure 3). The growth habit of wild rice accessions varied from annual to perennial types. Out of 90 accessions, 94.5% of the accessions exhibited annual growth habit and 5.5% of the accessions showed perennial growth habit. Among the morpho-qualitative traits, the highest variation was observed for culm angle. Five alternative forms of culm angles were erect, semi-erect, open, spreading and procumbent. Most of the accessions displayed semi-erect (40.0%) type of culm angle followed by erect type (31.1%). The procumbent type of culm angle was observed only in five accessions of *O. rufipogon*. The green color of basal-leaf sheath was the prominent one (48.9%) followed by light purple (30.0%) and purple (12.2%) colors (Figures 2b, 3a, b). The purple lines on basal-leaf sheath were observed mostly in *O. nivara* accessions (Figure 3c). Among the wild rice accessions studied, we observed four types of leaf angles in the germplasm. The most prominent type was horizontal (43.3%) followed by erect (28.9%) and

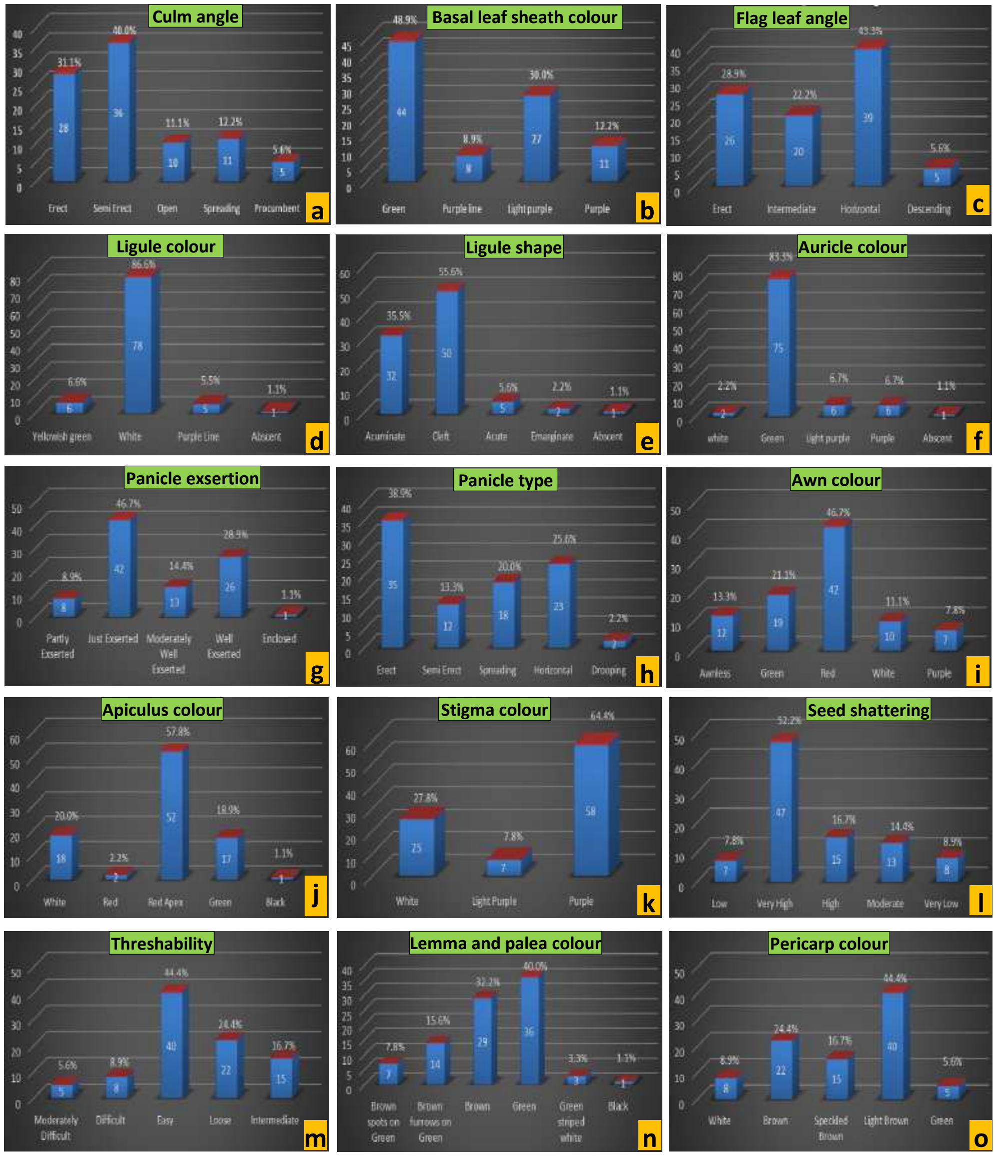

Figure 2: Bar diagrams displaying frequency distribution and phenotypic classes among 90 accessions of *Oryza species* for 15 qualitative traits. (a) Culm angle, (b) Basal-leaf sheath, (c) Flag-leaf attitude, (d) Ligule color, (e) Ligule shape, (f) Auricle color (g) Panicle exsertion, (h) Panicle type, (i) Awn color, (j) Apiculus color, (k) Stigma color, (l) Seed shattering, (m) Threshability, (n) Hull color and (o) Pericarp color.

intermediate (22.2%). The descending type (5.6%) of leaf orientation was the least observed. In cultivated rice, three types of leaf-blade pubescence are found namely, glabrous, intermediate and pubescent. However, the accessions in present study showed only two types with most of the accessions displayed pubescent (80.0%) type of leaf-blade pubescence followed by intermediate (20.0%).

Most of the wild rice accession exhibited white (86.7%) colored ligules followed by yellowish green (6.7%) and purple lines on ligules (5.5%) (Figures 2d, 3d-f). In AA-genome *Oryza* species studied, most of the accessions possessed green colored auricles (83.3%), whereas light purple and purple (6.7% each) (Figures 2f, 3g-i) and white (2.2%) colored auricles found in low frequency. Out of 90 accessions, we found one accession namely WR819 (*O. officinalis*) of liguleless type, while most of the accessions possessed 2-cleft (55.6%) ligule shape followed by acuminate (35.5%), acute (5.6%) and emerginate (2.2%) ligules shapes (Figures 2e, 3j-l). Most of the accessions showed just exserted (46.7%) type of panicle exsertion followed by well-exerted (28.9%) and moderately well-exserted (14.4%) panicles (Figure 2g). However, 8.9% accessions displayed partly exserted and 1.1% of accessions showed enclosed type of panicle exsertion. Erect type was the prominent panicle type and observed in 38.9% of the accessions followed by horizontal (25.6%) and spreading (20.0%) types of panicles (Figure 2h). The semi-erect (13.3%) and drooping type (2.2%, *O. officinalis*) of panicles were observed.

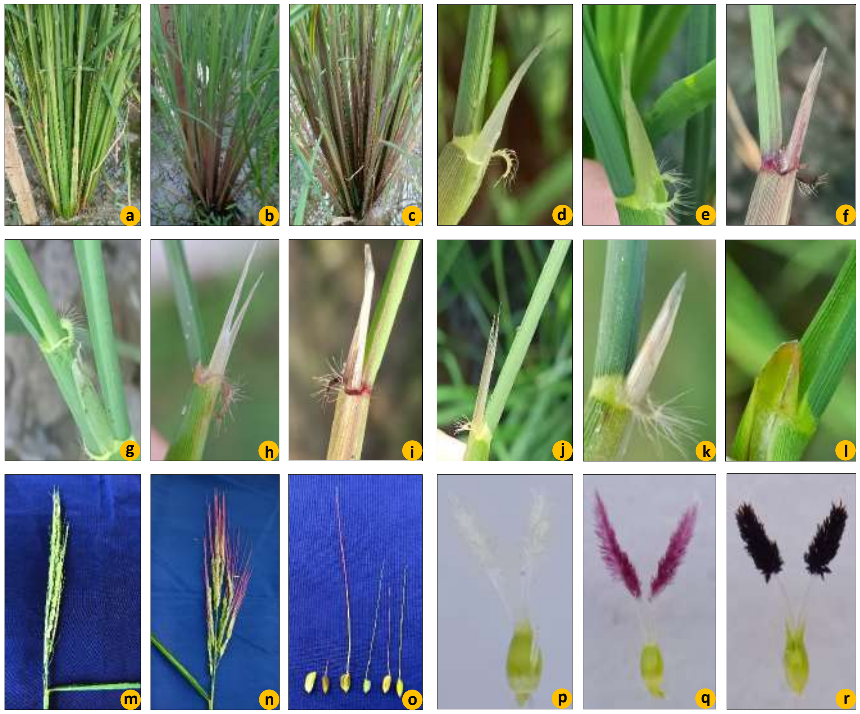

Figure 3: Basal-Leaf sheath color: (a) Green-WR732 (*O. nivara*), (b) Light Purple-WR809 (*O. glaberrima*), (c) Purple line-WR735 (*O. nivara*); Ligule color: (d) White-WR791 (*O. rufipogon*), (e) Yellowish green-WR733 (*O. nivara*), (f) Purple lines- WR-809 (*O. glaberrima*); Auricle color: (g) Green-WR 740 (*O. nivara*), (h) Light purple-WR809 (*O. glaberrima*; (i) Purple-WR781 (*O. rufipogon*); Ligule shape: (j) 2-Cleft-WR768 (*O. nivara*), (k) Acuminate-WR753 (*O. nivara*), (l) Acute-WR813 (*O. meridionalis*); Awn color: (m) Green-WR823 (*O. longistaminata*), (n) Red-WR782 (*O. rufipogon*), (o) Variations in awn size; Stigma color: (p) White-WR804 (*O. rufipogon*), (q) Light purple -WR743 (*O. nivara*), (r) Purple-WR809 (*O. glaberrima*)

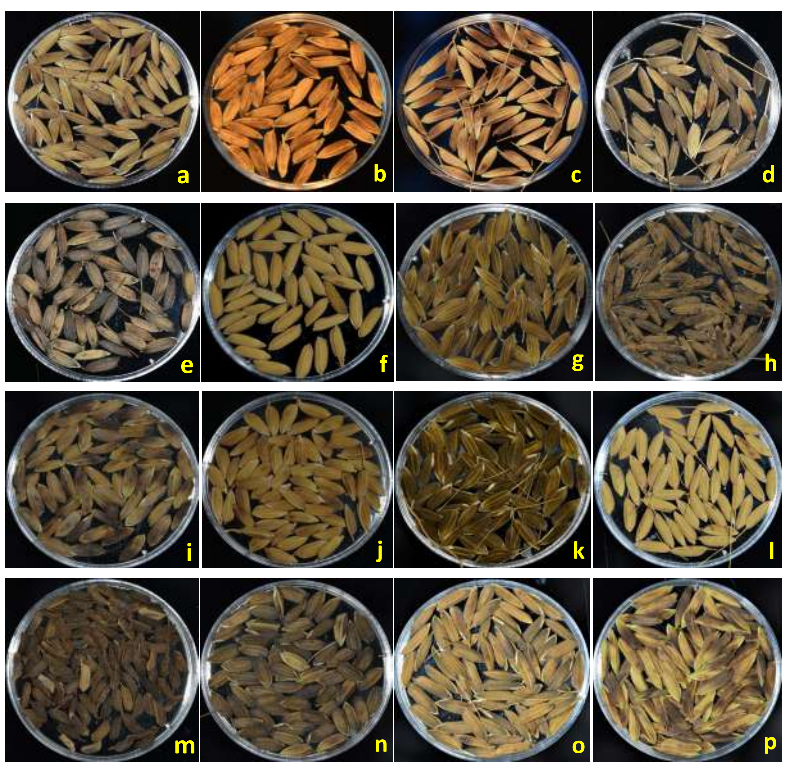

Figure 4: Phenotypic variability for hull color in *Oryza* species after harvest. (a-f) *nivara* (WR727, 728,729, 730, 757, 766), (g-i) *rufipogon* (WR773, 785, 804), (j-K) *glaberrima* (WR806, 807), (l) *meridionalis* (WR811), (m) *officinalis* (WR818), (n-o) *spontanea* (WR820, 821), and (p) *longistaminata* (WR823)

Figure 5: Phenotypic variability for pericarp color in *Oryza* species: (a) White in WR816 (*O. barthii*), (b) Brown in WR811 (*O. meridionalis*), (c) Light Brown in WR808 (*O. glaberrima*), (d) Speckled brown in WR732 (*O. nivara*) and w Green in WR799 (*O. rufipogon*)

Awning is a primitive character mostly found in CWRs of rice. The wild rice accessions exhibited awning phenotype (86.7%), whereas awnless phenotype (13.3%) was observed mainly in cultivated rice (Figure 3o). Amongst the four awn color variation observed, red colored awns (46.7%) were the most frequent followed by green (21.1%), white (11.1%) and purple (7.8%) (Figures 2i, 3m, n). Majority of the accessions displayed red apiculus color (57.8%) followed by white (20.0%) and green apiculus (18.9%) colors. However, red apex found in 2.2% accessions (WR731 and WR768; *O. nivara*) and black apiculus color in one accession (WR813; *O. meridionalis*). All the accessions produced yellow colored anthers. Three variants of stigma color were observed namely, purple in 64.4% accessions, the most frequent type, white in 27.8% and light purple in 7.8% of the accessions of *Oryza* species (Figures 2k, 3p-r).

Based on seed shattering pattern, most of the accessions displayed very high seed shattering (52.2%) followed by high (16.7%) and moderate (14.4%) (Figure 2l). The low (7.8%) and very low (8.9%) seed shattering observed mainly in cultivated rice accessions. After harvest, the threshability pattern of wild rice accessions was assessed by hand threshing. In most of the accessions, we observed easy threshability (44.4%) followed by loose (24.4%) and intermediate type (16.7%) (Figure 2m). The difficult (8.9%) and moderately difficult type (5.6%) of threshability were also found. Hull color observed at pre-maturity stage, showed a wide range of phenotypic variation (Figure 4). Green color recorded in 40.0% accessions, was the most frequent color followed by brown (32.2%), brown furrows on green (15.6%) and brown spots on green (7.8%) (Figure 2n). However, green striped white (3.3%) in WR746, WR767 (*O. nivara*) and WR815 (*O. glumaepatula)* and black (1.1%) found in WR813 (*O. meridionalis*). For lemma and palea pubescence, short hairs type (72.2%) was the prominent followed by hairs on upper portion (26.7%) and glabrous (1.1%) observed in WR806 (*O. glaberrima*). Pericarp color was recorded after dehusking rice grains (Figure 5a-e). Five pericarp colors observed based on the visual observation. Most of the accessions showed light brown (44.4%), followed by brown (24.4%), speckled brown (16.7%), white (8.9%) and green (5.6%) colored pericarp (Figure 2o).

Genetic Diversity in Wild Rice Germplasm

The Shannon-Weiner diversity index and Pielou's measure of evenness showed enormous variation within and between species for morpho-qualitative traits in *Oryza* germplasm accessions (Table 3). However, anthers displayed different shades of yellow colors, light yellow in immature to dark yellow in matured anthers with zero values of diversity index and evenness. The high level of diversity recorded in 11 morpho-qualitative traits namely, awn color, culm angle, panicle type, lemma and palea color, threshability, pericarp color, seed shattering, panicle exsertion, flag-leaf angle, basal-leaf sheath color and apiculus color. Six traits *viz*., ligule shape, stigma color, auricle color, lemma and palea pubescence, ligule color and leaf-blade pubescence showed

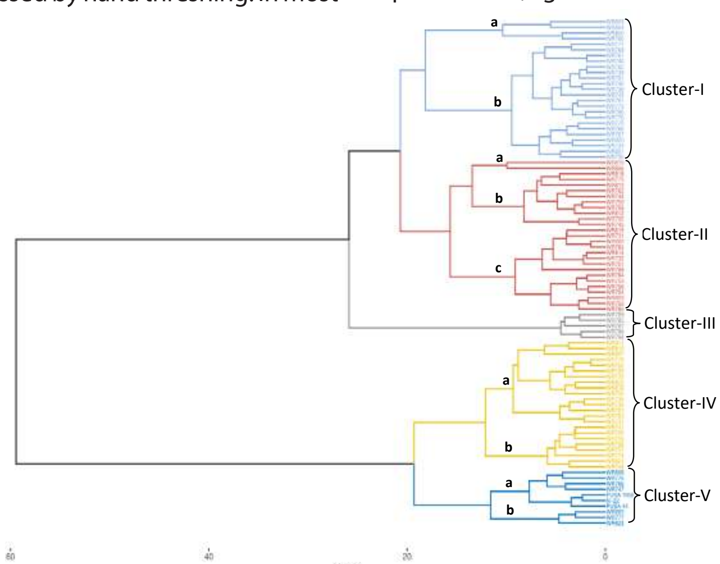

Figure 6: Dendrogram constructed using Ward's distance matrices based on data of 20 morpho-qualitative traits recorded in 90 accessions of *Oryza* species. Five clusters are shown on the right margin of the dendrogram with cluster-I divided into two subclusters (a-b), cluster-II into three subclusters (a-c), and cluster-IV and cluster-V each into two subclusters (a-b).

Table 3: Shannon -Weiner diversity index and evenness of 90 *Oryza* accessions based on qualitative data

Sl. No.	*Traits*	*Evenness (J)*			*Shannon-Weiner index (H')*		
		Between species	*Within nivara*	*Within rufipogon*	*Between species*	*Within nivara*	*Within rufipogon*
1.	Growth habit	0.31	0.00	0.66	0.22	0.00	0.66
2.	Culm angle	0.87	0.94	0.83	1.39	1.30	1.33
3.	Basal-leaf sheath color	0.85	0.95	0.83	1.18	1.32	1.15
4.	Flag-leaf angle	0.88	0.89	0.70	1.22	1.24	0.97
5.	Leaf blade pubescence	0.72	0.80	0.36	0.50	0.56	0.25w
6.	Ligule color	0.37	0.41	0.36	0.52	0.45	0.25
7.	Ligule shape	0.62	0.71	0.36	0.99	0.78	0.25
8.	Auricle color	0.40	0.35	0.52	0.65	0.49	0.52
9.	Panicle exsertion	0.78	0.86	0.83	1.26	1.20	0.83
10.	Panicle type	0.86	0.82	0.93	1.39	1.32	0.93
11.	Awning	0.57	0.17	0.58	0.39	0.12	0.58
12.	Awn color	0.87	0.80	0.63	1.40	1.29	0.63
13.	Apiculus color	0.68	0.82	0.68	1.09	1.13	0.68
14.	Stigma color	0.76	0.81	0.66	0.84	0.89	0.66
15.	Anther color	0.00	0.00	0.00	0.00	0.00	0.00
16.	Lemma and palea color	0.77	0.91	0.83	1.38	1.46	0.83
17.	Seed shattering	0.83	0.75	0.79	1.33	1.04	0.79
18.	Threshability	0.86	0.79	0.88	1.38	1.10	0.88
19.	Pericarp color	0.86	0.79	0.88	1.38	1.10	0.83
20.	Lemma and palea pubescence	0.58	0.90	0.85	0.64	0.63	0.85
	Average	0.67	0.67	0.66	0.96	0.87	0.69

Table 4: Distribution of 90 accessions of *Oryza* species into different clusters based on Wards' distance dendrogram

luster I (25 accessions)		*Cluster II (27 accessions)*		*Cluster III (5 accessions)*	*Cluster IV (23 accessions)*		*Cluster V (10 accessions)*
WR809	WR738	WR819	WR802	WR789	WR811	WR734	WR808
WR804	WR745	WR806	WR783	WR785	WR810	WR727	WR775
WR800	WR781	WR818	WR814	WR787	WR807	WR763	WR766
WR760	WR779	WR773	WR732	WR796	WR778	WR737	WR747
WR771	WR790	WR813	WR751	WR794	WR752	WR741	PUSA1850
WR768	WR776	WR762	WR799		WR729	WR728	N22
WR761	WR770	WR744	WR784		WR733	WR793	PUSA44
WR746	WR769	WR759	WR753		WR822	WR798	WR805
WR782	WR767	WR758	WR756		WR816	WR757	WR777
WR739	WR820	WR812	WR754		WR750	WR774	WR823
WR791	WR735	WR792	WR803		WR736	WR821	
WR740	WR801	WR743	WR755			WR748	
	WR730	WR815	WR780				
		WR731					

moderate level of morphological diversity, whereas two traits like growth habit and awning displayed low diversity amongst 90 accessions of 10 *Oryza* species. The maximum evenness observed in the traits *viz.*, flag leaf angle/attitude (0.88), awn color (0.87) and culm angle (0.87). The minimum evenness found in growth habit (0.31) followed by ligule color (0.37).

We observed within species high variability in *O. nivara* accessions for 11 traits namely, lemma and palea color, basal-leaf sheath color, panicle type, culm angle, awn color, flag-leaf angle, panicle exsertion, apiculus color, threshability, pericarp color and seed shattering. The moderate within species variability observed for stigma color, ligule shape, leaf-blade pubescence, and lemma and palea pubescence, while it was low for auricle color, ligule color and awning. However, there was no variation for two qualitative traits *viz*., growth habit and anther color for 41 *O. nivara* accessions. For *O. nivara* accessions, high evenness observed for 11 traits *viz*., basal leaf sheath color, culm angle, lemma and palea color, lemma and palea pubescence, flag-leaf angle, panicle exsertion, panicle type, apiculus color, stigma color, awn color and leaf-blade pubescence. Low evenness observed in auricle color, ligule color and awning.

Within species high diversity observed in *O. rufipogon* accessions for two traits *viz*., culm angle and basal-leaf sheath color, while the moderate level of variation found for 14 traits namely, flag-leaf angle, panicle type, threshability, lemma and palea pubescence, panicle exsertion, pericarp color, lemma and palea color, seed shattering, apiculus color, stigma color, growth habit, awning, auricle color and awn color. However, traits such as ligule shape, ligule color and leaf-blade pubescence revealed low within species diversity. The maximum evenness observed in panicle type (0.93) followed by threshability (0.88) and pericarp color (0.88). The minimum evenness was observed for ligule shape, ligule color and leaf- blade pubescence.

Genetic Inter-relationships in Wild Rice Germplasm

The dendrogram based on Ward's distance grouped the 90 accessions into five major clusters (Figure 6). Cluster-I included 25 accessions, of which 14 accessions belonged to *O. nivara*, 9 to *O. rufipogon*, one to *O. glaberrima* and one to *O. sativa* var. *spontanea* (Table 4). Cluster-II grouped 27 accessions consisted of 12 of *O. nivara*, 8 of *O. rufipogon*, two accessions each of *O. officinalis*, *O. meridionalis* and *O. glumaepatula*, and one accession of *O. glaberrima*. Cluster-III contained five accessions of *O. rufipogon* only. Cluster-IV separated 23 accessions consisting of 13 of *O. nivara*, four of *O. rufipogon*, two of *O. glaberrima* and one accession each of *O. meridionalis*, *O. barthii*, *O. sativa* var. *spontanea* and *O. longistaminata*. Cluster-V grouped 10 accessions, two accessions of *O. nivara*, three of *O. rufipogon*, three of *O. sativa* (checks), and one accession each of *O. glaberrima* and *O. longistaminata*. In four clusters, 85 accessions of nine *Oryza* species were grouped, each cluster containing accessions of three or more species because of shared similarities in morphological traits between accessions of different species. The cluster-II grouped the maximum accessions (27) of six different species including two accessions of CC-genome species, *O. officinalis* and was divided into three sub-clusters (a-c). Similarly, cluster-I the second largest cluster included 25 accessions of four species mainly *O. nivara* and *O. rufipogon* (23 accessions), and cluster-IV grouped 23 accessions of seven species of AA-genome. The second smallest cluster, cluster-V separated 10 accessions of five species. Thus, the 19 morpho-qualitative traits, which revealed phenotypic variations, shared the similarities amongst eight different species of AA-genome of genus *Oryza*.

Discussion

The utilization of genetic diversity from wild genetic resources is crucial for rice improvement. Accurate phenotypic characterization and organization of diversity would help to determine breeding strategies and facilitate appropriate choices for germplasm conservation (Khush and Virk, 2000). Traditionally, the identification and classification of species have been done mainly through morphological characterization (Vaughan and Morishima, 2003). The qualitative traits have been used for identification, characterization and classification of wild species germplasm, landraces and varieties of rice because these traits are stable and express in all environmental conditions (Singh *et al*., 2018; Tiwari *et al*., 2020; Sathishkumar *et al*., 2021). However, characterization of species diversity could help to decode their dynamic relationship and identification of suitable breeding material for rice improvement (Samal *et al*., 2018; Brar and Khush, 2018). Awning, seed shattering and dormancy are the key characteristics present in rice CWRs (Vaughan *et al*., 2008; Sun and Xu, 2021; Ishikawa *et al*., 2022). The anthocyanin pigmentation traits namely, basal leaf-sheath color, ligule color, auricle color, awn color, apiculus color, stigma color, anther color, hull color and pericarp color could distinguish cultivated species from wild rice species for classification and identification of species (Vaughan *et al*., 2003; Sun *et al*., 2018). The aim of the present study was to harness the genetic variation in morpho-qualitative traits available within and between the crop wild relatives of AA-genome species of rice.

In India, CWRs of rice occur mainly in temperate regions of Eastern and Western-Himalayas, sub-tropical regions of Indo-Gangetic-Brahmaputra plains, Central and Western parts, and tropical peninsular regions (Sharma and Patra, 2010). The annual wild progenitor (*O. nivara*) of Asian cultivated rice (*O. sativa*) occurs in seasonal pools, edges of ponds and tanks, ditches and swampy areas (Singh *et al*., 2018; Tiwari *et al*., 2020). It also grows sympatrically with *O. rufipogon* (perennial wild progenitor of *O. sativa*) and naturally hybridizes with both *O. sativa* and *O. rufipogon* forming *nivara-sativa-rufipogon* complex. These natural hybrids and their introgressed hybrids are commonly referred as *O. sativa* f. *spontanea* (Sharma, 2003; Sweeney and McCouch, 2007). We recorded data on 20 qualitative traits in 88 accessions of *Oryza* AA-genome species, of which polymorphism observed in 19 traits except for anther color. The morpho-qualitative traits *viz*., flag-leaf attitude, panicle type, panicle exsertion, awning, seed shattering, and panicle threshability are analysed mainly for genetic diversity assessment. However, traits namely, growth habit, culm angle, basal leaf-sheath color, leaf-blade pubescence, ligule shape and color, auricle color, awn color, stigma color, anther color, apiculus color, lemma-palea color and pubescence, and pericarp color are normally utilized for taxonomic and classification purposes (Vaughan, 1994). These traits varied between accessions of the same species (intra-species) and of different species (inter-species) in the present study. Our results corroborate with the findings on qualitative trait studied in wild rice species of AA-genome by earlier researchers in India (Singh *et al*., 2018; Samal *et al*., 2018; Tiwari *et al*., 2020) and abroad (Zamora *et al*., 2003, Arrieta-Espinoza *et al*., 2005; Dong *et al*. 2010).

In the present study, the differentiating traits among wild species were culm angle, basal-leaf sheath color, flag-leaf angle, panicle types and exsertion, ligule shape, awning, awn color and seed shattering. The accessions of *O. nivara* possessed annual growth habit and displayed moderate to high level of within species variation for 18 qualitative traits. In *O. nivara*, the prominent traits were semi-erect/erect culm angle (64.8%), green/light purple basal-leaf sheaths (68.2%), erect/intermediate flag-leaf attitude (61.0%), white ligule color (87.8%), acuminate ligule shape (65.8%), green auricles (87.8%), just exserted panicles (51.2%), red awns (46.3%), high to very high seed shattering (80.9%), purple stigma (63.4%) and light brown to brown pericarp (80.4%). Similarly, *O. rufipogon* accessions expressed annual to perennial growth habit, semi-erect to procumbent (89.6%), green to light purple basal leaf-sheath color (79.3%), horizontal flag-leaf attitude (65.5%), white ligules (93.1%), 2-cleft ligule shape (93.1%), green auricles (82.75), well-exserted panicles (44.8%), spreading/ horizontal panicle type (68.9%), red awns (68.9%), purple stigma (68.9%), high to very seed shattering (72.4%) and light brown to brown pericarp color (62.0%). Similar observations on morpho-qualitative traits were also made by Samal *et al*. (2018) after studying a large collection of *O. nivara* and *O. rufipogon* from Odisha and West Bengal. They reported a high level of morphological variation in both *O. nivara* and *O. rufipogon* accessions. The high variability was detected for basal-leaf color, leaf angle, culm attitude, internode color and flag leaf attitude in both Asian wild rice species of AA genome. In *O. nivara*, green basal-leaf sheath was predominant (52%), while majority (47%) of *O. rufipogon* accessions produced purple basal-leaf sheath. The present study revealed the existence of higher variation in *O. nivara* (H' = 0.87) than *O. rufipogon* (H' = 0.69) may be due to larger sample size of *O. nivara* (n=41) than *O. rufipogon* (n=29). The accessions of *O. sativa* f. *spontanea* were identified having erect culm, green basal-leaf sheath color; erect panicle type, purple stigma with brown pericarp (Samal *et al*., 2018). The dendrogram based on morphological traits clearly separated *O. nivara* and *O. rufipogon* accessions into two different classes. Singh *et al*. (2018) studied a large germplasm of 418 accessions of CWRs collected from diversity hotspot regions of India and reported tremendous variability for awning, awn color, leaf-blade color, basal-leaf color, leaf angle, growth habit, culm angle, panicle type and panicle exsertion. They also emphasized that the *O. nivara* was predominant morpho-taxonomic species across India.

In a study of Srilankan wild rices, Sandamal *et al*. (2021) reported that the *O. nivara* accessions were identified by semi-erect to spreading culm angle, green basal-leaf sheath color, intermediate flag-leaf attitude, acuminate ligule shape, low to high seed shattering; while, *O. rufipogon* accessions were identified with procumbent culm angle, horizontal flag leaf attitude, purple basal-leaf sheath color, 2-cleft ligule shape, purple stigma, open panicle type and red awns. The Asian cultivated rice (*O. sativa*) and African cultivated rice (*O. glaberrima*) produced non-shattering, awnless, erect culm angle and flag-leaf attitude, white stigma color and white pericarp color phenotypes, which clearly distinguished these cultivated species from wild species of *Oryza* AA genome. In our dendrogram, 85 accessions of nine *Oryza* species grouped in four clusters with each including accessions of three to seven species of AA genome because of shared similarities in morphological traits by *O. sativa* species complex.

Conclusion

Huge morphological variability detected for 19 morpho-qualitative traits in 88 germplasm accessions of CWRs of rice consisting of eight species of AA-genome. Shannon-Weiner diversity index revealed both within and between species variability for all traits except anther color. Within species variability analysis revealed the presence of higher diversity in *O. nivara* than the *O. rufipogon* for most of the traits. Cluster analysis grouped the accessions of CWRs into five major clusters with each cluster possessing accessions of multiple species showing morphological similarity between accessions of different species *O. sativa* species complex. The vast phenotypic variability revealed in the accessions of the progenitor species of cultivated Asian rice could be utilized for genetic improvement programmes of rice.

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