

RESEARCH ARTICLE

Functional trait diversity of wild rice species in Sri Lanka: implications for field identification and application

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Abstract: The study assessed the variations in morphologically, physiologically and anatomically distinct traits of wild rice species in Sri Lanka; *O. nivara*, *O. rufipogon*, *O. eichingeri*, *O. rhizomatis* and *O. granulata*, which could be useful in rice breeding. The wild rice species were grown in a common garden, and the morphological traits were measured soon after heading. The results showed qualitative parameters such as the panicle type, awning, stigma colour, lemma and palea pubescence, seed coat colour, blade pubescence and ligule shape, are distinctive among the five species and are promising characters in their field identification. ANOVA revealed that the quantitative traits, such as flag leaf length, flag leaf width, culm length, culm diameter, panicle length, 100 grain weight and plant height are useful for further confirmation of species. The highest net photosynthetic rate ($5.86 \mu\text{mol m}^{-2} \text{s}^{-1}$), high cluster width of the base ($61.4 \mu\text{m}$), and trichome density (184.33 per 25 mm^2 area) were observed in *O. rufipogon* compared to the rest, and such desirable traits are effective in rice breeding. Moreover, transpiration rates, stomatal conductance and substomatal CO_2 concentration are ideal physiological traits to be considered in super rice breeding. Significant correlations were observed between transpiration and photosynthesis processes. Thus, our study provides a clear picture on habitat preferences, life cycle, distinctive morphologies and diverse functional traits to be effectively used in field identification and future utilisation of wild relatives of rice in the plant breeding programmes.

Keywords: : Field identification, functional traits, genetic resources, species divergence, variation, wild rice.

INTRODUCTION

The global rice (*Oryza sativa*) production is expected to increase in the next few decades, with special focus on productivity enhancement, owing to limited land and increase in demand (Lim *et al.*, 2013; Tan & Norhaizan, 2020). Improving the yield potential of rice varieties has been the main breeding objective in many countries for several decades to meet this challenge. The ideotype breeding is a key approach for crop improvement. ‘Crop ideotype’ is an idealised plant type with a specific combination of characteristics favourable for photosynthesis, growth, and grain production based on knowledge of plant and crop physiology and morphology (Khan *et al.*, 2015). In this context, wild species of rice provides a wide range of favourable characters and is a valuable reservoir of genetic resources (Khush, 1997). Moreover, improving rice varieties by incorporating desirable traits from wild relatives may lead to advances in rice breeding, as the wild species of rice seems to harbour significantly higher genetic and phenotypic diversity than the cultivated rice (Sarala *et al.*, 2003). Consequently, the knowledge of functional trait diversity among wild relatives will largely enhance their efficient utilisation, in addition to effective conservation (Lu *et al.*, 2002; Ren *et al.*, 2003).

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Consequently, the knowledge of functional trait diversity among wild relatives will largely enhance their efficient utilisation, in addition to effective conservation (Lu *et al.*, 2002; Ren *et al.*, 2003). However, our understanding of functional trait diversity, particularly in wild *Oryza* species, is still limited (Duan *et al.*, 2007; Rathore *et al.*, 2016). Identifying traits and characterising their variation under different environmental factors is important to understand the functional trait diversity among species (Micol & Hake, 2003; Itoh *et al.*, 2005; Kadioglu & Terzi, 2007; Alvarez *et al.*, 2008; Tian *et al.*, 2012). The variability of functional traits among species is indicative of their important physiological processes including photosynthesis. Of the functional traits of wild rice, net photosynthetic rate (net assimilation rate), transpiration, and stomatal conductance are significant parameters to regulate the plant growth and development (Rathore *et al.*, 2016). Determination of diversity among the wild relatives of cultivated *Oryza* spp. based on functional traits will be needed in directing future efforts to discover desirable traits and thus facilitate effective germplasm conservation and utilisation in rice breeding (Zhu *et al.*, 2014).

The genus *Oryza* contains approximately 24 species distributed in Asia, Africa, Australia, and America. Of the species, only two are cultivated species and the remaining 22 species are wild relatives of rice (Vaughan, 1989; Khush, 1997). Moreover, Sri Lanka is one of the secondary diversity centres for rice genetic resources (Ikeda & Vaughan, 1991). Five wild species of *Oryza* viz. *O. nivara* (AA genome), *O. rufipogon* (AA genome), *O. eichingeri* (CC genome), *O. rhizomatis* (CC genome) and *O. granulata* (GG genome) are known in Sri Lanka and *O. rhizomatis* is considered endemic to the country. Among these, *O. nivara* and *O. rufipogon* are closely related to Asian cultivated rice (Banaticla-Hilario, 2012). The pest and disease resistance in these wild rice species are well-documented (Liyanage & Senanayake, 2010; Madurangi *et al.*, 2012). Their distribution, habitats, pollination and flowering patterns are also well described under Sri Lankan conditions (Liyanage *et al.*, 2002; Liyanage & Senanayake, 2010; Rajkumar *et al.*, 2015). Such information directs rice breeders to identify genetically diverse parents to gain desired traits when developing new rice cultivars.

However, the understanding of morphological diversity, particularly in wild *Oryza* species is still limited and sometimes contradictory. Thus, identification of wild species in their natural habitat is difficult or misleading to draw proper conclusions. In this regard, distinct structures

in different plants of the same or different species need to be examined in detail and compared (Sattler & Hall, 1994). The characters that are used in plant identification, classification and description should be diagnostic or key characters that can be either qualitative or quantitative or both qualitative and quantitative. Plant growth habitat, growth patterns, seedling characters, leaf characters, inflorescence and flowers, fruit characters and seed characters are the major traits considered in the proper identification process. However, plant descriptions are often limited, and the morphological, physiological and anatomical distinctions among these species are often vague and not clear enough for field identification and species differentiation. Thus, the field identification of these species is difficult and often confusing based on the available information. Therefore, this study aimed at characterisation of Sri Lankan wild rice species to identify morphologically distinct traits to support field identification, and physiologically and anatomically distinct traits that could be useful in rice breeding.

METHODOLOGY

Sample collection and field establishment

Five wild rice species, namely, *O. eichingeri*, *O. granulata*, *O. nivara*, *O. rhizomatis* and *O. rufipogon* found in Sri Lanka were collected from their typical natural habitats (S1, S2, S3, S4 and S5, respectively) (Table 1).

Mature seeds or root stocks of ten individual plants of each wild rice species were collected from the naturally occurring populations keeping a minimum distance of 5 m between plants to prevent the collection of ramets from a single genet. Exact locations of samples collected were recorded by a Global Positioning System (GPS; Garmin Oregon 550). Thereafter, ten individuals from each wild rice population of the respective wild rice species were established in cement pots (40 cm length × 40 cm width × 45 cm height), using seeds or rootstocks having 10 pots per population, in a common garden at the Faculty of Agriculture of the University of Ruhuna (latitude 06.060337°N and longitude 80.5681455°E), Sri Lanka, from January to December 2016. Each pot per population was considered as a replicate. Pots were arranged in a completely randomised design and the morphological, physiological and leaf anatomical features were characterised.

Table 1: Geographical and ecological information of sampling sites of five wild rice species in Sri Lanka

Species name	Location	SL accession code	GPS values		Habitat Description
			Latitude	Longitude	
<i>O. eichingeri</i>	Wawulpane-Rathnapura	S1	06°25' 59.88"	80°43' 59.87"	Forest, stream banks
<i>O. granulata</i>	Walakada-Urubokka	S2	06°21' 10.44"	80°41' 31.55"	Secondary forests, shade
<i>O. nivara</i>	Vellavelly-Batticaloa	S3	07°30' 33.10"	81°43' 55.10"	Shallow-water lake
<i>O. rhizomatis</i>	Sirnawatkulum-Mannar	S4	08°54' 36.40"	79°57' 42.30"	Shrubs and weeds
<i>O. rufipogon</i>	Thihagoda-Matara	S5	06°00' 01.60"	80°33' 43.90"	Stream

Morphological characterisation

Morphological characters (qualitative and quantitative) from seedling to mature stage were measured as described in the list of descriptors for wild and cultivated rice (*Oryza* spp.) published by the Biodiversity International, International Rice Research Institute and West Africa Rice Development Association (BI-IRRI-WARDA, 2007). Morphological diversity was measured by 11 quantitative (Table 2) and 28 qualitative traits (Table 3). For each character, average measurements taken from three randomly selected tillers per plant, including the main culm was considered. As time of planting was same for all five species, measurements were taken soon after heading (except seedling height).

Functional trait characterisation

Photosynthetically active radiation (PAR), leaf transpiration rate (Evap), stomatal conductance (GS), leaf surface temperature (LT), net photosynthetic rate (PN) and sub-stomatal CO₂ concentration (C Int) were determined using TPS-2 (MA 01913, Portable Photosynthesis System, Amesbury, USA). The measurements were taken randomly from the fully expanded top five leaves of the main culm and matured tillers for each selected plant, and repeated in all replicates. The same leaves were sampled for anatomical investigations. The mid portion of each leaf blade was inserted in the leaf chamber for gas-exchange measurements. For all *Oryza* species except *O. granulata*, two leaf blades were used to fully cover the cuvette luminal surface area. The leaf width of *O. granulata* was higher than that of the cuvette diameter and thus, a single leaf blade was assembled. Data were obtained between 10 a.m. to 2 p.m. with an air temperature of around 30 °C. Measurements were taken after the plants were exposed to sunlight for

approximately 1 h and the leaf functional traits that were given by the leaf gas exchange were recorded.

Fixing, staining and observation of leaf anatomy

A 3 cm long section of the first fully expanded leaf blade from each sampled plant was separated for the leaf structural studies. The leaf sections were cleared and fixed as described by Huckelhoven & Kogel (1998) with slight modifications. The leaf sections were placed in a clearance solution [0.15 % trichloroacetate, (w/v) in ethanol:chloroform (4:1; v/v)] for 48 h while the solution were changed twice in between the time. Then the samples were washed twice (15 min each) with 50 % ethanol, twice (15 min each) with 50 mM NaOH, thrice (10 min each) with MilliQ H₂O and finally 30 min incubation in 0.1 M Tris/HCl (pH 8.5). Samples were then stained using 0.1 % (w/v) safranin for 5 min, washed thoroughly 2–3 times with MilliQ H₂O followed by staining with 0.5 % (w/v) aniline blue for 2 h. After several rinses with MilliQ H₂O, tissues underwent microscopic assessments. Leaf cross sections were made using a microtome knife to observe bulliform cells and were observed at 10×40 magnification with the Olympus BH-2 light microscope fitted with inbuilt digital camera, and were quantified by Image J software. Minimum of 5 bulliform cell clusters of five different leaves from five different plants were measured. Structural measurements were made only on the bulliform cell cluster. Leaf and cell structural traits were determined from light microscopy on leaf sections. Number of cells per one cluster (A), distance between two clusters (μm) (B), middle cell width (μm) (C), cluster width of the base (μm) (D), area of the cluster (μm²) (E), vein density (F), number of stomata (10×40 magnification) (G) and trichome density per 25 mm² area (10×4 magnification) (H), were the traits measured to characterise leaf anatomy.

Statistical analysis

Statistical analysis was performed using SAS version 9.2 (SAS Institute) and Minitab version 17 (Minitab, 2014). First, ANOVA was carried out to describe the variability of each structural and functional leaf trait, based on the entire three (morphology, physiology and anatomy) datasets for five wild *Oryza* species. Quantitative traits of different wild rice species were statistically described using means and standard error of the means of particular traits to figure out the general information related to different species. Quantitative variables were subjected to Pearson's correlation analysis at $p = 0.05$. Pearson correlation matrices were calculated based on the mean values of each structural and functional trait of each

leaf of each *Oryza* species, to evaluate the trait-to-trait associations.

RESULTS AND DISCUSSION

Qualitative and quantitative traits for field identification of wild rice species

The results revealed that the quantitative traits evaluated showed a distinct variation among the species (Table 2). The variation and unique morphological traits that could be useful in species identification in field level are illustrated in Figure 1.

Table 2: Means and standard error of means for the quantitative traits of five wild rice species

Variable	<i>O. eichingeri</i>	<i>O. granulata</i>	<i>O. nivara</i>	<i>O. rhizomatis</i>	<i>O. rufipogon</i>	LSD*
Seedling height (cm)	28.25 ± 0.323	33.2 ± 1.34	67.13 ± 1.53	42.63 ± 3.67	100.0 ± 2.65	8.2783
Ligule length (mm)	2.125 ± 0.125	1.75 ± 0.25	30.5 ± 1.04	3.75 ± 0.25	28.25 ± 0.854	1.7274
Flag leaf length (cm)	28.5 ± 0.736	15.25 ± 0.323	69.5 ± 2.18	35.25 ± 0.878	68.625 ± 0.826	3.6984
Flag leaf width (cm)	1.325 ± 0.0629	2.05 ± 0.0289	0.75 ± 0.0289	1.425 ± 0.0479	0.775 ± 0.025	0.1867
Culm length (cm)	42.0 ± 2.35	55.8 ± 1.29	97.88 ± 2.93	67.33 ± 1.14	152.25 ± 2.39	6.8981
Culm diameter (mm)	5.825 ± 0.118	3.0 ± 0.314	6.475 ± 0.197	5.475 ± 0.197	7.3 ± 0.332	0.4855
Plant height (cm)	88.25 ± 3.75	60.25 ± 1.96	123.0 ± 3.88	95.45 ± 2.07	184.75 ± 2.54	9.822
Panicle length (cm)	20.75 ± 1.01	8.85 ± 0.194	25.13 ± 1.16	29.13 ± 1.98	31.375 ± 0.898	5.7191
100-grain weight (g)	0.6625 ± 0.0175	1.08 ± 0.0129	1.955 ± 0.0646	0.7225 ± 0.0342	1.6875 ± 0.0427	0.1133
Grain length (mm)	6.3625 ± 0.0239	6.3625 ± 0.0315	8.288 ± 0.114	5.5625 ± 0.0125	8.2 ± 0.0645	0.1914
Grain width (mm)	2.125 ± 0.0323	2.325 ± 0.0323	2.625 ± 0.0433	2.125 ± 0.025	2.4125 ± 0.0239	0.0851

The values presented are the means ± standard error of means of 10 replicates in each of the five species. One way ANOVA was used to compare the mean values. Traits among species are significantly different at $p < 0.0001$. *LSD (least significant difference) at $p = 0.05$.

Among the quantitative traits, flag leaf length (FLL) and flag leaf width (FLW), culm length (CL) and culm diameter (CD), panicle length (PL), 100-grain weight (SW) and plant height (PH) are distinctive parameters in identifying species in the field. Among the species, *O. granulata* showed the lowest ($p < 0.0001$) PH (60.3 cm), FLL (15.3 cm) and PL (8.9 cm) and a higher FLW (2.1 cm) indicating that this species is more appropriate for shade environments (Table 2). *O. rufipogon* recorded the highest CL (152.3 cm), CD (7.3 mm) and PH (184.8 cm) ($p < 0.0001$) compared to other species (Table 2) indicating that the species has developed higher lodging resistance than the rest of the species. Further,

the presence of such characteristics may help survival in permanently inundated habitats (Banaticla-Hilario et al., 2013). Previous studies have indicated that the culm-related traits such as a wider culm diameter and less number of tillers, are directly associated with crop physiology and yield due to increase in lodging resistance of the plant (Chuanren et al., 2004). *O. nivara* showed the highest 100-grain weight ($p < 0.0001$) among studied species. The 100-grain weight was also positively correlated ($p < 0.001$) with the LL ($r = 0.929$), FLL ($r = 0.827$), CL ($r = 0.745$) and PH ($r = 0.696$) (data not shown). The quantitative traits reported significant variations among species are distinctive indicators that could be used for field identification of wild rice species.

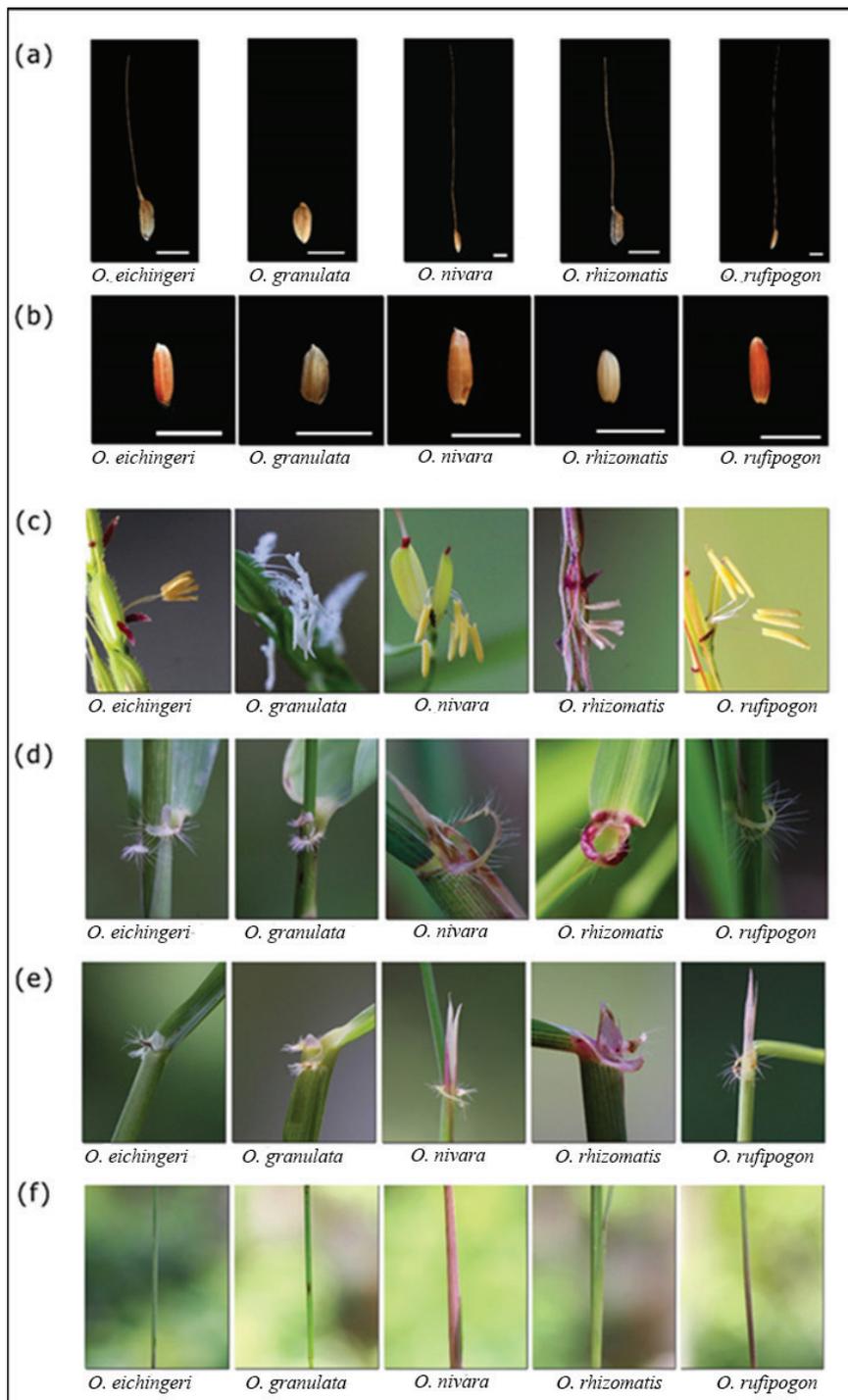


Figure 1: Variation Characteristic morphological traits found in five species of wild rice.
 (a) seed shape and awn; (b) pericarp colour; (c) androecium; (d) auricle shape and colour;
 (e) ligule shape and colour; (f) internode colour

The morphological differences among wild rice species are directly correlated to their natural habitats, life cycle and breeding system (Banaticla-Hilario *et al.*, 2013). Ammiraju *et al.* (2010) reported that the genus *Oryza* has experienced a rapid diversification within a short evolutionary time period. In Sri Lanka, wild rice species are niched to diverse eco-geographic environments (Liyanage & Senanayake, 2010; Sandamal *et al.*, 2018b). Most of the morphological traits are influenced by the environmental factors and thus, we evaluated them in the common garden under same environmental conditions as reported by Abhayagunasekara *et al.* (2018). Among them, traits linked with reproductive parts of the plant are the most important for identification and classification of wild *Oryza* spp. in Sri Lanka. Qualitative parameters such as the panicle type, awning, stigma colour, lemma and palea pubescence, seed coat colour, blade pubescence and ligule shape showed vast differences among the five species. Both *O. granulata* and *O. nivara* had compact panicles while open panicles were observed in *O. rufipogon*, *O. eichingeri* and *O. rhizomatis* (Figure 2).

Though *O. granulata* showed compact panicle, it is well exerted. Moreover, *O. rufipogon*, and *O. rhizomatis* had light secondary branching in the panicle, while there was no branching in *O. eichingeri*. An erect panicle was observed in all species except in *O. rhizomatis*. Open panicles may help to increase out crossing than self-pollination (Banaticla-Hilario *et al.*, 2013). *O. granulata* had no awns while other species had awns with different lengths (Figure 1a). Comparatively long awns were detected in *O. nivara* and *O. rufipogon*. The floral morphology among species showed many characteristic differences in stamens. *O. granulata* had plumose type stigma with white colour stamens, which was clearly divided into two parts at the base of the stamen (Figure 1c). *O. eichingeri* and *O. rhizomatis* had stamens of the same size (length and width) and shape but differed in colour, i.e. dark yellow and pale purple, respectively (*field observation*). Both *O. nivara* and *O. rufipogon* produced anthers of the same shape and colour (yellow) but *O. rufipogon* had longer anthers than *O. nivara* when compared to those reported by Banaticla-Hilario

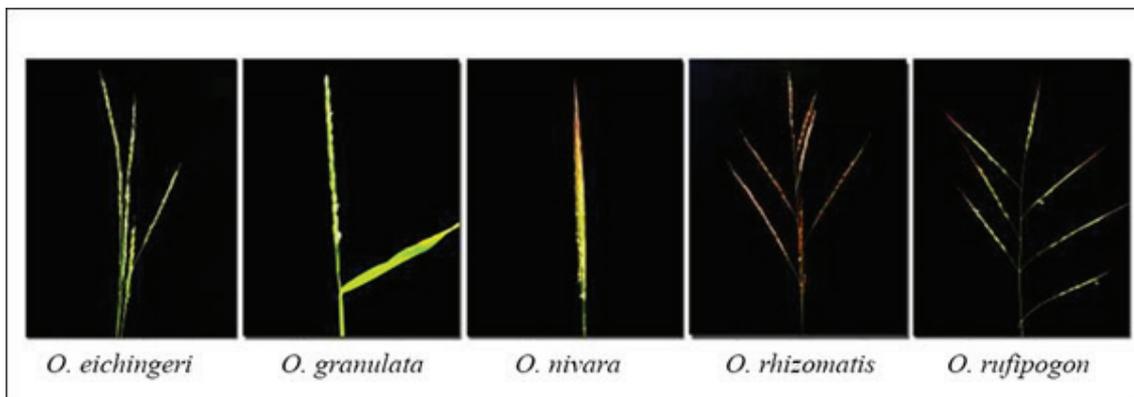


Figure 2: Panicle morphology of five wild rice species

et al. (2013). Presence of pubescence and the colour of lemma and palea, and other seed characteristics are good indicators to differentiate among wild rice species. Seeds of *O. granulata* were glabrous while the seeds of other species had pubescence (Table 3). Five wild rice species revealed large variations in seed coat colour, *viz.* red, light brown, brown and white (Figure 1b). Moreover, the dark green colour of lemma and palea was observed in the immature panicles of *O. granulata*. The size of the seeds (length and width) is one of the most stable

characteristics (Table 2), which has a high heritability and therefore, can be used to distinguish species (Jackson, 1995).

Marginal differences were observed in the shapes and colour of auricle and ligule (Figure 1d) in wild rice species, which is one of the key characteristics to identify *Oryza* species from other species in the family Poaceae. Further, a two-cleft ligule was observed only in *O. nivara* and *O. rufipogon* among all five wild *Oryza* species (Figure 1e).

Table 3: Diversity of qualitative traits among five wild rice species. All the measurements were taken according to the rice descriptor.

Variable	<i>O. eichingeri</i>	<i>O. granulata</i>	<i>O. nivara</i>	<i>O. rhizomatis</i>	<i>O. rufipogon</i>
Blade pubescence	Intermediate	Glabrous	Pubescent	Glabrous	Pubescent
Blade colour	Green	Green	Green	Green	Green
Basal leaf sheath colour	Green	Green	Purple lines	Green	Purple lines
Leaf angle	Intermediate	Horizontal	Intermediate	Horizontal	Intermediate
Ligule shape	Truncate	Truncate	2 - Cleft	Acute to acuminate	2 - Cleft
Ligule colour	White	White	Purple lines	Purple lines	Purple line - 50 %, white - 50 %
Collar colour	Pale green	Pale green	Pale green	Pale green	Pale green
Auricle colour	Pale green	Pale green - 50 %	Purple - 75 %	Pale green	Pale green - 75 %
Culm angle	Spreading	Purple - 50 %	Pale green - 25 %	Spreading	White - 25 %
Internode colour	Green	Procumbent	Open	Green	Intermediate
Flag leaf angle	Horizontal	Horizontal	Intermediate	Horizontal	Purple
Panicle type	Intermediate	Compact	Compact	Intermediate	Intermediate
Secondary branching	Absent	Absent	Light - 75 %, Absent - 25 %	Light-50 %, Absent-50%	Open
Panicle exertion	Well exerted	Well exerted	Moderately well exerted	Well exerted	Light - 75 %, Absent - 25 %
Panicle axis	Straight	Straight	Straight	Straight	Moderately well exerted
Awning	Short and fully awned	Absent	Long and fully awned	Short and fully awned	Straight
Stigma colour	Purple	White	Black	Light purple	Long and fully awned
Sterile lemma colour	Straw (Yellow)	Straw (Yellow)	Straw (Yellow)	Straw (Yellow)	Purple
Sterile lemma length	Short	Short	Long	Short	Straw (Yellow)
Panicle shattering	High (More than 50 %)	High (More than 50 %)	High (More than 50 %)	High (More than 50 %)	Long
Leaf senescence	Late and slow	Late and slow	Early	Late and slow	High (More than 50 %)
Spikelet fertility	Partly sterile	Fertile	Partly sterile	Highly sterile	Intermediate
Panicle threshability	Easy	Easy	Easy	Easy	Partly sterile
Apiculus colour	Brown	Straw (Tawny)	Red	Straw	Easy
Lemma and palea colour	Brown spots on straw	Straw	Brown (Tawny)	Brown furrows on straw	Brown (Tawny)
Lemma and palea pubescence	Short hairs	Glabrous	Hairs on upper portion	Short hairs	Brown (Tawny)
Seed coat colour	Red	Brown	Light brown	White	Hairs on upper portion
Endosperm type	Non-glutinous	Non-glutinous	Glutinous	Non-glutinous	Red

Generally, most rice varieties cultivated in Asia have pubescent leaves, and those in Africa and America are glabrous (Khush, 2001). Our observations indicated that both *O. nivara* and *O. rufipogon* had pubescent leaf blades while *O. granulata* and *O. rhizomatis* had glabrous leaf blades (Table 3). Glabrous trait may be selectively neutral in rice. However, trichomes are thought to be vital for plant defence against biotic and abiotic stresses. Thus, breeding for pubescent rice varieties is mainly targeted at the practical advantages of paddy production. Except for these qualitative traits, others showed minor variations among the species (Table 3). In contrast, no variations were observed for six qualitative parameters, viz. blade colour, collar colour, panicle axis, sterile lemma colour, panicle shattering and panicle threshability.

Functional trait diversity of five wild rice species

The physiological functions of the five wild rice species used in this study varied from each other, indicating the potential of using such functional traits in rice breeding. Photosynthesis forms an essential aspect of plant metabolism and the balance sheet of growth and development, which is sensitive to different abiotic stresses (Gupta *et al.*, 2002; Panda *et al.*, 2008; Gauthami *et al.*, 2014). Under the same environmental conditions, a remarkably high net photosynthetic rate was observed in *O. rufipogon* compared to the rest of the species (Table 4). The reduction in photosynthetic rates may be a result of the changes in stomatal and non-stomatal factors (Panda *et al.*, 2008; Mathobo *et al.*, 2017). Generally, a lower photosynthetic efficiency occurs due to the inhibition of photosynthetic enzymatic activity, and the decrease in chlorophyll and oxidative loads (Hayat *et al.*, 2012). The five wild rice species had transpiration rates

ranging from 0.7 to 2.1 mmol H₂O m⁻²s⁻¹. Although the majority of wild rice species (*O. granulata*, *O. nivara* and *O. rhizomatis*) had transpiration rates ranging from 0.722 – 0.778 mmol H₂O m⁻²s⁻¹ similar to that of cultivated rice, *O. eichingeri* and *O. rufipogon* showed higher ($p < 0.0001$) transpiration rates (1.618 – 2.06 mmol H₂O m⁻²s⁻¹). Transpiration is an important process for plants to create a negative pressure gradient that helps water and nutrient absorption from its roots. Results of the present study revealed a positive correlation ($p < 0.001$) between the stomatal conductance and transpiration ($r = 0.914$) (Supplementary table 01). Stomatal conductance directly influences regulation of gas flow and is known for its strong correlation with transpiration (Giuliani *et al.*, 2013). Further, it helps cooling a plant and promote cell enlargement (Crawford *et al.*, 2012). The highest stomatal conductance ($p < 0.0001$) in the present study was detected in *O. rufipogon*. The decrease of stomatal conductance was observed in species, except *O. rufipogon* under the existing environmental conditions, was may be due to the stomatal closure (Panda *et al.*, 2008; Gauthami *et al.*, 2014). The present study also reported a weak positive correlation between the photosynthetic rate and stomatal conductance. Siddique *et al.* (1999) reported that a strong relationship between net photosynthetic rate and stomatal conductance is an indication of the reduction in net photosynthetic rate, mostly due to stomatal closure, whereas a weak relationship indicates that the net photosynthetic rate is regulated by non-stomatal factors. An increase in sub stomatal CO₂ concentration (C_{int}) suggests the predominance of non-stomatal limitation to photosynthesis, whereas a decrease in C_{int} indicates the stomatal limitations dominated for the photosynthetic inhibition (Panda *et al.*, 2008). Results of the present

Table 4: Physiological characters of five wild rice species.

Variable	<i>O. eichingeri</i> (Mean ± SE)	<i>O. granulata</i> (Mean ± SE)	<i>O. nivara</i> (Mean ± SE)	<i>O. rhizomatis</i> (Mean ± SE)	<i>O. rufipogon</i> (Mean ± SE)	p value	LSD*
PAR	1777.0 ± 29.9	1638.0 ± 48.0	1612 ± 101	1749.4 ± 88.3	1372.6 ± 34.0	0.1938	391.27
Evap	1.618 ± 0.187	0.774 ± 0.0926	0.722 ± 0.0442	0.778 ± 0.0248	2.06 ± 0.121	< 0.0001	0.3623
GS	98.8 ± 13.8	65.2 ± 8.87	52.0 ± 3.66	52.0 ± 2.24	228.2 ± 19.0	< 0.0001	47.829
LT	34.74 ± 0.201	33.2 ± 0.0447	33.82 ± 0.24	34.86 ± 0.271	33.12 ± 0.0735	0.0134	0.9557
PN	0.28 ± 1.12	2.68 ± 1.29	0.96 ± 1.55	2.0 ± 1.39	5.86 ± 1.48	0.0622	4.6716
C Int	398.4 ± 25.6	324.4 ± 27.9	349.6 ± 47.1	336.2 ± 32.5	342.2 ± 15.1	0.6212	120.71

One way ANOVA was used to compare the mean values. PAR - photosynthetically active radiation (μmol m⁻²s⁻¹); (p=0.1938) Evap - leaf evaporation rate (mmol m⁻²s⁻¹); (p<0.0001), GS - stomatal conductance (mmol m⁻²s⁻¹); (p<0.0001), LT - leaf temperature (°C); (p=0.0134), PN net photosynthetic rate (μmol m⁻²s⁻¹); (p=0.0622), C Int - sub-stomatal CO₂ concentration (μmol mol⁻¹). (p=0.6212). *LSD (least significant difference) at p = 0.05.

study indicated that the sub stomatal CO₂ concentration was not significantly different ($p = 0.6212$) among species (Table 4).

The vascular tissues are the most important structural components in plant tissues, which are responsible for the transport of assimilates, minerals and water (Hose *et al.*, 2001; Cholewa & Griffith, 2004).

The present study showed that the vein density was not significantly different ($p = 0.0612$) among species (Table 5). However, the vascular bundle cell size and density are strongly correlated with the transpiration and photosynthetic rate of the species, which has a large culm (He & Zhang, 2003). Among the five wild rice species, *O. rufipogon* (Table 4) recorded the highest rate ($p < 0.0001$) of transpiration and stomatal conductance ($p < 0.0001$). Meanwhile, *O. rufipogon* showed a higher net photosynthesis rate among the tested species. *O. rufipogon* naturally grows and survives in environments such as deep-water habitats where water is not a limiting factor. Therefore, is an ideal species for super rice breeding when water is available at sufficient levels (Liu *et al.*, 2015). As found in *O. rufipogon*,. cultivars or

species with a large culm has shown a higher apoplastic transport ability (Gong *et al.*, 2006), which might help transfer water and nutrients more rapidly and efficiently thus, contributing to higher grain Bulliform cells are large, thin-walled and highly vacuolated cells that play a vital role in controlling leaf rolling in response to drought, salinity and high temperature (Itoh *et al.*, 2005). The efflux of water from bulliform cells induce adaxial leaf curling (Liu *et al.*, 2016). Expansion of the adaxial epidermal cells while an increase in bulliform cells, is closely related to abaxial rolling of the leaf. Furthermore, abaxial leaf rolling and their functions are bidirectional (Li *et al.*, 2010). The present study showed that *O. eichingeri* and *O. rufipogon* had the highest number of bulliform cells ($p=0.05$) per cluster (Figure 3) whereas *O. granulata* showed the lowest number. The largest bulliform cell cluster and the highest width of middle bulliform cells were found in *O. rhizomatis* (Table 5). Moreover, the wider bulliform cell cluster and higher distance between the two clusters in the *O. rufipogon* indicated a systematic modification in morphology and anatomy involved in the development of rice in terms of drought resistance.

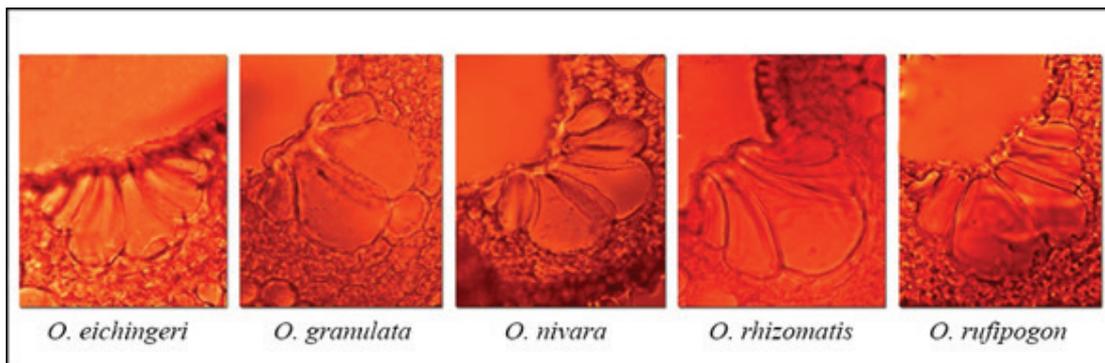


Figure 3: Light micrographs illustrating representative bulliform cell cluster of the five wild rice species (magnification 10×40)

Table 5: Anatomical characteristics of bulliform cell clusters in the leaves of five wild rice species.

Variable	<i>O. eichingeri</i> (Mean±SE)	<i>O. granulata</i> (Mean±SE)	<i>O. nivara</i> (Mean ± SE)	<i>O. rhizomatis</i> (Mean ± SE)	<i>O. rufipogon</i> (Mean ± SE)	LSD*
A	6.667 ± 0.333	4.333 ± 0.333	5.667 ± 0.882	5.333 ± 0.333	6.333 ± 0.333	1.5753
B	177.62 ± 8.52	159.8 ± 17.6	113.8 ± 23.4	212.2 ± 13.0	246.7 ± 43.0	84.826
C	34.11 ± 1.98	48.03 ± 1.43	47.73 ± 5.63	84.16 ± 7.07	53.39 ± 5.74	17.107
D	34.15 ± 4.04	33.26 ± 0.438	46.3 ± 11.1	39.44 ± 1.65	61.4 ± 13.7	27.664
E	4999 ± 591	5201 ± 386	4838 ± 1125	11696 ± 838	7831 ± 2133	3910.4
F	12.667 ± 0.333	11.00 ± 0.000	11.33 ± 0.333	11.00 ± 0.000	12.33 ± 0.333	0.6875
G	27.67 ± 1.45	63.33 ± 3.28	20.00 ± 1.000	32.67 ± 1.76	40.67 ± 1.45	5.5269
H	8.667 ± 0.667	8.667 ± 0.333	113.33 ± 6.98	23.33 ± 0.882	184.33 ± 6.98	13.2

Further, the number of bulliform cells per cluster had a positive significant correlation indicating that a relatively higher cell number or cluster area in a species might play an important role in the adaptation to dry conditions (Giuliani *et al.*, 2013). Stomata are microscopic apparatus in leaf epidermis enabling exchange of air and mainly contribute to the photosynthetic efficiency. The highest and the lowest stomatal density were recorded in *O. granulata* and *O. nivara*, respectively (Table 5). The stomatal density and stomatal size are the anatomical traits that contribute to gas diffusion (Giuliani *et al.*, 2013). Leaf gas exchange was controlled by different stomatal traits such as stomata number, density and size (Panda *et al.*, 2008). Stomatal density was influenced by the number of stomata per row, although on the abaxial surface, a greater number of rows across the leaf have also contributed to the stomatal density. The highest trichome density was observed in *O. rufipogon* (Table 5) indicating its enhancing antibiosis and antixenosis properties thus, reducing insect landing and feeding on leaf surface (Tian *et al.*, 2012). This character could be exploited by breeders in the selection of superior genotypes in terms of phenotypic performance.

Specific habitat information for field collectors

Information on habitat preference, geographical distribution and life history traits are the most important facts that drive efficient sampling of wild genetic resources in their natural habitats. Some of these habitats are threatened due to various human activities (Sandamal *et al.*, 2018a). Therefore, immediate actions are needed to conserve these valuable rice genetic resources (Abhayagunasekara *et al.*, 2018).

O. nivara was mainly confined to the low country dry and intermediate zones. It was not found in the wet zone or upcountry dry/wet regions (Liyanage & Senanayake, 2010). *O. nivara* is distributed extensively in the dry zone and approximately more than 2 ha area in certain natural habitats can be seen. Swampy areas, at the edges of ponds and lakes, and beside streams are the major natural habitats of *O. nivara*. It generally begins seedling emergence with monsoon rain and grows in shallow water. However, continuous water logging condition is not required throughout the life cycle (life cycle observations). It is an annual plant; flowering occurs from January to May and peak mature panicles were recorded from April to May (Ratnasekera *et al.*, 2019).

Natural populations of *O. rufipogon* were distributed mainly in the coastal belt from Puttalam to Matara in the intermediate and wet zones (Liyanage & Senanayake,

2010), thus differ in *O. nivara* habitats. The typical natural habitats of *O. rufipogon* were stream banks, marshy lands, swamps, and deep-water lake edges (Sandamal *et al.*, 2018a). It grows in water 10 cm – 5 m deep. Perennial *O. rufipogon* is photoperiod sensitive plant with a bimodal flowering pattern and peak mature panicles were observed in April and October, separately (Ratnasekera *et al.*, 2019). The reproductive stage of *O. rufipogon* occurred over a longer period than in *O. nivara*.

O. eichingeri is mainly distributed in evergreen forests and dry, mixed evergreen forests located in the intermediate and dry zones. The habitat included forest margins, disturbed and undisturbed forests, and stream banks under shaded or open conditions with well-draining soil condition (Liyanage, 2002). *O. eichingeri* shows a year-round flowering pattern. It was found in association with other wild rice species such as *O. nivara*, *O. granulata* and *O. rhizomatis* (*field observations*).

O. granulata is distributed in the intermediate zone of Sri Lanka. It grows in shady or partially shady habitats often in sloping upland. Moreover, it is well established in the degraded primary or secondary forest area. *O. granulata* shows a year-round flowering pattern.

O. rhizomatis grows naturally in the intermediate and dry zones of Sri Lanka. It grows in periodically flooded areas in the open or under partial shade in primary and secondary forests in dry and intermediate zones. Plants can be seen during the period of late December to May. Mature panicles were observed during the February and March and seed shattering happened in late March.

CONCLUSION

This study has dissected the morphological, leaf anatomical and physiological traits of wild relatives of cultivated rice in Sri Lanka (*O. nivara*, *O. rufipogon*, *O. eichingeri*, *O. rhizomatis* and *O. granulata*) and reports a significant morpho-physiological and anatomical diversity of the traits. Qualitative parameters such as the panicle type, awning, stigma colour, lemma and palea pubescence, seed coat colour, blade pubescence, and ligule shape showed vast differences among the five species and are useful and promising characters in field identification. Quantitative traits such as flag leaf length, flag leaf width, culm length, culm diameter, panicle length, 100-grain weight, and plant height are distinctive parameters among five species that could be used for further confirmation of species. The plant physiological characters such as net photosynthetic rate, transpiration rate, stomatal conductance and sub-stomatal CO₂

concentration are ideal to be considered in super rice breeding. Anatomical traits such as bulliform cells per cluster, stomatal density, and trichome density could be useful in addressing biotic and abiotic stresses in rice breeding. The leaf morphology, physiology and anatomy are not always inter-related. Hence, recombining those traits could open up new avenues to re-engineer new leaf types of genus *Oryza*. Increasing desirable traits are also possible using re-engineered trait from appropriate wild species.

Physiological functions and anatomical features of the five wild rice species vary from each other indicating the potential of using such functional traits in rice breeding programmes. There were significant correlations between several functional and structural traits, and physiological traits such as transpiration and photosynthesis. The findings of the present study help clear the way for field identification, conservation of the existing rice gene pool as well as provide useful information on important traits of the five rice genotypes for further utilisation.

Conflict of Interest

All authors declared that there is no conflict of interest involved in this work.

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Supplementary Table 1: Simple linear correlation coefficient between pairs of all the traits

	SH	LL	FLL	FLW	CL	CD	PH	PL	100GW	GL	GW	NCC	DBC	CBW	SD	TD	PAR	Evap	GS	LT	
LL	0.887*																				
FLL	0.855*	0.966*																			
FLW	-0.713*	-0.876*	-0.834*																		
CL	0.986*	0.861*	0.831*	-0.680*																	
CD	0.731*	0.717*	0.831*	-0.689*	0.705*																
PH	0.975*	0.85*	0.853*	-0.650*	0.988*	0.755*															
PL	0.622*	0.531*	0.698*	-0.353	0.623*	0.778*	0.719*														
100GW	0.752*	0.929*	0.827*	-0.811*	0.745*	0.461	0.696*	0.244													
GL	0.873*	0.957*	0.901*	-0.746*	0.875*	0.580*	0.851*	0.468	0.944*												
GW	0.611*	0.836*	0.731*	-0.707*	0.597*	0.341	0.549*	0.116	0.952*	0.877*											
NCC	0.253	0.238	0.351	-0.420	0.247	0.613*	0.293	0.497	0.068	0.057	-0.088										
DBC	0.340	-0.017	0.030	0.118	0.332	0.357	0.343	0.302	-0.224	-0.019	-0.286	-0.055									
CBW	0.66*	0.559	0.563	-0.448	0.683*	0.514*	0.715*	0.529*	0.486	0.556*	0.409	0.525*	-0.050								
SD	-0.153	-0.386	-0.557*	0.408	-0.084	-0.662*	-0.179	-0.586*	-0.170	-0.209	-0.172	-0.479	0.136	-0.124							
TD	0.979*	0.940*	0.905*	-0.805*	0.967*	0.762*	0.952*	0.588*	0.826*	0.904*	0.677	0.287	0.244	0.616*	-0.224						
PAR	-0.651*	-0.543*	-0.477	0.431	-0.663*	-0.295	-0.597*	-0.212	-0.569*	-0.626*	-0.498	-0.183	0.008	-0.570*	-0.162	-0.603*					
Evap	0.471	0.250	0.288	-0.444	0.449	0.569*	0.464	0.377	0.025	0.078	-0.142	0.606*	0.461	0.442	-0.077	0.448	-0.219				
GS	0.723*	0.457	0.440	-0.508	0.716*	0.574*	0.712*	0.445	0.276	0.363	0.099	0.476	0.484	0.640*	0.081	0.673*	-0.450	0.914*			
LT	-0.505	-0.455	-0.255	0.431	-0.511	0.015	-0.385	0.195	-0.622*	-0.552*	-0.570	0.120	0.046	-0.260	-0.436	-0.494	0.696*	0.017	-0.263		
PN	0.516*	0.288	0.296	-0.164	0.506	0.242	0.502	0.441	0.123	0.290	-0.036	-0.113	0.510	0.007	0.125	0.470	-0.306	0.380	0.487	-0.170	

*Significant at probability = 0.05; SH: seedling height; LL: ligule length; FLL: flag leaf length; FLW: flag leaf width; CL: culm length; CD: culm diameter; PH: plant height; PL: panicle length; 100GW: 100 grain weight; GL: grain length; GW: grain width; NCC: number of cells per cluster; DBC: distance between two clusters; CBW: cluster base width; SD: stomatal density; TD: trichome density; PAR: photosynthetically active radiation; Evap: transpiration rate from the leaf; GS: stomatal conductance; LT: leaf temperature; PN: net photosynthetic rate.