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Neglected wild rice (*Oryza* spp.) in Sri Lanka

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ABSTRACT: In Sri Lanka, five wild rice species are reported; *Oryza nivara* [AA], *O. rufipogan* (AA) *O. eichengeri* [CC], *O. rhizomatis* (CC) and *O. granulate* (GG). Wild rice is the presumed progenitor/s of cultivated rice and is distributed in tropical wetland ecosystems of the world. Wild rice specie often thrives and evolve in wetlands are now neglected. Out of the total of five wild rice species, three are commonly grown in wetlands. The present review describes five wild rice species individually and their treats have also been documented. Wild rice species in the wetlands of Sri Lanka indicates a decreasing trend. Several factors contribute to the decrease in wild rice communities. Degradation of habitat due to the environmental pollution, national developmental projects, agricultural activities and climatic change associated drought and floods stands as dominant factors. Building awareness among the communities living associated with the wetlands is one of the important activities to reduce further deterioration of the reduction of the wild rice species in Sri Lanka. Restoration of wild rice communities introducing community participatory programmes with relevant authorities are recommended.

Although five wild rice species are reported, the precise number of wild rice species in Sri Lanka becomes uncertain and thus, need to assess *Oryza* species complexes in Sri Lanka using morphological, anatomical, and molecular characterization information to enumerate number of species within each *Oryza* complex and of species and species complexes. Characterization of wild rice species was attempted through agro-morphological, anatomical and molecular characterizations. *O. rhizomatis* and *O. eichingeri* (CC) are well separated from the rest of the wild rice species (AA). Molecular data revealed, *O. nivara* and *O. rufipogon* have undergone independent evolution within Sri Lanka. Well separated five wild rice species are present in Sri Lanka. The findings led to conclude that wild rice species in Sri Lanka are "ecological swarms" and represents allopatric or sympatric populations. A comprehensive knowledge on genetic diversity and population structure of wild rice germplasm in Sri Lanka is useful to incorporate them in rice breeding programs.

KEY WORDS: *Oryza* species, Wild rice, Wetlands, Sri Lanka

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INTRODUCTION

Rice (*Oryza sativa* or *Oryza glaberrima*) is the most widely consumed staple food for a large part of the world's human population, especially areas in Asia. Rice can be categorized as cultivated rice and as wild rice, which belongs to the genus *Oryza*. The genus *Oryza* L. is classified under the tribe Oryzeae subfamily Oryzoidea of the grass family (Poaceae). This genus included two cultivated species *Oryza sativa* and *Oryza glaberrima*are and 20 wild species (Morishima *et al*., 1992; Brar and Khush, 1997) distributed in Asia, Africa, Australia, and the America (Vaughan, 1989; Vaughan and Morishima, 2002). Out of the two species, *Oryza sativa* is cultivated in Japan and Southeast Asian countries, the most common species of cultivated rice and *Oryza glaberrima*, which is grown in savanna zones of West Africa and southern edge of the Sahara. The intricate morphological variation in the genus *Oryza*, has been explained by introducing a system of grouping the species under several complexes (Takeoka, 1962, 1963, 1964).

Rice is the result of domestication and genetic improvement of wild rice. The ancestors of cultivated rice are referred to a wild rice which have been played an indispensable role biodiversity of the lakes and rivers. The dense culms of wild rice provide roosting

and loafing areas and brood cover for a variety of aquatic bird species proving habitats for nesting. The nutritious grains of the wild rice are a considerable part of the diet of many migrant birds. Beside bird species, a greater number of mammals also utilize the tender stalks of wild rice for both food and in the creation of their lodges. Thus, wild rice benefits many species due to the structure, cover, or food sources it contributes to the wetland.

The wild species of the genus *Oryza* L. serve as a virtually available reservoir of genetic diversity because these wild species can be used to improve rice. These species have been categorized into ten different genome types, such as six diploids (AA, BB, CC, EE, FF, and GG) and four allotetraploid species (BBCC, CCDD, HHJJ, and HHKK) (Aggarwal *et al*., 1997; Ge *et al*., 1999)

Wild rice spices have contributed tremendously through providing a greater number of abiotic resistant rice varieties (salinity, drought, etc.) and biotic (pest and disease resistance varieties) by donating important traits in rice breeding programs (Chang, 1976; Sitch *et al*., 1989; Vaughan, 1989; Khush, 1997). Nevertheless, the sterility barriers limit the use of *Oryza* germplasm in rice breeding programs (Khush, 1997; Piegu *et al*., 2006) and genetic resources of the AA- genome group (Oryza complex) have long been a core of the rice breeders. These wild *Oryza* species are, grass-like plants which are phenotypically inferior in agronomic traits -such as poor plant type, low grain yield, poor grain type, and are shattering in nature. The wild species show adaptation to different habitats and agronomic traits and remarkable diversity in morphological traits, height, tillering, flowering, growth habit, panicle, leaf, culm, and seed characteristics.

a) Systematics of Wild rice

The *Oryza sativa* complex consists of eight diploid species (Vaughan, 1989) meanwhile the Asian counterpart of the cultivated rice includes two subspecies, *O. sativa* ssp. *indica* and *O. sativa* ssp. *japonica* (Nayar, 1973; Oka, 1988; Morishima *et al*., 1992) of which are originated in Asia and cultivated in elsewhere in the world. In this regard. The two postulated wild progenitors; the perennial *O. rufipogon* is distributed throughout tropical Asia and Oceania, whereas the annual *O. nivara* is distributed in tropical continental Asia. In addition, one of the cultivated species in the genus, *O. glaberrima*, have been domesticated in parallel in West Africa where it becomes an endemic (Vaughan, 1989). There are two additional wild rice species, endemics to Africa, *O. barthii* and *O. longistaminata*. *O. barthii*is the annual wild progenitor of *O. glaberrima*, while *O. longistaminata*is a perennial, rhizomatous and partially self-incompatible grass species (Ghesquiere, 1987).

A total of five of wild rice grow in Sri Lanka and these aquatic plants grow in the soft substrate of shallow water, particularly in slow moving streams. They are falling into two species complexes, the *O. sativa* complex which consists of all the AA genome species and the *O. officinalis* complex of CC genome (Tateoka, 1962; Vaughan and Morishima, 2002). These two complexes are both pan tropical and have very similar distribution in wetland a slow running stream. *O. rhizomatis* is one of the species of the *O. officinalis* complex. The taxonomy of *O. officinalis* complex in Sri Lanka has been confusing and yet to be resolved. The morphological variation of these complex has led Biswal and Sharma (1987) to retract the name *O. collina* and considered this wild rice species as synonymy of O. *eichingeri*. It is evident that Biswal and Sharma (1987) has supported the view of both Bor (1960) and Tateoka (1962) that *O. eichingeri* is the sole representative of *O. officinalis* complex in Sri Lanka. *O. offocinalis* grows in both shaded and open habitats in Sri Lanka, whereas *O. eichingeri* grows in the shade of forests in Uganda (Tateoka, 1964). However, taxonomists were not able to give much weight to the habitat of this taxon since field notes are generally scanty.

The recent collections made by Vaughan (1989) have revealed distinct morphological and habitat differences in *O. eichingeri* and it is a widely distributed taxon in the drier habitats in Sri Lanka. This larger rhizomatous taxon has previously been called *O. latifolia* and *O. officinalis*. *O. latifolia* is a large non-rhizomatous tetraploid from South and Central America with broader leaves and whorled panicle branches. *O. officinalis* which usually has rhizomes, has smaller spikelets, shorter palea tip, more branches of approximately equal length from the lowest panicle node, and spikelets inserted away from the base of primary branches. *O. officinalis* is also genetically different from this Sri Lankan taxon with which it can form sterile hybrids. However, Sri Lankan taxon belongs to the same genome group as both *O. officinalis* and *O. eichingeri*, which is CC (Jena and Khush, 1985).

There are two diploid CC genome species in Sri Lanka, *O. eichingeri* and *O. rhizomatis* (Biswal and Sharma, 1987; Vaughan, 1990). Previously *O. collina* was the name used for Sri Lankan germplasm of the *O. officinalis* complex (Sharma and Shastry, 1965). *O. collina* has been used for both O. *eichingeri* and *O. rhizomatis.* However, *O. rhizomatis* is readily distinguished from *O. eichingeri* by its larger plant stature and rhizome formation. *O. rhizomatis* appears to be intermediate between *O. officinalis* and *O. eichingeri*. Analysis of the nuclear and chloroplast genome of *O. rhizomatis* by RFLP and SSR reveals that *O. rhizomatis* differed from *O. eichingeri* and *O. officinalis* (Dally and Second, 1990; Proven *et al.*, 1997; Wang *et al*., 1992).

The nomenclature and the taxonomy of the elements of these complexes have been studies and nomenclatural changes have been suggested and certain de novo species was described to disentangle the problem within the complexes. Due to this reason, the exact number of wild rice species in Sri Lanka becomes uncertain and detailed studies specially, on morphological, anatomical and molecular aspect of the Sri Lankan wild rice are needed for the delimitation of *Oryza* complexes in Sri Lanka. Thus, there is a controversy over the nomenclature of the species in the *Oryza* complexes in Sri Lanka. Therefore, due attention should be paid during reporting of the number of wild rice species in Sri Lanka. However, as far as ecology is concerned, there are discernible differences in the wild rice taxon in the country.

According to the recent literature on the wild rice species in Sri Lanka, there is a total of five species; O. *rufipogon*, *O. nivara*, *O. eichingeri*, *O. granulata* and *O. rhizomatis.* They are distributed in the wetland/periodically drying /slow running stream of the different climatic zone in the country (Figure 1). The agronomically important traits of the Sri Lankan wild rice species are shown in Table 1.

Figure 1. Distribution of the wild rice species in Sri Lanka.

b) Agronomic importance of Wild rice in Sri Lanka

The wild rice species possess agronomically important traits (Table 1) which can be utilized in rice breeding programs for developing disease resistant as well as salinity tolerant and drought tolerant rice varieties. However, wild rice species seems to be underutilized in Sri Lanka. Further, wild rice species occur in the wetland might provide a valuable germplasm which has been neglected.

Table 1. **Wild rice species reported in Sri Lanka and their genomes and agronomically important traits (BHP - Brown Plant Hopper resistant, SAL – Salinity tolerant, DR – Drought tolerant)**

Wild rice species	Genome	Trait
O. nivara	AA	BHP
O. rufipogon	AA	SAL
O. eichingeri	CC	BHP
O. rhizomatis	CC	BHP
O. granulata	GG	DR

c) Ecology of Wild rice species in Sri Lanka

The wild rice species are well distributed in different climatic zones in Sri Lanka, at least one wild rice species for a climatic zone (Table 2). The *O. nivara* is commonly occur in periodically drying water bodies in the dry and intermediates zones. Meanwhile, species *O. ruffipogon* commonly thrive well in the wetland and slow running streams in the wet zone. The species *O. eichingeri* and *O. rhizomatis* of which easily characterized by their well- recognized rhizomes are distributed in the dry and intermediate zone. The only species, *O. granulata*is reported to be restricted to the intermediate zone.

Table 2. Distribution of wild rice species in Sri Lanka.

Wild rice species	Ecological zone
O. nivara	Dry and intermediate zones
O. rufipogon	Costal belt
O. eichingeri and *O. rhizomatis*	Wet, dry and intermediate zones of Sri Lanka
O. granulata	Only in intermediate zone

d) Description of wild rice species in Sri Lanka

Oryza nivara

An annual grass, reaching short to intermediate height (often < 2 m); panicles are frequently compact, rarely open; spikelets are large, 6-10.4 mm long and 1.9-3.4 mm wide, with strong awn (4–10 cm long); anthers 1.5–3 mm long. Widely distributed in the periodically drying water bodies in the wet zone or rarely intermediate zone (Figure 2). It is commonly found in Bangladesh, Cambodia, China, India, Laos, Malaysia, Myanmar, Nepal, Sri Lanka, Thailand, and Vietnam.

A **B**

Figure 2. *Oryza nivara* growing in an irrigation canal running through a periodically drying marshland in Ranna, Hambantota District in dry zone, Sri Lanka.
A - Plant and associated habitat condition, B - part of the panicle.

Oryza rufipogon

A perennial aquatic grass with elongated rhizomes reached up to 2 m tall. It is an erect, tufted, 150-400 cm tall, with spongy culms which have glabrous and hollow nodes. Grows in swamps, channels, marshes, and by the boundaries of ponds and lakes. Often, it indicates running habit, creeping on the ground and rooting at internodes. Plant shows adaptive habit, erect and lax panicles that stand narrow and oblique, and beaked spikelets with awns.

This wild rice species is widely spread in southern China, South and South East Asia, Palua New Guinea, and Northern Australia. In Sri Lanka it is commonly found in wetlands in the wet zone (Figure 3).

A **B**

Figure 3. *Oryza rufipogon* growing in a broader of a marshland in Akuressa, Marata District, wet zone. A - Plant with habitat conditions, B - Part of the panicle.

Oryza rhizomatis

Perennial herb reaching 1.2-1.5 m tall, often with adventitious roots and prominent firm branched underground rhizomes from which a number of thick roots develop; erect to semi-erect with culm usually lacking tillers from above-ground nodes. Flag leaf linear-lanceolate, 1.89-2.95 cm long, 13-18 mm in breadth; ligule truncate, 3-8 mm in lower leaves, 1-2 mm long in flag leaf. Inflorescence a panicle with widely spreading branches, 20-28 cm long; base of panicle branches pubescent, slightly swollen, in general purple in color. Spikelets shattering, mottled black at maturity. Endemic to Sri Lanka (Figure 4).

O. rhizomatous was discovered in 1989 for the first time. This species is endemic to Sri Lanka and it is distributed in the dry zone low country of Sri Lanka such as Yala National Park, Wilpattu National Park and Tirappane (Anuradhapura).The distribution of *O. rhizomatous* overlaps *O. eichingeri* and populations can be found within about 20km of each other in the north central dry zone in Sri Lanka (Vaughan and Morishima, 2002).

This wild rice species thrives well in seasonally dry habitats in open grassland or partial shade at the edge of low forest. This species has been collected from reported from Hambantota, Puttalam, Vavuniya, Trincomalee, Monaragala, and Hambantota districts, Sri Lanka. *O. rhizomatous* usually grown in primary and secondary forests and open, tall scrub with grassy openings. It is grown in swampy or periodically flooded areas, usually in full sun or partial shade. Plants can be seen during the period of late December to May (Somaratne *et al*., 2018).

A **B**

C **D**

Figure 4. ***Oryza rhizomatis:*** **Collected from Puttalam District, Dry zone.**
A and B - Plants with conspicuous rhizome, C - Prominent collar, D - Part of the panicle.

Oryza eichingeri

An annual grass; Culms erect, reach up to 60–120 cm. high, spongy, usually 4 mm. or more in diameter. Leaf-blades up to 30 cm. long and 1 cm. broad; ligule 3–10 mm. long, soft, whitish, splitting longitudinally when dried, glabrous. Panicle loose, with spreading branches. Spikelets elliptic, 4.9–6.2 mm. long and 1.9–2.6 mm. broad (2.5 times longer than broad), hispidulous; sterile lemmas acute, triangular; awn 1–7 cm. long, straight or somewhat flexuous, hispidulous.

In general, this wild rice species grows in forest margins or openings in the forest. Frequently occur along boarders of the forest streams or around wet places in the forest. In Sri Lanka, it is common in the wet, dry or intermediate zone (Figure 5). This species indicates disjunctive distribution in East Africa, Uganda and Ivory Coast.

Figure 5. ***Oryza echingeri*** **showing habitat condition.**

granulata

A short perennial herb reaching 40-90 cm high, loosely tufted or solitary, erect. Leaves 10-25 cm long and 1-2 cm in breadth, lanceolate, glabrous; sheath with long hairs at mouth; ligule 1-3 mm long, membranous. Panicle 5-10 cm long, hardly branched; branches angled, smooth. Spikelets few, 5-6 long and 2.5 mm in breadth (Figure 6).

It is distributed in South Asia including Cambodia, China, India, Indonesia, Laos, Myanmar, Nepal, Philippines, Sri Lanka and Thailand. In Sri Lanka, this species is commonly occurred in the intermediate zone and often grows the forest canopy where soil moisture is abundant.

A

B

C

Figure 6. ***Oryza granulata***

A - Plant growing in a pot, B – Plant growing in a natural habitat in a forest, C - part of the panicle

e) Agro-morphological, Anatomical and Molecular differentiation of wild rice species in Sri Lanka

The following methodologies were adopted (Weerakoon, 2021) to differentiate and assess the relationships among wild rice species in Sri Lanka

Wild rice species; *O. rufipogon*, *O. nivara*, *O. eichingeri* and *O. rhizomatis* were collected from different localities of the Districts, Puttlam, Anuradhapura, Vavuniya, Trincomalee, Hambantota, Matara and Ampara in Sri Lanka.

Agro-morphological studies:

The agro-morphological characterization of each species collected was based on the Plant Genetics Resource Centre (PGRC), Sri Lanka Characterization Catalogue of Rice (1999). (Table 3). The leaf, culm, and rhizomes if available were collected and processed for micro sectioning. Temporary and permanent slides were prepared for cross sections of leaves, culm and rhizomes.

Table 3. Characters Observed for characterization of wild rice

Character	**Abbreviations**
Morphological characters	
Plant Height (cm)	PLH
Leaf blade length (cm)	LBL
Leaf blade wigth (cm)	LBW
Leaf blade pubescence at late vegetative stage	LBP
Leaf blade color at late vegetative stage	LBC
Basal leaf sheath color at late vegetative stage	BLSC
Ligule length at late vegetative stage (cm)	LiguleL
Ligule color at late vegetative stage	LiguleC
Ligule shape at late vegetative stage	LiguleS
collar color at late vegetative stage	CollorC
Auricle color at late vegetative stage	AuricleC
Culm length (cm)	CulmL
Culm angle after flowering	CulmA
Internode color after flowering	IINCAF
Culm strength	CulmS
Panicle length	PanicleL
Panicle type	PanicleT
Panicle excretion	Panicleex
Awning after full heading	AWNAFH
Awn color at maturity	AAWNC
Apiculuscolor	ApiculeC
Seed coat (bran) color at maturity	SeedCC
Leaf senescence	LeafS
Lamina Anatomical characters	
Vein diameter (µm)	VD
Inter Venial distance (µm)	IVD
Vein width (µm)	VW
Vein height (µm)	VH
Leaf thickness (µm)	LTH
Height of mesophyll layer (µm)	MESOH
Width of mesophyll layer(µm)	MESOW
Bundle cell length(µm)	BCLEN
Bundle cell width (µm)	BCWIDT

Anatomical studies:

The free hand sections of leaves and culms of collected specimens were taken and observed under the light microscope. Measurements of anatomical characteristic features were made using standard methods.

Molecular studies:

Total genomic DNA was extracted from 7-day old seedlings of wild rice species; *O.rufipogon*, *O. nivara*, *O. eichingeri* and *O. rhizomatis* respectively using Promega Plant DNA extraction kit. A total of ten SSR primer pairs were used (Table 4) for molecular study. SSR markers were obtained from Gramene (http://www.gramene.org/). All SSR PCR amplification reactions were carried out in a total volume 30µl of which consist 1 x PCR buffer, 1mM dNTPs, 2µM SSR primers, 2mM $MgCl_2$, 50ng of genomic DNA and 0.5 Units of *Taq* DNA polymerase. SSR alleles will be resolved on Poly Acrylamide Gel. The SSR banding patterns were identified using Poly Acrylamide Gel Electrophoresis (PAGE).

Table 4. SSR markers used for the molecular studies

SSR	Chr	Fw5′–3′	Rev5′–3′
RM11	7	tctcctcttcccccgatc	atagcgggcgaggcttag
RM14	1	ccgaggagaggagttcgac	gtgccaatttcctcgaaaaa
RM19	12	caaaaacagagcagatgac	ctcaagatggacgccaaga
RM21	11	acagtattccgtaggcacgg	gctccatgagggtggtagag
RM44	8	acgggcaatccgaacaacc	tcgggaaaacctaccctacc
RM55	3	ccgtcgccgtagtagagaag	tcccggttattttaaggcg
RM84	1	taagggtccatccacaagatg	tgcaaatgcagctagagtac
RM211	2	ccgatctcatcaaccaactg	cttcacgaggatctcaaagg
RM219	9	cgtcggatgatgtaaagcct	catatcggcattcgcctg
RM253	6	tccttcaagagtgcaaaacc	gcattgtcatgtcgaagcc
RM280	4	acacgatccactttgcgc	tgtgtcttgagcagccagg
RM289	5	ttccatggcacacaagcc	ctgtgcacgaacttccaaag

Analysis of Data:

Gathered data were analyzed with univariate, bivariate and multivariate statistical procedures. Suitable statistical software was employed in the analysis of data. In addition, data mining analysis were also attempted for the data gathered to reduce the noise in the data set.

Molecular data were analyzed using Genemapper 4.1 software and SSR profiles were analyzed using PowerMarker 3.25.

Findings of Agro-morphological studies:

The mean values of the parametric morphological measurements of wild rice species have indicated that *O. rufipogon* has the highest mean for plant height (153.23cm) and culm length (94.11cm) and minimum plant height was observed in *O. eichingeri* (99.25 cm). Similarly, the highest leaf length and breadth were found in *O. nivara* and narrow leaves were occurred in *O. rufipogon*. The variation of ligule length indicated that *O. nivara* possessed a higher ligule length with respect to other species included in the study. The summary of the ANOVA (data not shown) carried out on the parametric lamina morphological characters indicated that, except ligule length and panicle length, the rest of the characters are significantly ($p > 0.05$) varying across the wild rice species.

The association of the non-parametric characters with wild rice species showed that, the characters such as leaf blade pubescent, awn after full heading and intermodal color after full heading are not significantly differ across the species ($p> 0.05$). However, the rest of the characters are significantly associated with the wild rice species and are of potential characters in separating wild rice species.

The cluster analysis of agro-morphological characters (Figure 7) and species were grouped under each cluster with respect to their similarities. The samples of *O. nivara* and *O. rufipogon* were intermingled and separated into two groups. Meanwhile the samples of *O. eichingeri* and *O. rhizomatis* were well-separated from 80% similarity level and from rest of the clusters representing two populations. However, one sample of *O. eichingeri* was grouped with O. *rhizomatis.* The phylogenetic tree (Figure 6) constructed by agro-morphological characters clearly showed a well separated cluster of *O. rhizamatis*. The samples of *O. nivara* and *O. rufipogon* were intermingled and separated into four groups. Findings of the study led to conclude that wild rice species in Sri Lanka are “ecological swarms” and represents allopatric or sympatric populations.

Findings of Anatomical studies:

The variation of anatomical characters, especially the laminar anatomical features across the wild rice species indicated that, comparatively, the magnitude of mean values of bundle sheath cell width indicated a considerable variation between the wild rice species *O. eichingeri* (11.77 µm) and *O. rufipogon* (10.74 µm). The summary of the ANOVA (data not shown), indicated that all the anatomical characters except mesophyll height and bundle sheath height. The anatomy of the leaf sheath of wild rice species indicated that characteristic features of the structures reflect the habitat conditions of the wild rice species.

The result of the cluster analysis of anatomical characters of wild rice species is shown in Figure 8. Comparatively, the Dendrogram resulted from the anatomical features indicated that anatomical characters well-separate the samples of each wild rice species. The samples of *O. rhizomatis* formed a unique group at similarity level of 80%. The pattern of the sample grouping was similar to the results obtained from the cluster analysis of agro-morphological characters. However, samples were homogenized representing each wild rice species by pure tree branch.

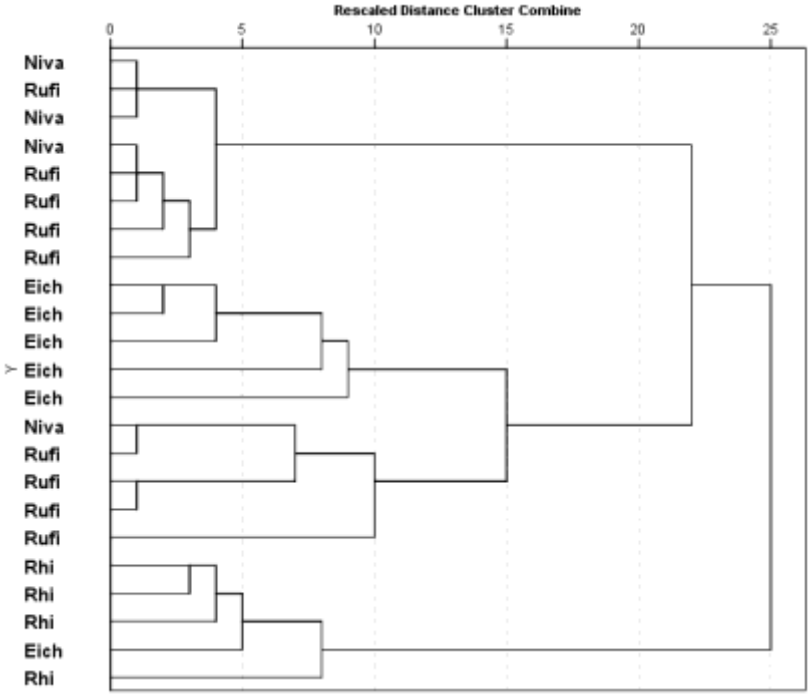

Figure 7. Dendrogram produced by cluster analysis of 22 agro-morphological characters of wild rice species, *O. nivara* (Niva), *O. rufipogan* (Rufi), *O. rhizomatis* (Rhi) and *O. eichingeri* (Eich) (Weerakoon, 2021)

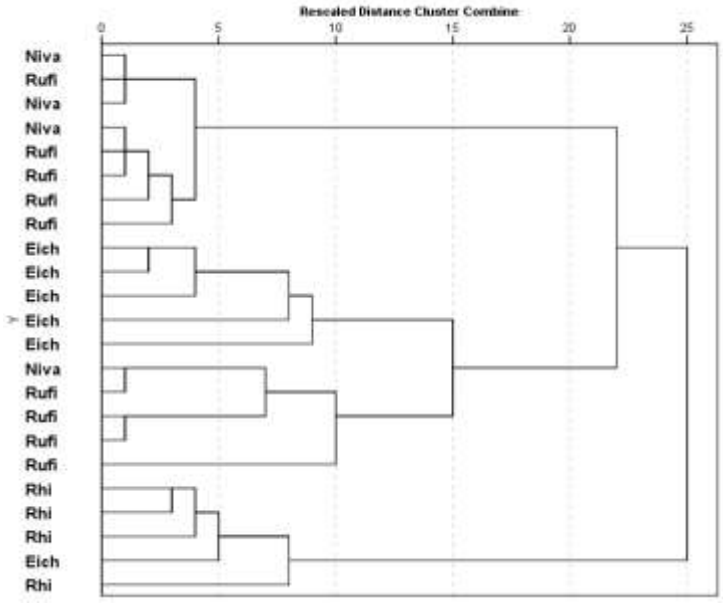

Figure 8. Dendrogram produced by cluster analysis of anatomical characters of wild rice species, *O. nivara* (Niva), *O. rufipogan* (Rufi), *O. rhizomatis* (Rhi) and *O. eichingeri* (Eich) (Weerakoon, 2021)

Findings of Molecular studies:

A total of three clusters were resulted from the cluster analysis of molecular data (Figure 9) and species were grouped under each cluster with respect to their genetic similarities. The samples of *O. nivara*, *O. rufipogon* and *O. rhizomatis* were very well separated from 40% similarity level confirming their distant relationship with each other and of independent evolution within Sri Lanka.

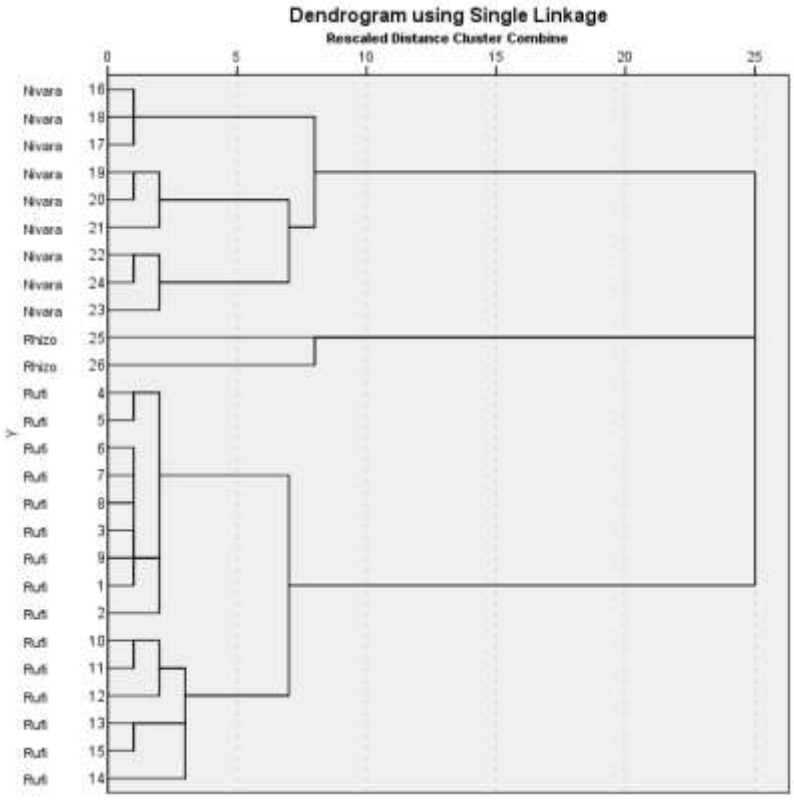

Figure 9. Dendrogram produced by cluster analysis of molecular data of wild rice species, *O. nivara* (Niva), *O. rufipogan* (Rufi), *O. rhizomatis* (Rhi) (Weerakoon, 2021)

f) Conservation status of wild rice in Sri Lanka

O. rhizomatous is endemic to Sri Lanka, therefore conservation is important. This species is present in protected areas as well as in many other unprotected areas. *Ex-situ* conservation is carried out in seed gene bank of the Plant Genetic Resources Centre (PGRC) and Rice Research Development Institute (RRDI), Bathalagoda.

Similarly, *ex-situ* conservation is carried out in seed gene bank of the Plant Genetic Resources Centre (PGRC) and Rice Research Development Institute (RRDI), Bathalagoda for *O. nivara*, *O. rufipogon*, *O. eichingeri* and *O. granulate*.

DISCUSSION

A decline in wild rice in wetlands has been observed in Sri Lanka due to several contributive factors. Three out of five wild rice species are found in the wetlands that currently showing varying densities in wetlands of Sri Lanka. The chemical and physical factors and aquatic plant densities play an important role in the reduction of wild rice species in wetlands. Wetlands that exhibited the greatest loss of wild rice species tended to have higher levels of residential development within their watershed. The aquatic plant communities, specially the invasive species, also led to shift the wild rice species to verge of extinct due to the competition for light and nutrients. Increasing agricultural activities in the vicinity of water sheds and wetlands increase the nutrient levels and increases with floating plants that can acquire nutrients directly from the water bodies. The wild rice species requires optimal conditions which are shared by the aquatic plant communities that are associated with those conditions. Declines in historic wild rice communities have occurred in the wetlands in the country due to many factors, including construction of dams, pollution, recreational boat rides, and invasive plant species. Reviving interest in the wild rice community will be led to large-scale restoration of threatened wild rice community to reintroduce wild rice in wetland of Sri Lanka.

Wild rice often prefers clear water since very dark or turbid water preclude the reaching sunlight to reach the young plant. In addition, fluctuation of water levels in wetlands in general determine the occurrence of wild rice since they prefer minimal annual fluctuations in water level and stable or gradually receding water levels during the growing season. Occurrence of dense competing invasive plant communities such as water hyacinth (*Eichhornia crassipes*), water ferns (*Slavonia molesta*), and *Hanguana malayana* prevent the establishment of wild rice seedlings. Further, the negative impacts of browsing by wildlife such as mammals including

elephants, wild buffaloes, extensively fed on wild rice species. Ranching animals also a cause severe threat to the wild rice communities if larger farms maintained at the vicinity of the wetlands.

The agro-morphological, anatomical and molecular characters were investigated in relation to the species identification and delimitation of wild rice species complex in the country. The results of the agro-morphological characters implied that they were useful in identification of wild rice species. However, the ecological resilience of the agro-morphological characters is to be investigated before reaching a firm conclusion on the diagnostic value of the agro-morphological characters. Compared to the agro-morphological characters, the anatomical characters especially, lamina and culm anatomical characters indicted, higher potential identification of species and delimitation of the wild rice species in each complex. Both morphological and anatomical characters can be used to separate the *O. rhizomatis* and *O. eichingeri* (CC) from the rest of wild rice species (AA). Further, based on both morphology and anatomy, *O. rhizomatis* can be distinguished from *O. eichingeri.* The finding suggests that species status of these two species deserved to maintain for further confirmation by molecular characterization. As far as the samples of two wild rice species of AA, *O. nivara* and *O. rufipogon* is concerned, there were considerable overleaps with respect to morphology and anatomy. However, the analysis of molecular data revealed that samples of *O. nivara*, *O. rufipogon* and *O. rhizomatis* have a distant relationship with each other and undergone independent evolution within Sri Lanka (Weerakoon, 2021).

The study revealed that wild rice species in the island are "ecological swarms" and represents allopatric or sympatric populations. This finding is further supported by the connotations made by Nelson on the genus *Oryza* and its species in Sri Lanka (Harriman., 1994).

CONCLUSIONS

Wild rice in wetlands of Sri Lanka are disappearing unnoticeably and requires taking timely action to prevent further loss in remaining wild rice communities in the country. Conservative measures should be taken during the developmental activities in the vicinities or even in distant areas that connected to the wetlands. Revival of public interest and the importance of the wild rice species required to restore among the young members since most of them is unaware about these valuable plant species reside in wetland.

Studies on the ecological resilience of agro-morphological characters in combination with anatomical and molecular studies are very useful for species delimitation of wild rice complexes in Sri Lanka. The finding led to conclude that wild rice species in Sri Lanka are "ecological swarms" and represents allopatric or sympatric populations.

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