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RESEARCH ARTICLE

A Note on Taxonomy and Genetic Resource Potential of *Oryza meyeriana* var. *indandamanica*, a Rare Wild Relative of Rice from the Andaman Islands

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Abstract

The narrow endemic taxon, *Oryza meyeriana* (Zoll. & Moritzi) Baill. var. *indandamanica* (J.L.Ellis) Veldkamp, was often merged with either *O. meyeriana* (Zoll. & Moritzi) Baill. or *O. granulata* Nees & Arn. ex Watt. or all three taxa were subsumed to *O. meyeriana*. A critical analysis of protologues, herbarium specimens and earlier taxonomic works pertaining to these three taxa coupled with the detailed morphological study of germplasm (IC641181) of var. *indandamanica*, which was originally collected from the Saddle Peak National Park of the North Andaman Island, was undertaken. Morphological studies indicated that var. *indandamanica* fell well within the circumscription of var. *meyeriana*. However, chloroplast genome sequence data reveals its closeness towards var. *granulata*, compared with var. *meyeriana*. Our views agree to the merger of all these three taxa into one are presented. Also, the genetic resource potential of the studied accession is discussed.

Keywords: Andaman wild rice, Chloroplast genome sequence, *Oryza meyeriana* complex, Narrow endemic taxon, Saddle Peak National Park.

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Introduction

A trekking for crop wild relatives' survey and their germplasm collection was carried out at the Saddle Peak National Park (SPNP) of North Andaman Island in January 2020. In the underneath of the evergreen forest canopy at an altitudinal range of 330 to 590 m above MSL for a stretch of around two kilometers (13.1637–13.1780° N, 93.0063–93.0125° E), the authors came across a dwarf but stiff bambusoid tuft-forming grass with erect culms and apparently unbranched spicate inflorescence with small, awnless spikelets. This population with over 100 clumps/plants was spotted in highly shady steep-sloped stony terrain, having well-drained loamy soils with rich litter. It was identified as wild rice, *Oryza meyeriana* (Zoll. & Moritzi) Baill. var. *indandamanica* (J.L.Ellis) Veldkamp, based on the earlier floristic records of this park. Known in just two localities, Rutland (type locality) and SPNP, the germplasm of this narrow endemic taxon was collected for the first time from the latter area. The collected germplasm (IC641181) is maintained in the Field Genebank (FGB) at Thrissur, and seeds were also conserved in the National Genebank at NBPGR, New Delhi, for long-term storage.

O. indandamanica J.L.Ellis was often considered an ecotype/variant/synonym of *O. granulata* Nees & Arn. ex Watt (e.g., Rao, 1989; Vaughan, 1989, 1994; Khush *et al.*, 1990) or identical with var. *meyeriana* (Veldkamp, 1988) or often all 3 infraspecific taxa names subsumed/merged under *O. meyeriana* (Zoll. & Moritzi) Baill. (Clayton *et al.*, 2006; POWO, 2023) or *O. granulata* (Gong *et al.*, 2000;

Lu *et al*., 2001). Nevertheless, using the total genomic DNA hybridization approach, Aggarwal *et al*. (1997) concluded that the genomes of these three species in *meyeriana* complex were highly divergent and distinguishable. Biogeographically, *Oryza granulata* is distributed in South Asia, SE Asia and SW China, while *O. meyeriana* is confined to SE Asia only. These species together with the Neo Caledonian endemic species, *O. neocaledonica* Morat, form the *Oryza meyeriana* complex (2n=24) designated with the GG genome group.

JL Ellis, the original author of *O. indandamanica* had distinguished the species only with *O. granulata* (and not with *O. meyeriana*), that too on feeble, and sometimes on arbitrary characters (e.g., almost unbranched panicle, absence of fertile lemma awn) (Ellis, 1985). It appears that he relied heavily on expert opinion (vide Ellis, 1994) rather than studying the protologue of allied species and associated herbarium specimens and literature. JF Veldkamp reduced this taxon to a varietal status under *O. meyeriana*, citing that the differences observed were insufficient to maintain as a distinct species (Veldkamp, 1991). Establishing the correct identity of the collected germplasm is crucial as misidentification of species and/or instability or non-universality in the application of taxon names greatly limits the value of plant genetic resources.

With the advent of next-generation sequencing technologies, chloroplast genome sequences had proven to be effective molecular resources for aspects related to species identification and phylogenetic studies (Dong *et al*., 2012), owing to their uniparental inheritance (occasionally paternal as well), lacking recombination, and lower rates of adaptive evolution than that of nuclear genomes (Neale and Sederoff, 1989; Rogalski *et al*., 2015; Daniell *et al*., 2016; Chung et al., 2023; Heinke, 2023). The highly variable regions in the chloroplast genomes can serve as DNA markers for the accurate and reliable identification of plant species. Therefore, both the morphological studies and molecular tools using chloroplast genome sequences were employed to arrive at a reasonable conclusion about the taxonomy of *Meyeriana* complex, in general, and the identity of the collected germplasm, in particular.

Material and Methods

A detailed morphological study of field-grown germplasm accession IC641181 was made and compared with the botanical description given in the protologue of *O.*

Table 1: Comparison of key morphological characters of studied accession with three taxa (Ellis 1985; Duistermaat 1987; Chang 1988; Veldkamp 1991; Vaughan 1994)

#	*Characters*	*IC641181*	*var. indandamanica*	*var. meyeriana*	*var. granulata*
1.	Fruiting spikelet	Oblong; 6.93 mm long; Length Width ratio 4.61	-----	Oblong-ovate lanceolate; more than (6.00–) 6.4 (–7) mm long; LW ratio 3.10–6.40	Elliptic; less than (6.00–6.4) (–7) mm long; LW ratio 2.10–2.70
2.	Caryopsis length (mm)	5.21	5.00	4.10–7.30	3.40–4.10
3.	Blade width (cm)	1.26	0.80	0.80–3.20	0.70–2.00
4.	Ligule	Collar-shaped, erose; c. 1.50 mm	1.00 mm; erose	Collar-shaped, erose; 1.00–5.00 mm	Collar-shaped, erose; 0.50–2.00 mm
5.	Auricle size (mm)	0.90 × 0.20	-----	1.00 × 0.20	0.50–3.00 × 0.20
6.	Sterile lemma length (mm)	1.76	1.50–2.50	0.15–2.45	0.05–1.40
7.	Sterile lemma shape	Deltoid - triangular	Linear-acuminate	Deltoid - triangular	Deltoid - triangular

Table 2: Evolutionary divergence estimates (distance matrix) for the multiple sequence alignment performed using the whole chloroplast genome among the six taxa of *Oryza* spp.

	1	2	3	4	5	6	7
1	0.010219						
2	0.000832	0.010621					
3	0.010904	0.014880	0.011181				
4	0.000228	0.010225	0.000868	0.010941			
5	0.000221	0.010292	0.000898	0.010956	0.000302		
6	0.010219	0.000000	0.010621	0.014880	0.010225	0.010292	
7	0.011035	0.015011	0.011312	0.000252	0.011072	0.011087	0.015011

1: *O. meyeriana* var. *indandamanica* (135,917 bp, Acc. No. OX485189, developed through this study); 2: *O. brachyantha* (134,644 bp, MT726939); 3: *O. neocaledonica* (135,950 bp, NC_053276 / MT726926); 4: *O. australiensis* (135,193 bp, MT726929); 5: *Oryza meyeriana* (136,133 bp, NC_034765 / KF359921); 6: *O. granulata* (135,942 bp, KF359920); 7: *O. brachyantha* (134,644 bp, KF359917); 8: *O. australiensis* (135,222 bp, KF359916). Here, *O. australiensis* and *O. brachyantha* were used as out groups.

indandamanica. Observation and data collection was done on ten plants by taking the average of three measurements for each plant for the characters of taxonomic significance (Table 1) to the genus *Oryza*. Simultaneously, critical analysis of protologues, the herbarium specimens of var. *granulata*, var. *meyeriana* and var. *indandamanica,* including type(s) housed at Kew (K), Leiden (L), Kolkata (CAL) and Port Blair (PBL) and taxonomic revisionary works (Duistermaat 1987; Chang 1988; Veldkamp 1991; Vaughan 1994) was made.

Chloroplast Assembly and Multiple Sequence Alignment

Freshly harvested leaves from the field-grown germplasm accession IC641181 were used for DNA extraction. In brief, approximately 1.5 g of the leaf samples were taken in a porcelain mortar and ground to a fine powder using liquid nitrogen. These powdered tissues were homogenized using 15 mL of 2% CTAB extraction buffer and the high-quality DNA was extracted using the protocol as reported earlier (Stewart and Via, 1993). The quality and quantity of the DNA was estimated using a 0.8% agarose gel electrophoresis and a Qubit 4.0 fluorometer. Quantified DNA was utilized thereafter for library preparation and sequencing using Novaseq6000 platform of the Illumina to obtain 2x150 bp paired-end data. The raw data obtained was subjected to adapter removal using TrimGalore v0.6.7 (Martin, 2011), and the quality check was done using FastQC v0.11.9 (Andrews, 2010) for the generated data. This short read data was used to generate the chloroplast assembly for the reported taxon using GetOrganelle v1.7.4.45 (Jin *et al*., 2020). Chloroplast genome annotation was performed using the GeSeq tool (Tillich *et al*., 2017); annotated information was used to derive the organelle genome map using OGDRAW v1.3.1 (Greiner *et al*., 2019). The generated whole chloroplast genome was aligned using ClustalW of the MEGA v11.0 with default parameters (Tamura *et al*., 2021), along with whole chloroplast genome sequence of other taxa of *Meyeriana* complex (downloaded from public domain) *viz*., *Oryza meyeriana* (136,133 bp; NC_034765.1), *O. granulata* (135,942 bp; KF359920.1), and *O. neocaledonica* (135,950 bp; NC_053276.1). Additionally, the whole chloroplast genome

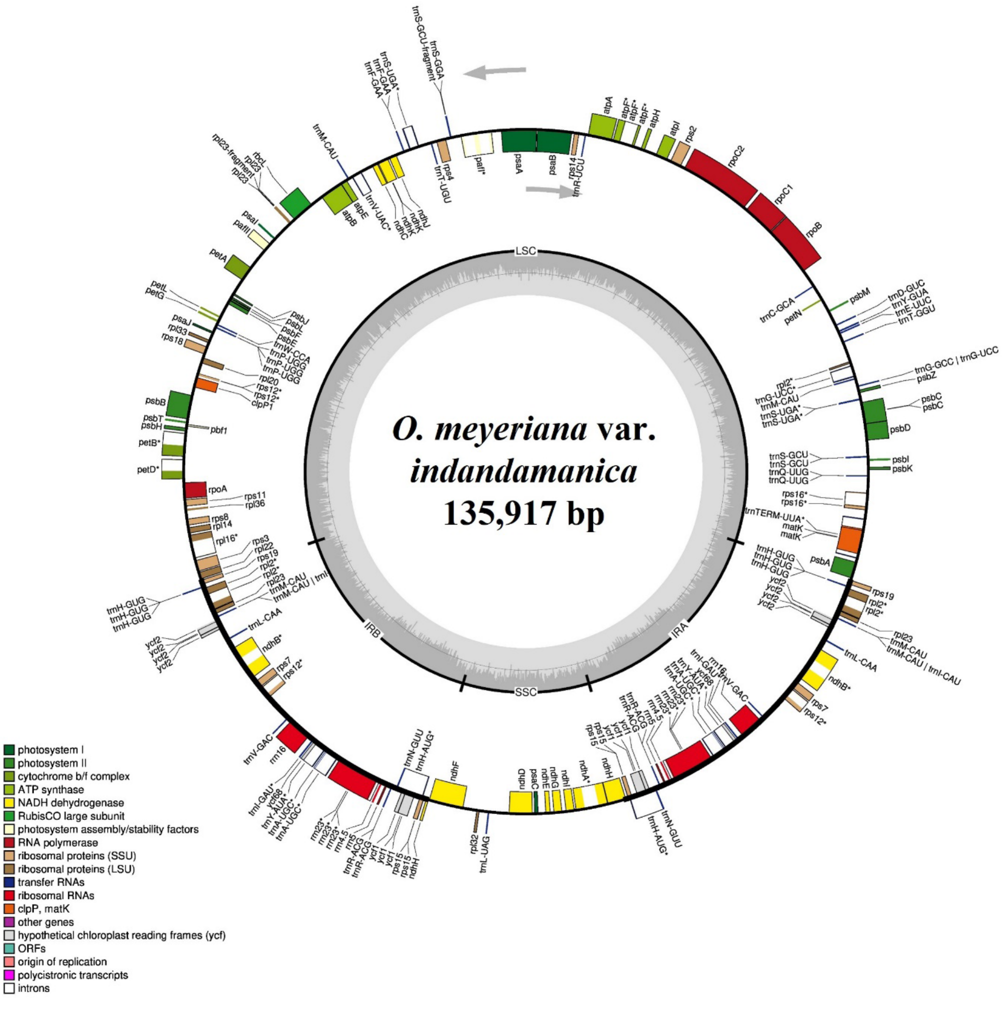

Figure 1: Single contig circular assembly of the chloroplast genome of 135,917 bp length for *Oryza meyeriana* var. *indandamanica* (IC641181). For sequence information please refer to the public database with accession number OX485189.1.

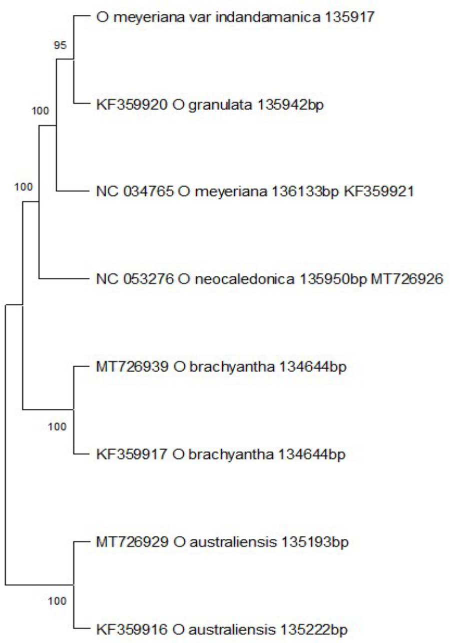

Figure 2: Cladogram derived using multiple sequence alignment for the whole chloroplast genome sequence for the represented taxa. Values at nodes indicate bootstrap values in percentage. For *O. meyeriana* var. *indandamanica*, the chloroplast genome sequence has been generated through the present study.

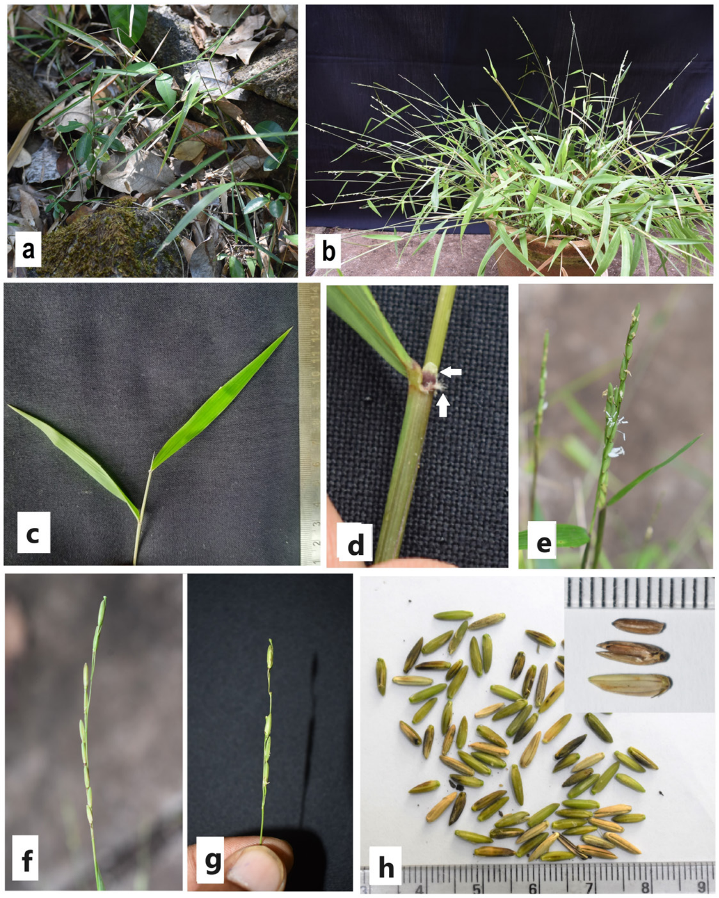

Plate 1: *Oryza meyeriana* var. *indandamanica* from SPNP. a. Plants in natural habitat; b. *Ex-situ* regeneration at Thrissur; c. Leaves - lower surface (left one) and upper surface (right one); d. Ligule and auricle of leaf; e. Inflorescence at anthesis; f. Inflorescence at dough stage; g. Inflorescence at spikelet maturity; h. Physiologically mature (pale green) and mature dry seeds (inset: seed, hull and kernel).

sequence of *O. australiensis* (135,222; KF359916.1 and 135,193 bp; MT726929.1, EE genome of *Officinalis* complex) and *O. brachyantha* (134,644 bp; MT726939.1 and KF359917.1, FF genome of *Officinalis* complex) were used as outgroup for the *Meyeriana* complex in this study. The evolutionary divergence estimates generated in the software was used to derive a cladogram using the maximum likelihood method and general time reversible model (Nei and Kumar, 2000). The confidence for the generated tree was assessed through the bootstrap option with 2000 replicates (Felsenstein, 1985).

Results and Discussion

A comparison of key morphological characters of studied accession with three taxa (Table 1) indicated that the SPNP population of var. *indandamanica* falls well under the circumscription of var. *meyeriana*, instead of var. *granulata*. The observed key characters, *viz*., fruiting spikelets oblong, 4.61 times as long as wide, and caryopsis 5.21 mm long, readily differentiate from var. *granulata* (having an elliptic fruiting spikelet which is only 2.10–2.70 times as long as wide, and caryopsis 3.40–4.10 mm long) (vide Duistermaat, 1987) (Plate 1). The characters given in the protologue of var. *inandamanica* (Ellis, 1985) also fit well within the range of characters recognized for var. *meyeriana*, except for the shape of the sterile lemma which is the case for the usage of inappropriate terminology. However, the character 'ribs in lower internode' as mentioned for the *meyeriana* complex (Duistermaat, 1987) was not evident in both the Andaman populations. Also, spikelets of Andaman materials are nearly smooth and hardly granulate, unlike that of the other two species (vide Vaughan, 1994) while Duistermaat (1987) didn't give weightage for this micromorphological trait. A few taxonomic characteristics of the SPNP population such as length of inflorescence (excl. peduncle) (7–9 *vs.* 5 cm in var. *inandamanica*) and floret (6.93 *vs.* 5 mm) and tufted habit (in natural habitat) do not match with the protologue information indicating the role of environmental factors modifying the phenotypic expression of plants. Herbarium studies also revealed that the characteristics such as habit, leaf shape and size, panicle length and branching nature used to study *meyeriana* complex had limited taxonomic value as the environment often modifies their expression.

The single-contig circular chloroplast genome of size 135,917 bp generated is given in Figure 1. Functional annotation using GeSeq tool revealed the presence of 125 genes comprising four rRNA genes, 34 tRNA genes and 87 mRNA genes. The list of unique genes among the annotated genes exhibited a conserved nature when compared with the other *Oryza* chloroplast genomes reported by Gao *et al*. (2019). Although our annotation had revealed the presence of *ycf1* and *ycf2* (Supplementary material 1), the absence of the genes *ycf1*, ycf2, and *ycf15* in the *Oryza* chloroplast genomes as documented by Gao *et al*. (2019) underscores the need for functional validation of *ycf1* and *ycf2*. Positively selected genes for shade tolerance in *Oryza* genus *viz*., *ndhD*, *rbcL*, *ndhH*, *psbD*, and *psbH* (Gao *et al*., 2019), were also identified in this taxon which is a shade tolerant one. The whole chloroplast genome sequence alignment as represented in the cladogram (Figure 2) indicated the closeness of var. *indandamanica* with var. *granulata* than var. *meyeriana* among the *Meyeriana* group. Also, the evolutionary divergence estimates (distance matrix) used to derive this cladogram suggests that the three taxonomical varieties of *O. meyeriana*, *viz*., var. *meyeriana*, var. *granulata*, and var. *indandamanica* were not sufficiently divergent from an evolutionary perspective (Table 2). This is in corroboration with the earlier report (Kumagai *et al*., 2010) on the phylogenetic study of the genus *Oryza* using chloroplast DNA sequences.

Foregoing studies indicated that the studied accession matches with var. *meyeriana* morphologically, but clusters with var. *granulata* at molecular level, indicating that a separate taxonomic identity as var. *indandamanica* is untenable. This further stems from the key taxonomic character 'spikelet size and shape', which showed continuous variation and inconsistency (Gong *et al*. 2000; Vaughan 2005). Different workers have set varied spikelet length values for distinguishing *O. meyeriana* from *O. granulata*, *viz*., > 6.4 mm (Vaughan, 1994), > 6.8 mm (Duistermaat, 1987) and >7 mm (Chang, 1988) indicating the lack of consensus owing to continuous variation exhibited for this trait. Wide hybridization experiments (Gong *et al*., 2000) between *granulata* and *meyeriana* resulted in high crossability (34–39% seed set) with relatively high fertility of interspecific hybrids, which denotes a very high genomic affinity and very limited reproductive isolation. Earlier, Khush *et al*. (1990) undertook morphological and cytological studies of the F_1 hybrids between *O. granulata* and *O. indandamanica* and concluded that both had identical genomes, and the latter could be regarded as an ecotype of *O. granulata* only. Based upon the principle of priority of ICN, the name *O. meyeriana* has precedence over *O. granulata*, therefore, become the currently accepted name.

With reference to the genetic resource potential of the studied accession, the habitat ecology of the studied population indicates the potential for tolerance to shade (as thriving in almost full shade) and drought (as occurring on steep stony slopes, blades roll inwards in response to stress). Mandal *et al*. (1989) reported the presence of wax-like coating over the culm and densely fibrous root system with abundant xylem strands in *O. indandamanica* contributing to drought tolerance. It takes only 16 days from anthesis to seed maturity (while rice takes about 30 days), and afterwards, the color of the lemma and palea changes from pale green to straw yellow within a day and sheds immediately. For this reason, it is very difficult to find mature grains on culms and therefore needs white clothing underneath to collect seeds. At Thrissur, the plant produced abundant tillers (up to 40), and flowers year-round, and they open at 10 AM throwing out far protruding white stigmas. The *meyeriana* complex is known for higher seed protein, including glutelin, and is a known source of bacterial leaf blight resistance. The studied accession has potential for studies involving biotic stress tolerance traits, for which only limited variability in rice is available (e.g., false smut, neck blast and stem borer). Nevertheless, it was found infected with brown spot (*Biploris oryzae*) under Thrissur conditions. Although *meyeriana* complex forms only a tertiary genepool of rice, advances in molecular biological techniques hold promise to broaden the crop genepool. Understanding the physiological, genetic and molecular basis of stress tolerance and its application in rice improvement programme is urgently required.

Though being a major food crop genus, general disagreement between rice researchers and taxonomists regarding the issues pertaining to synonymy in a few species of *Oryza* needs an urgent relook. A few Indian examples were *O. nivara vs O. rufipogon*; *O. minuta vs O. officinalis*; *O. malampuzhaensis vs O. officinalis*; and ambiguous taxa such as *O. perennis* and *O. spontanea*. The integrative taxonomy approach using information from various sources such as ecological data, molecular data, and detailed morphological characters would be helpful in solving species limits.

Sequence Availability

The assembled chloroplast genome sequence for *Oryza meyeriana* var. *indandamanica* (IC641181) of 135,917 bp is available through the public database on the accession number OX485189.1 (PRJEB61731 or INRP000060).

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