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Assessment of Morphological Diversity within Wild Rice (*Oryza rufipogon* Griff.) Germplasm of NBU Campus (West Bengal) for *In Situ* Conservation – A Case Study

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Natural population of *Oryza rufipogon* Griff. in the North Bengal University campus was characterized based on phylogeographic and morphological parameters for conservation purposes. Twenty-six different morphological characters were considered for genetic relatedness analysis within the population. Diagnostic characters were provided to delineate the species morphologically. Plants were annual ecotype having short to intermediate culm height (64-145 cm), and 0.5-1.6 cm thickness. Profuse fibrous roots of 2 mm thick and 3-9 cm long creamy-white colour emerged from the floating nodes, that was solid and pinkish (5-7 mm long). Flag leaf length ranged from 13-40 cm and 0.3-1.8 cm in width. Flag leaf blade attitude varied from deflexed to horizontal to semierect. Auricle was hard, curved, glabrous and 13-15.5 mm. Two-cleft ligule length ranged 17-35 mm. Panicle length varied from 15.4-30 cm with 1-8 cm wide with 5-13 primary branches. Attitude of primary branches was spreading, well-exserted (100%), alternately arranged on the wavy axis. Length of the lemma varies 6-11 mm and 1.4-2.3 mm wide, lemma-awn junction marked by a pinkish pubescent constriction with 23-80 mm long red awn. Yellow colour anthers varied in length 4-8 mm long. Stigma was bifurcated (> 2 mm) and purple comes out from the spikelets. Seeds (blackish) varied in length (7.5-10 mm). Density of fertile spikelets ranged from 15-82. Grain length/breadth ratio was 3.73 and average kernel length/breadth ratio was 3.99. The results suggested that the studied population were genetically diversified, heterogeneous and should be conserved *in situ* to protect precious genetic resource as separate ecological race (ecotype or intergrade) for germplasm enhancement.

Key Words: Eco-habitat, Diagnostic morphology, Genetic variation, *In situ* conservation, *Oryza rufipogon*

Introduction

The genus *Oryza* (Poaceae) includes two cultivated species (*Oryza glaberrima* Steud. and *O. sativa* L.) and 22 wild species. *O. glaberrima* is restricted only Western Africa, but *O. sativa* is cultivated globally (Vaughan, 1994; Kovach *et al*., 2007) due to its wide adaptability to different habitats and growing conditions. The species *O. sativa* can be grouped into two subspecies, *Oryza sativa* ssp. *japonica* and *O. sativa* ssp. *indica*, based on a number of physiological and morphological traits (Second, 1982; Oka, 1998). *Oryza sativa* ssp. *indica* was domesticated within a region south of the Himalaya mountain range, lowland of tropical Asia likely eastern India, Myanmar, and Thailand, whereas *Oryza sativa* ssp. *japonica* was domesticated from wild rice in the upland hills of southern China, southeast Asia, and Indonesia, and also outside of Asia (Africa, North America, and South America) (Khush, 1997; Londo *et al*., 2006). *Oryza sativa* L., represents the world's most important staple food crop, feeding more than half of the human population (supplying 20% of the world's total caloric intake). Despite this essential role in world agriculture, the history of cultivated rice's domestication from its progenitors (ancestors) and evolutionary pathways remains unclear. Phylogeographic studies indicate that India and Indochina may represent the centre of diversity of one of the 22 wild rice species *O. rufipogon* Griff. Wild rice species, *O. rufipogon*, grows across this entire range. *O. sativa* L. and *O. rufipogon* Griff. share the similar vegetative growth and other eco-geographical characters including the same AA genome and is widely recognised as a progenitor of cultivated rice (*O. sativa* L.) (Khush, 1997; Londo *et al*., 2006; Vaughan, 2008). *O. rufipogon* is a perennial species and cultivated rice is an annual species, it has been proposed that the annually occurring form of *O. rufipogon*, *O. nivara* (Sharma *et* Shastry, 1965), may represent the most recent ancestor of *O. sativa*. During the course of domestication from

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wild rice to cultivated rice, many genes were lost through natural and human selection, leading to the lower genetic diversity of the cultivated rice (Zhu *et al*., 2007). These wild species are the reservoirs of many useful genes/ QTLs particularly for resistance to major biotic and abiotic stresses (Xiao *et al*., 1996; Sanchez *et al*., 2013). The most successful examples are the use of genes from *Oryza nivara* to breed rice varieties with long-lasting resistance to grassy stunt virus (Khush, 1977) and the use of wild abortive cytoplasmic male sterility from *O. spontanea* to hybrid rice (Lin and Yuan, 1980). Broad spectrum blast resistance gene(s) and yield improving potentials have been introgressed for the development of new cultivar, Dhanarasi using *O. rufipogon* as donor (Ram *et al*., 2007a; 2007b). Green leafhopper resistance gene has been introgressed into cultivated species through molecular breeding from *O. rufipogon* (Fujita *et al*., 2003). Many useful genes from *O. rufipogon* have been successfully introgressed into the cultivated background (Wright and Gaut, 2005). Yield-enhancing traits/QTLs/ genes were mapped and identified from *O. rufipogon* and incorporated into the genetic background of IR64/ other cultivars for yield increase (Septiningshih *et al*., 2003; Thomson *et al*., 2003; Tian *et al*., 2006; McCouch *et al*., 2007). Today, the wild species exists in only a few severely fragmented populations that may be remnant of a formerly more continuous widespread range (Gao, 2004; Londo *et al*., 2006). Populations of *Oryza rufipogon* have gradually reduced due to ecological stresses.

Due to long term neglect, wild rice population in NBU campus (North Bengal University) are being lost at an alarming rate. NBU campus has received little attention to *Oryza rufipogon* populations. It needs immediate attention with high priority before agriculturally important *Oryza* genepool is lost.

After exhaustive reviewing of the literature and considering the importance of origin of problem of wild rice genetic resources, the present investigation was made for the first time to characterize the germplasm of *O. rufipogon* from the campus of North Bengal University (NBU) based on ecological and morphological parameters to provide much needed data to maintain and implement *in situ* conservation strategies.

Material and Methods

Plant Material

Natural population of wild rice *Oryza rufipogon* Griff. in the campus of the University of North Bengal (NBU) was surveyed for the present study including the growing conditions, ecological environments, water availability and morphological characteristics. Some specific ecological environments are supporting wild rice *Oryza rufipogon* Griff. population in the NBU campus as natural habitat (Fig. 1), which is located at Latitude of 26º 84′ North and Longitude of 88º 44′ East, near Siliguri, Dist-Darjeeling, WB, India. The area is seasonally dry and water logged. During summer this region is completely dry but during monsoon June to September there is plenty of water in the ponds and ditches.

Soil Testing

Soil samples were collected from the rice field during March (2014) according to the standard protocol and sent to the Tea Science Department, NBU for analysis (Tea Board approved soil testing lab) on payment basis.

Methodology for Morphological Trait Analysis

Plant materials (*Oryza rufipogon* Griff.) were directly used for morphological trait measurement and analysis in the natural field conditions in the NBU campus. Twenty-five plants were selected for recording twenty-six morphological features for genetic variation study. All the phylogeographic and morphological observations were recorded and measured as per standard protocol for wild rice characterization (IBV/IRRI). Eighteen quantitative morphological traits were considered for the present study *viz.*– panicle length, flag leaf length/ width, spikelet number per panicle, seed length/width, awn length, stigma length, anther length and plant height, uppermost internode distance, primary branching, ligule, auricle, palea length, sterile lemma, kernel length/ breadth, etc. The average quantitative trait value for each variable from all individuals in a population was generated and used for analysis. Eight qualitative traits were considered in the present study – attitude of panicle branches, panicle curvature, panicle exsertion, attitude of flag leaf, spikelet anthocyanin, keel edge, stigma colour, and lemma colour at maturity.

Results and Discussion

Wild rice of NBU campus were identified as *Oryza rufipogon* Griff. based on data on phylogeography and morphological traits analysis. Morphological characteristics measurements were statistically analysed using SPSS-15 software and summarized in Table 1 and graphically represented in Fig. 3.

Fig. 1. Location of surveyed population of wild rice *Oryza rufipogon* **in the NBU campus**

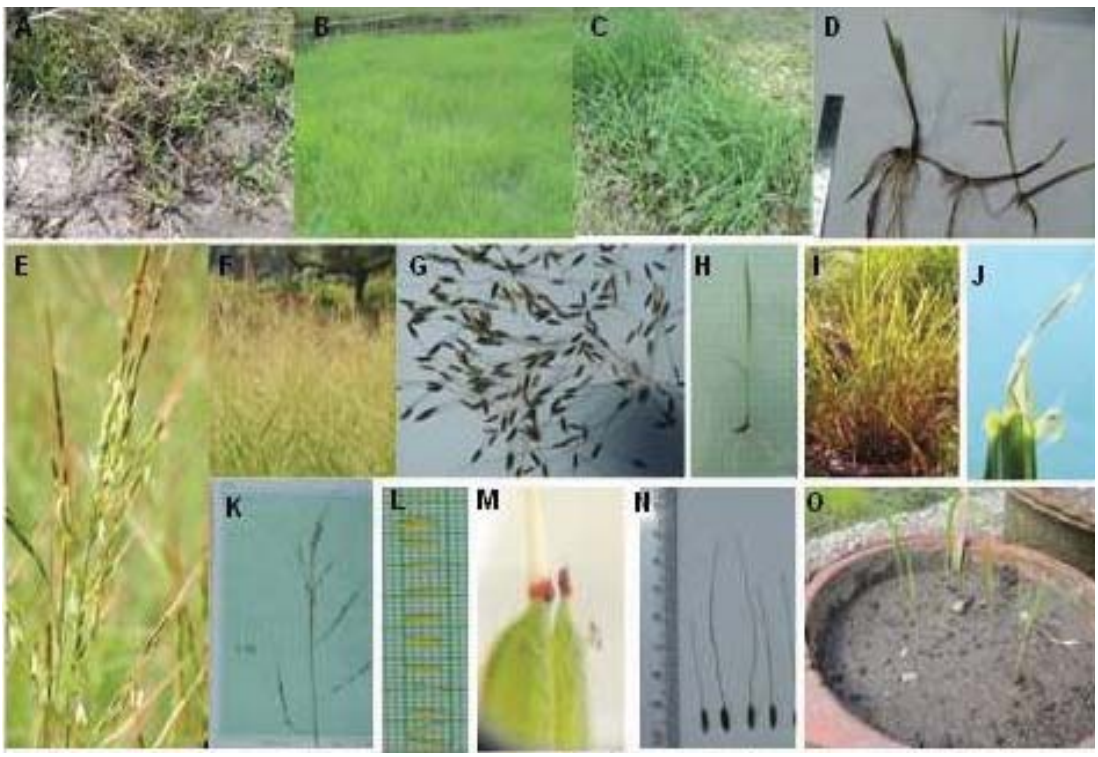

Fig. 2. Different morphological traits and measurement for characterization of wild rice *Oryza rufipogon* Griff. of NBU campus. A : Plants with stem stolon survive in dry summer, B: Vegetative propagation in monsoon, C: Stem stolon grown in water tanks for propagation and conservation, D: Tillering with roots from nodal region, E: Panicle with spikelets showing protruding anthers and stigmas, F: Starts seeds shattering during maturity, G: Mature black seeds with long awn, H: Mature seed germinated and measured seedling length, I: Profuse tillering from node of a stem part, J: Showing auricle and ligule, K: Panicle length measurement, L: Anther length, M: Dark red point at awn base, N: Seed length, O: Germinated seeds in pot during *kharif* 2014

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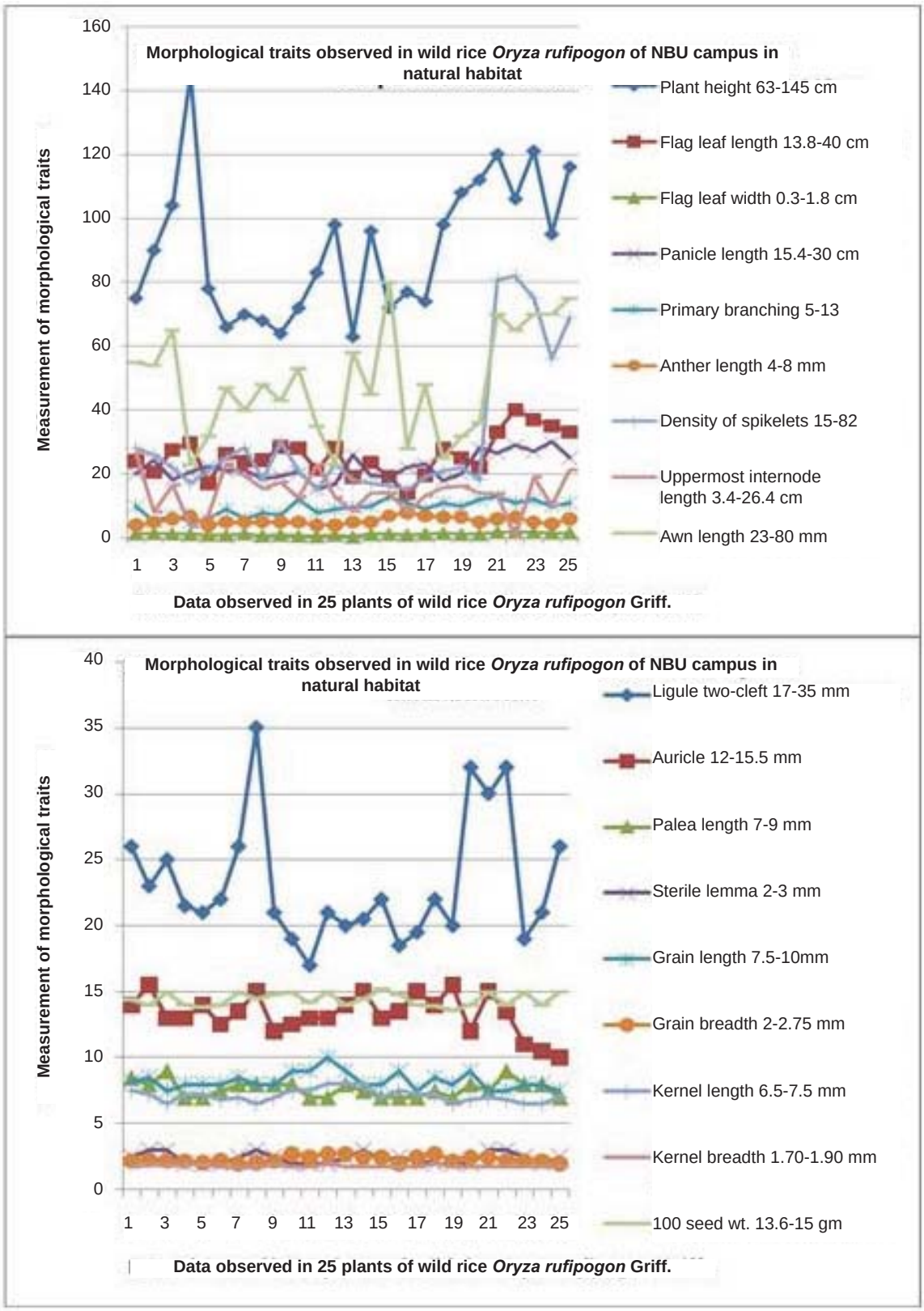

Fig. 3. Graphical representation of some morphological traits observed in *Oryza rufipogon* Griff. of NBU campus in natural habitat

Habitat and Ecology

Plant is annual ecotype, sometimes rhizomatous, short to intermediate height (64-145 cm). It is found growing in swampy areas (shallow water, up to 0.3 m), at edge of ponds and tanks, in ditches around the river of Magurmari which flows through the campus of the University of North Bengal, which is seasonally dry and open habitats. Plants start flourishing during the rainy season June-July of every year mostly through clonal propagation, flowering in September-October and seed shedding November-December and then dried up (Fig.1 & 2). Low population are due to destruction by human activities (poor women collect wild green vegetables along with wild rice during monsoon period) and over grazing.

Morphological Diversity

Culms: Geniculately ascending/sub-erect or decumbent (prostrate), 64-145 cm long, 0.5-1.6 cm thick, spongy lower part floating on the water surface with profuse fibrous rooting at the node region often with rhizomes, roots are 2 mm thick and 3-9 cm in length, creamy-white in colour present in the subsoil culms at the node region, node region is solid, hard and pinkish in colour (5–7 mm).

Roots: Roots fibrous, often with rhizomes. Fibrous root produces from nodal region of floated stems/stolons (Fig. 1 D).

Leaf Morphology: Leaf blades linear, lanceolate, flat, somewhat glaucous, scabrid on margins and main nerves, base rounded, margin tentate, apex acuminate, leaf blade surface scabrous or smooth, flag leaf 13-40 cm long and 0.3-1.8 cm width. Flag leaf blade attitude- deflexed/ horizontal/semierect. Leaf sheath loose, cylindrical, smooth, glabrous on surface, generally cover the whole intermodal region with distinct auricle at the junction with the blade. Auricles 13 mm-15 mm in size, narrow, curved, hard, glabrous or lined with long hairs to 2 mm long. Ligule- two-cleft, V-shaped, triangular, an unfringed membrane up to 17-35 mm long.

Inflorescence: Panicle, open, linear, nodding, 15-30 cm long, 1-8 cm wide; primary branches 5-13; attitude of branches –spreading, well exserted (100%). Panicle before anthesis erect and compact but after anthesis spikelets primary branches spread from the panicle axis, primary branches alternately arranged on the axis and axis is wavy. Flowering time September-October.

***Fertile Spikelets*:** Spikelets oblong, laterally compressed, 5-9 mm long, 1.6-3.5 mm wide with pedicel.

***Functional Florets*:** Lemma 6-11 mm long, 1.4-2.3 mm wide, straight or curved, lemma-awn junctions marked by a purplish, pubescent construction, awns 23-80 mm long. Awn bicolour, hard and rough surface, lower part creamy-white before anthesis and upper part reddish colour at maturity. Palea–elliptic, 7 mm long, coriaceous, 3-veined, 1-keeled, surface scabrous, apex acute. Glumes 2-3 mm long, ovate, and unequal.

Flowers: Lodicules 2, membranous, stamens 6, anthers 4-8 mm long, yellow in colour, stigma bifurcated, purple-brown in colour comes out from the spikelets through lemma and palea, ovary 1 mm long.

Fruits: Caryopsis with adherent pericarp 7.5-10 mm long, 2-2.75 mm wide, broadly elliptic or oblong, reddish-brown to dark blackish-red, embryos 1-1.5 mm. Most of the seeds are shed before harvesting due to shattering nature. Seeds harvested during November-December.

Wild rice of NBU campus is considered as a variant ecotypic race of *Oryza rufipogon* Griff. due to distinct characteristics specially anther length (4-8 mm), stigma >2 mm and sterile lemma 2-3 mm long. In most cases, individuals within populations showed different levels of morphological variability (Table 1 and Fig. 2). It is consistent with earlier report (Dong *et al*., 2010). In case of plant height and spikelet density Sd value is 21.74, and 21.87, respectively, which clearly indicating that morphological data are highly variable (Table 1). There was no report of secondary panicle branching in the present observation that was not supporting the earlier observation (Dong *et al.,* 2010). Morphological variation existed within the populations may be due to the existence of variety of ecological parameters include temperature, water, soil and associated plants communities, among others (Gao, 2004). Soil is loam type containing 40% silt, 20% clay and 40% sand, with acidic pH 5.69. Proportion of organic matter was 2.41 %, organic carbon 1.40 %, nitrogen 0.12 %, K_2O 89.8 ppm, P_2O_5 18 ppm and sulphur 40.5 ppm. Panicle is less than 30 cm in length with 15-80 spikelets per panicle. Spikelet tip was marked with a deep pink colouration. Plenty of fibrous roots were evident in many individuals. Morphological variation were observed in the present study may be due to the introgression of genetic factors from *Oryza sativa* to *O. rufipogon* (Dong *et al*., 2010). The two taxa (*O. nivara* and *O. rufipogon*) are recognized as distinct

Table 1. Morphological data of *Oryza rufipogon* of NBU campus

		Plant height (cm)	Flag leaf length (cm)	Flag leaf width (cm)	Panicle length (cm)	Primary branching	Anther length (mm)	Spikelets density	Upper most internode (cm)	Awn length (mm)
N	Valid	25	25	25	25	25	25	25	25	25
	Missing	0	0	0	0	0	0	0	0	0
Mean		90.8400	25.8720	1.1400	22.0440	9.3800	5.4800	31.3200	13.9840	48.8000
SEm		4.34921	1.28398	0.08062	0.79103	0.47546	0.22076	4.37596	1.23994	3.43511
SD		21.74603	6.41992	0.40311	3.95517	2.37732	1.10378	21.87982	6.19971	17.17556

		Ligule size (mm)	Auricle size (mm)	Palea length (mm)	Sterile lemma (mm)	Grain length (mm)	Grain breadth (mm)	Kernel length (mm)	Kernel breadth (mm)	100 seed wt. (g)
N	Valid	25	25	25	25	25	25	25	25	25
	Missing	0	0	0	0	0	0	0	0	0
Mean		23.2000	13.3200	7.6600	2.3960	8.2480	2.3364	7.1160	1.8120	14.4280
SEm		0.94074	0.29563	0.12490	0.08072	0.12585	0.04907	0.09340	0.01589	0.10042
SD		4.70372	1.47817	0.62450	0.40361	0.62923	0.24537	0.46698	0.07943	0.50210

species (Sharma *et* Shastry, 1965; Banaticla-Hilario *et al*., 2013) but others considered *O. nivara* to be the annual ecotype or subspecies of *O. rufipogon* (with *O. rufipogon sensu stricto* as the perennial ecotype) due to their continuous variation (morphological and genetic) including interfertility. Presence of stolons and long, strong, and spreading culms make *O. rufipogon* suitable for permanently inundated habitats. Stigma and anther are longer and panicles are more exerted and open in this outcrossing *O. rufipogon* than in inbreeding *O. nivara* (Table 2). *O. rufipogon* is photoperiod sensitive (Banaticla-Hilario *et al*., 2013). Gene flow between populations of *Oryza rufipogon* (*sensu lato*) and *Oryza sativa* is highly probable due to their overlapping distribution in the same area (Oka, 1998; Vaughan *et al*., 2008). However, hybrid populations between *O. nivara* and *O. rufipogon* are rarely reported as they are hardly found growing side by side in the same area. There are some intermediate populations (morphologically and genetically) of the two wild species in some part of Thailand and Vietnam but not considered as a hybrid of *O. nivara* and *O. rufipogon*, instead they may be hybrid of *O. sativa* with *O. nivara* or *O. rufipogon* (Banaticla-Hilario *et al*., 2013). The wild rice taxon of NBU campus has been identified as *Oryza rufipogon* based on morpho-ecological parameters although it is not matching all the characteristics of its previous reports (Table 2). Deviation from the previously reported morphological features may be due to its adaptation in different environmental factors. Before the establishment of the University (1962), the lands were used for rice cultivation due to lowland habitat, ditches, and ponds around the small flowing River Magurmari. During that period (1962s) there may be some kind of gene flow between *O. sativa* and *O. rufipogon*, and thus the wild taxon of this NBU campus showing deviated morphological features from *O. rufipogon* and may be considered as intermediate form (intergrade) (Table 2). Morphological differentiation and variation within and among population has been studied to diagnosing species boundary (Elizabeth *et al*., 2008). Genetic diversity assessment within the species has been basis to utilize and manage germplasm resources (Wright and Gaut, 2005) and particularly crucial to its conservation. Many rice scientists have studied the genetic structure within and among the *O. rufipogon* based on morphology, ecology and DNA markers (Sun *et al*., 2001; Dong *et al*., 2010).

Conclusion

The present ecogeographical and morphological characteristics showed that high level of genetic diversity in the populations of *O. rufipogon* in NBU campus. Thus, wild rice population of NBU campus is considered as separate ecological race (ecotype) and may carry some important genes/alleles and hence considered as precious germplasm for crop genetic improvement. Ecological

Table 2. Comparison of NBU wild rice with reference data of other wild rice species

Morphological traits	*Oryza rufipogon*	*Oryza nivara*	Wild rice NBU
Plant height (cm)	136 – or >170	50-150 or <170	64 – 145
Flag leaf length (cm)	7 – 20	30 – 49	13 – 40
Flag leaf width (cm)	0.4 - 0.95	1.1 - 1.8	0.3 - 1.8
Panicle length (cm)	19.8-37.6	14-16	15.4-30
Attitude of panicle branches	Spreading	Compact/spreading	Compact/spreading
Panicle exsertion	Well exserted	Well exserted	Well exserted
Uppermost internode length (cm)	1.6 – 2.6	0.9 – 1.8	3.4 – 26
Attitude flag leaf blade	Horizontal	Semierect	Deflexed/Horizontal/ Semierect
Ligule (mm)	18 – 30	21 – 44	17 – 35 two-cleft
Awn length (mm)	40 – 67	50 – 88	23 – 80
Anther length (mm)	> 3.7	< 3.7	4 – 8
Pollination	Cross pollination	Self pollination	Cross pollination
Stigma length (mm)	1.8 – 2.8	1.4 – 1.8	>2
Sterile lemma (mm)	0.5 – 0.76	0.83 – 1.2	2 – 3
Grain length (mm)	7 – 10	6 – 10.4	7.5 – 10
Grain breadth (mm)	1.8 – 2.3	2.8 – 3.4	2 – 2.75
Kernel length (mm)	5 – 7	5.5 -8.5	7.5 – 8
Kernel breadth (mm)	2.2 – 2.7	0.65 – 0.92	1.7 – 2
Life cycle	Perennial/annual	Annual	Annual

diversity of the wild rice species should be protected and maintained. The population should be conserved *in situ* conditions to understand the morphological diversity within the populations and make convenient habitat for evolution. In addition, competitive associated plant species in protected areas should be controlled and the level of intra- and inter-population gene flow in protected populations should be monitored. This type of well-defined eco-phenotypes based species delineation will help gene bank people in dealing with misidentified accessions and in maintaining intermediate forms. Population of *Oryza rufipogon* of NBU campus should be maintained at *in situ* condition to conserve this valuable wild rice germplasm as important genetic resources.

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