NBU Journal of
Plant Sciences

Vol. 8, No. 1 March 2014 p. 53-64
ISSN No. 09749027

Morphological characterization of wild rice (*Oryza rufipogon* Griff.) of NBU campus (West Bengal) for *in situ* conservation and germplasm enhancement

Subhas Ch. Roy
Plant Genetics & Tissue Culture Laboratory, DRS Department of Botany, University of North Bengal, PO- NBU, Siliguri-734013, WB, India.

Abstract

Asian wild rice *Oryza rufipogon* Griff. (Poaceae) is considered as a progenitor of the cultivated rice (*Oryza sativa* L). It is a source of many biotic and abiotic stress tolerance genes and these are utilized for genetic improvement of cultivated rice. Thus it needs immediate attention with high priority so that we could not loss this agriculturally important Oryza gene pool. Natural population of *Oryza rufipogon* in the North Bengal University campus is characterized based on phylogeographic and morphological parameters for conservation purposes. Twenty-five plants were considered randomly to measures twenty-six different types of morphological passport data for genetic relatedness analysis within the population. Diagnostic characters are provided to delineate the species morphologically. Plant is annual ecotype having short to intermediate culms height (64 cm -145 cm), and 0.5 – 1.6 cm in thickness. Profuse fibrous roots of 2 mm thick and 3 – 9 cm long creamy-white in colour grows from the floating nodes, node region is solid and pinkish in colour (5-7 mm long). Flag leaf length ranges from 13 cm – 40 cm with 0.3 cm – 1.8 cm width. Flag leaf blade attitude is varying from deflexed to horizontal to semierect. Auricle was hard, curved, glabrous and 13 – 15.5 mm in size. Two-cleft ligule length ranges 17 mm to 35 mm. Panicle length varies from 15.4 cm – 30 cm with 1 cm – 8 cm wide with 5-13 primary branches. Attitude of primary branches is 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 vary in length 4-8 mm long. Stigma was bifurcated (> 2 mm) and purple in colour comes out from the spikelets. Seeds (blackish) are harvested during November-December and vary in length (7.5 mm – 10 mm). Density of fertile spikelets ranges from 15 to 82. Grain length/breadth ratio was 3.73 (average) and average kernel length/breadth ratio was 3.99. The results suggest that the population of NBU campus are genetically diversified, heterogeneous and should be conserved *in situ* to protect this precious genetic resource as separate ecological race (ecotype or intergrade) for germplasm enhancement.

Key-words: *Oryza rufipogon*, eco-habitat, diagnostic morphology, *in situ* conservation, genetic resources.

Introduction:

Rice (*Oryza sativa* L.) is the staple food for half the world population, particularly for the poorest of Asia. Rice played a major role in the Green Revolution in the 1960s, a well-known story of modern plant breeding that contributed significantly to global food security. Sustaining rice production relies on the intelligent use of rice diversity. Now, rice production must increase by at least 25% by 2030 to keep pace with predicted population growth. This has to be achieved using less land, less water and under more severe environmental stresses expected, due to the effects of climate change and disease pressures. Much of this increase must come from genetic improvement of rice. Wild rice can be appropriately used to widen the gene pool of cultivated rice and introgressed the desired agronomic traits. Only two cultivated species are available in the world, *Oryza glaberrima* and *O. sativa*. Species *O. glaberrima* is cultivated in restricted areas of Western Africa, whereas *O. sativa* is cultivated globally. But the genus *Oryza* includes 21 wild species such as *Oryza rufipogon, O. nivara, O. minuta, O. longistaminata, O. officinalis, O. latifolia, O. alta, O. australiensis, O. granulate, O. brachyantha.* and *O. granulata etc*. Since the time of its initial domestication, Asian cultivated rice (*O. sativa* L.) has been moved across the globe

Corresponding author:
E-mail: subhascr@rediffmail.com

with migrating human populations. Rice (*O. sativa*) cultivation can now be found on all continents except Antarctica (Kovach *et al*., 2007). *O. sativa* is cultivated globally due to its adaptability to different habitats and growing conditions. Cultivated rice, *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 (Londo *et al*., 2006). Rice domestication is thought to have begun ~9,000 years ago within a broad geographic range spanning eastern India, Indochina, and portions of Southern China. The majority of rice cultivars can be placed within two subspecies or races of rice, *Oryza sativa* ssp. *japonica* and *Oryza sativa* ssp. *indica*, based on a number of physiological and morphological traits such as drought tolerance, potassium chlorate resistance, phenol reaction, plant height, and leaf color (Oka, 1998) and growth habitat as well. *O. sativa* ssp. *japonica* can be further differentiated into tropical (*javanica*) and temperate (*japonica*) forms, with the temperate form appearing to be a derivative of tropical *japonica* (Second, 1982). *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, as well as rice outside of Asia (Africa, North America, and South America) (Khush, 1997). In addition to the two major races, several other minor rice types have been identified with genetic markers (Second 1982) and include the upland drought-tolerant Aus cultivars of India and Bangladesh, the deep-water Ashina varieties of Bangladesh, and the aromatic basmati rice of India as well as many others. Whether all these different rice types have arisen from a single domestication event or are the result of multiple domestications remains unknown. Phylogeographic studies indicate that India and Indochina may represent the center of diversity of one of the 21 wild rice species *O. rufipogon* Griff. This wild rice species, *O. rufipogon*, grows across this entire range. Both the species, *O. sativa* L. and *O. rufipogon* Griff. (Poaceae) share similar vegetative growth and other eco-geographical parameters including the same AA genome and is widely recognized as a progenitor of cultivated rice (*O. sativa* L) including its two subspecies *O. sativa* ssp *japonica* and *O. sativa* ssp *indica* (Khush, 1997; Vaughan *et al*., 2008). This putative ancestor of Asian cultivated rice (*O. sativa* L.), is a perennial, out-crossing and widely distributed species in the tropics and subtropics of Asia, including the northern parts of Australia (Londo *et al*., 2006; Vaughan *et al*., 2008). This species represents an important reservoir of genetic biodiversity for cultivated rice because it shares the same AA genome with cultivated rice. This weedy taxon *Oryza rufipogon* can hybridize readily with *O. sativa*, and can produce partially fertile hybrids. Compared to the other wild species, *O. rufipogon* is studied more extensively for its compatibility of genetic introgression into the cultivated rice *O. sativa*. Incidence of natural intercrossing between the two species is very high resulting in establishment of numerous morphological intergrades. It has been suggested that the existence of 'pure' forms of *O. rufipogon* is only conceptual, because of the continual crossing among cultivated and wild rice types, resulting in a conglomerate of hybrids. Thus confusion exists taxonomically about the delineation of species. This may be why some authors consider the name *O. rufipogon* to be that of a perennial grass with rhizomes, while others say it is an annual. *Oryza rufipogon* is native to Southeast Asia and Australia, where it grows in shallow, standing or slow-moving water, along irrigation canals, and as a weed in rice fields. *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*, *Oryza nivara* (Sharma and Shastry, 1965), may represent the most recent ancestor of *O. sativa*. Through the process of domestication, cultivated rice has lost many of the traits associated with other wild grass species; in addition to a shift from perennial to annual populations, cultivated rice differs from

O. rufipogon in its non-shattering seeds, lack of awns (so long), erect habit, and high grain yield (Londo *et al.*, 2006). As a progenitor of cultivated rice, this species has proven to be a valuable reservoir of genes for genetic improvement in cultivated rice. Notably, those improvements include: resistance to blast resistant to brown plant hopper. While this wild rice species is the most agriculturally important, most populations, which represent the primary gene pool of rice, are seriously endangered by introgression (gene flow from cultivated rice) and human activity that has led to the extinction of the species throughout the areas of distribution (Gao, 2004). Today, the species exists in only a few severely fragmented populations that may be remnants of a formerly more continuous wide spread range. *O. rufipogon* populations are highly diverse for morphology and for genetic markers and they often segregates for agronomically important traits such as pathogens or insects resistance. Natural gene flow from the cultivated rice may be a cause of the high genetic diversity of *O. rufipogon* populations in some region. Many useful genes from *O. rufipogon* have been successfully introgressed into the cultivated background. Successful use of this wild rice genetic resource included the identification of several yield-improving quantitative trait loci (QTLs) alleles and a virus resistance gene (Xiao *et al.*, 1996). Maintenance of broad genetic diversity is important to explore elite genes resources to develop high yielding, good quality and multi-resistant rice cultivars and so ensures high and stable rice production. During the course of domestication from 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). It is feasible to explore and utilize the gene resources in wild rice to solve the problem faced in modern rice production. 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, 1997) and the use of wild abortive cytoplasmic male sterility from *O. spontanea* to hybrid rice. It is served as a rich genetic resource providing favourable alleles/genes to improve cultivated rice yield and quality. It is important for the germplasm which has many resistant genes, and hence is collected and conserved *in situ* and *ex situ* in various places of the world. Genetic diversity assessment within species has been the basis to utilize and manage germplasm resources (Wright and Gaut, 2005) and particularly crucial to its conservation. Genetic erosion is threatening the integrity of wild rice germplasm resources in some part of the world. During domestication process from wild species to cultivated rice, selecting desirable agronomic traits to keep achieving high yield allows many genes to be either directly selected or filtered out, resulting in a significant reduction of genetic diversity in rice gene pool (Brar *et al.* 2003). Sun *et al.* (2001) revealed that the number of alleles in cultivated rice had been reduced by 50-60% compared to wild rice. Thus, it is necessary to broaden the gene pool in rice breeding from diverse sources, especially from wild rice. Common wild rice (*Oryza rufipogon* Griff.), due to its long term growth in the wild conditions, possesses numerous advantages such as genetic diversity, excellent agronomic traits, and resistance against various biotic and abiotic stresses, proved to be an important resource for genetic improvement of rice (Song *et al.*, 2005). It is for certain that many valuable traits exist in the wild rice species, but the most challenges to us are how to explore the valuable genes from wild rice and effectively transfer them into the cultivated rice for diversifying genetic basis of cultivated rice. Recently, many genes and QTLs have been mined from the wild rice, which functions include disease and insect resistances, abiotic stress tolerances, high yield, and so on. Therefore, there is an urgent need to broaden the rice gene pool from diverse resources, of which the wild rice is an ideal option.

Li *et al.* (2009) evaluated blast resistance for 21 progenies from crossing with common wild rice, and obtained three stably resistance progenies. Preliminary analysis showed that the rice blast resistance was controlled by dominant genes. Geng *et al.* (2008) cloned rice blast resistance gene *Pi-ta+* from Jinghong erect type of common wild rice (*O. rufipogon*). Bacterial blight resistance gene against causal organism *Xanthomonas oryzae pv.*

oryzae named as *Xa23(t)*, was first detected from *O. rufipogon* by Zhang (2005), showing resistance to race 6 of bacterial blight in the Philippines. Jin *et al.* (2007) identified a rice bacterial blight resistance germplasm (Y238) from the wild rice species *Oryza rufipogon*, and then they transferred the resistance locus into the cultivated rice to breed near isogenic line. The common wild rice (*Oryza rufipogon*) could be precious resource for genetic improvement of drought resistance in cultivar improvement (Xie *et al.*, 2010; Hu *et al.*, 2013). The positive yield QTLs from *O. rufipogon* may be potentially useful for breeding high yield cultivar if the disadvantage linkage drag could be broken through careful selection (Brondani *et al.*, 2002; Cho *et al.*, 2003; Fu *et al.*, 2010; Wu *et al.*, 2012). Molecular mapping of these good genes will help discover and make full use of the elite resources of wild species to broaden the genetic base of modern cultivars.

Due to long term neglect, wild rice population in NBU campus (University of North Bengal, Siliguri-734013, Bagdogra, Dist-Darjeeling, India) are being lost at an alarming rate. NBU campus has received little attention in regards to its *Oryza rufipogon* populations. It needs immediate attention with high priority so that we could not loss this agriculturally important Oryza gene pool.

After exhaustive reviewing the literature and considering the importance of origin of problem, the present investigation will be made first time to characterize the population of *Oryza rufipogon* Griff. exists in 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 for this precious ecotype for crop improvement. Population of *Oryza rufipogon* of NBU campus should be maintained *in situ* condition to conserve this valuable wild rice ecotype as important genetic resources for germplasm enhancement.

Material and methods:

Plant material

Wild rice (*Oryza rufipogon* Griff.) in a natural eco-habitat condition of the University of North Bengal (NBU) campus 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 26° 84A North and Longitude of 88° 44A East, near Siliguri, Dist- Darjeeling, WB, India. The area is seasonally dry and water logged. During summer this region is completely dry and during monsoon June to September plenty of water in the ponds and ditches. There was no *O. rufipogon* populations during summer but their stems remains viable as dried conditions, they stars germination/ propagation as soon as they receive rain water during May-June. Soil type is loam based on textural class containing 40% silt, 20% clay and 40% sand, organic matter 2.41 %, organic carbon 1.40 %, 0.12 % nitrogen, K_2O 89.8 ppm, P_2O_5 18 ppm and sulphur 40.5 ppp with pH range 5.65 to 5.70.

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

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 without collecting any one of the plants. This germplasm is preciousness to the rice breeder. Twenty-six morphological features were considered to this genetic variation study within the wild rice population. All the phylogeographic and morphological observations were recorded and measured as per standard protocol for wild rice characterization (IBV/IRRI). Eighteen quantitative morphological traits are considered for the present study are as follows- 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 in the analysis (Table 1). Eight qualitative traits are as follows- attitude of panicle branches, panicle curvature, panicle exsertion, attitude of flag leaf, spikelet anthocyanin, keel edge, stigma colour, and lemma colour at maturity.

Seed germination

Seeds were harvested during November 2013 by bagging (thin cotton bag) immature panicle before seed shattering. Seeds were preserved in the Plant Genetics & Tissue Culture Lab, Department of Botany, NBU for future use. For germination, seeds were soaked in tap water for 24 hours and next placed on soil in an earthen pot keeping seeds submersed (date 18.06.2014). Germination initiation starts on 24.06.2014 and 90% seeds were germinated.

Results and discussion

Wild rice of NBU campus has been identified as *Oryza rufipogon* Griff. based on the study of phylogeography and morphological traits analysis. Morphological characteristics features are summarized in table 1, fig 2 and data are graphically represented in figure 3.

Habitat and ecology: Plant is annual ecotype, sometimes rhizomatous, short to intermediate height

Table 1. Morphological traits observed in wild rice (*O. rufipogon*) of NBU campus.

Number of plants studied	Plant Height (cm)	Flag leaf Length (cm)	Flag leaf width (cm)	Panicle length (cm)	Primary Branching	Anther length (mm)
1	75.0	24.0	1.3	20.0	10.0	4
2	90.0	20.5	1.4	24.2	5.0	5
3	104.0	27.5	1.3	18.2	7.0	6
4	145.4	29.7	1.1	20.6	5.0	7
5	78.0	17.0	0.9	22.4	6.0	4
6	66.0	26.4	0.8	21.0	9.0	5
7	70.0	23.8	1.3	21.3	6.0	5
8	68.0	24.3	0.7	18.4	8.0	5
9	64.0	28.5	0.9	19.3	7.0	5
10	72.0	28.0	0.6	20.5	12.0	5
11	83.0	21.3	0.5	15.4	8.0	4
12	98.0	28.1	0.9	16.8	9.0	4
13	63.0	18.9	0.3	26.0	9.5	5
14	96.0	23.5	1.1	19.5	10.0	5
15	72.0	19.0	1.1	19.0	13.0	7
16	77.0	13.8	0.9	22.0	11.0	8
17	74.0	19.5	1.2	23.0	9.0	7
18	98.0	28.0	1.4	18.0	11.0	6.5
19	108.0	25.0	1.1	20.0	10.0	6.5
20	112.0	22.0	1.1	28.0	12.0	5
21	120.0	33.0	1.8	26.5	13.0	6
22	106.0	40.0	1.8	29.0	11.0	6.5
23	121.0	37.0	1.8	27.0	12.0	5
24	95.0	35.0	1.6	30.0	10.0	4.5
25	116.0	33.0	1.6	25.0	11.0	6

Continued table 1

Density of Spikelets	Internode Length, cm (uppermost)	Ligule (mm), two-cleft	Auricle (mm) Curved, hard, glabrous	Palea length (mm)	Awn length (mm)	Sterile lemma (mm)
28.0	26.4	26	14	8.50	55	2.5
26.0	8.0	23	15.5	8.00	54	3
22.0	16.3	25	13	9.00	65	3
17.0	3.4	21.5	13	7.00	23	2
21.0	7.0	21	14	7.00	32	2
25.0	23.0	22	12.5	7.50	47	2
28.0	19.2	26	13.5	8.00	40	2.5
18.0	15.2	35	15	8.00	48	3
30.0	17.3	21	12	8.00	43	2.5
21.0	12.4	19	12.5	8.00	53	2
15.0	22.8	17	13	7.00	35	2
23.0	12.4	21	13	7.00	23	2
18.0	8.2	20	14	8.00	58	2.5
17.0	14.0	20.5	15	7.50	45	3
16.0	14.0	22	13	7.00	80	2.2
15.0	8.0	18.5	13.5	7.00	28	2.5
19.0	13.0	19.5	15	7	48	2.2
21.0	15.5	22	14	7.5	25	2
22.0	16.0	20	15.5	7	32	2
18.0	14.0	32	12	8	36	2
81	13.5	30	15	7.5	70	3
82.0	0.0	32	13.5	9	65	3
75.0	19.0	19	11	8	70	2.5
56.0	10.0	21	10.5	8	70	2
69.0	21.0	26	10	7	75	2.5

Grain length (GL) mm	Grain breadth (GB) mm	GL/GB ratio	Kernel length KL(mm)	Kernel breadth KB (mm)	KL/KB ratio	100 Seed wt. (g)
8.2	2.2		7.5	1.8		14.4
8.5	2.3		7.2	1.9		14
7.5	2.21		6.5	1.85		15
8	2.2		7.2	1.9		14
8	2.1		7.2	1.9		13.8
8	2.3		6.8	1.8		14
8.5	2.0		7	1.7		15
8	2.1		6.5	1.75		14.5
8	2.2		7	1.9		14.8
9	2.75		7.5	1.75		15
9	2.5	3.73	7.5	1.8	3.99	14.2
10	2.75		8	2		15
9	2.75		8	1.75		14
8	2.5		8	1.75		14.5
8	2.5		7	1.8		15.2
9	2		7.5	1.8		14.8
7.5	2.5		7.2	1.75		14
8.5	2.75		7.2	2		13.9
8	2.2		6.5	1.75		13.6
9	2.5		6.8	1.8		14
7.5	2.5		7	1.75		15
7.5	2.2		6.8	1.8		14
8	2.2		6.5	1.75		15

(64 – 145 cm). It is found growing in swampy areas (shallow water, up to 0.3 m), at edge of ponds and tanks, besides streams, 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. *Oryza rufipogon* starts flourishing during the rainy season June-July of every year, flowering in September-October and seed shedding November-December and then dried up (Fig.1). It is noted that the NBU species is restricted to shallow-water pond and summer-parched ecosystem. The populations within this ecotype exhibited preference for shallow-water ditches, ponds, paddy-side pond. Wild rice of NBU campus face viability challenges in terms of eco-environments. Because eco-environment has suffered some form of destruction by human activities (poor women collect some green vegetables along with wild rice during monsoon period) and over grazing. So, the association with other plant species is compromised and as a result loss of suitable habitat.

Morphological diversity in *Oryza rufipogon*

Seed germination: Ninety percent seeds were germinated in the present study with healthy shoot and roots (Figure 2 O). Shoot length was ranges from 6 cm to 14 cm after 7 days of germination (dates: 24.6.14 to 01.07.14).

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 and pinkish in colour (5 - 7 mm).

Roots: Roots fibrous, often with rhizomes. Fibrous root produces from nodal region of floated stems/ stolons.

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: It is 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 angular, rough on the angles and axis is wavy. Flowering time was recorded during the month of September-October.

Fertile spikelets: Bisexual spikelets oblong, always awned, easily shed, laterally compressed, articulated on top of the stalk, which is more or less distinctly 2-lobed, 5 – 9 mm long, 1.6 – 3.5 mm wide with pedicel up to 2 mm long.

Functional florets: Lemmas 6-11 mm long, 1.4 – 2.3 mm wide, straight or curved boat-shaped, lemma-awn junctions marked by a purplish, pubescent construction, awns 23 – 80 mm long. Awn is bicolour, hard and rough surface, lower part creamy-white before anthesis and upper part reddish colour at maturity. Paleas – elliptic, 7 mm long, coriaceous, 3-veined, 1-keeled, surface scabrous, apex acute. Lemma and palea green to yellowish, often dark red at apex, covered with stiff transparent hairs. Glumes 2-3 mm long, ovate, unequal.

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

Fruits: Caryopsis narrow 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, enclosed by a stiff lemma and palea. Most of the seeds shed before harvesting due to shattering nature. Seeds are harvested during

November-December.

Wild rice of NBU campus is *Oryza rufipogon* because they exhibited similar characteristics of *O. rufipogon*, these are as follows- a prostrate habit, an exposed purple bifurcated stigma, long red awns, slender grains (length/width > 3.5) that are easily shattered, black chaff, red rice, few seeds per panicle, yellow anther > 3.7 mm (4-8 mm), and the absence of secondary branches. It is considered as a variant ecotypic race of *Oryza rufipogon* Griff. available in the NBU campus due to distinct characteristics specially anther length (4-8 mm), stigma >2 mm and sterile lemma 2-3 mm long. Eight qualitative (8) characteristics found in NBU wild rice were as follows- attitude of panicle branches –spreading, panicle curvature – straight, panicle exsertion- well exserted (100%), attitude of flag leaf blade- deflex/horizontal/semierect, spikelet anthocyanin- pinkish-red, keel edge- pubescent, stigma colour- purple brown bifurc ated, lemma colour at maturity- blackish with 3 nerved. *Oryza nivara* Sharma and Shastry is considered as annual ecotype of perennial *Oryza rufipogon* Griff. can be distinguished between two species from the phylogeography and morphological traits, which is represented in table 2. Table 2 also shows the characteristics feature of wild rice prevailed in NBU campus, which is *O. rufipogon* Griff. In most cases, individuals within populations showed different levels of morphological variability (Table 1 and Fig. 2). It is consistent with some earlier reports (Dong *et al.*, 2010). Individual exhibited longer and wider flag leaves ranges from 13-40 cm and 0.3-1.8 cm respectively. Attitude of flag leaf blade varies from horizontal or semi-erect but maximum is deflexed. Uppermost internode distance was invariably different in each individual which was ranged between 3.4 cm to 26.4 cm. No difference in panicle curvature, exsertion and attitude of primary branching was detected among individuals. There was no report of secondary panicle branching in the present observation that was not supporting the observation of Dong *et al* (2010). 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. The results are consistent with the report of some other publication that the genetic variation of *Oryza rufipogon* is mainly within the populations (Gao, 2004). Morphological variation existed within the

Table 2. Comparison of some morphological diversity among different 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		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

Figure 2. Showing 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: Start vegetative propagation as monsoon begins, C: Stem stolon grown in water tanks for propagation and conservation, D: Showing 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, I: Profuse tillering from node of a stem part, J: Showing auricle and ligule, K: Panicle length measurement, L: Anther length, M: Showing dark red point at awn base, N: Seed length, O: Germinated seeds on pot during kharif 2014.

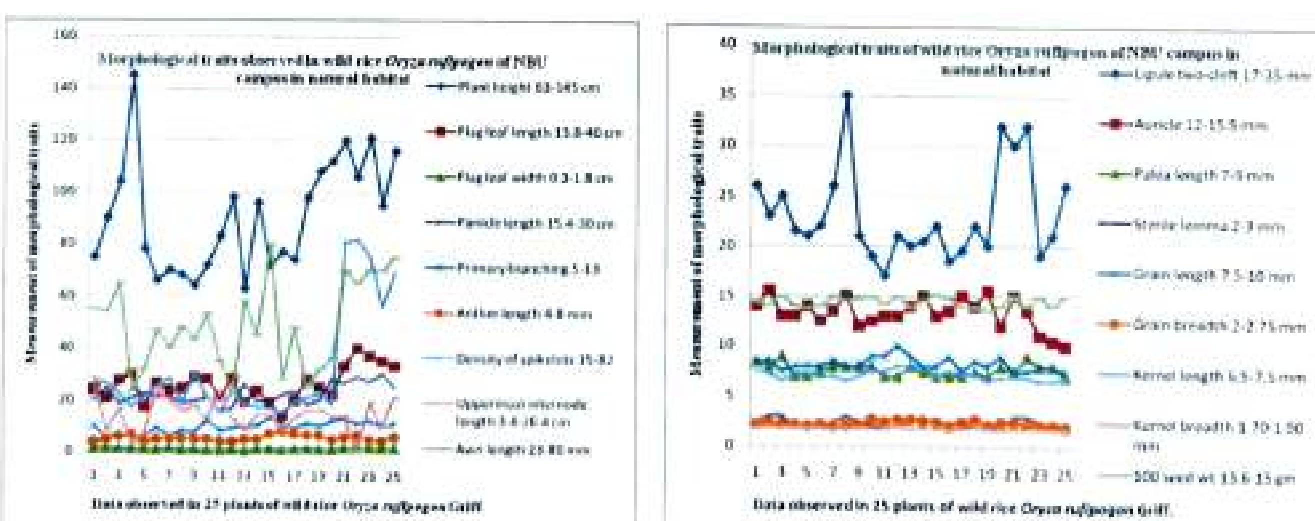

Figure 3. Graphical representation of some morphological traits observed in *Oryza rufipogon* Griff. of NBU campus in natural habitat.

populations may be due to the existence of variety of ecological parameters include temperature, water, soil and associated plants, among others. Soil of NBU campus was loam type with pH 5.6. Morphological variation were observed in the present study may be due to the introgression of genetic factors from *Oryza sativa* to *O. rufipogon*, including growth habit, panicle length and other traits (Dong *et al*., 2010). The two taxa (*O. nivara* and *O. rufipogon*) are recognized as distinct species (Sharma and 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. Natural population of these two taxa can be easily distinguished by flag leaf length and width, panicle branching type and distance from the panicle base to the lowest spikelet insertion. The presence of stolons and long, strong, and spreading culms make *O. rufipogon* suitable for permanently inundated habitats while the shorter, less decumbent culms of *O. nivara* are probably more suited for seasonally dry habitats. Stigmas and anthers are longer and panicles are more exserted and open in the out-crossing *O. rufipogon* than in inbreeding *O. nivara*. *O. rufipogon* is photoperiod sensitive but *O. nivara* is not as photoperiod sensitive in this annual species (Banaticla-Hilario *et al*., 2013). For adaptation to different hydrological habitats, two taxa led to the differentiation of morphology, reproductive system and life cycle (annual/perennial). *Oryza nivara* and *Oryza rufipogon* have undergone and are still undergoing ecological speciation (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; Khush, 1997). 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 Vientnam 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. Deviation from the previously reported morphological features may be due to its adaptation in different hydrological regimes in this campus around the Magurmari River. 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). The existed population may be remnants of a formerly more continuous widespread range of populations in this campus. Morphological classification is a traditional means of characterizing species and remains a fundamental approach in systematic. Morphological differentiation and variation within and among population has been studied to diagnosing species boundary (Elizabeth *et al*., 2008). These morphological data may not be sufficient to adequately assess divergence and variability in this population. It is necessary to use molecular data combined with morphologic and phytogeographic data before confirmation of the status. This should be confirmed by molecular technique using single nucleotide polymorphism (SNP) genotyping, whether they are really hybrid or non-hybrid populations, which can provide more detailed information on the migration of genes between *O. nivara* and *O. rufipogon* (Banaticla-Hilario *et al*., 2013). 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 been studied the genetic structure within and among the *O. rufipogon* based on morphology, ecology and DNA markers (Dong *et al*., 2010).

Conclusion

The present ecogeographical and morphological characteristics show that a high level of genetic diversity exists in the populations of *O. rufipogon* of 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 genetic resources for crop germplasm enhancement. Therefore, effective conservation management of the genetic resources of *O. rufipogon* of NBU campus is urgently needed. Ecological diversity of the wild rice species should be protected and be maintained in proper way. Human disturbances preferably overgrazing should be avoided in this area. The population should be conserved *in situ* conditions to understand the morphological diversity within the populations and make convenient habitat for evolution. In order to preserve the genetic diversity of *Oryza rufipogon* in protected areas, an isolation zone should be established to avoid potential introgression from *O. sativa* to *O. rufipogon*. 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.

References

Banaticla-Hilario et al (2013) Ecogeographic variation in the morphology of two asian wild rice species, *oryza nivara* and *oryza rufipogon*. *Int J Plant Sci.* 74: 896–909

Brar DS and Khush GS (2003) Utilization of wild species of genus *Oryza* in rice improvement. In: Nanda JS and SD Sharma (eds). Monograph of genus *Oryza*. Science Publishers Inc, UK, pp. 283–310

Brondani C, Rangel PHN, Brondani RPV, Ferreira ME (2002) QTL mapping and introgression of yield-related traits from *Oryza glumaepatula* to cultivated rice (*Oryza sativa*) using microsatellite markers. *Theor Appl Genet*.104:1192"1203

Cho YC, Suh JP, Choi IS, Hong HC, Baek MK, Kang KH, Kim YG, Ahn SN, Choi HC, Hwang HG, Moon HP (2003) QTLs analysis of yield and its related traits in wild rice relative *Oryza rufipogon*. *Treat of Crop Res* 4: 19–29

Dong Yibo et al, (2010) Ecological, morphological and genetic diversity in Oryza rufipogon Griff. (Poaceae) from Hainan Island, China. *Genet. Resour. Crop. Evol.* 57: 915-926

Elizabeth AV et al (2008) Morphoagronomic genetic diversity in American wild rice. *Braz. Arch Biol. Technol.* 51: 95–104

Fu Q, Zhang PJ, Tan LB, Zhu ZF, Ma D, Fu YC, Zhan XC, Cai HW, Sun CQ (2010) Analysis of QTLs for yield-related traits in Yuanjiang common wild rice (*Oryza rufipogon* Griff.). *J. Genet. Genomics.* 37: 147"157

Gao L Z (2004) Population structure and conservation genetics of wild rice Oryza rufipogon (Poaceae): a region-wide perspective from microsatellite variation. *Molecular Ecology.* 13: 1009–1024

Hu BL, Yang P, Wan Y, Li X, Luo SY, Luo XD and Xie JK (2013) Comprehensive assessment of drought resistance of BILs population derived from Dongxiang wild rice (*Oryza rufupogon* Griff.) at seedling stage and its genetic analysis. *Journal of Plnat Genetic Resources.* 14: 249–256

Khush GS (1997) Origin, dispersal, cultivation and variation of rice, *Plant Molecular Biology* 35: 25–34

Kovach M J et al (2007) New insights into the history of rice domestication. *Trends in Genetics.* 23: 578–587

Londo JP et al (2006) Phylogeography of Asian wild rice, *Oryza rufipogon*, reveals multiple independent domestications of cultivated rice, *Oryza sativa*. *Pros Nat Acad Sci USA* 103: 9578–9583

Oka HI (1998) Origin of cultivated rice. Developments in Crop Science 14, Tokyo, Japan Scientific Societies Press, Japan

Second G (1982) Origin of the genetic diversity of cultivated rice (*Oryza* spp.): study of the polymorphism scored at 40 isozyme loci. *Japanese Journal of Genetics*. 57: 25–57

Sharma and Shastry (1965) Taxonomic studies in the genus *Oryza* L. III. *O. rufipogon* Griff. sensu stricto and *O. nivara* Sharma et Shastry *nom. Nov. Indian J. Genet Plant Breed.* 25:157–167

Song ZP, Li B, Chen JK and Lu BR (2005) Genetic diversity and conservation of common wild rice (*Oryza rufipogon*) in China. *Plant Spec. Biol.* 20: 83–92

Sun CQ, Wang XK, Li ZC, Yoshimura A, Iwata N (2001) Comparison on the genetic diversity of common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (*O. sativa* L.) using RFLP markers. *Theor. Appl. Genet* 102: 157–162

Vaughan DA et al (2008) The evolving story of rice evolution. *Plant Science* 174: 394–408

Wright SI and Gaut BS (2005) Molecular population genetics and the search for adaptive evolution in plants. *Mol. Biol. Evol.* 22: 506–519

Wu B, Han ZM, Li ZX and Xing YZ (2012) Discovery of QTLs increasing yield related traits in common wild rice. Yi Chuan 34: 215"22

Xiao J, Grandillo S, Ahn SN et al (1996) Genes from wild rice improve yield. *Nature* 384: 223–224

Xie JK, Hu BL, Wan Y, Zhang T, Li X, Liu RL, Huang YH, Dai LF and Luo XD (2010) Comparison of the drought resistance characters at Seedling Stage between Dongxiang Common Wild rice (*Oryza rufipogon* Griff.) and cultivars (*Oryza sativa* L.). *Acta Ecologica Sinica* 30: 1665–1674

Zhu Q et al (2007) Multilocus analysis of nucleotide variation of *Oryza sativa* and its wild relatives: Severe bottleneck during the domestication of rice. *Mol. Biol. Evol.* 24: 875-888