PLOIDY LEVEL AND PHENOTYPIC DISSECTION OF NEPALESE WILD SPECIES OF RICE

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Abstract: Determination of ploidy level and tagging of species specific useful agronomical traits in wild relatives of crop plants are of crucial value to introgress alien gene/s of interest into cultivated background through wide hybridization. Four Nepalese wild species of rice viz. *Oryza nivara, O. rufipogon, O. officinalis and O. granulata* were collected and grown in the green house during 2001-2002 at IAAS, Rampur, Chitwan, Nepal to assess the ploidy level of these species and characterize the species specific different phenotypic traits. Chromosome number of pollen mother cells was recorded at Diakinesis and Metaphase I by harvesting and fixing young florets at suitable stages following usual technique of chromosome preparation. Morphological and seed characteristics of each species were recorded from early vegetative stage to until harvest and after harvest, respectively, from randomly selected ten sample plants. Chromosome counting revealed that all the wild species of rice had consistent chromosome number 2n = 24. The species exhibited specific variations in the observed traits. Additionally, they had also common wild characteristics like spreading growth habit, high stigma exertion rate, high spikelet shattering, dark green leaves with purple to dusky brown basal leaf sheath colour and 2-3 branching of culm. Based on the growth habit and panicle characteristics, *O. rufipogon* exhibited marked ecotypic differentiation. The present study confirmed that all wild species of Nepal are essentially diploid with an array of potential traits for the improvement of cultivated rice.

Key words: Ploidy; Ecotypic differentiation; Wild species. *Oryza*.

INTRODUCTION

Nepal is rich in rice genetic resources. It is estimated that Nepal has over 2000 local landraces and four wild species viz. *Oryza rufipogon, O. nivara, O. granulata* and *O. officinalis* out of 22 wild species around the world (Upadhyay *et al.,* 2002). According to the gene pool classification of rice (Khush, 2000), these four species constitute the all three basic gene pool of rice. The former two species are abundantly observed throughout the terai and inner terai of Nepal, but the distribution of the later two species is restricted to specific niche (Niroula, 2003). Nepal also harbors weedy rice: *Oryza sativa f. spontanea,* and *Oryza* related genera: *Leersia hexandra*, and *Hygroryza aristata* (Shrestha and Vaughan, 1989).

At present utilization of wild taxa of cereal crops is becoming increasingly important among cereal breeders due to specific traits that constitute the excellent insurance against the genetic vulnerability (Chang, 1976). The taxa can be used as gene/s source against the biotic and abiotic stresses (Khush, 1977; Brar and Khush, 1997; Soriano *et al.,* 1999), to provide alternative cytoplasm for inducing new cytoplasmic sterility systems (Brar and Khush, 1998; Li and Yuan, 2000), widen adaptation (Brar and Khush, 1995), improve stature, increase crossability between species (Vaughan, 1994; Stalker, 1980), and enhanced yield (Frey *et al.,* 1984; Xiao *et al.,* 1998; Martinez *et al.,* 2002). It is also noteworthy to mention that some cultivated forms attained commercial status only with genetic background of their wild species (Harlan, 1976). These wild species of rice are widely used in breeding and improvement programmes (Brar *et al.,* 2002). Broadening of gene pool of rice is necessary to extend the rice crop into new management regimes, habitats, or regions of marginal climate, and bridging the yield gap (Brar and Khush, 1997).

Evolutionary relationship is a crucial aspect for directing efforts to search for useful genes in wild species of rice. Kuwada (1910) for the first time reported that the diploid number of chromosome of rice is 24. Since then a large number of rice cytogeneticist reported diploid to tetraploid chromosomes with ten different genomic constituents in 22 different wild species of rice (Khush and Brar, 2001). Lu *et al.* (1997) documented that variation in ploidy number within *O. officinalis* collected from different geographical area. They reported that two of the accessions of *O. officinalis* out of 138, collected from

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Table 1. Description of germplasm collection during study period (2001).

Name of Species	Site of collection					Type of collection	Collection date
	District	Location	Altitude (amsl)	Latitude	Habitat		
Oryza nivara	Banke	Nepalgunj-7	160	28^0 01' 0'' N 81^0 36' 0'' E	Road side ditches	Plants and seeds	May 22-24
	Bardiya	Kumber forest	204	28^0 11' 31'' N 81^0 29'19'' E	Forest pond		
		Maina Pokhari	198	28^0 10'27'' N 81^0 29'13'' E	Swamp		
	Surkhet	Latikoili-2	661	28^0 34'39'' N 81^0 37'0'' E	Road side canal		
O. rufipogon	Kapilbastu	Agighara	145	27^0 31' 2'' N 82^0 52' 3'' E	Lake		
	Surkhet	Bulbule park	671	28^0 34'45'' N 81^0 37'04'' E	Road side canal	Plants	May 27-28
O. granulata	Chitwan	Piple-6	456	27^0 31'21'' N 84^0 27'31'' E	River side forest	Plants and rhizomes	May 19-20
O. officinalis	Agriculture Botany Division, NARC, Kathmandu		-	-	-	Seeds	April 15-18

India, had 48 chromosomes instead of 24. However, the ploidy level of four wild species available in Nepal is still unknown. Therefore, the present study was carried out to determine ploidy level and record the diagnostic phenotypic characters of wild rice species of Nepalese origin.

MATERIALS AND METHODS

Seeds as well as live plants and rhizomes of wild species were collected from different parts of Nepal during 2001 (**Table 1**). Collected live plants and rhizomes were planted in plastic buckets filled with well-fertilized soil sterilized by formaldehyde. Collected seeds were dehulled and treated with 1% sodium hypochlorite for 15 minutes and were incubated at 33^{0}C for germination (Vaughan, 1994). The germinated seeds were planted during May-June, 2001 at Institute of Agriculture and Animal Science, Tribhuwan University, Rampur, Chitwan, Nepal (IAAS), and these were reared upto maturity in the green house. IAAS is located at 84^0 29' E and 27^0 37' N and 224 m above sea level.

Observation on morphological traits were recorded from vegetative to maturity stages white seed characteristics after harvest. Observations on each character were taken based on the standard rice descriptor (IBPGR-IRRI, 1980). More than 40 characters were observed to be distinctly different. Each observation was taken from random sample of size ten i.e. 1 sample from each bucket.

For the determination of ploidy level, young spikelets from the plants when the distance between boot and juncture of the flag leaf ranged from 0 to 6.5 cm depending upon the species were fixed in acetic alcohol (1:3 v/v) to which traces of ferric chloride was added to intensify the staining of the chromosomes. The materials were kept in the fixative for 24 hours at low temperature (14^0-25^{0}C) and then transferred to 70 % ethanol until used for smearing. Fixed anthers were removed from the spikelets and smeared in 1 or 2 drops of 1 %

Table 2. Meiotic chromosome number and behavior at Diakinesis and Metaphase I from four wild species

Wild species	Total no. of cells	Meiotic observation (%)		
		12 II§	2-4 I + 10-11 II	8-10 II + 1-2 IV
O. nivara	158	93.04	5.06	1.90
O. officinalis	149	97.99	2.01	-
O. granulata	220	93.63	1.82	4.55
O. rufipogon	168	96.43	3.57	-

§ 12 II chromosomes = 24 chromosomes

(Vaughan, 1994). In this study too minor meiotic irregularities, such as univalent and quadrivalent formations, were observed in all the species, however, the frequency was too small. Based on the meiotic Metaphase I analysis, Lu *et al.* (1998) also reported that four species AA genome viz., *O. nivara, O. rufipogon, O. glumaepatula* and *O. meridionalis*, from different geographical origin of world also had consisted 2n=24 number of chromosomes.

Oryza granulata was mostly found in upland field (aerobic soils) under the shade of partial to dense vegetation in its natural habitat. It was found completely photoperiod insensitive and flowered round the year. Its height ranged from 29 to 68 cm and number of spikelets per panicle varied from 4-28. Morphologically, plants were

Table 3: Diagnostic phenotypes of four Nepalese wild species of rice recorded during study period 2001-2002.

Note: TNPB = total number of panicle branches, DPWT = dark purple with white tip, DGLPT = dark green with light purple tip, SB = secondary branching, BLSC = basal leaf sheath coloration, SLC = sterile lemma colour, MNBLNP = maximum number of branches at the lowest node of panicle.

short in stature with broad lanceolate leaf, small bamboo like miniature, perennial with rhizomes in its roots, short panicle (5-14 cm), spikelets completely sessile, granulate texture on its spikelet and ovate type grain, minute rachilla, small culm diameter with sturdy stem, 2-4 culm branches, high panicle shattering and spreading growth habit. Its diagnostic features were found to be upland (prefers aerobic soils), photoperiod insensitive, large stigma and anther with white in color **[Plate 2 (2)]**. Seed color was found to be dark brown to brownish white, high seed shattering even in pot experiment. Other morphological traits are shown in **Table 3**. Sharma and Sampath (1985) and Vaughan (1994) observed similar characters in accessions from various geographical regions of Asia.

Owing to the direct seed collection from NARC (Botany Division), the natural state of *O. officinalis* was not observed. Its morphological features observed during pot experiment were plant with dark green leaf, tall stature (height ranges from 90-125 cm), small flat spikelet, large panicle with well exertion, profuse tillering, brown to black seed coat colour, long flowering period than *O. sativa*, perennial with underground rhizomes, phenotypic outlook better under partial shade condition, sturdy stem, profuse secondary branching in its spikelets etc **[Plate 2 (1)]**. Its diagnostic features were small spikelet with partially short awned, reddish purple stigma with white tip, high number of spikelets per panicle, light yellow with greyish anther and perennial herb. In case of seed propagation this species flowered within 6.30-8.30 am at Rampur environment. Alike results on anthesis of this species was also reported by Gopalkrishinan (1962). Other morphological characters are presented in **Table 3** and similar results have also been reported by Sharma and Sampath (1985) and Vaughan (1994).

Based on morphological study of *O. nivara*, no distinct geographical differentiations was detected. Most of the collections showed short stature with short duration of life cycle (75-85 days after sowing), photoperiod insensitive, bold grain type with long and fully awned (5.21-11.1 cm), long dormancy period with few secondary branches in its panicle and dark brown to black seed coat with distinct rachilla (**Table 3)**. Its diagnostic features were short stature, small anther size (1.5-2 mm), dark purple stigma with long and fully awned, dark leaf with purple tip, purple lines in basal leaf sheath, bold grain etc. **[Plate 2 (4)]**. Shrestha and Vaughan (1989) reported similar observations in its natural conditions except its response to photoperiod. They recorded three types of populations within specific locations of Nepalgunj and reported that photoperiod insensitive to strongly sensitive traits existed in *O. nivara* population. However, in this collection all the ecotypes from Banke, Bardiya, and Surkhet districts showed photoperiod insensitivity. Other phenotypic features were found to be similar as reported by Sharma and Sampath (1985) and Vaughan (1994). Long dormancy and very long awn were noted as the characteristics features of this species. Similar such conspicuous characters have also been reported in the literatures (Sharma and Shastry, 1965; Sharma, 1986; Vaughan, 1994). Vaughan (1994) reported awn length up to 10 cm in *O. nivara*, while, in this study awn length was recorded up to 11.1 cm in Surkhet collection.

In contrast to *O. nivara*, the widely distributed *O. rufipogon* was perennial, tall (height ranges from 75 to 130 cm), late flowering, strongly photoperiod sensitive. Jackson *et al.* (2000) reported that all the populations of *O. rufipogon*, collected from Nepal and evaluated at International Rice Research Institute (IRRI) were strongly photoperiod sensitive. In this study, variations were recorded particularly for panicle type (open-compact), and growth pattern (procumbent-creeping). Additional morphological features were long and cylindrical spikelet with long and fully awned, dark purple stigma with large anthers (4-5.1 mm), profuse tillering, procumbent to creeping type growth habit (**Table 3**). Seed coat colour was found to be dark brown to completely black. It was interesting to note that the collection from Surkhet had open panicle with creeping nature of culm while from Kapilbastu compact panicle with procumbent type of culm **[Plate 2. (3)]**. The range of variation within traits suggests that the species has quite high phenotypic diversity within the geographical distribution. Phenotype based ecotype divergence observed for *O. rufipogon* might be either due to inbreeding/genetic drift followed by geographical isolation or recovery of separate mutant rather than chromosomal structural changes. It is because of the population of the *O. rufipogon* at Bulbule Park was very small, scattered and completely isolated as compared to the large and continuous populations at Ajighara swamp (i.e. > 50 hectares). However, the present study was not sufficient to generalize the exact cause of morphological divergence in terms of observed characteristics in this species. Therefore, to pinpoint the causes of divergence in *O. rufipogon* collected from two diverse geographical areas, it is suggested that detail cytogenetic, genetic, and molecular analysis across the geographical areas followed by Geographical Information System (GIS) based survey should be undertaken. Further emphasis should also be given to the collection, evaluation (screening against the reactions to several biotic and abiotic stresses), utilization, and conservation aspects to use gene/diversity from jungles and swamps into farmers' field for combating biotic and abiotic stresses.

Specific traits (**Table 3**) such as photoperiod insensitive and adoption to aerobic soils in *O. granulata*, shade loving and sturdy stem traits in *O. officinalis,* short stature, early maturity, drought avoidance and photoperiod insensitive in *O. nivara* and large anthers, high stigma exertion rate, perennial nature, and source of cytoplasmic male sterility in *O. rufipogon* are of great importance and can be used to improve present day cultivated rice through hybridization and repeated backcross breeding procedure. Such breeding works would be effective and possible since more than dozens of economic genes such as BPH resistance gene from *O. officinalis*, grassy stunt virus resistance gene from *O. nivara*, CMS source, tungro resistance, and acid sulphate tolerance from *O. rufipogon* have already been transferred into *indica* background (Brar and Khush 1997; Brar *et al.,* 2002). Characters in *O. rufipogon* will cruciably be important to develop three line breeding systems of hybrid rice seed production and development of perennial rice cultivars.

The present cyto-morphological study revealed that all the wild species of Nepal are diploid with immense phenotypic diversity. The untapped diversity and diploidy features of wild rice can be exploited to expand the gene pool of rice, through the technique of wide hybridization coupled with the approaches of simple plant tissue culture techniques (embryo rescue, ovary and anther cultures), for sustainable rice production within the country.

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