Oryza Vol. 45. No.2, 2008 (98-102)

Exploration, collection, characterization, evaluation and conservation of wild rice germplasm of east India

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ABSTRACT

The wild species of the cultivated rice are a good reservoir of genetic variability for various biotic and abiotic characters. They harbour significantly higher genetic diversity than the cultivated species. A devastating super cyclone had hit the Orissa coast during 29-30 October, 1999 and about 10,000 people and a lot of properties including plant genetic resources were lost. To salvage the rice genetic resources from the region special collection missions were made during 1999-2001. A total of 483 accessions of two wild rice germplasm, namely Oryza nivara and O. rufipogon were collected from south east India – where they occur abundantly. They were then multiplied for seed increase, characterized, evaluated and conserved. Among the wild rice accessions which showed high variability, 24 accessions were resistant to bacterial blight and two accessions of O. nivara (IC-330470 & IC-330611, were tolerant) to moisture stress.

Key words: *Exploration, Germplasm, Oryza nivara, O. rufipogon, east India, bacterial blight*

Apart from the two cultivated species, the genus *Oryza* contains 21 wild species around the world (Vaughan *et al*., 2003). The Asian cultivated rice is *Oryza sativa* L. and the African cultivated rice is *O. glaberrima* Steud. The wild species are widely distributed in the pantropics and subtropics and they constitute an exceptionally valuable gene pool. In India there are four valid wild species of *Oryza*. These are *O. nivara* Sharma *et* Shastry, *O. rufipogon* Griff., *O. officinalis* Wall. ex Watt and *O.granulata* Nees *et* Arn. ex Watt. Out of these, *O. nivara* and *O. rufipogon* are widely distributed throughout east India. Three new species described from India at different times by different workers were *O. jeyporensis, O. indandamanica* and *O. malampuzhaensis*. Later, these were treated as synonyms of *O. sativa*, *O. granulata* and *O. officinalis* respectively.

Wild species are an excellent reservoir of variability for several traits including resistance to biotic and abiotic stresses; quality and productivity traits (Tanksley and McCouch 1997). The most successful example of utilising wild *Oryza* species is the development of hybrid rice, in which the male sterility gene was introduced from the wild rice (*Oryza rufipogon* L.) found in Hainan Island of China (Yuan, 1993). Another prominent example is the incorporation of grassy stunt virus resistance gene from one accession (IRRI AC 101508) of the annual wild rice (*nivara*) collected from Basti region of eastern Uttar Pradesh, India (Khush, 1977, 1989). Many disease and insect resistant genes, high yielding genes and abiotic stress tolerant genes have also been found in the wild species of *Oryza* (Brar and Khush 1997). In areas where the wild species grow sympatrically with the cultivated species, many problems are encountered as wild rices have shattering habit and grow as problematic weeds. A clean crop approach leads to elimination of several wild species in areas where these grow sympatrically with the cultivated species. Natural calamities especially in the east coast of India also lead to washing away of a large magnitude of variability present in the germplasm. Therefore, the present explorations and collections were made after the super cyclone to conserve the wild rice germplasm (Table 1).

MATERIALS AND METHODS

Soon after the super cyclone, which hit the Orissa coast in October, 1999 the Central Rice Research Institute (CRRI), Cuttack and National Bureau of Plant Genetic

Table 1. Inventory of wild rice germplasm collected under NATP (Plant Biodiversity)

Period of collection	Areas explored	State	No. of accessions	
			O. nivara	*O. rufipogon*
16.11.99 to25.11.99	Khurda, Nayagarh, Phulbani,Boudh, Sonepur, Bolangir, Kalahandi, Rayagada, Gajapati & Ganjam districts	Orissa	30	25
16.11.99 to3.12.99	Sundargarh & Sambalpur districts	Orissa	25	15
16.10.99 to 6.12.99	Cuttack, Khurda, Jagatsinghpur & Puri districts	Orissa	16	9
2.12.99 to 9.12.99	Bhadrak & Balasore districts	Orissa	1	3
16.12.99 to 23.12.99	Balasore, Bhadrak, Jajpur, Dhenkanal & Cuttack districts	Orissa	1	5
16.12.99 to23.12.99	Ganjam district	Orissa	1	3
2.1.00 to 9.1.00	Bhadrak, Balasore, Mayurbhanj & Keonjhar districts	Orissa	2	38
27.1.00 to 29.1.00	Jagatsinghpur district	Orissa	2	1
27.10.00 to 02.11.00	Bhadrak, Balasore, Mayurbhanj, Keonjhar, Sundargarh, Jharsuguda, Sambalpur & Midnapore districts	Orissa & WB	39	20
27.10.00 to 04.11.00	Koraput, Malkangiri, Nawarangpur & Kalahandi districts	Orissa	43	12
31.10.00 to 1.11.00	Cuttack, Dhenkanal, Angul, Sambalpur, Jharsuguda, Sundargarh & Bargarh districts	Orissa	19	18
25.11.00 to 03.12.00	Midnapore, Bankura, Purulia, Burdwan, Nadia & Hooghly districts	West Bengal	2	24
10.10.01 to 16.10.01	Purulia, Bankura and Midnapur districts	West Bengal	29	14
4.11.01 to 13.11.01	North and South 24 Parganas districts	West Bengal	16	2
4.11.01 to 13.11.01	Malda, South Dinajpur, North Dinajpur, Darjeeling, Jalpaiguri & Cooch Bihar districts	West Bengal	5	15
9.11.01 to 16.11.01	Hooghly, Nadia, Burdwan, Murshidabad, Birbhum & Malda districts	West Bengal	19	25
29.11.01	Puri district	Orissa	-	4
			250	233

Resources (NBPGR), New Delhi made collaborative efforts to salvage and conserve the multi-crop germplasm from the devastated areas. These special collection missions were termed as Rescue Mission (RM). A total of 17 exploration trips were undertaken for collecting wild rice germplasm in eastern India comprising the states of Orissa and West Bengal (Fig.1). Both the states lie approximately between 18^{o} N to 26^{o} N latitude and 82^{0} E to 90^{0} E longitude. Each site was thoroughly explored and the germplasm were collected either in the form of seed or stubbles, wherever seed was not available. The salient information like habit, habitat, abundance etc. of each collection was noted in passport data book in the field.

As the wild rices exhibit a strong dormancy, the seeds were dehusked and put in the germination paper in petri dishes to facilitate quick germination and raise seedlings. Once the seeds sprouted, they were planted in small earthen pots containing sterilized soil. Preliminary observations on percentage of germination, days to 2^{nd} leaf emergence, days to 3^{rd} leaf emergence, 15 days seedling height and leaf sheath pigmentation were recorded. The seedlings from the earthen pots were transferred to the experimental field after 30-35 days. These accessions comprising of 223 samples of *O. nivar*a and 203 samples of *O. rufipogon* were grown in the field with two replications following Complete Randomized Block Design (CRBD) and evaluated for various traits. Data were recorded on 5 random plants for each character in each replication**.** Mean values of different characters were taken for analysis.

Three hundred accessions of *O. nivara* and *O. rufipogon* were grown in the field at Punjab Agricultural University, Ludhiana with row-to-row distance of 1m and plant-to-plant distance of 45cm under stagnant water conditions. Nitrogenous fertilizer dose was applied as recommended for cultivated rice to

ensure luxuriant growth that is congenial for Bacterial Blight development. These were evaluated against six pathotypes for two years (2001 and 2002) and the resistant accessions were evaluated for another three years (2003-05) against the most virulent pathotypes. Highly susceptible *O. sativa* cv. TN1 was used as check. Two plants from each accession were inoculated with each pathotype by clipping 5–10 fully expanded leaves, with scissors dipped in inoculum (Kauffman et al., 1973). Lesion length was recorded 14 days after inoculation on five fully expanded and randomly chosen leaves from each pathotype–accession combination. Accessions with mean lesion lengths 0–5.0 cm were scored as resistant(R), 5.1–10.0 cm as moderately resistant (MR) and >10 cm as susceptible (S).

Screening for drought was done during dry season at CRRI farm in an augmented design with four tolerant checks namely, Vandana, Vanaprabha, Salumpikit, CR 143-2-2 and one susceptible check IR 20 randomized once in every 30 lines. Dry seeding was done in the first week of February and the crop was grown with adequate soil moisture for 30 days with sprinkler irrigation system. On 31 days after sowing, irrigation was suspended until the susceptible check showed permanent wilting and then irrigated for plant recovery. The soil moisture content during drought period decreased from 34% to 3% in the soil profile of 45cm. Data on leaf rolling and recovery were recorded using SES of IRRI.

RESULTS AND DISCUSSION

The entire project resulted in collection of 483 accessions consisting of 250 samples of *O. nivara* and 233 samples of *O. rufipogon*. The observations showed that there was rare or no occurrence of wild rice from Katoya in Burdwan district of West Bengal to Jalangi (border of Bangladesh), the northern side of Murshidabad and beyond. Similarly there was very scanty population of wild rice in Malda and beyond, towards northern side of West Bengal. Wild rices did not grow in coastal saline areas/swampy places at least 5 km along the sea coast. Among all the districts, Burdwan and Birbhum in West Bengal and Cuttack, Bhadrak and Balasore in Orissa state were having profuse occurrence of wild rice Yogesh and his associates (2007) reported that maximum numbers of wild rice accessions were susceptible to bacterial blight (Yogesh *et al*., 2007). However, few accessions were found resistant to all the pathotypes and the details of the accessions are presented in Table 2.

Table 2. Wild rice accessions resistant to Bacterial Blight pathotypes

Species	Acc. Nos.	IC Nos.
O. nivara	CR-100268	309815
	CR-100311	282947
	CR-100312	282948
	CR-100426	330651
	CR-100427	330652
	CR-100428	330653
	CR-100431	330656
	CR-100432	330657
	CR-100448	330583
	CR-100450	330585
	CR-100454	330589
	CR-100460	330595
	CR-100463	330598
	CR-100477	330612
	CR-100481	330616
	CR-100482	330617
	CR-100486	330621
O. rufipogon	CR-100444	330669
	CR-100445	330670
	CR-100462	330597
	CR-100464	330599
	CR-100466	330601
	CR-100488	330623
	CR-100490	330625

Data on various morpho-agronomic traits were recorded as per the IRRI-IPGRI descriptor. The morpho-agronomic observations on 39 characters were noted. The vegetative characters like leaf pubescence, blade colour, leaf angle, ligule colour, ligule shape, collar colour, auricle colour, number of days to 50% flowering, culm angle, internode colour, culm strength, flag leaf angle, panicle type, secondary branching of the panicle, panicle exsertion, panicle axis, awning, awn colour, apiculus colour, stigma colour, sterile lemma colour etc. were recorded. In all the accessions irrespective of species diversity, the leaves were pubescent, shape of ligule 2-cleft, panicle axis straight, secondary branching of panicle low, sterile lemma colour straw, awning

pattern was long and fully awned. The blade colour was mostly green, collar and auricle colour light green, panicle type mostly open and well-exserted.

Substantial variability for different agronomic characteristics was observed. In *O. nivara* (Table 3), days to 2nd leaf emergence and 3rd leaf emergence were lowest in AC 100023 and highest in AC 100128; 15 days old seedling height was lowest in AC 100385 and highest in AC 100228; leaf length varied from 11.4cm in AC 100353 to 72.68cm in AC 100406; leaf width from 0.56cm in AC 100400 to 1.7cm in AC 100404. Maximum ligule length of 3.32cm was expressed in AC 100422 and minimum of 0.59cm was expressed in AC 100313. The culm length ranged from 31.22cm (AC 100353) to 159.5cm (AC 100452). Days to 50% flowering were minimum (94 days) in AC 100024 and maximum (168days) in AC 100044. Maximum of 147 ear-bearing tillers (EBT) in AC 100387 and minimum of 5 was observed in AC 100091. A maximum of 35.42cm panicle length was observed in AC 100414 and minimum of 10.7cm observed in AC 100353. Awn length varied from 1.15cm (AC 100150) to 13.35cm (AC 100418). Highest L/B ratio (4.45) was expressed in AC 100452 where as lowest (2.31) expressed in AC 100423. The weight of 100 grains was highest of 2.76g in AC 100023 and lowest of 1.04g was observed in AC 100450. Similarly in *O. rufipogon* (Table 4), the variability in different characteristics like the days to 2nd leaf emergence was lowest in AC 100055 and highest in AC 100138 and 3rd leaf emergence was lowest in AC 100201 and highest in AC 100147; 15 days old seedling height was lowest in AC 100018 and highest in AC 100062; leaf length varied from 13.04cm in AC 100151 to 69.85cm in AC 100035, leaf width from 0.58cm in AC 100159 to 1.52cm in AC 100443. Maximum ligule length of 1.38cm was observed in AC 100189 and minimum of 0.55cm was expressed in AC 100151. The culm length ranged from 46.13cm in AC 100309 to 176.8cm in AC 100173. Days to 50% flowering were minimum (83 days) in AC 100310 and maximum (172 days) in AC 100259. Maximum of 101.6 ear-bearing tillers (EBT) in AC 100173 and minimum of 5.5 was observed in AC 100168. A maximum of 37.93cm panicle length was observed in AC 100173 and minimum of 12.62cm observed in AC 100156. Awn length varied from 1.98cm in AC 100151 to 11.24cm in AC 100453. Highest L/B ratio of 4.64 was observed in AC 100401 where as lowest of 2.12 was observed in AC 100152. The weight of 100 grains was lowest (0.86g) in AC 100160 and highest (2.64g) in AC 100265.

Table 3. Mean and range values for major characteristics of *Oryza nivara* germplasm based on 223 accessions

Characters	Range	Mean	CV (%)
Days to 2nd leaf emergence	5.4-26	10.67	26.18
Days to 3rd leaf emergence	10-30.33	15.44	24.01
15-days seedling height	4.5-20.3	11.21	27.01
Leaf length (cm)	11.44-72.68	39.50	31.39
Leaf width (cm)	0.56-1.7	0.82	22.16
Ligule length (cm)	0.59-3.32	1.59	32.66
Culm length (cm)	31.22-159.5	80.64	31.47
Culm diameter (cm)	0.22-0.65	0.39	19.84
Culm No.	5.0-147	60.23	-
Days to 50% flowering	94-168	125.9	13.69
Panicle length (cm)	10.7-35.42	22.33	21.72
Grain length (mm)	6.94-9.52	8.43	5.27
Grain breadth (mm)	1.83-3.24	2.62	8.89
l/b ratio	2.31-4.45	3.23	10.04
Awn length (cm)	1.15-13.35	8.1	21.82
100-grain weight (gm)	1.04-2.76	1.94	14.63

Table 4. Mean and range values for major characteristics of *Oryza rufipogon* based on 203 germplasm accessions

Characters	Range	Mean	CV (%)
Days to 2nd leaf emergence	6-14.25	9.81	17.08
Days to 3rd leaf emergence	10.6-22	14.59	16.91
15-days seedling height	3.8-22.45	10.25	33.21
Leaf length (cm)	13.04-69.85	36.24	29.97
Leaf width (cm)	0.58-1.52	0.90	21.63
Ligule length (cm)	0.55-3.6	1.80	39.13
Culm length (cm)	46.13-176.8	99.78	32.67
Culm diameter (cm)	0.23-0.56	0.37	17.66
Culm No.	5.5-101.6	37.33	-
Days to 50% flowering	83-172	136.6	12.48
Panicle length (cm)	12.6-37.9	23.81	26.13
Grain length (mm)	7.05-9.27	8.25	4.94
Grain breadth (mm)	1.75-3.25	2. 33	12.44
l/b ratio	2.12-4.64	3.55	13.49
Awn length (cm)	1.98-11.24	6.98	24.58
100-grain weight (gm)	0.86-2.64	1.59	23.63

The lines with early leaf rolling after suspension of sprinkler irrigation showed higher score for drought tolerance (7-9). Lines with delayed leaf rolling recovered faster after irrigation. Two accessions of wild rice *O. nivara* i.e. IC-330470 & IC-330611 collected from West Bengal were found to have high degree of tolerance to drought with an SES score of 0 & 1. The scoring details of these two tolerant wild rice lines are presented with their corresponding check varieties (Table 5). Both the accessions are morphologically same except in a few characters like ligule, collar, internode and auricle colour, culm and flag leaf angle, culm strength and panicle exsertion. Days to 50% flowering also differed from each other being 107 days for IC-330470 and 118 days for IC-330611. It is of significance as the habitat of wild species is usually swampy places like seasonal ditches. Root length of *O. nivara* was 20.2cm

Table 5. Wild rice accessions identified for drought tolerance

Genetic resources	Species	Drought score ('SES')	Recovery score ('SES')
Vandana (Tolerant check)	*O. sativa* (HYV)	1	1
IR 20 (Susceptible check)	*O. sativa* (HYV)	9	9
IC-330470 (AC-100374)	*O. nivara*	1	1
IC-330611 (AC-100476)	*O. nivara*	1	1

under normal condition while it was 25.7cm under moisture stress. However, in *O. rufipogon* root length was 11.7cm under normal condition and 14.3cm under mosture stress. Root penetration in *O. nivara* was deeper while in *O. rufipogon*, it was shallow and adventitious.

The seeds of each of the accessions were dried for reducing the moisture content up to 10-12% and kept in 3-layered aluminium foil pouches for medium term storage. The outer layer of the pouch is polyester of 12 micron; intermediate layer is aluminium of 12 micron and the innermost layer is polythene of 250 gauge. These aluminium foil pouches have been stored in cold module at a regulated temperature of 4° C and 33 % relative humidity (RH) at CRRI Cuttack. The seeds are stored in the medium term storage for 8-10 years. Another set of these wild rices is conserved in the long-term storage at NBPGR, New Delhi at -20^0 C with 3-4% RH.

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