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Screening and identification of new sources of resistance to sheath blight in wild rice accessions

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Abstract

A total of 218 wild rice accessions including *O. rufipogon* and *O. nivara* were evaluated under artificial inoculation for resistance to sheath blight disease for two years under field conditions. The germplasm identified as resistant to sheath blight were further evaluated through artificial inoculation for an additional year under glass house conditions. Based on three years of testing, two *Oryza rufipogon* accessions, namely, IC336719 and IC336721 were identified as resistant to sheath blight disease. *In vitro* characterization of resistant genotypes revealed that the size of sheath blight lesion formation was small along with comparatively lesser number of infection cushions and penetration pegs as compared to the susceptible checks. The *O. rufipogon* accessions identified in the present study are very valuable genetic resource, which can be utilized in the development of introgression lines and mapping QTL(s) governing resistance to sheath blight of rice.

Key words: Rice, sheath blight, resistance, *Rhizoctonia solani*, *Oryza rufipogon*

Introduction

Sheath blight is one of the most economically important diseases of cultivated rice (*Oryza sativa* L.) across the world. The causal agent is the necrotrophic fungus *Rhizoctonia solani* Kühn [(teleomorph *Thanatephorus cucumeris* (A. B. Frank) Donk)] anastomosis group (AG) 1, intraspecific group IA (Webster and Gunnell 1992). *R. solani* is soil-borne and survives in the absence of a host as asexually produced sclerotia. Small, water-soaked spots first appear on the leaf sheath within 3 inches above the water line. These spots enlarge rapidly under favorable conditions, progress longer up and down the plant, and have grayish-white centers with a tan-to-brown margin. If unchecked, the disease progresses upto the whole plant causing white-to-gray lesions on the leaves. On an average, sheath blight causes yield loss ranging from 20 to 50% in rice (Marchetti and Bollich 1991).

Fungicides can reduce sheath blight severity (Kiesling 1985; Videma de and Kohli 1998), but repeated applications represent a significant cost and health hazard for farmers and the ecosystem. Further, extensive fungicide use is also associated with the emergence of fungicide resistance in the target pathogen (Golembiewski et al. 1995). Hence, the search for effective, non-fungicide control of sheath blight is of the utmost importance.

Wild relatives of rice (*Oryza* spp.) are a valuable source of important agronomic traits as well as genes for biotic and abiotic stress tolerance, and have been used in rice improvement programmes, worldwide (Brar et al. 1991; Brar and Khush 1997; Jena and Khush 2000). Although, complete field resistance to the rice sheath blight pathogen has not been identified, partial resistance has been reported (Amante et al. 1990; Pan et al. 1999). Sheath blight resistance genes were identified in *O. minuta* J. S. Presl. ex C. B. Presl. (IRGC101089) and *O. rufipogon* Griff. (IRGC100907) accessions (Amante et al. 1990). Rice sheath blight resistance genes from *O. officinalis* Wall ex Watt were transferred into cultivated rice through the backcross breeding approach (Lakshmanan 1991). These results suggest that wild rice relatives are important sources of resistance for sheath blight disease and could be

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useful in developing resistant cultivars or genetic stocks in an adapted genetic background. The present study was carried out with the objective of identifying new sources of resistance to sheath blight disease primarily from the A genome wild relatives of cultivated rice namely, *Oryza rufipogon* and *O. nivara,* and characterization of the disease resistance in the resistant accessions.

Materials and methods

Sources of wild rice germplasm

A set of two hundred and eighteen wild rice accessions belonging to A genome species namely, *Oryza rufipogon* and *O. nivara* were evaluated for their resistance to sheath blight disease. Details of the collection sites of the wild rice accessions were presented in an earlier report by Chouhan et al. (2014). Single seedlings of 21 days old were transplanted with a spacing of 20 × 15 cm in a plot size of 3.0 × 1.5 m in randomized block design with three replications at the research farm of ICAR-Indian Agricultural Research Institute, New Delhi. Recommended agronomic practices were followed to raise a healthy crop.

Isolation and multiplication of inoculum of Rhizoctonia solani

A highly virulent isolate of *R. solani* "Kapurthala isolate" (ITCC No-7479) was used for screening the wild rice germplasm accessions in the present study (Susan et al. 2015). The inoculum was multiplied by following the procedure described by Bhaktavatsalam et al (1978). Shoots of water sedge (*Typha angustata*) were cut into pieces of 4-5 cm long washed thoroughly and soaked in Typha medium (Peptone: 10.0 g, Sucrose: 20 g, K_2HPO_4: 0.1g, $MgSO_4$: 0.1g, Distilled water: 1L) for 5 minutes. The pieces were drained of excess water and later these were filled loosely to one third volume of 250 ml conical flask and sterilized in autoclaved at 1.05 kg/cm^2 for 20 min each for two consecutive days. The sterilized typha was inoculated with 5 mm diameter disc of actively growing mycelium of the isolate and incubated for 15 days at 28±2°C. These colonized typha pieces were used as inoculum (Dubey et al. 2014).

Method of inoculation

Plants of each accession were inoculated at the maximum tillering stage (30-35 days after transplanting) with three colonized typha pieces, which were placed between tillers in the central region of the hills of the genotypes, just above the water level. After inoculation, water level of 5-10 cm was maintained constantly by irrigation for ensuring enough humidity to promote disease development.

Observations recorded

Relative lesion height was recorded at two stages, first observation 15 days after inoculation and second observation at 30 days after inoculation. The total height of lesion spread (cm) was recorded from base of the plant to the tip of the top most lesions on the stem. The lesion height and plant height were measured. Relative lesion height (RLH) was calculated using the following formula given by Sharma et al (1990).

$$\text{RLH}(\%) = \frac{\text{Lesion height}}{\text{Plant height}} \times 100$$

Rice sheath blight grade chart 0-9 (IRRI, 1996) was used for recording reaction and lesion height (Table 1). Rainfall (mm) data of *kharif* 2012 and 2013 was obtained from the Division of Agricultural Physics, ICAR-IARI, New Delhi.

Table 1. Standard Evaluation System (SES) for sheath blight of rice

Disease score	Disease reaction	Description (based on relative lesion height-RLH %)
0	Immune	No infection
1	Resistant	Vertical spread of lesion up to 20 % of plant height
3	Moderately resistant	Vertical spread of lesion up to 21-30 % of plant height
5	Moderately susceptible	Vertical spread of lesion up to 31-45 % of plant height
7	Susceptible	Vertical spread of lesion up to 46-65 % of plant height
9	Highly susceptible	Vertical spread of lesion up to 66-100 % of plant height

Microscopic studies on selected resistant and susceptible wild rice genotypes

Two genotypes showing consistent resistant reaction during both the years of evaluation were taken for further detailed microscopic studies along with known moderately resistant, IR24 and IR64 (Xing et al. 2013**)** and susceptible check, Pusa Basmati 1 (Bashyal et al. 2011a). Penultimate leaves of these genotypes were inoculated with 2 mm diameter mycelia disc in moist chamber. The chlorophyll of infected leaves was removed by overnight soaking in 70 % glacial acetic acid solution and ethanol (Bashyal et al. 2011b). The leaves were washed in distilled water, dried for five minutes and stained with Trypan Blue 0.5 % solution in Dulbecco's Phosphate Buffer Saline (Himedia). The leaves were again rinsed in distilled water to remove excess stain. These leaves were observed under simple light microscope for the number of infection cushion and penetration peg after 48 hrs of inoculation. Number of infection cushions in a 0.5 cm^2 area, 1 cm above the point of inoculation, 48 h after inoculation were observed. Five samples from each individual were considered as one replication and mean of three replications were taken for each observation.

Results

Evaluation of wild rice accessions under field conditions

Out of 218 accessions screened for sheath blight resistance in the year 2012, 15 accessions were found to show resistant reaction (Supplementary Table S1 http://epubs.icar.org.in/journal/index.phb/IJGPB). However, during evaluation during subsequent year (2013), only five of the accessions were found to show consistently resistant reaction, which included *O. rufipogon* accessions, IC336690, IC336719 and IC336721, and *O. nivara* accession, IC336696 and IC336716. Out of them two accessions of *O. rufipogon* accessions, IC336719 and IC336721 have shown relative lesion height up to 15% only (Fig. 1, Supplementary Table S1), Eleven accessions including *O. nivara* accessions namely, IC336684, IC336685, IC336699, IC336700, IC336705 and *O. rufipogon* accessions, NKSWR 36, NKSWR 37, NKSWR 53, NKSWR 80, NKSWR 81 and NKSWR 82 were found to show consistently moderately resistant reaction. Out of 218 accessions evaluated maximum (45.87%) genotypes have shown moderately susceptible (MS) reaction followed by 33% genotypes showing susceptible (S) reaction. Only 7% genotypes have shown moderately resistant (MR) reaction and 2% genotype have shown resistant (R) reaction (Fig. 2; Table 2).

Fig. 1. Sheath blight disease development in susceptible and resistant genotypes of rice. a-Pusa Basmati 1; b-*O. rufipogon* IC336719; c-*O. rufipogon* IC336721

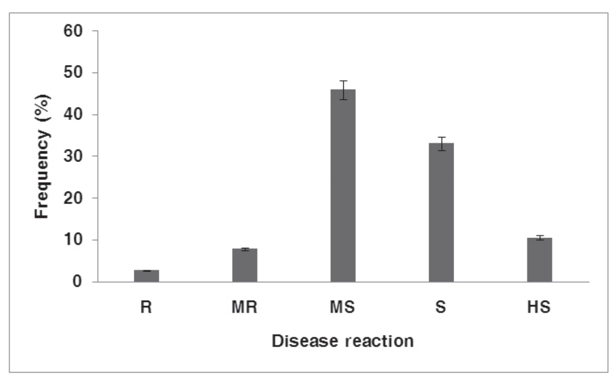

Fig. 2. Frequency distribution of *Oryza* spp. accessions showing different reaction to sheath blight disease (R-Resistant; MR-Moderately resistant; MS-Moderately Susceptible; S-Susceptible and HS-Highly susceptible)

Sheath blight disease is influenced by environmental conditions where high humidity and rainfall plays major role. Rainfall data taken during the evaluation period indicated that 2012 was comparatively dry year with no rainfall during June and rainfall of 150 mm in July followed by a maximum rainfall of 250 mm in the month of August and only 50 mm of rain in September. In the year 2013, a rainfall

Table 2. Classification of the wild Oryza spp. based on their response to sheath blight disease during two consecutive years of screening under artificial inoculation

Disease response	No. of genotypes	Genotypes
R	5	*O. rufipogon* (IC336690), *O. rufipogon* (IC336719), *O. rufipogon* (IC336721), *O. nivara* (IC336696), *O. nivara* (IC336716)
MR	18	*O. nivara* (IC336684), *O. nivara* (IC336685), *O. nivara* (IC336695), *O. nivara* (IC336699), *O. nivara* (IC336700), *O. nivara* (IC336713), NKSWR13, NKSWR14, NKSWR36, NKSWR37, NKSWR47, NKSWR53, NKSWR80, NKSWR81, NKSWR82, NKSWR83, NKSWR93, NKSWR94
MS	100	*O. rufipogon* (IC336679), *O. rufipogon* (IC336687), *O. rufipogon* (IC336707), *O. rufipogon* (IC336708), *O. nivara* (IC336676), *O. nivara* (IC336680), *O. nivara* (IC336682), *O. nivara* (IC336683), *O. nivara* (IC336693), *O. nivara* (IC336694), *O. nivara* (IC336697), *O. nivara* (IC336698), *O. nivara* (IC336705), *O. nivara* (IC336706), *O. nivara* (IC336715), *O. nivara* (IC336724), *O. nivara* (IC330639), *O. nivara* (IC330646), *O. nivara* (IC330647), *O. nivara* (IC330648), *O. nivara* (IC330649), *O. nivara* (IC330650), NKSWR1, NKSWR2, NKSWR3, NKSWR7, NKSWR8, NKSWR12, NKSWR16, NKSWR17, NKSWR18, NKSWR20, NKSWR21, NKSWR23, NKSWR24, NKSWR25, NKSWR26, NKSWR27, NKSWR28, NKSWR29, NKSWR30, NKSWR31, NKSWR32, NKSWR33, NKSWR34, NKSWR35, NKSWR38, NKSWR39, NKSWR41, NKSWR42, NKSWR45, NKSWR46, NKSWR48, NKSWR50, NKSWR51, NKSWR52, NKSWR54, NKSWR55, NKSWR56, NKSWR60, NKSWR62, NKSWR63, NKSWR64, NKSWR65, NKSWR66, NKSWR67, NKSWR68, NKSWR69, NKSWR70, NKSWR71, NKSWR72, NKSWR75, NKSWR77, NKSWR78, NKSWR79, NKSWR84, NKSWR85, NKSWR86, NKSWR87, NKSWR88, NKSWR89, NKSWR90, NKSWR91, NKSWR92, NKSWR95, NKSWR101, NKSWR103, NKSWR127, NKSWR128, NKSWR129, NKSWR130, NKSWR134, NKSWR136, NKSWR138, NKSWR142, NKSWR143, NKSWR146, NKSWR149, NKSWR159, NKSWR160
S	72	*O. rufipogon* (IC336701), *O. rufipogon* (IC336703), *O. rufipogon* (IC336712), *O. rufipogon* (IC336714), *O. rufipogon* (IC336723), *O. rufipogon* (IC336727), *O. rufipogon* (IC336728), *O. nivara* (IC336681), *O. nivara* (IC336689), *O. nivara* (IC336726), *O. nivara* (IC330631), *O. nivara* (IC330644), *O. nivara* (IC330645), *O. nivara* (IC330651), *O. nivara* (IC330654), *O. nivara* (IC330657), *O. nivara* (IC330617), *O. nivara* (IC330621), NKSWR5, NKSWR6, NKSWR9, NKSWR10, NKSWR11, NKSWR15, NKSWR19, NKSWR22, NKSWR40, NKSWR43, NKSWR44, NKSWR49, NKSWR57, NKSWR58, NKSWR59, NKSWR61, NKSWR73, NKSWR74, NKSWR76, NKSWR96, NKSWR98, NKSWR99, NKS100, NKSWR102, NKSWR104, NKSWR109, NKSWR110, NKSWR114, NKSWR115, NKSWR116, NKSWR120, NKSWR121, NKSWR123, NKSWR124, NKSWR125, NKSWR126, NKSWR131, NKSWR132, NKSWR133, NKSWR137, NKSWR140, NKSWR141, NKSWR144, NKSWR145, NKSWR147, NKSWR148, NKSWR150, NKSWR152, NKSWR153, NKSWR154, NKSWR155, NKSWR156, NKSWR157, NKSWR158
HS	23	*O. rufipogon* (IC336692), *O. nivara* (IC330628), *O. nivara* (IC330629), *O. nivara* (IC330630), *O. nivara* (IC330641), *O. nivara* (IC330642), *O. nivara* (IC330643), NKSWR4, NKSWR97, NKSWR105, NKSWR106, NKSWR107, NKSWR108, NKSWR111, NKSWR112, NKSWR113, NKSWR117, NKSWR118, NKSWR119, NKSWR122, NKSWR135, NKSWR139, NKSWR151

of 150 mm was observed in June, 480 mm in July, 500 mm in August, 100 mm in September followed by 100 mm rain in October (Fig. 3).

***In vitro* evaluation of sheath blight resistance**

Two *O. rufipogon* accessions identified to be resistant in the present study namely, IC336719 and IC336721, along with other moderately resistant genotypes IR64 and IR24, and susceptible check, Pusa Basmati 1 were evaluated against *R. solani* under *in vitro* conditions. Minimum lesion size of 3 mm^2 appeared in *O. rufipogon* accessions after 48 hrs of inoculation, while in the susceptible check, Pusa Basmati 1, it was found to be 8 mm^2. Minimum number of infection cushions were observed in *O. rufipogon* accession, IC336719 (10), whereas it was maximum (45) in Pusa

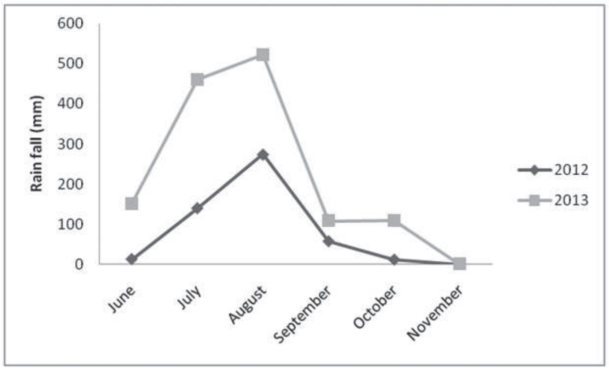

Fig. 3. Rainfall recorded during the *kharif* season of year 2012 and 2013 at New Delhi

Table 3. Evaluation of promising wild rice genotypes for the different characters under *in vitro* conditions

Genotype	Lesion length (mm)	Infection cushion (No.)*	Penetration pegs (No.)*
O. rufipogon (IC336719)	3.0 (±0.5)	10 (±1.5)	3.6 (±1)
O. rufipogon (IC336721)	3.0(±0.5)	15 (±1.5)	5.3 (±1.5)
IR 64	5.0 (±1.0)	22 (±1.0)	8.5 (±1.5)
IR 24	6.0 (±1.5)	25 (±1.5)	9.0 (±1.2)
Pusa Basmati 1	8.0 (±1.5)	45 (±2.0)	13.2 (±2.0)

*= Mean, after 48 hours of inoculation; Values in parenthesis-standard deviation; * Area = 0.20mm^2

Basmati 1. The moderately tolerant genotypes, IR64 and IR24 produced 22 and 25 infection cushions, respectively. Significant differences were observed in genotypes for the penetration peg number. Maximum numbers of penetration pegs (13.2) were observed in Pusa Basmati 1 whereas, minimum number of penetration pegs (3.6) were observed in *O. rufipogon* accession, IC226719 (Table 2).

Discussion

Several studies have been conducted to identify source for sheath blight resistance in rice genotypes but only partial resistance to rice sheath blight has been identified till date, as evidenced by a survey of 6,000 rice cultivars from 40 countries from which no cultivar exhibiting a major gene for rice sheath blight resistance was identified (Hashiba 1984). Breeding for resistance to sheath blight has been very limited (Singh et al. 2016; Dubey et al. 2014), due to lack of a perfect donor for resistance to this dreaded disease. In the present study, two *O. rufipogon* accessions were identified resistant and eleven accessions were moderately resistant. Reddy et al. (1997) tested 457 breeding lines for sheath blight resistance and found two lines as resistant. Prasad and Eizenga (2008) also evaluated 73 *Oryza* spp. accessions and identified seven accessions of *O. nivara*, *O. barthii*, *O. meridionalis* and *O. officinalis* as moderately resistant. Ram et al. (2008) reported sheath blight resistance in three accessions of *O. rufipogon* WR 105, WR 135 and DRW 220 with average lesion height of 9.7, 12.6 and 5.1, respectively. However, they have not included the present accessions in their evaluation and continuous screening of these accessions were not presented. Under favorable conditions of low sunlight, high humidity and warm temperature, the infection spreads rapidly by means of runner hyphae to upper plant parts (Rush and Lee 1992) which might be responsible for the high disease severity during 2013 as compared to 2012. Groth (1992) reported that selection on the basis of number of infection cushions and infection type was effective to maintain resistance in rice genotypes against sheath blight disease. The number of cushions in *O. rufipogon* accessions were significantly lower as compared to the susceptible check, Pusa Basmati 1 and moderately tolerant genotypes, IR64 and IR24, which is indicative of the higher level of resistance expressed in these accessions. Further, the number of penetration pegs were also lesser in *O. rufipogon* accessions as compared to the susceptible check, Pusa Basmati 1 reconfirming the effectiveness of the resistance in these accessions.

The two *O. rufipogon* accessions namely, IC336719 and IC336721, identified as resistant and characterized for their resistant phenotype based on infection cushion and penetration pegs in the present study offers new sources of resistance to sheath blight disease in the A genome wild relative of rice. Since, there are very limited number of genotypes identified as resistant to sheath blight and very low utility of the already identified genotypes in breeding for resistance to sheath blight disease due to very less recombination between *O. sativa* and other genome (other than A genome), these accessions offers a valuable source for developing germplasm lines with enhanced sheath blight resistance in an adapted background. Introgression lines have been developed using one of the *O. rufipogon* as source for sheath blight resistance and research is underway to study the genetic nature and also for molecular mapping of QTL(s)/genes governing resistance to sheath blight disease in this wild rice accession.

Authors' contribution

Conceptualization of research (BMB, SGK, AKS, RA); Designing of the experiments (BMB, SGK, RA); Contribution of experimental materials (BMB, AKS, NKS); Execution of field/lab experiments and data collection (BMB, KR, DS); Analysis of data and interpretation (BMB, SGK); Preparation of manuscript (BMB, SGK).

Declaration

The authors declare no conflict of interest.

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Supplementary Table S1. The response of the wild *Oryza* spp. to sheath blight infections during two years of screening under artificial inoculation

S.No.	Wild rice genotype	Year 2012-13			Year 2013-14			Mean	
		Relative lesion height (RLH)	Disease score	Disease reaction	Relative lesion height (RLH)	Disease score	Disease reaction	Disease score	Disease reaction*
1	*O. rufipogon* (IC336679)	33.30	5	MS	32.53	5	MS	5	MS
2	*O. rufipogon* (IC336687)	25.00	3	MR	31.00	5	MS	4	MS
3	*O. rufipogon* (IC336690)	17.70	1	R	20.00	1	R	1	R
4	*O. rufipogon* (IC336692)	58.00	7	R	70.00	9	HS	8	HS
5	*O. rufipogon* (IC336701)	66.60	9	HS	45.00	5	MS	7	S
6	*O. rufipogon* (IC336703)	42.80	5	MS	47.70	7	S	6	S
7	*O. rufipogon* (IC336707)	46.00	7	S	22.03	3	MR	5	MS
8	*O. rufipogon* (IC336708)	21.00	3	MR	54.30	7	S	5	MS
9	*O. rufipogon* (IC336712)	53.50	7	S	38.60	5	MS	6	S
10	*O. rufipogon* (IC336714)	66.60	5	HS	14.00	7	R	6	S
11	*O. rufipogon* (IC336719)	12.50	1	R	15.00	1	R	1	R
12	*O. rufipogon* (IC336721)	11.50	1	R	14.60	1	R	1	R
13	*O. rufipogon* (IC336723)	40.00	5	MS	51.40	7	S	6	S
14	*O. rufipogon* (IC336727)	46.00	7	S	57.50	7	S	7	S
15	*O. rufipogon* (IC336728)	48.50	7	S	35.00	5	MS	6	S
16	*O. nivara* (IC336676)	21.60	3	MR	55.70	7	S	5	MS
17	*O. nivara* (IC336680)	45.00	5	MS	36.50	5	MS	5	MS
18	*O. nivara* (IC336681)	32.00	5	MS	45.00	7	S	6	S
19	*O. nivara* (IC336682)	25.00	3	MR	46.80	7	S	5	MS
20	*O. nivara* (IC336683)	33.30	5	MS	22.50	3	MR	4	MS
21	*O. nivara* (IC336684)	24.00	3	MR	26.50	3	MR	3	MR
22	*O. nivara* (IC336685)	20.00	1	R	23.50	3	MR	2	MR
23	*O. nivara* (IC336689)	75.00	9	HS	38.80	5	MS	7	S
24	*O. nivara* (IC336693)	36.00	5	MS	38.80	5	R	5	MS
25	*O. nivara* (IC336694)	33.30	5	MS	12.30	3	MR	4	MS
26	*O. nivara* (IC336695)	12.00	1	R	26.60	3	MR	2	MR
27	*O. nivara* (IC336696)	16.00	1	R	19.60	1	R	1	R
28	*O. nivara* (IC336697)	20.00	1	R	51.50	7	S	4	MS
29	*O. nivara* (IC336698)	50.00	7	S	46.00	7	S	5	MS
30	*O. nivara* (IC336699)	25.00	3	MR	23.20	3	MR	3	MR
31	*O. nivara* (IC336700)	23.00	3	MR	24.00	3	MR	3	MR
32	*O. nivara* (IC336705)	23.50	3	MR	24.00	5	MS	4	MS
33	*O. nivara* (IC336706)	22.00	3	MR	31.30	5	MS	4	MS
34	*O. nivara* (IC336713)	20.00	1	R	35.00	5	MS	3	MR
35	*O. nivara* (IC336715)	33.30	5	MS	25.40	3	R	4	MS
36	*O. nivara* (IC336716)	12.00	1	R	18.30	1	R	1	R
37	*O. nivara* (IC336724)	23.30	3	MR	52.00	7	S	5	MS

38	*O. nivara* (IC336726)	50.00	7	S	42.00	7	S	7	S
39	*O. nivara* (IC330628)	50.00	7	S	70.80	9	HS	8	HS
40	*O. nivara* (IC330629)	76.60	9	HS	65.00	7	S	8	HS
41	*O. nivara* (IC330630)	66.60	9	HS	59.10	7	S	8	HS
42	*O. nivara* (IC330631)	50.00	7	S	44.40	5	MS	6	S
43	*O. nivara* (IC330639)	37.10	5	MS	21.70	3	MR	4	MS
44	*O. nivara* (IC330641)	50.00	7	S	68.50	9	HS	8	HS
45	*O. nivara* (IC330642)	66.60	9	HS	55.70	7	S	8	HS
46	*O. nivara* (IC330643)	66.60	9	HS	58.00	7	S	8	HS
47	*O. nivara* (IC330644)	50.00	7	S	49.00	7	S	7	S
48	*O. nivara* (IC330645)	46.00	7	S	37.20	5	MS	6	S
49	*O. nivara* (IC330646)	48.00	7	S	27.70	3	MR	5	MS
50	*O. nivara* (IC330647)	26.00	3	MR	31.40	5	MS	5	MS
51	*O. nivara* (IC330648)	37.50	5	MS	21.70	3	MR	4	MS
52	*O. nivara* (IC330649)	38.50	5	MS	32.60	5	MS	5	MS
53	*O. nivara* (IC330650)	43.30	5	MS	45.00	5	MS	5	MS
54	*O. nivara* (IC330651)	43.30	5	MS	71.60	9	HS	7	S
55	*O. nivara* (IC330654)	37.50	5	MS	72.40	9	HS	7	S
56	*O. nivara* (IC330657)	33.30	5	MS	52.10	7	S	6	S
57	*O. nivara* (IC330617)	36.00	5	MS	51.00	7	S	6	S
58	*O. nivara* (IC330621)	50.00	7	S	40.80	5	MS	6	S
59	NKSWR1	29.60	3	MR	38.00	5	MS	4	MS
60	NKSWR2	32.50	5	MS	42.60	5	MS	5	MS
61	NKSWR3	25.00	3	MR	55.50	7	S	5	MS
62	NKSWR4	50.00	7	S	67.20	9	HS	8	HS
63	NKSWR5	62.50	7	S	48.50	7	S	7	S
64	NKSWR6	43.60	5	MS	62.30	7	S	6	S
65	NKSWR7	60.00	7	S	28.10	3	MR	5	MS
66	NKSWR8	23.20	3	MR	53.50	7	S	5	MS
67	NKSWR9	57.50	7	S	54.40	7	S	7	S
68	NKSWR10	62.50	7	S	56.00	7	S	7	S
69	NKSWR11	55.00	7	S	46.30	7	S	7	S
70	NKSWR12	44.40	5	MS	26.30	3	MR	4	MS
71	NKSWR13	25.00	3	MR	21.30	3	MR	3	MR
72	NKSWR14	20.00	1	R	31.40	5	MS	2	MR
73	NKSWR15	66.60	9	HS	25.50	3	MR	6	S
74	NKSWR16	20.00	1	R	51.00	7	S	4	MS
75	NKSWR17	50.00	7	S	21.40	3	MR	5	MS
76	NKSWR18	30.00	3	MR	51.50	7	S	5	MS
77	NKSWR19	54.50	7	S	35.00	5	MS	6	S
78	NKSWR20	33.30	5	MS	28.00	3	MR	4	MS
79	NKSWR21	15.00	1	R	58.10	7	S	4	MS
80	NKSWR22	54.50	7	S	40.50	5	MS	6	S
81	NKSWR23	16.60	1	R	47.50	7	S	4	MS
82	NKSWR24	40.00	5	MS	32.30	5	MS	5	MS
83	NKSWR25	32.80	5	MS	32.80	5	MS	5	MS

84	NKSWR26	29.40	3	MR	32.60	5	MS	4	MS
85	NKSWR27	33.30	5	MS	32.40	5	MS	5	MS
86	NKSWR28	27.10	3	MR	43.80	7	S	5	MS
87	NKSWR29	36.60	5	MS	30.00	3	MR	4	MS
88	NKSWR30	29.00	3	MR	31.30	5	MS	4	MS
89	NKSWR31	27.20	3	MR	40.00	5	MS	4	MS
90	NKSWR32	31.60	5	MS	34.30	5	MS	5	MS
91	NKSWR33	26.00	3	MR	45.00	5	MS	4	MS
92	NKSWR34	41.10	5	MS	26.60	3	MR	4	MS
93	NKSWR35	23.70	3	MR	30.40	5	MS	4	MS
94	NKSWR36	30.00	3	MR	26.00	3	MR	3	MR
95	NKSWR37	26.20	3	MR	30.00	3	MR	3	MR
96	NKSWR38	28.00	3	MR	33.30	5	MS	4	MS
97	NKSWR39	27.50	3	MR	50.00	7	S	5	MS
98	NKSWR40	47.80	7	S	57.50	7	S	7	S
99	NKSWR41	44.20	5	MS	42.80	5	MS	5	MS
100	NKSWR42	36.10	5	MS	37.60	5	MS	5	MS
101	NKSWR43	33.00	5	MS	51.00	7	S	6	S
102	NKSWR44	46.00	7	S	41.60	7	S	7	S
103	NKSWR45	38.70	5	MS	27.00	3	MR	4	MS
104	NKSWR46	26.00	5	MS	35.80	5	MS	5	MS
105	NKSWR47	26.60	3	MR	27.30	3	MR	3	MR
106	NKSWR48	25.00	3	MR	45.40	7	S	5	MS
107	NKSWR49	50.00	7	S	37.50	5	MS	6	S
108	NKSWR50	33.30	5	MS	32.60	5	MS	5	MS
109	NKSWR51	33.30	5	MS	34.00	5	MS	5	MS
110	NKSWR52	32.00	5	MS	25.00	3	MR	4	MS
111	NKSWR53	21.80	3	MR	23.00	3	MR	3	MR
112	NKSWR54	22.70	3	MR	32.60	5	MS	4	MS
113	NKSWR55	27.70	3	MR	38.00	5	MS	4	MS
114	NKSWR56	37.50	5	MS	42.70	5	MS	5	MS
115	NKSWR57	36.70	5	MS	48.60	7	S	6	S
116	NKSWR58	46.60	7	S	51.70	7	S	7	S
117	NKSWR59	50.00	7	S	53.30	7	S	7	S
118	NKSWR60	43.40	5	MS	41.20	5	MS	5	MS
119	NKSWR61	35.70	5	MS	50.00	7	S	6	S
120	NKSWR62	41.60	5	MS	40.00	5	MS	5	MS
121	NKSWR63	38.40	5	MS	40.60	5	MS	5	MS
122	NKSWR64	34.40	5	MS	44.00	5	MS	5	MS
123	NKSWR65	33.30	5	MS	42.60	5	MS	5	MS
124	NKSWR66	37.10	5	MS	32.90	5	MS	5	MS
125	NKSWR67	20.50	3	MR	42.60	5	MS	4	MS
126	NKSWR68	37.50	5	MS	30.00	3	MR	4	MS
127	NKSWR69	27.30	3	MR	33.70	5	MS	4	MS
128	NKSWR70	28.50	3	MR	38.80	5	MS	4	MS
129	NKSWR71	30.00	3	MR	50.00	7	S	5	MS

130	NKSWR72	44.40	5	MS	45.00	5	MS	5	MS
131	NKSWR73	40.00	5	MS	50.00	7	S	6	S
132	NKSWR74	47.10	7	S	33.00	5	MS	6	S
133	NKSWR75	25.00	3	MR	52.60	7	S	5	MS
134	NKSWR76	41.60	5	MS	50.00	7	S	6	S
135	NKSWR77	42.80	5	MS	29.10	3	MR	4	MS
136	NKSWR78	27.20	3	MR	33.70	5	MS	4	MS
137	NKSWR79	26.00	3	MR	38.00	5	MS	4	MS
138	NKSWR80	25.00	3	MR	26.00	3	MR	3	MR
139	NKSWR81	22.00	3	MR	29.00	3	MR	3	MR
140	NKSWR82	28.00	3	MR	22.80	3	MR	3	MR
141	NKSWR83	10.00	1	R	29.00	3	MR	2	MR
142	NKSWR84	20.50	3	MR	32.50	5	MS	4	MS
143	NKSWR85	32.50	5	MS	35.70	5	MS	5	MS
144	NKSWR86	30.20	5	MS	31.50	5	MS	5	MS
145	NKSWR87	28.00	3	MR	31.40	5	MS	4	MS
146	NKSWR88	26.60	3	MR	37.50	5	MS	4	MS
147	NKSWR89	24.70	3	MR	31.20	5	MS	4	MS
148	NKSWR90	27.80	3	MR	36.53	5	MS	4	MS
149	NKSWR91	33.30	5	MS	39.40	5	MS	5	MS
150	NKSWR92	38.80	5	MS	21.90	3	MR	4	MS
151	NKSWR93	22.80	3	MR	21.90	3	MR	3	MR
152	NKSWR94	19.40	1	R	42.50	5	MS	2	MR
153	NKSWR95	39.50	5	MS	46.60	5	MS	5	MS
154	NKSWR96	36.00	5	MS	51.20	7	S	6	S
155	NKSWR97	50.00	7	S	68.00	9	HS	8	HS
156	NKSWR98	63.00	7	S	36.30	5	MS	6	S
157	NKSWR99	35.00	5	MS	63.60	7	S	6	S
158	NKSWR100	50.00	7	S	31.40	5	MS	6	S
159	NKSWR101	26.60	3	MR	40.00	5	MS	4	MS
160	NKSWR102	33.30	5	MS	48.00	7	S	6	S
161	NKSWR103	42.00	5	MS	41.40	5	MS	5	MS
162	NKSWR104	40.00	5	MS	52.90	7	S	6	S
163	NKSWR105	51.00	7	S	70.70	9	HS	8	HS
164	NKSWR106	65.00	7	S	75.00	9	HS	8	HS
165	NKSWR107	61.80	7	S	68.50	9	HS	8	HS
166	NKSWR108	70.00	9	HS	62.30	9	HS	9	HS
167	NKSWR109	56.00	7	S	31.33	5	MS	6	S
168	NKSWR110	30.00	3	MR	85.70	9	HS	6	S
169	NKSWR111	60.60	7	S	71.30	9	HS	8	HS
170	NKSWR112	68.00	9	HS	62.80	7	S	8	HS
171	NKSWR113	66.60	9	HS	61.90	7	S	8	HS
172	NKSWR114	53.30	7	S	65.00	7	S	7	S
173	NKSWR115	40.00	5	MS	57.10	7	S	6	S
174	NKSWR116	47.00	7	S	58.10	7	S	7	S
175	NKSWR117	50.40	7	S	77.50	9	HS	8	HS

176	NKSWR118	54.50	7	S	72.00	9	HS	8	HS
177	NKSWR119	54.50	7	S	70.00	9	HS	8	HS
178	NKSWR120	64.70	7	S	59.20	7	S	7	S
179	NKSWR121	56.50	7	S	61.30	7	S	7	S
180	NKSWR122	56.00	7	S	65.70	9	HS	8	HS
181	NKSWR123	64.70	7	S	61.20	7	S	7	S
182	NKSWR124	42.70	5	MS	52.30	7	S	6	S
183	NKSWR125	45.00	5	MS	53.30	7	S	6	S
184	NKSWR126	46.60	7	S	39.00	5	MS	6	S
185	NKSWR127	31.50	5	MS	45.00	5	MS	5	MS
186	NKSWR128	33.00	5	MS	37.50	5	MS	5	MS
187	NKSWR129	37.10	5	MS	36.60	5	MS	5	MS
188	NKSWR130	28.50	3	MR	33.30	5	MR	4	MS
189	NKSWR131	29.30	3	MR	66.20	9	HS	6	S
190	NKSWR132	42.80	5	MS	66.00	9	HS	7	S
191	NKSWR133	54.70	7	S	55.50	7	S	7	S
192	NKSWR134	46.60	3	MR	32.00	5	MS	4	MS
193	NKSWR135	30.00	9	HS	72.00	9	HS	9	HS
194	NKSWR136	66.60	3	MR	45.00	5	MS	4	MS
195	NKSWR137	29.80	5	MS	71.20	9	HS	7	S
196	NKSWR138	32.00	5	MS	32.00	5	MS	5	MS
197	NKSWR139	31.20	7	S	77.70	9	HS	8	HS
198	NKSWR140	47.00	7	S	57.10	5	MS	6	S
199	NKSWR141	56.30	7	S	60.00	7	S	7	S
200	NKSWR142	50.40	3	MR	50.00	7	S	5	MS
201	NKSWR143	30.00	5	MS	44.60	5	MS	5	MS
202	NKSWR144	37.50	7	S	50.00	7	S	7	S
203	NKSWR145	50.00	5	MS	45.60	7	S	6	S
204	NKSWR146	38.40	3	MR	33.50	5	MS	4	MS
205	NKSWR147	30.00	7	S	55.70	7	S	7	S
206	NKSWR148	50.00	7	S	62.50	7	S	7	S
207	NKSWR149	54.00	5	MS	42.00	5	MS	5	MS
208	NKSWR150	32.80	7	S	62.00	7	S	7	S
209	NKSWR151	55.00	7	S	89.40	9	HS	8	HS
210	NKSWR152	65.00	7	S	45.70	7	S	7	S
211	NKSWR153	45.40	7	S	52.00	7	S	7	S
212	NKSWR154	53.50	7	S	59.00	7	S	7	S
213	NKSWR155	50.00	7	S	57.50	7	S	7	S
214	NKSWR156	48.67	7	S	52.00	7	S	7	S
215	NKSWR157	51.33	7	S	56.20	7	S	7	S
216	NKSWR158	55.00	7	S	33.30	5	MS	6	S
217	NKSWR159	31.60	5	MS	38.06	5	MS	5	MS
218	NKSWR160	34.40	5	MS	34.13	5	MS	5	MS
CD (0.5%)		1.58			3.96				

*Values 2, 4, 6 and 8 were designated as MR, MS, S and HS respectively as their score was more compared to the designated reaction