Fundam. appl. Nematol., 1998, **21** (4), 307-317

Screening for resistance to *Heterodera sacchari* in the two cultivated rice species, *Oryza sativa* and *O. glaberrima*

Georges REVERSAT and Denis DESTOMBES

Laboratoire d'Écologie des Sols Tropicaux, Centre ORSTOM d'Ile-de-France, 32, Av. Henri Varagnat, 93143 Bondy Cedex, France.

Accepted for publication 6 August 1997.

Summary - A total of 73 rice accessions belonging to the two species of cultivated rice, *Oryza sativa* and *O. glaberrima*, and one wild species, *O. breviligulata*, were screened in pot experiments for resistance to a Congolese population of the cyst nematode *Heterodera sacchari*. All 43 accessions of *O. sativa* were susceptible, although they were presumably genetically different as they came from various parts of the world. In contrast, fifteen of the 21 accessions of *O. glaberrima* and seven of the nine accessions of *O. breviligulata* were resistant whereas the others were susceptible. All of these 30 accessions originated from Africa, as did the parasite itself. These results were verified with two other populations of the parasite from Chad and Senegal. These results are analysed and their possible use is discussed. © Orstom/Elsevier, Paris

Résumé - ***Criblage pour la résistance à* Heterodera sacchari *des deux espèces de riz cultivé*, Oryza sativa *et* O. glaberrima.** - Un total de 73 accessions de riz appartenant aux deux espèces cultivées, *Oryza sativa* et *O. glaberrima*, et une espèce sauvage, *O. breviligulata*, ont été criblées, dans des expériences en pots, pour leur résistance vis-à-vis d'une souche congolaise du nématode à kyste *Heterodera sacchari*. Les 43 cultivars testés appartenant à l'espèce *O. sativa* sont sensibles, bien que présumés génétiquement différents, comme provenant d'origines géographiques très variées. Par contre, parmi les 21 accessions testées de l'espèce *O. glaberrima*, d'origine africaine, quinze sont résistantes et deux sont intermédiaires, tandis que les quatre autres sont sensibles. Il en va de même pour l'espèce de riz sauvage, *O. breviligulata*, également d'origine africaine et proche d'*O. glaberrima*, pour laquelle sept des neuf accessions testées sont résistantes, tandis que les deux autres sont sensibles. Ces résultats ont été confirmés pour deux autres souches du parasite, provenant du Tchad et du Sénégal. Leur interprétation et leur utilisation possible sont discutées. © Orstom/Elsevier, Paris

Keywords: *Heterodera sacchari*, nematodes, *Oryza breviligulata*, *Oryza glaberrima*, *Oryza sativa*, resistance.

The nematode *Heterodera sacchari* was described as a parasite of sugarcane in Congo (Brazzaville) (Luc & Merny, 1963). It was later found in Africa on sugarcane in Burkina Faso (Cadet & Merny, 1978), Nigeria (Babatola, 1983*a*), and Chad (Reversat & Nayalta, 1991) and on rice in Nigeria (Jerath, 1968), Ivory Coast (Merny, 1970), Senegal and Gambia (Fortuner & Merny, 1973), and Liberia (Vovlas *et al.*, 1986). Outside Africa, it was reported on sugarcane in India (Swarup *et al.*, 1964), Trinidad (Singh, 1974), Pakistan (Maqbool, 1981), and Thailand (Anon., 1987). *H. sacchari* was shown to be highly pathogenic on rice (Babatola, 1983*a*), and the selection of resistant cultivars could be a valid control method for this parasite. Among 50 rice cultivars in Nigeria, Babatola (1983*b*) found only differences in degrees of susceptibility, which did not exactly prove resistance. There are three other species of *Heterodera* parasitising rice (*H. oryzae*, *H. oryzicola*, and *H. elachista*) and, although the distribution of these four species is at present restricted, their potential economic impact could be important (Bridge *et al.*, 1990).

There are two species of cultivated rice in the world: *Oryza sativa* L. that originated from Asia and is cultivated in all the tropical and sub-tropical areas of the world and *O. glaberrima* Steud that originated from Central Africa and is cultivated only in western tropical Africa (Bezançon & Second, 1984). The objective of the present study was to screen for resistance to *H. sacchari* within these two rice species. The screening was based on the collections of seeds stored in Montpellier (France) by plant geneticists of CIRAD (Centre International de Recherche Agricole pour le Développement) and ORSTOM. In addition, some accessions of *O. breviligulata* A. Chev. & Roehr, a wild rice close to *O. glaberrima*, were also tested. A total of 73 accessions were screened against a Congolese population of *H. sacchari*. Some accessions were also tested against two other populations of *H. sacchari*, one from Chad and the other from Senegal. Some resistant accessions were found in *O. glaberrima* and *O. breviligulata*, but none in *O. sativa*. The invasion rates of second stage juveniles (J2) in roots of susceptible and resistant accessions were compared. These experiments were performed at the ORSTOM Research Centre of Pointe Noire (Congo-Brazzaville) from 1989 to 1993 and have been briefly reported elsewhere (Reversat & Destombes, 1995).

Table 1. *Screening for resistance to* Heterodera sacchari *in accessions of* Oryza sativa: *numbers of living J2 released from 60 cysts dissected in potassium permanganate.*

Accessions			Population(1)	Number of J2(2)
No.	Name	Country	(Numb. exp.)	
1	63-104	Senegal	C (9)	15 240±901
2	Bansi	India	C (11)	21 940±1 771
3	Basmati 370	India	C (10)	17 760±850
4	Chianan 8	Taiwan	C (10)	17 480±1401
5	Gambiaka	Guinea	T (12)	17 440±684
–	–	–	S (14)	14 600±504
6	IR5	Philippines	C (5)	14 080±1 133
7	IR 8	Philippines	C (5)	20 280±848
–	–	–	T (13)	18 640±1 287
–	–	–	S (15)	14 775±472
8	IR 52	Philippines	C (11)	20 280±921
9	IR 1529/680	Philippines	C (5)	19 590±1 432
–	–	–	T (13)	18 140±966
–	–	–	S (14)	13 393±1 561
10	IR 1545/339	Philippines	C (6)	15 540±867
11	IR 1552	Philippines	C (6)	18 080±576
12	IR 1561	Philippines	C (6)	15 990±1 148
13	IRAT 104	Ivory Coast	C (2)	19 800±1 081
14	IRAT 112	Ivory Coast	C (4)	21 660±1 000
15	IRAT 120	Madagascar	C (2)	18 520±2 030
16	IRAT 124	Madagascar	C (5)	17 370±1 697
17	IRAT 128	Madagascar	C (1)	21 360±637
18	IRAT 133	Ivory Coast	C (3)	13 802±1 637
19	IRAT 144	Burkina Faso	C (1)	25 760±1 011
–	–	–	T (12)	21 180±1 258
–	–	–	S (14)	11 700±1 288
20	IRAT 170	Ivory Coast	C (2)	18 780±1 437
21	IRAT 177	French Guyana	C (9)	14 300±671
22	IRAT 212	Ivory Coast	C (1)	22 440±1 643
23	IRAT 216	Ivory Coast	C (4)	13 420±1 261
24	IRAT 262	Ivory Coast	C (5)	16 420±830
25	IRAT 268	Ivory Coast	C (1)	20 060±707
26	IRAT 283	French Guyana	C (1)	24 240±2532
27	IRAT 291	French Guyana	C (6)	16 700±1 020
28	IRAT 308	Ivory Coast	C (3)	14 780±904
29	IRAT 310	Ivory Coast	C (2)	19 760±605
30	IRAT 312	French Guyana	C (2)	16 480±937
31	IRAT 316	Ivory Coast	C (6)	18 530±1 099
32	IRAT 318	Ivory Coast	C (4)	16 120±1 179
33	IRAT 319	Ivory Coast	C (7)	16 940±959

End of Table 1 next page

Table 1. *(End).*

Accessions			Population(1)	Number of J2(2)
No.	Name	Country	(Numb. exp.)	
34	IRAT 330	French Guyana	C (6)	20 060±1 698
35	IRAT 347	French Guyana	C (2)	23 400±1 738
–	–	–	T (12)	19 560±679
–	–	–	S (15)	12 800±888
36	IRAT 349	Philippines	C (1)	20 280±2 008
37	ITA 222	Nigeria	C (10)	17 620±1 089
38	Lung Sheng 1	Taiwan	C (10)	23 680±1 173
39	P 175	Liberia	C (9)	18 840±900
–	–	–	T (13)	20 940±1 131
–	–	–	S (14)	15 575±2 050
40	Rikuto Norin 24	Japan	C (10)	16 940±867
41	Tek Si Chut	Taiwan	C (11)	20 920±1 306
42	Tjempo Welut	Idonesia	C (11)	25 500±1 123
73	Moroberekan	Ivory Coast	all (all)	Fig. 1

(1) Population of *H. sacchari*: [C = Congo; T = Chad; S = Senegal]. (2) Mean ± SD of five replicates.

Materials and methods

RICE ACCESSIONS

A total of 73 accessions of rice, numbered from no. 1 to no. 73, were screened: 43 *O. sativa* (Table 1), 21 *O. glaberrima* (Table 2), and 9 *O. breviligulata* (Table 3). Specifications included the name of the accession and the originating country. All accessions of *O. sativa* and some accessions of *O. glaberrima* were true cultivars whereas most *O. glaberrima* and all *O. breviligulata* accessions were wild populations collected and stored for genetic studies. Two accessions (no. 45 and no. 53) were heterogeneous regarding the colouration of the seed: half seeds were dark coloured whereas the other half were light coloured. Each kind of seeds was treated as a distinct accession during the experiments (Table 2).

In order to use the culture tube sites fully, when rice seeds sown in a tube did not germinate, this tube was used to screen some other plants: *Sorghum vulgare* Pers. (cv. IRAT 207 and cv. IRAT 327), *Zea mays* L. (cv. SIAEB LG 60), *Vigna radiata* L., and *Glycine max* Merr.

H. SACCHARI POPULATIONS

Most experiments were performed with a Congolese population (population C) of *H. sacchari*, collected in 1986 at Nkayi (Congo-Brazzaville), in the sugarcane plantation of SUCO (Sucrière du Congo). Some experiments were carried out with a population from Chad (population T) of *H. sacchari*, collected in 1989 at Sarh (Chad), in the sugarcane plantation of SONASUT (Société Nationale Sucrière du Tchad), and a Senegalese population (population S) of *H. sacchari*, collected on rice in Casamance (southern part of Senegal) in the 70's. As described below, nematodes were multiplied on rice (*O. sativa*, cv. Morobérékan) and kept in a quiescent state, induced by osmotic pressure (Dropkin *et al.*, 1958), in Dropkin's solution (NaCl, 0.3 M) for weeks or months between two successive multiplication periods.

SCREENING EXPERIMENTS

Rice seeds were germinated on coarse filter paper soaked with distilled water for 5-7 days. Some batches of seeds developed fungal contamination and were disinfected by calcium hypochlorite for 5 min (1 g of CaClO in 20 ml of distilled water) without rinsing. When shoots were well developed, after 5-7 days, rice seedlings were planted in 100 ml culture tubes made of PVC cylindrical tubing (26 mm inner diameter and 210 mm in length). The bottom of each tube was closed with a 0.25 mm mesh stainless steel sieve welded in the PVC, and the top was provided with an external PVC ring that was used to suspend the tube through a hole in the cover of a thermostatically-controlled wooden box (Baujard, 1995). The box could hold 72 tubes. The tube was first lined with a sheet of transparent polyethylene and filled with about 100 ml of heat-sterilized sandy soil. The plastic sheet projected slightly (5 mm) over the top of the PVC tube, which made it easy to pull the cylinder of soil out of

Table 2. *Screening for resistance to* Heterodera sacchari *in accessions of* Oryza glaberrima: *numbers of living J2 released from cysts dissected in potassium permanganate (°) or/and from roots and debris in the mistifier (*).*

Accessions			Population[1]	Number of J2[2]	Status[3]
No.	Name	Country	(Numb. exp.)		
43	Kpécékré	Ivory Coast	C (8)	23 240 ± 1 167 °	s
–	–	–	T (13)	17 660 ± 919 °	s
–	–	–	S (15)	16 075 ± 1 478 °	s
44	MG 03	Mali	C (8)	278 ± 70 *	R
–	–	–	T (13)	40 ± 6 *	R
–	–	–	S (15)	230 ± 151 *	R
45	MG 022 [4]	Mali	C (9)	1 270 ± 291 °*	?
–	– [5]	–	C (9)	1 198 ± 310 °*	?
46	MG 023	Mali	C (9)	984 ± 139 °*	R
–	–	–	T (12)	24 ± 11 *	R
–	–	–	S (15)	10 ± 10 *	R
47	UG 38	Cameroon	C (8)	22 300 ± 720 °	s
–	–	–	T (12)	19 580 ± 1 270 °	s
–	–	–	S (15)	19 025 ± 907 °	s
48	UG 67	Cameroon	C (10)	404 ± 192 °*	R
49	CG 18†	Senegal	C (8)	166 ± 32 *	R
–	–	–	T (13)	55 ± 10 *	R
–	–	–	S (15)	30 ± 12 *	R
50	CG 74†	Senegal	C (8)	184 ± 44 *	R
–	–	–	T (12)	28 ± 11 *	R
–	–	–	S (14)	122 ± 73 *	R
51	TG 017	Chad	C (8)	183 ± 94 *	R
–	–	–	T (13)	33 ± 12 *	R
–	–	–	S (14)	22 ± 13 *	R
52	OG 17	Senegal	C (9)	2 784 ± 420 °*	?
53	YG 189[4]	Guinea	C (10)	200 ± 74 °*	R
–	– [5]	–	C (10)	480 ± 141 °*	R
54	YG 275	Guinea	C (8)	104 ± 33 *	R
–	–	–	T (12)	44 ± 6 *	R
–	–	–	S (14)	18 ± 11 *	R
55	BG 04	Guinea Bissau	C (11)	157 ± 129 °*	R
56	CG 13†	Senegal	C (7)	294 ± 57 *	R
57	CG 60	Senegal	C (4)	161 ± 96 *	R
58	HG 9	Burkina Faso	C (7)	471 ± 156 *	R
59	LG 45	Mali	S (14)	24 375 ± 1 540 °	s
60	LG 46	Mali	C (6)	20 650 ± 1 348 °	s
60	LG 46	Mali	C (6)	20 650 ± 1 348 °	s
61	LG 118	Mali	C (3)	0 ± 0 *	R
62	YG 62	Guinea	C (5)	107 ± 56 *	R
63	YG 140	Guinea	C (3)	59 ± 58 *	R

(1) Population of *H. sacchari*: [C = Congo; T = Chad; S = Senegal]. (2) Mean ± SD of five replicates. (3) Status: R = resistant; S = susceptible; ? = intermediate. (4) dark-coloured seeds. (5) light-coloured seeds. †: resistant to *Meloidogyne incognita* (Diomandé, 1984).

Table 3. *Screening for resistance to* Heterodera sacchari *in accessions of* Oryza breviligulata: *numbers of living J2 released from cysts dissected in potassium permanganate (°) or from roots and debris in the mistifier (*).*

Accessions			Population[1]	Number of J2[2]	Status[3]
No.	Name	Country	(Numb.exp.)		
64	HB 3	Burkina Faso	C (7)	370±101 *	R
65	MB 311	Mali	C (7)	229±71 *	R
66	MB 321	Mali	C (4)	199±77 *	R
67	TB 32	Chad	C (5)	113±43 *	R
68	TB 51	Chad	C (3)	10 740±1 183 °	s
69	UB 2-1	Cameroon	C (7)	382±84 *	R
70	UB 35	Cameroon	C (7)	589±199 *	R
71	UB 40	Cameroon	S (14)	0±0 *	R
72	UB 41	Cameroon	C (11)	21 300±1 879 °	s

(1) Population of *H. sacchari*: [C = Congo; T = Chad; S = Senegal].
(2) Mean ± SD of five replicates.
(3) Status: R = resistant; s= susceptible.

the tube without damaging the roots and nematodes at the end of the experiment. The soil used was collected in the savanna near Pointe Noire and was composed of 5.1% clay, 2.9% silt, 34.2% fine sand, 58.3 % coarse sand, and 0.7% organic matter. One rice seedling was planted in each culture tube. There were nine replicates for each rice accession. Inside the boxes, the temperature was kept at 28°C minimum during the experiments. The shoots were kept at ambient temperature ranging from 20 to 30°C. Soil from each tube was watered daily with excess water, which was then drained off. Each tube was fertilised weekly with 20 ml of Hoagland's mineral nutritive solution. Each experiment consisted of one box with eight accessions, including the reference accession: Morobérékan, with 72 tubes completely randomized. A total of fifteen experiments, numbered 1–15, were conducted from 1989 to 1993.

Inoculation

Rice seedlings were grown for 3-4 weeks before inoculation. A few days before inoculation, batches of cysts were taken from the Dropkin's solution, crushed on a 0.25 mm stainless steel sieve and the egg content was washed with distilled water into beakers where hatching of J2 occurred. When necessary, hatching in the beakers was increased by adding potassium permanganate to a final concentration of 4 mM for 24 h (Reversat, 1981). Each culture tube was inoculated three times, at one-week interval, with a total of 1000 freshly hatched J2 (age < 5 days). Each inoculum of 330 J2, in a volume of 1 ml, was introduced into the soil at a depth of 3 cm, at root level.

Cyst recovery

At 10-12 weeks after the last inoculation, five tubes from each accession were treated as follows. The shoots were discarded and the cylinders of soil were extracted from the tubes and placed on a 2 mm mesh stainless steel sieve. The soil was then washed away with a gentle flow of water. The soil-free roots were spread out on a 1 mm stainless steel sieve fitted on a 0.16 mm stainless steel sieve. Roots were sprayed with a high pressure stream of tap water, which separated white females and cysts from roots and washed them down onto the lower sieve where they were retained. For each replicate, roots and debris containing cysts and white females were kept separately in the Dropkin's solution for at least 4-5 weeks to allow all white females to become cysts and all eggs to develop into J2 stage. Finally, debris from each replicate were spread out on a 0.1 mm stainless steel sieve and examined under binocular microscope.

When cysts were present and numerous, a batch of 60 cysts was hand-picked and placed in a drop of water on a 0.25 mm stainless steel sieve. Cysts were then gently crushed with the round bottom part of a hemolysis plastic tube to free eggs. Eggs and debris of crushed cysts were then poured into a beaker where the volume was made up to 40 ml with distilled water. Artificial hatching was triggered by adding 1 ml of 160 mM potassium permanganate to each beaker. One day later, the suspension was diluted to 0.5 l and hatched J2 were counted in several aliquots of 5 ml. Since the batch of 60 cysts represented only a part of the cysts present, the final population was assumed to be greater than the count. The presence of numerous cysts indicated that the accession was susceptible to

H. sacchari and the content of the cysts was checked only to ascertain that they were filled with viable eggs in numbers comparable to those in the reference accession.

When no cysts were present, the accession was considered to be possibly resistant. Debris and roots were placed in a mistifier (Seinhorst, 1950) on 0.1 mm plastic sieves for 4 weeks, and hatched J2 were recovered and counted every week.

When very few cysts were present, the two treatments were combined: cysts were crushed and treated with potassium permanganate and roots and debris were placed in the mistifier. This was also done for selected susceptible accessions of the two cultivated rice species to obtain an estimation of the final population.

The total number of J2 obtained by dissection of cysts with potassium permanganate and/or by spontaneous hatching in the mistifier was recorded for each replicate. An accession was considered to be susceptible when the number of recovered J2 was higher than the number of inoculated J2 and it was considered to be resistant if this was not the case.

ROOT INVASION

Seedlings of *O. sativa* (Morobérékan), *O. glaberrima* (LG 118, no. 61), *S. vulgare* (cv. IRAT 207), and *Vigna radiata* were planted in plastic Petri dishes (diameter 9 cm, one seedling per dish) with about 10 cm^3 wet soil. The shoot of the seedlings emerged from the dish through a hole in the cover lid. The dishes were kept at room temperature (25°C) and were under fluorescent lighting for 12 h each day. Twelve replicates were made for each plant and the 48 dishes were completely randomized. Ten days later, when roots were 5 cm long, each seedling was inoculated with 50 freshly hatched J2 of *H. sacchari,* population C. Eight days after inoculation, the roots were harvested, washed in water, and fixed in boiling colourless lactophenol, then stained in cold cotton blue lactophenol (de Guiran, 1966). Ten days later, roots were pressed firmly between two glass plates and the stained J2 counted under the binocular microscope.

Results

GENERAL

During the fifteen experiments carried out over 5 years, the reference accession Morobérékan always gave many cysts. Mean numbers of J2 released from 60 cysts from this accession, dissected in permanganate, ranged from 11 750 to 24 280 (Fig. 1). Means, however, were significantly different between experiments (ANOVA, F=7.5, P=0.0001). Moreover, variations in the final populations (sums of numbers of J2 recovered from cysts and from the mistifier), recorded in only two selected experiments, were even more pronounced (see, *e.g.*, on Fig. 2, accession no. 73, Morobérékan in experiments no. 7 and 8). There were no contradictory results among the 72 other accessions tested: all replicates of one accession had the same results.

Sorghum vulgare (cvs IRAT 207 and IRAT 327), *Vigna radiata,* and *Glycine max* were resistant: no cysts were visible in the debris and no J2 were found in the mistifier. An average of twelve J2 per replicate was found in the mistifier for maize (*Zea mays* cv. SIAEB LG 60).

ORYZA SATIVA

The 43 *O. sativa* accessions tested, including the reference accession Morobérékan, were all highly susceptible (Table 1). Numerous cysts were recovered from each replicate and on 60 dissected cysts an average of 11 700–25 760 J2 were recovered. The numbers of J2 recovered from the mistifier were similar to or higher than the numbers obtained from the dissection of 60 cysts, depending on the experiment and on the accession (see, *e.g.*, Fig. 2: accession no. 33, IRAT 319). When the J2 released from the dissected cysts and those recovered from the mistifier were added together, the multiplication rate on Morobérékan ranged from 34.9 (experiment No. 7) to 48.8 (experiment No. 8) and was equal to 63.5 on IRAT 319 (Fig. 2).

ORYZA GLABERRIMA

Of the 21 accessions tested for this rice species, fifteen were resistant, two were moderately susceptible (accessions no. 45 and 52), and four were highly susceptible to the Congolese population of *H. sacchari* (Table 2). In the resistant accessions, no cysts were visible on the roots or in the debris and the number of J2 recovered from roots and debris in the mistifier was always less than the inoculum level. On the contrary, results from susceptible accessions of *O. glaberrima* were similar to those from the reference accession Morobérékan (see, *e.g.*, Fig. 2, experiment 8: accessions no. 73 - Morobérékan, no. 43 - Kpécékré, and no. 47 - UG 38). Six of the fifteen resistant accessions were also resistant to the other two parasite populations, T (Chadese population) and S (Senegalese population). Results from accessions no. 45 and 53, with seeds either dark or light coloured, were independent of seed colouration (Table 2).

ORYZA BREVILIGULATA

Seven of the nine accessions tested were resistant, the other two were susceptible to *H. sacchari.* (Table 3).

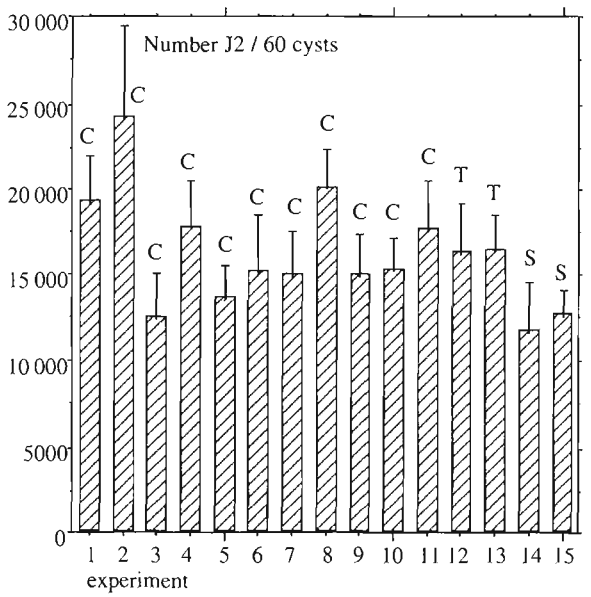

Fig. 1. *Numbers of living J2 of* Heterodera sacchari *released from 60 cysts dissected in potassium permanganate for the reference accession Morobérékan during the fifteen experiments (C: Congolese population; T: Chadese population; S: Senegalese population. Mean + SD of five replicates for each experiment).*

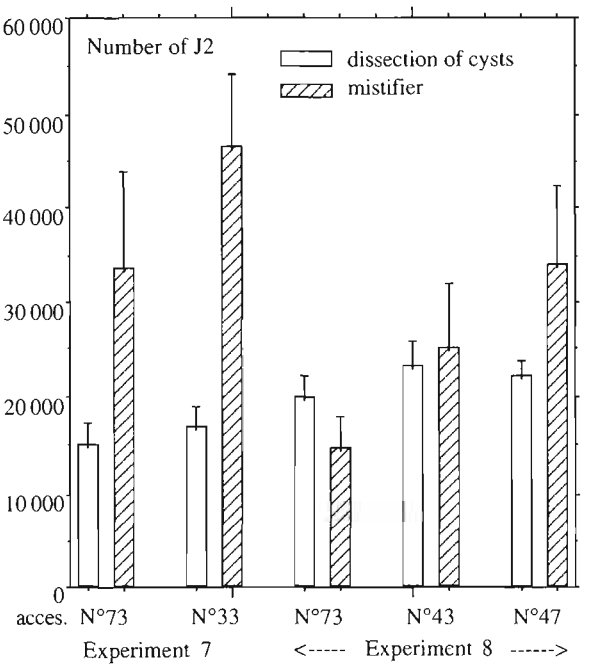

Fig. 2. *Comparison of the numbers of living J2 of* Heterodera sacchari *released from 60 cysts dissected in potassium permanganate (dissection of cysts) and from roots and debris in the mistifier in susceptible accessions of rice: no. 73 and 33 in experiment no. 7 and no. 73, 43 and 47 in experiment no. 8 (Mean + SD of five replicates for each experiment).*

PENETRATION OF J2 IN RICE ROOTS

Almost 50 % of the J2 inoculated invaded roots of the reference accession Morobérékan. At the time of fixation, 8 days after inoculation, most of the invading juveniles were at the 3rd juvenile stage. In roots of the resistant rice accession LG 118 and of *S. vulgare*, nearly all invading juvenile remained at the second stage and the rate of invasion was low: 20 and 32 % in these two plants, respectively (Fig. 3). In *V. radiata*, no J2 were observed in the roots and this plant can be considered as immune.

Discussion

The variation in the mean numbers of J2 released from 60 cysts with the reference accession Morobérékan from one experiment to another (Fig. 1) is possibly related to external conditions in experiments 1-11, for which the Congolese population was used, and to the population itself (C, T, or S) in experiments 1-15. Considering the effect of external conditions, the ambient temperature to which shoots were submitted varied from 20 to 30 °C according to the season, whereas soil and roots were maintained at a temperature of 28°C or above. Light intensity was also very dependent on the season. Other causes of variation included length of the experiment, from J2 inoculation to cysts recovery, and quality of the inoculum, depending on the duration of cyst quiescence in Dropkin's solution before hatching and/or use of potassium permanganate for artificial hatching, since artificially hatched J2 seem to be less invasive than naturally hatched ones. The variability of the reference accession from one experiment to the other compelled us to consider each of the fifteen experiments independently, with the reference accession Morobé-rékan used as a qualitative control of the inoculum.

It would be interesting to grow cultivars of *O. glaberrima* or interspecific hybrid cultivars in which the resistance gene has been introduced on soils infested by *H. sacchari* , since resistance was found in *O. glaberrima* and not in *O. sativa*. The breeding of rice for pest resistance is increasingly used and some successes have been reported, especially for diseases caused by fungi and bacteria (Bonman *et al.*, 1992). The agronomic performances of *O. glaberrima*, however, are lower than those of *O. sativa* (Clément & Koffi Goli, 1987) and the use of interspecific hybrid cultivars with *O. sativa* seems to be a more promising approach. Introgression between *O. sativa* and *O. glaberrima* appears rather difficult since F1 is male sterile

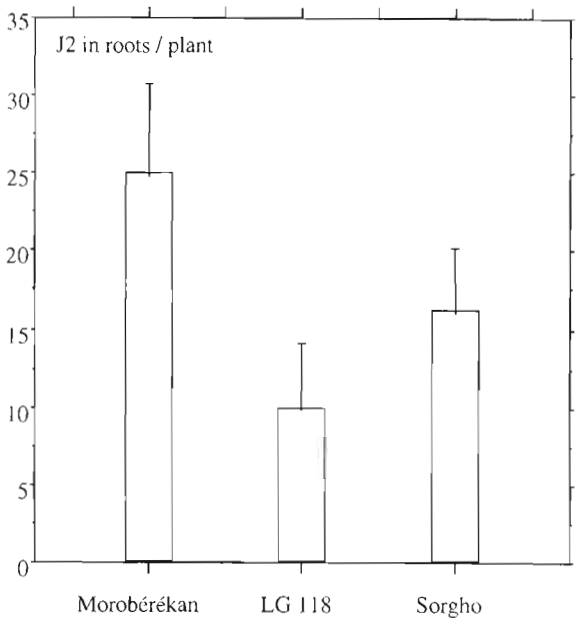

Fig. 3. *Penetration of J2 of* Heterodera sacchari *in roots of* Oryza sativa *(accession no. 73, Morobérékan),* O. glaberrima *(accession no. 61, LG 118) and* Sorghum vulgare *(cv. IRAT 207) (Inoculum: 50 J2; duration of contact between roots and inoculum: 8 days; Mean + SD of twelve replicates for each plant).*

and it is necessary to introduce a backcross with the *O. sativa* parent (Ghesquière *et al.*, 1995). The same strategy, however, was used to develop wheat lines resistant to *Heterodera avenae* by breeding *Triticum aestivum* L. with resistant lines of the wild resistant related species, *Aegilops ventricosa* Tausch (Rivoal *et al.*, 1993).

In the case of rice and *H. sacchari*, the next step will be to characterize the resistance found in *O. glaberrima* during the present experiments by studying the inheritance of resistance in the progeny of *O. sativa* and *O. glaberrima* crosses. It is important to determine if this resistance is monogenic or polygenic and dominant or recessive. *O. breviligulata* is considered to be a direct wild ancestor of the domesticated species *O. glaberrima* (Bezançon *et al.*, 1989) and its use as genitor for breeding purposes now seems to be unnecessary since the resistance character is well represented in *O. glaberrima*.

Part of the screening strategy used in the present work was based on the diversity of geographical origins that exists among accessions in our *O. sativa* collection (Table 1). Because of this diversity, the accessions were presumed to be genetically different, which was considered to increase the chance for selecting resistant accessions. The results clearly showed that either this assumption was wrong or the diversity of the collection used was insufficient. Another possible approach for future screening on this rice species is to consider the occurrence of enzymatic groups in *O. sativa* and their possible linkage with susceptibility to pathogens. In an analysis of this rice species based on fifteen isozyme loci among 1688 accessions, six varietal groups (I - VI) were strongly demonstrated and characterized by contrasting multilocus types with dissimilar environmental and macrogeographic distributions (Glaszmann, 1987, 1988). In a further survey of 288 rice accessions, it was found that all accessions of group IV were resistant to the fungus *Gerlachia oryzae* (Hashioka & Yogoki) W. Gams that causes the leaf scald disease of rice in Asia (Bonman *et al.*, 1990). This group IV of *O. sativa* accessions consists of floating rice of Bangladesh with unusually long life cycle, during which they are probably subjected to strong selection pressure for disease resistance. It would be interesting to examine the susceptibility to *H. sacchari* among these six groups and especially in group IV.

This study did not reveal any pathotypes in *H. sacchari*. It must continue with others populations of *H. sacchari*, including: *i*) other African populations of this species, *ii*) populations from Asia, *iii*) related species of rice cyst nematodes, especially *H. oryzae*, which is considered by Netscher (1969) as a potential ancestor of *H. sacchari*. Both species of nematodes are very close to each other but have different behaviour during rice root invasion (Reversat & Bois, 1982).

A preliminary study comparing the behaviour of the two species *H. oryzae* and *H. sacchari* (population S) on the reference accession Morobérékan and on an unidentified accession of *O. glaberrima* showed that the *O. glaberrima* accession was susceptible to *H. oryzae* and resistant to *H. sacchari* (Reversat, unpubl.).

Contrary to the conditions prevailing in tropical areas, soils in temperate areas are typically infested by only one cyst nematode species, which supports the use of resistant cultivars against these parasites. The success of this strategy is now well established against such cyst nematodes as *Globodera rostochiensis*, *Heterodera schachtii,* and *H. avenae* (Evans *et al.*, 1993). On the contrary, multispecific nematode complexes are the rule in tropical areas (Luc & Reversat, 1985) and a cultivar resistant to one species of the complex is not necessarily resistant to the other species. Therefore, sources of resistance against these other species are needed.

In Africa south of the Sahara, rice is cultivated either in lowland or in upland fields and *H. sacchari* occurs in both cropping systems (Merny, 1970; Fortuner & Merny, 1973).

The simultaneous occurrence of one of the two main African *Hirschmanniella* species (*H. oryzae* or *H.*

spinicaudata) with *H. sacchari* is common in lowland rice fields (Merny, 1970; Fortuner & Merny, 1973). Unfortunately, references on sources of true resistance to *Hirschmanniella* sp. among rice accessions remain rather scarce (Sahu & Das, 1988; Bridge *et al.*, 1990).

The presence of root knot nematode, mainly *Meloidogyne incognita* and *M. javanica* , in sub-Saharan Africa is frequent in upland rice fields (Bridge *et al.*, 1990). *M. incognita* is highly pathogenic against rice (Diomandé, 1981) and some *O. glaberrima* accessions exhibited a high level of resistance against this nematode during laboratory tests (Diomandé, 1984). Three of these, CG 13, CG 18 and CG 74, were also resistant to *H. sacchari* (Table 2). This suggests that the genetic support of resistance against the two nematode species could be the same. On the contrary, none of Indian rice accessions (*O. sativa*), resistant to another rice cyst nematode, *Heterodera oryzicola*, were resistant to *Meloidogyne graminicola*, an important pest of flooded rice in Asia (Prasad *et al.*, 1986).

Accessions resistant to *H. sacchari* were found only in *O. glaberrima* and *O. breviligulata,* two rice species with a natural distribution area identical to that of *H. sacchari*. Thus, the resistance observed could be due to coevolution. The susceptibility of some *O. glaberrima* accessions could be due to spontaneous introgression from *O. sativa*, which has been cultivated for several centuries in the same Africa regions as *O. glaberrima* (Bezançon & Second, 1984). On the other hand, we suggest that, in *O. glaberrima*, susceptibility with tolerance might represent the alternative to resistance as the result of coevolution.

The technique used here for the screening of resistant accessions is rather time consuming. Collection and dissection of 60 cysts from susceptible plants were made to check that female growth and egg production were never dissociated under certain feeding stresses on particular accessions of rice. Despite some variations observed in the J2 content of 60 cysts (Tables 1-3), clear evidence of a strong dissociation between these phenomena never occurred. In future studies, the routine test in resistance selection for cyst nematodes, the 'root-ball' observation, would probably be sufficient: the root-ball with soil is extracted from the culture pot and the qualitative presence of cysts is checked by direct naked-eye observation, or cysts are counted with the help of a low-power lens (Southey, 1986).

The technique used in this work has been developed only for the screening of resistant accessions. Nevertheless, the yield of cysts from susceptible accessions of the three rice species was very variable, despite the uniformity of inoculum level and development time after inoculation. For some accessions, the sampling of 60 cysts was performed within minutes, whereas a lengthy exploration of a significant part of the debris was necessary to obtain this number of cysts with other accessions. We suggest that this could be due to interaction between susceptibility and tolerance because the duration of the experiments (10-12 weeks) was more than twice as long as the life cycle of *H. sacchari* (5 weeks), which allowed the pathogenic effect to appear. In tolerant accessions, the root system would be in good condition at the end of the first nematode generation, and able to produce a great yield of cysts at the end of the second generation. In contrast, the bad condition of the root system in non-tolerant accessions at the end of the first nematode generation would result in a weak yield of cysts at the end of the second generation. Thus, some of the susceptible accessions might be tolerant when others might be truly susceptible (*i.e.*, non-tolerant), and it would be interesting to classify the susceptible rice accessions according to the actual value of the final nematode population. For this purpose, the hand-picking of every cyst in debris and roots for further dissection in potassium permanganate is too time-consuming, whereas the treatment of the whole material (debris and roots) in the mistifier probably underestimate the final population, since the hatching of all eggs contained in cysts cannot be completed within 4 weeks. This means that a new technical approach will be needed. Tolerance is not necessarily a useful property on infested soils with a monocropping system, but could explain the population dynamics of *H. sacchari* in the field.

The mechanism of resistance of *O. glaberrima* to *H. sacchari* remains unknown. In most of the resistant accessions, except accessions no. 61 and no. 71, some J2 were recovered in the mistifier (Tables 2, 3), which indicates that J2 invaded rice roots and developed to the female stage, but at a drastically lower rate than in susceptible accessions. This was confirmed by the results of the host roots invasion study: the rate of invasion was 20% for LG 118 compared with 50% for Morobérékan (Fig. 3). It is possible that *H. sacchari* J2 invade the roots of resistant rice accessions, then leave because they are unable to get established, as demonstrated in the case of *M. incognita* J2 in roots of different resistant plants (de Guiran, 1960).

Acknowledgments

The authors are fully indebted to G. Delbosc, J.C. Glaszmann, J.L. Notteghem, C. Poisson, and R. Vandevenne from CIRAD and G. Bezançon, J.C. Clément, A. Ghesquières, and M. Lorieux from ORSTOM for kindly supplying rice seeds and helpful comments. The authors thank E. Goma and S. Diabasana for the making of culture tubes and thermostatically controlled boxes and for technical assistance during the study.

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