Fungi on roots of dryland rice continuously cropped in the Philippines

Although dryland rice soil sickness caused by continuous cropping is well known in Japan, there have been no clear reports on its occurrence outside Japan for many years. Recently, however, its occurrence was confirmed in dryland rice fields of the International Rice Research Institute, Los Baños, Philippines. The fields were well irrigated, fertilized, and continuously cropped twice a year. The yield reduction caused by continuous cropping was much more remarkable than in Japan.[8] Because fungi seemed the most likely cause[1], we examined fungal flora on roots of dryland rice (IR-2061-464-2-4) grown continuously or in rotation at IRRI. Cultural treatments[8] of the plots are briefly shown in Table 1.

1) Mycelial length on the root surface of dryland rice

If fungus is a causal agent, it should be more abundant on and in roots of the affected plants than in roots of the control plants. Therefore, mycelial length on the root surface of the crown region of the basal roots was examined.

Basal roots longer than 5 cm, excluding seed roots, were gently shaken to remove soil particles in distilled water. The crown regions were sectioned along the center with a shaving blade. The slices, approximately 2 cm long, were composed of the epidermis and cortex; some also had a part of stele. After being stained with phenolic aniline blue for 5 min, the mycelia on the root surface and part of the epidermis was measured for length by the line intercept technique under the microscope (×256).

At 17 days after seeding, there was no significant difference in mycelial length on roots, nor in plant growth between the continuously cropped and the control plants (Table 2). However, the 47-day-old plants in the continuously cropped plot were significantly shorter and less green (Plate 1), and had 3.5 times denser fungal mycelia than the plants in the control plot. The greater density of mycelia observed, similar to that obtained in Japan (Nishio and Kusano, unpublised), suggested fungi as a causal agent.

Fungi of brown mycelia attract special attention, because their length increased by 10 times during an additional 30 days of cultivation in the continuously cropped plot. Most of them were 2.5 μm in diameter and septated (Plate 2). Some had penetrated into the cortex and stele.

Table 1. Cultural treatments of continuously cropped dryland rice plots, IRRI, 1979

Plot No.	Date continuous cropping began	Previous crops	Sowing date
1 a	June, 1974	11 DR+DR*	June 9
2 a	June, 1974	3 F+1 DR+2 F +1 DR+5 F+DR*	June 9
3 a	June, 1974	4 S+3 M+2 F +1 M+DR*	June 9
1 b	June, 1974	12 DR+DR*	July 13 & 14
2 b	June, 1974	3 DR+1 M+1 F +2 DR+1 F+1 DR +3 F+DR*	July 13 & 14

* Present crop
DR: Dryland rice, F: Fallow, S: Sorghum, M: Mungbean
All plots were in Block IV of 4 replications.

Table 2. Mycelial length on roots of dryland rice determined under the microscope

Mycelia	Mycelial length* (mm/mm^2)			
	17 days after sowing		47 days after sowing	
	Control plant (Plot 2b)	Continuously cropped plants (Plot 1b)	Control plant (Plot 2a)	Continuously cropped plant (Plot 1a)
Brown	0.73	0.33	0.80	3.61
Hyaline	2.38	1.86	1.17	3.85
Total	3.11	2.19	1.97	7.46

* Mycelial length was determined by observing 288 microscopic fields (=8 plants×3 roots×12 microscopic fields).

Plate 1 Dryland rice 47 days after sowing
Right: the continuously cropped plants
Left: the control ones

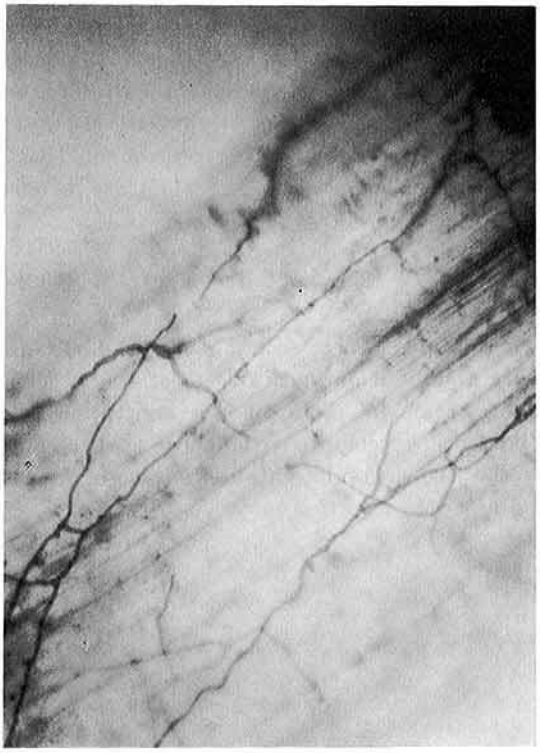

Plate 2 Brown mycelia creeping on root-surface of continuously cropped dryland rice

2) Fungal genera on the root surface of dryland rice

Serially washed root segments from 45- and 53-day-old plants were planted on several kinds of agar plates.

About 40 basal roots, excluding seed roots, were randomly selected from 15 plants on each plot, and 3-cm samples were collected from the crown region. Each of the halves of the root sample was serially washed with 17 changes of sterilized water. Each washing involved vigorous shaking by hand for 1 min. The roots were aseptically cut into 2-mm segments with scalpel. Each segment was planted on the agar plate and incubated at room temperature (25–30°C) for 4–10 days to determine the genus of the fungi grown from it. Most of the fungi isolated by this method are considered to have been present on the root surface not as spores but as active mycelia. The frequency of occurrence of each genus was expressed as the fungal activity: the total number of isolates of each genus×100/total number of segments planted.

The dominant genera on roots of dryland rice were found considerably simple in the Philippines. In Japan the dominant genera are *Fusarium*, *Pyrenochaeta*, and *Pythium* as well as sterile forms[5,6]; in the Philippines only *Fusarium* and sterile forms were abundant (Table 3). *Pyrenochaeta* and *Pythium* were not detected at all, even when a total of 900 root segments from the continuously cropped and the control plants were planted on Czapek-Dox agar supplemented with rose-bengal and streptomycin, potato dextrose agar (Difco) with streptomycin, and water agar. Neither was *Phytophtora*, uncommon on roots of dryland rice in Japan found on 360 root segments planted on the medium selective for it.[4]

Table 3. Frequency of occurrence of fungi isolated from washed root segments of dryland rice using Czapek-Dox agar medium

Fungi	Fungal activity*		
	Control plants**		Continuously cropped plants** (Plot 1a)
	Plot 2a	Plot 3a	
Fusarium	23.3	9.8	12.9
Curvularia	0.8	3.6	0.8
Monocillium	3.3	3.6	3.0
Aspergillus	1.7	0.9	0
Penicillium	1.7	0	0
Other genera***	0	1.8	0.8
Sterile hyaline	38.3	33.0	24.2
Sterile brown	18.3	21.4	31.8
Unidentified	2.5	0.9	0
Segments planted (No.)	120	112	132
Total No. isolates	109	84	97
Segments colonized (%)	87.5	71.4	72.7

* Total No. isolates of each genus × 100 / Total No. segments planted

** 47 days after sowing.

*** Other genera included *Cephalosporium*, *Cylindrocarpon*, *Mucoraceae*, and *Trichoderma*.

The absence of *Pyrenochaeta* on roots in IRRI fields does not mean its complete absence in the tropics, because the fungus was recently found in Bogor, Indonesia. It occurred abundantly on the root surface of dryland rice cultured in pots containing soil from the field successively cropped with dryland rice for 2 growing seasons (Nishio, unpublished). The distribution of *Pyrenochaeta* on roots of dryland rice in the tropics and the difference in dominant fungal genera between Japan and the Philippines offer interesting problems.

Sterile fungi of brown mycelia tended to increase with continuous cropping (Table 3). This tendency agrees with the increase in length of brown mycelia (Table 2), suggesting involvement of an unidentified fungus (or

Table 4. Frequency of occurence of *Fusarium* isolated from washed root segments of dryland rice using selective medium

	Fungal activity*	
	Control plant** (Plot 2a)	Continuously cropped plant** (Plot 1a)
F. oxysporum	18.8 (83.3%)	2.5 (11.5%)
F. solani	1.7 (7.4%)	1.7 (7.7%)
F. moniliforme	2.1 (9.2%)	17.5 (80.8%)
Segments planted (No.)	240	240
Total No. *Fusarium* isolated	54 (100.0%)	52 (100.0%)
Segments colonized (%)	22.0	20.4

* Total No. isolates of each genus × 100 / Total No. segments planted

** 47 days after sowing.

fungi) of brown mycelia in dryland rice soil sickness.

4) Species composition of Fusarium on the root surface of dryland rice

The species composition of *Fusarium*, the only dominant genus identified, in addition to sterile forms, on roots of dryland rice in IRRI fields was examined on washed root segments planted on an agar plate selective for Fusarium.[3] Table 4 shows that the species composition of *Fusarium* was distinctly influenced by previous cropping history: *F. oxysporum* was predominant on roots of the control rice, *F. moniliforme*, on roots of continuously cropped rice.

This result strongly suggests the involvement of *F. moniliforme* in dryland rice soil sickness, because *F. moniliforme* is known as a pathogen not only of bakanae of wetland rice but also of foot rot of dryland rice: the latter caused a serious damage in the 1950's in Japan.[2] *F. moniliforme* is well known as a seed-borne pathogen, although it may also be soil-transmitted.[9] However, in the IRRI fields where dryland rice was continuously cropped with a shorter interval between crops than in Japan, the fungus can probably survive on the plant residues in soil between two growing seasons and inhibit the growth of dryland rice. *F. moniliforme*, which causes foot rot of dryland rice, survived on heavily infested plant residues on and in soils.[2,7] The pathogenicity of *F. moniliforme* found in IRRI fields is now being studied.

In conclusion, dryland rice soil sickness in IRRI fields seems to be caused by fungi different from those of Japan. An unidentified fungus of brown mycelia and *F. moniliforme* were suspected as causal agents.

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