

台湾産サトウキビ黒穂病 VI

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Culmicolous Smut of Sugar Cane in Taiwan (VI) New Pathogenic Strain Obtained by Artificial Hybridization and Further Studies on Compatibility of *Ustilago scitaminea* Sydow*

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呂 理榮** : 台湾産サトウキビ黒穂病(VI)人工交配で得た *Ustilago scitaminea* Sydow の新病原性系統並びにその親和性の再研究

Abstract

A new pathogenic strain No. 3 was obtained by artificial hybridization of sporidia of *Ustilago scitaminea* between pathogenic strains No. 1 and No. 2. Pathogenic strain No. 3 attacked both NCo310 and F134, which were susceptible only to strain No. 1 and strain No. 2, respectively.

If the inoculated cuttings were kept in room temperature (26-28 C) for a few days before planting, only one of the two strains (either No. 1 or No. 2) could be recovered from the newly developed whips resulting from the inoculation with a mixture of teliospores of both strains on the compatible varieties.

Further studies on the compatibility of *U. scitaminea* revealed that all tested 5 sets of sporidia isolated from 5 teliospores showed two mating types with two allele systems. Compatible matings resulted in mycelial growth on colonies.

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Introduction

Two pathogenic strains of *Ustilago scitaminea* Sydow have been reported in Taiwan^{2,4)} and more recently in Hawaii¹⁾. In Taiwan strains No. 1 and No. 2 induce whiplash-like structure on sugarcane varieties NCo310 and F134, respectively. However, some varieties, such as F160 and F173, could be induced to produce whips by both strains. Thus the possibility of raising a new pathogenic strain has been studied²⁾. The present paper reports the successful development of a new pathogenic strain by artificial hybridization of *U. scitaminea* and results of further studies on the compatibility of this fungus.

Materials and Methods

Single sporidia were isolated by a glass needle from single promycelia of germinated teliospores on 3.5 percent water agar incubated at 18 C. Teliospores were collected from the whips of either NCo310 or F134. All sporidia isolated were those of small size and derived from each of the 4 cells of the promycelium. The sporidia were cultured on potato dextrose agar (PDA) and incubated at 26 C. Thick sporidial

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suspension was prepared with sterilized water and inoculation was made to the bud on single-eye cuttings. The bud, 1-2 cm in height, was first pricked by a needle to make 10 holes and then smeared with cotton balls wetted with the sporidial suspension. In case of hybridization, the bud was inoculated twice using separate cotton ball respectively wetted with the two different test isolates.

Teliospores from single whips of NCo310 and F134 plants originally inoculated with the sporidial cultures were also used directly for inoculation. The teliospores from either NCo310 or F134, or a mixture of same amounts of teliospores from NCo310 and F134, were inoculated to NCo310, F134, F160, and F173. After the whips appeared, the teliospores from a single whip were collected and inoculated to NCo310, F134, F160, and F173.

In each case, 10 single-eye cuttings of each variety were inoculated. After inoculation, the cuttings were put in a plastic bag and incubated at 26-28 C for 2 to 3 days. They were then planted in 12.7 cm pots, usually 2 to 3 cuttings to a pot, and kept in the greenhouse or in the field. Inspections were conducted regularly.

Only those which produced whips were recognized as a criterion for the disease.

For compatibility test *in vitro*, the isolates of the single sporidial cultures were paired in all possible combinations by mixing 2 isolates on PDA plate. The plates were then incubated at 26 or 30 C and examined daily up to 4 days.

Results

Inoculation with sporidial cultures

Compatibility of strain No. 1 and strain No. 2 is shown in Tables 1 and 2, respectively, while the compatibility among the sporidial cultures obtained from strain No. 1 and No. 2 in inducing whips on F160 is shown in Table 3.

The teliospores from the single whips on F160 which had been induced by the mating of the sporidial cultures of strains No. 1 and No. 2 were further used for inoculation. In all cases, whips appeared on NCo310, F134, F160, and F173 from the teliospores collected from the same whip. Thus a new pathogenic strain No. 3 was obtained.

Inoculation with teliospores

Teliospores from NCo310 and F134 obtained from the above tests (Table 1 and 2) were mixed in equal amounts and inoculated. After the whips appeared, the teliospores from single whips were collected and inoculated to NCo310, F134, F160, and F173. The teliospores collected from the whips on NCo310 induced whips only on NCo310, F160, and F173 but not on F134. Those from F134 induced whips only on F134, F160, and F173 but not on NCo310. While the teliospores from the whips of F160 and F173 induced whips on F160 and F173 in all cases and either on NCo 310 or

Table 1. Compatibility of strain No.1 isolated from whip produced on NCo310 and tested on the same variety

Isolates	1-102-1	1-102-2	1-102-3	1-102-4
1-102-1	-	+	+	-
1-102-2		-	-	+
1-102-3			-	+
1-102-4				-

+ Whips produced, - No pathogenicity

Table 2. Compatibility of strain No.2 isolated from whip produced on F134 and tested on the same variety

Isolates	2-101-1	2-101-2	2-101-3
2-101-1	-	+	-
2-101-2		-	+
2-101-3			-

+ Whips produced, - No pathogenicity.

Table 3. Compatibility between strain No. 1 and strain No.2 tested on F160

Isolates	1-102-1	1-102-2	1-102-3	1-102-4
2-101-1	-	+	+	-
2-101-2	+	-	-	+
2-101-3	+	-	-	+

+ Whips produced, - No pathogenicity.

Compatibility test in vitro

After the sporidial isolates were paired on PDA, white mycelium started to grow in 1-4 days if they were compatible. If incompatible, the paired isolates as well as those of single sporidial cultures, still grew by budding which gave yeast-like colonies. Both strain No. 1 and strain No. 2 showed two mating types with two allele systems of compatibility, and so was it for all combinations among the isolates of

F134 alone but never on both of them. Five whips were used in each case.

Whips formation was noted on over one-half and in some cases all of the inoculated plants. Under conditions of this experiment, mixtures of the teliospores of strains No.1 and No. 2 failed to breed new pathogenic strain.

Table 4. Compatibility of strains No. 1 (101-x, and 102-x) and strain No.2 (201-x) isolated, respectively, from NCo310 and F134 on potato dextrose agar

Iso-lates	101-1	101-2	101-3	101-4	102-1	102-2	102-3	102-4	201-1	201-2	201-3	201-4
101-1	-	+	-	+	+	+	-	-	+	+	-	-
101-2		-	+	-	-	-	+	+	-	-	+	+
101-3			-	+	+	+	-	-	+	+	-	-
101-4				-	-	-	+	+	-	-	+	+
102-1					-	-	+	+	-	-	+	+
102-2						-	+	+	-	-	+	+
102-3							-	-	+	+	-	-
102-4								-	+	+	-	-
201-1									-	-	+	+
201-2										-	+	+
201-3											-	-
201-4												-

+ Mating type where mycelia grew from paired isolates.

- No mycelia grew, colonies remained to be yeast-like.

No. 1 and No. 2 strains (Table 4). All compatible combinations induced whips on F160 after inoculation, while incompatible matings did not.

Discussion

Four single sporidial cultures of *U. scitaminea* which were derived from 4-cell promycelium that germinated from single teliospores could not induce smut on sugarcane if inoculated alone. Thus it is feasible to inoculate sugarcane artificially by combining sporidial cultures originated from different strains. The pathogenicity of the resultant teliospores from the whips was proved to be positive to both varieties which are thus far susceptible only to either strain No. 1 or No. 2, while each of them could induce whips only on NCo310 and F134, respectively. However, using the 1:1 mixture of teliospores of the two strains failed to develop a new pathogenic

strain. It is reasonable to speculate that since the inoculated buds were incubated at 26-28 C, at which the teliospores germinated directly with the formation of hyphae or 2 big-size sporidia, which have been proved to be solopathogenic³⁾. Any chance of matings between different strains by small-size sporidia would have been eliminated. Incubating the inoculated buds at lower temperatures to test the possibility of raising new pathogenic strains, therefore, is worth trying. As discussed before⁴⁾, it is possible that many pathogenic strains of *U. scitaminea* already exist somewhere in the world. More new pathogenic strains could be raised as with other smuts⁵⁾ if a precaution is not taken to prevent the introduction of foreign strains to the country. Although this investigation discovered that a new pathogenic strain No. 3 could be bred by mating strains No. 1 and No. 2 through artificial inoculation, there is no proof that this new strain also exists in the field. Recently culmicolous smut has been found on NCo310 in Taitung area, where only strain No. 2, which does not attack NCo310, has been recorded. Preliminary studies revealed that the teliospores collected from NCo310 in Taitung area were identical to those of strain No. 1 (Wang, Z. N., personal communication). This is the first time that presence of both strains in the same area was detected in Taiwan. Whether a new pathogenic strain will arise in the field needs further investigation.

Mating types of 5 sets of isolates revealed two allele systems with two mating types of compatibility, as reported earlier with the use of 33 sporidia isolates from 12 teliospores²⁾. This is different from the tetrapolar system³⁾ reported before. Whether tetrapolar system is a rare occasion needs to be further studied. In vitro test, it is possible to elucidate compatibility within a few days and furthermore, the dikaryotic mycelia could grow in an artificial media. Thus the fungus is an ideal organism for this type of study.

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和 文 摘 要

台湾産サトウキビ黒穂病 (VI) 人工交配で得た *Ustilago scitaminea* Sydow の新病原性系統並びにその親和性の再研究

呂 理 榮

Ustilago scitaminea の1号菌と2号菌の担胞子を人工交配すると新しい病原性系統3号菌が得られる。3号菌は品種NCo310及びF134を侵す。親の1号菌はNCo310に、2号菌はF134に病原性が有るだけである。

1号菌及び2号菌の黒穂胞子を等量混合し、感受性品種の芽に接種後、室温(26-28C)に数日置き、それから植えると、出て来た黒穂からは1号菌あるいは2号菌のどちらか一方だけが検出された。

本菌親和性の再研究のため、5個の黒穂胞子から5組の単一担胞子を分離、培養後交配した結果、本菌の親和性は一対の対立因子から成る二極性である事が示された。親和性の組合せではPDA上で菌糸の生長が見られた。不親和性の組合せでは酵母状の粘質集落の状態にとどまった。