ON THE SEXUALITY OF THE WHEAT SCAB FUNGUS, GIBBERELLA ZEAE (SCH.) PETCH*

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I. Introduction

Dodge (1) demonstrated that *Neurospora tetrasperma*, which developed asci with four bisexual spores, was normally homothallic; while *Neurospora sitophila* and *N. crassa*, in which the asci were eight spored, was heterothallic. The heterothallism of some species of *Ceratostomella* and *Glomerella* has been shown by Buisman and others. The sexuality of *Gibberella Zae* (Sch.) Petch=*G. Saubinetii* was studied by Eide, who secured 72 isolates from the hyphal tips cut of the germ tubes of germinating conidia and ascospores. As the isolates produced perithecia, with 2 exceptions, he concluded *Gibberella Saubinetii* to be homothallic. The present writers (6) have also studied on the sexuality of the wheat scab fungus, *Gibberella Zeae*.

II. Materials and Methods

In the present experiments, single ascospore isolations were made from the perithecia secured from the following four sources:

A) Perithecia formed on steamed rice straw cultured in a reagent glass bottle by the fungus No. 2352 of the Ohara Institute. This line was originally isolated from a scabbed wheat ear collected in the prefecture of Tokushima, July 1951.

B) Perithecia produced on a diseased ear of Huron Ottawa wheat grown in the experimental field of the Institute.

C) Perithecia found on a rice straw pile of a field near the Institute.

D) Perithecia formed on an ear of an unknown variety of wheat grown in a greenhouse of the Institute.

From the sources A, B, C and D, the writers isolated 24, 12, 30 and 58 single spores respectively. For isolating the spores, the method devised by the senior writer (3) was used, in which the perithecia, first washed carefully with repeated changes of sterile water, were crushed with a sterilized needle on a sterilized slide to release the ascospores. These ascospores were diluted and smeared over the surface of agar plates in petri dishes.

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By placing the petri dishes in an inverted position on the stage of the microscope, each ascospore that has shown germination was marked at the bottom of the dish with ink. The portions of the agar thus selected with single spores were cut with a platinum micro-ring 2mm in diameter and finally fished out with a micro-spatula into potato-dextrose agar slants.

To test the formation of perithecia, the method also reported by the senior author (4) was used. Each single ascospore culture or mixed culture of two isolates was inoculated to the steamed rice straw stalks placed in reagent glass bottles and allowed to grow for about two weeks at 30°C. When the stalks were fully covered with the mycelium of the fungus, they were transferred to a beaker 18cm in height and 5.5cm in diameter for the formation of perithecia. These beakers, after adding a small amount of water and covering with a thin Japanese rice paper, were placed near a window partially exposed to the sunshine.

III. Experimental Results

The perithecium formation in the mixed cultures of two single ascospore isolates was studied by the method described above. The results are given in table 1. In this table the perithecium formation is expressed by the number of plus signs for density and plus minus sign for a very poor formation.

Table I. *The perithecium formation in the mixed cultures of each two of the single ascospore isolates of Gibberella Zeae* Petch.

A_1 ×		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
		+*	++*	++*	+++	+++	++	++	++*	++*	++	++	±	+	±*	+*	++	+	+*	+*	+*
	B								C												
		5	6	7	8	10	11	12	18	19	20	21	22	23	24	25	26	27	28	29	30
		+	+	±*	+	+	+	+	±	±*	±	±*	+*	±	±	+	±*	±	±	+	±*
	D																				
		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
		+++	+*	+	+	++	++	++	+	++*	±	++	+*	++*	+*	++	++	±*	±*	±	±
	A													B							
		1	2	3	4	5	6	7	8	9	10	11	12	4	5	6	7	8	9	10	12
		±*	+	+	+	++	+	+	+	+	±*	+	±*	±*	±	+*	±*	±*	+	±*	++
	C																				
B_1 ×		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
		±*	±*	±	±*	±	±	+	±*	±*	++	+	+	+	+	±*	±*	±*	±*	±*	±
	D																				
		21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
		++	+	++	++	+	±	++*	±*	±*	+	±*	+	++	++	+	++	+	++	±	±
	A													B							
		1	2	3	4	5	6	7	8	9	10	11	12	5	6	7	8	9	10	11	12
		++	+	±	++	±*	++	±*	+*	++	+	+	+*	+	+	+	++	+	+	±*	+
C_1 ×	C																				
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	22
		±*	±*	±	++*	±	+	+	±	±	+	++	+	+	±*	±*	+	++	++	++	±

Remarks: $A_1 \times B_{5-12}$, $B_1 \times B_{4-12}$ are the results observed after 2 days' incubation in the tall beakers; $A_1 \times C_{13-30}$, $B_1 \times A_{1-12}$, $B_1 \times C_{2-15}$, $C_1 \times A_{1-12}$, $C_1 \times B_{5-12}$, $C_1 \times C_{2-22}$ are the results after 3 days' incubation; and $A_1 \times A_{2-21}$, $A_1 \times D_{21-40}$, $B_1 \times D_{21-40}$ are the results after 4 days' incubation.

* An asterisk shows the culture, in which pretty good perithecium formation was observed in a week's incubation in the regent glass, after the medium was transferred from the tall beaker.

Resuts on similar experiments for the individual ascospore isolates are shown in table 2. As in the case of the mixed cultures, the single spore isolates also produced perithecia, but their density varied with the isolate. Mature ascospores were observed in all the perithecia formed.

Table II. *The perithecium formation in the pure cultures of each of the single ascospore isolates of Gibberella Zeae* Petch.

A																			
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
++*	+	++*	±	++*	++*	+	++	±	+	++*	++*	+	+	+*	+*	+	++	++	+*	+
A			B												C					
22	23	24	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6
+	±	++	+++	++*	++*	++	+++	++*	++	+*	++*	++*	++*	++	+*	+	±	±	+	±*
C																				
7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
+*	++*	±*	+	+++	+	±*	+	++*	+*	+*	+	+*	±*	+*	+*	+	+*	+	+*	+*
C			D																	
28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
±*	±*	+	++	++*	++	++*	++	+++	++	+	++*	++	++	++	++	++	++	++	++	+
D																				
19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
++	+*	++*	++*	+	+*	+*	++	+	++	++	±	±	++*	+*	+*	±*	++*	++*	++	++
D																				
40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58		
+*	++	+*	+	+*	+*	+	+	+	±	±*	±	++	+*	++	+*	±*	++*	+*		

Remarks: A_1—A_{24}, B_1—B_{12}, D_1—D_{38} show the results observed after 4 days' incubation in the tall beakers and C_1—C_{30} those after 3 days' incubation.

* An asterisk shows that the culture produced the perithecia pretty well after a week's incubation in the reagent glass used.

The results of the experiments given above show that all of the 124 single ascospore isolates without exception produced perithecia and mature ascospores. They lead to the conclusion that *Gibberella Zeae* Petch is homothallic, as shown by Eide (2).

IV. Summary.

1. The present paper deals with the sexuality of the wheat-scab fungus, *Gibberella Zeae* (Sch.) Petch=*Gibberella Saubinetii* (Mont.) Sacc.

2. 124 single ascospore isolations were made from four different sources of perithecia.

3. The perithecium formation was tested on the isolates or mixed cultures of two of them by first growing them on steamed rice straw stalks in reagent glass bottles, and then transferring the stalks to a tall beaker when they have shown a growth covering the entire surface of the stalk with the fungus mycelium after filling with a small amount of water and covered with a sheet of thin Japanese rice paper. The beakers were set near a window partially exposed to sunshine.

4. By this method, mixed cultures as well as individual ascopsore isolates produced perithecia and matured ascospores. This demonstrated that the isolates of *Gibberella Zeae* (Sch.) Petch is homothallic, as shown by Eide.

Literature Cited

(1) Dodge, B. O. (1927) Nuclear phenomena associated with heterothallism and homothallism in the Ascomycete *Neurospora*. Jour. Agr. Res. 35 (4) : 289-305.

(2) Eide, C. G. (1935) Pathogenicity and genetics of *Gibberella saubinetii* (Mont.) Sacc. Univ. Minn., Agr. Exp. Sta., Tech. Bull. 106, 67pp.

(3) Nisikado, Y. (1938) On a simple method for isolating single spore culture of fungi. Agriculture and Horticulture 13 (1) : 96-102. (In Japanese)

(4) Nisikado, Y., K. Hirata & K. Kimura (1938) On the perithecium formation of the wheat scab fungus, *Gibberela saubinetii* (Mont.) Sacc. Nogaku Kenkyu (Japanese Edition of the Report of Ohara Instit. for Agr. Res.) 30 : 415-443.

(5) Nisikado, Y. (1940) Reviews on the knowledge of the sexuality of plant pathogenic fungi. Plants and Animals 8 : 167-172. (In Japanese)

(6) Nisikado, Y. & T. Inouye (1953) On the sexuality of the wheat scab fungus, *Gibberella Zeae*. Nogaku Kenkyu (Japanese Edition of the Ohara Institute for Agr. Research) 40 (4) : 191-194. (In Japanese)