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Mass Inoculation Technique of *Fusarium* Disease in Rice

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褐色葉枯病의 人工接種法에 關한 硏究

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적 요

갈색엽고병(*Fusarium nivale*)의 효과적인 접종방법을 찾아내기 위해 본 시험을 수행하였던 바 다음과 같은 결과를 얻었다.

1) 본 균의 분생포자의 증류수 현탁액을 벼 유묘에 분무접종할 때, 접종 후 습실에 1일 보존했을 경우에는 거의 발병이 되지 않았으나 2일간 보존한 경우에는 어느정도 병반이 나타났다.
2) 벼 유묘에 바람이나 또는 나무막대기로 물리적인 상처를 입힌 다음 증류수 포자현탁액을 분무한 결과 상당한 병반이 형성되었다.
3) 포자현탁액에 벼잎추출액, glucose, polypeptone, yeast extract 등의 1% 용액을 첨가 분무접종했을 때 병반형성이 양호하였다.
4) 포자발아상태는 증류수중에서는 불량하였으나 영양분을 첨가한 용액중에서는 발아력도 좋았고 균사의 융합도 양호하게 일어났다.
5) 본 접종시험의 결과 통일품종은 재래 장려품종인 풍광에 비해 본 병에 대해 약했다.

Introduction

Greyish or dark brown spots together with physiological discoloration on the tips of leaves at ear forming stage were observed in the new rice variety Tong-il cultivated in Radiation Research Institute in Agriculture experiment farm located at Kimpo and Keumgok in 1971. From the diseased spots fungus was isolated and identified as *Fusarium nivale* which is not reported in Korea yet.

However, studies on this fungus made slow progress, since adequate inoculation technique was not established. Development of artificial inoculation technique will play an important role in race identification, breeding for disease resistance, and practical application in mass screening.

Kawai and Mori[3] employed an inculation technique using mixture of spores with pollen and maintaining the plant considerably long at high temperature. Various attempts were made to develop inoculation technique by Hashioka and Ikegami[1] and a good result by soaking the leaves in spore suspension was also obtained by Koshimitzu *et al*[4].

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The purpose of this study was to find effective inoculation method. In an experiment to do this, artificial wounds to the leaves and the spore suspension supplemented with various nutrients for inoculation were adopted. This paper reports a newly developed effective inoculation method for *Fusarium nivale* obtained from this experiment.

Materials and Methods

Two rice varieties, Tong-il and Pung-Kwang, were employed throughout the study to clarify the relationship between wounds and inoculation. Seedlings at three-leaf stage grown in the green house were used for inoculation tests. To give the injury on leaves electric fan (for one hour) and wooden stick(flourishing on both sides) were used and the disease occurrence due to these was compared with that of control. For the preparation of spore suspension, the fungus was cultured on Potato Dextrose Agar (PDA) for about two weeks and then the surface of the colony was brushed with a hair brush to collect spores. The spore suspension was adjusted to contain 50 to 100 spores under the×300 microscopic field, adding 100 ppm of Tween 20. For inoculation, glass sprayer connected with air compressor was used and 100 m*l* of spore suspension was sprayed on 100 plants. Thereafter the inoculated plants were placed in a moist chamber at 20-28°C for two days. Spots showing evidence of disease development were counted 3 days after inoculation.

Some varieties and inoculation methods were adopted in the study of effect of nutrients added to the spore suspension on disease development. The spores suspension used in this study included distilled water, rice leaf extract solution (2 gr of leaves were chopped, and then agitated in 100m*l* distilled water), 1% glucose solution, 1% polypeptone solution, and 1% yeast extract solution. For the evaluation of disease development, spots on the second leaves showing clear symptoms were examined 3 days after inoculation.

An experiment on spore germination in various nutrients solution was made. The spore suspension was dropped on the surface of slide glass and incubated at 26°C for 7 and 14 hours, respectively. The germination of 500 spores were examined under the microscope and the germination rate was compared among the treatments.

Results and Discussion

In the summer of 1971, isolates of *Fusarium nivale* were obtained from the diseased leaves collected at a paddy field in Kimpo. They were cultured on PDA for two weeks to form spores. The collected spores were suspended in distilled water to inoculate on the leaves of rice cultivars Tong-il and Pung-kwang. The inoculated plants were kept at 25-30°C moist chamber for one or two days. No disease symptoms have appeared at all when the inoculated plants were maintained for one day in a moist chamber, whereas disease symptoms were found with the incubation for two days. Some results were observed in the repeated experiments using dense spore suspension and the pathogenicity was also maintained. From these results, it was concluded that ordinary inoculation technique are not to be applied to obtain sufficient disease development in the *Fusarium. nivale.*

Hashioka and Ikekami[1], and Koshimitzu *et al.*[4] employed an inoculation technique soaking the leaves in spore suspension by which they could induce disease. It suggests that the penetration of this fungus into leaf takes a considerably long time. On the other hand, Kawai and Mori[2] showed the possibility of disease development by using spore suspension mixed with pollens as well as making wounds with them for inoculation. It is assumed that penetration of germ tube take place easily through the wounds of leaf, and partly because of the nutrients exuded from pollen and wounds. After due consideration of these two points inoculation method was investigated.

After the leaves are wounded by wind or wooden stick, artificial inoculation was practiced and diseased lesions were counted. As shown in Table 1, only negligible disease symptoms were induced in control, while considerable infections were observed when the seedlings were treated with either wind or wooden stick. In this experiment, remarkable increase in disease occurrence due to wounds coincides well with result of Koshimitzu[5] indicating that penetration can take place

Table 1. Number of lesions on rice seedlings appeared by inoculation of water suspension of *Fusarium* spores.

Variety	Treatment	Wind		Injury		Control	
	Plot	1st leaf	2nd leaf	1st leaf	2nd leaf	1st leaf	2nd leaf
Tong-il	I	3.0	6.3	4.7	6.2	1.0	1.7
	II	2.5	3.8	6.0	6.8	0.0	1.0
	Ave.	2.8	5.1	5.4	6.5	0.05	1.4
Pung-kwang	I	3.3	—	3.2	4.0	0.3	0.1
	II	2.0	3.5	3.0	4.7	0.3	0.2
	Ave.	2.7	1.8	3.1	4.4	0.3	0.2

through stomata or wounds.

Relationship between disease occurrence and nutrients added to the suspension are shown in Table 2. In general, nutrients added spore suspension shows satisfactory development of disease symptoms in order of yeast extract, polypeptone, and leaf extract solution. From these investigations, marked varietal difference between Tong-il and Pung-kwang was observed and the variety Tong-il turned out to be susceptible to this disease.

Table 2. Number of lesions on the 2nd leaf inoculated by various *Fusarium* spore solutions in rice seedlings

Variety	Plot	Distilled water	Leaf extract solution	Glucose 1% sol.	Polypeptone 1% sol.	Yeast ext. 1% sol.
Tong-il	I	0.8	17.3	4.0	9.1	28.0
	II	1.7	26.4	8.0	41.1	45.0
	Ave.	1.3	21.9	6.0	25.1	36.5
Pung-kwang	I	0.7	17.4	6.3	7.6	14.3
	II	1.4	14.8	4.2	6.3	11.0
	Ave.	1.1	16.1	5.3	7.0	12.7

From the inoculation experiments conducted so far, it has established that either addition of nutrients or injury on leaves, or both when inoculating resulted in increase in disease occurrence. On the assumption that this increase may be ascribed to the degree of spore germination and germ tube development in suspension, spore germination test after adding various nutrients was performed. As a result, in distilled water as shown in Tables 3 and 4 poor germination were observed; 3.4% during first 7 hours and 29.3% during 14 hours in germination ratio were indicated with most of spores having short germ tube and insufficient anastomosis. Accordingly it was presumed that with distilled water-spore suspension induction to disease was difficult

Table 3. Germination ratio of *Fusarium* spores in various solutions.

Hours after begining of germ. test.		Distilled water	Leaf extract solution	Glucose 1% sol.	Polypeptone 1% sol.	Yeast extract 1% sol.
7hrs.	I	6.6%	78.6%	74.0%	76.8%	74.8%
	II	0.2	76.0	81.8	95.6	91.6
	Ave.	3.4	77.3	77.9	86.2	83.2
14hrs.	I	36.2	61.4	69.4	95.0	100
	II	22.4	70.6	61.0	95.2	100
	Ave.	29.3	66.0	65.2	95.1	100

500 spores per treatment were examined.

Table 4. Length of germination tubes and their anastomosis of *Fusarium* spores in various solutions.

Spore-suspension		Distilled water	Leaf extract solution	Glucose 1% sol.	Polypeptone 1% sol.	Yeast ext 1% sol.
Length of germ.tubes	I	1.9	3.3	2.8	2.7	3.0
after 7 hrs.	II	2.4	3.5	3.2	2.4	3.6
	Ave.	2.2	3.4	3.0	2.6	3.3
Anastomosis of germ.tubes after	14 hrs.	+	+	++	++	++−+++
	24 hrs.	+～++	+～++	+～+++	++～+++	+++～+++

Remark: grade of anastomosis

+ ——————— +++

low high

because of insufficient germination ratio and anastomosis necessary for penetration. On the contrary, good germination rate and anastomosis resulted from nutrient addition to the spore suspension. It seems that this made penetration into stomata easy, and consequently a lot of diseased spots were formed. Particularly treatment with yeast extract or polypeptone showed good disease development as well as good spore germination and anatomosis.

As clearly demonstrated in inoculation experiment, easy penetration into host plant was mainly due to the injury to the leaves and leaf sheaths and also the reasults of this study agree with reports that in Japan when continuous wind and rain prevail, this disease occurs most severely. Nutrient substances exuded from wounded area appear to be the another factor for good spore germination and mycelial anastomosis. Susceptibility of Tong-il to this fungus could be explained by the fact that this variety is apt to be wounded and to cause physiological yellowish red discoloration. The physiological conditon related to disease occurrence should be further investigated.

Negligible result obtained when the inoculated plants were maintained in a moist chamber for one day means that it takes long time for this fungs spore to germinate and penetrate into host plant tissue, also suggesting that this fungus spore itself, unlike rice blast or Helminithosporium leaf blight, has weak germinability. But it was believd that increased germinability, mycelial fusion, and consequent vigorous propagation owing to the nutrient addition made an important role in infection through stomata and disease development. In conclusion, presumably this disease requires continuous rain or high humidity to occur most severely.

Abstract

In an effort to find effective inoculation method of *Fusarium nivale*, this experiment was conducted. The results obtained are summarized as follows:

(1) Distilled water-spore suspension of this fungus was inoculated on rice seedling and the result showed that negligible disease symptoms were observed when the inoculated plants were placed in a moist chamber for one day, whereas considerable symptoms were indicated when kept for two days.

(2) After giving physical wounds by wind or wooden stick to the rice seedling, distilled water-spore suspenion was sprayed on them and considerable diseased spots were formed.

(3) Sufficient disease occurrence was due to the addition of 1% rice leaf extract, glucose, polypeptone, and yeast extract solution to the spore suspension.

(4) In distilled water, spore germination was poor, while in nutrient added solution good germination rate as well as anastomosis was found.

(5) A new rice variety Tong-il turned out to be more susceptible to *Fusarium nivale* than Pung-kwang.

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