한국식물병리학회지 3(2) : 100~107(1987)
Korean J. Plant Pathol. 3(2) : 100~107(1987)

Effect of Soil Moisture on the Pre-Penetration Activity of *Pyricularia oryzae* Cav. on Rice Leaf Epidermis

Choong Hoe Kim

Department of Plant Pathology, Agricultural Sciences Institute, Suwon 170, Korea

벼 잎 表皮에서 稻熱病菌의 侵入前 行動에 對한 土壤水分의 效果

金 忠 會

農業技術研究所 病理科

ABSTRACT

Pre-pentration activity of *Pyricularia oryzae* Cav. from the stage of conidia germination to appressorium formation was examined on rice leaf epidermis under light and scanning electron microscopes to determine the causes for differences in blast susceptibility between plants grown under three different soil moisture conditions in the greenhouse. No significant differences were found in the external shape of leaf epidermal cells including bulliform cells between plants grown under different soil moisture conditions. Growth and orientation of germ tube and morphology and size of appressorium of *P. oryzae* did not vary with soil moisture treatment. Site of appressorium formation was consistent over soil moisture treatment with the highest frequency of bulliform cell (35-48%), followed by short cell (19-27%), and long and guard cells (13-20%). No appressorium was formed on trichome. This result suggests that the observed differences in blast susceptibility between plants grown under different soil moisture conditions were not due to the differences in the pre-pentration activity of *P. oryzae* on those plants.

Key words: pre-penetration activity, *Pyricularia oryzae*.

要 約

同一한 벼 品種을 水分含量이 다른 土壤에서 栽培했을 境遇, 稻熱病에 對한 感受性에 差異가 생기는데 그 原因을 調査하기 爲하여 잎 表皮에서 分生胞子發芽 및 付着器形成까지의 稻熱病菌 侵入前 行動을 光學顯微鏡과 走査電子顯微鏡(SEM)으로 觀察하였다. 水分含量이 다른 土壤에서 자란 벼 잎 사이에 表皮細胞의 外部形態, 잎 表皮에서의 分生胞子發芽管의 生長 및 生長方向, 付着器의 形態 및 크기는 差異가 없었다. 付着器는 土壤水分處理와 相關없이 機動細胞(35~48%) 위에 가장 많이 形成되었고 短細胞(19~27%), 長細胞 및 孔邊細胞(13~20%)의 順이었다. 毛茸上의 付着器形成은 觀察되지 않았다. 本試驗結果, 水分含量이 다른 土壤에서 자란 벼 사이에 나타나는 稻熱病에 對한 感受性의 差異는 稻熱病菌의 侵入前 行動에서 基因하는 것이 아닌 것으로 생각된다.

INTRODUCTION

Rice blast disease caused by *Pyricularia oryzae* Cav. is a major limiting factor of rice production throughout the world. It has long been known that rice plants become susceptible to blast when grown in dry soil, moderately resistant in moist soil and resistant under flooded conditions (3, 4). Susceptibility to blast is said to be inversely related to soil moisture, irrespective of variety and age of plant growth (6). Different degrees of silicification of epidermis as well as nitrogen absorption have been proposed as part of the reason by some workers (7). However, the nature of high susceptibility to blast in dry soil is still not fully understood.

One approach to study the reason for this phenomenon is to examine whether or not there is any difference in infection process of *Pyricularia oryzae* between plants grown in different soil moisture conditions. This has not been attempted yet. Therefore, the purpose of this study is to examine pre-pentration activity of *P. oryzae*, as a first step, on the plants grown under different soil moisture conditions. Post-penetration process of *P. oryzae* on those plants was also studied and published in a separate paper (5).

MATERIALS AND METHODS

Plant materials. The rice cultivar, Brazos, susceptible to blast disease was used in this experiment. Seeds were treated with 0.6% sodium hypochlorite solution for 10 min, rinsed with distilled water three times, and germinated in the water. Germinated seeds were planted six each in twelve Wagner pots 1/50,000a containing synthesized soil (sand: clay: peat=1:2:1). The plants were grown in the greenhouse at 22-38 C°. At the four leaf stage, plants grown in pots were subjected to three different soil moisture levels. Four pots (=replications) were flooded, four pots were watered every two days so as not to dry, and remaining four pots were maintained in a dry condition by watering when only necessary.

Inoculum and inoculation. An isolate of IH-1 of *P. oryzae* which was selected from a preliminary test and was highly virulent to Brazos, was grown on oatmeal agar (oatmeal 25g, agar 15g, sucrose 3g, and distilled water 1l) in 9 cm diameter petridishes as an inoculum. After 20 days of incubation, the cultures were scraped with a teaspoon to remove aerial mycelia and placed under two black light blue ultraviolet lamps (main wave length 250-350 nm) at 30 cm distance from the lamps to induce sporulation of the culture. The next day, conidia of *P. oryzae* on the cultures were harvested with small volume of water using a paint brush and filtered through cheese cloth to remove mycelial fragments. Prior to inoculation, conidial concentration was estimated with a haemacytometer and adjusted to 1.5 x 10^5 conidia per ml with sterile water. A drop of 0.01% solution of Tween-20 was added to 250 ml of conidial suspension as a surfactant.

The plants grown at different soil moisture regimes were inoculated 3 wks after soil moisture treatment with the conidial suspension using an atomizor. Inoculum was sprayed until leaves of plants are totally wet. The inoculation was performed in the evening of a cloudy day to prevent rise of temperature. After inoculation, the plants were kept in a square plastic covered chamber (ca. 2m x 2m x 5m) in the greenhouse to maintain high humidity. Temperature inside the plastic chamber ranged to 22-30 C° during the experiment period. The plants were removed from the plastic chamber the next day.

Leaf tissue preparation for microscopic observations. Four samples of fully expanded 6th leaves were collected at 24 hr after inoculation from one plant in each pot under each soil moisture regime. The leaf samples were cut into 1 x 1 cm pieces prior to microscopic preparation. Whole mounts were prepared by a modification of the method of Vance and Sherwood (8). Leaf pieces were cleared and stained by simmering for 3 min in aniline blue-lactophenol (1 ml lactic acid, 1 ml phenol, 8 ml ethanol, 4 mg aniline blue, 1 ml distilled water).

They were mounted in lactophenol for observation (1).

For SEM observation, leaf pieces were fixed in 3% glutaraldehyde in 0.1 mol phosphate buffer, pH 6.8 for 18 hr. They were washed in the buffer three times after fixation. The specimens were dehydrated with increasing concentrations of ethanol (30, 50, 70, 99%) for 15 min each. After that, the tissue samples were washed with 99% acetone 3 times for 15 min each. The specimens were dried with a Denton Critical Point Drier using aceton as a transition fluid. All specimens were then mounted on stubs with silver paint, coated with gold-palladium (100 A° thick) using a Hummer 1 Sputter Coater. Examination and photography were done with a Hitachi-Hiscan, Model S-500 scanning electron microscope.

RESULTS

Structure of rice leaf surface. The cross section and surface features of rice leaf are shown in Fig. 1A-F. Topography of rice leaf surface was wave-shape (Fig. 1A). Vein is up and bulliform cell is down as shown in Fig. 1B. Stomata are lined at both sides of vein. The rice leaf has a typical gramineous epidermis which contains five main types of cells: long cells, silica cells, cork cells, bulliform cells and guard cells. Silica cells and cork cells occur together in pairs and are collectively referred to as short cells. Sclerenchyma consist of a large number of short cells. Vascular bundles are located just below sclerenchyma (Fig. 1E). Stomata consist of guard cells. Long cells are mainly located between bulliform cells and guard cells (Fig. 1C). Bulliform cells form bands, usually several cells wide and were arranged parallel with the veins (Fig. 1A, B). In cross section (Fig. 1E, F), bulliform cells consist of two or three cells and appear as a fan, for the median cells are usually the largest and somewhat wedge-shaped. Bulliform cells are located at the bottom of V-shape feature of leaf surface. Groove-like topography of bulliform cell coming from its location on leaf surface is shown in Fig. 1D. The rice leaf surface is also characterized by the presence of numerous papillae. Papillae are epidermal cell projections arranged in rows along the long axis of the leaf (Fig. 1C).

Pre-pentration activity. The primary infection process of *P. oryzae* on leaf epidermis consists of several recognizable stages: conidial germination germ tube elongation, formation of appressorial initials, maturation of appressoria and formation of secondary hyphae. A picture of conidia germination to appressorium formation is shown in Fig. 2A.

Germ tube refers to the germination hyphae from the conidium to appressorium. The length of germ tubes varied greatly among conidia. Some conidia had long germ tubes (Fig. 2A, E) but some conidia had short germ tubes (Fig. 2C, D). Conidia often formed appressoria without substantial germ tube (Fig. 2F). No apparent consistency in germ tube length of conidia was observed between soil moisture treatments. Germ tubes emerged mostly from the one end of conidia (Fig. 2A). However, germ tubes were also observed emerging from both ends or middle parts of the conidia (Fig. 2D). There was no definite trend in the orientation of germ tubes on leaf epidermis between soil moisture treatments.

Appressoria varied in size and shape regardless of soil moisture treatment. Size of appressorium ranged to 4.6-8.7μ x 4.6-9.6μ in maximum length and maximum width, and averaged 6.9 x 7.9 μ. Appressoria were mostly well differentiated, round (Fig. 3B, D), horseshoe-like (Fig. 2C, D) or club-shaped (Fig. 2B). Shape of appressorium was easily affected by surrounding topography at the site of appressorium formation. For instance, papillae often affected shape of appressorium as shown in Fig. 3A and 3F. Most appressoria did not initiate additional hyphae (Fig. 2A-D), but in several cases, appressoria germinated (Fig. 3B) and produced one or more additional appressoria (Fig. 3D, E).

There were no significant differences in site of appressorium formation between soil moisture treatments (Table 1). Thirty five to 48% of appressoria were formed over bulliform cell, irrespective of soil moisture treatment. Appressoria were also fre-

Table 1. Site of appressorial formation of *Pyricularia oryzae* on leaf epidermal cells of plants grown under three different soil moisture conditions in the greenhouse

Soil treatment[a]	% appressorium formation on[b]				
	bulliform cell	short cell	long cell	guard cell	trichome
Flooded	37.0	27.0	17.4	18.6	0
Wet	48.2	18.9	13.3	19.6	0
Dry	34.7	26.9	17.8	20.2	0
L.S.D. (.95)	13.6	10.1	8.5	4.7	0

[a] Plants were subjected to each soil moisture condition for 3 weeks before inoculation of *P. oryzae*.

[b] Values are averages of 4 replications and were obtained from light microscope observations. Number of appressoria examined in each soil moisture treatment varied with replication and ranged from 97 to 120 per replication. Because of uneven sample size, percentage of appressorium formation, instead of number of appressorium, was used for statistical analysis.

quently formed on short cell with 19 to 27%, followed by guard cell and long cell with the frequency of 13 to 20%. Appressorium formation on trichomes was not observed in this study. Formation of appressorium on bulliform cell, short cell, guard cell and long cell is illustrated in Fig. 3C, 2B, 3E and 3D, respectively. Appressorium formation on cell juncture was also frequently observed in the study (Fig. 3F). Appressoria were rarely formed over stomata but usually on the edge of the stomata or on the guard cell (Fig. 3E).

DISSCUSSION

Bulliform cells have been recognized as the usual site of penetration for the blast fungus (9, 10). These cells are large, thin-walled and highly vacuolated. They are mainly water-storing epidermal cells which are concerned with the hygroscopic opening and closing movement of leaf blade through changes in cell tugor in response to water condition (2). The fact that these cells are directly influenced by water condition and are the usual entrance point for blast fungus leads to the hypothesis that high blast susceptibility in dry soil condition may be associated with physical changes in bulliform cells that allow easier penetration of the rice blast pathogen.

In the present study, no apparent differences in the external shape of leaf epidermal cells including bulliform cell were found between plants grown under three different soil moisture conditions. Growth and orientation of germ tube, and size and morphology of appressorium of *P. oryzae* did not differ with soil moisture treatment. Frequency of appressorium formation on each epidermal cell was consistent over soil moisture treatment. This suggests that the observed differences in blast susceptibility between plants grown under different soil moisture conditions (3, 4) were not due to the differences in the pre-penetration activity of *P. oryzae* on those plants. This result leads us to future researches on the post-penetration activity that may determine the association of bulliform cell with leaf blast susceptibility.

P. oryzae formed appressoria most frequently on bulliform cell irrespective of soil moisture treatment. Nevertheless, formation of appressoria on leaf epidermis appeared to be random. For instance, appressoria were formed on either the edge or guard cell of stomata while some germ tubes passed over the stomata without formation of appressorium. High frequency of appressorium formation on bulliform cell observed in this study can not be used as a direct evidence for that *P. oryzae* favors bulliform cell in appressorium formation. Groove-like topography around bulliform cell could give an advantage for the formation and maturation of appressoria. Nevertheless, relative proportion of each epidermal cell area on leaf epidermis may be considered in the studies on the site of appressorium formation and penetration as well. Bulliform cell area in leaf epidermis is larger than the areas of other epidermal cells. This may give more chances of formation of appressorium on the bulliform cell. In this context, number of appressorium formation per unit area of each epidermal cell would be more appropriate than percentage or number of appressorium forma-

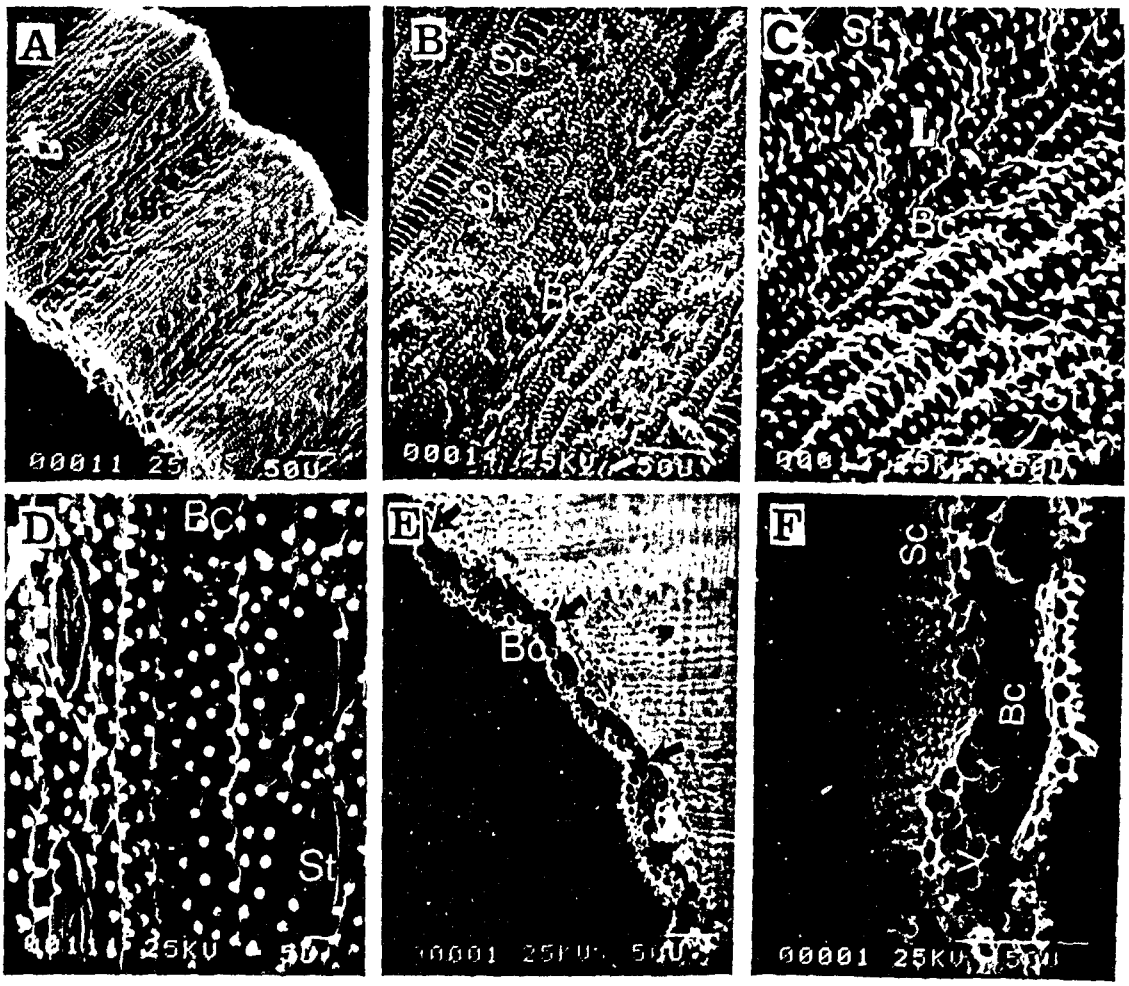

Fig. 1. *A.* Wave-like topography of rice leaf surface where sclerechyma (Sc, vein) is up and bulliform cell (Bc) is down. Bulliform cell forms bands usually several cells wide and is arranged parallel with the vein. *B.* A magnified picture of rice leaf surface. Stomata(St) are lined at both sides of sclerenchyma (Sc) that consist of a large number of short cells. *C.* A picture showing long cells (L) which are located between stomata (St) and bulliform cell (Bc). Wart-like projections are papillae. Papillae are arranged in rows along the long axis of leaf. *D.* Topography of bulliform cells (Bc) that are located at the bottom of groove-like feature of leaf surface. *E.* A cross section of rice leaf showing bulliform cells (Bc and arrows) which consist of two or there cells and appear as a fan. Vascular bundle (V) is located just below the sclerenchyma. *F.* A magnified cross section showing a bulliform cell (Bc) where the mdeian cells are largest. 'Sc' and 'V' indicate sclerenchyma and vascular bundle, respectively.

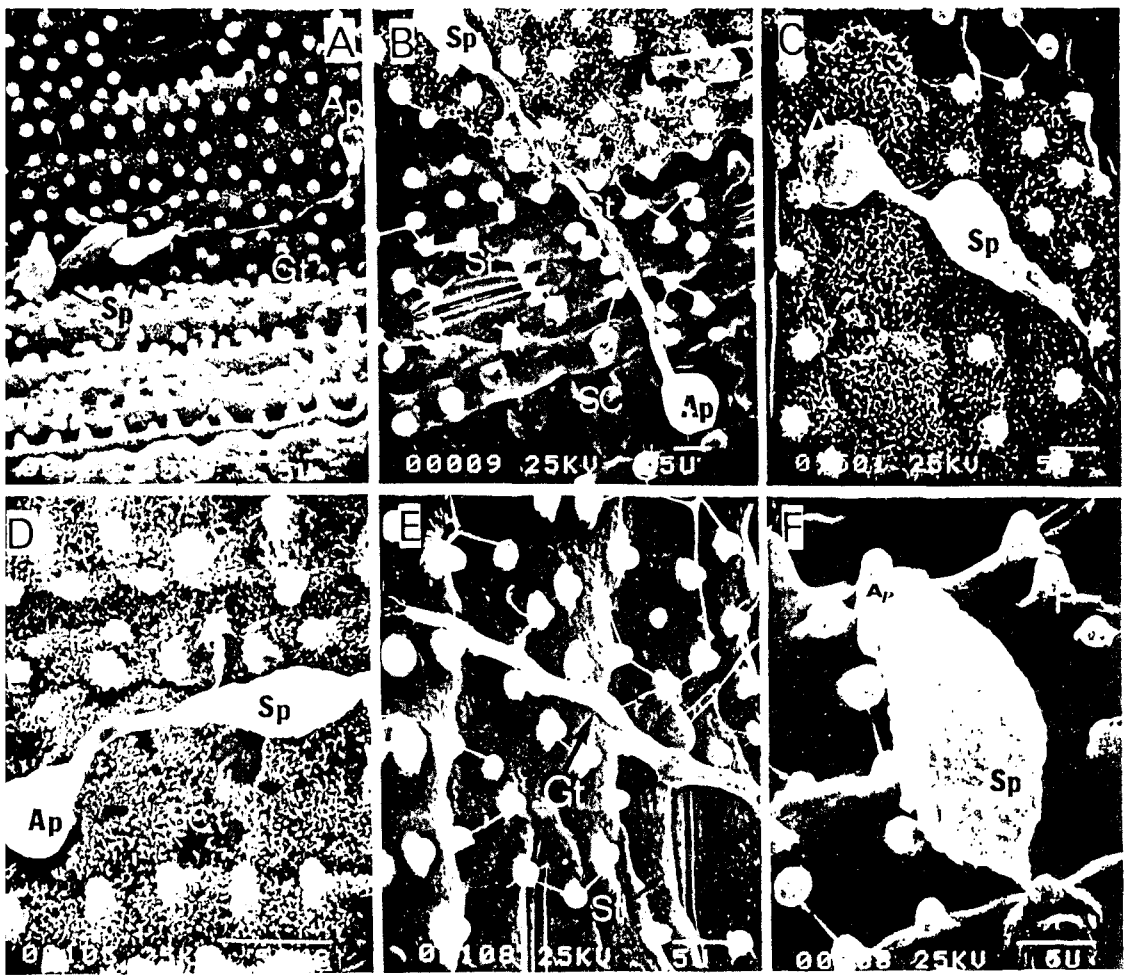

Fig. 2. *A.* A picture of conidia (Sp) of *Pyricularia oryzae* producing germ tube (Gt) and forming appressorium (Ap) at the tip of germ tube on rice leaf epidermis. *B.* A club-shaped appressorium (Ap) formed on sclerenchyma (Sc) after long germ tube growth from a conidium (Sp). *C.* Horseshoe-like appressorium (Ap) with short germ tube from a conidium (Sp). *D.* Germ tube growth emerging from both ends and middle part of a conidium (Sp). An appressorium (Ap) was formed on sclerenchyma (Sc). *E.* Germ tube (Gt) elongation that is crawling on leaf epidermis and passing over a stomatum (St). *F.* Appressorium (Ap) formation of a conidium (Sp) of *P. oryzae* wihtout any substantial germ tube growth. 'P' indicates papillae.

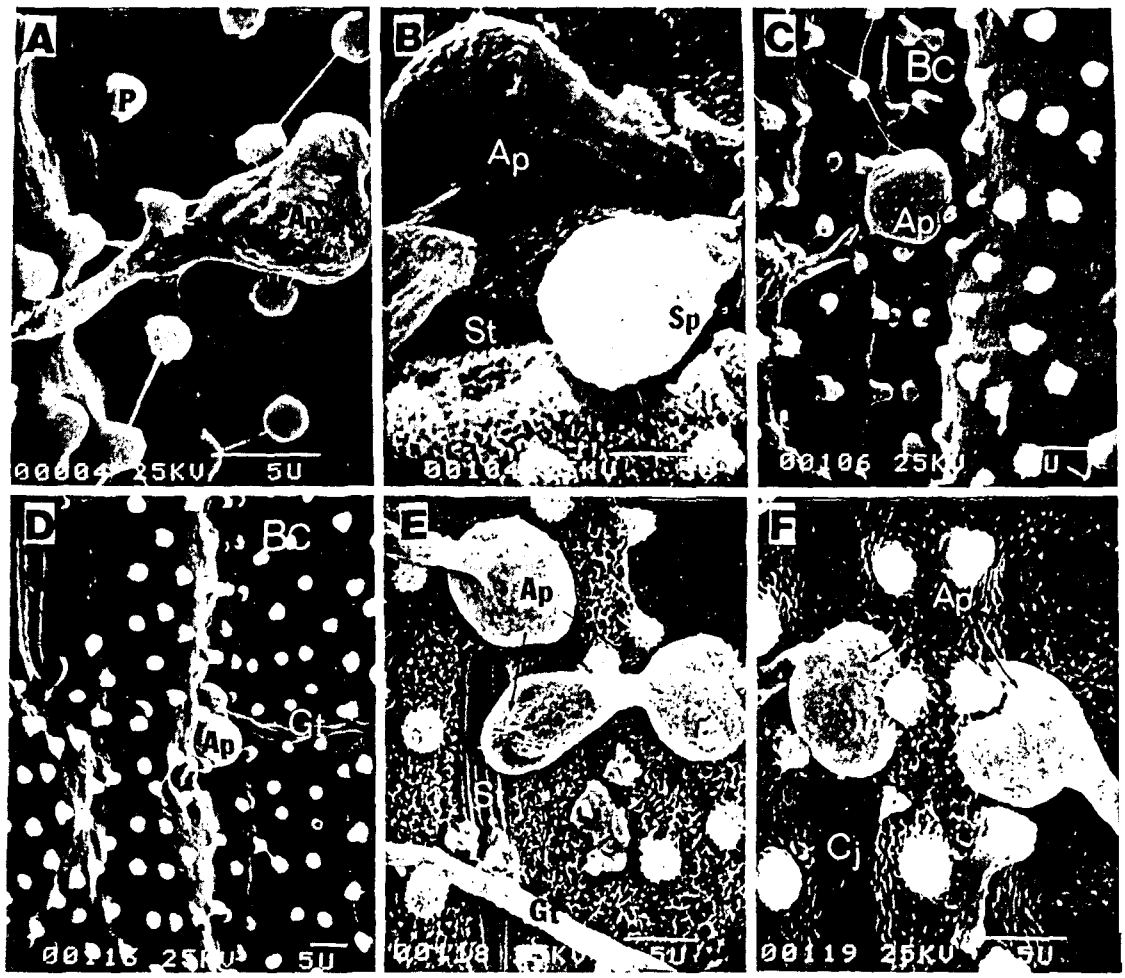

Fig. 3. *A.* Shape of appressorium (Ap) influenced by surrounding papillae (P). *B.* An appressorium (Ap) producing additional hyphae around a stomatum (St). 'Sp' indicates a conidium of *P. oryzae*. C. An appressorium (Ap) formed on bulliform cell (Bc). *D.* Appressoria (Ap) formed on long cell. An appressorium germinated and produced additional appressorium. 'Bc' indicates bulliform cell. *E.* Appressoria (Ap) formed on the edge of a stomatum (St) and on the guard cell. Some germ tube (Gt) is passing over the stomatum. *F.* Formation of appressoria (Ap) on cell juncture (Cj).

tion to describe frequency of appressorium fomation on leaf epidermis.

Appressorium formation on trichome was not observed in the present study. In other researches, it has been observed occationally that *P. oryzae* formed appressoria on trichome (5, personal communication with Dr. C. K. Kim).

REFERENCES

1. BERLYN, G. P. & MIKSCHE, J. P. (1976). *Botanical Microtechnique and Cytochemistry.* The Iowa State University press. Ames, 326 p.
2. ESAU, K. (1965). *Plant Anatomy.* John Wiley & Sons, New York. 767 p.
3. HEMMI, T. & ABE, T. (1932). Studies on the rice blast disease. II. Relation of the environment to the development of blast disease. *Bull. Dep. Agric. For. Japan* 47, 204 p.
4. KAHN, R. P. & LIBBY, J. L. (1958). The effect of environmental factors and plant age on the infection of rice by the blast fungus, *Pyricularia oryzae. Phytopathology* 48:25-30.
5. KIM, C. H., RUSH, M. C. & MACKENZIE, D. R. (1987). Effect of soil moisture on the histopathology of rice blast development. *Phytopathology* 77: (in press).
6. SUZUKI, H. (1934). Studies on the influence of some environmental factors on the susceptibility of the rice plant to blast and Helminthosporium disease and on the anatomical characters of the plant. I. Influence of differences in soil moisture. *J. Coll. Agric. Tokyo Imp. Univ.* 13:45-108.
7. SUZUKI, H. (1935). Studies on the influence of some environmental factors on the susceptibility of the rice plant to blast and Helminthosporium disease and on the anatomical characters of the plant. II. Influence of differences in soil moisture and the amounts of fertilizer and silica given. *J. Coll. Agric. Tokyo. Imp. Univ.* 13:23-31.
8. VANCE, C. P. & SHERWOOD, R. T. (1976). Cycloheximide treatments implicate papilla formation in resistance of reed canarygrass to fungi. *Phytopathology* 66:498-502.
9. YOSHII, H. (1936). Pathological studies of rice blast caused by *Piricularia oryzae.* II. On the mode of infection of the pathogen. *Ann. Phytopath. Soc. Japan* 6: 205-219.
10. YOSHINO, R. (1979). Ecological studies on the penetration of rice blast fungus, *Pyricularia oryzae* into leaf epidermal cells. *Bull. Hokuriku Nat'l. Agric. Exp. Stn.* 22:163-221.