DISCUSSION
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The blast disease of rice caused by *Pyricularia oryzae* Cav., responsible for its heavy losses in the field, transit and during storage, has received much more attention from the workers at present. Evidences so far obtained as a result of this investigation indicated that isolates of the fungus, *Pyricularia oryzae* Cav. exhibited a marked variations to such an extent as to distinguish different strains based on morphological and physiological characters. Among 66 isolates, isolated directly from different infected parts of rice plants collected from different localities of Sibsagar district of Upper Assam during 1985, 4 different strains were established basing on cultural characters and virulence of the isolates; and out of these 4, only 3 strains were found to be successful pathogenic species, viz., Bs, Cs and Ds. Tochinai and Shimamura (1932) classified 39 isolates into 9 forms based upon their cultural characteristics.

Inoculation experiments on different varieties of rice showed different degrees of disease reactions indicating thereby, a correlation between colour and texture of the mycelium, sporulation intensity and degree of virulence. The extent of germination of conidia, growth rate, enzyme reaction and toxic activity also vary with the type of isolates. It is interesting to note that virulence of the strain depends on...
the colour and texture of the mycelia and sporulation of the strains. In the strain A (group no. 4) the virulence was almost nil. So this strain was not taken for further experiments.

Three virulent strains (Bs, Cs and Ds) showed different colours, and texture of the colony, sporulation intensity, size of conidia on different solid and liquid media according to their degrees of virulence and with the composition of the media. It has been observed from the present experiments that the best solid medium is the "host-extract-agar medium" and the best liquid medium is the "czapek-dox-liquid medium". Kulkarni and Patel (1956), Ono and Nakazato (1958) also reported that conidia produced on culture media were longer than those on the host plants and it varied according to the kind of media.

The growth of the isolates of Pyricularia oryzae Cav. was markedly affected by the synthetic and nonsynthetic media. Among all these synthetic and nonsynthetic media, the best was the nonsynthetic medium, "Rice powder agar medium" which showed the highest vegetative growth and sporulation. Yoshii (1936), Tochinai and Nakano (1940), Ito (1943) also reported that the vegetative growth and sporulation of Pyricularia oryzae Cav. were sustained by the agar-agar media containing extracts of the plants and it was difficult to culture in synthetic media. Among all these synthetic media (medium A, B, C, D and E),
"medium E" supported the best vegetative growth and sporulation. This was perhaps due to the fact that the "medium E" contained Asparagine, the nitrogen sources, and Biotin and Thiamine, the vitamin sources besides the carbon sources, and other necessary (MgSO$_4$·7H$_2$O, KH$_2$PO$_4$) elements; whereas the "medium A" contained only Thiamine; "medium B" contained Biotin; "medium C" contained the Biotin and Nicotinic acid and "medium D" contained Biotin and Thiamine besides other necessary elements.

pH of the substratum is of vital importance in the growth and metabolism of fungi as it affects nitrogen uptake, mineral nutrition, and permeability of cell wall which in turn play significant role in disease physiology (Garret, 1956; Rubin and Artskhovskaya, 1963). The optimum pH for growth of the isolates of Pyricularia oryzae Cav. was found to be 6.5 and maximum sporulation occurred at pH 7.5. Henry and Anderson (1948) also studied the effect of initial pH of the substrate on sporulation by Pyricularia oryzae Cav. at 24°C using a pH range of 3.0 to 8.8, and found that growth and sporulation occurred from 4.1 to 8.8 with maximum sporulation between 4.9 and 7.5.

The growth of the isolates of Pyricularia oryzae Cav. was markedly affected by temperature. For all the 3 isolates of Pyricularia oryzae Cav. the optimum temperature for growth within a given length of time (10th and 12th day for solid and
liquid media respectively) was considered to be about 25-28°C. The strain "Bs" and "Cs" showed best growth at 25°C whereas the strain "Ds" showed the best growth at a temperature of 28°C. Nishikado (1926) found that the optimum temperature for the mycelial growth was between 27° and 30°C. Sueda (1928), Abe (1930b), Yoshii (1936) also reported that the optimum temperature for hyphal growth within a given length of time was considered to be about 28°C. Here in this present investigation, so far dry weight is concerned it was maximum at 25°C, in case of "Bs" and "Cs" and in case of "Ds" it was maximum at 28°C.

The percentage of relative humidity was also found to have influenced the growth of the fungus, Pyricularia oryzae Cav. and the best growth of all the three strains of Pyricularia oryzae Cav. occurred at 93% relative humidity beyond which gradual declining took place. Abe (1941) also reported that mycelium grows best at 93% air humidity and growth decreases at about or below this point.

In the study of carbon sources in culture medium, it was found that the best growth of mycelia of all the three strains of Pyricularia oryzae Cav. was supported by cellulose while manitol showed the minimum growth in all the three strains. Sucrose as carbon produced abundant sporulation in all the three strains of Pyricularia oryzae Cav. Among sucrose and cellulose as carbon sources, the 3 strains of
Pyricularia oryzae Cav. showed the maximum mycelial growth in Czapek's dox medium containing 1.5% of cellulose and 2.0% of sucrose respectively and any higher or lower concentrations than that, decreased the growth considerably. Tochinai and Nakano (1940) had reported that storage carbohydrates in the leaves of the host plant, namely, glucose, fructose, sucrose and starch are all favourable nutrients for blast fungus. Tanaka and Tsuji (1952) reported that even the constructive carbohydrates, xylan and cellulose were also found helpful to this microorganism. Of course, these polysaccharides are utilized by the fungus after being hydrolysed into monosaccharides. Using thirty-two (32) different carbon compounds and forty-seven (47) isolates Otsuka et al. (1965), however, showed that sucrose, glucose, maltose, fructose, lactose and xylose were the most suitable carbon sources.

Effect of different nitrogen sources on the growth of the isolates of Pyricularia oryzae Cav. was studied and it was seen that biotin, as nitrogen source, supported the best growth of all the three strains of Pyricularia oryzae Cav. while sodium nitrate and biotin also supported very good sporulation. Urea, however, produced no sporulation. Tamari and Ogasawara (1957a,b,c) involving 23 nitrogen compounds and 47 isolates of the fungus, showed that KNO$_3$, NaNO$_3$, L-aspartic acid, L-aspergine, L-arginine, L-alanine, L-proline, L-serine, glycine, L-histidine and L-glutamic acid were suitable for growth.
In case of mechanism of infection by *Pyricularia oryzae* Cav., the process might be divided in the following phases:

(a) the initiation of conidia to form germ tubes on the host surface;

(b) formation of infection hyphae at the contact surface; and

(c) development and spread of infection hyphae on the host tissue.

All these phases were influenced mainly by the nature of the host, physical environment of the infection court, nutritional status and physical, physiological and chemical nature of the host and host-pathogen interactions.

It was seen that conidia of *Pyricularia oryzae* Cav. started germination after 3 hours both in distilled water and in host extract. The extent of germination of conidia was found to be lower in water than in the host extract and the length of the germ tube of the germinated conidia of different isolates was also seen to be higher in host extract than in distilled water. Ono and Suzuki (1959a) also showed that conidia germinate freely in water. It was also observed that in host extract, the germ tubes were profusely branched after ten hours of incubation.
It was seen that the extent of germination of conidia and the length of the germtubes of the isolates of \textit{Pyricularia oryzae} Cav. varied with surface of different parts of the plant. It was found that the lowest rate of germination of conidia was shown by the mature leaf blades and highest, by the young stems; the highest length of the germtube was shown by the young stem and lowest by the mature leaf blade. It was thus suggested that different constituents deposited on different parts of the plants at different stages influenced the germination of conidia.

It was seen that conidia of \textit{Pyricularia oryzae} Cav. germinated much higher on dead leaf blades of rice than fresh living leaf blades. This might be due to the normal germination and growth of conidia on dead leaf surface, while in case of living blades it might happen that the germination and growth of the germtubes of conidia were checked by some kind of resistance offered by the host cells of the leaf blades.

The extent of infection on different parts of young and mature rice leaf (Aijung variety) showed that the penetration was through stomata and after germination of conidia, the germtube produced dark brown appressoria which remained attached on the surface. The extent of infection was higher and faster in young leaf blades than in mature leaf blades. As the young leaves were found to be more susceptible than
the mature leaves, it indicated that the cultivated plots got earlier infection would be more harmful and of greater damage.

The discolouration of the epidermal cells of leaf below the infection drop indicated the production of some antifungal substances in response to the attack of *Pyricularia oryzae* Cav. and it was probably associated with hypersensitive reactions of the host cells. Muller (1956) also reported about the host pathogen interaction leading to the production of antifungal substances of the nature of phytoalexin.

Present investigation also showed a reduction in grain yield of blast infected rice. It was seen that the loss varied with different varieties of rice according to their susceptibility or resistance quality. The greatest loss in grain yield from leaf and panicle blast was shown by the Aijung variety (very susceptible) amongst all the varieties that had been tested, while the variety, Pankaj (resistant) showed the smallest loss amongst all the varieties. The variety, Bora, which was moderately resistant, showed 54.3% and 53.5% loss in grain yield from leaf blast and panicle blast respectively. It was also found that in comparison to panicle blast, over-all leaf blast caused greater losses than panicle blast. This may be attributed to be due to the inhibition of photosynthesis in diseased leaves. Although panicle infection was always much more destructive than the infection of leaves, but here, in our experiment inoculation was made
simultaneously both in the panicles and the leaves. Within the limit of this specific period panicle infection did not come to the fullest extent within this specific period, whereas infection on the leaf took place in full success. As a result, the ultimate reduction of the yield in panicle infection was found to be lesser in this piece of investigation than the infection caused in leaf blades. Similar result had also been reported by Izumi in 1955. Goto (1965a) showed that the time of infection and other factors influenced the disease severity and eventually yield. Padmanabhan (1965a) estimated the yield losses from blast, comparing yields in (1) non-epidemic and epidemic areas; (2) resistant and susceptible varieties which have similar potential yielding ability; and (3) plots protected and unprotected by fungicides. Strict comparisons were not possible because many other factors are involved, but he reported that 1% neck infection reduced the yield by 1.4% in one case, and by 17.4% and 0.4% in other cases.

The incidence of rice blast disease in the field, on being analysed, showed that it was intimately connected with three primary factors, viz., (i) source of primary inoculum; (ii) spread of the disease in the crop field, and (iii) survival of the pathogen in soil.

From the findings of separate experiments, it was observed here that the surface soil served as the reservoir of
microplankton of fungal spores and their distribution was governed by the depth of the soil, complex meteorological and biological conditions. Moreover, the variation of microbial population was mainly due to the variation in the organic matter content, moisture level and pH of the soil. Moisture content of the soil and the influence of vegetation also played a major role in determining the microbial population of rice field soil. The surface layer of the soil showed the occurrence of highest number of types of fungi. It was also found that the number of fungi gradually decreased according to the increasing depth of the soil level. This may probably be due to low organic matter, poor oxygen content, and higher carbon dioxide concentration in the deeper layers of soil. Warcup (1957), Eicker (1969) and Konger (1969) also supported this view. The presence of *Aspergillus* at different depth levels besides the surface layer indicates that it can grow easily at different climatic, biotic and nutritional factors. The presence of *Pyricularia oryzae* Cav. at surface layer, 1 inch, 3 inches and 6 inches of soil depths in its vegetative and conidal form might be due to its saprophytic behaviour.

The influence of different seasons also played a major role in determining the microbial population of rice field soil. Water-logged period, pre-dry period, dry period, pre-water logged period determined the fluctuation of microbial population to a very great extent. This supports the findings of Venkatesan and Rangaswami (1965), Chetia (1965)
and Mishra (1966).

In water-logged condition soil microflora was found to be lowest. This might be due to water-logged condition of the soil. Similar result was also reported by De and Bose (1938). This is probably because, the submergence causes rapid curtailment of normal gaseous exchange between soil and atmosphere and it leads to an anaerobic condition. Probably, the decrease of $O_2$ and increase of $CO_2$ in the water-logged fields had a profound effect in soil microflora and due to this factor the decrease of microbial population was recorded. The microbial population during the dry period (January to March) was found to be increased. This was probably because of the fact that during this period, the rice field was found to be rich in organic matter; moreover the soil gets sufficient moisture from the air during this period.

Experiment on airspora catch over rice field was also carried out by nutrient-plate method and slide-exposure method respectively. It was observed that there was a considerable variation in number of fungal colonies in nutrient plates and the number was also found to increase with time. The cold season (November, December and January) showed the highest number of catch of airspora. This might probably be due to the effect of some biotic factors and the presence of rice crop in the fields which served as the basic source. Minimum number of spores was found in the cold to warm season.
(February, March and April) when there was no paddy crop in the field. Seasonal fluctuation in the population of microorganisms present in the air depends upon the environment factors such as temperature, humidity, rainfall and direction of wind and the presence of rice crop in the fields. In India, studies of the air spora relating to seasonal variations were conducted by Sreeramulu and Ramalingam (1964) at Vishakhapatnam, Chetia and Barua (1966) over paddy fields and Konger (1969) over the capsicum plantation in Gauhati University campus. Catch of air spora by the slide exposure method showed that Pyricularia oryzae Cav., the fungal pathogen of rice plant was found in all the seasons of the year. This might perhaps be due to the fact that Pyricularia oryzae Cav. can survive as a symbiont on the soil, air and as parasites on collateral hosts.

Seeds are always found to play an important role in the dissemination of pathogens associating both externally and internally which may affect the crops either at early or at later stage of growth. The infection of seeds by different fungi results in the reduction of germination and depends on many factors, viz., age of the seeds and storage period of the seeds, temperature, moisture content of the surrounding environment etc. Present investigation regarding the fungi associated with the one year, two years and three years stored rice seeds showed that the rate of germination of the seeds decreased along with the age of storage. It was also noted that the infection percentage increased along with the
age of the seeds. The results indicated that invasion of seeds by fungi in storage can drastically reduce the germination percentage. This decrease in percentage of germination might be due to the mycotoxins produced by different fungi associated with the seeds. Dange, Rathore and Sing (1978) worked on maize seed and found that Aspergillus flavus produced mycotoxin, aflatoxin B$_1$, B$_2$, G$_1$ and G$_2$ which were highly poisonous for human as well as animal consumption. The fungi isolated here from the seeds were mainly Helminthosporium, Fusarium, Curvularia, Aspergillus, Penicillium, Pyricularia, Rhizopus, Epicoccum, Corynespora, Alternaria, Microspora, Mucor and Cladosporium. Vaidehi and Ramarao (1972) reported similar findings.

Macerating enzymes were also produced by the isolate of Pyricularia oryzae Cav. The different tissues, viz., Apple, Potato, Pear and Rice showed considerable variation in maceration. Apple and pear tissues were found to be macerated quickly by these enzymes; while potato stem tissues did not show any indication of maceration. Rice tissues were found to be completely macerated after 24 hours. The pectin methyl esterase activity was found to be very negligible in all the enzyme extracts.

Studies on the relationship of the chemical constituents of the infected host showed that the infected rice
plants showed:

(a) reduction of total nitrogen content than the healthy plants. This decrease of nitrogen content during infection was probably due to its utilization by the pathogen concerned during its growth and development in host tissues.

(b) a reduction of total sugar content in infected parts than the healthy plants. This also might be due to the fact that the pathogen utilized a certain amount of sugar from the host during infection period.

The silica content in rice leaf blades was found to increase along with the increase of their age. In susceptible varieties, the silica content was found to be lower than the resistant varieties. It was also found that during infection, the infected plants showed slightly higher silica content than the healthy ones. This indicated clearly that resistance and susceptibility was linked with the amount of silica and perhaps this increase of silica during pathogenesis was only a defensive mechanism trying to create internal resistance towards infection and to save the host. Similar result was also reported by Tasugi and Yoshida (1958).

The reduction of chlorophyll in infected plants was found to be associated with this fungal infection. The chlorophyll degradation resulted in destruction of the
lamellar arrangement in the chloroplast. The formation of new plastids in the growing tissues was affected, and as such, the rate of photosynthesis got reduced.

The possibility of toxin production during pathogenesis, leading to inactivation of the host protoplast was also investigated. It appeared that the pathogen, Pyricularia oryzae Cav. was capable of toxin production in vitro and in vivo. This toxin was usually leached out of the fungal body into the substratum; otherwise the metabolite would not have shown deleterious effects like wilting, denaturation and coagulation of the protoplasm on the test plants. Differences in time for wilting in different test plants (Table 52A, B, C and D) might be due to either:

(i) the differences in the nature and strength of secreted toxins; or,

(ii) the variable response of test plant treatments which might be governed by the genetic constituents.

The results of chromatographic (Paper) analysis clearly showed that the isolate, Bs of Pyricularia oryzae Cav. of different age periods did secrete one toxin with the same R<sub>f</sub> value. Experimental results showed that the time taken to develop wilting symptom in a particular species of test plant was inversely proportional to the concentration of the toxin in the metabolite. It had been found that dilution of the
metabolite delayed wilting time. Hoitink, Pelletier and Coulson (1966) also showed that a dilution series of the toxin produced by the bacteria, P. phaseolicola gave a positive correlation with halo areas produced as a function of concentration. It was also found that the toxins produced by the three strains of Pyricularia oryzae Cav. were thermostable. Further, it was clear that the toxicity was not dependent on the hydrogen ion concentration; and so the wilting of the test plants were not due to the change of pH in the medium but was due to the toxins secreted by the pathogen.

Histological studies on the hypocotyl tissues of wilting plant seedlings and hypocotyl tissues of the control plant seedlings revealed that the xylem vessels of the vascular bundle in wilting plants were blocked by brownish gummy material. This blockage was perhaps created by the action of the toxic metabolite of the fungi by depositing gummy substances inside xylem vessels. Wilting of plants might, therefore, be due to the blockage of xylem vessels which obstructed the upward flow of water and mineral salts from the roots.

In histological study of leaf tissue of wilting plants it was found that stomata were found to be closed. This "water stress" symptom is due to the water deficiency in the guard cells caused by the blockage of water transport through xylem vessels. The deficiency in water resulted in shrinkage
of guard cells leading to the closure of the stomata. Arntzen, Haugh and Bobick (1973) observed similar stomatal closure by Helminthosporium maydis pathotoxin in wilting hosts.

The toxic metabolite of Pyricularia oryzae Cav. was also found to play important roles on the growth, respiration of rice plants and also on the germination of rice seeds. The effect on stunting rice seedlings may perhaps be due to the fact that the toxic metabolites inhibited the rice seedlings to get their nutritional requirements for their usual growth.

The seeds that had been soaked in toxic metabolites of Pyricularia oryzae Cav. failed to germinate and this failure might perhaps be due to the inhibitory effect of this fungal metabolite which destroyed the viability of the seeds to germinate. The metabolite of Pyricularia oryzae Cav. affected respiration of rice seedlings; because the iron and copper enzymes (as reported by Ogasawara, Kaji and Tamari, 1975) which play important role in respiratory system might perhaps be affected by the toxic metabolite.

The pathogenicity and sporulation test of the three strains of Pyricularia oryzae Cav. on plants, viz., Chichory and Citranella was also carried out. It was found that all the three strains of Pyricularia oryzae Cav. were pathogenic to Citranella plants while the same three strains of Pyricularia oryzae Cav. were moderately pathogenic to Chichory plants. Kuribayashi, Ichikawa and Terazawa (1953)
also found that isolates from barnyard grass (grown on wild on an experiment station farm) were moderately pathogenic to rice and barley but only slightly pathogenic to corn. With the help of this investigation, it can be inferred that the initial pathogenicity of the rice blast fungus to rice was not lost by passing through other gramineous plants, like Citronella and Chichory. Similar results were also reported by Narita, Iwata and Yamanuki (1956).

The present investigation involved the studies about the efficacy of different antibiotics (Streptomycin, Actidione, Grisovin and Tetracycline) and fungicides (Zineb, Thiram, Tricyclazole and Bordeaux mixture) against blast infected rice seeds. It was found that among the eight chemicals, the antibiotic, Actidione and the fungicide, Tricyclazole were found to be most satisfactory in controlling the blast infection; because the seeds treated with these chemicals did not show any infection. Moreover, it was also observed that the antibiotic Streptomycin and the fungicide, Bordeaux mixture had shown their fairly high efficacy against blast infection. So, from the present investigation, it can be inferred that both these two chemicals (Streptomycin and Bordeaux mixture) can also be recommended for the purpose of controlling blast infection of rice seeds. However, it needs further investigation. Among the eight chemicals, the test antibiotic in regard to seed germination was found to be Actidione, while
the best fungicide in regard to seed germination was found to be Tricyclazole.

It thus, appeared that blast infection of rice seeds caused by *Pyricularia oryzae* Cav. might be controlled in the laboratory with antibiotics, viz., Actidione, Streptomycin and fungicides, viz., Tricyclazole and Bordeaux mixture.

Further experiments were carried with the same eight chemicals, spraying at different times and under different conditions, to control the blast disease and to minimize the yield loss. The aforesaid study provided some valuable information in this regard.

It was found that 4 sprayings in a season brought about a significant reduction in panicle infection and a significant increase in yield of rice. Complete control of the disease could not, however, be affected even by 4 sprayings. All the chemicals used in the present study were more or less equally effective. But differences among fungicidal and antibacterial treatments were not significant.

Another experiment was conducted with the fungicide, Tricyclazole to find out the most effective period and number of sprayings for preventing the loss in yield due to blast. It was found from the analysis of data that 4 sprayings given after flowering of the crop might be enough to control the
Of course, this evidence was not conclusive; therefore, it requires further investigations. Metcalf (1906), Lin (1936) also claimed that blast could be controlled by spraying Bordeaux mixture. Woloshuk and Sisler (1982) also reported that the activity of Tricyclazole inhibited colonization in epidermal penetration by *Pyricularia oryzae*.