PROGRESS AND TREND OF RESEARCH ACTIVITIES IN THE ASIAN REGION IN RELATION TO VIRUS DISEASES OF RICE

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ABSTRACT

The rapid spread of the high-yielding varieties of rice in Asia has been associated with outbreaks of several virus diseases. Insect transmission studies were mainly carried out during the 1970s. The five-year Research Project on Virus Diseases of Rice and Legumes in the Tropics which was initiated in 1978 by the Tropical Agriculture Research Center in collaboration with the Insitute for Plant Virus Research and others, involved a sub-center under the Department of Agriculture, Thailand. TARC provided a set of equipment including an electron microscope, and Japanese long-term visiting researchers were dispached to work in collaboration with the Thai researchers. Several Japanese short-term visiting researchers carried out surveys overseas and brought back virus materials to Japan to undertake more precise studies using the facilities in Tsukuba. The properties of the viruses, distribution, mode of transmission by vectors, epidemiology, and serology, etc. were studied. Serological diagnosis of the diseases were developed and is being used at IRRI at present. Rice ragged stunt, rice tungro, rice gall dwarf, rice transitory yellowing, and rice grassy stunt diseases are important and the etiology and properties of the causal viruses were studied. Rice gall dwarf virus was identified and studied in some detail before the epidemic of 1983. Cell lines of insect vectors have been established.

Introduction

Studies on rice virus diseases by Japanese researchers were carried out in collaboration with IRRI. Also a collaborative research project on plant protection was initiated between Japan and Indonesia in addition to the dispatch of long-term and short-term visiting researchers by the Tropical Agriculture Research Center to several South and Southeast Asian countries, etc. Recently rice virus diseases which are known to occur in tropical Asia such as rice waika disease (Furuta, 1977), rice transitory yellowing disease (Saito *et al.*, 1978), rice grassy stunt disease (Iwasaki and Shinkai, 1979), and rice ragged stunt disease (Shinkai *et al.*, 1980) have been reported in Japan. A five-year research project on virus diseases of rice and legumes in the tropics was initiated in 1978 by the Tropical Agriculture Research Center in collaboration with the Institute for Plant virus Research and other organizations. A great deal of progress has been made as a result of these studies.

Research project on virus diseases of rice and legumes in the tropics

This research project was implemented by the Tropical Agriculture Research Center from 1978 to 1983, in collaboration with the Institute for Plant Virus Research as the main collaborative Institute and other National Agricultural Experiment Stations. Two years before the start of the research project, an international symposium on virus diseases of crops in the tropics was organized by TARC. It was pointed out at this symposium that a large number of virus diseases affecting various crops are widely distributed in the South and Southeast Asian countries, becoming a major threat to production. However, few researchers were studying these diseases and few facilities and items of equipment such as electron microscopes, ultracentrifuges, various kinds of spectrophotometers, etc. which are necessary for these studies were available in

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these countries.

It was strongly recommended by the participants in the symposium that technical assistance and financial support should be extended to the South and Southeast Asian countries to overcome these problems. Also it was hoped that further international meetings on similar themes would be held in the near future.

Beside these facts, the occurrence of several rice virus diseases which were thought to originate from the tropics were reported in Japan, and research on these diseases was deemed necessary for the promotion of agriculture in Japan.

The Department of Agriculture, Bangkhen, Thailand was selected as a sub-center for this research project, and a set of equipment for virus research including an electron microscope, an ultracentrifuge, a spectrophotometer, etc. was sent by TARC. Long-term visiting researchers were dispatched by TARC to Thailand to work in collaboration with the Thai researchers. Also, several short-term visiting researchers carried out surveys and research abroad and brought back virus materials to Japan for more precise studies, using the facilities of the Institute for Plant Virus Research at Tsukuba. The properties of the viruses, their distribution, mode of transmission by vectors, epidemiology, etiology and serology, etc. were studied. Serological diagnosis of the diseases was developed successfully and is being used at IRRI at present. A new rice virus, the rice gall dwarf virus was identified during the course of the investigations of this research project and studied in detail. It was undoubtedly exceptional that information had been accumulated before the major epidemic outbreak of the disease in 1983.

This research project was characterized by two aspects:

1) The research activities were concentrated in one country, in this case Thailand. The results of the detailed studies conducted in Thailand were then applied to the surveys of other countries in South and Southeast Asia, based on the assumption that some of the conditions may be common to the region;

2) Research on the properties of the viruses was not carried out in the respective countries, but in Japan where qualified researchers and adequate facilities are available.

Distribution of rice virus diseases in Asia

Previously it was thought that the kinds of virus diseases of rice in the temperate zone such as Japan and Korea are quite different from those in the tropics. However, it became clear that there are many exceptions.

Rice stripe virus, which had never occurred in the northern part of Japan, was discovered in 1968 in paddy fields of Hokkaido, the northernmost island of Japan, causing a decrease of rice yield. The disease was also identified in Taiwan in 1969 (Chiu, 1982). It has since occurred in some localities in the central and southern parts of Taiwan.

Rice dwarf virus was distributed in Japan, Korea and mainland China. Its occurrence was recently reported in Nepal, and the main insect vector in this region was thought to be *N. nigropictus*.

Occurrence of rice necrosis mosaic virus which was reported in Japan was also recorded in India in 1980 (Ghosh, 1981).

In contrast, the occurrence of tropical rice viruses has often been reported recently in the temperate zone. Occurrence of rice tungro spherical virus, rice grassy stunt virus, and rice ragged stunt virus in Japan was reported in 1971, 1978, and 1979, respectively.

Rice transitory yellowing virus which was reported only in Taiwan, is now distributed in Okinawa, Japan, mainland China, and the northern part of Thailand (Inoue *et al.*, 1980).

Thus, it is considered that rice virus diseases may occur in a region if the vectors are distributed in this particular area.

Etiology of some rice virus diseases

Some misunderstanding or confusion had occurred in the etiology of several virus diseases, due to the difficulty in studying these diseases as most of the rice virus diseases are transmitted by insects and not by mechanical means.

1 Rice tungro disease

This is one of the most important rice virus diseases in Asia. Previously the causal agent of this disease was thought to be the rice tungro virus with spherical particles about 30 nm in diameter. However, it became clear that two kinds of viruses are associated with the disease (Satito *et al.*, 1975; Saito, 1979). One is the rice tungro spherical virus and the other is the rice tungro bacilliform virus (Saito *et al.*, 1981).

Although both viruses are transmitted by the leafhoppers in a non persistent manner, the rice tungro bacilliform virus is transmitted by the leafhoppers only in the presence of the rice tungro spherical virus which acts as a helper (Hibino *et al.*, 1979). The symptoms of the disease on rice plants are severe in the case of double infection with the viruses, moderately severe in single infection with rice tungro bacilliform virus, and no clear symptoms are evident in single infection with rice tungro spherical virus.

2 Rice waika disease

Waika disease of rice has occurred since 1971 in Kyushu, Japan. Characteristic symptoms on diseased rice plants consist only of stunting of the plants and in a few cases, discoloration of the leaves occurs. Spherical particles about 30 nm in diameter, are observed in dip-preparations and in purified preparations of samples of the diseased plants. The virus was identified as rice tungro spherical virus on the basis of serological relationships, particle morphology, particle properties, characteristics of ultrastructural alterations and vector relationships.

The disease caused by single infection of the rice tungro spherical virus was widely distributed in the Philippines and possibly in Indonesia where tungro incidence was very low or inexistant. Tungro outbreaks occurred in various locations especially in Indonesia when IR varieties which had been introduced became subsequently susceptible to the disease, presumably due to the adaptation of the insect vectors. As a result, the varieties which had been previously infected with only the rice tungro bacilliform virus became susceptible to both RTBV and RTSV transmitted by adapted insect vectors (Hibino, personal communication).

3 Rice ragged stunt disease

Rice ragged stunt disease was described for the first time by Hibino *et al.* (1977) and designated as rice ragged stunt by K. C. Ling (1977). The properties of the virus were studied at the Institute for Plant virus Research and at the University of Hokkaido in collaboration with IRRI by E. Shikata and his associates (Shikata *et al.*, 1979). The properties of the virus were also studied by Milne and his associates in Italy on the materials mailed from the Philippines. The virus was considered to be a possible member of the plant reovirus sub-group 2, fijivirus, mainly due to the presence of 8 segments in the double-stranded ribonucleic acids it contains (Boccardo and Milne, 1980). However, it became clear that RNAs of rice ragged stunt virus could be separated into 10 segments (Omura *et al.*, 1983), hence confirming the assumption that the virus belongs to the fijivirus sub-group.

4 Rice grassy stunt disease

The causal agent of rice grassy stunt disease had not been identified for a long time although the disease was considered to be caused by a virus or mycoplasma. Recently, Shikata *et al.* (1978) reported that purified fractions consisting of small particles about 20 nm in diameter obtained from RGS-infected rice plants were infective by micro-injection techniques. Beside this fact, a

large quantity of filamentous particles 6–8 nm in diameter, 950–1350 nm long, and often circular in shape were purifed from diseased plants. The filamentous particles comprised ribonucleoprotein and were serologically distantly related to rice stripe virus (Hibino *et al.*, 1983). Antiserum against the filamentous particles reacted specifically with diseased plants or viruliferous insect vectors. The name rice grassy stunt virus was proposed for the filamentous particles.

5 Rice gall dwarf disease

A previously undescribed virus disease of rice was discovered in central Thailand and named rice gall dwarf disease (Omura *et al.*, 1982). The symptoms consisted of the formation of small galls along leaf blades and sheaths, dark green discoloration of leaves, twisted leaf tips, reduction in the number of tillers, and severe stunting of rice plants. The disease was transmitted by *Nephotettix nigropictus, N. cincticeps, N. malayanus, N. virescens,* and *Recilia dorsalis* in a persistent manner and was also transmitted through the eggs of *N. nigropictus* (Inoue and Omura, 1982; Morinaka *et al.*, 1982). Double-shelled polyhedral particles about 65 nm in diameter were purified from the diseased rice plants, and the nucleic acid of the purified virus was composed of double-stranded RNA divided into 12 segments (Hibi *et al.*, 1984). The virus is a new member of phytoreovirus in the plant reovirus group. Virus antigens both in the infected plants and individual insect vectors were detected by latex flocculation test. A severe epidemic of the disease in the western part of Guandong Province in South China was reported in 1983.

6 Rice dwarf disease

The establishment of cell lines from the culture of embryonic tissues of *N. cincticeps* and infection of the growing cell sheets with rice dwarf virus were reported. These materials can be used for the research on rice virus diseases transmitted by leafhoppers (Omura *et al.*, 1982).

7 Rice grassy stunt disease (new RGS strain)

A new RGS strain causing tungro-like symptoms occurred in the Philippines and was designated as RGSV 2 (Hibino *et al.*, 1983). The strain shows a pathogenicity to rice cultivars with a resistance gene. Similar strains also occurred in India, Thailand, etc.

Serological studies on rice viruses

Rice tungro spherical virus and rice tungro bacilliform virus were separately purified from doubly infected plants and antisera specific to each virus were prepared (Omura *et al.*, 1983). Latex flocculation test was employed to detect rice tungro bacilliform virus and rice tungro spherical virus (Omura *et al.*, 1984). Latex flocculation test is simple, rapid and is sensitive. Sensitized latex is stable and can be preserved for a long time. LF is an excellent, convenient and practical serodiagnostic method. Transmission of RTSV and RTBV can be specifically blocked by feeding the antisera before the inoculation feeding (Hibino, personal communication).

Rice ragged stunt virus was purified from diseased rice plants, and antiserum against RRSV was prepared and used for virus detection by the enzyme-linked immunosorbent assay (ELISA) (Hibino and Kimura, 1982). However, strong nonspecific reactions occurred in extracts of virus-free female planthoppers carrying eggs. These non-specific reactions disappeared when phosphate buffer, pH 6.5, containing 2% polyvinylpyrrolidone was used as diluent. RRSV was detected efficiently even from dead and stored single planthoppers by ELISA and the assay was found to be more sensitive than insect transmission tests. Antisera against rice gall dwarf virus and the filamentous particles of rice grassy stunt disease were also prepared and are used successfully for the detection of the viruses by latex flocculation test, ELISA, etc.

Conclusion

The progress achieved in rice virus research in recent years has clearly shown that the problems of rice virus diseases should be approached on a global scale and not solely at a national level. Therefore, international collaboration in rice virus research should be strengthened in future, together with the promotion of surveys in areas located between the temperate zone and the tropics such as South China and Taiwan.

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Discussion

Tantera, D.M. (Indonesia): Is it possible to send virus materials to Japan to carry out special studies? Are there any special formalities required?

Answer: A permit is required to import infected virus materials in Japan. The authorization can be granted if research is carried out in institutes provided with good facilities, including isolation greenhouses.

Kishimoto, R. (Japan): You mentioned several examples of regional spreading of viruses from the tropical zone to the temperate region. Do you consider that the spread of viruses from the temperate region to the tropics could also take place? Are there any examples of such cases?

Answer: It is possible to consider that rice viruses could be propagated from the temperate zone to the tropics if the vectors transmitting the viruses are distributed in the region. Examples include the occurrence of rice stripe virus in Taiwan and the southern part of China and that of rice necrosis mosaic virus in India.

Shikata, E. (Japan): It appears that in most of the cases the viruses reach the temperate zone after originating in the tropics, as in the case of rice stripe which occurs severely in Hokkaido.

Answer: However, presently in Taiwan, rice stripe is a major problem.

Mochida, O. (IRRI): I would like to add that in the southern part of Taiwan black-streaked dwarf virus has become very important. This is an example of North-South propagation.

Dollet, M. (CIRAD): In the case of the reovirus, in the serological reactions, was the antiserum prepared from purified double-shelled particles against ds DNA? Were there any cross-reactions? Did you test the method using antiserum against synthetic Poly A and Poly C?

Answer: Yes it was. The titer was very low and the reaction was not very specific.

Mochida, O. (IRRI): In your review you did not include data on the research activities relating to the breeding and use of resistant varieties. This aspect is extremely important for rice virus research as well as for the control of rice virus diseases.