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Studies on Purification and Serology of Rice Dwarf Virus

Soon Hyung Lee · Key Woon Lee · Bong Jo Chung and R.H. Halliwell*

Department of Plant Pathology, Institute of Agricultural Sciences,
Office of Rural Development, Suweon, Korea.
*: Department of Plant Sciences, Texas A & M University, College Station,
Texas 77843, U.S.A. FAO/UNDP Consultant, July-December, 1974

벼 오갈병 바이러스의 純化와 抗血淸 製造에 關한 硏究

李淳炯 · 李起運 · 鄭鳳朝 · R. H. Halliwell*

農業技術硏究所 植物病理科

ABSTRACT

Yield losses from rice dwarf virus infection are significant in Korea. Rice dwarf virus(RDV) was purified and RDV-antiserum was produced.

The purified virus, mixed with an adjuvant(1 : 1)was injected every 10 to 14 days into rabbits. Three injections were sufficient to produce an antiserum of 1/4,096 titer.

The produced antisera will be used to facilitate the detection and identification of RDV in rice plants and in the RDV leafhopper vectors.

INTRODUCTION

Rice dwarf virus disease is prevalent in the southern province of Korea. Losses resulting from infected rice plants are severe. There are RDV resistant cultivars, but these do not include both the common and Tongil lines now grown extensively throughout the Republic. However, disease incidence has been abated hrough clever manipulation of planting dates and inect vector monitoring and control.

Kimura[5] reported the virus concentration in infected ice plants attains a maximum 40 days after inoculaion.

Fukushi, Shikata and Kimura[4, 7, 10] isolated chara-eristic virus like particles, icoshedrons of 70 nm in iameter, from both rice RDV infected rice plants and ruliferous leafhoppers by purification in differential centrifugation techniques.

Kimura et al.[4, 5, 10] reported on the isolation of RDV particles by differential centrifugation techniques utilizing chloroform.

Antiserum produced as purified virus was injected intramuscularly in rabbits with Freud's adjuvant.

This research was conducted to identify rice dwarf virus disease occurence by use of produced antiserum in identification of infected rice plants and leafhopper vectors in eventual hope of being able to suppress this disease through more effective control programms.

MATERIALS AND METHODS

Purification Procedures:

Procedures involved mincing of the RDV infected plant in phosphate buffer(pH 6.8) with 0.1% thioglycollic acid and then removing large fragments of cells

by centrifugation in chloroform at low speed. Sedimentation of the virus was next obtained by centrifuging at high speed, with the pellet resuspended in a phosphate buffer solution.

Density-gradient centrifugation was conducted with swing bucket tubes in 10–40% gradient sucrose buffer.[3]

Concentration of purified virus was determined by spectrophotometric optical density measurements.

Antiserum production:

As shown in Table 1, RDV antiserum was produced on injection with purified virus, mixed with an adjuvant (1:1) injected two times intramuscularly and three times intravenially in rabbits.

Trial bleedings were carried out after the third injection, with complete bleedings for antiserum production 10 days after the last fifth injection.

Table 1. Injection purified rice dwarf virus required for antiserum production in rabbits

Injection time	Injection site	Amount of purified RDV (ml)
The 1st	Intramuscular	4
The 2nd	〃	3
The 3rd	Intravenial	2
The 4th	〃	1
The 5th	〃	1

Determination of antiserum titer and serology:

Titer of produced antiserum was determined by micro-precipitin tests with petridishes[2,8,11]. Serological experiments were conducted Ouchterlony agar gel-diffusion tests. Antigen were used 4 fold solution of purified RDV in Tris-HCl buffer.

RESULTS AND DISCUSSION

The RDV purification was modified from procedures of Toyda, Kimura, and Suzuki (Fig. 1)[10]. The results of this experiment indicated the concentration of purified RDV was 3.12mg/ml.

In our procedure given in Figure 1, 0.1M phosphate buffer was employed in mincing diseased tissue and dissolving pellets, while 1/30M buffer was used in Fukushi et al's[4] procedures. Purification was ensured by repeating the high and low speed differential centrifugation. A more efficient separation of virus resulted with density gradient centrifugation in our studies, as opposed to the treatment of fractions with phospholipase as in the procedures of Toyoda et al's[10].

Frozen infected plants (100g)
- Maceration in 500 ml of PO_4 buffer (0.1M pH 6.8) Containing 0.1% thioglycollic acid

Sap
- Clarification 1. 6,500 g for 20min.
- Discard pellet

Supernatant
- To make 20% solution with cold chloroform
- Stir. for 5 min.
- Clarification 2. 2,000 g for 20 min. Discard pellet

Supernatant
- Differential cfg. 30,000 g for 60 min. — Repeat
- Discard supernatant. 2,000 g for 20 min. —

Unfrac. preparation
- Sucrose density gradient cfg. 22,000 g for 60 min. (10–40%)

Aqueous phase
- 26,000 g for 60 min.

PPT
- Resuspend in PO_4 buffer. 2,000g for 20 min.

Virus (Storage in 10 ml of Tris-HCl buffer)

Fig 1. Procedures for rice dwarf virus isolation and purification.

When rabbits were injected intramuscluarly with an emulsion of the partially purified virus in Freud's adjuvant, a high titer antiserum was obtained as previous noted in Kimura's[4] report.

As shown in Table 2, three injections were sufficient to produce an antiserum of 1/4,096 titer according to the determination titers of antiserum separated in trial bleedings.

The Ouchterlony agar-gel diffusion test also, showed a good reaction between RDV antigen and produced antiserum.

摘　要

우리나라 中部以南 地域에서 벼 오갈병에 依한 被害가 顯著히 늘어나고 있는 實情이다. 本試驗에서는 媒介虫을 使用, 純粹分離하고 이를 接種하여 增殖한 後 Toyoda 等의 純化方法을 改善하여 純化하였다. 그 結果 純化된 바이러스 含量은 ml當 3.12 mg이었다. 純化된 바이러스를 Adjuvant와 함께 토끼에 10~14일 간격으로 5회 注射하여 抗血淸을 製造한 結果 1/4,096의 높은 力價를 나타내는 抗血淸이 製造되었다.

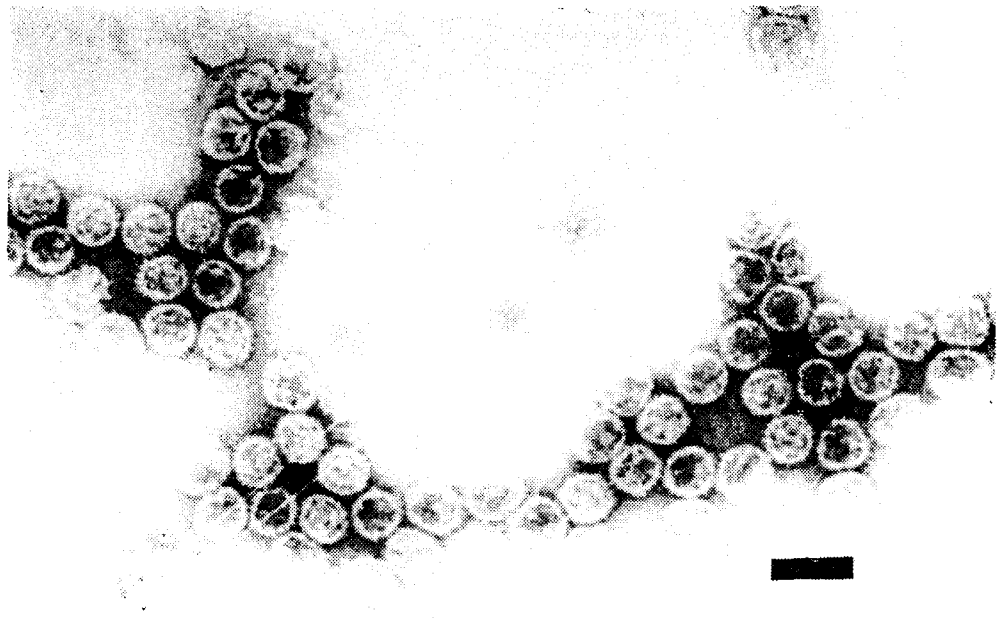

Fig 2. Virus particles from a purified preparation, stained with phosphotungstate. Bar represents 100nm.

Table 2. Titer determination of produced rice dwarf virus antiserum by micro-precipitin tests

Antiserum										
1/8	1/16	1/32	1/64	1/128	1/256	1/512	1/1024	1/2048	1/4096	1/8192
++++	+++	+++	++	++	++	+	+	+	+	−

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