

## ISOLATION AND CHARACTERIZATION OF SOME VIRUSES ASSOCIATED WITH WHEAT PATHOGEN *GAEUMAN- NOMYCES GRAMINIS* VAR. *TRITICI*

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### Abstract

Isometric virus particles measuring 35–40 nm in diameter with double stranded RNA genomes of molecular weight  $(1.48 - 6.1) \times 10^6$  dalton was detected in the field isolates of the wheat take all fungus *Gaeumannomyces graminis* var. *tritici*. Out of three isolates of *Ggt* four different viruses based on particle size, size of RNA and molecular weight of coat protein belonging to three groups of mycoviruses were detected.

### Introduction

Lapierre *et al.*, (1970) discovered the first isometric virus particles in two weakly pathogenic isolates of *Gaeumannomyces graminis* var. *tritici* (Sacc) Arx. oliv. (*Ggt*) the cause of take all disease of wheat. Previous work has established four groups of *Ggt* viruses based on physical and serological properties and on their double stranded RNA (dsRNA) and capsid polypeptide mol. wt. (Buck *et al.*, 1981; Buck, 1984). The size distribution of these viruses within four group were: group I and II virus particles 35 nm, group III particles 40 nm and group IV particles 29 nm in diameter. These viruses are unusual in that they are transmitted only intracellularly, during growth in asexual and sometimes in sexual spores and via heterokaryosis. Pryor *et al.*, (1988) have isolated a dsRNA mycovirus from maize rust fungus *Puccinia sorghi*. Presence of isometric virus particles in germinated uredospores of the fungus has presented the evidence of intracellular horizontal transmission of virus. Association of these virus particles with pathogenicity of the fungi has not been established, although it is becoming increasingly clear that dsRNA can affect a fungus phenotype in other ways. For example, protein toxin secreted by Killer strains of *Saccharomyces cerevisiae* and of *Ustilago maydis* are encoded by specific dsRNA molecule (Bostian *et al.*, 1980, Koltin & Kendel, 1978). Similarly Rogers *et al.*, (1986) reported a clear association between specific segments of dsRNA and a cytoplasmically transmitted disease in the aggressive strain. Yeast Killer viruses, has been reported to be transmitted only by cytoplasmic mixing during division, mating or other induced forms of cell fusion. El-Sherbeni & Bostian (1987) have demonstrated the extracellular transmission of these virus particles. The present report describes the isolation and characterization of some viruses associated with *Gaeumannomyces graminis* var. *tritici*, the cause of take all disease of wheat.

### Materials and Methods

**Fungal Isolates:** Isolates of *G. graminis* from wheat crops in Highfield, Rothamsted Experimental station, Harpenden, U.K. were kindly provided by Mr. D. B. Slope.

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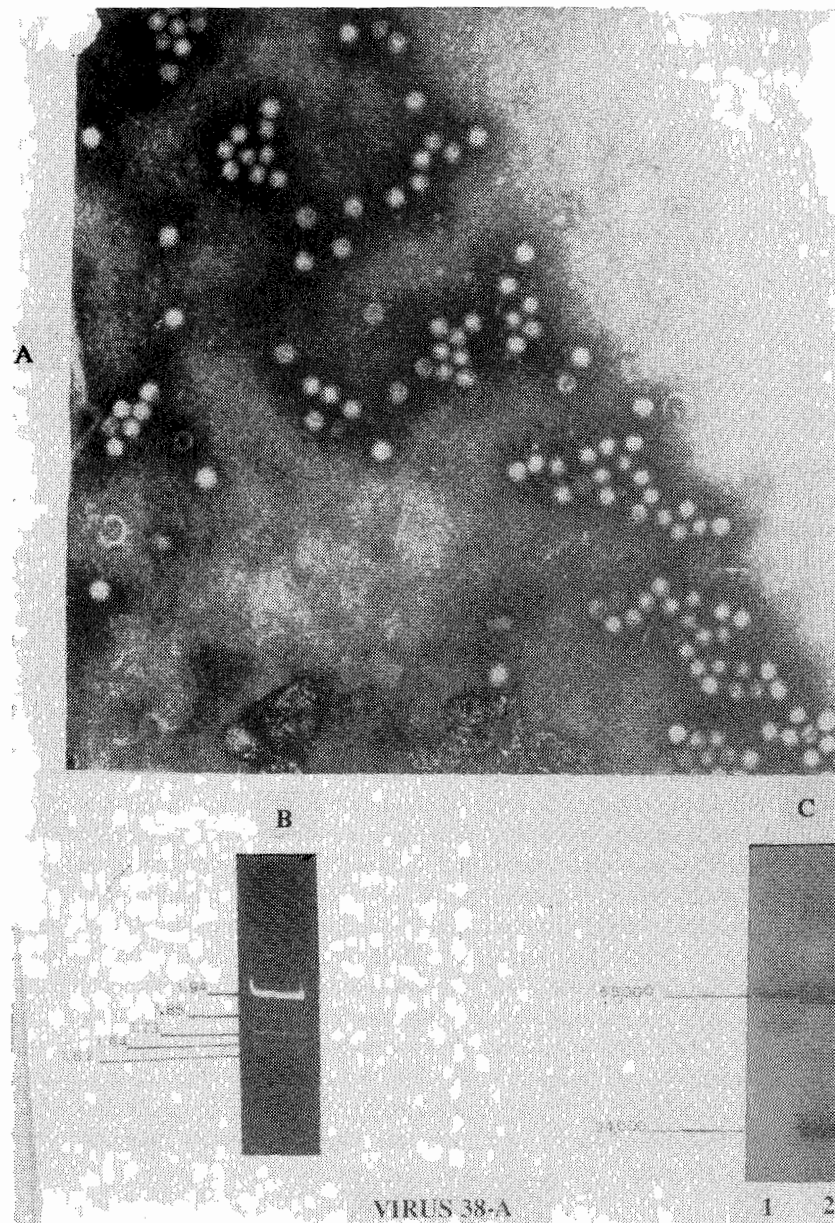


Fig.1. A. Electron Micrograph of Ggt virus 38-a (x 105.000 Magnification).  
 B. dsRNA banding pattern of virus 38-a in 46 Polyacrylamide gels (Sizes of dsRNA are in Kbp).  
 C. SDS Polyacrylamide gel Electrophoresis.  
 Lane 1 = Capsid polypeptide; mol. wt. 55.000.  
 Lane 2 = Human globulin: mol. wt. of heavy chain 55,000; mol. wt. of light chain 24,000.

**Isolations and purification of virus:** *G. graminis* isolates were grown in 60 litre fermenters for 3 days at 24°C in the glucose corn steep liquor medium (Jamil *et al.*, 1984; Banks *et al.*, 1971). Preparation and purification of virus by polyethylene glycol precipitation, ultracentrifugation and sucrose density gradient centrifugation were carried out as described by Buck *et al.*, (1981). The two viruses in isolates 87-1 were separated by CsCl density gradient centrifugation as described by Jamil & Buck (1984).

**Electron microscopy:** Purified virus samples were negatively stained with 1% potassium phosphotungstate pH 7. The grids were examined in a Siemens Elmiskop Ia electron microscope and photographs were taken on Ilford EM plates. A magnification of x 32743 was used, followed by x 3.5 in printing to give an over all magnification of x 105000.

**Isolation and analysis of dsRNA:** Virus RNA was prepared by phenol extraction and shown to be double stranded by S1 nuclease treatment. Analysis and molecular weight determinations of dsRNA component were carried out by polyacrylamide gel electrophoresis (PAGE) as described by Buck & Ratti (1977) using internal dsRNA standards covering the mol. wt. range,  $1.11 \times 10^6$  to  $6.3 \times 10^6$  from the following viruses: *Aspergillus foetidus* viruses S and F (Buck & Ratti, 1977); *Helminthosporium maydis* virus (Bozarth, 1977); *Ggt 3 bla* virus (Buck *et al.*, 1981).

**Analysis of capsid polypeptide:** Viral proteins was denatured by heating viral suspensions in 1% SDS and 0.1% 2-mercaptoethanol for 3 min, at 100 C, then analyzed by electrophoresis at 60-100V for 16-18 h in 4% - 10% SDS PAGE as described by Laemmli (1970). Denatured polypeptide samples were co-electrophoresed with denatured protein markers. The following standards were used; phosphorylase A subunit mol. wt. 94000; Bovine Serum albumin mol. wt. 68,000; Lactate dehydrogenase mol. wt. 36,000; glutamate dehydrogenase mol. wt. 55,490 human gamma globulin heavy chain mol. wt. 55,000 light chain 24,000; oval albumin mol. wt. 47,000 Pyrovate Kinase 60,000.

### Results and Discussion

Buck *et al.*, (1981) defined the properties of *Ggt* groups I and II viruses as follows: group I particle diameter 35 nm, capsid polypeptide mol. wt. 54,000 to 60,000, two to four dsRNA segments 1.53 to 1.94 Kbp; group II particle diameter 35 nm, capsid polypeptide mol. wt. 68,000 to 73,000, two to four dsRNA segments in the group II range. Confirmation of the grouping was obtained for isolated virus particles from four isolates (87-1, 38, 74 and 16).

Viruses 38-A and 87-1-L can be accommodated in group I, since their particle diameter, capsid polypeptide mol. wt. and size of dsRNA components are in good agreement with the other members of this group (Table 1). This was confirmed for 38-A virus which was found to be weakly serologically related to a previously described group I virus (F6-C) although no sequence homology could be detected between virus 87-1-L and several other group I viruses. However the study was not comprehensive and distant serological relationships cannot be excluded, since the titres of some of the sera used were only moderate (1:64 to 1:16). 38A and 87-1-L viruses are distinct viruses since no sequence homology was detected between their dsRNAs.

The number of dsRNA segments (Six) associated with 38-A virus (Fig.1) is greater than that of previously described group I viruses two to four (Buck *et al.*, 1981). Although the virus has a single capsid polypeptide mol. wt. species, it could still be a mixture of virus strains viruses 3bla-B1 and 3bla - B2, both present in isolates 3bla, have the same capsid polypeptide mol. wt. but have distinct dsRNA components (Mcfadden *et al.*, 1983;

**Table 1. Properties of viruses from *Ggt* isolates 34, 78, 87-1.**

Virus	Particle dia (nm)	Size of dsRNA components (kbp)	Mol. wt. of capsid polypeptide ( $\times 10^{-3}$ )
38 - A	35	1.94, 1.85, 1.82, 1.73, 1.64, 1.62	55
74 - A	35	2.33, 2.24	73
87 - 1 - L	35	1.85, 1.76, 1.48	60
87 - 1 - H	41	6.1	84, 78

Stanway & Buck; 1984). Originally it was believed that they were a single virus (Buck *et al.*, 1981). Furthermore in agarose gel electrophoresis intact particles of 38-A virus gave two closely spaced bands which stained with both coomassie Blue and ethidium bromide, suggesting the presence of two viruses (Fig. 2).

Virus 74-A has characteristic features of group II viruses. The capsid polypeptide mol. wt. of 73,000 and the size of the two genomic dsRNAs (2.33 Kbp and 2.24 Kbp). Fig.3 is the same as those of a previously described group II virus, 3 bla-B1 (Mcfadden *et al.*, 1983; Stanway & Buck, 1984). Furthermore, gel transfer hybridization under conditions of both low and high stringency showed that the dsRNAs of the two viruses were closely related (unpublished). Both isolates come from Rothamsted: 3bla from Little Knottfield in 1970 and 74 from Highfield in 1981. Interestingly isolate 74 was infected with only one virus whereas isolate 3bla was infected with four viruses, encompassing group I, II, and III. However virus B1 was the only one to be transmitted into ascospores (Mcfadden *et al.*, 1983).

Sequence homology was also detected between 74-A dsRNA and a dsRNA of 2.17 from virus OgA-A, another group II virus (unpublished). However in this virus the dsRNA was slightly smaller than either of those of 74-A. Isolate OgA came from a different location. Small differences of size in dsRNA segments are of doubtful significance since they can arise for example, during ascospore formation (Mcfadden *et al.*, 1983). Hence of the five group II viruses described by Buck *et al.*, (1981) and Mcfadden *et al.*, 1983), virus 74-A is related to three closely to 3 bla -B1 and distantly to OgA-A and F6-B. No relationship was detected to group II virus T1-A, from an Australian isolate of *Ggt*, T1-A virus was previously found to be only distantly related to the group II viruses from European *Ggt* isolates.

*Ggt* virus groups I and II have been placed in the Partitiviridae family of dsRNA mycoviruses (Brown, 1985). Partitiviridae have genomes of two monocistronic dsRNA segments, one encoding the capsid polypeptide and the other probably encoding a dsRNA - dependent RNA polymerase. Some viruses in groups I and II have additional dsRNAs and these could be satellites, similar to a satellite dsRNA associated with a previously described *Ggt* virus (Romanos *et al.*, 1981). Evidence to support came from

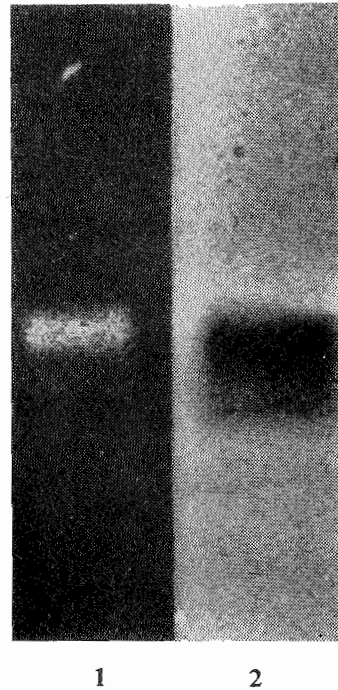


Fig.2. Agrose gel Electrophoresis of intact virus particles  
 Lane 1 = Gel stained with ethidium bromide.  
 Lane 2 = Gel stained with Coomassie blue

individual dsRNAs. Virus 87-I-L Fig. 4, has three dsRNA and probes from each RNA did not hybridize with the other two (Jamil *et al.*, 1984). Hence the third RNA is probably a satellite, rather than a defective RNA which would be expected to be related to one of the genomic RNAs (Huang & Baltimore, 1977). Similar results were obtained from the dsRNAs of virus 38-A, although complete separation of all the smaller dsRNA segments was not possible. Some or all of the dsRNAs with sizes below the group I i.e., 1.48 to 1.35 Kbp range was found in some of the field isolates.

Buck *et al.*, (1981) defined the properties of *Ggt* group III viruses as follows: Particles 40 nm diameter, two dsRNA components 4.7 to 6.3 Kbp and three capsid polypeptides, mol. wt. 87,000, 83,000 and 78,000. Virus 87-I-H has similar properties (Fig. 5) to the group III viruses and was found to be closely related serologically to virus F6-A (antiserum to virus 3 bla A was not available) (unpublished). However, viruses 87-I-H had only two capsid polypeptide species with mol. wt. 84,000 (P1) and 78,000 (P11). It has been noticed that molar ratio of these polypeptides is 1 : 1 in fresh virus preparation purified by CsCl gradient centrifugation, but on storage, P1 starts degrading into P11, hence the ratio of P1 and P11 in old preparation becomes 1 : 2. Tryptic and peptic fingerprints and amino acid analysis of P1 and P11 indicated that P1 is derived from P11, either *in vitro* or *in vivo* (Jamil & Buck, 1984). The more rapid conversion of P1 into P11 on aging of 87-I-H virus preparation, purified by CsCl gradients compared to sucrose

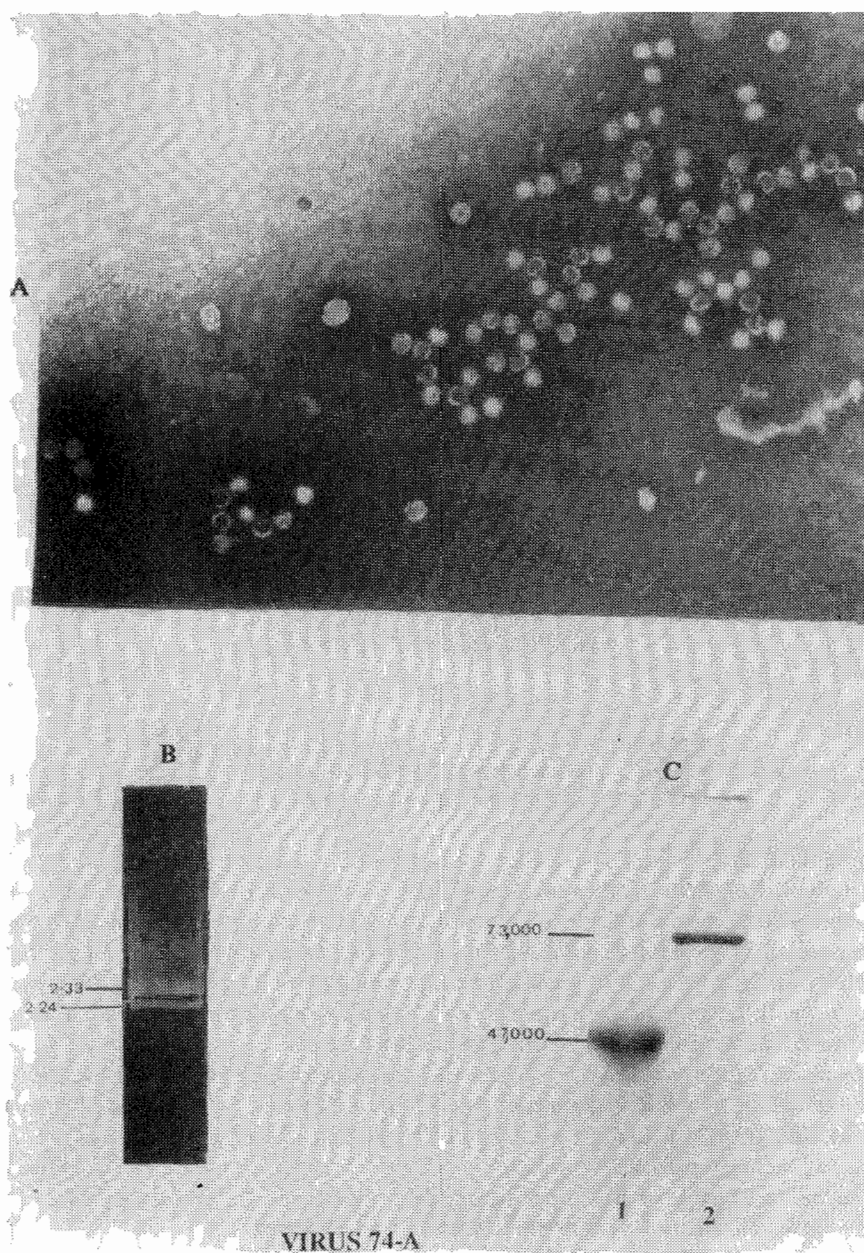


Fig.3. A. Electronmicrograph of Ggt virus 74-A ( $\times 105,000$  Magnification).  
B. dsRNA Banding Pattern of virus 74-A in 4% Polyacrylamide gels. (Sizes of dsRNA are in Kbp).  
Lane 1 = EGG albumin Mol. Wt. 47,000 SDS Polyacrylamide gel Electrophoresis.  
Lane 2 = Capsid Polypeptide of virus 74-A.



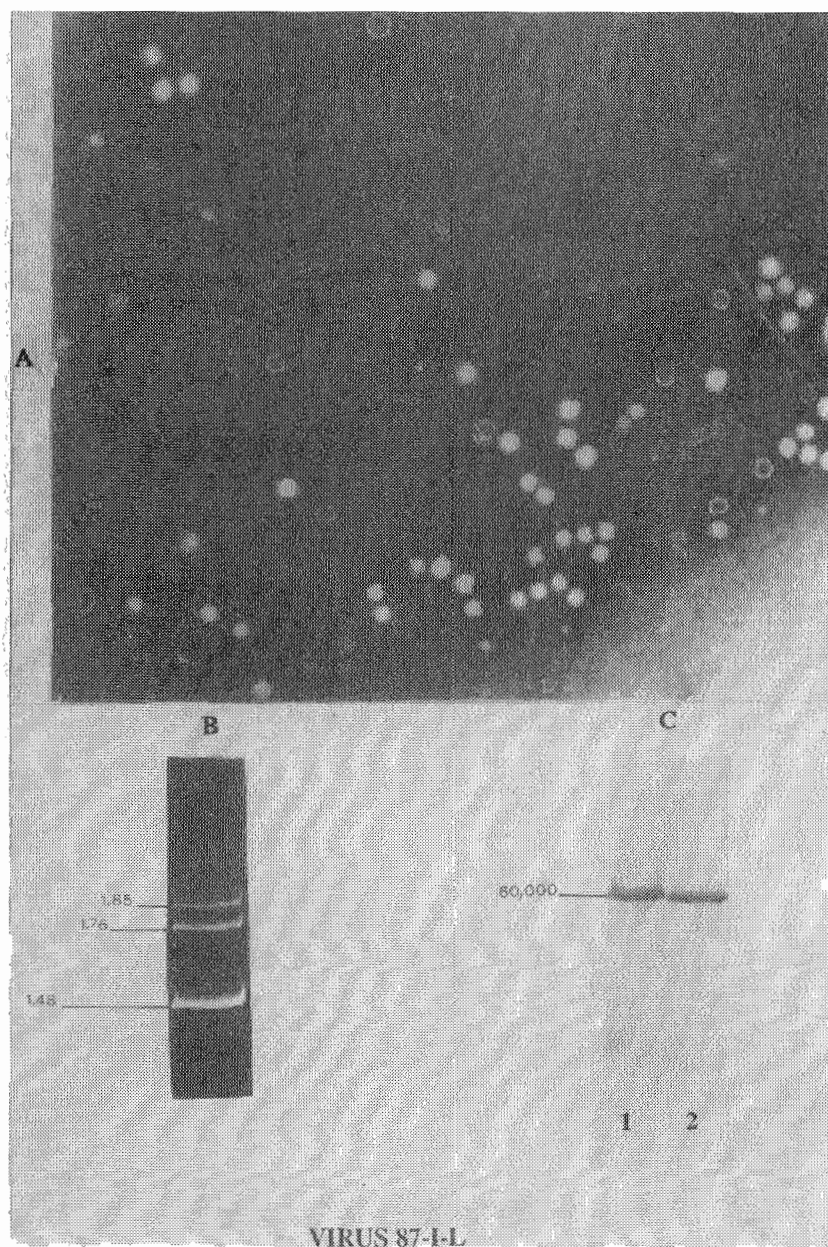


Fig.4. A. Electronmicrograph of Ggt virus 87-1 L (x 105,000 Magnification).  
B. dsRNA banding pattern of virus 87-1-L in 4% Polyacrylamide gel Electrophoresis.  
Lane 1 = Pyrovate Kinase mol. wt. 60,000.  
Lane 2 = Capsid polypeptide of virus 87-1-L: mol wt 60,000.

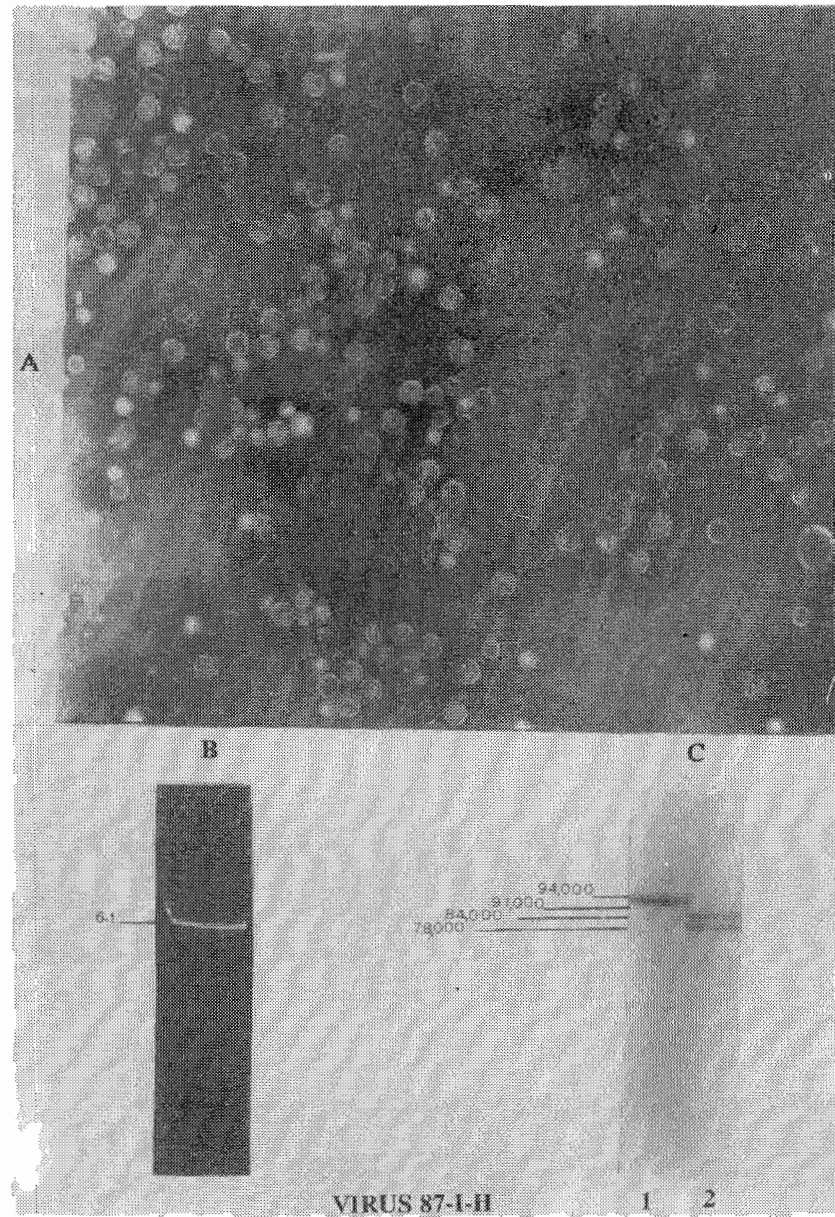


Fig.5. A. Electronmicrograph of Ggt Virus 87-1-H (x 105.000 Magnification).  
 B. dsRNA banding pattern of virus 87-1-H in 4% Polyacrylamide gels. (Size of dsRNA in Kbp)  
 C. SDS - Polyacrylamide gel Electrophoresis.  
 Lane 1 = Phosphorylase A. mol. wt. 94,000.  
 Lane 2 = Capsid polypeptides of virus 87-1-H Preparation purified by CsCl density gradient.



gradient purified virus, supports the possibility that PI cleaved into PII after virion disruption *in vitro*. Since the virus was less stable in CsCl Proteolytic cleavage of capsid polypeptide has been reported for *Aspergillus foetidus* virus S in which the major capsid polypeptide O-1 was converted to O-2 by a protease both during virus preparation and on subsequent storage (Buck & Ratti, 1975). It is likely, therefore, that virus 87-I-H (and probably also viruses 3bla-A and F6- A) has only one major capsid polypeptide. However, it has been noticed that the molar ratio of PI and PII in 87-I-H virus, which was purified by sucrose gradient only, remained constant. Hence the possibility can not be eliminated that some conversion of PI into PII takes place *in vivo*, because of post translational modification, analog one to reovirus M2 protein which is a cleavage product of U2 *in vivo* (Silverstein *et al.*, 1976).

Another apparent difference between virus 87-I-H and the other two group III viruses, F6-A and 3bla-A, was that 87-I-H apparently had only one dsRNA species (Fig. 5). In this context it is noteworthy that dsRNA of 87-I-H has shown moderate and weak sequence homology with larger and smaller dsRNAs of 3bla-A virus respectively (unpublished). There are two possible interpretations of these results:

- 1) The two 3 bla-A dsRNAs could be related to each other, the larger being the virus genomic dsRNA and the smaller a defective yeast strains (Bruenn & Kane, 1978).
- 2) 87-I-H dsRNA could consist of two components which comigrate in gel electrophoresis and each of which is related to one of the 3bla dsRNAs. Some dsRNAs in yeast strains have been shown to consist of at least two different components with different nucleotide sequences, but which migrate as a single band in gel electrophoresis (Sommer & Wickner, 1982; Field *et al.*, 1982). Molecular cloning of 87-I-H dsRNA would resolve which of these two possibilities is valid.

Members of the *Totiviridae* family of dsRNA mycoviruses (Brown, 1985) have the following properties:

Virus particles, 40-43 nm ; single molecule of dsRNA 4.8 to 6.1 Kbp, capsid polypeptide mol. wt. 75 to 88 x 10<sup>-3</sup>, buoyant density in CsCl, 1.40-1.42 gm. If virus 87-I-H is confirmed to have only one dsRNA component and one major capsid polypeptide species as seems likely from the results presented here, it could be assigned to the *Totiviridae* family.

#### References

- Banks, G.T., K.W. Buck and A. Fleming. 1971. The isolation of viruses and viral ribonucleic acid from filamentous fungi on a pilot scale. *Chem. Engineer.* 251: 259-261.
- Bostian, K., W. Bussey, Q. Elliot, V. Burn, A. Smith and D.J. Tipper 1984. Sequence of the pretoxin in dsRNA gene of type I Killer yeast: multiple processing events produce a two component toxin. *Cell.* 36: 741-751.
- Bozarth, R. F. 1977. Biophysical and biochemical characterisation of virus-like particles containing high molecular weight dsRNA from *Helminthosporium maydis*. *Virology*, 80: 149-157.
- Brown, F. 1985. *Classification and nomenclature of viruses*. Fifth Report of the International Committee on Taxonomy of viruses. S. Karger, Basel.
- Bruenn, J. and W. Kane. 1978. Relatedness of the dsRNAs present in yeast virus like particles. *J. Virol.*, 26: 762-772.
- Buck, K. W. and G. Ratti. 1975. Biophysical and Biochemical properties of two viruses isolated from *Aspergillus foetidus*, *J. Gen. Virol.*, 27: 211-224.
- Buck, K.W. 1984. A new double-stranded RNA virus from *Gaeumannomyces graminis*. *J. Gen. Virol.*, 65: 987-990.
- Buck, K.W. and G. Ratti. 1977. Molecular weight of double-stranded RNA: a re-examination of *Aspergillus foetidus* virus dsRNA components. *J. Gen. Virol.* 37: 215-219.
- Buck, K. W., M.R. Almond, J.J. Mcfadden, M.A. Romanos and C.J. Rawlinson. 1981. Properties of thirteen viruses and virus variants obtained from eight isolates of wheat take all fungus, *Gaeumannomyces graminis var tritici*. *J. Gen. Virol.* 53: 235-245.

- El-Sherbeni, M. and K.A. Bostian. 1987. Viruses in fungi: Infection of yeast with K1 and K2 killer viruses. *Proc. Natl. Acad. Sci.*, 84: 4293-4227.
- Field, L.J., L.A., Bobeck, V.E. Breman, J.D. Reilly and J.A. Breunn. 1982. There are at least 2 yeast viral dsRNAs of the same size. *An Explanation for viral Exclusion cell.*, 31: 193- 200.
- Huang, A.S. and D. Baltimore. 1977. Defective interfering animal viruses. In *Comprehensive virology* 10 (Ed. H. Fraenkel-Conrat and P. R. Wagner : 73-116 New York.
- Jamil, N., K.W. Buck and M.J. Carlile. 1984. Sequence relationship between virus dsRNA from Isolates of *Gaeumannomyces graminis* in different Vegetative compatibility groups. *J. Gen. Virol.*, 65: 1741-1747.
- Jamil, N and K.W. Buck. 1984. Apparently identical viruses from *Gaeumannomyces graminis* var. *tritici* and *Phialophora* sp. (lobed hyphopodia). *Trans. Brit. Mycol. Soc.*, 83: 519-522.
- Jamil, N. and K.W. Buck. 1986. Caspid Polypeptide in Group III virus from *Gaeumannomyces graminis* var. *tritici* are related. *Gen. J. Virol.*, 67: 1717-1720
- Kotlin, Y and J.S. Kandel. 1987. Killer phenotype in *Ustilago maydis* the organization of viral genome. *Genetics*, 88: 267- 276.
- Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, Lond., 227: 680-685.
- Lapierre, H., J.M., Lemare, B. Jouan and G. Molin 1970 Mise en evidence des particules virales associees a une perte de pathogenicite chez le pielin-echuadage des cereales. *Ophiobolus graminis* Sacc. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences D* 271. 1833- 1836.
- Mcfadden, J.J.P., K.W. Buck and C.J. Rawlinson. 1983. Infrequent transmission of double stranded virus particles but absence of DNA provirus in single ascospores cultures of *Gaeumannomyces graminis*. *J. Gen. Virol.*, 64: 927-937
- Pryor, A. and M.G. Boelen. 1987. A dsRNA mycovirus from maize rust *Puccinia sorghi*. *Canad. J. Bot.*, 65: 2380-2383.
- Rogers, H.J., K.W. Buck. 1986. Transmission of double stranded RNA and a disease factor in *Ophiostoma ulmi*. *Plant Pathology*, 35: 277-287.
- Romanos, M.A K.W. Buck, C.J. Rawlinson. 1981 A satellite dsRNA in a virus from *Gaeumannomyces graminis*. *J. Gen. Virol.*, 57: 375-385
- Stanway, C.A. and K.W. Buck. 1984. Infection of protoplasts of the wheat take-all fungus, *Gaeumannomyces graminis* var. *tritici*, with double stranded RNA viruses. *J. Gen. Virol.*, 65: 2061-2065.
- Silverstein, S.C and J.K. Christman. 1976. The reovirus replication cycle. *Ann. Rev. Biochem.*, 45: 375-408
- Sommer, S.S. and R.B. Wickner. 1982 Co-curing of plasmids affecting Killer dsRNA of *S. cerevisiae*. HoK, NEX, and the abundance of L are related and further evidence that M require L. *J. Bacteriol.*, 150: 545-551.

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