Pak.J.Bot., 23(2): 160-164, 1991.

EFFECT OF VEGETATIVE INCOMPATIBILITY ON DOUBLE STRANDED RNA AND MYCOVIRUS TRANSMISSION IN *GAEUMANNOMYCES GRAMINIS* VAR. *TRITICI*

NUSRAT JAMIL* AND K. W. BUCK

Department of Pure and Applied Biology,
Imperial College of Science and Technology,
London SW7 2BB, U.K.

Abstract

The vegetative incompatibility of *Gaeumannomyces graminis* (wheat plant pathogen) was examined by physical interaction of field isolates on potato dextrose agar. Due to multi-allelic system 31 isolates of *Gaeumannomyces graminis* var. *tritici* were divided into 18 vegetative incompatibility (V-C) groups. The genetic mechanism of somatic incompatibility appears to be heterogenic. Fusion incompatibility was rarely found. The dsRNA components from these isolates belonged to different V-C group e.g., 38 (V-C group A) and 43 (V-C group B) are of the same size. Similar ly dsRNA components from isolates 87-1(V-C group F) and isolates 83(V-C group G) have same size range. The close similarity of the dsRNAs present in isolates from different V-C group suggests that somatic incompatibility cannot restrict the transmission of virus or viral genome (dsRNA).

Introduction

Several fungi can be divided into numerous vegetative compatibility (V-C) groups; hyphal fusion between strains from V-C groups results in vegetative (somatic) incompatibility reactions in which fusion is terminated by localized death of cell through a clear zone containing few living hyphae and often banded on each side by a narrow zone of intense black pigmentation (Lane, 1981; Todd & Rayner, 1980). Caten (1972) has suggested that this kind of incompatibility may serve to protect fungi against genetic infection by preventing the spread of viruses and other suppressive cytoplasmic determinants from strain to strain in nature.

In *Endothia parasitica* some vegetative incompatibility pairings allow the transmission of hypovirulence determinants (Anagnostakis & Day 1979). The presence of the dsRNA, found in hypo-virulent strains, may overcome vegetative incompatibility or some genetic differences leading to vegetative incompatibility may allow more cytoplasmic exchange than others. Even in an incompatible interaction there may be sufficient cytoplasmic contact to permit transfer of dsRNA before cell death occurs (Anagnostakis & Day, 1979; Ghabrial, 1980). Hence vegetative incompatibility is not a complete barrier for virus transmission. Virulent (V) strains of *E. parasitica* were converted to hypo-virulent (H) strains by hyphal anastomosis and dsRNA transfer (Anagnostakis, 1981).

*Department of Microbiology, University of Karachi, Karachi-75270, Pakistan.

Vegetative incompatibility between isolates of *Gaeumannomyces graminis* var *tritici* has been observed by several workers(Davis, 1925; Chambers *et al.*, 1967; Cunningham, 1975). However, no attempts were made to classify isolates into V-C groups or to study effect of the phenomenon on virus transmission. The present work was undertaken with the aim of understanding the effect of vegetative incompatibility by classifying the range of field isolates of *Ggt* into different V-C groups and to screen dsRNA components from these isolates.

Materials and Methods

Isolates of *Ggt* used in the present study were provided by Dr. Slope from Rothamsted Experiment Station.

Assignments of fungal Isolates to V-C groups: Interaction between pairs of *Ggt* isolates on PDA plates were examined by inoculating two pairs of 2.5 mm diameter discs of agar carrying mycelium from different isolates. Incubation at 25°C resulted in four colonies which came into contacts giving test encounters and also control encounters between the colonies of the same isolates.

Microculture of cellophane films: To examine the interaction between *Ggt* isolates by high-power microscopy, isolates were grown on cellophane strips (300 PT grade) of 2 x 4 cm. The strips were first boiled in water for 20 min., to remove the plasticizer and then autoclaved in test tubes containing water. A 2 cm agar pieces were cut from the center of plates containing Lilly and Barnett agar. Sterile strips of cellophane were then laid on the medium across the slit, 2.5 mm diameter plugs of each isolate were incubated until fungal colonies just made contact. The cellophane strips were then removed and examined microscopically.

Microculture Technique: On a microscope glass slide, 3 x 1", two strips of Whatman 3 mm filter paper (0.2 cm) were stuck just parallel to each other on to them a glass coverslip was stuck with Araldite. This slide was placed in a glass Petri plate and autoclaved. A 0.5 ml of H_2O was poured in between the surface of the slide and coverslip with the help of a Pasteur pipette , so that the whole surface was covered and allowed to set.

To see the interaction between different strains, 2 fungal cultures were inoculated with sterile needles at both open ends of the agar. Some H_2O soaked filter paper strips were placed in the Petri dish to keep damp conditions, and the plate was incubated at 24 °C for 3-4 days until the hyphae of both cultures confronted each other. Then the interaction was observed under the microscope.

Nucleic Acid extraction

Preparation of dsRNA from fungal Mycelium: *G. graminis* isolates were grown for 10 to 14 days at 24 °C in shaken flasks in a medium containing 1% (w/v) glucose monohydrate, 3% (w/v) corn steep liquor, 0.1% (w/v) K_2HPO_4, 0.05% (w/v) $MgSO_4$, 0.001% w/v $FeSO_4$. $7H_2O$ adjusted to pH 6.0 with NaOH). Mycelium was harvested by filtration and disrupted by grinding in liquid nitrogen. The RNA was then isolated by phenol extraction and cellulose chromatography as described by Morris & Dodds (1979). 4% Polyacrylamide gel elect- rophoresis of dsRNA was done as described by Jamil & Buck (1984).

Results

Thirty one isolates of *G. graminis* were classified into 18 V-C groups by testing all pairwise combinations. Growth of each isolate occurred symmetrically from the plugs of inoculum, and after 7 to 10 days when hyphae from opposing colonies had intermingled, two different types of interactions were observed.

(1) A dark black line appeared at the region of confrontation of the opposing mycelia which developed into a barrage (Fig. 1) similar to the incompatibility reactions shown by other fungi (Esser , 1971).

(2) Colonies from the two isolates merged freely without any apparent adverse reaction. Isolates were assigned to V-C groups on the basis that within each groups yielded barrage reactions. The results of interaction are shown in (Table 1) and the classification of the isolates into 18 V-C groups A to S is given in (Table 2).

***Microcultures and Culture of Cellophane films*:** Both types of cultures were examined microscopically (X 400 and X 1000). Hyphal strands were growing sparsely in the barrage region. Hyphal cells were found to be highly vacuolated or empty. Mycelium was growing rapidly at the peripheral region. Hyphal fusion or anastomosis were observed only between lateral hyphae. The cell wall of macrohyphae was found to be more refractile and black in color than the microhyphae. Cells involved in apparent anastomosis were collapsed, or distorted, indicating post-fusion death. Contortion of the hyphae gave cells that seemed to be healthy. Essentially similar results were obtained from the following pairs of isolates : (1) 37 x 75 (2) 39 x 75 (3) 38 x75 (4) 30 x 75 (5) 74 x 75.

***Analysis of the dsRNA content of G.graminis field isolates*:** Total nucleic acids were extracted from the mycelium of each of the 31 *Ggt* field isolate; dsRNA was then separated from single -stranded RNA and DNA by chromatography on Whatman CF II cellulose and analyzed by PAGE as described in Method. Bands were identified as dsRNA by their resistance to DNAase I and by their resistance to RNAase A. Molecular weights of dsRNA components were determined by reference to standard dsRNAs of known molecular weights included in same gel. Plots of log molecular weight versus electrophoretic mobility gave a smooth curve (Fig.2) similar to the results of Bozarth & Harley (1976) and Buck & Ratti (1977). Molecular weights were converted to sizes in Kbp using a value of 687 for the molecular weight of 1 bp (Field *et al.*, 1983).

Double stranded RNA patterns are shown in (Fig.3) No dsRNA could be detected in ten isolates , and only trace amounts in a further seven isolates (Table 2). The fields of dsRNA in the remaining 14 isolates were from 2-5 μg/g (wet weight) of mycelium.

Discussion

Incompatibility results from the interaction of specific alleles at different loci (Fincham *et al.*, 1979). Juxtaposition of mycelia carrying incompatible alleles results in the formation of a barrage between them, meaning a sparsely colonized "no mans land" with abortively heterokaryotic and degenerating hyphae.

Thirty one isolates of *Ggt* from Highfield, Rothamsted were divided into 18 V-C groups, when they were paired in all possible combinations on PDA medium. The genetic mechanism of somatic incompatibility seems to be heterogenic in nature. It can be suggested that this incompatibility system involves multialleles and possibly more than one locus, in other words this system refers to one possible explanation from this V-C grouping. (a) At one extreme if only allelic incompatibility at one locus "a" is involved there would be 18 different allelic and each isolate would have a different allele, eg., a1 , a2, a3, a18. Genetic crosses between pair of isolates would result only in the parental V-C genotypes. (b) At the other extreme 18 V-C groups could be explained by fine biallelic V-C genes which could give 32 V-C groups. This would be analogous to *Podospora anserina* incompatible strains which have nine unlinked loci each with bialleles (Bernet, 1967 ; Bernet & Begueret, 1968), and *Endothia parasitica* in which seven V-C genes with alternative alleles can account for the multiple V-C groups (Anagnostakis, 1983). In such cases genetic crosses between isolates give rise to new V-C phenotypes in the progeny resulting from genetic combinations which produce new combinations of V-C genes.

The number of V-C groups in *Ggt* isolates from two plots in a single field at Rothamsted indicates a diverse population of individual *Ggt* propagules. This was further emphasized by the differing patterns of dsRNA segments in different isolates (the same pattern was found only twice in 14 isolates examined). However,since minor changes of size of dsRNA segment have been reported on transmission e.g., into ascospores of *Ggt*, (Mcfadden *et al.,* 1981) more significant was the observation that sequence homology between dsRNA from different isolates was comparatively rare. For example sequence homology with dsRNA from virus 89-I-L (group I) was detected in only two out of twelve isolates having dsRNA in the group I size range. Furthermore no homology could be detected between dsRNA from virus 74-A (group II) and dsRNA in the group II range from six other isolates. (Jamil & Buck, 1984).

The *Ggt* which has to respond both to alternating parasitic and saprophytic phases and to changing cropping patterns with their associated changes of competing soil microflora. Cunningham (1975) has postulated that the population of *Ggt* in first cereal crops consists of highly aggressive individuals selected on the basis of their parasitic ability, whereas during several years of cereals monoculture the population shifts towards less aggressive isolates with greater competitive saprophytic ability.

For many ascomycetes including *Ggt*, the dominant phase of the life cycle is the true vegetative phase. Genetic diversity in a population would be created slowly, by mutation and more rapidly by genetic crossing of mutants which have already evolved. The role of ascospores in the life cycle is still uncertain, but they could be the source of genetic diversity which arises in fields newly colonized by *Ggt*. Although *Ggt* is homothallic, out crossing can also occur (relative heterothallism) and perithecia are known to be formed at the stem base of heavily infected plants in the fields. Vegetative incompatibility, as well as preventing or reducing the spread of potentially harmful cytoplasmic elements such as viruses (Caten, 1972), would also serve to maintain the integrity of individuals within a population.

Although fusion incompatibility would not have been distinguished from compatibility in the test carried out, the small number of isolates within individual V-C groups suggests that it is rare in *Ggt*. Nevertheless the finding of completely different patterns of dsRNA from isolates within a V-C group; e.g., isolates 38 and 62 in group A, suggests that either these isolates do not fuse or else they have not come in contact with each other. Either way that serve to emphasize the heterogeneity of *Ggt* populations.

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(Received for Publication 11 February 1991)