A Uninucleate Wheat-infecting Strain of the Stem Rust Fungus Isolated from Axenic Cultures

By D. J. MACLEAN AND K. J. SCOTT

*Department of Botany, University of Queensland,
St Lucia, Queensland 4067, Australia*

AND INEZ C. TOMMERUP

*Department of Botany, University of Cambridge,
Downing Street, Cambridge, CB2 3EA*

(Accepted for publication 14 January 1971)

SUMMARY

Four monokaryotic axenic cultures derived from a dikaryotic isolate of *Puccinia graminis tritici* race 126-ANZ-6, 7 were tested for pathogenicity on wheat leaves. Only two rust cultures were pathogenic, one of which was propagated on wheat for six uredial generations. Cytological examination showed that cells of this culture were uninucleate and apparently haploid. This isolate may have arisen by somatic recombination.

INTRODUCTION

The growth of plant rust fungi (Uredinales) in artificial culture media (axenic culture) has now been firmly established (Scott & Maclean, 1969). Such cultures should prove useful for biochemical, physiological, and genetical studies of the fungus and of the host-parasite system. However, first we must characterize any spontaneous variation which occurs in rusts grown in axenic culture. In some of our experiments (Maclean & Scott, 1970), the incubation of wild-type uredospores of *Puccinia graminis tritici* race 126-ANZ-6, 7 on an artificial medium has given rise to colonies which are morphologically distinct from those described previously (Williams, Scott & Kuhl, 1966; Williams, Scott, Kuhl & Maclean, 1967; Bushnell, 1968; Coffey, Bose & Shaw, 1969). These colonies were isolated at an erratic and low incidence, and it was found that the mycelium consisted of uninucleate cells (Maclean & Scott, 1970) in contrast to the bi- or multi-nucleate cells in mycelium formed after infection of wheat plants by wild-type uredospores (Nelson, Wilcoxson & Christensen, 1955). The nuclear condition and pathogenicity of four uninucleate isolates are described below.

METHODS

Wheat plants (variety: Little Club) fully susceptible to *Puccinia graminis* (Pers.) f. sp. *tritici* (Eriks. & E. Henn.) race 126-ANZ-6,7, were grown in pots containing a sand + soil mixture in a growth cabinet (16 h. light régime: 24 to 26° light, 20° dark). Axenic cultures of monokaryotic strains (v1, v2, v3 and v4) of the above race of rust were isolated and maintained as described previously (Maclean & Scott, 1970).

Wheat leaves were inoculated with mycelium using methods similar to those of

Williams *et al.* (1967). Mycelial colonies (3 to 5 mm. diameter) were grown on an agar medium overlaid with Cellophane (Maclean & Scott, 1970). After partial removal of the abaxial epidermis of primary leaves of 9 to 10 day sown wheat, single fungal colonies were applied so that aerial hyphae were in contact with exposed mesophyll. The inoculum was held in position with square self-adhesive labels of the type generally used for affixing to microscope slides (Stickfast Labels Pty Ltd, Sydney, Australia). Pots containing inoculated plants were covered with polythene bags (to keep humidity high) and replaced in the growth cabinet. The polythene bags were removed after 3 to 4 days.

For cytological examination, mycelium or infected leaves were fixed in lactic acid + acetic acid + ethanol (1 + 1 + 6, by vol.) (Singleton, 1953), for 12 to 24 h., then stored in 70 % ethanol. Small pieces of mycelium, or frozen sections (10 μ m.) of infected leaves were placed in N-hydrochloric acid for 8 min. at 60°, then stained by the Giemsa technique (Tommerup, 1969). Alternatively, nuclei could be observed by phase contrast microscopy in unfixed teliospores mounted in water.

Wheat leaves were inoculated with uredospores using a small, sterile scalpel or by rubbing the spores onto the leaf with clean fingers. The plants were incubated in a glass tank in the dark at 100 % humidity and 22 to 23° for 20 to 24 h. Better infection with VIC uredospores often resulted if the glass tank was lined with wet filter-paper. After incubation the plants were replaced in the growth cabinet.

RESULTS AND DISCUSSION

Pathogenicity of isolates

Under natural conditions, the uninucleate state is restricted to the diploid fusion nucleus in mature teliospores formed on wheat, and to the haploid mycelium initiated by basidiospore infection of barberry (Buller, 1950). We carried out a series of tests to investigate any interaction between wheat leaves and uninucleate mycelium of V1, V2, V3 and V4. After 6 to 12 days incubation, sori containing uredospores or teliospores or both ruptured the epidermis opposite an inoculum of V1 or V3, but not V2 or V4. In some sori as many as 50 % of teliospores were single-celled. Isolate V3 differed from V1 in that spores took longer to develop, and most cells in the sporophore layer of V3 remained sterile. The pathogenicity of V1 and V3 has been demonstrated on many leaves on at least four separate occasions; however, repeated attempts to infect wheat leaves with mycelium of V2 and V4 all failed.

Tests were made of the ability of uredospores of V1 formed on wheat leaves under the above conditions to infect intact wheat plants. In March 1969 uredospores were placed on the primary leaf of two plants growing in a pot containing about 30 plants. A single rust pustule was subsequently formed on one of the inoculated leaves; the non-inoculated plants remained uninfected. This isolate (designated VIC) was propagated on intact plants for 3 to 4 generations; uredospores from the later generations were used to inoculate a nutrient agar medium (Williams *et al.* 1967). The axenic cultures of VIC so obtained were maintained by serial subculture. Such cultures of VIC showed growth characteristics different from those of V1, the parental culture. Similar infection tests were not carried out with V3 because few uredospores were formed after infection by the epidermis-removal method.

Experiments were performed to determine whether saprophytic mycelium of VIC

retained pathogenicity. Wheat leaves were readily infected by the epidermis-removal method of infection. The uredospores so formed were applied to 13 leaves on intact plants of which eight developed 1 to 5 pustules per leaf.

Disease symptoms caused by uredospores of VIC and the wild-type were readily distinguished. For example, flecking appeared 7 to 9 days after inoculation by VIC, compared with 5 to 6 days for the wild-type. Pustules of the wild-type were more elongated and produced a greater profusion of uredospores than did VIC. Whereas wild-type infections rarely formed teliospores under the conditions in our growth cabinets, VIC infections often entered a telial stage 1 to 3 weeks after rupture of the host epidermis.

Nuclear condition

The nuclear condition of fungal cells in plants infected by uredospores of VIC was studied. Uredospores and teliospores, together with their pedicels and stalk cells, were uninucleate at all stages of development (Pl. 1, fig. 1, 2), although occasional binucleate spores of each type were found. The proportion of binucleate spores varied from 0 to 3 % in samples of up to 500 spores. The incidence of teliospores containing binucleate cells was about the same as that of binucleate uredospores.

In all VIC infections examined, binucleate cells were usually restricted to spores rather than to vegetative hyphae; spores containing binucleate cells were almost invariably subtended by uninucleate pedicels, and such spores did not occur in sectors; anastomosis was not detected between cells of VIC in the immediate vicinity of dikaryotic spores; occasional late mitotic division occurred in teliospores which had thick dark walls, and so appeared to be mature. This evidence is consistent with VIC being a haplont in which all nuclei are haploid and identical, but nuclear division and septation may sometimes get out of phase, with the consequent formation of spores containing two daughter nuclei. The view that VIC is a haplont is further supported by its derivation from a single pustule on a wheat leaf, the parent uredospore inoculum having arisen from a monokaryotic culture (VI). The uninucleate condition has remained stable for at least six generations of VIC propagated from plant to plant.

The specificity of heteroecious rusts for their primary or alternate host species is thought to be related to the nuclear condition of their mycelium. This idea is consistent with the results of reinfection studies carried out by Cutter (1959; Scott & Maclean, 1969) on his seven axenic isolates of *Gymnosporangium juniperi-virginianae*. These isolates varied in nuclear content: the number of nuclei per cell determined whether the alternate host (*Pyrus*) or the primary host (*Juniperus*) could be infected, and also determined the type of fungal fructification formed. However, this habit does not apply to our uninucleate isolate of *Puccinia graminis tritici* (VIC) since it is able to complete the uredial stage of development on wheat. The work of Newton & Johnson (1939) and Buller (1950) supports our findings. They obtained abnormal strains of *P. graminis tritici* by sowing some races on barberry. Some abnormal strains were propagated on wheat plants and produced uredial sori in some of which all uredospores were uninucleate (Buller, 1950). Buller (1950) thought the uninucleate uredospores were haploid, although direct evidence was not available.

We have examined dividing nuclei of VI, V2, V3 and V4 when grown on agar medium, of VIC and V3 when mycelium had invaded mesophyll cells of wheat after the epidermis had been removed, and of VIC when grown on plants infected by

uredospores. In Giemsa-stained squashes or sections, six or approximately six chromosomes were counted per nucleus in many nuclei of each isolate. Concomitant examination of nuclei in the dikaryotic wild-type organism has also revealed $n = 6$ chromosomes per nucleus, in agreement with previous work (McGinnis, 1953).

Despite circumstantial evidence for the nuclear homogeneity of v1c, the status of our other monokaryotic isolates is less clear because we have recently observed binucleate sectors in saprophytic mycelium of v2 and v3. At present, we are further characterizing the variant forms we have observed in our cultures. It seems possible that at least v1c could have arisen by some mechanism of somatic recombination during the isolation or maintenance of axenic cultures. This is supported by the isolation of similar variants during the selfing experiments of Newton & Johnson (1939).

This work was supported in part by grants from the Wheat Industry Research Council of Australia, and by the Australian Research Grants Committee. D.J.M. held a Commonwealth Post-graduate Award, and I.C.T. received support from the International Federation of University Women and the South Australian Women Graduates Association.

REFERENCES

- BULLER, A. H. R. (1950). *Researches on Fungi*, vol. 7, pp. 293–296. Toronto, Canada: University of Toronto Press.
- BUSHNELL, W. R. (1968). *In vitro* development of an Australian isolate of *Puccinia graminis* f. sp. *tritici*. *Phytopathology* **58**, 526–527.
- COFFEY, M. D., BOSE, A. & SHAW, M. (1969). *In vitro* growth of gelatin suspensions of uredospores of *Puccinia graminis* f. sp. *tritici*. *Canadian Journal of Botany* **47**, 1291–1293.
- CUTTER, V. M. JUN. (1959). Studies on the isolation and growth of plant rusts in host tissue cultures and upon synthetic media. I. Gymnosporangium. *Mycologia* **51**, 249–295.
- MCGINNIS, R. C. (1953). Cytological studies of chromosomes of rust fungi. I. The mitotic chromosomes of *Puccinia graminis*. *Canadian Journal of Botany* **31**, 522–526.
- MACLEAN, D. J. & SCOTT, K. J. (1970). Variant forms of saprophytic mycelium grown from uredospores of *Puccinia graminis* f. sp. *tritici*. *Journal of General Microbiology* **64**, 19–27.
- NELSON, R. R., WILCOXSON, R. D. & CHRISTENSEN, J. J. (1955). Heterokaryosis as a basis for variation in *Puccinia graminis* var. *tritici*. *Phytopathology* **45**, 639–643.
- NEWTON, M. & JOHNSON, T. (1939). Variation and hybridization in *Puccinia graminis*. *Proceedings of the Third International Congress for Microbiology, New York*, p. 544.
- SCOTT, K. J. & MACLEAN, D. J. (1969). Culturing of rust fungi. *Annual Review of Phytopathology* **7**, 123–146.
- SINGLETON, J. R. (1953). Chromosome morphology and the chromosome cycle in the ascus of *Neurospora crassa*. *American Journal of Botany* **40**, 124–144.
- TOMMERUP, I. C. (1969). *Studies on Some Biotrophic Fungi Associated with Heteropogon contortus*. Ph.D. Thesis, University of Queensland.
- WILLIAMS, P. G., SCOTT, K. J. & KUHL, J. L. (1966). Vegetative growth of *Puccinia graminis* f. sp. *tritici* *in vitro*. *Phytopathology* **56**, 1418–1419.
- WILLIAMS, P. G., SCOTT, K. J., KUHL, J. L. & MACLEAN, D. J. (1967). Sporulation and pathogenicity of *Puccinia graminis* f. sp. *tritici* grown on an artificial medium. *Phytopathology* **57**, 326–327.

EXPLANATION OF PLATE

Fig. 1. Uninucleate uredospores of isolate v1c (*Puccinia graminis tritici*) at different stages of development $\times 700$.

Fig. 2. Single-celled and two-celled uninucleate teliospores of isolate v1c subtended by uninucleate stalk cells $\times 700$.