Table 1 *Effect of burial of sclerotia at different depths in soil on the formation of apothecia*

Depth (cm)	Number of apothecia observed* December 1986**	January 1987**	February 1987**	Mean
0.0	–	–	–	–
0.5	14	18	5	12.3
1.0	23	19	13	18.3
2.0	15	13	21	16.3
3.0	9	12	14	12.3
4.0	–	3	–	1.0
5.0	–	–	–	–

– Apothecia did not form; * Average of 3 replicates containing 10 sclerotia each; ** Number of apothecia (total of 4 weeks).

erect and live-appearing apothecia were counted.

The results indicate that numerous apothecia developed in December (figure 1) from field collected sclerotia buried at 1 cm followed by 2 cm depth. However; the maximum number of apothecia developed in February at 2 cm depth. A few apothecia developed in December from sclerotia buried at 3 cm depth, but their number increased in January and February. Very few apothecia developed during January from sclerotia buried at 4 cm depth. Sclerotia buried at 5 cm depth did not produce apothecia even up to February (table 1). Apothecia were also not formed from sclerotia produced in culture.

Burial of sclerotia at >3 cm depth greatly reduced the number of apothecia. The formation of apothecia was abundant as early as December and ceased by March. These findings were supported by earlier workers[2,6]. Varying number of apothecia at different depths indicates the possibility of apothecial production in the next season from sclerotia buried up to 5 cm depth when they come up with the tillage practices.

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A MODIFIED TECHNIQUE FOR DIFFERENTIAL STAINING OF VESICULAR-ARBUSCULAR MYCORRHIZAL FUNGUS AND ROOT TISSUES

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SYMBIOTIC association of vesicular-arbuscular mycorrhizal (VAM) fungi with plant roots has drawn considerable attention in the recent past. The most commonly used method for the study of mode of infection and the type and degree of root colonization by the VAM fungi is the staining of these fungi in association with the roots. Several stains viz. cotton blue, Sudan IV[1], Trypan blue[2] etc. have been tried but acid fuchsin[3] is reported to be most satisfactory. It stains fungal structures dark red against light to medium red root tissues background. A double staining (toluidine blue-O-acid fuchsin) system, which gives differential colour with root tissues and fungal structures, was developed during the present study and this is found to be superior to the acid fuchsin method.

In order to obtain roots colonized by the mycorrhiza, surface-sterilized (2 min dipping in 1:1000 W/V $HgCl_2$; 4 washings in sterilized distilled water), mungbean (*Vigna radiata* L.) seeds were sown in plastic pots containing sterilized sand mixed with chlamydospores of *Glomus mosseae* (Nicol. & Gerd.) Gerdemann and Trappe comb. nov. and incubated at 25 ± 3°C. Hoagland's solution[4] with half level of phosphorus was periodically applied as a source of nutrients. Two-month-old mungbean plants were uprooted and their roots were washed carefully under running tapwater. The method described by Phillips and Hayman[5] was followed for clearing of roots. Roots (1 cm) were boiled in 10% KOH at 90°C for 45 min, rinsed with distilled water and acidified with dilute HCl. For staining, acidified roots were washed with distilled water, dipped in aqueous solution of toluidine blue-O (0.1%) for 5 min, dehydrated through alcohol series, stained in acid fuchsin for 10 min, cleared in xylene and mounted in D.P.X. mountant.

As a result of the proposed staining procedure, the fungal mycelium attained medium red colour,

whereas the vesicles and arbuscules showed dark red stain against a contrasting background of green to blue coloured host tissues. Even the mycelial strands were clearly discernible from host tissues due to differential staining. This dual stain (toludine blue-O-acid funchsin) system was better than the acid fuchsin alone, where it was comparatively difficult to differentiate thin mycelial strands from host tissues due to light to medium red colour of both. Differential staining of the mycelium is essential for the study of mechanism of root colonization by the VAM fungus.

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A NEW VIRULENCE 104-1(21R31-1) OF *PUCCINIA RECONDITA TRITICI* IN INDIA

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PREVALENCE and distribution of virulences of *Puccinia recondita* f. sp. *tritici* Rob. Ex. Desm, the causal agent of leaf rust of wheat, is being continuously monitored in India for well over five decades. A constant vigil is kept by analysing and identifying the frequency of leaf rust pathogenic forms. Uptil now 29 virulences of *P. recondita tritici* have been reported. Here we present the occurrence of yet another new virulence designated as 104-1 (21R31-1).

Virulence 104(17R23) was recorded in 1972[1] and later two variants 104-A(21R31) in 1975[2] and 104B (29R23) in 1983[3] were detected. During 1985 a leaf rust sample from Kalyansona collected from Rajasthan yielded a virulence similar to 104-A(21R31) but had additional pathogenicity on IWP 94, a line in Set-O of the currently used set of differentials[4].

Table 1 *Comparative reactions of various pathotypes of 104 group on differentiating lines*

Virulence Name		Year recor-	Differentiating Lines			
Old	New	ded	*Lr13*	*Lr18*	*Lr20*	*IWP 94*
104	17R23	1972	O;−2	O;−2	O;−1	O;
104-A	21R31	1975	O;−2	4	4	O;−2
104-B	29R23	1980	2-3	4	O;−1	4
104-1	21R31-1	1985	O;−1	4	4	4

Lr13 = Egret, *Lr18* = Timvera, *Lr20* = Thew.

As the major pathogenicity difference is on Set-O comprising the lines with unknown specific genes so the new pathotype is denoted by a subscript as −1. Isolations from IWP-94 were multiplied on Agra local and used to inoculate the set of differential to confirm the identity of the sample.

On Set-A and Set-B there was no perceptible difference between the new isolate and 104A (21R31) but on Set-O, IWP-94 was susceptible, while it was resistant to virulence 104-A(21R31).

The major difference between 104-1(21R31) and 104B(29R23) that is also virulent on IWP 94 is given in table 1. The avirulence/virulence formula of the new virulence 104-1(21R31) is: *Lr9*, *Lr10*, *Lr13*, *Lr15*, *Lr18* (Sabikei), *Lr19*, *Lr24*, *Lr25*, *Lr26*, *Lr28*, *Lr29*/*Lr1*, *Lr2a*, *Lr2b*, *Lr2c*, *Lr2d*, *Lr3*, *Lr11*, *Lr12*, *Lr14a*, *Lr14b*, *Lr16*, *Lr17*, *Lr18* (Timvera), *Lr20*, *Lr21*, *Lr22*, *Lr23*, *Lr27* and *Lr30*.

Gene *Lr2a* (Webster), a line in Set-B, showed temperature sensitivity against virulences of the 104 group. The reaction types tended to change towards susceptibility with increase in temperature[5]. This fact is to be considered when recordings are done in cooler months.

During 1984–85 the new virulence 104-1 (21R31-1) was detected in 5 samples out of 453 analysed and in 1986–87 in 22 out of 748 evaluated for pathogenicity test. The wheat lines hitherto resistant to leaf rust at seedling stage were tested against 104-1(21R31-1). Of these, CPAN 1800, CPAN 1874, CPAN 1946, CPAN 1961, CPAN 1962, CPAN 1967, CPAN 1973, HNP 8643, HP 1452, HUW 37, HUW 117, HUW 199, HUW 202, M 13, M 14, Raj 1865, Raj 2232 and WG 2109 were still resistant and can be used as donor lines. Also the genes that accord immunity to leaf rust infection such as *Lr9*, *Lr18* (Sabikei), *Lr19*, *Lr24*, *Lr25*, *Lr28* and *Lr29* can be used in the breeding programme

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