

Effect of Rust Infection of Oat Leaves on Cytoplasmic and Chloroplast Ribosomal Ribonucleic Acids

T. Tani, M. Yoshikawa, and N. Naito

Laboratory of Plant Pathology, Faculty of Agriculture, Kagawa University, Kagawa 761-07, Japan. Present address of second author: Department of Plant Pathology, University of California, Riverside 92502.

Supported in part by Grant No. 46004 from the Ministry of Education of Japan. We thank T. Hirai, H. Oku, J. Shishiyama, S. Ouchi, and A. Hirai for their suggestions, and K. Yamanaka for use of Gilford instrument. We also thank C. J. Mirocha for his advice in preparation of the manuscript.

Accepted for publication 2 October 1972.

ABSTRACT

In the susceptible oat cultivar, 'Victoria', the ratio of cytoplasmic ribosomal ribonucleic acid (rRNA) between inoculated and noninoculated control leaves increased slightly from 1.10 to 1.17, respectively, 2 and 3 days after inoculation, followed by a more pronounced increase of 1.62 and 2.14, 4 and 6 days after. By contrast, the chloroplast rRNA increased to 1.02 and 1.06, respectively, 2 and 3 days after inoculation, then decreased to 0.70 and 0.53, 4 and 6 days afterwards. The ratio of incorporation of ^{32}P into rRNA increased to about 1.3 for both cytoplasmic and chloroplast fractions

2 days after inoculation, but at 4 days, the cytoplasmic fraction increased to 1.81, whereas the chloroplast fraction remained unaltered. In the highly resistant cultivar 'Shokan 1', the ratios of cytoplasmic and chloroplast rRNA between inoculated and noninoculated controls did not change during the period when the resistant reaction occurred (28 and 48 hr after inoculation). The incorporation of ^{32}P into rRNA, however, increased in both fractions 1.57 to 1.81 times that of the noninoculated control.

Phytopathology 63:491-494

Rust infection generally increases the amount of ribonucleic acid (RNA) in a host-parasite association (6). In regard to the changes in ribosomal RNA (rRNA), Wolf (22) reported a rise of the synthetic activity in susceptible wheat leaves infected with stem rust. Heitefuss & Bauer (8) observed an increased synthesis of rRNA in rust-infected bean leaves. With crown rust of oats, we (20) found that infection stimulates the synthesis of rRNA in susceptible and resistant leaves. The possible significance of such an increase has been discussed by Hirai (10) in relation to virus-infected plants. Little has been done, however, to evaluate quantitative changes in the proportion of different rRNA components following rust infection. By the use of polyacrylamide gel electrophoresis, we have been able to characterize the alteration of molecular species of rRNA by rust infection. This paper reports the periodical changes, after inoculation with *Puccinia coronata* Corda, in the amount of cytoplasmic and chloroplast rRNA as well as in the ability to incorporate ^{32}P into the rRNA fractions of the susceptible and resistant oat leaves.

MATERIALS AND METHODS.—Methods for growing the plants, inoculating the primary leaves with uredospores, and supplying ^{32}P -orthophosphate (carrier-free) for 4 hr to the detached leaves were identical to those described previously (20). The oat cultivars 'Victoria' and 'Shokan 1' were sources of susceptible and highly resistant leaves, respectively. Detailed explanations for the fungal development and appearance of the symptom on leaves of these cultivars have been reported elsewhere (14).

Nucleic acid was extracted using the Na-lauryl sulfate-diethyl pyrocarbonate method of Solymosy et al. (17) with the modification described previously (20), except that the final nucleic acid preparation was dissolved in the buffer solution containing 40 mM Tris [tris(hydroxymethyl) amino methane], 40

mM Na-acetate, 4 mM EDTA, and 10% sucrose (ribonuclease-free). Acetic acid was used to adjust the pH to 7.8 at 4 C. Polyacrylamide gel electrophoresis was carried out according to the method of Loening (13). About 25 μg of the nucleic acid sample were layered on a 2.5% gel, and a current of 5 ma/gel was applied for 300 min at 0-5 C, by which time high resolution of rRNA fractions could be obtained, although by this time the soluble RNA had run off the gel. For quantitative estimation of RNA content, the gel was placed in quartz tubes and scanned directly at 265 nm using a Gilford linear transport attachment coupled to a Beckman DU spectrophotometer. The relative proportions of the RNA were calculated by weight of the recording paper under each RNA peak, and were expressed as the ratio to deoxyribonucleic acid (DNA) value in the same gel. The DNA content of inoculated and noninoculated leaves during the experimental periods is constant for each of the two cultivars (T. Tani, unpublished data).

When ^{32}P was included, about 100 μg of nucleic acid sample were layered on the gel. After electrophoresis under the same condition as above, the gel was stained with 0.2% azure B as described by Peacock & Dingman (16). Each rRNA band that appeared was cut into 3-mm slices, and the heavy rRNA was placed together with the light rRNA bands and dried on a planchet. Radioactivity was counted by a thin-window, gas flow counter (Aloka, Model PC-10 E), and the count was corrected for background by subtraction of the radioactivity measured in the 6-mm slice of gel found between the DNA and 25-S bands.

RESULTS.—rRNA content.—A typical profile of the constituents separated by electrophoresis is shown in Fig. 1. Based on the measure of the sedimentation coefficient, using the linear relation

between relative electrophoretic mobility and the log of the molecular weight as described by Bishop et al. (1), these RNA peaks are identified as follows: cytoplasmic heavy rRNA, 25 S; chloroplast heavy rRNA, 23 S; cytoplasmic light rRNA, 18 S;

chloroplast light rRNA, 16 S. A minor peak electrophoresing faster than the 16-S peak gives a value of 13 S, which has been referred to as a degradation product of the 23-S RNA (12).

The profile for noninoculated and inoculated leaves of the two oat cultivars all had the same DNA and four major RNA peaks and, occasionally, one minor peak (13 S). The ratio of the heavy rRNA to total RNA was estimated from the elution profiles and was in the range of 0.65-0.72 and 0.55-0.66 for the cytoplasmic and chloroplast rRNA's, respectively. The relative value of rRNA content for Victoria and Shokan 1 is given in Table 1. The value of the 13 S was omitted because it was always less than 10% of the 23 S.

The rRNA content of both cytoplasm and the chloroplasts in Victoria leaves was at a maximum on the day of inoculation. In noninoculated leaves, the rRNA decreased continuously with age, and at 6 days, the content of cytoplasmic and chloroplast rRNA fell to 34 and 15%, respectively. In inoculated leaves, the content of cytoplasmic rRNA similarly decreased by 3 days, and then remained fairly constant until 6 days after inoculation, giving the inoculated/noninoculated ratio of 1.10, 1.17, 1.62, and 2.14 at days 2, 3, 4, and 6, respectively. On the other hand, the content of rRNA from chloroplasts of inoculated leaves declined up to 3 days, but was followed by a large decrease 4 to 6 days after inoculation. The inoculated/noninoculated ratio was 1.02, 1.16, 0.70, and 0.53 at days 2, 3, 4, and 6, respectively. Consequently, the total rRNA content in susceptible leaves appeared to increase continuously up to 1.74 times that of the healthy control as a result of rust infection.

The content of both cytoplasmic and chloroplast rRNA in noninoculated leaves of Shokan 1 also showed a maximum at the time of inoculation, but decreased to about 70% at 28 hr, then remained roughly constant until 48 hr after inoculation. There was no appreciable change in rust infection.

Incorporation of ^{32}P into rRNA fractions.—Figure 2 illustrates a typical profile showing the coincidence of RNA peaks absorbing at 620 nm and radioactivity. Table 2 shows radioactivity of ^{32}P in rRNA fractions from inoculated and noninoculated leaves at various stages of infection.

In Victoria, the ratio of rRNA of the cytoplasmic fraction of the inoculated to noninoculated control increased to 1.34 by day 2, then to 1.81 at day 4. For the chloroplast fraction, the ratio increased to about 1.3 by day 2, but no obvious difference was noted between days 2 and 4.

In Shokan 1, the ratio of rRNA of the cytoplasmic and chloroplast fractions of the inoculated and noninoculated control increased slightly up to 14 hr, then increased to 1.6-1.8, after which it remained constant until 48 hr after inoculation. The ratio for the total rRNA of the inoculated and noninoculated was similar in proportion to the above.

DISCUSSION.—Our previous study showed that in the susceptible cultivar, Victoria, the incorporation

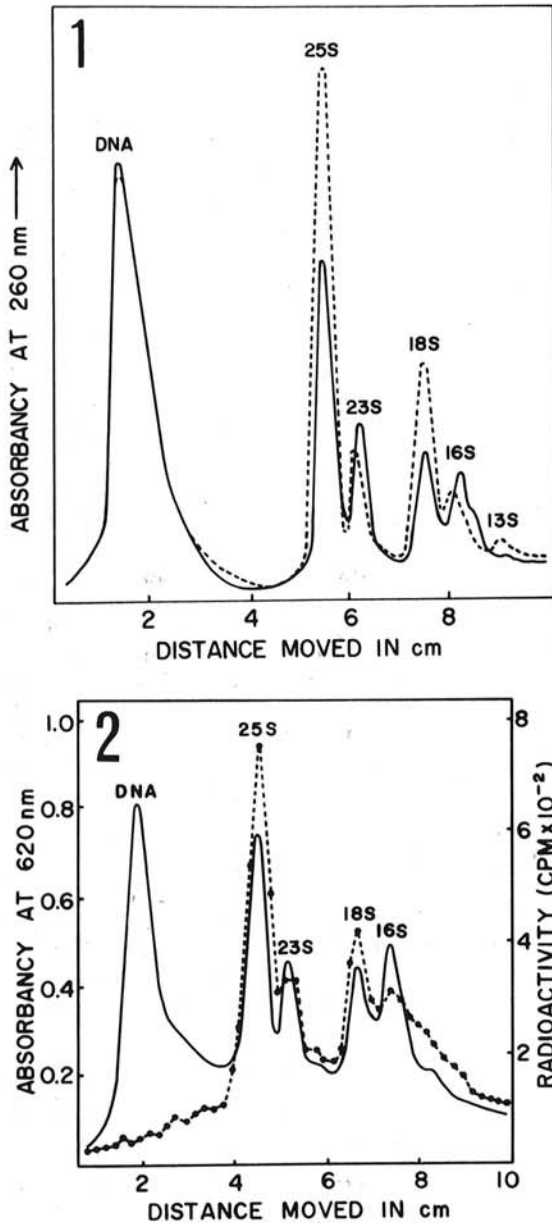


Fig. 1-2. 1) Disc electrophoretic profiles typical of RNA extracted from Victoria leaves 4 days after inoculation with *Puccinia coronata*. Noninoculated (—); inoculated (----). 2) Disc electrophoretic profile illustrating the coincidence of the area of rRNA and radioactivity. The absorbancy was determined by a Toyo Densitator-2. RNA was extracted from the primary leaves of 9-day-old Victoria seedlings. The gel was frozen at $-20^{\circ}C$ and cut into 1-mm slices using a gel slicer. Absorbancy (—); radioactivity (----).

TABLE 1. RNA/DNA ratio for cytoplasmic and chloroplast rRNA in oat leaves inoculated with *Puccinia coronata*

Time after inoculation	Cytoplasmic rRNA		Chloroplast rRNA		Total rRNA	
	H ^a	R ^a	H	R	H	R
Victoria (susceptible)						
day						
0	1.50		1.11		2.61	
2	1.05	1.16	0.53	0.54	1.58	1.70
3	0.80	0.94	0.37	0.43	1.17	1.37
4	0.66	1.07	0.36	0.25	1.02	1.32
6	0.51	1.09	0.17	0.09	0.68	1.18
Shokan 1 (resistant)						
hr						
0	1.50		0.94		2.44	
28	1.05	1.07	0.58	0.55	1.63	1.62
48	0.99	1.21	0.63	0.55	1.62	1.76

^a H = noninoculated; R = inoculated.

of ³²P into the rRNA fraction of inoculated leaves increased to 124% of the noninoculated control at an early stage of infection; i.e., 2 days after inoculation (20). In the present study, a similar increase was associated with a slight rise in rRNA content in both the cytoplasmic and chloroplast fractions. The healthy leaves used in these experiments appeared to be in a senescent stage (Table 1) in which a rapid decrease of rRNA and ribosomes is usually noted (5, 10, 15, 18). It is likely, therefore, that the crown rust

TABLE 2. Incorporation of ³²P into cytoplasmic and chloroplast rRNA fractions from oat leaves inoculated with *Puccinia coronata*

Time after inoculation	cpm/gel ^a of rRNA bands					
	Cytoplasmic rRNA		Chloroplast rRNA		Total rRNA	
	H ^b	R ^b	H	R	H	R
Victoria (susceptible)						
day						
1	3,135	3,968	1,639	2,000	4,771	5,968
2	2,068	2,772	1,142	1,472	3,210	4,244
4	1,615	2,926	1,035	1,406	2,650	4,332
Shokan 1 (resistant)						
hr						
14	7,360	8,830	4,470	4,955	11,830	13,785
28	4,174	7,550	3,123	4,975	7,297	12,525
48	4,235	7,080	2,718	4,360	6,953	11,440

^a Nucleic acid sample extracted from 2 g leaves was dissolved in 0.7 and 0.35 ml of the buffer solution for Victoria and Shokan 1, respectively, and 50 μ liter of the solution was layered on the gel.

^b H = noninoculated; R = inoculated.

infection has a juvenile effect on the host tissues during the vegetative stage of fungal growth. This is in accord with the description of green islands surrounding rust pustules, where acceleration of RNA synthesis and an increase of RNA content have been observed (7). In the next period of infection on Victoria; i.e., up to 4 days after inoculation, chlorotic flecks appear and the fungus begins to develop uredosorus mother cells (14). Our previous studies showed that, at this stage, the RNA content (19) and ³²P incorporation into the rRNA fraction (20) increase to 120 and 170-200% of the noninoculated control, respectively. The results in Table 2 are in accord with those for the total and cytoplasmic rRNA, whereas for chloroplast rRNA, the content is reduced and the incorporation of ³²P is not increased. A large increase of cytoplasmic rRNA during this stage could be mainly attributable to the increased synthesis of the fungus as was indicated previously from the analysis of the purine and pyrimidine bases of the ³²P-labeled rRNA (20). An abundance of ribosomes in the cytoplasm of rust fungi may also support this view (9, 21). The acceleration of RNA metabolism in nuclei of wheat affected by stem rust (2, 3), and the data on chloroplast rRNA obtained in the present study, suggest the possibility that once reproductive action of the fungus is initiated, the metabolic shift of host tissues declines toward senescence despite the fact that RNA metabolism in the fungus is becoming more active. This may result in the destruction of subcellular organelles prior to the loss of integrity of the haustorium as observed by Ehrlich & Ehrlich (4). Thus, the results for the susceptible reaction in the present study support the idea of Király (11), who stated that rust infection has a double effect on host tissues; i.e., a "juvenescence effect" in an early stage of infection and a "senescence effect" in the chlorotic and sporulation stages.

With the highly resistant reaction of Shokan 1, our early report (19) showed no essential change in total RNA content. This was confirmed in the present study for both cytoplasmic and chloroplast rRNA's. Another report (20) indicated a remarkable increase in the synthesis of RNA species during the period of the resistant reaction (28 to 48 hr after inoculation), and it was attributed to the host response against infection. A large stimulation in RNA synthesis similarly occurred for both cytoplasmic and chloroplast fractions (Table 2). It could be concluded, therefore, that hypersensitive response is accompanied by a remarkable acceleration of rRNA synthesis in both host nuclei and chloroplasts. The fact that the enhancement of rRNA synthesis in highly resistant leaves does not result in an increase of RNA content may be due to an increased turnover of rRNA.

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