Cellular Resistance in Rice to Cercosporin, a Toxin of *Cercospora*

R. B. Batchvarova, V. S. Reddy, and J. Bennett

Research associate, research fellow, and senior scientist, International Centre for Genetic Engineering and Biotechnology, NII Campus, Shaheed Jeet Singh Marg, New Delhi 110067, India.
Present address of first author: Institute of Genetic Engineering, Kostinbrod-2, Bulgaria.
We thank A. Krikorian for norflurazon, M. C. Rush for seeds and fungal strains and for advice, and K. V. S. Rao for HPLC analyses.
Accepted for publication 18 January 1992.

ABSTRACT

Batchvarova, R. B., Reddy, V. S., and Bennett, J. 1992. Cellular resistance in rice to cercosporin, a toxin of *Cercospora*. Phytopathology 82:642-646.

Four rice cultivars differing in resistance to *Cercospora oryzae* were examined for resistance to purified cercosporin, the red toxin secreted by the fungal mycelium. The cultivars were red rice (most resistant to pathovars of *C. oryzae*), Lemont and Leah (intermediate resistance), and Labelle (most susceptible). Resistance to the toxin was assessed in seedlings (inhibition of growth), leaves (chlorosis and necrosis), callus (inhibition of growth and hydrolysis of fluorescein diacetate), and cell suspensions (ion leakage). Toxicity of cercosporin depended on illumination. Labelle proved to be the most sensitive cultivar in each test, whereas red rice showed extreme resistance to the toxin and grew in the presence of cercosporin concentrations that were completely toxic to Labelle. Red rice cells contained about one-tenth as much cercosporin as cells of Labelle; this suggests that resistant cells have a mechanism for excluding, exporting, or destroying the toxin. Treating cell suspensions of red rice with norflurazon abolished the cercosporin resistance in this cultivar, and thus carotenoids are implicated in the resistance mechanism. These results show that plants can display cellular resistance to illuminated cercosporin and suggest a method of screening cells of other crop plants for variants resistant to the toxin.

Additional keyword: *Oryza sativa*.

Fungi of the genus *Cercospora* infect many crop plants, including maize, groundnut, sugar beet, coffee, banana, soybean, and rice (7,12,20,27). Important to symptom development is the secretion of a red toxin, cercosporin, by fungal mycelium. On illumination, cercosporin is excited and generates free radicals that damage cell membranes and cause cell death (8). Cercosporin is almost universally toxic to living cells and has been proposed as an agent for photodynamic cancer chemotherapy (25). In most crop species, resistance to *Cercospora* is poor or nonexistent; attempts to generate somaclonal variants resistant to the toxin have been unsuccessful (8).

Rice is unusual in that high levels of resistance to *C. oryzae* Miyake have been deployed commercially since the 1940s (22,26). Louisiana red rice is resistant to almost all known races of the pathogen. Other cultivars of rice are resistant to some races but not to others. Lemont and Leah are resistant to 40–50% of the known races of *C. oryzae*, whereas Labelle is resistant to only about 2% of races (23). Although the basis of the complex pattern of resistance is not understood, cellular resistance to cercosporin might be involved. Accordingly, we used purified toxin to screen calli and suspension cells of the above four rice cultivars for resistance to cercosporin. The results establish not only that plant cells display resistance to cercosporin but also that carotenoids are involved in the resistance mechanism.

MATERIALS AND METHODS

Biological materials. Plants and fungi were supplied by M. C. Rush, Department of Plant Pathology, Louisiana State University, Baton Rouge. Three commercial rice cultivars were used: Labelle, which is highly susceptible to *C. oryzae*; and Leah and Lemont, which have intermediate resistance. The cultivar resistant to all races of the pathogen is the annual weed known as Louisiana red rice (23). Eleven isolates of three races (LA, LB, and LD) of *C. oryzae* were maintained on potato-sucrose agar (PSA) and Czapek-Dox broth. The cultures were grown at 28 C in 12-h alternating light and darkness at 4,000 lux, provided by warm white fluorescent tubes. *C. oryzae* was also grown in liquid medium: Czapek-Dox broth (100 ml) in a 250-ml flask at 28 C with the same light conditions. In the preparation of PSA, peeled and sliced potatoes (1.8 kg) were boiled for 10 min in 4.5 L of water, strained through cheesecloth, and diluted 1:1 with water before the addition of 20 g each of sucrose and agar. The mixture was heated to dissolve the agar, the pH was adjusted to 6.4 with $CaCO_3$, and the solution was autoclaved. Czapek-Dox broth (1 L) contained $NaNO_3$ (2 g), K_2HPO_4 (1 g), $MgSO_4 \cdot 7H_2O$ (0.5 g), KCl (0.5 g), $FeSO_4$ (10 mg), and sucrose (30 g), with 1 ml of 1% (w/v) $ZnSO_4$ and 0.5% (w/v) $CuSO_4$. For solid medium, the broth was supplemented with agar (20 g/L).

Cercosporin extraction. The toxin was extracted and purified according to the procedure of Balis and Payne (2), with some modifications. Cercosporin was extracted with acetone from 6-wk cultures of *C. oryzae* grown on PSA. Acetone was removed under vacuum, and the toxin was extracted in diethyl ether. The ether was evaporated under vacuum, and the dry residue was dissolved in a small volume of chloroform. The red fraction of cercosporin was purified on a hydroxyapatite column, and purity was checked by thin-layer chromatography on silica gel G plates. The solvent system was ethyl acetate/methanol (4:1), and the R_f for cercosporin was 0.68. Column fractions containing cercosporin were combined in acetone and stored at −20 C. We established the concentration of pure cercosporin by using a molar extinction coefficient of 23,600 $M^{-1}cm^{-1}$ at A_{473nm} (33). All treatments of leaves, calli, or cell suspensions with cercosporin in acetone were compared with controls treated with acetone alone. The levels of acetone used had no discernible effect on any of the genotypes.

Treatment of rice seedlings with cercosporin. Seeds were germinated under 12-h light/dark cycles (4,000 lux). They were placed in glass petri dishes on filter paper containing 200 μM cercosporin in 4 ml of water. Control plates contained 4 ml of water. After 5 days, root and shoot length were measured in six replicates.

Treatment of rice leaves with cercosporin. Mature leaves of 30-day-old plants were punctured with a fine needle at various locations. Cercosporin in acetone (20 μl, 100 μM) was applied to the top surface. Control leaves were treated with acetone alone. After treatment, plants were covered with polyethylene bags and

kept in the light (4,000 lux) at 28 C for up to 24 h.

Initiation of callus and cell suspension cultures. Callus cultures were established from mature seeds of cultivars Labelle, Leah, Lemont, and red rice according to Lee et al (18). MS and N6 media were used with 2.5 mg L^{-1} of 2,4-dichlorophenoxyacetic acid (2,4-D). The pH was adjusted to 5.7, and agar (6 g L^{-1}) was added. Cultures were incubated at 25 C in the dark and routinely subcultured at 4-wk intervals. To obtain cell suspensions, we transferred 6-wk-old calli to 50 ml of liquid N6 medium in 250-ml flasks. Initially, the medium was changed every alternate day; after 2 wk, it was changed once a week. Flasks were shaken at 120 rpm in the dark at 25 C.

Testing toxicity of cercosporin on callus. Six pieces of callus (about 50 mg each) were placed on agar plates containing MS medium with either cercosporin in acetone or acetone alone. The fresh weight increase of each piece was determined over a 4-wk period.

Callus viability test. Fluorescein diacetate (0.5 mg ml^{-1} in acetone) (30) was added to MS medium at a concentration of 5 μl ml^{-1}. Small fragments of calli were added to the solution and examined under a Nikon (Tokyo, Japan) fluorescence microscope (excitation was at 450–490 nm, and the emission cut off was 520 nm).

Carotenoid determination. The herbicide norflurazon (4-chloro-5-methylamino-2-[α,α,α-trifluoro-*m*-tolyl]-3[2*H*]-pyridazinone) dissolved in ethanol was used to block carotenoid biosynthesis (3,9,24). Final concentration of norflurazon in MS medium was 400 μM, and, every 2 days, total carotenoids were extracted from rice cells. After 6 days of growth in the presence or absence of norflurazon, cell suspensions were treated with 20 μM cercosporin and assayed, as described below, for cell damage manifested by ion leakage. We extracted carotenoids by homogenizing cells in ethanol and measuring absorbance at 453 nm. We calculated total carotenoid content by assuming the specific absorption coefficient of β-carotene ($A^{1\%}_{cm} = 2{,}620$ at 453 nm; ref. 13).

Cercosporin content of suspension cells. Suspension cells (1 g) of Labelle and red rice were grown for 6 h in the presence of 20 μM cercosporin, separated from medium by filtration on Millipore (Bedford, MA) membranes (1 μm pore size), and extracted with 1 ml of 90% acetone. Aliquots (100 μl) were analyzed by reverse-phase high-performance liquid chromatography (HPLC) with a Microsorb C18 column (Rainin, Emeryville, CA) (4.5 × 250 mm, 5-μl particle size, 0.1 μm pore size, 1 ml min^{-1} flow rate). Solvent A was methanol/water (4:1); solvent B was ethyl acetate. The column was equilibrated with solvent A. A 100-μl sample was injected through a 2-ml loop, and the following gradient profile was used for elution: isocratic for 2 min with 100% solvent A; linear gradient from 100% solvent A to 50% solvent A for next 20 min; isocratic for 5 min; linear gradient to 100% solvent B for 5 min; isocratic for 5 min. The peak corresponding to cercosporin was identified and quantified by comparison with purified cercosporin of known concentration. Lutein and β-carotene were identified by co-chromatography with authentic samples extracted from etiolated rice, as described (11).

Electrolyte leakage. Ion leakage was used as an indicator of cell membrane damage in response to the presence of cercosporin and light (4,000 lux) (15,21). Suspension cells (150 mg fresh weight) were added to 10 ml of MS medium, and the conductivity was determined with a type 4010 meter from Jencons (Leighton Buzzard, U.K.). The conductivity measured immediately after addition of cells was usually 6.0–6.5 mS. Cercosporin (20 μM in acetone) or a control volume of acetone was added, and, 6 h later, conductivity was again measured. The difference in conductivity (Δ, in mS) is the net ion leakage over the 6-h period and is the mean of four replicates. We used MS medium here rather than water (as used with leaf disks [15]) in case the sudden osmotic disturbance impaired metabolism in the cells.

Statistical analysis. Six replicates were made of each measurement of seedling growth, callus growth, and carotenoid content. Ion leakage was measured in four replicates. Analysis of variance was conducted according to Duncan's new multiple range test.

RESULTS

Cercosporin resistance in plants. The effects of purified cercosporin on the growth of young rice seedlings are shown in Table 1. Toxin was supplied from the start of imbibition to the time of analysis 5 days later and is presumed to have entered the plants through the germinating seeds and the roots. At a concentration of 200 μM, cercosporin reduced root number and total root length by 50–75%; both effects were more marked in the cultivar Labelle than in red rice. Shoot growth was also inhibited in Labelle, but it was unaffected in red rice.

To examine toxin resistance in the shoot more directly, we treated leaves of Labelle, Leah, Lemont, and red rice with purified cercosporin by using a needle-puncture technique. Punctured leaves were painted with cercosporin in acetone or with solvent alone. Within 24 h of treatment with acetone alone, leaves of all four cultivars showed a strictly localized white necrosis that was restricted to within 1 mm of the sites of wounding. Red rice treated with cercosporin showed exactly the same type of highly localized necrosis, indicating that cercosporin itself is without effect on red rice. In contrast, Lemont and Leah, after treatment with cercosporin, developed chlorotic lesions 20–40 mm in length, whereas Labelle showed an intense but delimited brown necrosis surrounding the sites of wounding (about 5 mm in diameter). This difference in response between Lemont and Leah and Labelle is reproducible but is not understood. No cultivar showed toxic symptoms in the dark, confirming the general observation that excited cercosporin produces toxic symptoms (8,9).

Cercosporin resistance in calli. To determine if the resistance of red rice to cercosporin would also be manifested in undifferentiated cells, we generated callus from each rice cultivar and exposed it to cercosporin. Growth in the presence and absence of toxin (100 μM) was assessed after 4 wk in the light (Table 2). The four cultivars grew at different rates and differed in their response to cercosporin. Growth of Labelle was prevented, and the tissue became necrotic. Cell death was confirmed by the observation that fluorescein diacetate was not hydrolyzed within the cercosporin-treated callus cells (results not shown). Callus from

TABLE 1. Effect of 200 μM cercosporin on root and shoot growth of 5-day-old rice seedlings[z]

Cultivar and treatment	Root length (cm)	Root number	Shoot length (cm)
Labelle			
Control	3.5	5.0	4.6
+ Cercosporin	0.9 a	2.6 a	2.4 a
Red rice			
Control	4.7	6.8	4.3
+ Cercosporin	2.4 b	4.6 b	4.5 c

[z] a = Treatment significantly different from control at $P = 0.001$; b = treatment significantly different from control at $P = 0.01$; c = treatment not significantly different from control at $P = 0.05$.

TABLE 2. Effects of 100 μM cercosporin on 4-wk growth of calli in light and dark[z]

Cultivar	Treatment	Callus growth (mg)
Labelle	Light	12 a
	Light + cercosporin	0.0 b
	Dark + cercosporin	11 a
Leah	Light	6.2 c
	Light + cercosporin	2.2 d
Lemont	Light	73 e
	Light + cercosporin	36 f
Red rice	Light	20 g
	Light + cercosporin	20 g

[z] Values with different letters differ at $P = 0.001$; values with the same letter do not differ at $P = 0.05$. Initial mass of callus = 50 mg.

Leah or Lemont continued to grow in the presence of the toxin but at reduced rates (33–49%). When fluorescein diacetate was supplied to cercosporin-treated callus of Lemont, hydrolysis of accumulated fluorogen was observed in the younger tissue at the peripery of the callus but was absent from older tissue in the center of the callus. Thus, the toxin caused slow death of cells of this cultivar. Growth of red rice, by contrast, was not significantly affected by 100 μM cercosporin (Table 2), and all callus cells accumulated and hydrolyzed fluorescein diacetate. These results establish that red rice shows cellular resistance to illuminated cercosporin, whereas Lemont, Leah, and Labelle show decreasing levels of resistance. Cercosporin did not affect growth of rice calli in the dark. Table 2 illustrates this point for callus from Labelle.

Coloration of rice calli and cell suspensions in the presence and absence of cercosporin. Calli of Labelle and Leah were very pale yellow during normal growth but became dark red-purple when grown on agar containing 200 μM cercosporin. By contrast, calli of Lemont and red rice are more distinctly yellow in the absence of toxin and tend to remain so in its presence. Three explanations for this difference in toxin content might be (not mutually exclusive): red rice cells do not permit entry of cercosporin; toxin enters red rice cells but is actively pumped out; or red rice detoxifies cercosporin by a mechanism that involves decoloration.

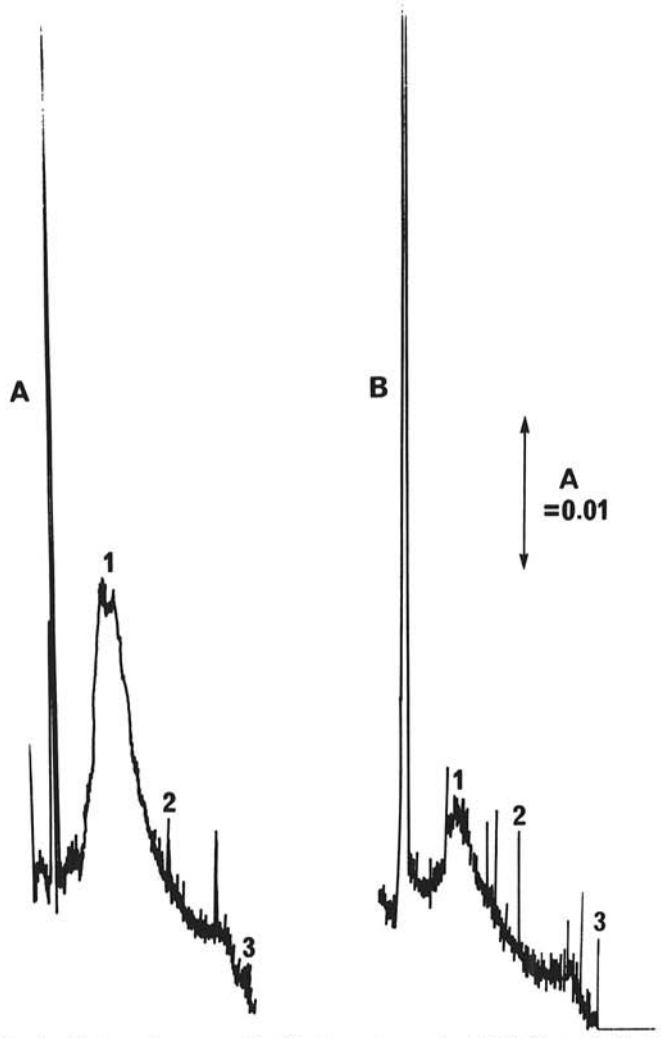

Fig. 1. High-performance liquid chromatography (HPLC) analysis of acetone extracts of suspension cells derived from **A,** Labelle and **B,** red rice. The A_{475nm} profile shows the broad peak of cercosporin (1) at 11.5 min and the sharp peaks of carotenoids, lutein at 22.6 min (2) and β-carotene at 35.9 min (3).

To investigate this difference in more detail, we turned from calli to cell suspensions. The latter grow more rapidly and are more suitable for experimental manipulations than calli. Cell suspensions of the four rice cultivars varied in their carotenoid content. Red rice contained the highest carotenoid content (2.0 μg/g fresh weight), and Labelle had the lowest (0.9 μg/g fresh weight). Cercosporin content of suspension cells exposed to 20 μM cercosporin was measured by extraction of pigments into 90% acetone followed by HPLC. Whether pure or extracted from cells, cercosporin chromatographed as a much broader peak (peak 1 in Fig. 1) than did carotenoids. Labelle contained 7.3 nmol toxin per gram of cells, whereas cells of red rice contained 0.65 nmol toxin per gram. Thus, the trends in carotenoid content and cercosporin accumulation noted for calli were also seen with cell suspensions. The carotenoids of cercosporin-treated cell suspensions of red rice appear to be more diverse than those of cercosporin-treated cell suspensions of Labelle. Lutein and β-carotene have been identified (peaks 2 and 3 in Fig. 1), but the other peaks have not been characterized.

Cercosporin resistance in cell suspensions. Cercosporin-induced damage to suspension cells may be determined rapidly by measurements of ion leakage into the growth medium (MS medium) (15,21). The change in conductivity (Δ) of the medium over a 6-h incubation period was used as the measure of ion leakage. Table 3 shows how ion leakage depends on plant cultivar, light, and the presence of cercosporin. Labelle showed highly significant ion leakage only in the light and in the presence of 20 μM cercosporin; neither light alone nor the toxin alone caused significant ion leakage. By contrast, red rice cell suspensions showed no significant ion leakage, even in the presence of illuminated cercosporin; this confirms the resistance of red rice cells to the toxin. The light-dependent increase in conductivity for cercosporin-treated Labelle cells was about 1.4 mS per 6 h for 150 mg of cells in 10 ml of MS medium. This is comparable to a value of 0.8 mS per 6 h for 200 mg of cercosporin-treated leaf disks of corn in 10 ml of water (15).

Carotenoids and cercosporin resistance. Because illuminated cercosporin produces active oxygen species (9) and carotenoids quench such species (14), we examined whether carotenoids are involved in resistance to the toxin. Suspension cells were treated with norflurazon, an inhibitor of carotenoid biosynthesis (3,24). The herbicide reduced the carotenoid content of cells derived from red rice and Labelle (Table 4). After 6 days of exposure to 400 μM norflurazon, the carotenoid level fell to 1–2% of the initial value. At this point, the cells were examined for sensitivity to cercosporin.

Cells were exposed to 20 μM cercosporin in the light, and leakage of ions into the medium was measured after 6 h (Table 5). Cell suspensions of Labelle showed marked cercosporin-dependent ion leakage irrespective of whether the cells had been grown in the presence or absence of norflurazon. Red rice, by contrast, showed little cercosporin-dependent ion leakage, unless norflurazon was present in the medium during growth. Exposure of cells to norflurazon in the absence of cercosporin did not induce ion leakage. These results implicate carotenoids in the mechanism that causes resistance to cercosporin in red rice.

TABLE 3. Differential cercosporin-induced ion leakage from cell suspensions of cultivars Labelle and red rice[y]

Treatment	Δ Conductivity (mS)[z]	
	Red rice	Labelle
Control suspension cells in light	−0.2 a	−0.3 a
Control suspension cells in dark	0.0 a	−0.2 a
Cercosporin-treated cells in light	−0.1 a	1.3 b
Cercosporin-treated cells in dark	−0.4 a	−0.1 a

[y] Ion leakage was measured as the change in conductivity of MS medium over 6 h of incubation of cells in the light or dark and in the presence or absence of 20 μM cercosporin.

[z] a = Treatments not significantly different at $P = 0.05$; b = treatment significantly different at $P = 0.001$.

DISCUSSION

This is the first report of cercosporin resistance in plant cells. High levels of resistance were seen for leaves, calli, and cell suspensions of the annual weed, red rice. A lower level of resistance was observed in the commercial cultivars Lemont and Leah. Another commercial cultivar, Labelle, was susceptible to the toxin. These responses parallel the degree of resistance shown by the four cultivars to the fungus itself (23).

In each test of cercosporin toxicity (with leaves, calli, or cell suspensions), light was required for the development of symptoms. This suggests that cercosporin toxicity was due to photo-induced formation of active oxygen species (7,8) rather than to inhibition of protein kinase C, which is a target of cercosporin in mammalian cells (25). Norflurazon converts red rice cell suspensions from resistant to susceptible, which implicates carotenoids in the protection mechanism. Carotenoids protect plant cells in several ways, such as by quenching singlet oxygen (14), quenching excitation energy in chlorophyll (10), or acting as a light filter that absorbs excessive radiation (4). Carotenoids might participate in a similar range of mechanisms to protect cells against excited cercosporin, which produces singlet oxygen (8). Resistant calli and cell suspensions contain more carotenoid than do susceptible cells. We are currently identifying and estimating the concentrations of individual carotenoids in resistant and susceptible cultivars to determine if the increase in carotenoid content is general or restricted to particular molecular species.

Susceptible cells accumulate high levels of cercosporin, whereas resistant cells do not; this difference could be explained in terms of regulation of cercosporin transport into and out of cells or by detoxification with concomitant decoloration. However, the high cercosporin content of susceptible cells might be misleading. Because cercosporin is highly lipophilic, it might partition directly into accessible membrane bilayers even after cell death. Red rice cells treated with norflurazon accumulate high levels of cercosporin (results not shown). Thus, susceptibility to cercosporin is associated with massive intracellular accumulation of toxin, but it is not yet clear whether such accumulation is the cause or consequence of cell death. The resistance of red rice to cercosporin might depend on the synergistic effects of an elevated carotenoid content and the presence of an active exclusion-detoxification system. Treatment of red rice cells with norflurazon would abolish carotenoid-mediated protection completely and thereby prevent the cells from excluding or detoxifying cercosporin.

The high affinity of Labelle cells for cercosporin may explain why the necrosis on Labelle leaves is more intense and delimited than on leaves of Leah and Lemont. As the limited amount of cercosporin diffuses from the sites of application, it becomes bound to cells. Higher affinity means greater damage but also reduced radius of diffusion.

The use of plant tissue culture as an aid to the selection of new cultivars resistant to disease has frequently been advocated and has been successful in obtaining plant cell lines resistant to fungal genera such as *Phytophthora*, *Phialophora*, *Fusarium*, *Alternaria*, *Phoma*, *Helminthosporium*, and *Puccinia* (6,19,28, 31,32). The past failure of this approach with *Cercospora* spp. may be due to the difficulty of generating a clone that produces an adequate carotenoid content and an exclusion-detoxification mechanism. Cercosporin-resistant somaclonal cultivars of crops other than rice might be obtained if a two-step screening procedure is adopted. In the first step, genotypes with elevated carotenoid content in tissue culture are identified. In the second step, cells of the carotenoid-rich genotypes are screened in the light and in the presence of cercosporin for clones that fail to accumulate the toxin and remain yellow. Such clones would presumably contain protective carotenoids and an exclusion-detoxification mechanism. In fungi, a general connection between cercosporin resistance and total carotenoid content is observed, but the presence of carotenoids is not a guarantee of resistance (8,9).

One of the most vulnerable parts of the cell is the plasma membrane, the first subcellular component likely to encounter the toxin. However, carotenoids do not appear to be found in plasma membranes; extremely pure preparations of plasma membranes from etiolated shoots of barley and corn (29), from inflorescences of cauliflower (5), from green leaves of spinach and barley (17), and from roots of various plants (16) do not contain carotenoids. Unless cercosporin-resistant genotypes of rice are exceptional in this respect, we must assume that the plasma membranes of resistant cells do not contain carotenoids and are protected indirectly by carotenoids located elsewhere in the cell, most likely in plastids (9).

In most cases in which adequate genetic information is available, the resistance of rice cultivars to *C. oryzae* is governed by one or two genes (22). To better understand the mechanism of cercosporin resistance in rice, we have studied the F1 and F2 progeny from a cross between red rice and Labelle. We will determine whether resistance to *C. oryzae* cosegregates with resistance to cercosporin and whether the latter cosegregates with elevated levels of particular carotenoids and with the ability of calli or cells to exclude or detoxify cercosporin.

During purification of cercosporin from cultures of *C. oryzae* race LD, we detected, in a second hydroxyapatite fraction, a substance toxic to all four cultivars of rice (results not shown). This indicates that a second toxin is produced by this strain of *C. oryzae* in culture. Preliminary characterization of the second toxin suggests that it may be related to the *C. beticola* toxin (CBT), although this toxin is absent from *C. oryzae* (1). Red rice is susceptible to the CBT-like toxin but is resistant to almost all pathovars of *C. oryzae*, which indicates either that production of the toxin is not widespread among pathovars or that pathovars produce sublethal amounts of the toxin in vivo.

TABLE 4. Progressive decline in total carotenoid content in rice cells of cultivars grown in the presence of 400 μM norflurazon[z]

No. of days with norflurazon	Labelle	Red rice
	(μg/g fresh weight)	
0	0.7 a	1.4 d
2	0.54 a	0.84 d
4	0.20 b	0.22 e
6	0.015 c	0.017 f

[z] For each cultivar, values with same letters differ at $P = 0.01$; values with different letters differ at $P = 0.001$.

TABLE 5. Effect of cercosporin on ion leakage from suspension cells of cultivars after inhibition of carotenoid biosynthesis by norflurazon[y]

Treatment	Δ Conductivity (mS)[z]	
	Red rice	Labelle
Control suspension cells − cercosporin	−0.2 a	−0.1 a
Control suspension cells + cercosporin	0.2 a	1.0 b
Norflurazon-treated cells − cercosporin	0.1 a	−0.2 a
Norflurazon-treated cells + cercosporin	1.2 b	1.1 b

[y] Ion leakage was measured as the change in conductivity of MS medium over 6 h of incubation of cells in the light and in the presence or absence of 20 μM cercosporin.

[z] a = Treatments not significantly different at $P = 0.05$; b = treatment significantly different at $P = 0.001$.

LITERATURE CITED

1. Assante, G., Locci, R., Camarda, L., Merlini, L., and Nasini, G. 1977. Screening of the genus *Cercospora* for secondary metabolites. Phytochemistry 16:243-247.
2. Balis, C., and Payne, M. G. 1971. Triglycerides and cercosporin from *Cercospora beticola*: Fungal growth and cercosporin production. Phytopathology 61:1477-1484.
3. Bartels, P. G., and Watson, C. W. 1978. Inhibition of carotenoid synthesis by fluridon and norflurazon. Weed Sci. 26:198-203.
4. Ben-Amotz, A., Shaish, A., and Avron, M. 1989. Mode of action of the massively accumulated β-carotene of *Dunaliella bardawil* in protecting the alga against damage by excess radiation. Plant Physiol.

91:1040-1043.
5. Caubergs, R., Widdell, S., Larsson, C., and de Greef, J. A. 1983. Comparison of two methods for the preparation of a membrane fraction of cauliflower inflorescences containing a blue light reducible b-type cytochrome. Physiol. Plant Pathol. 57:291-295.
6. Chaleff, R. S. 1981. Genetics of Higher Plants. Applications of Cell Culture. Cambridge University Press, New York.
7. Daub, M. E. 1982. Cercosporin, a photosensitizing toxin from *Cercospora* species. Phytopathology 72:370-374.
8. Daub, M. E. 1987. The fungal photosensitizer cercosporin and its role in plant diseases. Pages 271-280 in: Light-Activated Pesticides. J. R. Heitz and K. R. Downum, eds. American Chemical Society, Washington, DC.
9. Daub, M. E., and Payne, G. E. 1989. The role of carotenoids in resistance of fungi to cercosporin. Phytopathology 79:180-185.
10. Demming-Adams, B., Adams, W. W., Heber, U., Neimanis, S., Winter, K., Kruge, A., Czygan, F., Bilger, W., and Bjorkman, O. 1990. Inhibition of zeaxanthin formation and of rapid changes in radiationless energy dissipation by dithiothreitol in spinach leaves and chloroplasts. Plant Physiol. 92:293-301.
11. Eskins, K., and Harris, L. 1981. High-performance liquid chromatography of etiolated pigments in red kidney bean leaves. Photochem. Photobiol. 33:131-133.
12. Fajola, A. O. 1978. Cercosporin, a phytotoxin from *Cercospora* spp. Physiol. Plant Pathol. 13:157-165.
13. Goodwin, T. W. 1980. The Biochemistry of the Carotenoids. 2nd ed. Chapman and Hall, London. 377 pp.
14. Goodwin, T. W., and Mercer, E. I. 1983. Introduction to Plant Biochemistry. 2nd ed. Pergamon Press, Oxford, England. 677 pp.
15. Gwinn, K. D., Stelzig, D. A., and Brooks, J. L. 1987. Effects of corn plant age and cultivar on resistance to *Cercospora zeae-maydis* and sensitivity to cercosporin. Plant Dis. 71:603-606.
16. Hodges, T. K., and Mills, D. 1986. Isolation of the plasma membrane. Meth. Enzymol. 118:41-54.
17. Kjellbom, P., and Larsson, C. 1984. Preparation and polypeptide composition of chlorophyll-free plasma membranes from leaves of light-grown spinach and barley. Physiol. Plant Pathol. 62:501-509.
18. Lee, L., Schroll, R. E., Grimes, H. D., and Hodges, T. K. 1989. Plant regeneration from Indica rice (*Oryza sativa* L.) protoplasts. Planta 178:325-333.
19. Lepoivre, P. H., and Carels, N. 1980. Selection of sugarbeet calluses in order to obtain plant resistant to *C. beticola*. IAEA-SM 282:51-56.
20. Lynch, F. J., and Geoghegan, U. J. 1977. Production of cercosporin by *Cercospora* species. Trans. Br. Mycol. Soc. 69:496-498.
21. Macri, F., and Vianello, A. 1979. Photodynamic activity of cercosporin on plant tissues. Plant Cell Environ. 2:267-271.
22. Ou, S. J. 1987. Rice Diseases. 2nd ed. C.A.B. International, Slough, U.K. 380 pp.
23. Sah, D. N., and Rush, M. C. 1988. Physiological races of *C. oryzae* in the southern United States. Plant Dis. 72:262-264.
24. Sandman, G., Bramley, P. M., and Bogar, P. 1980. The inhibitory mode of action of the pyridazinone herbicide norflurazon on a cell-free carotenogenic enzyme system. Pestic. Biochem. Physiol. 14:185-191.
25. Tamaoki, T., and Nakano, H. 1990. Protein and specific inhibitors of protein kinase C of microbial origin. Biotechnology 8:732-737.
26. Tullis, E. C. 1937. *Cercospora oryzae* on rice in the United States. Phytopathology 27:1007-1008.
27. Venkataramani, K. 1967. Isolation of cercosporin from *Cercospora personata*. Phytopathol. Z. 58:379-382.
28. Wenzel, G. 1985. Strategies in unconventional breeding for disease resistance. Annu. Rev. Phytopathol. 23:172-179.
29. Widell, S., Lundborg, T., and Larsson, C. 1982. Plasma membranes from oats prepared by partition in an aqueous polymer two-phase system. Plant Physiol. 70:1429-1435.
30. Widholm, J. M. 1972. The use of fluorescein diacetate and phenosaframine for determining viability of cultured plant cells. Stain Technol. 47:189-194.
31. Willmont, D. B., Nickell, C. D., Widholm, J. M., and Gray, L. A. 1989. Evaluation of soybean resistance to *Phialophora gregata* culture filtrate in tissue culture. Theor. Appl. Genet. 77:227-232.
32. Witsenboer, H. M., Carla, E., Raoul, B., Loffler, H., Nijkamp, H. J., and Hille, J. 1988. Effects of *Alternaria alternata* f. sp. *lycopersici* toxin at different levels of tomato plant cell development. Plant Sci. 56:253-260.
33. Yamazaki, S., and Ogawa, T. 1972. The chemistry and stereochemistry of cercosporin. Agric. Biol. Chem. 36:1707-1718.