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Short Communication

Antiviral activity of tiazofurine against barley stripe mosaic virus

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Summary

Tiazofurine (2-β-D-ribofuranosylthiazole-4-carboxamide) was found to inhibit replication of barley stripe mosaic virus (BSMV) in barley and wheat plants. Treatment with this nucleoside analogue delayed and inhibited symptom development and suppressed virus multiplication. The most effective concentration applied twice as a foliar spray 3 h and one day after inoculation, was 10^{-3} M. Decreased virus multiplication was obtained without marked phytotoxicity. Three weeks after treatment the antiviral effect declined.

Plant virus; Barley stripe mosaic virus; Tiazofurine

Introduction

The definition (Gáborjányi and Tóbiás, 1986) of antiphytoviral substances should be restricted to those compounds that (*i*) efficiently inhibit virus multiplication and spread of virus infection in systemically infected plants, (*ii*) suppress and delay the development of symptoms, (*iii*) may have a curative effect, especially if they are applied after inoculation, and (*iv*) do not cause harmful effects to the host. From previous experience it has proven very difficult to find any compound that interferes with viral replication without seriously affecting the host plants (Dawson, 1983). Exceptions are Virazole (ribavirin, 1-β-D-ribofuranosyl-1,2,4-triazole-3-

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carboxamide) and its analogues (Byhan et al., 1978; Dawson, 1983; Dawson and Lozoya-Saldana, 1984; Lerch, 1987), which inhibit virus replication at doses that are not overtly toxic.

Tiazofurine (2-β-D-ribofuranosylthiazole-4-carboxamide), a compound that is structurally related to ribavirin, has proved effective against both human and plant viruses (Caner et al., 1984; Streissle et al., 1985). In experiments (Caner et al., 1984) with tomato spotted wilt virus (TSWV) in tomato plants, the severity of symptoms was reduced and the fruit yield of infected plants was enhanced after spraying with tiazofurine. Tomato spotted wilt is an unusual virus and we wished to determine whether tiazofurine had similar effects against a completely different plant virus, i.e. barley stripe mosaic virus, and, also, whether it was effective in monocotyledonous plants.

Barley stripe mosaic virus (BSMV) is an economically important pathogen that infects barley, wheat, and other species of Gramineae. This rod-shaped virus contains 3 genomic RNAs, having a cap structure at the 5′ end, an internal poly(A) sequence and a tRNA-like structure at the 3′ end of each RNA (Agranovsky et al., 1982; Gustafson et al., 1982).

Materials and Methods

Plants and viruses

Barley (*Hordeum vulgare* cv. GK Omega) and wheat (*Triticum aestivum* cv. Martonvásári 8) plants that are highly susceptible to BSMV infection, were used in all experiments. Plants in their two leaf stage were mechanically inoculated with crude sap of systemically BSMV-infected plants diluted twofold with 0.1 M Sörensen's phosphate buffer (pH 7.0) using Celite as abrasive. We used the Braunschweig (Br) strain of BSMV (originally obtained from Dr. W. Huth, Institut für Viruskrankheiten der Pflanzen, Braunschweig, F.R.G.). This strain produces severe stripe mosaic symptoms and leaf necrosis.

Treatment

Tiazofurine (a kind gift of Dr. P.C. Srivastava, Oak Ridge National Laboratory, Tennessee, U.S.A.) was dissolved in 25 ml of distilled water in 5×10^{-3}, 10^{-3}, 10^{-4} and 10^{-5} M concentrations and sprayed onto the leaf surface (ca. 1 ml/10 plants) after adding a drop of Triton X-100. Tiazofurine was applied at various time intervals (see Table 1) before and after inoculation. Each experiment was repeated three or four times. Fifteen to twenty greenhouse plants were used in each experiment.

Inhibition of symptom development and virus multiplication

Without treatment, the BSMV-Br yield reached its peak value 10–14 days after inoculation of the barley plants (data not shown). The most severe symptoms were also observed at that time. The BSMV yield and the severity of symptoms decreased three weeks after inoculation. The number of plants showing symptoms

TABLE 1

Inhibitory effect of tiazofurine (10^{-3} M) on symptom development and BSMV-Br multiplication in barley plants as a function of time of application

Treatment[a]	Phytotoxic effect	% of plants exhibiting symptoms[b]	Virus content (μg BSMV/g leaf material)[b]
−2 d, −1 d, +3 h, +2 d, +4 d	+++[c]	37.8	83
−2 d, −1 d	+	80.8	304
+3 h		72.4	242
+3 h, +1 d		57.4	139
+3 h, +2 d, +4 d	++	56.2	90
+2 d, +4 d	+	60.0	169
+4 d		86.2	204
No treatment		84.0	277

[a]− and + denote pre-inoculation and post-inoculation, respectively.
[b]At 12 days post-inoculation.
[c]Phytotoxic effects: + = slight, ++ = moderate, +++ = severe.

was recorded and samples were taken for the measurement of virus yield 12 days post inoculation (p.i.).

The amount of virus coat protein was measured by rocket immunoelectrophoresis, as described before (Gáborjányi and Tóbiás, 1984). In each experiment all plants with or without symptoms were harvested separately and cut into pieces. Randomly chosen pieces (0.5 g) were homogenized in 1 ml 0.1 M Sörensen's phosphate buffer (pH 7.0). After low speed centrifugation (10000 rpm for 10 min), 5 μl samples of the supernatant were assayed for virus content. To determine virus yield, standard curves were prepared with purified BSMV.

Results

Optimum time of treatment

The greatest inhibition of symptom development, measured as a decrease in the number of plants showing symptoms and the greatest decrease in virus yield were obtained when plants were treated with tiazofurine five times from 2 days before until 4 days after inoculation (Table 1). A similar inhibition in virus multiplication was achieved when treatment was applied three times (+3 h, +2 d and +4 d) after inoculation. However, in both experiments plants were severely damaged. As the number of treatments increased, especially during the p.i. period, the number of symptom-bearing plants gradually decreased. Pretreatment, applied twice (−2 d and −1 d) was not effective. Similarly, treatment at 4 days after infection caused only a slight inhibition in virus content. The optimal effect (inhibition of virus content without phytotoxicity) was achieved by treatment at +3 h and +1 d after inoculation.

TABLE 2

Inhibition of symptom development and BSMV-Br multiplication in barley plants as a function of tiazofurine concentration[a]

Tiazofurine concentration	Phytotoxic effect	% of plants showing[b] symptoms	Virus content (μg BSMV/g leaf material)[b]
$10^{-2.5}$ M	+++[c]	12.0	67
10^{-3} M	+	55.5	140
10^{-4} M		95.0	204
10^{-5} M		91.7	218
No treatment		96.1	283

[a]Tiazofurine was applied twice, at +3 h and +1 d post-inoculation.
[b]At 12 days post-inoculation.
[c]Phytotoxic effect: + = slight, ++ = moderate, +++ = severe.

Optimum concentration

With the optimal treatment regimen (+3 h and + 1 d p.i.), a series of tiazofurine concentrations were evaluated to establish the concentration that inhibited virus replication and did not cause marked phytotoxicity (Table 2). A concentration of 10^{-3} M gave the best results. Almost half of the inoculated plants remained disease-free. In plants showing symptoms of the disease, the virus coat protein content was reduced to half of that in the untreated controls.

Using wheat plants, the concentration of tiazofurine could be increased to 5×10^{-3} M without causing severe phytotoxicity (Table 3). At this concentration a 70% decrease in virus content was achieved in those plants that exhibited symptoms.

Discussion

Tiazofurine is structurally related to ribavirin but less active against human and animal DNA and RNA viruses in vitro (Streissle et al., 1985). In plants, tiazofurine has previously been tested only against tomato spotted wilt virus (Caner et al., 1984), which is an important pathogen but quite distinct from any other plant virus (Francki and Hatta, 1981; Francki et al., 1985). Treatment with tiazofurine p.i.

TABLE 3

Inhibition of symptom development and BSMV-Br multiplication in wheat plants as a function of tiazofurine concentration[a]

Tiazofurine concentration	% of plants showing[b] symptoms	Virus content (μg BSMV/g leaf material)[b]
$10^{-2.5}$M[c]	47.8	71
10^{-3} M	44.8	111
No treatment	88.5	235

[a]Tiazofurine was applied twice, at +3 h and +1 d post-inoculation.
[b]At 12 days post-inoculation.
[c]At this concentration slight phytotoxic effect was observed.

suppressed symptoms due to TSWV (Caner et al., 1984). This is a good example of the chemotherapeutic effect of the substance; however, virus yield in the plants was not measured.

In our experiments tiazofurine caused partial (50–70%) inhibition of BSMV replication. This reduction in virus yield may be considered as an important achievement since the chemical was sprayed onto and not infiltrated into the plants. Furthermore, tiazofurine suppressed the development of symptoms in about half of the BSMV-infected plants without causing overt phytotoxicity.

The optimal effect was obtained if the plants were treated twice, at 3 h and 1 day post-infection. This suggests that tiazofurine interfered with BSMV replication. At which step of the BSMV replicative cycle tiazofurine interacts remains to be elucidated.

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