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BRIEF COMMUNICATION

Influence of auxin-like herbicides on tobacco mosaic virus multiplication

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Abstract

Tobacco (*Nicotiana tabacum* L., cv. Samsun) leaf discs inoculated with tobacco mosaic virus (TMV) were treated with auxin-like herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), 2-methyl-4-chlorophenoxyacetic acid (MCPA), 3-amino-1,2,4-triazol (Amitrol) and 6-chloro-2-ethylamino-4-isopropylamino-1,3,5-triazine (Atrazin). All herbicides in the concentration of 10^{-7} M enhanced the virus content (MCPA to 227.4 %, Amitrol to 218.1 % and Atrazin to 257.3 % of values found in TMV-infected, herbicide untreated discs). The 2,4-D alone did not affect the activity of the glucose-6-phosphate dehydrogenase and ribonucleases, but the 2,4-D treatment together with TMV infection raised their activities twice as high as in the untreated control discs. Polyacrylamide gel electrophoresis of acidic extracellular proteins washed from leaf discs treated with 2,4-D did not prove the induction of PR-proteins.

Additional key words: Amitrol, Atrazin, 2,4-dichlorophenoxyacetic acid, glucose-6-phosphate dehydrogenase, MCPA, *Nicotiana tabacum* L., pathogenesis-related proteins, ribonucleases, TMV.

Auxins are believed to enhance the biosynthesis of RNA especially rRNA and mRNA (Macháčková 1997). According to Ladonin *et al.* (1970) the increased RNA content after application of synthetic auxin 2,4-D was not caused by a decrease in ribonuclease activity because the herbicide did not inhibit this enzyme. A similar finding was reported in virus infected plants by Cheo (1969). A number of exogenously applied chemicals, salicylic acid, polyacrylic acid, inorganic salts, chitosan, free oxygen radicals producing compounds such as paraquate, ozone, and others have been shown to induce extracellular pathogenesis related proteins (PR-proteins) and/or resistance to subsequent infection with viruses, fungi or bacteria (Van Loon 1985, Tamás *et al.* 1997, Burketová *et al.* 1999). In contrast to findings that auxins can induce the biosynthesis of PR proteins (Antoniw *et al.* 1981, Ohashi and Matsuoka 1987) involved in host resistance, we observed that synthetic auxin 2,4-D and other auxin-like herbicides enhanced the TMV multiplication in tobacco. In tobacco mesophyll protoplasts, the concentrations of 10^{-3} - 10^{-5} M 2,4-D decreased the TMV level (90 - 92 %) as opposed to 10^{-8} - 10^{-10} concentrations which enhanced the TMV content up to 123 % (Šindelář and Šindelářová 1994). Šindelář and Makovcová (1975), Makovcová and Šindelář (1981a) and Šindelář and Šindelářová (1994) also followed the effect of 2,4-D on the TMV multiplication in whole plants of *Nicotiana tabacum*. In whole plants, the TMV content was increased by 20 % after herbicide application whereas in leaf discs the amount of virus was double in comparison with herbicide untreated discs. Activities of enzymes galactokinase and galactose-1-phosphate uridyltransferase converting galactose to glucose-1-phosphate, the intermediate of glycolysis, were not affected by 2,4-D treatment but the increase in their activities was induced by virus infection. In pea infected with BYMV, the 2,4-D significantly increased both BYMV content (twofold) and activity of glucose-6-phosphate dehydrogenase (G6P DH)

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Abbreviations: Amitrol - 3-amino-1,2,4-triazol, Atrazin - 6-chloro-2-ethylamino-4-isopropylamino-1,3,5-triazine, BYMV - bean yellow mosaic virus, CMV - cucumber mosaic virus, 2,4-D - 2,4-dichlorophenoxyacetic acid, G6P DH - glucose-6-phosphate dehydrogenase, IF - intercellular fluid, MCPA - 2-methyl-4-chlorophenoxyacetic acid, PR-proteins - pathogenesis related proteins, RNase - ribonuclease, TMV - tobacco mosaic virus.

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in contrast to the selective herbicide cyanazine inducing their decrease (Makovcová *et al.* 1979). Makovcová and Šindelář (1981b) observed that application of 2,4-D resulted in a decrease in the activity of carbohydrate catabolising enzymes in leaves of cucumber plants in contrast to CMV-infected plants where the activities were enhanced. The application of 2,4-D to CMV-inoculated plants promoted the increase so that the activities were twice as high as control.

The aim of this paper was to study the effect of auxin-like herbicides (especially 2,4-D) on TMV multiplication, on activity of control enzymes of two metabolic pathways tending to precursors biosynthesis for TMV-RNA replication (oxidative pentosephosphate pathway and pathway of host RNA degradation), and on PR-protein biosynthesis in tobacco leaf discs.

Two-month-old tobacco (*Nicotiana tabacum* L. cv. Samsun) plants grown in soil, at an irradiance of 60 μmol m^{-2} s^{-1}, 16-h photoperiod, and average temperature of 25 °C were used. Leaf discs 1 cm in diameter were mechanically inoculated with purified TMV at a concentration of 100 μg cm^{-3} and cultivated on half strength Vickery nutrient solution with or without herbicides in continuous illumination (60 μmol m^{-2} s^{-1}) and 25 °C (Šindelář and Šindelářová 1994). They were used for the extraction of intercellular fluid (IF) using the slightly modified method as described by Pierpoint *et al.* (1987). Discontinuous nondenaturing polyacrylamide gel electrophoresis (PAGE) in 1 mm thick 10 % resolving gel and 4 % stacking gel was performed to analyse acidic (Laemmli system) proteins (Hames and Rickwood 1990), using the *Mighty Small II* apparatus (*Hoefer Scientific Instruments,* San Francisco, USA). The amount of proteins loaded to each well corresponded to IF derived from 10 leaf discs (6 - 12 μg of proteins). Gel was silver stained (Hames and Rickwood 1990). The TMV content was determined by the quantitative DAS-ELISA method (Clark and Adams 1977) with rabbit anti-TMV antibodies and alkaline phosphatase labelled antibodies. Proteins content was determined according to Bradford (1976) using bovine serum albumin as a standard. G6P DH and RNases activities were determined according to Šindelářová *et al.* (1997, 1998). The *t*-test was employed to characterise the differences. Chemicals were purchased from *Sigma Chemical Company* (St. Louis, USA).

The leaf discs were analysed on the 8th day post inoculation (8 dpi), when the culmination of multiplication curve of TMV, of G6P DH-activity and of RNases were observed (Šindelářová *et al.* 1988). Addition of different concentrations of 2,4-D (from 10^{-10} to 10^{-4} M) into incubation medium enhanced TMV multiplication (Fig. 1). The most efficient concentration was 10^{-7} M, which resulted in the increase of TMV content to 168.7 % in comparison with 2,4-D untreated discs (Fig. 1, Table 1). Other auxin-like herbicides under study (MCPA, Amitrol and Atrazin, 10^{-7} M) also enhanced the content of TMV (Table 2).

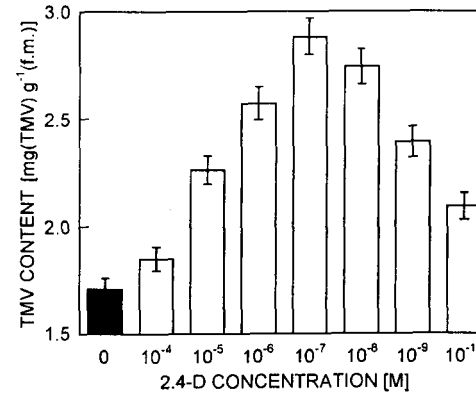

Fig. 1. TMV content in 2,4-D treated discs from tobacco leaves.

Table 1. TMV content [mg g^{-1}(f.m.)], the activity of glucose-6-phosphate dehydrogenase (G6P DH) [nmol mg^{-1}(proteins) s^{-1}] and ribonucleases (RNases) [U mg^{-1} (proteins)] in healthy, TMV-infected, 2,4-D (10^{-7} M) treated, and TMV-infected and 2,4-D (10^{-7} M) treated tobacco leaf discs 8th day post inoculation (means ± SE). Percentage values related to healthy discs are shown in parentheses.

	Healthy discs	TMV infected discs	Healthy discs + 2,4-D	TMV infected discs + 2,4-D
TMV	-	1.82 ± 0.04 (100.0)	-	3.07 ± 0.34*** (168.7)
G6P DH	0.57 ± 0.07 (100.0)	0.78 ± 0.07** (137.0)	0.55 ± 0.07 (97.4)	1.19 ± 0.14*** (209.7)
RNase	90.81 ± 13.78 (100.0)	152.29 ± 14.82*** (167.7)	94.99 ± 8.97 (104.6)	177.90 ± 20.03*** (195.9)

G6P DH and complex of RNases are control enzymes of two main metabolic pathways leading to biosynthesis of virus-RNA precursors: oxidative pentosephosphate pathway and pathway of host rRNA degradation (Šindelářová *et al.* 1997). 2,4-D treatment alone did not affect activity of G6P DH and RNase, but the 2,4-D treatment together with TMV infection raised theirs activities twice as high as in the untreated control discs (to 209.7 and 195.9 %, respectively) (Table 1). The rise of activity of G6P DH could be more enhanced by higher content of $NADP^+$ in 2,4-D treated plant as observed by Golebski *et al.* (1988).

Antoniw *et al.* (1981) and Ohashi and Matsuoka (1987) found that auxins could induce the biosynthesis of PR-proteins. In our experiments, the electrophoretic separation of extracellular proteins in discontinuous nondenaturing polyacrylamide gel did not prove the induction of PR-proteins in 2,4-D treated discs (Fig. 2),

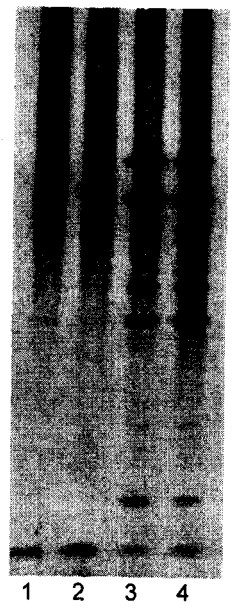

Fig. 2. Analysis of acidic extracellular proteins (silver stained) extracted 8th day post inoculation from: *lane 1* - mock inoculated tobacco leaf discs, *lane 2* - 2,4-D treated leaf discs, *lane 3* - TMV infected discs, *lane 4* - TMV infected and 2,4-D treated discs by electrophoresis in 10 % polyacrylamide gel under native conditions (Laemmli system).

Table 2. TMV content in tobacco leaf discs treated with auxin like herbicides (10^{-7} M) 8th day post inoculation.

Herbicide	TMV content [mg g^{-1} (f.m.)]	[% of control]
control	1.71 ± 0.03	100
MCPA	3.89 ± 0.07	227.4 ***
Amitrol	3.73 ± 0.06	218.1 ***
Atrazin	4.40 ± 0.09	257.3 ***

which corresponded with the fact that 2,4-D treatment did not inhibit the TMV infection. The massive increase of TMV observed in leaf discs inoculated with TMV and simultaneously treated with 2,4-D is probably caused by enhancement of biosynthesis of RNA induced by 2,4-D.

All these observations lead to the conclusion that auxin-like herbicides enhance the TMV content in leaf discs. The 2,4-D treatment of healthy discs does not affect the activity of G6P DH and RNase, respectively, in contrast to treatment of virus inoculated discs where the 2,4-D induces considerable increase in the activity of both enzymes. The 2,4-D neither induces the synthesis of PR-proteins nor the resistance against the TMV infection.

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