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# Inhibition of Acquired Resistance to Tobacco Mosaic Virus by Actinomycin D

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SUMMARY

In tobacco (*Nicotiana tabacum* cv. Xanthi-nc) actinomycin D inhibits the resistance to tobacco mosaic virus that is induced by polyacrylic acid or by earlier infection with potato virus Y. Formation of the additional proteins associated with this resistance is also prevented.

Heller (1963) found that actinomycin D inhibited the production of interferon in animals. As actinomycin D inhibits DNA-dependent RNA synthesis, his finding implies that interferon production and the resistance that it causes are dependent on DNA, i.e. the genetic information for interferon production is present in the cellular genome but does not express itself until induced to do so by a virus infection. Also, Gicherman & Loebenstein (1968) found that the resistance to tobacco mosaic virus (TMV) caused by injecting yeast RNA into leaves of a hypersensitive tobacco variety, was inhibited by actinomycin D. Recently, Gianinazzi & Kassanis (1974) demonstrated that injecting polyacrylic acid (PA) into intercellular spaces of tobacco leaves cv. Xanthi rendered them completely resistant to infection with TMV and tobacco necrosis virus and that injected leaves produced three, or sometimes four, additional proteins. This paper shows that resistance and the production of additional proteins are inhibited by actinomycin D.

Opposite halves of lower leaves of *Nicotiana tabacum* cv. Xanthi-nc were injected as before (Gianinazzi & Kassanis, 1974) with 50 $\mu$g/ml PA, mol. wt. 3500, with or without 5 $\mu$g/ml actinomycin D. Three days after injection the leaves were manually inoculated with a solution containing 2 $\mu$g/ml of TMV. No lesions developed in the half-leaves injected with PA alone but an average of 14 lesions appeared in the 9 halves (three lower leaves of three plants) injected with PA and actinomycin D. In two other tests, when the leaves were inoculated 2 days after injection, the average lesion numbers were 0 after injecting PA alone and 57 and 67 with the mixture. When actinomycin D was injected alone into half-leaves and water into the opposite halves, the average lesion numbers following inoculation with TMV were 38 and 30. Slight leaf injury appeared 6 days after injecting actinomycin D at 5 $\mu$g/ml but by that time the lesions had been counted.

Two days after injecting PA, sap from injected leaves contained the $B_1$ protein, which was the first new protein to appear following PA injection (Gianinazzi & Kassanis, 1974). No extra protein was detected in sap from the half-leaf injected with PA and actinomycin D during this period. However, tests 4 days after injection showed the $B_1$ protein was in both half-leaves, although there was less in the half injected with PA and actinomycin D than in the half injected only with PA. Leaves injected with PA and actinomycin D showed some resistance to TMV inoculation 4 days after injection. Gicherman & Loebenstein (1968) found that actinomycin D is effective in the plant for about 2 days.

We have shown that infecting tobacco cv. Xanthi with potato virus Y, or certain other viruses causing systemic infections, makes plants resistant to infection with TMV (Kassanis, Gianinazzi & White, 1974). Inoculating half-leaves with potato virus Y it was possible to

show resistance by inoculating the opposite halves with sap from a healthy plant. Four or 6 days later both sides were inoculated with 4 $\mu$g/ml of TMV. When the challenge inoculation was made 4 days after the first inoculation there was an average of 60 lesions/half-leaf when the first inoculation was with healthy sap and 37 when it was with potato virus Y (38 % reduction). When the challenge inoculation was made 6 days after the first inoculation the lesion numbers were respectively 82 and 34 (59 % reduction). The resistance of leaves to TMV decreased after injecting actinomycin D. Three lower leaves of three plants were inoculated with potato virus Y and 2 days later half-leaves were injected with water and the opposite halves with actinomycin D at 3·5 $\mu$g/ml, a concentration that does not injure leaves. Four days later, both halves were inoculated with TMV at 3 $\mu$g/ml. An average of 27 lesions developed in the half-leaves injected with water and 47 in half-leaves injected with actinomycin D (74 % increase). Loebenstein, Rabina & Van Praagh (1968) reported that the resistance of TMV-infected tobacco cv. Samsun NN to a second infection with TMV is also inhibited by actinomycin D.

It seems that animals and plants have a similar cellular resistance mechanism that is activated by virus infection or injecting synthetic polyanions, such as polyacrylic acid (De Clercq, Eckstein & Merigan, 1970). In both plants and animals resistance seems to be controlled by a DNA-dependent RNA but animals apparently produce only one protein, whereas plants produce 3 or 4 proteins, which could be aggregated forms of a single protein. This possibility is supported by the fact that two days after injection, when resistance has developed, only one protein is detectable.

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