

Leaf senescence and increased virus susceptibility in tobacco: The effect of abscisic acid

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(Accepted for publication January 1973)

Previous reports associating leaf senescence with increased susceptibility to virus infections were supported by the results of experiments with abscisic acid. The aging effect of ABA increased both the number of lesions produced in tobacco leaves when infected by tobacco mosaic virus and also increased multiplication of the virus in the leaves.

INTRODUCTION

Király *et al.* [6] showed that increasing the rate of senescence of leaves of tobacco (*Nicotiana tabacum*) increased their susceptibility to tobacco mosaic virus (TMV). Similar results have been reported with inoculated leaves after their excision [10, 11, 13] and after treatment with chloramphenicol [6, 17], actinomycin D [8, 9] and ethylene [3, 5, 12].

The present study was made to determine the effects on tobacco leaf susceptibility to TMV after treatment of the leaves with abscisic acid (ABA).

MATERIALS AND METHODS

The U₁ strain of TMV was cultured in *N. tabacum* cv. Samsun. Infectivity of virus preparations was determined by inoculating leaves of *N. tabacum* cv. Xanthi-nc and counting the local lesions produced. These plants were used at the 8 to 10 leaf stage.

Abscisic acid (ABA) at 1, 10 or 100 µg/ml tap water was applied either by infiltrating one half of attached leaves [7] or by placing leaf disks (15 mm in diameter) on a layer of granulated polystyrene floated on an ABA solution. The latter procedure permitted good aeration of the leaves [2].

To minimize differences in the physiological condition of test leaf disks, all disks were taken from one leaf and randomized between experimental treatments as shown in Fig. 1.

Virus multiplication in leaves of Samsun tobacco was determined either by local lesion assay or by estimating spectrophotometrically ribonucleic acid (RNA) in crude extracts of the infected leaves [16]. The effect of ABA was also estimated by measuring the diameter of local lesions [4, 15].

The influence of ABA on the synthesis of RNA was determined by estimating the incorporation of [¹⁴C]orotic acid into the acid insoluble fraction of Samsun tobacco leaves. ABA-treated and untreated leaf disks were floated for 4 h on a solution of [¹⁴C]orotic acid with an activity of 50 µCi/100 ml. The specific activity was 1·0 mCi/mmol. The leaf disks were then homogenized in 10% trichloroacetic acid (TCA)

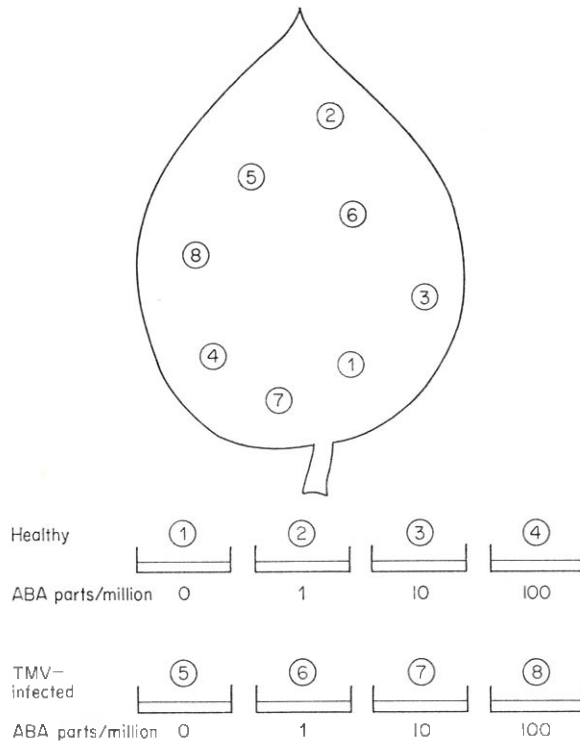


FIG. 1. Method of sampling leaf disks from tobacco leaves.

at 5 °C and twice centrifuged at 8000 rev/min. The pellet was washed in distilled water, re-centrifuged and re-suspended in water. Samples (0.8 ml) representing 100 mg fresh weight of leaf were pipetted in planchettes, carefully dried and their radioactivity determined in cts/min.

RESULTS AND DISCUSSION

Effect of ABA on leaf susceptibility to TMV

Xanthi tobacco leaf disks were floated on a solution of ABA for 1 day, inoculated with a purified preparation of TMV (120 mg/ml) and floated on test solutions for a further 2 days. The effect of ABA on local lesion production is shown in Table 1.

TABLE 1
Effect of ABA on the susceptibility of Xanthi tobacco leaf disks to TMV^a

ABA treatment ($\mu\text{g/ml}$)	Total number of lesions/44 cm ² leaf	Relative number of lesions
0	1158	100
1	1560	135
10	1752	150

^a Values represent the mean of five replications. Leaf disks were floated on water or ABA 1 day before and 2 days after inoculation. The number of lesions on 30 replicate disks (15 mm in diameter) were counted, representing a total leaf area of 44 cm². Disks were inoculated by using a glass rod. No abrasive was added to the inoculum.

In further experiments with leaves attached to the plant, half-leaves of Xanthi tobacco were infiltrated by injection with water or ABA solutions and, at various subsequent intervals, inoculated with TMV. Solutions containing 10 or 100 $\mu\text{g/ml}$ ABA were toxic to the leaves. The results obtained after infiltration with ABA solutions containing 1.0 $\mu\text{g/ml}$ are shown in Table 2.

The results demonstrated that ABA increased the susceptibility of excised leaf disks and attached leaves to infection by TMV.

TABLE 2

Effect of ABA infiltration of attached half-leaves of Xanthi tobacco on susceptibility to TMV^a

Time between ABA infiltration and inoculation (h)	Total number of lesions/44 cm ² leaf		Increase of lesion numbers (%)
	Control (water)	ABA (1 $\mu\text{g/ml}$)	
48	937	1173	25
72	1086	1393	28
96	1045	1360	30

^a Values represent the mean of five replications. One half of the leaf was injected with ABA and the opposite half with water as control. The whole leaf was then inoculated at various intervals with TMV by using a glass rod. No abrasive was added to the inoculum. The number of lesions on 30 disks (15 mm in diameter) were counted, representing a total leaf area of 44 cm².

Effect of ABA on virus multiplication

Samsun tobacco leaf disks were inoculated with TMV and immediately floated on water or on ABA solutions for 84 h, as shown in Fig. 1. To remove virus on the surface of the disks, they were washed in 2% NaOH, rinsed in tap water for 5 min and extracted in 0.15 M-phosphate buffer, pH 7.0 (100 mg/ml). The extracts were diluted 1/10 in the buffer and assayed by inoculation to Xanthi tobacco half-leaves. As shown in Table 3, more virus was detected in the disks floated on ABA solutions than in disks floated on water.

TABLE 3

Effect of ABA on the multiplication of TMV in Samsun tobacco. Values calculated from relative virus replication of homogenates of TMV-inoculated leaf disks tested on Xanthi tobacco^a

ABA concentration ($\mu\text{g/ml}$)	Relative number of lesions
0	100 ^b
1	162
10	180
100	148 ^c

^a Samsun leaf disks were inoculated with TMV and immediately floated on water or ABA for 84 h. A 100 mg leaf was extracted in 1 ml 0.15 M-phosphate buffer and then diluted 1/10 in the buffer. Half-leaves of Xanthi were inoculated with the diluted extracts by using a glass rod. No abrasive was added to the inoculum. Values represent mean of five replications.

^b Mean number of lesions per half-leaf for the water control was 550.

^c In most cases leaf damage occurred.

Evidence of increased virus multiplication in ABA-treated leaf disks was further obtained by determining (i) the absorbance of the RNA fractions of healthy and infected leaf disks after ABA treatment, (ii) the incorporation of [^{14}C]orotic acid (a precursor of uracil) into the acid insoluble fractions of untreated and ABA-treated healthy leaf disks and (iii) the effect of ABA on lesion size of inoculated leaf disks.

The spectrophotometric absorbance of the RNA fractions of healthy and infected Samsun tobacco disks after 84 h treatment with ABA is shown in Fig. 2. Results showed increased levels of RNA in virus-infected compared to healthy leaf disks.

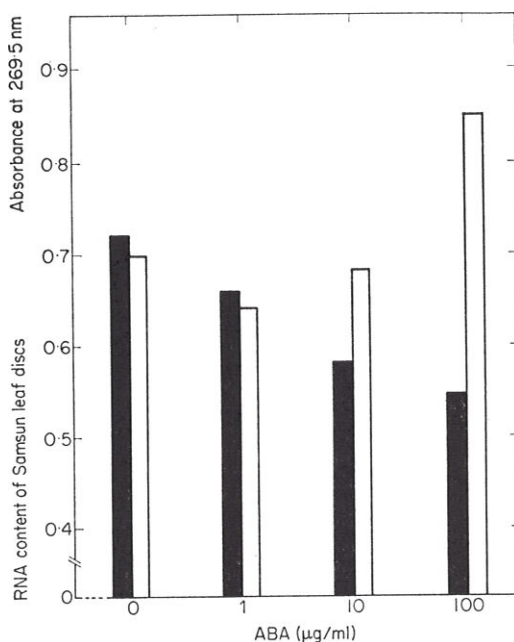


FIG. 2. The effect of treatment with abscisic acid on the absorbance at 269.5 nm of RNA fractions of healthy (■) and virus-infected (□) Samsun tobacco leaves 84 h after inoculation.

To determine the effect of ABA on RNA synthesis in a healthy leaf, disks of Samsun tobacco were floated on a 10 µg/ml ABA solution for 20 and 96 h at 22 °C and then transferred to [^{14}C]orotic acid. The results (Table 4) showed that whereas

TABLE 4

Incorporation of [^{14}C]orotic acid into the acid insoluble fraction of leaves of Samsun tobacco after treatment with abscisic acid^a

Abscisic acid (µg/ml)	Mean cts min ⁻¹ per 100 mg fresh wt ^b	
	20 h	96 h
0	848	583
10	744	165

^a Thirty disks of healthy leaves from the upper third of 8 to 10 leaf stage Samsun tobacco were floated on abscisic acid or on water (control) for 20 or 96 h, respectively.

^b Values are corrected for background and represent the mean of five replications.

RNA was not markedly reduced after 20 h, it was after 96 h. This result, when considered in relation to the virus multiplication in ABA-treated leaf disks, showed that although ABA reduced RNA in uninfected leaf disks it significantly increased it in ABA-treated infected disks. This type of control seems to be indispensable in such experiments, because the effect of ABA on the healthy plant may be influenced by the state of maturity of leaves as well as by excision [1, 14].

The effect of treatment with ABA on the size of local lesions in Xanthi tobacco leaf disks after inoculation with TMV is shown in Table 5. As shown, the diameter

TABLE 5
Effect of treatment with abscisic acid on TMV lesion size in Xanthi tobacco leaf disks^a

Abscisic acid ($\mu\text{g/ml}$)	Lesion size (mm)	Relative lesion size
0	0.50	100
1	0.48	96
10	0.57	114
100	0.64	128

^a Values represent the mean of ten replications. Leaves were inoculated with TMV by using a glass rod. No abrasive was added to the inoculum. Disks from the inoculated leaves were floated on water or on ABA for 92 h immediately after inoculation. The mean diameter of lesions on 30 replicate disks (15 mm in diameter) was calculated after measurement under a stereoscopic microscope equipped with an ocular micrometer.

of the lesions increased when the leaf disks were floated on solutions of 10 and 100 $\mu\text{g/ml}$ ABA for 84 h.

From the results of the foregoing experiments, therefore, it was concluded that leaf senescence was correlated with increased susceptibility to infection by TMV.

We are grateful to F. Hoffman-La Roche and Co Ltd, Basle, Switzerland, for a sample of abscisic acid.

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