inositol., 1mg per liter each of thiamine. HCl, naphthalene acetic acid, and benzyladenine., 80 mg per litcr adenine sulfate (.2H20), 100 *mgjl* L-tyrosine, 0.17 g/l NaH2 P04 yH20 and 0.8% Bacto-Agar (Difco). Culture plates were placed under 16 h light (high intensity fluorescent tubes, flux density 180 I..LE m^{-2} s^{-1}) at a constant temperature of 27C. Fresh weight and dry weight of individual disks were recorded after 0, 7, 14, 21, and 28 days of culture. Numbers of regenerated plant lets containing at least 2 leaflets were counted at 21 and 28 days. Each data point was based on measurements using eight disks, and the entire experiment was replicated four times.

RESULTS AND DISCUSSION

Two sets of healthy or virus infected *Nicotiana sylvestris* plants (four plants per set) were allowed to grow for four weeks after inoculation. Leaf disks were taken randomly from equivalent positions in the rosette and on the stem axis of systemically infected and healthy leaves and placed into culture.

In less than 10 days, the stem axis leaf disks from uninfected plants had expanded and grown much more rapidly than either class of virus-infected disks or healthy disks from the rosette leaves. The differences in growth were more pronounced among the disks taken from axis leaves as compared to disks taken from rosette leaves (Fig. 1). Thus, the disks taken from leaves of different developmental states responded differentially to virus infection despite the fact that virus titters in both types of leaves were the same at the time of initiation of tissue cultures.

To investigate this phenomenon further, two additional experiments were performed that included disks from leaves of different developmental state from healthy and infected plants. The data presented in Fig. 1 and 2 indicate that significant differences occurred among several treatments. For the first three weeks of culture, disks taken from uninfected leaves from the stem axis grew considerably faster in terms of fresh and dry weight accumulation than disks from virus-infected leaves on the stem axis. Disks taken from rosette leaves at the base of the plant grew more slowly still, but infection by the virus did not affect their growth during the first two weeks of culture. In the fourth week of culture, the growth of disks from axis leaves of infected plants reached about the same level as that of the uninfected axis leaf disks. Curves for accumulation of fresh and dry weight were similar in shape. Thus, although the growth rate of infected axis leaf disks was reduced significantly by virus infection, the weight at which further growth ceased was the same for uninfected and infected tissues.

Table 1. ElTects of leaf age and viral infection on regeneration of plantlets from cultured leaf disks of Nicotiana sylvestris.

Days Cultured	Uninfected		Infected	
	Rosette	Stem Axis	Rosette	Stem Axis
21	7 ± 1	54 ± 4	12 ± 2	25 ± 3
28	34 ± 3	77 ± 5	46 ± 4	65 ± 3

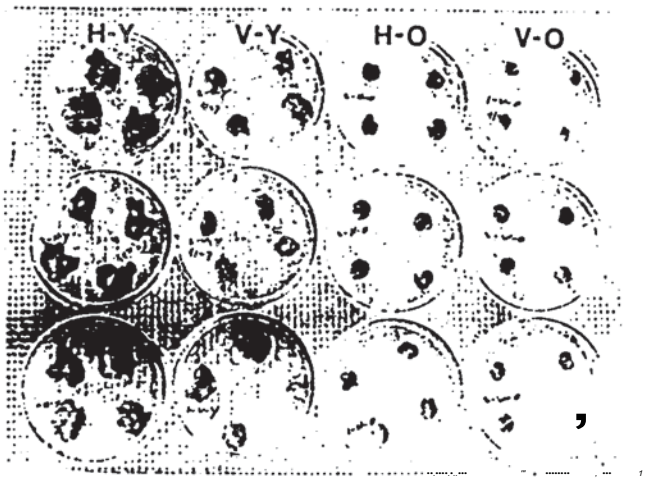

Fig. 1. Ten day old leaf disk cultures from TMV infected *Nicotiana sylvestris* plants. H-Y: disks from uninfected stem axis leaves; V-V. disks from infected stem axis leaves; H-O disks from uninfected rosette leaves; V-O: disks from infected rosette leaves.

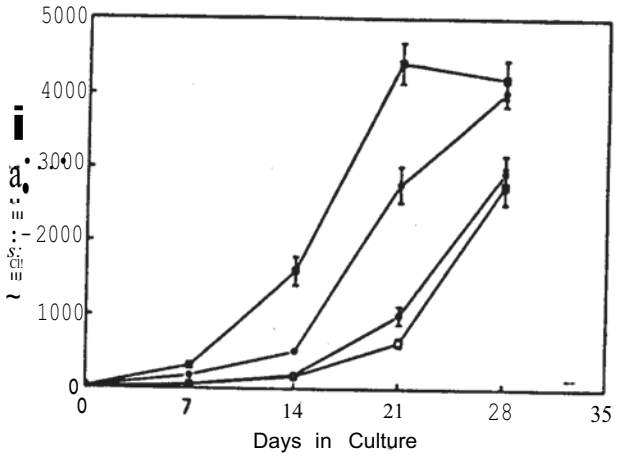

Fig. 2. Fresh weights of leaf disks at various times after initiation of cultures. Closed squares: disks from uninfected stem axis leaves; closed circles: disks from infected stem axis leaves; open squares: disks from uninfected rosette leaves; open circles: disks from infected rosette leaves. Data are presented as means ± standard errors of means.

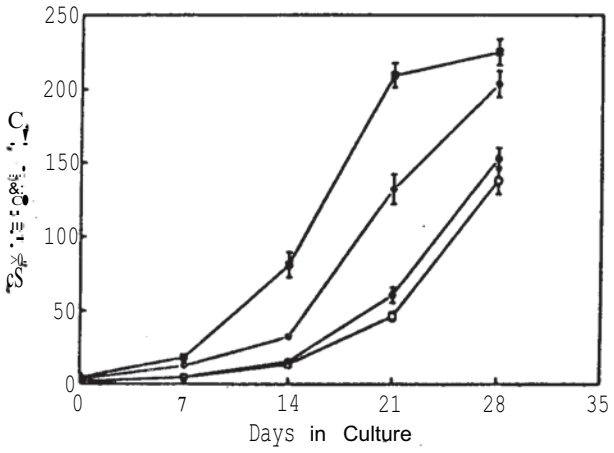

Fig. 3. Dry weights of leave disks at various times after initiation of cultures. Closed square: disks from uninfected stem axis leaves; closed circles: disks from infected stem axis leaves; open squares: disks from uninfected rosette leaves; open circles: disks from infected rosette leaves. Data are presented as means ± standard errors of means.

The number of plant lets produced from each type of leaf disk is a parameter of practical interest. The results summarized in Table Lshow that the presence of the virus significantly slowed the development of plant lets on disks from axis leaves. After three weeks, the number of plantlets on virus infected disks from axis leaves was only about 50% of the number of disks from uninfected axis leaves. Plant lets developed even more slowly on disks from rosette leaves, but virus infection appeared to have little effect on regeneration from disks of this type.

These experiments show that both infection by TMV and the developmental stage of the host explant are important factors affecting the growth and regeneration of plantlets from cultured leaf disks of this species. Under conditions used here, a clear distinction between infected and uninfcctcd tissues from axis leaf cxplants could be discerned, but no significant difference was observed between infected and uninfected rosette leaf cxplants. How the virus reduces disk growth and plant regeneration is not yet known.

The differential effects of the virus on rapidly developing tissues of stem axis leaves might have implications for shoot tip culture methods for deriving virus-free plants. In other work, we have shown that, during invasion of rapidly developing tissues as well as the regeneration of plantlets in tissue culture, the genetic structure of a virus population can be dramatically altered, which does not occur in fully developed tissues (Khan and Jones 1989 a,b). Perhaps some instances in which shoot tip culture is not successful occur because a genetically distinct subpopulation of the virus can successfully invade the rapidly developing meristematic regions. Use of cultured explants from dcvcloprnentally different tissues of the species in question might be of value in investigating such occurrences.

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