Rhabdovirus Infection of Taro, Colocasia esculenta (L.) Schott, from Papua New Guinea: Comparison of Symptomatic and Asymptomatic Material

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ABSTRACT

Samples of symptomatic and asymptomatic leaves of taro, Colocasia esculenta (L) Schott, from Papua New Guinea, were fixed, embedded and sectioned for electron microscopy. Symptomatic material showed marked bobone disease virus (BDV) symptoms.

Cells of infected, symptomatic tissue contained inclusions, of virions and viroplasm. The cytoplasm appeared granular and contained large numbers of polyribosomes. The chloroplasts had reduced membrane contents and increased amounts of starch. General anatomy of the leaf was altered with an increase in cell number and decrease in size of the palisade cells.

In similar samples from the same plant at a later, asymptomatic, time no cellular evidence of viral infection could be detected. The cytoplasm contained fewer polyribosomes and plastids, less starch and more extensive granal membranes. Leaves had palisade anatomy like that in uninfected material.

Introduction

Taro, Colocasia esculenta, has been shown to be affected by three different viruses (Jackson and Pelomo, 1980; Jackson and Firman, 1982; Shaw, Jackson and Plumb, 1979). Dasheen Mosaic Virus, a long flexous rod, has been assigned to the potato Y virus group. (Zettler, et al, 1970; Kenten and Woods, 1973). The other two viruses are bacilliform particles, associated with the "alomae" and "bobone" diseases of taro in the Solomon Islands (Jackson and Firman, 1982; Gollifer and Brown, 1972; Kenten and Woods, 1973). The larger of these bacilliform particles is restricted to the Solomon Islands and Papua New Guinea (Jackson, 1978; Jackson and Firman, 1982; Shaw, et al., 1979), and is considered to be a rhabdovirus (Francki and Randles, 1980; James, Kenten and Woods, 1973; Shaw, et al., 1979). The smaller particle, similar to cocoa swollen shoot virus, appears to be distributed throughout many taro growing areas (Jackson and Firman, 1982; James, et al., 1973; Shaw, et al., 1979).

Few papers have been published regarding the nature of the bacilliform particles of taro. Classification of the large particle is based primarily on evidence from a single study (James, et al., 1973; Kenten and Woods, 1973). Instability and difficulty of isolation have even further hampered efforts to purify the rhabdoviral particle (James, et al., 1973). Little information is

available regarding localization of these viruses in infected material. The passage of time has only served to accentuate the need for better understanding and control of these diseases (Jackson and Firman, 1982). This is a preliminary report of efforts to determine the distribution of the viruses of taro in infected material and the effects of these viruses on normal structure. Work has been primarily concerned with the rhabdovirus, recently designated Bobone Disease Virus (BDV) (Francki and Randles, 1980; Francki, Kitajima and Peters, 1981).

Materials and Methods

Samples of taro displaying symptoms of viral infection were received from Papua New Guinea. These plants were maintained in an environmental chamber under 16 h photoperiods at 28° to 30°C and 70 to 90% relative humidity. Alternately, plants were placed in 21 plastic pots at the Burlington Botanical Research Station, Woburn, Massachusetts. Ambient light in the greenhouse was supplemented with Sylvania Gro-lux fluoroescent lights to produce continuous light conditions.

Portions of leaves, from symptomatic and asymptomatic plants, were fixed in glutaraldehyde, post fixed in osmium, embedded and examined under the electron microscope by procedures described elsewhere in this volume (Stein, Strauss and Scheirer). Three to five micron sections were afixed to glass slides, stained with toluidine blue (Richardson, Jarret and Finke, 1960) and examined using a bright field Olympus Light Microscope.

Results and Discussion

Several cellular differences are notable between symptomatic and asymptomatic leaves (Table 1). One difficulty in interpreting the results as BDV effects is that nearly all cells containing the rhabdovirus virions also contain the flexous rods of Dasheen Mosaic Virus (DMV) (Figure 1). In these regions of virus production prominent polyribosomes are evident. Viroplasms, like those associated with other plant rhabdoviruses (Francki and Randles, 1980) have also been noted occasionally, but only in symptomatic material (Table 1).

Table 1. Summary of cellular differences noted in symptomatic and asymptomatic *Colocasia esculenta* var *esculenta* cv K268 plants infected with dasheen mosaic virus (DMV) and bobone disease virus (BDV).

	General Appearance	
Structure	Symptomatic Plants	Asymptomatic Plants
Virion	present	absent
Viroplasm	present	absent
Polyribosomes	numerous	few
Chloroplasts		
membranes	reduced	grana/stroma
	grana	structure typical
Starch	abundant	few grains

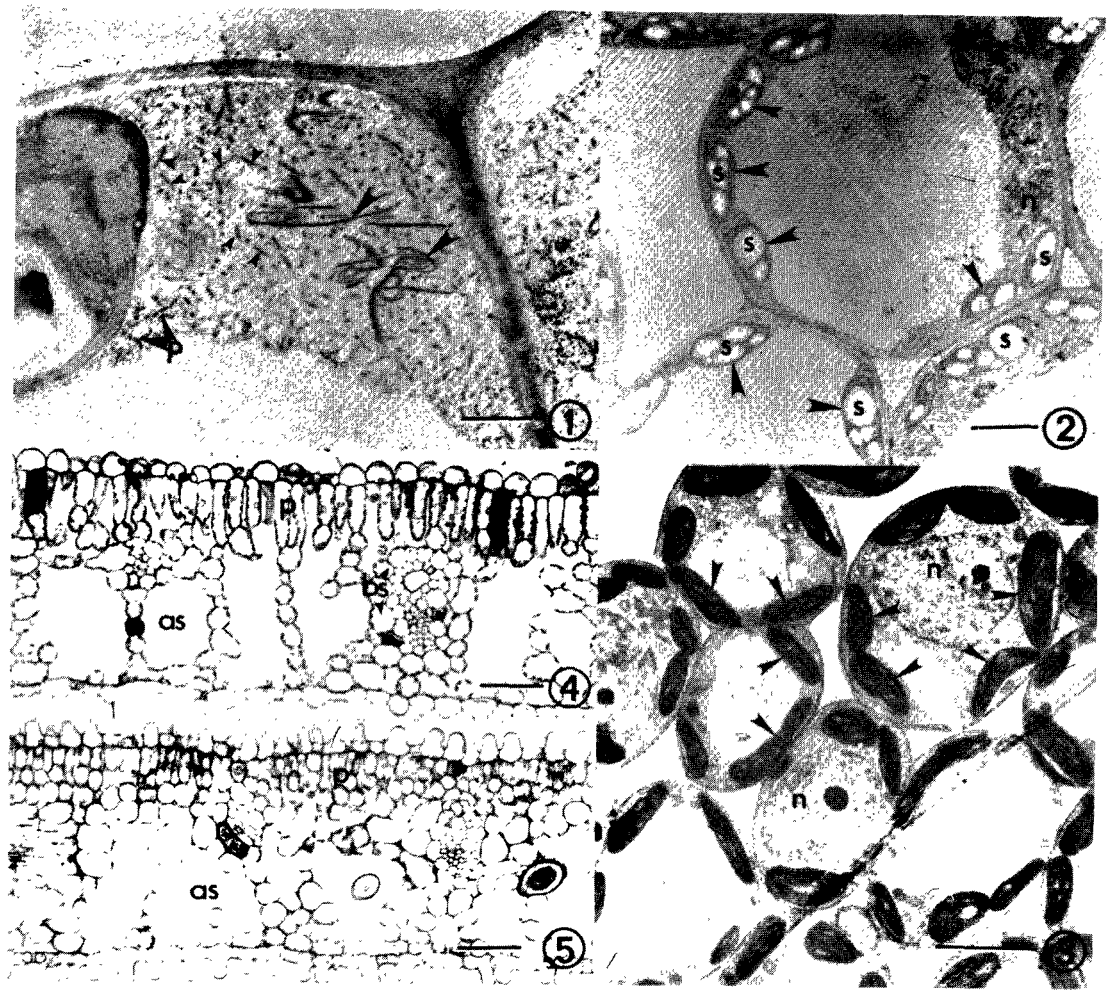

Figure 1. Cytoplasm of leaf mesophyll cell from infected, symptomatic taro, Colocasia esculenta var esculenta cv K268. Note bacilliform particles of Bobone Disease Virus (BDV) (small arrows), flexous rods of Dasheen Mosaic Virus (DMV) (large arrows), and polyribosomes (p). Bar = 1 micron.

Figure 2. Transverse section through palisade parenchyma from infected, symptomatic taro, Colocasia esculenta var esculenta cv K268. Chloroplasts (arrow) contain considerable starch (s). Bar = 5 microns.

Figure 3. Transverse section through palisade parenchyma from infected, asymptomatic taro, Colocasia esculenta var esculenta cv K268. Chloroplasts (arrows) contain little starch and numerous membranes n, nuclei. Bar = 5 microns.

Figure 4. Transverse section of leaf from infected, asymptomatic taro, Colocasia esculenta var esculenta cv K268. Note rectangular air spaces (as), prominent bundle sheath (bs), and palisade (p). Bar = 50 microns.

Figure 5. Transverse section of leaf from infected, symptomatic taro, Colocasia esculenta var esculenta cv K268. Palisade (p) cells are more numerous and of a smaller average length. Note lack of a distinct bundle sheath. There are still large air spaces (as) in the leaf. Bar = 50 microns.

Chloroplasts in the palisade parenchyma of symptomatic plants often contain fewer internal membranes and usually display considerable starch accumulation, when compared (Figures 2 and 3) to the same plant during an asymptomatic period. Thus though the virus appears to be restricted to the cytoplasm, it has profound effect on chloroplast biochemistry. One review reports virus particles being noted as budding from the nucleus (Francki, et al., 1981). We have been unable to confirm this in material from Papua New Guinea.

Examination of distribution and morphology of cell types in the leaf reveals a profound effect on cells of the palisade parenchyma and bundle sheath (Figures 4 and 5). In the former, cells appear to have undergone multiple division, as their numbers are increased and average size decreased. The normally distinct bundle sheath is poorly visible in the symptomatic plant. This is consistent with findings that all of these cells contain virus in the symptomatic plant. It is not clear, though, where the virus (or its genetic material) is during an asymptomatic period.

This preliminary report points up the need for considerable further study of this virus. Little success is evident in isolating virus particles. However, the dense number of polyribosomes suggests that it might be possible to use these in a cell-free protein synthetic system to produce viral proteins for antisera production. Feasibility of such an approach remains to be determined. Distribution of this virus is needed to understand its development and survival in taro germplasm.

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