J. Phytol. Res. 5 (1 & 2): 55-57, 1992

AZURE B IN THE DIANGNOSIS OF MLO INFECTION IN *CATHARANTHUS ROSEUS* (L.) DON

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Several NA specific, histochemical, light microscopic methods have been in use for the detection of MLO infections in plants. Use of Azure B under this category has been presently evaluated for this purpose.

Keywords : Azure B; NA-specific; Histochemical stain; MLO- detection.

Introduction

For the diagnosis of yellows diseases of presumptive MLO association several rapid light microscopic methods have been used. Most of these methods have relied upon the histochemical method of detection of the nucleic acids, DNA or RNA or of both simultaneously (Sharma *et al.*, 1986). These methods are useful not only in the case of newly recorded diseases but also for the selection of MLO free plants from a lot.

This paper outlines a method that is rapid, simple and reliable for the diagnosis of yellows diseases. It involves the use of Azure B, which is very specific for nucleic acids (Kramer and Windrum, 1955; Schubert and Hamerman, 1956), and as such could be used in case of a material already known for or suspected to be carrying the natural infection of mycoplasma-like organisms.

Material and Methods

Samples from the healthy and 'yellows' affected - *Catharanthus roseus* (L.) Don plants, latter with virescent flowers (Misra *et al.*, 1987), were collected from a locality within the University campus.

Pieces of stem of the healthy and diseased samples were taken separately for sections to be cut with the help of a single-edged razor blade. The sections were stained with Azure B (0.2% solution in 0.05 citrate buffer at pH 4.0) at 50°C for 1 hr, thereafter washed in water and placed in pure tertiary butyl alchohol (TBA) for 30 minutes to differentiate (Flax and Himes, 1952). Sections with a deep stain were left in TBA for over-night and mounted in glycerine.

As per usual practice in Histochemistry, sections to be taken as control were run through the process of differential extraction of nucleic acids with perchloric acid (Erickson *et al.*, 1949), prior to staining with Azure B as above. As such control sections of both healthy and diseased samples were placed in 1N perchloric acid for 12 hr at 4°C in a refrigerator. This step removed the RNA. The sections were washed thoroughly in running water and again placed in 0.5N perchloric acid at 70°C

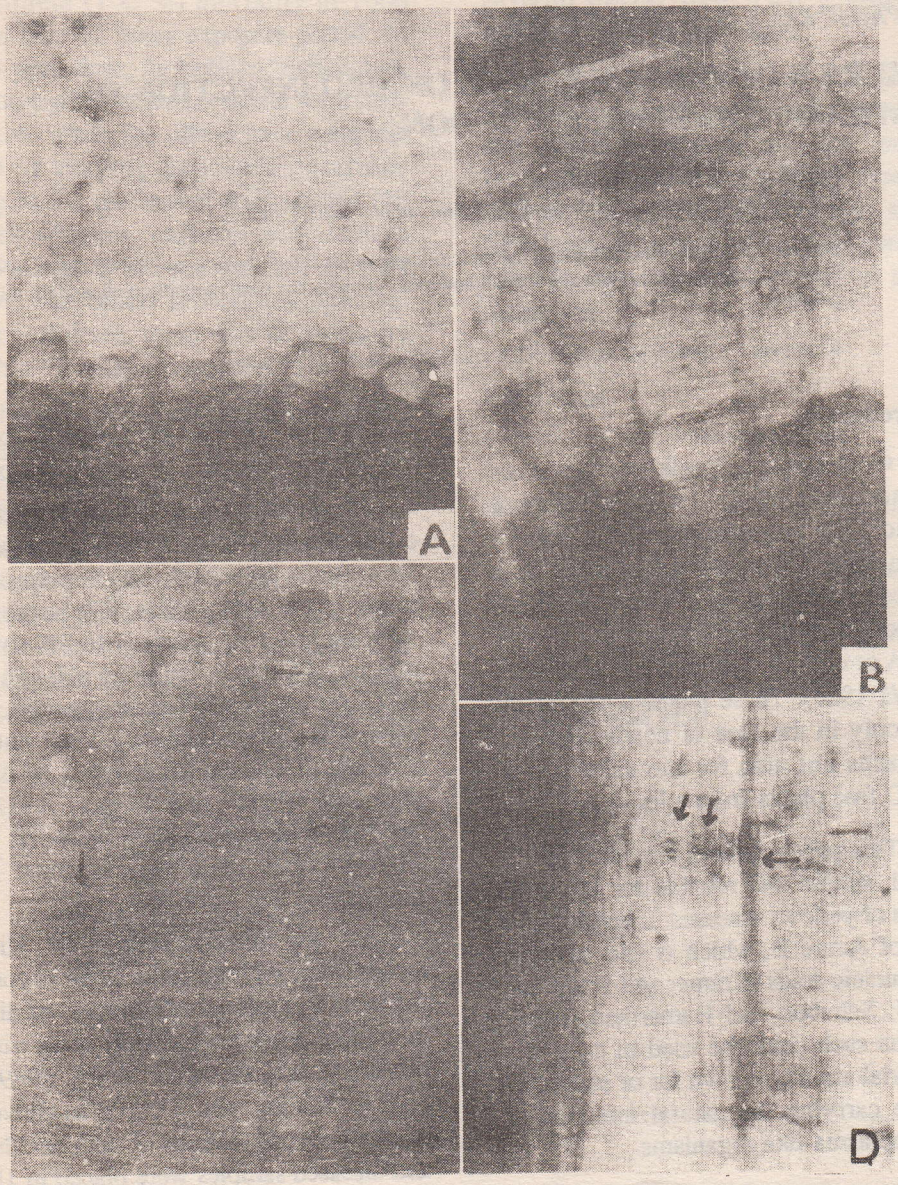

Figure 1 A-D Micrographs showing transverse and longi-sections of stem internodes of *Catharanthus roseus* stained with Azure 'B'.
A. T. S., Healthy stem, a portion showing no stain in phloem region;
B&C. T.S., Diseased stem, a portion showing blue stain in sieve tube (arrow);
D. Longi-section stem, showing blue stain in full length of sieve tube; also showing blue stain at sieve plates (arrow).

for 20 minutes. This step removed the DNA. The sections were then immersed in sodium carbonate for 5 minutes and again washed in running water. Staining with Azure B was done after the digestion of both the nucleic acids from the sections.

Results

Both undigested and control sections of the healthy and diseased, stained with Azure B, were observed under the bright field light control microscope. In all the sections the lignified cell walls stained green and phloem of the healthy stem sections, undigested as welll as control, remained almost unstained, but phloem of the diseased stem in the case of undigested sections were found to contain regularly distributed areas that stained distinctly blue (Fig.1B & C). Under high magnification, these areas resolved as groups of phloem sieve cells.

Similarly, the undigested longi-sections of the diseased stem, showed a consederable number of mature sieve tubes with blue stain and the stain condensed along the sieve areas (Fig.1D). Some of the sieve tubes, having stain throughout their whole length, appeared as articulated bones. On the contrary the sieve tubes in the healthy counter parts remained unstained.

In the controls only the lignified cell walls stained green. The substance that stained blue or bluish-green in the undigested material and which did not occur in the extracted tissue was the nucleic acid DNA and/or RNA.

The above procedure using Azure B gave convincing results as regards detection of nucleic acid (NA) containing bodies the mycoplasmas - inside sieve tubes. And hence, could be quite useful in the diagnosis of MLO - infections in plants in which morphological symptoms were also characteristic.

Acknowledgement

The authors are very much thankful to the Head, Department of Botany, University of Rajasthan, Jaipur for providing laboratory facilities.

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