

Banana streak badnavirus research at the International Institute of Tropical Agriculture

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The International Institute of Tropical Agriculture (IITA) is working on the following aspects of banana streak badnavirus (BSV) as part of IITA's core research agenda, with supplementary funding from the World Bank (CFC/BIP), Gatsby Charitable Foundation, UK and BADC, Belgium.

- Natural and experimental transmission of BSV
- Epidemiology of BSV
- Yield loss assessment
- Detection of BSV
- Virus-host interactions
- Control of BSV
- BSV elimination from infected plant material
- Safe movement of *Musa* germplasm in Africa

Other IITA scientists involved in these studies at IITA Ibadan (Nigeria), IITA High Rainfall Station, Onne (Nigeria) and IITA's East and Southern Africa Regional Centre (ESARC, Uganda) are J. Ikea, S.Y.C. Ng, M. Pillay, A. Tenkouano, G. Thottappilly and D. Vuylsteke. J. Crouch, H. Crouch, G. Dahal, F. Gauhl, R. Ortiz and C. Pasberg-Gauhl are no longer at IITA but were involved with the initiation of the research at IITA on BSV and the subsequent development and implementation of the research programme.

Natural and experimental transmission of BSV

BSV can be transmitted experimentally by the mealybugs *Planococcus citri* and *Saccharicoccus sacchari* (Lockhart & Autrey 1988).

Mealybugs are found on banana and plantain growing in the field in West Africa. In Nigeria the main species are predominantly *Ferrisia virgata*, *Dysmicoccus brevipes* and *D. grassii*. The numbers and proportion of nymphs of these species varies according to the time of year. In spite of large numbers of mealybugs (including a new species *Planococcus musae*, described by Matile-Ferrero & Williams, 1995) on the bracts, pseudostems and roots, there is no evidence from IITA of field spread of BSV by these possible vectors.

There is some evidence of seed-transmission of BSV in *Musa* (Daniells *et al.* 1995). However, at IITA it has not been possible to confirm conclusively that seed transmission

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does occur. Preliminary studies, albeit with seedlings which were not grown in an insect-proof environment, suggest that seed transmission of BSV in hybrids does occur. Further studies are in progress using seed derived from BSV-infected and virus-tested mother plants to determine if seed transmission occurs and, if so, whether this occurs through the male or female parent.

Epidemiology of BSV

Trials at IITA with foci of BSV-infected material showing severe symptoms planted in the middle of blocks of 'healthy', symptomless plants of two IITA hybrids do not show any evidence of radial spread of the disease or new outbreaks which would be expected through mealybug transmission. The main means of transmission of BSV in Nigeria appears to be through dissemination, by farmers, of BSV-infected suckers.

Water stress and sustained cooler temperatures (Dahal *et al.* 1997b) seem to be the cause of localized 'outbreaks' of symptoms. Where there remains an adequate supply of water and other stresses are minimised, the plants generally appear to be healthy with few if any symptoms, except during the cooler season in southern Nigeria. Some varieties/hybrids appear to be more susceptible to the stress and cooler temperature induction of symptom expression than others.

Yield loss assessment

Studies are in progress at IITA-Ibadan to determine the relative yield losses incurred by banana and plantain landraces and hybrids which have been recorded as exhibiting symptoms of BSV compared to plants which have not been recorded as exhibiting symptoms at any time during the season.

Detection of BSV

Several detection methods are used at IITA to confirm the presence of BSV in germplasm. Usually the tests are not done singly; several tests are applied to each plant. The following tests are used:

Visual

The symptoms of BSV are fairly characteristic although they may occasionally be confused with those caused by CMV. Where distinct symptoms are seen, BSV can usually be implicated although it may be necessary to confirm the diagnosis through other techniques. The use of a controlled temperature room at approximately 22°C (compared with ambient temperatures of about 28°C - 32°C) promotes symptom expression in 2-5 months and concurrently promotes a higher virus titre in the plant tissue enabling more reliable detection by serological tests and electron microscopy.

Triple antibody sandwich enzyme-linked immunosorbent assay (TAS-ELISA)

Several different TAS-ELISAs are used at IITA depending on the purpose of the tests and the antibodies used. Both rabbit (B.E.L. Lockhart, University of Minnesota) and mouse (G. Thottappilly, IITA) polyclonal antibodies are used in one TAS-ELISA. In a second, the rabbit polyclonal antiserum is combined with one raised in chickens (two antisera available: B.E.L. Lockhart, University of Minnesota and G. Thottappilly, IITA). In addition, monoclonal antibodies against a Nigerian isolate of BSV are being produced at IITA.

Electron microscopy

Electron microscopy, using sap and/or partially-purified preparations, can often be useful for making a preliminary diagnosis of BSV infection in symptomatic, and some asymptomatic, plants. CMV will not be readily seen by electron microscopy without the use of immunosorbent electron microscopy (ISEM).

Immunosorbent electron microscopy (ISEM)

ISEM is routinely used for BSV diagnosis either from sap extracts or partially-purified preparations. Generally, sap extracts are first tested and, if negative, re-tested using partially purified preparations. To detect BSV in samples from Nigeria, mouse polyclonal antiserum (G. Thottappilly, IITA) is used, with random back-up tests using rabbit polyclonal antiserum (B.E.L. Lockhart, University of Minnesota). For leaf samples from outside Nigeria, BSV test results are always confirmed using the rabbit polyclonal antiserum (B.E.L. Lockhart, University of Minnesota) which is known to be effective in detecting a wide range of BSV isolates.

Immunocapture polymerase chain reaction (IC-PCR)

IC-PCR is carried out on sap extracts. The efficiency of IC-PCR using sap rather than partially purified preparations as well as the specificity of the test for episomal BSV at IITA has been confirmed. Rabbit polyclonal antiserum (B.E.L. Lockhart, University of Minnesota) is usually used to trap the virus (although the other antisera can also be used) and the primer obtained from R. Hull (John Innes Centre) is subsequently used for the PCR. The IC-PCR is reliable, detecting BSV in those plants exhibiting symptoms as well as in some symptomless plants. The presence of BSV in the symptomless plants can later be confirmed using other techniques such as ISEM after transfer of the plants to 22°C to promote an increased virus titre.

Polymerase chain reaction (PCR)

PCR is also carried out on extracted DNA. The results from these tests are however not conclusive after confirmatory testing with other diagnostic methods. Because the

significance of detection of integrated BSV sequences in *Musa* is not fully understood, PCR for diagnosis of BSV at IITA remains a research technique and is not, as yet, a diagnostic tool.

Virus-host interactions

It has been noted in the past that tetraploid hybrids seem to be more 'susceptible' to BSV. Studies at IITA on micropropagated banana and plantain hybrids confirmed that plantain hybrids had a high incidence of symptomatic plants which were negligible or absent in their parental lines and other landraces (Dahal *et al.* 1997a). In addition it has been noted that while plantain hybrids express symptoms under cooler (22°C) as well as ambient temperatures, most cooking bananas did not express symptoms under either regime although virus could be detected by ISEM in some of the plants (Dahal *et al.* 1997b, Dahal *et al.* 1998).

Control of BSV

Studies on controlling BSV are centred around aspects of phytosanitation, good management of the plants and their environment, the possible use of resistant or tolerant germplasm and use and distribution of healthy planting material.

Phytosanitation

Roguing is traditionally used to remove plants which are apparently unhealthy so that healthy plants can be substituted. In the case of BSV-infected plants, severely affected plants may not produce acceptable yield. It can therefore be useful to remove plants exhibiting severe symptoms and replant with 'healthy' suckers. It must be noted however that symptomless suckers can be obtained from mother plants showing severe symptoms, and that suckers with symptoms can grow from apparently symptomless mats. In the former case, the symptomless suckers are virus-infected and may later develop symptoms.

Good management

Good farm management appears to allow plants to attain a high percentage of their yield potential, despite infection with BSV. Field trials using severely BSV-infected suckers of many cultivars and hybrids, when well-managed result in plants with reduced symptom expression and apparently much improved yields, as compared to plants of the same cultivars and hybrids exhibiting severe symptoms under poor management conditions.

The major factors which seem to reduce symptom expression and thus have a subsequent effect on yield are: reduced water stress, mulching, reduced weed competition and minimal stress from other pests and diseases. Mulching is likely to be effective in reducing the effect of BSV on the plants by helping to reduce water loss and therefore also reducing water stress. Removing or reducing other stresses (such as

weeds competing for available nutrients, and other pests and diseases) also appear to be beneficial in reducing the effects of BSV.

Resistant/tolerant germplasm

IITA has identified several hybrids which are apparently resistant to BSV based on symptom expression. These hybrids are described by Ortiz and Vuylsteke, 1998a; Ortiz and Vuylsteke, 1998b and Ortiz *et al.*, 1998. Some recent results show that the incidence of BSV symptom expression in tetraploid *Musa* hybrids in South-eastern Nigeria varies from over 43% in one hybrid to less than 4% in another. This may indicate a degree of resistance or tolerance of the germplasm to BSV.

Healthy planting material

To produce healthy planting material, national tissue culture laboratories are being encouraged to micropropagate virus-tested germplasm for distribution. Additionally, training in the aetiology of BSV has enabled national programme scientists to be able to make decisions on the health status of banana and plantain and make appropriate recommendations for extension workers and farmers.

BSV elimination from infected plant material

The elimination of BSV from infected plants has been attempted by both meristem-tip culture and the use of embryonic cell suspension culture.

In vitro meristem-tip culture

Meristem-tip culture alone, with chemotherapy and/or thermotherapy has been unsuccessful in eliminating BSV from infected plants.

Embryonic cell suspension (ECS) derived plants

ECS derived plants of three lines (Three Hand Planty, Bise Egome and Nakitengwa) have been produced by the Katholieke Universiteit Leuven (KUL). However, it appears unlikely that any of the plants will be virus-free as all plants have exhibited symptoms at some time. In addition, many have tested positive by ELISA, ISEM and/or IC-PCR. Some plants, sent to the John Innes Centre for fluorescent *in situ* hybridization studies, showed positive signals of integrated BSV genome. Final testing of the ECS plants is currently in progress.

Safe movement of *Musa* germplasm in Africa

IITA has considered the following points in taking the decision to distribute improved hybrids, upon request, within Africa:

- There is no evidence of natural transmission of BSV under field conditions,

- There is no direct evidence of field spread of BSV,
- There is uncertainty over the significance of the results of direct PCR (*c.f.* IC-PCR) and the consequences/detection of BSV genome integrated within the *Musa* genome,
- The effects of BSV in Nigeria appear to be relatively mild and can be managed by good farming practices,
- The risks associated with moving virus-tested germplasm from Nigeria are relatively low,
- The need for Sigatoka-resistant material and improved hybrids of banana and plantain with other characteristics to alleviate poverty in sub-Saharan Africa is very high,
- National programme scientists have been trained in BSV symptom recognition and BSV diagnostics and there are national laboratories with diagnostic capability in Ghana, Nigeria and Uganda,
- There is a strong commitment to good field sanitation of imported IITA germplasm in quarantine plots in receiving countries,
- There will be a follow-up programme of visits by IITA scientists to monitor the germplasm in the quarantine plots in the receiving countries,
- The consignments are always sent with safety notes and in future will be accompanied by further explanatory documentation, similar to that in Appendix 3.

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