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## CURRENT KNOWLEDGE AND PRACTICES RELATED TO SEED TRANSMISSION OF SUGARCANE PATHOGENS AND MOVEMENT OF SEED

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### ABSTRACT

Sugarcane breeding programs benefit from sharing genetic resources. Traditionally, this has been accomplished by exchanging vegetative planting material of clones of interest. Diseases can be spread during this process, and quarantines were established to enable continued sharing of germplasm while minimizing the risk of pathogen introduction. The inclusion of sensitive pathogen assays in quarantine operations has greatly reduced this risk, but sugarcane quarantines are expensive and time consuming. The exchange of seed offers another means to obtain genes of interest. There has been minimal movement of seed because plant growth, crossing and selection are required to introgress new genes, and there is uncertainty about the threat of seed transmission of pathogens. A survey of different sugarcane industries was undertaken to determine the current practices and experience with seed movement and pathogen transmission. Some industries have decided that the potential benefits of seed exchange outweigh the risks of seed transmission of pathogens. Levels of precautions being taken to attempt to prevent pathogen introduction vary widely. There is currently no evidence of seed transmission of pathogens in sugarcane. Most pathogens detected have been external contaminants. The morphology of sugarcane seed facilitates external contamination by microorganisms. Research is attempting to expand knowledge about the potential for actual seed transmission by different viruses and bacteria.

## INTRODUCTION

Commercial sugarcane cultivars are interspecific hybrids of *Saccharum* species that are clonally propagated. The genomes of modern cultivars are highly polyploid, aneuploid, and have from 80-140 chromosomes (D'Hont, 2005; Grivet and Arruda, 2002; Ming et al., 1998). *Saccharum officinarum* provides approximately 80% of the chromosomes with 10-15% contributed by *S. spontaneum* and the rest resulting from recombination between the two species. Progeny from crosses between hybrids exhibit a high degree of variability, and this provides an opportunity for improvement through breeding and selection. Sugarcane germplasm has been extensively shared among industries in different regions as stalk/bud cuttings. However, true seed or “fuzz” from crosses between parents of interest offers an additional means to share genetic resources.

The movement of seed might provide a safer, more extensive and rapid alternative to vegetative material. In some cases, seed exchange might provide an opportunity to obtain genetic material from sources where it is not possible to get vegetative material. However, the utilization of new genetic resources in the form of seed requires planting and selection then crossing with current germplasm and further selection. In addition, knowledge about seed transmission of sugarcane pathogens is limited.

Many important diseases of sugarcane are caused by pathogens that are systemic within the plant. These pathogens are a risk to movement of vegetative material, and diseases were spread around the world in this way. Now, many countries have sophisticated quarantine facilities that allow continued movement of germplasm while minimizing the threat of introducing new pathogens. This process requires considerable resources and time, and space limitations limit the amount of material that can be moved. Currently, it is not known whether the different systemic pathogens can be transmitted through seed and how seed should be handled in quarantine.

There are some known fungal pathogens of the sugarcane inflorescence, primarily causing smuts and ergot (Robinson, 1964). However, seed for exchange would not be collected from obviously infected flowers. Fungi have been reported as external contaminants of seed (Wahid et al., 1988). Fungal spores and bacteria can become entrapped by the long hairs or bristles originating at the base of the glumes, and infection of the glumes might occur. In Brazil (Martins et al., 2009; Sanguino and Tokeshi, 1980), it was demonstrated that numerous fungi could be isolated from the seed surface. The leaf scald pathogen, *Xanthomonas albilineans*, is a bacterial pathogen that was demonstrated to be externally seed-borne (Duttamajumder, 1990). Seedling blights and damping-off are common problems in the initial seed germination stage of cultivar selection programs (Byther and Steiner, 1972); however, it is not certain whether the pathogens were associated with the seed prior to planting.

Viruses and some bacterial and fungal pathogens systemically distributed within the sugarcane plant could be the greatest threat to accomplish actual seed transmission. There is no direct evidence that any systemic pathogen is seed transmitted in sugarcane, but research is lacking.

There is no evidence for seed transmission of *Sugarcane mosaic virus* (SCMV) and *Sorghum mosaic virus* (SrMV) in sugarcane. However, seed transmission has been reported for some potyvirus strains related to SCMV and SrMV. In maize (*Zea mays* L.), very low potyvirus transmission rates were detected, ranging from one or two out of 10,000 to 30,000 seedlings for *Maize dwarf mosaic virus* (MDMV) (Hill et al., 1974; Mikel et al., 1984; Williams et al., 1968), 14 of 22,925 seedlings (Zhu et al., 1983) and 17 of 9,485 seedlings (Shepard and Holdeman, 1965) for *Sugarcane mosaic virus*-MDB. Another example of a low potyvirus transmission rate through seed was 0.047% transmission for *Zucchini yellow mosaic virus* in squash (Schrijnwerkers et al., 1991). In the case of potexviruses and machlomoviruses, 0.026% transmission to seedlings from tomato plants infected by *Pepino mosaic virus* (Hansen et al., 2010) and 21 of over 42,000 (0.05%) seed for *Maize chlorotic mottle virus* (Jensen et al., 1991) were reported, respectively.

The low incidence of seed transmission of some viruses has been associated with low production of viable seed by infected plants. For example, few viable seed are produced from *Zucchini yellow mosaic*-infected plants of field pumpkin (*Cucurbita pepo* L.) (Desbiez and Lecoq, 1997). Also, detection of virus in seed may not be a good indicator of the probability of seed transmission. Viruses located in tissues outside the embryo rarely survive seed maturation, and viruses located in the embryo may be inactivated during seed development, maturation or storage (Johansen et al., 1994). Even though many virus-infected plants produce few viable seed or transmission rates are low, even a small number of virus-infected seedlings could result in the introduction of exotic virus into new geographical areas.

Another viral disease of sugarcane is yellow leaf caused by the polerovirus *Sugarcane yellow leaf virus* (SCYLV) (Vega et al., 1997). There is currently no evidence for seed transmission within the Luteoviridae (Mink, 1993) including the seed transmission of *Barley yellow dwarf virus* which is closely related to SCYLV.

Contamination of seed, particularly the glumes, is a common means of seed transmission for bacteria. Actual seed infection has been reported in other crops for some species of *Xanthomonas* and *Acidovorax* (Elango and Lozano, 1980; Lessl et al., 2007; Walcott et al., 2003). Detection of phytoplasma in seed of lime (*Citrus* spp.), tomato (*Lycopersicon esculentum* Mill.), alfalfa (*Medicago sativa* L.), and coconut (*Cocos nucifera* L.) has suggested the possibility of seed transmission for this type of pathogen; however, no experimental results have been published demonstrating transmission to seedlings (Bertaccini and Duduk, 2009; Cordova et al., 2003).

Fungi causing smut, *Sporisorium scitamineum*, and downy mildews, *Peronosclerospora sacchari* and *P. philippinensis*, are systemic within the plant. Seed transmission of the downy mildew pathogens has been demonstrated in corn; however, no transmission was detected when seed were fully dried (Bonde, 1982; Smith and Renfro, 1999).

A survey was conducted among sugarcane pathologists in different countries to determine any unpublished information concerning seed transmission of pathogens in sugarcane, exotic pathogens representing the greatest threat, the extent of movement of seed and practices

associated with it. The survey was sent to pathologists in 16 countries, and responses were received from eleven.

## SURVEY RESULTS

The survey was devised to determine actual experiences with seed transmission of pathogens; whether the transmission was internal in the seed or by surface contamination; whether movement of seed is allowed and precautions taken against seed transmission; how long and extensively seed has been imported; what pathogens represent the greatest quarantine risk; and whether plants infected with these pathogens successfully flower and set seed. The survey questions and a summary of responses follow.

Question 1. Are you aware of any seed-borne pathogens of sugarcane from personal experience?  
Question 2. Were the pathogens internally borne or surface contaminants?

Responses to Questions 1 and 2 combined (by country):

- Brazil: Multiple fungi have been detected on the exterior of seed. In one study, species of *Fusarium*, *Helminthosporium*, *Monilia*, *Pestalotia*, *Phoma*, and *Pithomyces* were detected. In another study, *Bipolaris*, *Cladosporium*, *Curvularia*, *Fusarium*, and *Phoma* were isolated. Some fungi were detected infecting the caryopsis.
- Colombia: Fungi including *Curvularia*, *Fusarium*, and *Helminthosporium* spp. were detected superficially on the seed. Therefore, a sodium hypochlorite treatment was employed.
- India: *Xanthomonas albilineans* was detected externally on seed.
- Mauritius: Seedlings were attacked by damping-off pathogens including *Curvularia* and *Helminthosporium* spp.; however, it was not determined whether the fungi came from the seed.
- Pakistan: Species of *Alternaria*, *Cladosporium*, *Curvularia*, *Dreschlera*, *Fusarium*, *Epicoccum*, *Nigrospora*, and *Phomopsis* were detected on the seed exterior.

Question 3. Do you import seed from foreign sources; if so, what precautions are taken?

Question 4. How long have you been importing seed?

Responses to Questions 3 and 4 combined:

- Australia: Multiple importations during last 10 years with the following precautions.
  1. Pre-shipment:
    - a. De-fuzz seed and treat with broad-spectrum fungicide.
    - b. Pack seed in new, properly labeled containers.
    - c. Issue phytosanitary certificate with treatment details and specifying that “the seed has been sourced from an area where sugarcane Sereh disease, sugarcane spike disease, sugarcane green grassy shoot phytoplasma, Western x-disease phytoplasma, and *Anguina spermophaga* are not known.”
  2. Post-shipment quarantine:
    - a. Seed inspection for freedom from live insects, soil, prohibited seeds and other plant materials; treat if necessary.
    - b. Treatment of seed in mesh bags by immersion in sodium hypochlorite solution (0.5-1% available Cl) for 30 minutes, followed by several rinses

in water over a period of 10 minutes under supervision at a quarantine facility.

- c. Seed forwarded for growth in closed quarantine facility.
  - d. Grow plants for one growth cycle or 3 months, whichever is longer.
  - e. Inspect plants during quarantine at seedling emergence and at least three other times.
  - f. Test plants for SCMV by PCR or other appropriate method.
  - g. Grow plants in open quarantine for one growth cycle during which plants must be inspected four times.
  - h. In the event an exotic pest or disease is detected, contact quarantine officer.
  - i. Plants found to be free of diseases may be released from quarantine.
  - j. After harvest, properly dispose of all residues, all derivatives, and all materials that came in contact with the imported material.
- Barbados: Regular exportation of seed for over 70 years to countries including Guyana, Dominican Republic, Jamaica, Belize, Trinidad, Guadeloupe, Guatemala, Panama, Costa Rica, Brazil, Senegal, Sudan, Mauritius, South Africa, and Tanzania. Fuzz is dried and sealed in foil packages and dusted with insecticide and fungicide as required by the recipient country. No reports of disease problems in the resulting seedlings have been made following germination.
  - China: No importation of seed.
  - Colombia: Yearly importation from Mexico for more than 10 years; approximately 500 g. Precautions taken:
    1. De-fuzz seed.
    2. Dry seed treatment with carboxin fungicide.
    3. Visual inspection.
    4. Sowing of insect-free seed in sterile medium in an insect-free facility.
    5. Observation for at least 1 month.
    6. Transfer of plants to an open area for at least 3 months.
    7. Rouging of infected plants.
    8. Transfer of healthy plants to the field.
  - France: CIRAD (Centre de coopération internationale en recherche agronomique pour le développement) quarantine does not coordinate any seed movement.
  - Guatemala: Irregular, infrequent importations since 1994; no precautions are taken.
  - India: No importation of seed.
  - Mauritius: One introduction in 2008 of 34 g of seed. Precautions were pre-treatment with broad-spectrum fungicide and sowing of seed in a confined glasshouse for 3 months.
  - Pakistan: Regular seed importation from multiple sources since 1984 and domestic movement. Precautions taken:
    1. Inspection by quarantine officer to verify free of injurious pests and diseases, including mosaic, Fiji leaf gall, Sereh, pineapple disease, gumming, ratoon stunt, and grassy shoot.
    2. Fumigation prior to storage at 4 C.
    3. Fungicide treatment (trifloxystrobin and tebuconazole) for 5 minutes at sowing.
    4. Spray seedlings with fungicides (mefenoxam and chlorothalonil) at 10 day interval.

- 5. Fungicide treatment (trifloxystrobin and tebuconazole) during transplanting.
- 6. Post-entry quarantine.
- Philippines: No importation of seed.

Question 5. Do plants infected with systemic pathogens successfully flower and set seed?

- Australia: In Australia, Fiji leaf gall disease-infected plants of susceptible cultivars are severely stunted and unlikely to flower. Tolerant cultivars might flower but will not produce viable pollen. Successful flowering and seed set possible for Fiji-infected plants in Papua New Guinea and Fiji.
- Colombia: Plants infected with SCMV, SCYLV, *Sugarcane bacilliform virus* (ScBV), *Leifsonia xyli* subsp. *xyli*, and *Xanthomonas albilineans* do flower and set seed.
- Guatemala: Plants infected with *Xanthomonas albilineans* and SCMV can flower and set seed.
- India: Virus infected plants do flower with less intensity.
- Mauritius: Plants infected with SCYLV, *Xanthomonas axonopodis* pv. *vasculorum*, *Leifsonia xyli* subsp. *xyli*, and *Xanthomonas albilineans* do flower and set seed.
- Philippines: Downy mildew infected plants can flower and set seed under favorable environmental conditions.
- United States:
  1. Florida: Plants infected with SCYLV, and *Leifsonia xyli* subsp. *xyli* flower and set seed.
  2. Louisiana: SrMV-infected plants will flower and set seed. Plants infected by *Xanthomonas albilineans* occasionally flower, but few viable seed are produced.

Question 6. What systemic pathogen in your country would present the greatest quarantine risk?

- Australia: FDV represents a quarantine threat to other countries. SCMV strain A is a threat within portions of the country.
- Barbados: *Sporisorium scitamineum*, *Leifsonia xyli* subsp. *xyli*, and *Xanthomonas albilineans*.
- Colombia: Viruses including SCMV, SCYLV, ScBV and *Leifsonia xyli* subsp. *xyli*, and *Xanthomonas albilineans*.
- Guatemala: *Xanthomonas albilineans* and SCMV.
- India: Viruses including ScBV, SCMV, *Sugarcane streak mosaic virus*, and SCYLV and phytoplasmas.
- Mauritius: *Fiji disease virus*, *Xanthomonas axonopodis* pv. *vasculorum*, *Xanthomonas albilineans*, *Sporisorium scitamineum*, *Leifsonia xyli* subsp. *xyli*, and SCYLV.
- Pakistan: *Fiji disease virus*, *Xanthomonas axonopodis* pv. *vasculorum*, *Leifsonia xyli* subsp. *xyli*, grassy shoot/white leaf phytoplasmas, and *Peronosclerospora sacchari*.
- Philippines: *Peronosclerospora sacchari* and *Sporisorium scitamineum*.
- United States: SCYLV, *Leifsonia xyli* subsp. *xyli*, and *Xanthomonas albilineans*.

Question 7. What systemic pathogens in other countries represent the greatest quarantine risk during germplasm importation?

- Australia: *Peronosclerospora sacchari*, *Ramu stunt virus*, and *Sugarcane streak mosaic virus*.

- Barbados: *Sugarcane mosaic virus* and other viruses.
- Brazil: *Fiji disease virus*.
- Colombia: *Fiji disease virus*.
- Guatemala: *Fiji disease virus*, *Peronosclerospora sacchari*, and grassy shoot phytoplasma.
- India: *Fiji disease virus*, *Xanthomonas axonopodis* pv. *vasculorum*, white leaf phytoplasma, Sereh, *Peronosclerospora sacchari*, *Herbaspirillum rubrisubalbicans*, *Sugarcane streak virus*, and *Sugarcane mild mosaic virus*.
- Mauritius: *Sugarcane mosaic virus*, *Sugarcane streak mosaic virus*, *Fiji disease virus*, downy mildew, white leaf phytoplasma, grassy shoot phytoplasma, and green grassy shoot phytoplasma.
- Pakistan: *Colletotrichum falcatum* and *Sporisorium scitamineum*.
- Philippines: Grassy shoot and white leaf phytoplasmas, *Fiji disease virus*, and *Xanthomonas albilineans*.
- United States: *Fiji disease virus*, *Sugarcane streak mosaic virus*, white leaf phytoplasma, grassy shoot phytoplasma and other phytoplasma infecting sugarcane, *Sugarcane streak virus* and other Mastreviruses, *Ramu stunt virus*, *Sugarcane striate mosaic virus*, and downy mildew.

## CONCLUSIONS

The survey results provide an improved framework for considering the risks associated with the exchange of seed. Experience and practices related to the movement of seed vary considerably among the industries/countries that allow its importation. The level of precautions taken to prevent possible seed transmission of pathogens varies from those requiring multiple years in quarantine and isolation along with pathogen testing to requiring no precautions. There has been no report of the introduction of a new systemic pathogen into a country linked to seed transmission. Some sugarcane industries without well developed breeding programs have relied on receiving seed produced by other programs. Most notably, the breeding program at Barbados has produced seed and shipped it to other industries for many years without incident. The overall perceived risk of importing seed is less than for importing vegetative material.

Decontamination of fuzz should be a regular part of any movement of seed. In almost all cases, seed is de-fuzzed to ease handling and treated with sodium hypochlorite and/or fungicides to eliminate microorganisms contaminating the seed exterior. Fumigation also has been successfully employed with fuzz. If possible, a quarantine step to allow observation of seedlings for exotic disease symptoms has been included as a precaution. However, the large numbers of plants associated with using seed as a means of sharing germplasm can make this process difficult. Consensus methods for seed movement were developed previously (Frison and Putter, 1993) that consisted of defuzzing and applying fungicide prior to shipment, visual inspection upon arrival, growth in a screened greenhouse for at least 3 months, followed by transplanting to an isolated field location for one growth cycle. Applying diagnostic tests would require bulking of seedlings, and guidelines based on research are not currently available for any pathogen.

The failure of plants infected with some systemic pathogens to flower or produce viable seed has been cited as a factor that reduces the risk of pathogen transmission. This is



undoubtedly so, but it is not uniform across all pathogens. In cases where infected plants are known to flower and set seed, it would be desirable to have research results related to the potential for seed transmission.

Research on actual seed transmission of different pathogens is lacking partly because of the difficulty in proving a negative but also because of the lack of proper controls, the sensitivity of pathogen detection methods that would affect sample size are unknown when applied to seed, and the difficulty associated with preventing natural infection of seedlings from occurring. Despite these obstacles, research is in progress with multiple pathogens of importance to attempt to detect seed transmission. The failure to detect transmission may not provide proof that it does not occur, but it will increase confidence that the risk associated with seed importation is low. In the event of positive detection of transmission of one or more pathogens, it will have to be evaluated for the degree of threat posed. However, even if a seed-transmitted pathogen is found, research can develop protocols to assay young seedlings for that specific pathogen. These protocols can be used to release only seedlings that tested pathogen-free for transplanting to the field.

There is no doubt that seed represents a valuable genetic resource and sharing it among industries could positively impact the ability of breeding programs to continue to develop improved cultivars. Each industry will have to make a determination whether the potential benefits of sharing seed will outweigh any risk of pathogen introduction. A determination of acceptable risk will be coupled with and affected by the development of regulations to regulate seed movement based on feasible precautions and testing protocols.

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