Assessing Efficiency of Different Methodologies in Sweetpotato Sample Processing and Virus Detection

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Why?

- Collection of good samples and proper preservation is an important step in extraction of nucleic acids from plants
- Field studies require transportation of samples over long distances
  - Can result in degradation and reduction of purity and quantity of nucleic acids if samples are not properly preserved
- Degraded nucleic acids can compromise the success of intended analyses e.g. applications that require high molecular weight DNA

Some methods for SP sample processing

- **Leaf samples**

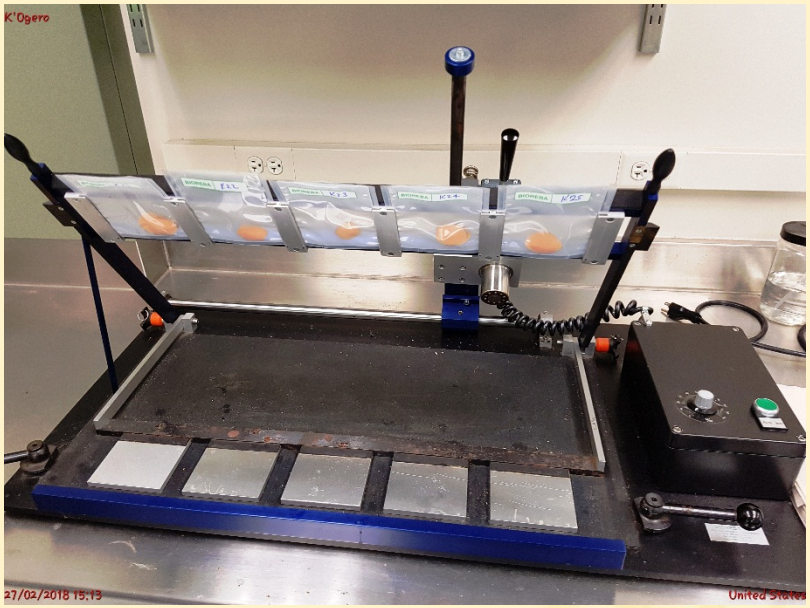
a) Freezing methods: liquid nitrogen and dry ice

b) Desiccants: silica gel and blotter paper

c) Preservatives: CTAB, ethanol, and isopropanol

Freezing in liquid N and drying in silica gel are the most common

- **Root samples**
  - Homogenizing in a Bioreba Homex 9 machine



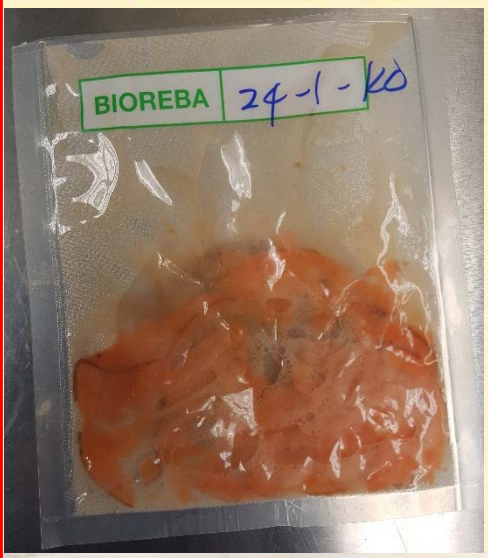
What we did

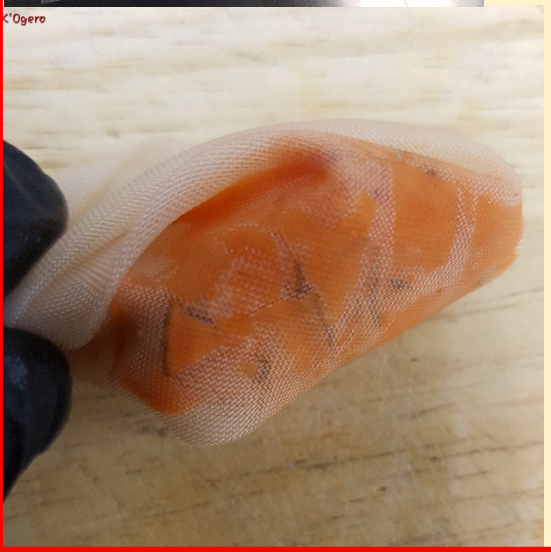
a) Assessed efficiency of silica gel & liquid N in preserving leaf samples for potyvirus (SPFMV, SPVC, SPVG & SPV2) detection via multiplex PCR

b) Compared use of normal PCR and qPCR in testing mericlones for sweet potato leaf curl virus (SPLCV)

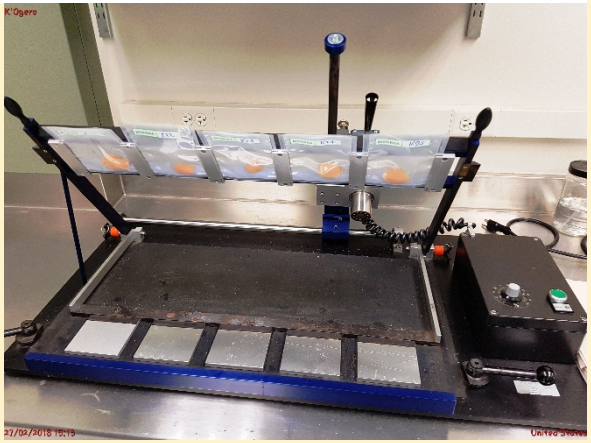
c) Assessed efficiency of four methods in processing sweetpotato storage roots for nucleic acid extraction and potyvirus-testing via PCR

A. Methods evaluated for processing fresh roots

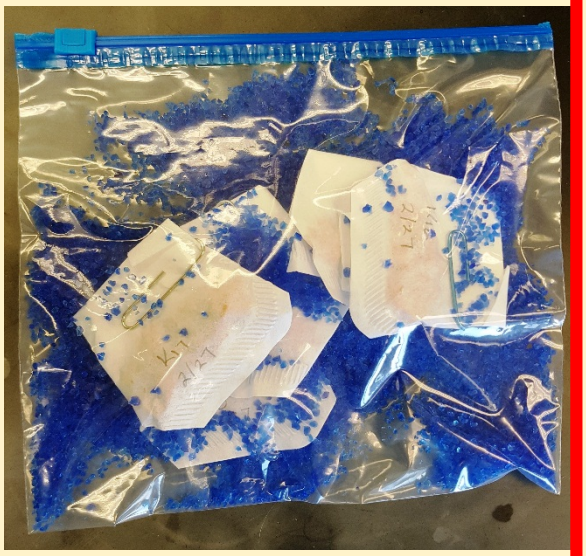


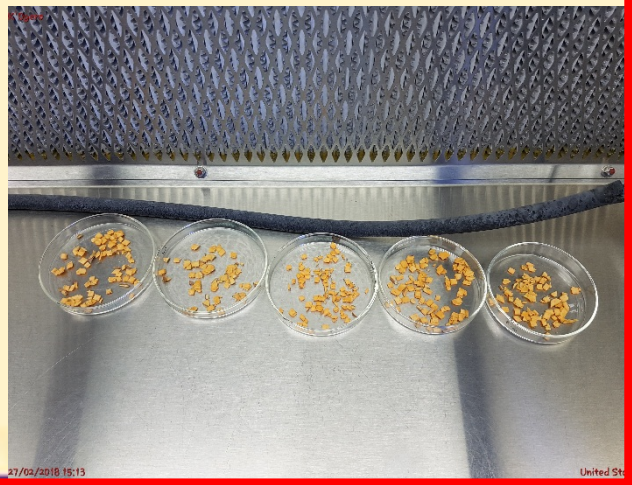


C. Control



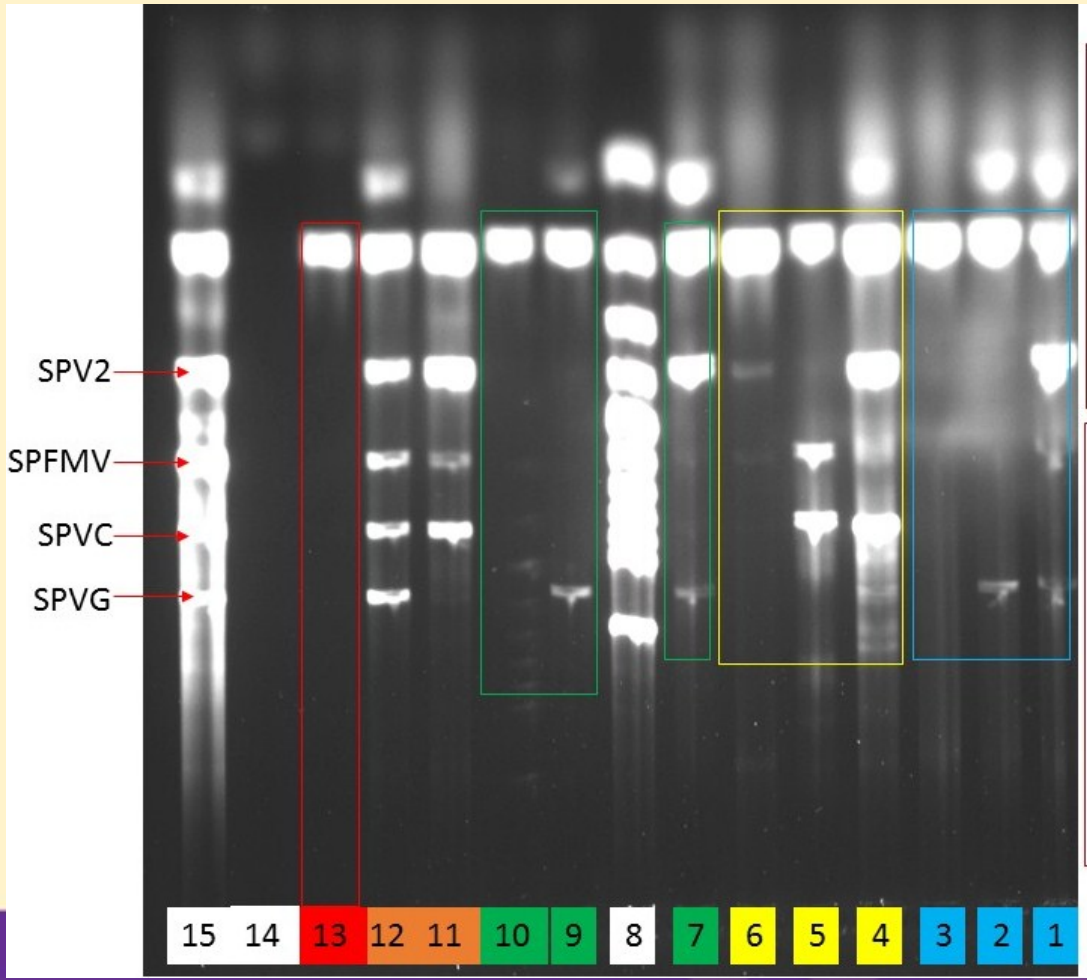
B. Methods evaluated for drying SP root samples





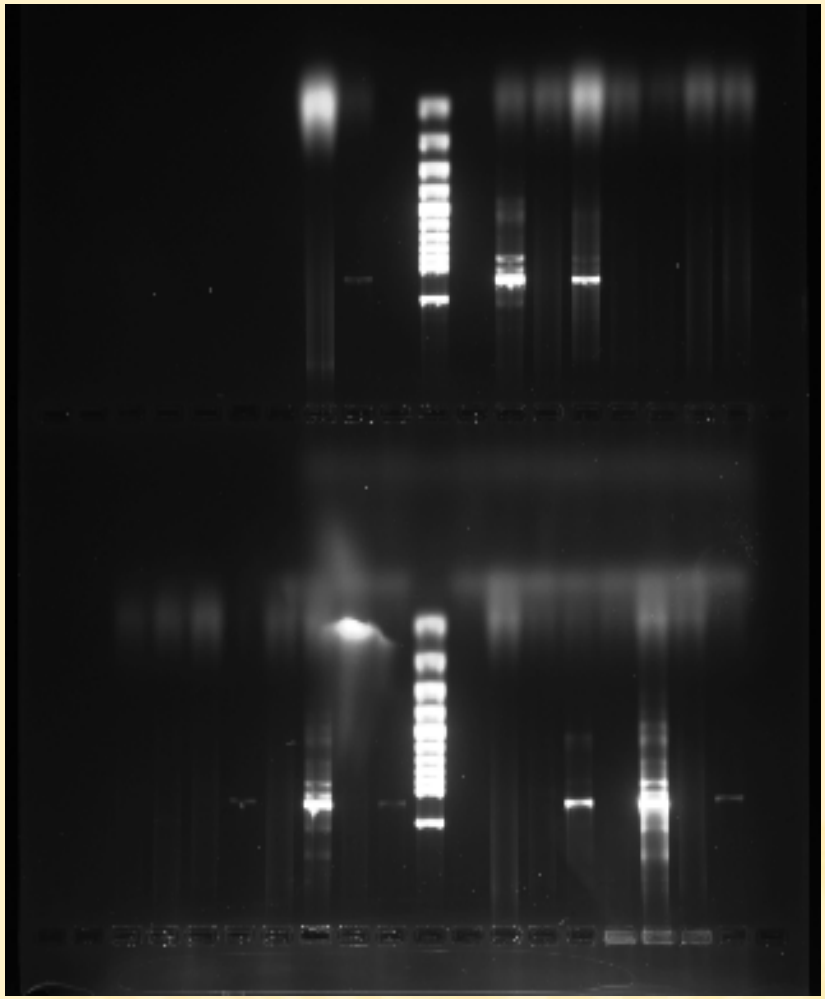
What we learnt

a) Detection of the potyviruses (SPFMV, SPVC, SPVC & SPV2) via multiplex PCR on leaf samples frozen in liquid N and those dried in silica gel not different.



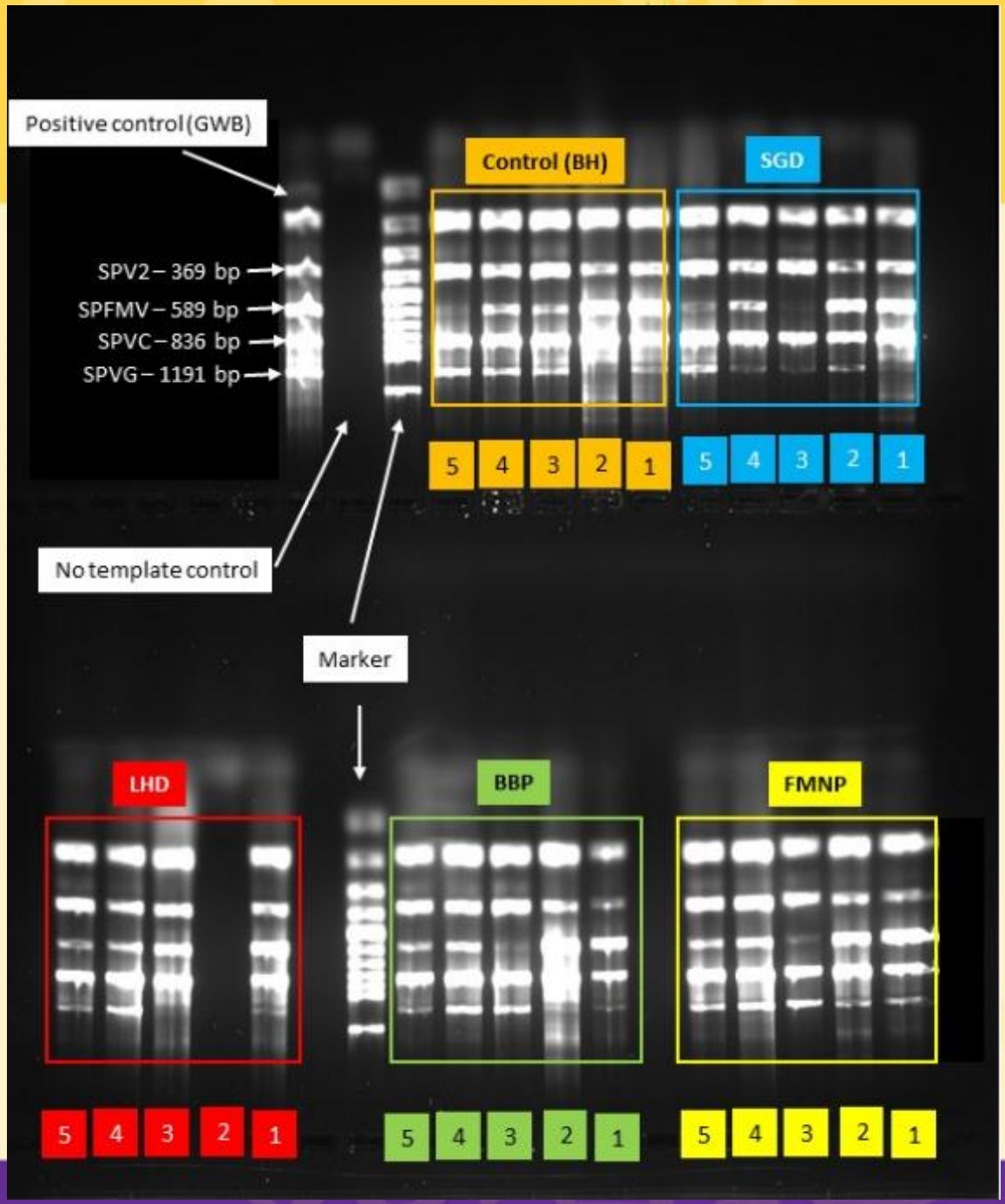
b) SPLCV- testing via normal PCR can result in false negatives.

- *Only 5 of the 26 samples tested turned positive when normal PCR was used*
- *Verification via qPCR showed that 21 samples were infected with SPLCV*
- *Previous work has shown that the internal standard amplification may compete or interfere with the geminivirus amplication*

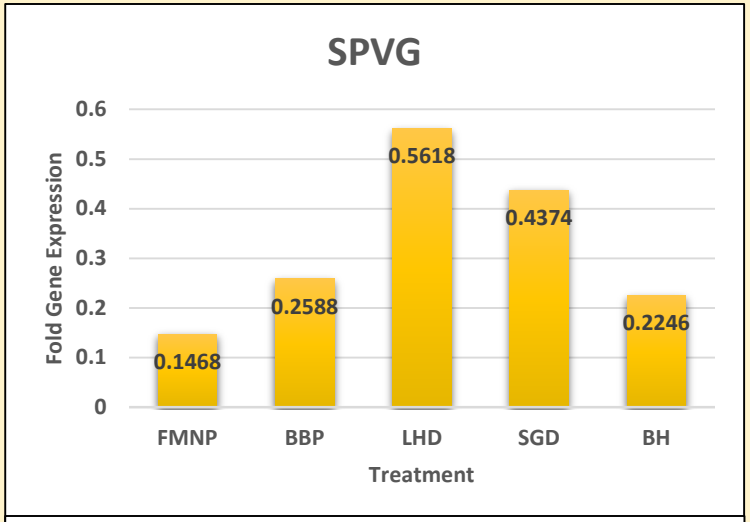


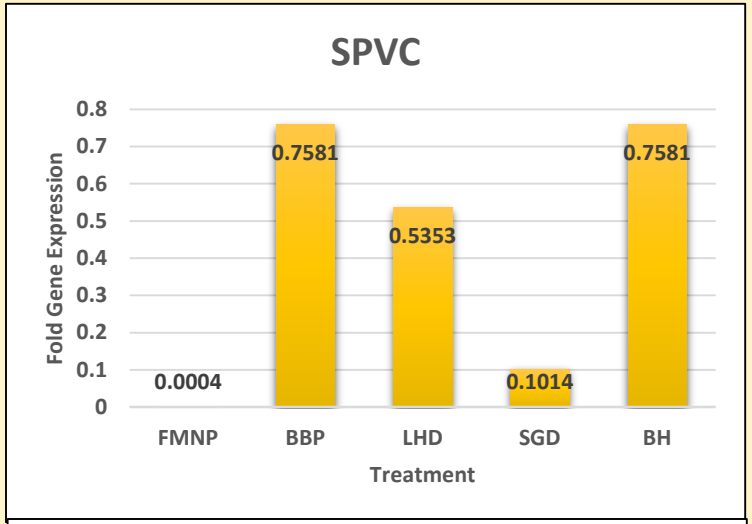
c) Choice of any of the four methods evaluated for processing storage root samples will depend on ease and convenience of use

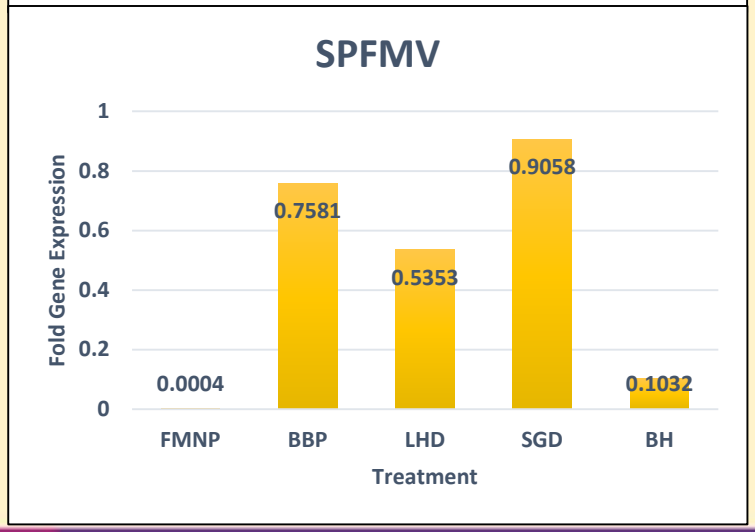
- There were no significant differences in the quality of nucleic acids extracted
- Detection of potyviruses through multiplex PCR was similar

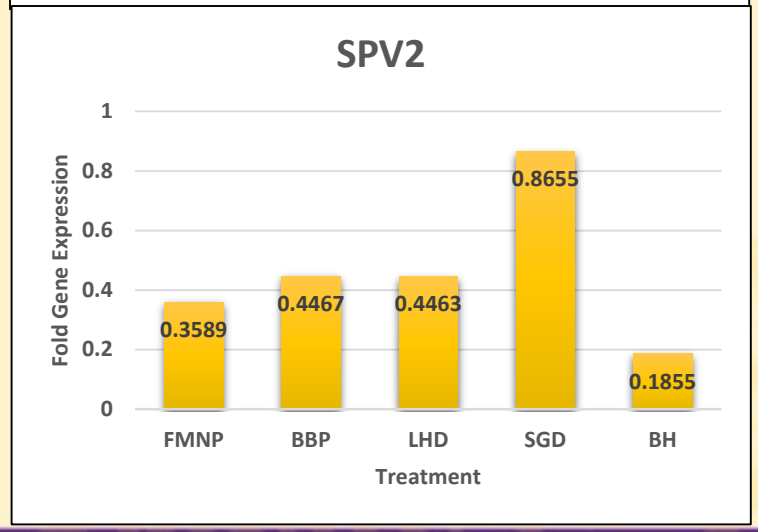


- Quantification via qPCR showed no significant differences in the virus titers between the different treatments
- Titers of the various viruses varied with treatments



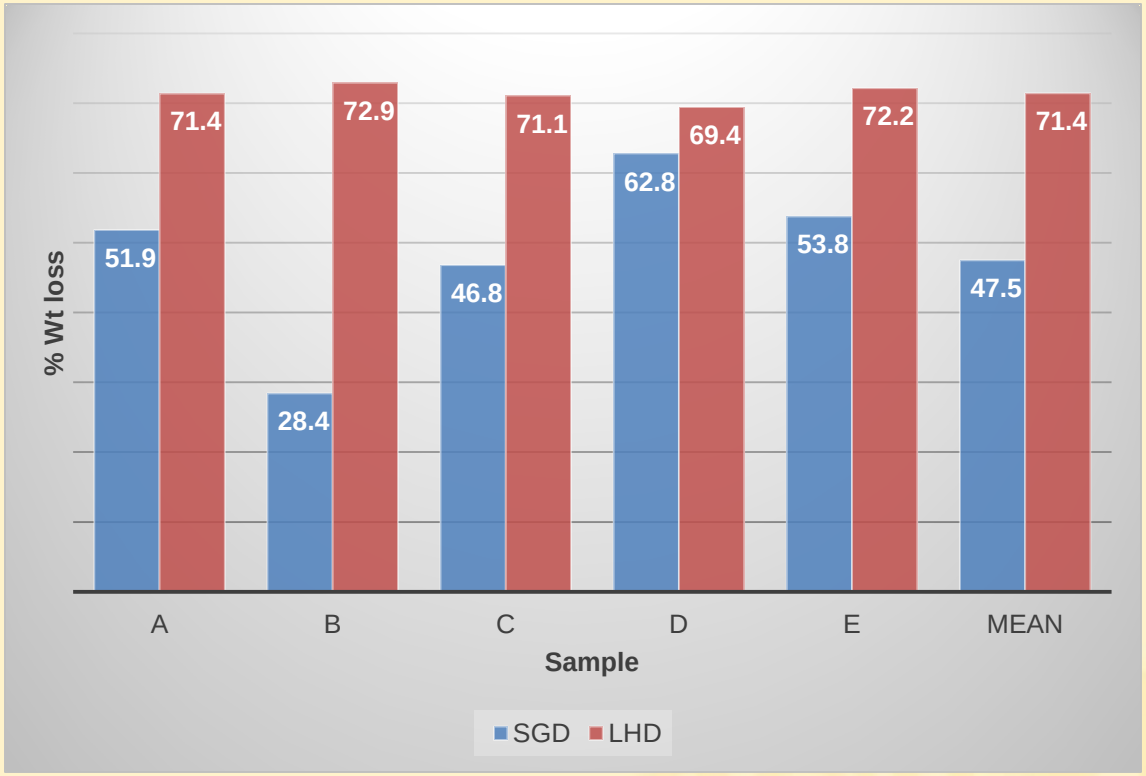






d) Air-drying root samples in a laminar hood was better than drying in silica gel

- Root samples air-dried in the laminar hood lost an average of 23.9% more weight compared to those dried in silica gel



- Samples air-dried in the laminar hood were crispier and easier to grind into fine powder as compared to those dried in silica gel

A. Samples dried in silica gel

A. Samples air-dried in a laminar hood

Conclusions

a) If properly used, silica gel can efficiently preserve leaf samples where liquid N is not available

b) Normal PCR should be used with caution when testing for SPLCV due to the possible false negatives.

c) It is possible to use root samples for SP virus-testing even when there is no Bioreba Homex 6 homogenizer

- Several methods of sample processing are available depending on ease and convenience.

Acknowledgements

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