Investigations for confirmation of country freedom from certain quarantine significant pests in Sri Lanka

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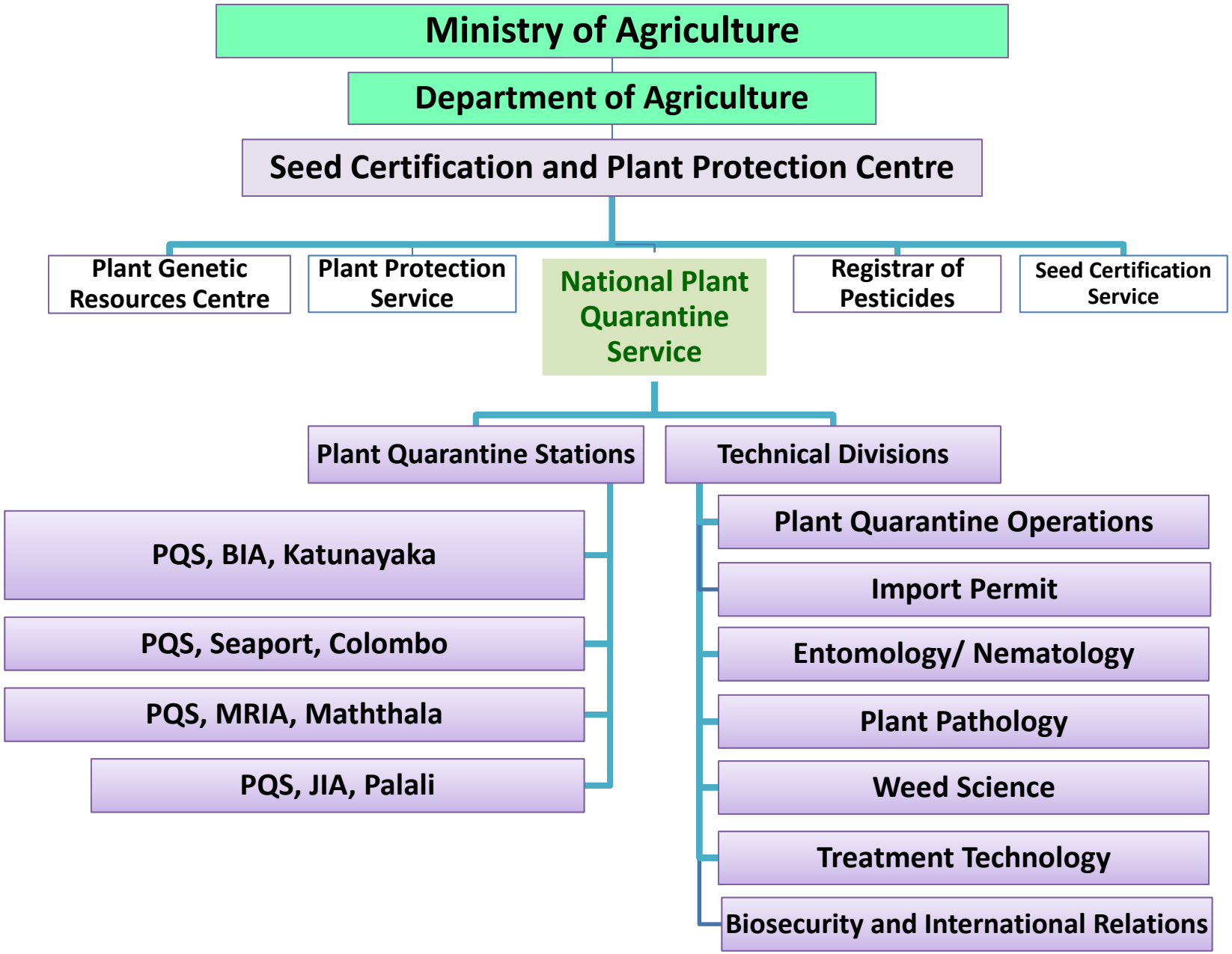
National Plant Quarantine Service (NPQS)
Sri Lanka

Organogram


Ministry of Agriculture
Department of Agriculture
Seed Certification and Plant Protection Centre
Plant Genetic Resources Centre
Plant Protection Service
National Plant Quarantine Service
Registrar of Pesticides
Seed Certification Service
Plant Quarantine Stations
Technical Divisions
PQS, BIA, Katunayaka
PQS, Seaport, Colombo
PQS, MRIA, Maththala
PQS, JIA, Palali
Plant Quarantine Operations
Import Permit
Entomology/ Nematology
Plant Pathology
Weed Science
Treatment Technology
Biosecurity and International Relations

Vision

- Facilitate the International movement of healthy plants and plant products for the development of national agriculture and related industries

Objectives

- Prevention of introduction, establishment and spread of dangerous alien pests within the country
- Involvement in domestic pest control programmes
- Development of treatment technologies to eradicate pests of quarantine importance
- Promotion of export of healthy plants and plant products

Mandate

- Responsibility of enforcing and implementation of **Plant Protection Act No.35 of 1999** and Regulations made there under in relation to plant quarantine activities. It also conducts research and development activities in plant quarantine aspects

Recent concerns in trade of plants and plant products to EU from Sri Lanka

- EU banned the imports fresh curry leaves from third countries due the citrus greening disease as per Commission Implementing Directive 2014/78/EU of 17 June 2014
- EU restricted importation of *Xylella fastidiosa* host plants from third countries until country freedom is assured as per Commission Implementing Regulation (EU) 2020/1201 of 14 August 2020

Investigating the presence of *Candidatus* Liberibacter *asiaticus* CLas) in *Murraya koenigii*

Significance of Citrus Greening (CG) = Huanglongbing (HLB)

- The most serious citrus diseases in the world
- Caused by non-culturable fastidious phloem limited bacterium know as *Candidatus* Liberibacter *asiaticus* (CLas)
- **Symptoms**: Yellow shoots, blotchy mottling and chlorosis, reduced foliage and tip dieback of citrus plants, eventually declining and die
- CG is widespread in Asia, Africa, and the Saudi Arabian Peninsula
- DNA hybridization studies revealed the presence of CLas in Sri Lanka (Nakashima *et al*, 1998)

Transmission of CG

- It can be transmitted by
  - Asian Citrus Psyllid-ACP (*Diaphorina citri*)
  - African Citrus Psyllid (*Trioza erytreae*)
  - Grafting
  - Dodder
  - Possibly by seed
- ACP is harmful both as the insect vector of the CG and as a significant citrus pest in its own
- Identification of ACP
  - 45^0C feeding angle of adults
  - Full of eggs and nymphs on new flushes
  - Lot of excreted wax like substances
  - Notched leaves
- Alternate hosts of ACP
  - *Murraya koenigii*
  - *Murraya paniculata*
  - *Calamondins*
  - Orange jasmine (Chinese box) – *Murrya exotica*
- Curry leaves are exported to EU as spice crop

Hosts of ACP

Curry leaves *(Murraya koenigii)*

Murraya paniculata

Calamondins

Orange jasmine (Chinese box)
Murrya exotica

Transmission of CG

- Due to the serious and destructive nature of citrus greening disease, EU has restricted the movement of plants that are hosts of CG and ACP
- Host plants for both CG and ACP are considered regulated articles in USA
- In 2015, EU banned importing of fresh curry leaves from third countries due the citrus greening disease
- Plants that are host of ACP but not host of CG be accepted in USA a compliance agreement

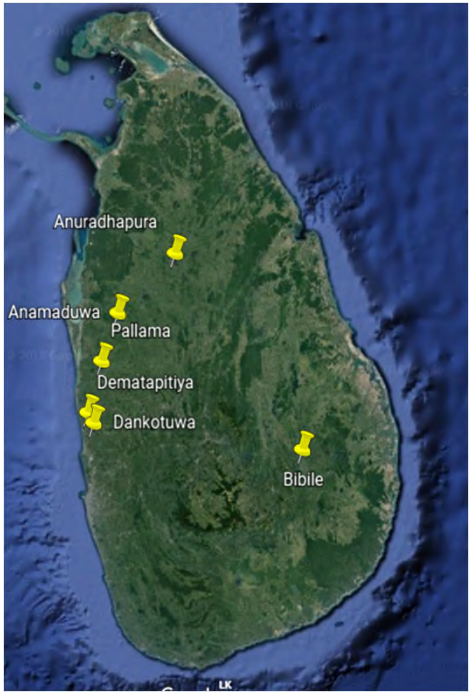
Investigation of presence of CLas in *Murraya koenigii*

Objectives

- To investigate the presence of *Candidatus* liberibacter *asiaticus* (CLas) in *Citrus* spp and *Murraya Koenigii* from selected areas in Sri Lanka
- To confirm of the absence of Clas) in *Murraya koenigii*

Methodology

- 66 samples of citrus (35) and curry leaves (31) were collected from 6 locations of major citrus growing area
- DNA extracted from each sample were tested by PCR using specific primers which amplified the 1160 bp fragment of 16S rRNA gene of CLas {EPPO Diagnostic Protocols -PM 7/121 (1) }



Investigation of presence of CLas in Murraya koenigii

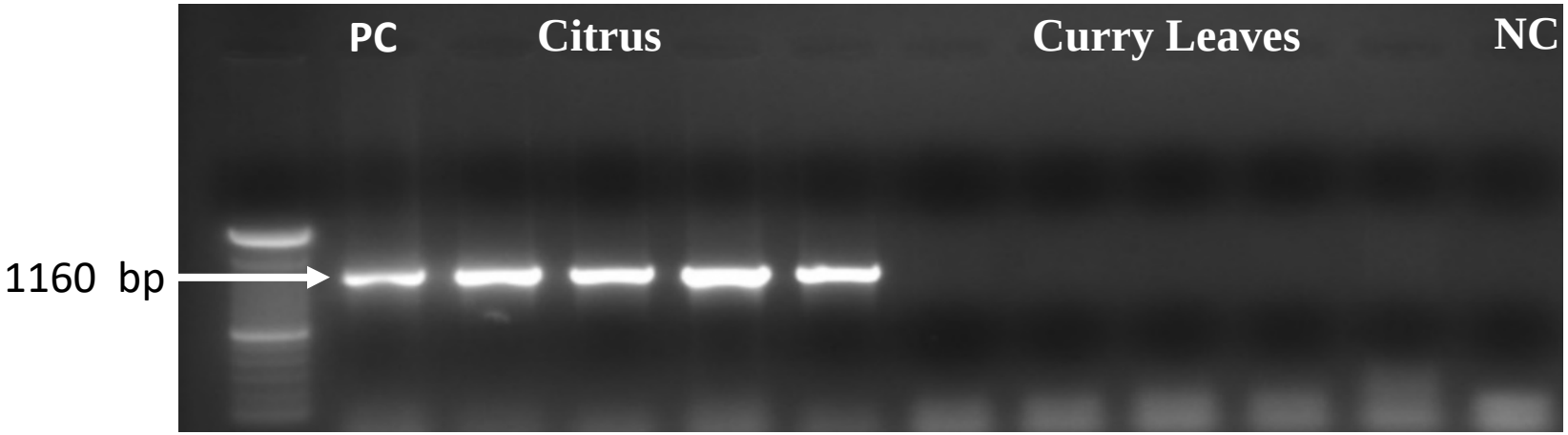
…Methodology

- Viruliferous ACP were introduced into health citrus and curry leaves (10 from each) plants in a insect proof cage
- After I month of inoculation, DNA from each plant were tested for presence of CLas

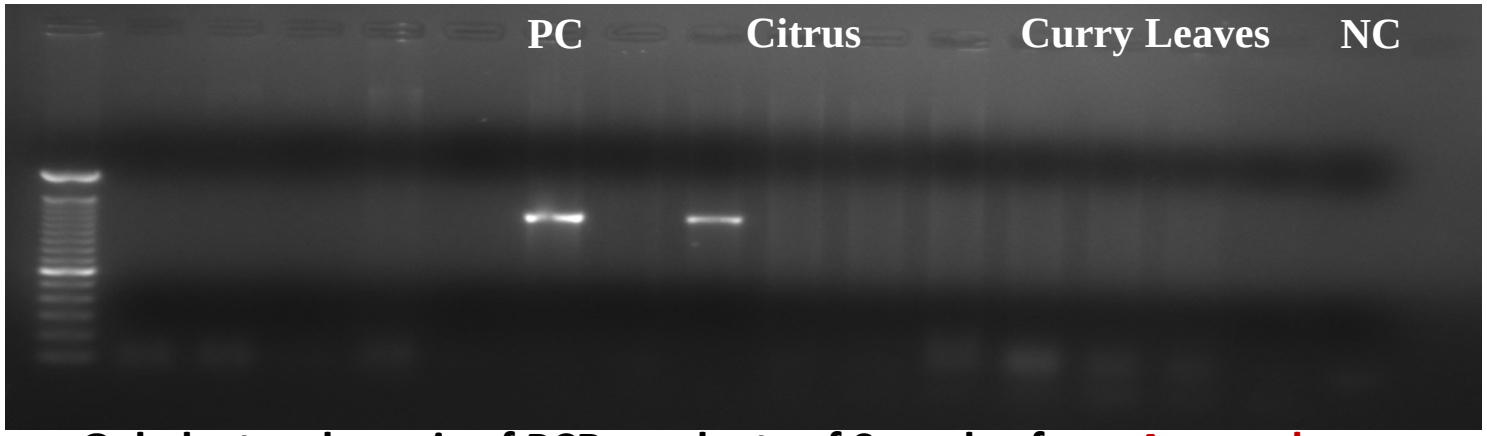
Healthy citrus and *Murraya* plants were inoculated with viruliferous CLas in a insect proof cage

Results and discussion

- Out of 35 samples of citrus, 15 were found infected with CLas where as all 31 samples of *Murraya* found uninfected with CLas



Gel electrophoresis of PCR products of Samples from Pallama. Infected samples showed amplification of 1160 bp fragment



Gel electrophoresis of PCR products of Samples from Anamaduwa. Infected samples showed amplification of 1160 bp fragment

…Results and discussion

Summery of the PCR results of artificially innoculated citrus and *Murraya* plants using viruliferous CLas

Gel image number	Plant numbers	Results
2.1	M 01	Negative
2.2	M 02	Negative
2.3	M 03	Negative
2.4	M 04	Negative
2.5	M 05	Negative
2.6	M 06	Negative
2.7	M 07	Negative
2.8	M 08	Negative
2.9	M 09	Negative
2.10	M 10	Negative
2.11	M 11 (control)	Negative

Gel image number	Plant number	Results
2.12	C 01	Negative
2.13	C 02	Negative
2.14	C 03	Negative
2.15	C 04	Negative
2.16	C 05	Negative
2.17	C 06	Negative
2.18	C 07	Negative
2.19	C 08	Negative
2.20	C 09	Positive
2.21	C 10	Negative
2.22	C 11 (control)	Negative

- Only one citrus plant artificially inoculated was found positive for CLas where as no single plant of *Murraya* found positive
- It was generally found that ACP was shown more attraction towards *Murraya* plants

…Results and discussion

- Beloti *et al*, 2018 could not observe the successful transmission of of CLas to *Murraya* by ACP and it was treated as immune to CLas
- Curry leaves plants are proved to be more attractive to the ACP than citrus plant (Beloti et al, 2017)
- Curry leaves is not a host of CLas, but is good host for the ACP insect vector (Teck *et al*, 2011 and Westbrook *et al*, 2011)
- The presence of CLas in leaf samples of citrus were identified by amplification of 1160 bp fragment of 16S rRNA (Athukorala *et al*, 2020)

Conclusion

- CLas is in association with citrus plants in Sri Lanka. Thus country is not free from CLas.
- *Murraya* is not a host of CLas though it is a good host of ACP. Thus, *Murraya* is treated as immune to CLas
- There is a potential to export fresh curry leaves due to the absence of CLas in curry leaves under a special compliance agreement with EU

Confirmation of country freedom from *Xylella fastidiosa* in Sri Lanka

Significance of *Xylella fastidiosa (Xf)*

- Xylella fastidiosa is a Union quarantine pest that is known to occur in the EU. It is regulated in the EU as a harmful organism under Plant Health Regulation (EU) 2016/2031
- Xf lives in the xylem vessels of plants. The xylem vessels can become blocked by biofilms of the bacteria exudates leading to leaf scorch diseases characterized by the desiccation of leaves and dieback
- Infections may be asymptomatic but can produce a range of disease symptoms from minor leaf scorch through to extensive die-back and plant death
- Has a wide range of potential intraspecific hosts including over 220 species which can be infected naturally
- Target host species more likely to be infected with several subspecies of *Xylella fastidiosa*
  - Prunus dulcis, Prunus avium, Polygala myrtifolia, Spartium junceum, Nerium oleander, Rhamnus alaternus and Rosmarinus officinalis

Vectors of Xylella fastidiosa

- natural spread of the bacterium is exclusively through xylem-feeding insect, Philaenus spumarius
- The most likely pathway for introduction of Xf is the importation of plants for planting and / or infected insects (vectors) originating from areas where the pest is present
- Following detection of Xf outbreaks in Europe, the EU has implemented several risk reduction options to combat this plant disease and prevent its entry and spread
- Xf is quarantine listing pest in EU thus all hosts are prohibited to import into EU
- All nonEuropean Cicadellidae (leafhoppers) that are known to be vectors of Xf such as Carneocephala fulgida, Draeculacephala minerva and Graphocephala atropunctata are also regulated in EU

Hosts of Xf

Plum leaf scald

Cherry leaf scorch

Polygala leaf tip burn

Coffee leaf scorch

Pierce's disease of grape

Citrus Variegated Chlorosis

Almond leaf scorch

Confirmation of country freedom from *Xylella fastidiosa* in Sri Lanka

Objectives

- To investigate the presence of Xf in Sri Lanka
- To study the presence of Xf in potential host plants in Sri Lanka

Methodology

- Site Inspection of places of production for *Xylella fastidi*osa was conducted in accordance with the guidelines of PM 3/82 (2)
- Sixty seven fresh leaf samples of 23 species were collected from Colombo, Gampaha, Kandy, Nuwara Eliya, Kurunegala, Puttlalam and Anuradhapura districts in Sri Lanka
- Samples were subjected to PCR with forward RST31 5' – GCGTTAATTTTCGAA / reverse RST33 GTGATTCGATTGC-3' (Amplicon size- 733 bp.) primers which amplify the 733 bp fragment of *rpo*D gene (Appendix 4 of EPPO Protocol for Xylella fastidiosa)
- Positive samples of test DNA of *Xylella fastidiosa* was procured from the French collection of Plant Associated Bacteria, CIRM-CFBP

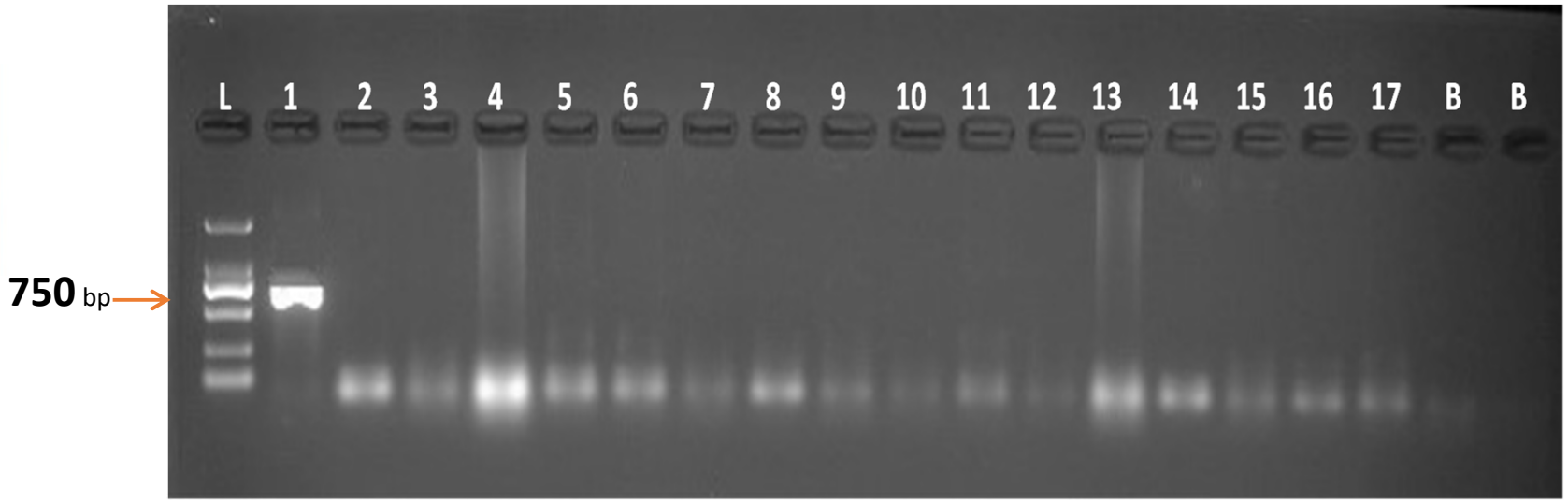
Results and discussion

Photographs of some samples collected for the study

Cinnamomum zeylanicum, Garcinia morella, Elettaria cardamomum, Coffea spp., Citrus reticulate, Piper nigrum, Averrhoa carambola,Muntingia calabura, Rosa spp., Persea Americana, Citrus spp., Senna auriculate, Coffea spp.

Results and discussion

- The desired band with the size of 733bp, was not observed from any of the samples studied
- The positive control of *Xylella fastidiosa* produced the band with the size 733bp as expected



Gel electrophoresis of PCR products of some samples collected. 733 bp amplified in positive control (L:2000D DNA Marker, 1:Positive control (*Xf,733bp*), 2&16:NPQS 282, 3&13:NPQS 327, 4&10:NPQS 350, 5&14:NPQS 270, 6&11:NPQS 284, 7&15:NPQS 265, 8&17: NPQS 342, 9&12:NPQS 262, B:Negative controls)

Results and discussion

- This conventional PCR is suitable for the detection and identification of Xf and the test is based on Minsavage *et al*. (1994).
- This primer pair can specifically amplify the 733bp fragment of *rpo*D gene (Appendix 4 of EPPO Protocol for Xylella fastidiosa)

Conclusion

- Xylella fastidiosa is not known to present in Sri Lanka on the basis of inspection, sampling and molecular testing carried out by NPPO, Sri Lanka using a test listed in Annex IV of Regulation (EU) 2020/1201
- However, the investigations will be continued periodically to confirm the freedom of Xf
- Work in progress with DG-SANTE to obtain a conditional permission for resume the trade of Xf host plants to EU

Thank you