## Detection of Symptoms Characteristic of Viral Infection on the Clone BR 25

## **Background**

The clone BR 25 (RUQ 1739) was received as budwood from the Malaysian Cocoa Board on 17<sup>th</sup> April 2016. The clone had been received the previous year (2015) but since it had remained dormant for a very long period of time with no signs of growing out we had decided to re-receive it in 2016. Since receipt of the clone in 2016, the previously established clone (RUQ 1738) started to grow out. However as RUQ 1739 was more vigorous it was decided that this one would be put through quarantine and RUQ 1738 was kept back as a reserve.

## Virus Detection

Virus indexing was started on 10<sup>th</sup> January 2017. On 2<sup>nd</sup> March 2017, Heather Lake noted viral-like symptoms on one of the tests plants and alerted Andrew Daymond to this (Figure 1). The plants were moved to the University campus and a number of leaves were detached for PCR testing by Andy Wetten. DNA extractions from symptomatic and non-symptomatic leaves both generated products when screened with degenerate PCR primers designed for badnavirus reverse transcriptase. Sequencing of the PCR products indicated that all the closest matches are badnaviruses. The viral sequence detected in BR 25 is however only distantly related to any published badnavirus reported in cocoa. Further tests are now underway to determine if the virus is mealybug transmissible.

We have written to the Malaysian Cocoa Board to advise them of this finding and have made a recommendation to them that they carry out a series of tests to see if they can detect viral symptoms in their stock plants. To do this we have suggested that they graft onto Amelonado seedlings and observe the flush leaves for viral symptoms, which they are currently carrying out. We have also offered to receive leaf material from stock plants at MCB to test using PCR and in response MCB sent samples to the University on 21<sup>st</sup> August 2017.



**Figure 1**: Symptoms characteristic of viruses on the clone BR 25 (Accession number RUQ 1739). Vein clearing can be seen in A. and red-vein banding is observed in B.

Following detection of the virus in RUQ 1739, we felt that it was prudent to also test RUQ 1738. Amelonado test plants were grafted and these were put into virus indexing on 23<sup>rd</sup> June 2017. Initial signs of viral symptoms were observed during the week of 17<sup>th</sup> July and these were much more clear by the week of 24<sup>th</sup> July (Figure 2). The plants were moved to the campus and PCR testing of these is being conducted.



**Figure 2**: Symptoms characteristics of viruses on the second tested plant of BR 25 (RUQ 1738). A. Shows red-vein banding and B. Vein clearing.