Response to APHIS questions

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1. One concern we have is for potential recombination between [**CBI**] and the [**CBI**]. To help us figure out the likelihood of recombination, we would like to know:

a. The function of [

CBI]

b. Is there sequence homology at the flanking regions of your gene of interest on the [**CBI**] with [**CBI**].

> [

CBI, our gene of interest is not flanked by sequences homologous to the [**CBI**]

Figure 4: Schematic description of the [**CBI**].

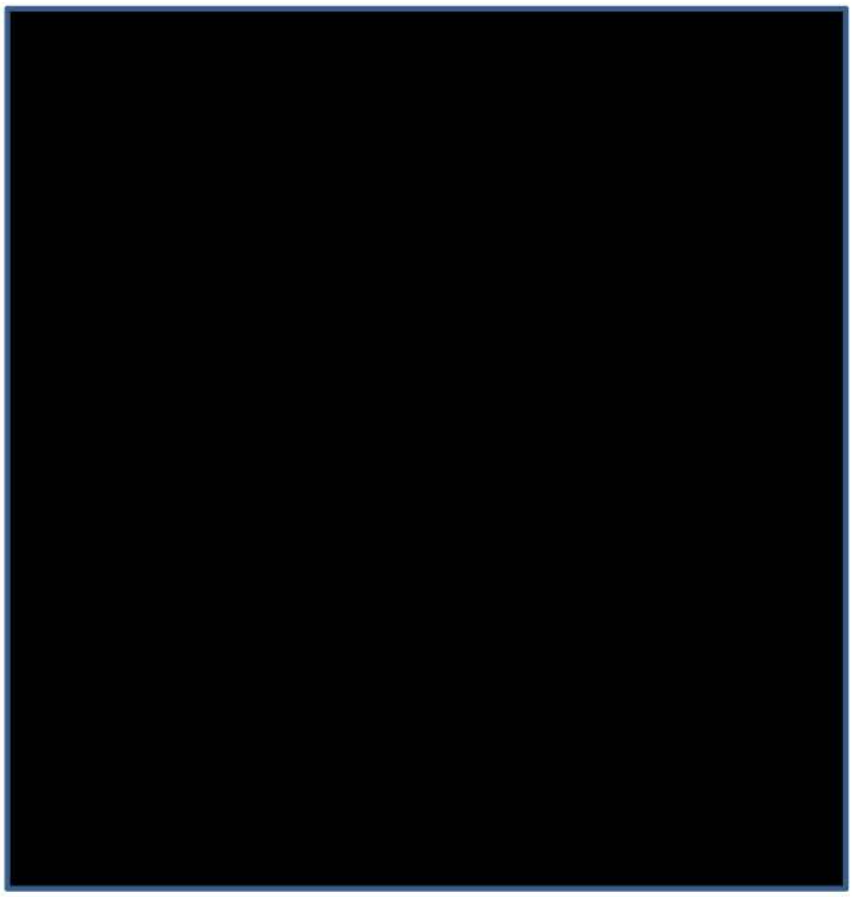
Schematic map of the [**CBI**] components:

[

–

CBI]

The numbers in bracket represent the size in base pairs.



c. How stable is the TraitUP combination throughout the lifetime of the plant?

> As mentioned by Peretz et al. 2007, TraitUp combination is durable and persists throughout the life span of the plant.

d. Is [**CBI**] transmitted by seed or pollen?

> Studies in Israel and in other countries provide evidence that [**CBI**] is not transmitted through the seeds. For your convenience the relevant articles are provided:

[

6.

CBI]

2. Transmissiblity

a. For the experiences on transmissibility by [**CBI**], we were expecting to see data verifying that the [**CBI**] you are using are transmitting [**CBI**] from infected to uninfected plants. Because that data aren't included in the paper, we don't know if the data showing lack of transmission TraitUP by [**CBI**] is due to a colony of [**CBI**] that are poor [**CBI**] or is transmission efficacy is low with your colony. Information on your positive controls would be most helpful.

> The transmissibility experiment was conducted at [**CBI**] and the [e v **CBI**] used in our experiments were part of a population used for tests of [**CBI**] (and other geminiviruses) infection. The service unit at the [**CBI**] reported to us that this population was tested for positive transmission of [**CBI**] in other experiments. In addition, Morflora performed several other experiments at the [wild te v **CBI**] before, during and after with the same [**CBI**] population to study [**CBI**] resistance using the TraitUP technology. In those experiments most of the plants (70 to 100%) exhibited [**CBI**] symptoms, therefore we conclude that this [**CBI**] population was effective at transmitting the [**CBI**]. The data of those experiments is provided to you in the attached report [**CBI**] resistance study using the TraitUP technology Spring 2011], summarizing 2 of those experiments). These other sets of data serve as a secondary form of positive control.

b. We were unable to follow your conclusion for your mechanical contact experiments based Figure 7. Could you walk us through that experiment? Did you conduct any positive controls for the Mechanical Contact or Plant Residue experiments?

> The Mechanical experiments were done as follows:

Group A- Leaves of untreated plants were rubbed with mix of sap extracted from a leaf of a positive treated plant (the treated plant was verified by PCR to harbor the TraitUPTM plasmid Figure 7A, lane 14). 0.3-0.4 grams of Positive plant leaf was ground with 3 ml of Buffer K2HPO4 1% and Carborundum (Silicon carbide – SiC) powder (~5%) was added to the solution.

Group B- Leaves of untreated plants were cut with scissors that were used to cut leaves of the positive treated plant.

The positive control for these experiments was the verification of the presence of the TraitUP plasmids in the same treated plant leaves that serves in these experiments by the same PCR analysis.

Figure 7A

Mx: Mix PCR with no DNA.
Mr: Size marker (MassRuler DNA ladder-Fermentas).
Lane 2-13: DNA from infected plants(Lane 14) rubbed with sap+carborundum.
Lane 14: DNA extracted from the TraitUP-FB100 treated plant(positive control).
Lane 15: Control plant.
Lane 16: [**CBI**]

Figure 7B

Mx: Mix PCR with no DNA.
Mr: Size marker (λDNA/Eco47I)..
Lane 2: control untreated plant.
Lane 3-22: DNA from treated plants cut with scissors.
Lane 23: DNA extracted from [**CBI**] infected plant (positive control).
Lane 24: [**CBI**].

In conclusion the PCR resulted in no marker related bands being present for any of the [**CBI**] (Figure 7A lane 2-13) or [**CBI**] inoculated plants (Figure 7B- lane while the positive control, which was the source of inoculum had a band (Figure 7A lane 14) which coincided with the band produced by the [**CBI**].

Also, the figure on page 13 (Efficacy report) – the y-axis should be healthy plants?? Right?

Yes, of course you right! Our apologies for the mistake. The corrected figure is below.