Bean Pod Mottle Virus (BPMV) Viral Inoculation Procedure in Common Bean (*Phaseolus vulgaris* L.)

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Summary of Q&A (last updated on 9/19/2016)

1. During the experiment

Q#1. *Why are the leaves always rinsed with water after sap inoculation?*

A. Leaves must be rinsed with tap water to remove all the carborundum. We tested without rinsing and the leaves died rapidly after inoculation.

Q#2. *Why the sap-inoculated leaves quickly die after inoculation in my experiment? Is some silencing still possible even if the leaves die back?*

A. Possible explanations:

1) Too much carborundum.
   Be careful not to powder too much carborundum on the leaves before rubbing. If you have many plants to inoculate with the same sap, powder only the first 6-10 plants with carborundum. The miracloth will be charged with carborundum and you can inoculate all the rest of your plants without powdering their leaves. In all cases, always rinse the leaves with tap water and dry them carefully! The inoculated leaves will appear "crumpled" the first 24 h but should recover their normal shape after.
2) Too hard rubbing.
   Did you observe some necrosis areas on the inoculated leaves? If so, it is possible that you rubbed too hard. The rubbing intensity is also genotype-dependent. Just try to rub more gently.
   The survival of the inoculated leaf is crucial for virus infection, multiplication and spreading in the whole plant and VIGS induction is dependent on these first steps.

2. Post the experiment

Q#3. *Is it possible to save tissue from the secondary inoculation to use as inoculum for further inoculations?*

A. Yes it is possible. However for our own experiments, we never do this because virus genome theoretically accumulates mutations while replicating and that’s why we prefer always use leaf sap derived from leaves of primary inoculated plants.