Appendix 2: Grading Guidelines for Plant Lab Report

These guidelines used by instructors outline items that should be included in each of the lab report sections.

Purpose/Introduction:

- silence su gene, magnesium chelatase, involved in chlorophyll formation (lack= yellow)
- mention viral delivery (geminivirus, A and B components) and B::su has 154 bp su and purpose of A/B reaction

Materials and Methods:

- Transplantation
- Plasmid preparation, quantification spec and gel
- Gene Gun-shot one A/B, three A/B::su , volumes used
- Observation of leaf phenotypes
- RNA purification (amt leaves used) and spectrophotometric quantification
- Real time RT-PCR (RT-qPCR): reaction setup and analysis

Results:

- Plasmids
  - gel picture (supposed to be 2.5 kb)
  - amounts and purity via spectrophotometric readings
- Leaf observations
  - chart of students results
  - class data
  - plant pictures
- RNA purification- concentration and purity of prep
- qRT-PCR
  - Values Ct, extent silencing (green and silenced as calibrators)
  - Differences between dilution series steps

Discussion:

- Expectation when use
  - A/B: wildtype virus lead to shorter plants, chlorosis irregular spots (yellowing), leaf curling
  - A/B::su: yellow circular spots at infection site, systemic effect
- Height of plants
- Silencing seen in A/B versus A/B::su
- Silencing seen over time
- Student's data compared to class- leaves and qRT-PCR
- Expected results for qRT-PCR reactions
  - purpose of Rubisco: housekeeping, normalizer
  - expect mature/green versus other leaves
- Results seen in qRT-PCR
  - Detect silencing? If not, why not?
  - Ct values increase by 3.3 for 10fold dilutions? Why not?

References: class protocols, Kjemtrup et al. 1998 paper