Quantification of Callose Deposition in Leaves
Adapted from L. Scalschi et al. (2015)

Introduction
The study of callose, a polysaccharide composed of β-1,3-glucan found in plants, has proved very useful for plant immunity research. Callose is known to be involved in multiple aspects of plant growth and development, from strengthening the plant cell wall to regulating cell-to-cell movement, and is produced in response to both biotic and abiotic stresses. When modifying plants using Agrobacterium tumefaciens, changes in callose structures can be observed when compared to non-modified plants. By staining callose with methyl blue you can visualize the plant structure and note changes between modified and unmodified plants to verify successful bacterium mediated T-DNA insertion. This protocol specifically describes the staining of Arabidopsis thaliana, Solanum lycopersicum, or citrus leaves. It should, however, be applicable to any higher plant species with β-1, 3-glucan present.

Materials
- Plant materials: Arabidopsis thaliana cotyledons and leaves, Solanum lycopersicum leaves, or Citrus leaves
- 96% ethanol
- Na$_2$HPO$_4$ · 2H$_2$O
- NaH$_2$PO$_4$ · 2H$_2$O
- Methyl Blue (GoldBio Catalog # A-298)
- Sodium phosphate buffer (instructions included below)
- Microscope possessing both a UV filter and camera, with epifluorescence capability band pass filter between 340 and 380 nm, and long pass filter set to 425 nm.
- 50 ml tubes (1 per sample)

Preparation of sodium phosphate buffer (0.07M, pH 9):
- Dissolve 12.46 g of Na$_2$HPO$_4$ · 2H$_2$O in 1 L of distilled water.
- Dissolve 0.966 g of NaH$_2$PO$_4$ · 2H$_2$O in 100 ml of distilled water.
- Adjust the pH of Na$_2$HPO$_4$·2H$_2$O solution to 9 using the solution of NaH$_2$PO$_4$·2H$_2$O. This will likely require all of the NaH$_2$PO$_4$ · 2H$_2$O, but use a pH meter to confirm.

Preparation of 10x Methyl Blue Stock Solution:
- Dissolve 0.5 g of Methyl blue in 100 ml of sodium phosphate buffer.
Preparation of Methyl Blue Working Solution:
- Add 10 ml of 10x Methyl Blue Stock to 90 ml of sodium phosphate buffer.

**Storage and Handling**
- Methyl blue should be stored at room temperature. Protect from light.

**Method**
This procedure can be used on the listed plant materials with little variation due to tissue toughness.

1. Sample the entire leaf or cotyledon from the plant and place in 50 ml tubes containing 96% ethanol for the removal of chlorophyll. Destain until all chlorophyll is removed. If the ethanol is saturated, it may be replaced.

   *Note: The speed of this process depends on the size of the leaf, with larger leaves requiring longer destaining. *Arabidopsis* leaves may only need 1 day while citrus leaves may require up to 1 week.*

2. Rehydrate samples in sodium phosphate buffer for 30 minutes.

3. Discard the phosphate buffer and cover the sample with Methyl blue working solution.

4. Incubate the samples for 30 minutes and discard the solution. Callose in *Arabidopsis* cotyledons will be observable after 30 minutes of incubation (can proceed to step 7). In other structures, callose can be observed after 3-4 hours.

5. After 3-4 hours of incubation, callose in larger samples will require covering with 0.5% Methyl blue solution, followed by a 24-hour incubation in darkness.

   *Note: The time may vary depending on the sample. Some samples, such as citrus leaves, may require a vacuum stroke of 2 minutes and up to 7 days of staining.*

6. For microscope observation, mount the samples on slides with the adaxial surface up using fresh 0.05% methyl blue.

7. Observe the samples with an epifluorescent microscope with a UV filter. Stained callose appears as fluorescent yellow spots.
Note: Be aware to use the same augmentation all the time.

**Associated Products**

**Methyl Blue (GoldBio Catalog # A-298)**

**References**
