

Vanillin Assay of *Arabidopsis* Seeds for Proanthocyanidins

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[Abstract] Proanthocyanidins (PAs) are colorless flavonoid polymers and deposit in *Arabidopsis* seed coat specifically. Oxidation of PAs gives rise to brown color of mature seeds. PA accumulation can be affected by a number of growth conditions, such as temperature and sun light. PAs, which are converted from anthocyanidins, can protect seeds from outer environment and have a positive effect in seed longevity (Debeaujon, 2003). Vanillin turns red upon binding to leucoanthoanthocyanidins, catechins and monomers and terminal subunits of PAs (Butler *et al.*, 1982; Deshpande *et al.*, 1986). Based on this principle, PA deposition in *Arabidopsis* seed coat can be visualized.

Materials and Reagents

1. *Arabidopsis* seeds (Col 0 and *tt* mutants)
2. 6 M HCl
3. Sterile distilled water
4. Dye solution (for coloration and reaction with PAs) containing 1% w/v vanillin (Sangon Biotech, catalog number: VT0974- 100g) and 6 M HCl stored in brown bottle
Note: The dye solution should be used after preparation as soon as possible (not longer than half an hour).
5. Vanillin reagent (see Recipes)

Equipment

1. Brown bottle (or glass bottle covered with aluminum foil)
2. 1.5 ml microcentrifuge tubes
3. Glass slides (25.4 mm x 76.2 mm) and coverslips (20 mm x 20 mm)
4. Dissecting needle and tweezer
5. An SZ61-zoom stereomicroscope (OLYMPUS, model: SZ61)
6. A compound light microscope (OLYMPUS, model: BX61)

Procedure

1. Intact seeds should be dipped into dye solution at room temperature.
2. For mature seeds, the incubation should last for approximately 1 h, whereas for immature seeds, the incubation time can be up to 10 min, depending on the degree of immaturity. Figure 2 indicates siliques at different developmental stages.
3. After the reaction with vanillin, seeds can be gently separated into embryos and seed coats with dissecting needle and tweezers under an SZ61-zoom stereomicroscope on glass slides.
4. Cover slides with coverslips. A little bit extra vanillin reagent should be left to avoid drying (water and glycerol will fade this red color).
5. Stained seed coats were observed and photographed with a compound light microscope.

Representative data

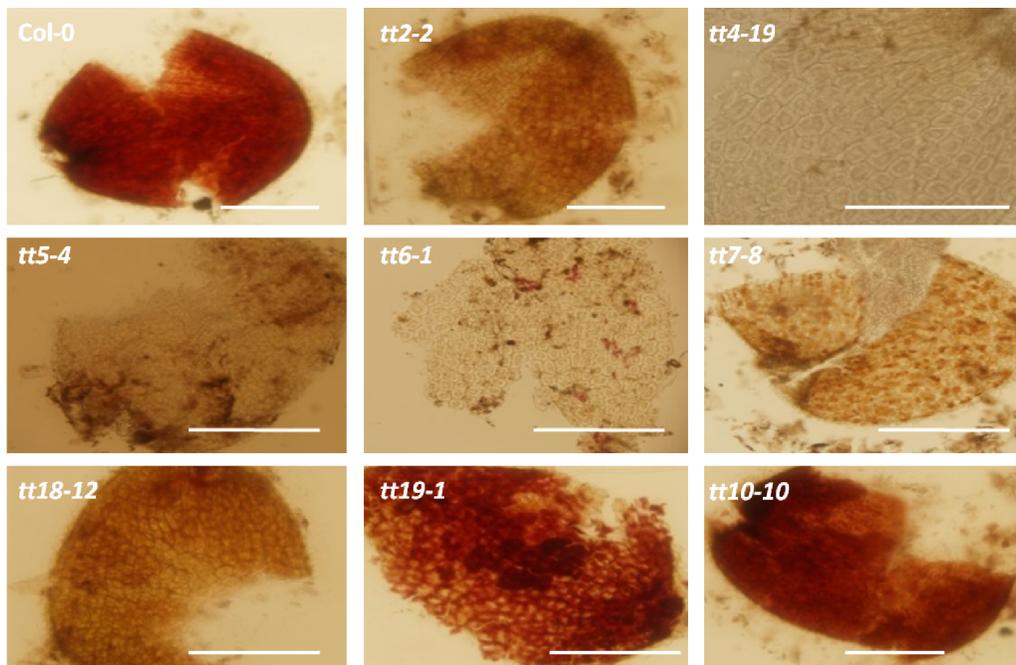


Figure 1. Comparison of the darkness of PA staining in seed coats among various *test transparent* mutants. Scale bars = 100 μ m (Wang *et al.*, 2014)

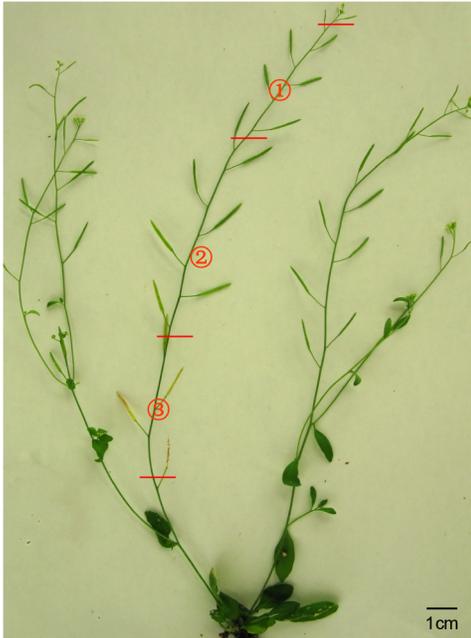


Figure 2. Comparison of *Arabidopsis* siliques between different developmental stages.

Stage 1: Young siliques: less than or about one week after pollination. 10 min is enough for reaction between PAs and vanillin. Stage 2: Immature siliques: 1-3 weeks after pollination. 20 to 40 min are required for the reaction. Stage 3: Mature silique: 3 weeks or more after pollination, color of siliques appears pale yellow. 1 h is necessary for the reaction.

Notes

1. Reaction between vanillin and PAs is affected by temperature. Make sure stain all the seeds at the same condition.
2. Do not try to rinse seeds, especially young seeds, with water after dying with vanillin. Red color can be rinsed away by water.
3. Make sure all the seeds were dipped into vanillin reagent at the same condition, and a quick centrifuge may help. For seed quantity, just cover the bottom of a 1.5 ml microcentrifuge tube (one or two layers).

Recipes

1. Vanillin reagent

The vanillin reagent should be fresh and 1 ml is quite enough for seed staining. Make sure vanillin reagent volume and seed number are the same in wildtype and mutant microcentrifuge tubes. Stained red color continues getting dark along with time duration. It is very important to photograph seed coats at the same light condition. Light intensity can

affect visual effects.

Acknowledgments

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References

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