

**FAMILY: VERBENACEAE**Genera: *Glandularia*, *Lantana*, *Verbena***1. PRECONDITIONING:**

METHOD	TIME (h)	TEMP (°C)
Soak in water	16-48*	20-25

Note: Push seeds down to immerse or wrap in filter paper and submerge. If seeds continue to float after soaking overnight, they may be empty. \*Referee tests on one sample of *V. hastata* showed that 48 hours of imbibition time (soaking) was necessary.

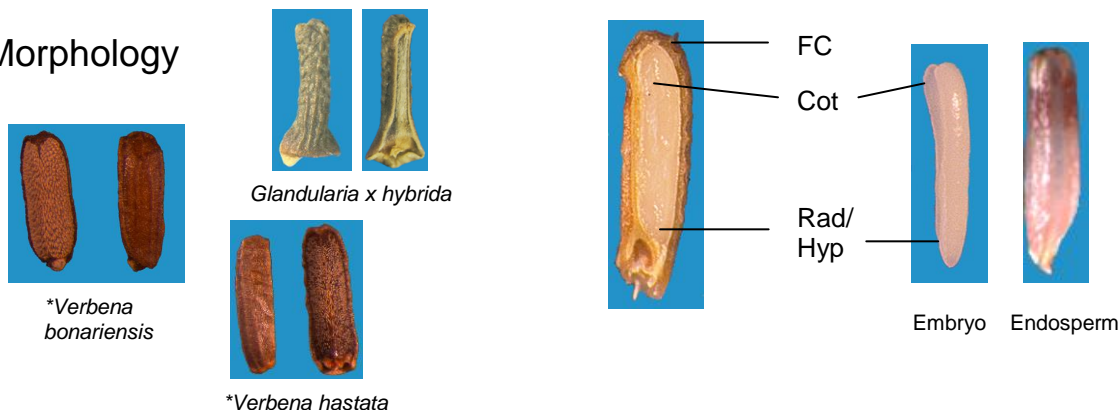
**Morphology**

Fig 1 External

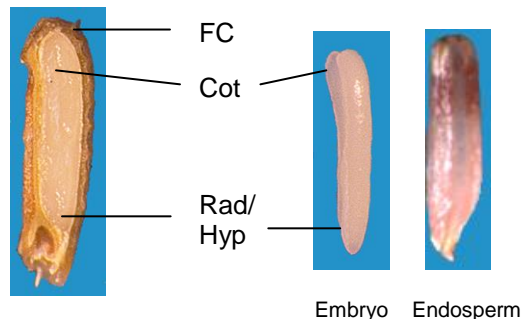


Fig 2 Embryo

Note: A thin layer of endosperm surrounds the embryo. \*Small-seeded *Verbena* (<2mm).

**2. PREPARATION AND STAINING:**

METHOD	TZ Conc (%)	TIME (h)	TEMP (°C)
Cut laterally at the distal end, just enough to see the embryo. (recommended for small-seeded <i>Verbena</i> <2mm).	1.0	16-48*	30-35
Cut longitudinally, leaving fruit coat or distal end intact to keep both halves together	0.1	4-16	30-35

Note: Rigid woody fruitcoat will cause artifacts at the cut site. Change blades frequently to reduce artifacts.

Seed may release an oily substance into TZ solution when staining.

A lateral cut will restrict artifacts to the distal end. Longitudinally cut seeds sometimes fail to stain either because of artifacts or seed constituents released from the cut that may interfere with the tetrazolium reduction reaction.

Ensure immersion of small-seeded *Verbena* in TZ solution by wrapping seeds in filter paper.

\*Referee tests on one sample of *V. hastata* showed that 48 hours of staining time was necessary.

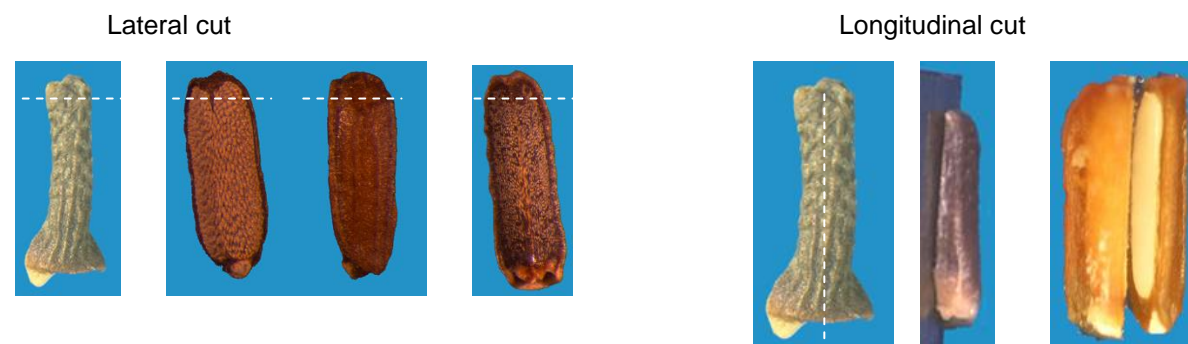


Fig 3 Preparation method

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**Post-staining notes:** If prepared with a lateral cut, either push the embryo out of the end by squeezing the base or cut longitudinally. Pull halves of longitudinally cut seed apart to evaluate.



Lateral cut with embryo beginning to push out of the fruit coat



**3. EVALUATION:**

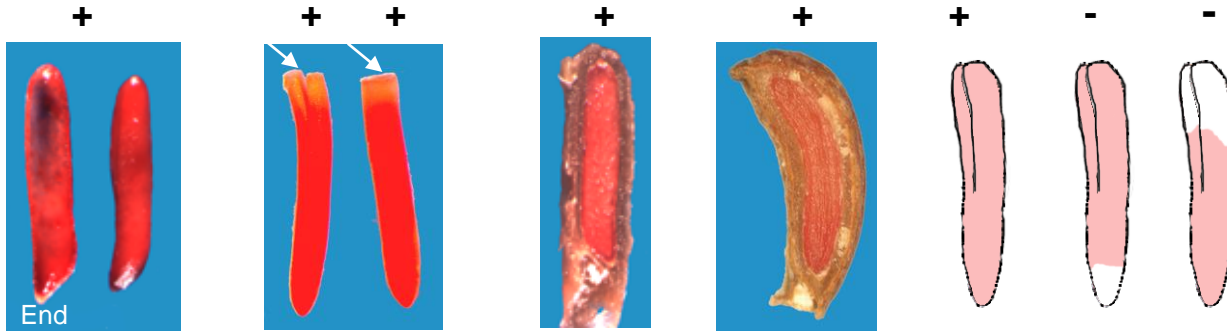
**VIABLE (NORMAL STAINING)**

- entire embryo evenly stained

**NON-VIABLE (ABNORMAL OR NO STAINING)**

- any part of embryo unstained  
 - empty or partially filled seed units.

Notes: Seed is subject to artifact damage (see sections 14.2 and 15.1.3). Surface pathogens will often cause germination results to be lower than TZ test results. Endosperm surrounding the embryo is living and will stain; however, it is not included in evaluation. For laterally cut seeds, the tissue may be white at the cut site.



Endosperm, left  
 Embryo, right.  
 Endosperm is a thin sheath around the embryo.

Embryos with white artifact at cut surface (distal end of cotyledons)



*V. hastata*  
 1 day imbibition,  
 1 day stain



2 day imbibition,  
 2 day stain

Fig 4 Seed stain evaluation

Photos:

Arnold Larsen, Fort Collins, CO: external view, *G. x hybrida*

Annette Miller, USDA/ARS NLGRP: Fig. 1 External *V. bonariensis*, *V. hastata*. Fig. 2 Embryo, endosperm, Fig. 3. cut with razor, Post stain: lateral cut view, Fig. 4 three evaluation views at left, drawings and *V. hastata* stained embryo images.

Sarah Dammen, SGS, Brookings SD: Fig. 3 right view of cut seed, Fig. 4 fourth evaluation view from left.