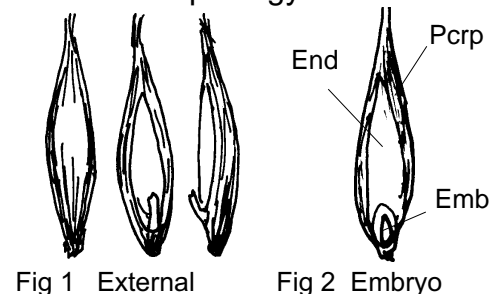


FAMILY: POACEAE II (Grass Family II - Small grasses)

Genera: *Achnatherum, Agropyron, Agrostis, Bouteloua* (see *Poaceae III* for *B. curtipendula*), *Bromus, Calamagrostis, Cynodon, Dactylis, Elytrigia, Elymus, Eragrostis, Eremochloa, Eriochloa, Festuca, Hilaria, Leptochloa, Leymus, Lolium, Nassella, Panicum, Pascopyrum, Paspalum, Piptatherum, Poa, Puccinellia, Schismus, Schizachyrium, Sorghastrum, Sporobolus, Stipa, Zoysia*

**1. PRECONDITIONING:**

METHOD	TIME (hrs)	TEMP (°C)
1. (larger seed) soak in beaker of water	overnight	20-25
2. (small seed) imbibe on moist blotters, filter paper, or paper towels	overnight	20-25

Morphology

Notes: Seed with soft caryopsis (*Dactylis*) do not need a preconditioning step and can be cut dry.

**2. PREPARATION & STAINING:**

METHOD	TZ Conc(%)	TIME (hrs)	TEMP (°C)
1. (large seed) bisect longitudinally through embryo and retain half for staining or leave seed intact at distal end	0.1-1.0	overnight	20-30
2. (small seed) cut laterally slightly above embryo or undercut laterally beneath embryo	0.1-1.0	overnight	20-30
3. (small or soft seed) pierce with a needle in central endosperm region	1.0	overnight	20-30

Notes: 1. Small grasses or seeds with a soft caryopsis or bisected seeds may stain more rapidly (4-6 hrs) at 35-40°C.
 2. TZ concentration will vary with cutting method and is dependent upon seed size, time, and temperature.
 3. Seeds that are pierced may be placed in a vacuum oven at 25 psi for 1 hr to facilitate TZ solution uptake and staining.

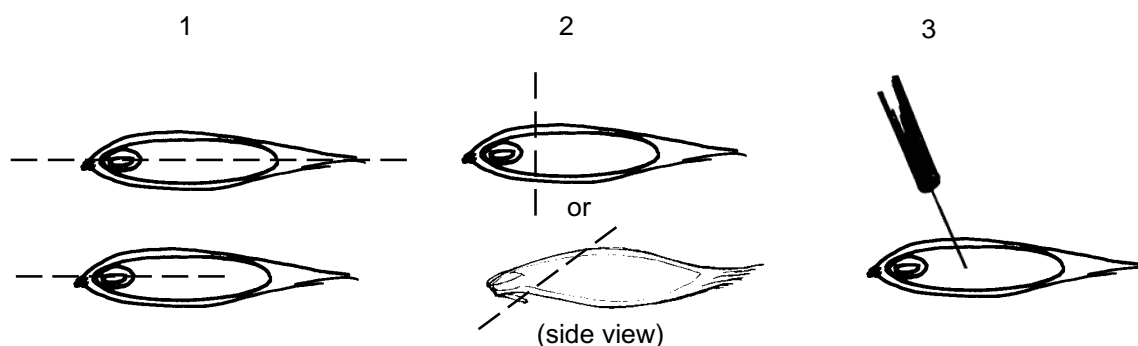


Fig 3 Preparation method

FAMILY: POACEAE II

Post Staining Notes: None for bisected method (1). If seeds cut laterally or pierced (methods 1 or 3) then clear lemma pigmentation with 85% lactic acid for 30 minutes at 25-35°C. If pigmentation remains a problem with microscopic evaluation, bisect seed longitudinally or remove lemma and palea to evaluate.

**3. EVALUATION:****VIABLE (NORMAL STAINING)**

- entire embryo evenly stained
- endosperm will not stain
- unstained outside edge of scutellar region acceptable
- greenish-colored pericarp acceptable if entire embryo stains as normal (particularly over embryonic region)

NON-VIABLE (ABNORMAL OR NO STAINING)

- any essential part of the embryo unstained
- greenish-colored pericarp with off-color or unstained embryo
- mottled or broken embryonic tissue
- soft or flacid stain over embryonic region

OTHER TISSUE/NOTES

Endosperm may have an orange or yellow coloration, but should not stain dark red like embryo. Immature seed which stains light or pale pink with yellow endosperm is considered 'viable' (see sections 15.1.3.2 and 15.1.3.4).

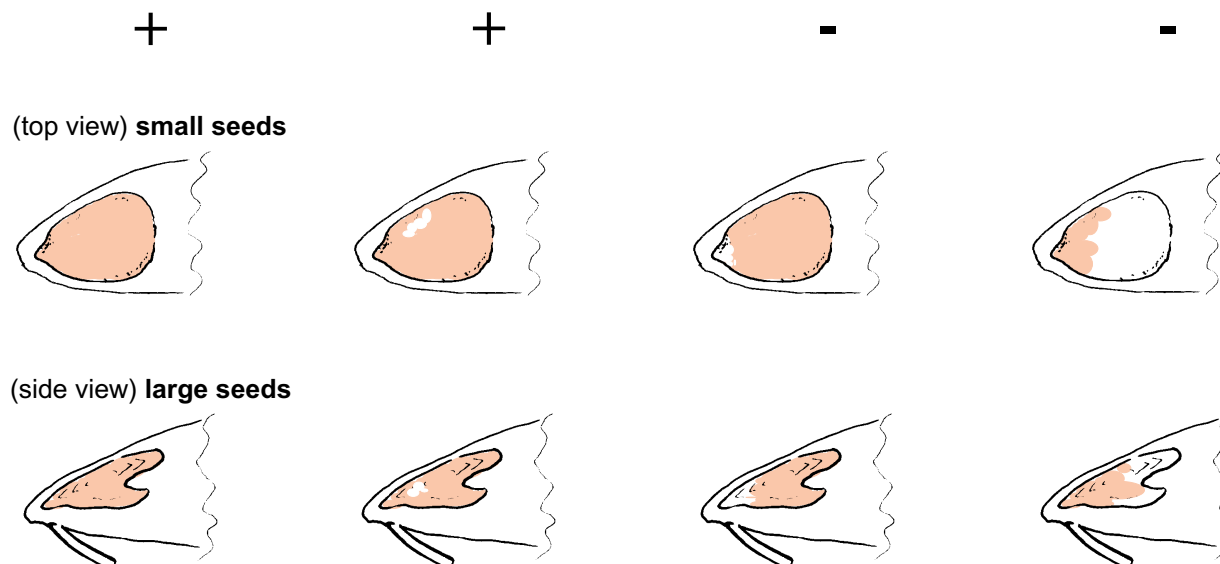


Fig 4 Seed stain evaluation

REFERENCES: 1, 2, 3, 4, 5, 6, 9, 10, 11