

AOSA and ISTA Tetrazolium Testing References:

Excerpts from methods and evaluation statements (Some paraphrased for easier comparison):

(Prepared in 2004 by Annette Miller, AOSA TZ Subcommittee Chairman)

	AOSA	ISTA
Amaranthaceae <i>Amaranthus</i>	imbibe on moist media overnight bisect (0.1% overnight 20-25) face cut (1% 6-18 hrs. 30-35°C) - may take up to 24 hrs V: entire embryo evenly stained slight radicle damage acceptable NV: any essential part of embryo unstained bruised embryo areas	soak 18 hours in water pierce near micropyle (1% 20 hrs 30°C) allowable damage: 1/3 radicle, measured from the radicle tip, 1/3 from the distal end of the cotyledons
Apiaceae <i>Daucus</i>	imbibe on moist paper towels longitudinal cut through basal end leaving seed intact at distal end (longitudinal edge cut - alternate method) 1%, overnight, 20 V: entire embryo and endosperm evenly stained "If turgid, unstained embryos are observed, retest by clipping the distal end and preconditioning with GA3)"	soak 18 hours in water longitudinal cut through distal end leaving seed intact at basal end 1%, 18 hrs, 30 V: no necrotic or unstained areas permitted
Asteraceae <i>Helianthus</i>	imbibe on moist blotters cut laterally and remove distal end of cotyledons 1%, overnight to 24 hrs, 30-35 remove seed from achenes after staining V: slight damage to root tip acceptable NV: cotyledons half or more damaged, or damaged near point of attachment	soak 18 hrs remove pericarp and seedcoat from the seed 1% 3 hrs, 30 cut longitudinally...observe both sides Allowable damage: V: 1/3 radicle measured from the radicle tip, 1/2 of the distal end of the cotyledons
Balsaminaceae <i>Impatiens</i>	soak overnight cut longitudinally, leaving seed intact at top of cotyledons 0.1% overnight 30-35 no damage allowable	soak 18 hrs cut longitudinally 1/2 through the distal end (flat side) 1% 18 hours 30 no damage allowable
Brassicaceae <i>Brassica</i>	imbibe on moist blotters remove seed coat or cut or nick through cotyledons 1% 8 hrs 30-35 or 1% overnight 20-25 (2nd method: remove seed coat after stain)	soak 18 hrs incise seed cross wise on cotyledon.... remove seed coat 1% 18 hrs., 30

	<p>V: radicle tip stained slightly darker Cots: isolated superficial necroses away from attachment point NV: radicle tip unstained, necrotic or fractured. (damaged) area at juncture of cots and emb. axis or at any point along axis.</p>	<p>allowable damage: 1/3 radicle measured from radicle tip 1/3 superficial necrosis on cots. (away from hyp.)</p>
<p>Campanulaceae <i>Campanula</i></p>	<p>imbibe on moist blotter pierce or cut longitudinally 1% overnight (pierce), or 0.1% overnight (cut) entire embryo and endosperm must stain</p>	<p>soak 18 hrs cut longitudinally 1/3 through the distal end 1% 18 hrs. entire embryo and endosperm must stain</p>
<p>Caryophyllaceae <i>Silene</i></p>	<p>imbibe on moist blotter longitudinal cut through embryo or longitudinal cut - thin slice off edge V: entire embryo evenly stained NV: any damage to radicle, hyp, cots</p>	<p>soak 18 hrs longitudinal cut through embryo</p> <p>allowable damage: 1/3 radicle, measured from the radicle tip 1/3 distal end of cotyledons</p>
<p>Chenopodiaceae <i>Beta</i></p>	<p>imbibe on moist media or soak overnight slice off cap 1% 16-24 hrs 20-25 C V: entire embryo stained NV: any essential part of the embryo unstained, especially the radicle. embryo flaccid.</p>	<p>soak 18 hrs remove cap 1% 24 hrs 30 C allowable damage: 1/3 radicle, measured from rad. tip 1/3 from distal end of cots</p>
<p>Convolvulaceae <i>Ipomoea</i></p>	<p>imbibe between moist blotters (scarify if hard) cut half longitudinally 1% overnight 30-35 C V: slight damage to radicle tip acceptable half or more cotyledons stained NV: damage to radicle damage to arch area of hypocotyl less than half of cots stained</p>	<p>soak 18 hrs cut half longitudinally 1% 24 hrs 30 C allowable damage: 1/3 radicle (from radicle tip) 1/3 distal area of cotyledons, 1/2 if superficial</p>
<p>Crassulaceae <i>Sedum</i></p>	<p>imbibe on moist media cut laterally or pierce pierce: 1% overnight 30-35 cut: 0.1% 6-18 hrs 30-35 entire embryo must be stained</p>	<p>soak 18 hrs no prep 1% 18 hrs 30 (remove seed coat after stain)</p> <p>allowable damage: 1/3 radicle tip, 1/2 distal end of cots.</p>
<p>Cucurbitaceae <i>Cucurbita</i></p>	<p>soak overnight distal lateral cut (1% 6-18 hrs, 30-35) longitudinal cut -edge, or bisect for both longitudinal cuts: 0.1% overnight 30-35</p>	<p>soak 18 hrs distal lateral cut 1% 18 hrs, 30 C</p>

V: less than half cots damaged and away
from point of attachment
NV: any damage to radicle
half or more of cotyledons damaged or
damage close to point of attachment.

allowable damage:
1/3 radicle, measured from radicle tip,
1/2 of the distal end of the cotyledons

Fabaceae <i>Glycine</i>	imbibe overnight on moist rolled paper towels stain intact seed or cut longitudinally, bisecting the embryo axis 0.5-1% 1-2 hrs 30-35 (intact seed) 0.1-1% 1 hour 30-35 (bisected seed) post stain: separate cotyledons, bisect embryo axis V: unstained....including tip of central conducting tissue NV: radicle (damaged) above tip of central conducting tissue	soak 18 hours between wet paper stain intact seed 1% 6 hrs, 30 C post stain: remove seed coat allowable damage: 2/3 radicle, measured from the radicle tip, 1/2 distal area of cotyledons
Fabaceae <i>Medicago</i>	imbibe between moist blotters stain intact (nick if hard seeded) 1% 2-72 hrs 30-35 clear 1 hr at 35C with glycerol or lactic acid or remove seed coat V: slight damage to radicle acceptable V: half or more of cots attached to embryonic axis and evenly stained NV: unstained radicle above tip of central conducting tissue	soak 18 hours stain intact 1% 18 hrs, 30 remove seed coat allowable damage: 1/3 radicle, measured from the radicle tip 1/3 distal area of cotyledons, 1/2 if superficial
Hydrophyllaceae <i>Phacelia</i>	imbibe on moist media longitudinally cut thin slice off edge of embryo 0.1% overnight 30-35 C entire embryo must stain (illus must be fixed to show stained end.)	soak 18 hours cut longitudinally (bisect) 1% 24 hrs 30 C allowable damage: none
Lamiaceae <i>Ocimum</i>	imbibe on moist media lateral cut (distal end of cots.), or longitudinal cut (bisected leaving seed intact at top of cots) 1% overnight 30-35 V: entire embryo evenly stained slight damage to cotyledons	soak 18 hours longitudinal side cut, open and extract embryo 1% 4 hrs. 30 C allowable damage: 1/3 radicle, measured from radicle tip, superficial necrosis at distal end of cots.

Liliaceae <i>Allium</i>	<p>soak in beaker of water or imbibe between moist blotters nick in hilar notch and then cut longitudinally, a thin slice off edge of seed</p> <p>1% 8-18 hrs 30-35 post stain: cut more tissue away from embryo for eval of folded type emb.</p> <p>V: entire embryo evenly stained endosperm completely stained</p>	<p>soak 18 hrs</p> <p>cut longitudinally through the linear side of the seed (thin slice) and longitudinally 2/3 to the endosperm near the middle of the seed between the radicle and cots, 1/3 into the endosperm (hilar notch?)</p> <p>1% 18 hrs 30 C post stain: cut longitudinally from the flat side through endosperm to expose the embryo</p> <p>allowable damage: none except small superficial necrosis on the outer part of the endosperm, not in connection with the embryo cavity</p>
Linaceae <i>Linum</i>	<p>imbibe on moist blotters (enough to soften but not for mucilage to form) or no preconditioning necessary (cut dry) lateral cut (remove distal end of cots or longitudinal thin slice off side) lateral cut: 0.1% overnight 30-35 longitudinal cut: 1% 6-12 hrs 30-35 V: entire embryo evenly stained, root tip slightly darker acceptable</p>	<p>soak 18 hrs</p> <p>lateral cut (1/3 of distal end of cots)</p> <p>1% 18 hrs 30 C</p> <p>allowable damage: 1/3 radicle, measured from the radicle tip, 1/3 of distal end of the cots.</p>
Malvaceae <i>Gossypium</i>	<p>acid delinted: imbibe on moist rolled towels linted: imbibe in water or on rolled towels remove seed coat and imbibe in water 15 min., remove membrane surrounding embryo 1% 1-2 hrs, 30-35 C V: slight necrotic tissue on cots acceptable radicle tip unstained, provided cots are well stained</p>	<p>no imbibition</p> <p>cut transversely 1/3 from distal end</p> <p>1% 18 hrs, 30 allowable damage: 1/3 radicle tip, 1/3 distal area of cots</p>
Papaveraceae <i>Papaver</i>	<p>soak in beaker of water or, imbibe on moist media pierce or cut longitudinally leaving seed intact at distal end</p> <p>pierce: 1% overnight 30-35 cut: 1% 6-18 hrs 30-35 C V: entire embryo must stain orange coloration inside seed coat, on periphery of endosperm, acceptable</p>	<p>soak 18 hrs</p> <p>cut longitudinally through the middle of the whole seed through embryo and endosperm.</p> <p>1% 24 hrs 30 C</p> <p>allowable damage: none</p>

Plantaginaceae <i>Plantago</i>	imbibe on moist media cut laterally (distal end) or longitudinally leaving seed intact at top of cots. lateral cut: 1.0% overnight 30-35 long. cut: 0.1% overnight 30-35 V: entire embryo & endosperm evenly stained	soak 18 hrs cut thin longitudinal slice to open embryo cavity 1% 18 hrs 30 allowable damage: none
Plumbaginaceae <i>Limonium</i>	imbibe on moist blotters cut longitudinally, completely through seed 0.1% overnight 30-35 V: entire embryo evenly stained	soak 18 hrs cut a small piece at distal end of seed 1% 18 hrs 30 allowable damage: none
Poaceae <i>Triticum</i>	imbibe on moist rolled paper towels or soak overnight bisect longitudinally, discard one half or cut leaving distal end intact and leave both halves together 0.1-0.5% 1-2 hrs 20-25 C V: at least 1/4 radicle stained near mesocotyl V: no more than 1/3 scutellum unstained at either end.	soak 4 hrs (embryo excision method) or soak 18 hrs (embryo bisected) excise embryo or bisect leaving distal end intact both methods: 1% 3 hrs. 30 C allowable damage: root area except one root initial. 1/3 extremities of scutellum, Examine scutellum for heat damage (unstained tissue at center of scutellum)
Poaceae <i>Sorghum</i>	imbibe on moist rolled paper towels overnight 20-25 C bisect longitudinally, discard one half or cut leaving distal end intact and leave both halves together 0.1-0.5% 1-2 hrs 20-25 C V: radicle, plumule/col, point of attachment of embryo axis to scutellum, completely stained. No more than 1/3 scutellum unstained at either end.	soak 18 hrs 7 C (both methods) cut through distal 1/2 of endosperm or cut longitudinally through embryo and 1/4 of endosperm (basal long.) distal cut: 1% 18 hrs, 30 C basal cut: 1% 3 hrs, 30 C allowable damage: 1/3 radicle, measured from radicle tip., 1/4 distal part of scutellum
Poaceae <i>Poa</i>	imbibe on moist media, overnight 20-25 C pierce in central endosperm area 1% overnight 20-30 C post stain: clear lemma with 85% lactic acid for 30 minutes at 25-35 C. Bisect long. or remove lemma if pigmentation remains a problem. V: entire embryo evenly stained, unstained outside edge of scutellar region acceptable NV: soft or flaccid stain over embryonic region	soak 2 hrs or 16 hrs between wet paper, 20 C pierce near embryo 1% 18 hrs, 30 C post stain: remove lemma allowable damage: 1/3 radicle measured from radicle tip., 1/4 of the area of the margin of the scutellum.

Poaceae Bromus	bisect long: 0.1-1% overnight, 20-30 C V: entire embryo evenly stained, unstained outside edge of scutellar region acceptable NV: soft or flaccid stain over embryonic region	bisect long: 1% 2 hrs., 30 C allowable damage: 1/3 radicle measured from radicle tip., 1/4 of the area of the margin of the scutellum.
Poaceae Elytrigia	bisect long: 0.1-1% overnight, 20-30 C V: entire embryo evenly stained, unstained outside edge of scutellar region acceptable NV: soft or flaccid stain over embryonic region	bisect long: 1% 2 hrs., 30 C allowable damage: 1/3 radicle measured from radicle tip., 1/3 of the extremity of the scutellum.
Polemoniaceae <i>Phlox</i>	imbibe on moist media cut longitudinally, completely through seed 0.1% overnight 30-35 V: cut surface must stain, endosperm must stain, in <i>Phlox</i> , embryo has yellowish color	soak 18 hrs cut longitudinally through the middle of seed 1% 18 hrs 30 allowable damage: none
Polygonaceae <i>Fagopyrum</i>	imbibe between moist blotters bisect longitudinally leaving seed intact at top of cotyledons (both halves together) 1% overnight 30-35 V: entire embryo evenly stained	soak 18 hrs cut longitudinally through fruit and seed coats and remove fruit and seed coats to expose embryo. 1% 18 hrs 30 allowable damage: none
Portulacaceae <i>Portulaca</i>	soak in beaker of water, overnight bisect longitudinally through curved back leaving halves attached or pierce endosperm bisect: 0.1% overnight 30-35 pierce: 1% overnight 30-35 V: entire embryo evenly stained	soak 18 hrs cut at the edge between radicle and cotyledons into the endosperm (hilar notch) 1% 18 hrs, 30 allowable damage: none
Primulaceae <i>Cyclamen</i>	imbibe on moist media, overnight cut longitudinally 1% overnight 30-35 (Page needs comments about post stain slicing and endosperm stain) V: embryo evenly stained	soak 4 hours cut 1/3 longitudinally through the seed near the hilum 1% 24 hrs, 30 post stain: expose the embryo by slicing the endosperm step for step allowable damage: none
Ranunculaceae <i>Consolida</i>	imbibe on moist media, overnight cut longitudinally leaving seed intact at distal end of seed 0.1 % 8-12 hrs, 30-35 V: entire embryo evenly stained, endosperm may be unstained	soak 18 hrs cut longitudinally 1/3 through distal end (endosperm) 1% 18 hrs, 30 allowable damage (unstained, flaccid, or necrotic tissue permitted): none

Scrophulariaceae <i>Digitalis</i>	soak in beaker of water 6-18 hrs or imbibe between moist blotters cut laterally and remove distal end of cotyledons 1% 24 hrs (or longer if necessary) 30-35 entire embryo and endosperm evenly stained	soak 18 hrs cut longitudinally 1/2 through the seed beginning at distal end 1% 18 hrs, 30 allowable damage: none
Solanaceae <i>Capsicum</i>	imbibe on moist media overnight pierce or bisect pierce: 1% 4-16 hrs, 30-35 bisect: 0.1% 8-12 hrs 30-35 post stain for pierce: clear with glycerol or lactic acid (or slice apart to evaluate) V: embryo and endosperm stained superficial blotchiness acceptable	soak 18 hrs cut a small piece of the seed coat near the base, only to open embryo cavity 1% 6 hrs, 30 C post stain: cut the seed at the flat side and observe embryo and endosperm allowable damage: none
Violaceae <i>Viola</i>	imbibe on moist media overnight cut longitudinally leaving seed intact at top of cotyledons 0.1% overnight 30-35 post stain: pull halves apart to evaluate V: entire embryo evenly stained	soak 18 hrs cut transversely a piece at the distal end only to open cavity 1% 24 hrs., 30 post stain: cut the seed longitudinally near embryo axis, expose embryo and endosperm allowable damage: none