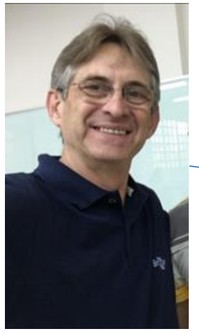
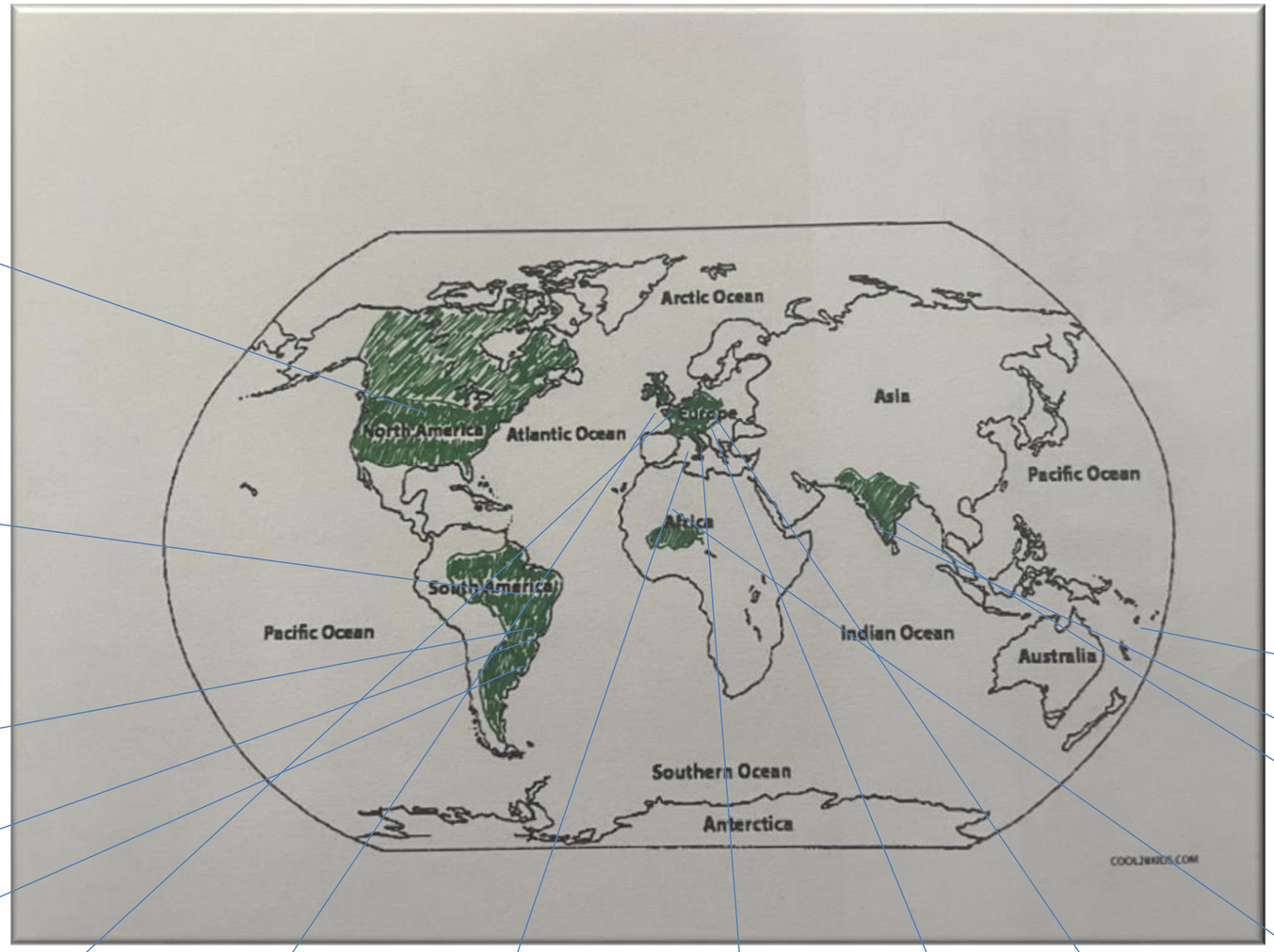
Tetrazolium Committee Report

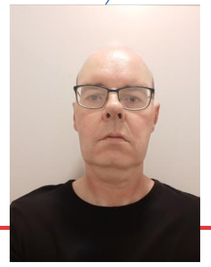
Dr. Sergio Pasquini, TEZ TCOM Chair

	ISTA Tetrazolium Committee Composition		
1	Chair: Sergio Pasquini		Italy
2	Vice-Chair: Steffi Krämer		Germany
3	2 nd Vice-Chair: Eoin O'Connor		Ireland
4	Ignacio Aranciaga		Argentina
5	Maria Belen Aranguren		Argentina
6	Alessandra Arioli		Italy
7	Jose de Barros Franca-Neto		Brazil
8	Sunita BH		India
9	Valerie Blouin		France
10	Edith Daboue		Burkina Faso
11	Veena Gouda		India
12	Augusto Martinelli		Argentina
13	Shaminder Miranpuri		USA
14	Asia Roberts-Yalland		United Kingdom
15	Adele Scott		New Zealand

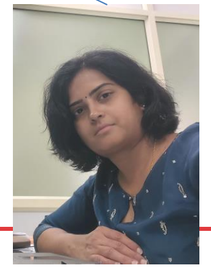
ISTA ANNUAL MEETING 2026




Arctic Ocean
Asia
Europe
North America
Atlantic Ocean
Pacific Ocean
Africa
South America
Pacific Ocean
Indian Ocean
Australia
Southern Ocean
Anterctica
COOLTHINGS.COM




CREA-DC
CENTRO DI RICERCA
DIFESA E CERTIFICAZIONE



Aims of Tetrazolium Committee:

Developing new tetrazolium tests to determine the viability of seeds for species not yet included in the ISTA Rules and re-evaluate existing methods

Providing theoretical and hands-on training on tetrazolium testing during workshops

Creating tetrazolium working sheets for many species with detailed and standardized descriptions to conduct and evaluate tetrazolium tests

Answering to the questions received by others about the TEZ test

Creating tutorial videos for e-learning for technicians in the labs

E-mail: sergiopasquini68@gmail.com

From Activity Report 2026-2027

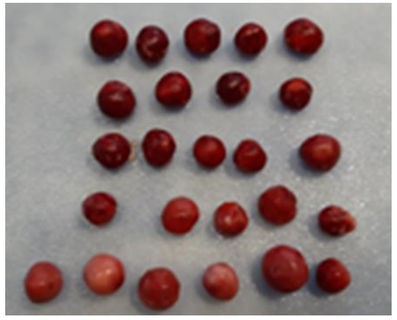
	A2. Introduction of New Species		
	Species and test	proposed	collaboration
		finalisation	
1	*Moringa oleifera* TEZ test	Jun 2027	
2	*Glycine max* TEZ test	Done!	

Validation test on *Glycine max L.*			
Test Leader	Ignacio Aranciaga		
Participants			
ISTA accredited labs	Argentina	Ignacio Aranciaga	ARDL01
	Canada	Tauhid Parvez	CAML12
	France	Valerie Blouin	FRDL02
	Germany	Stefanie Kraemer	DEDL04
	United Kingdom	Asia Roberts-Yalland	GBDL01
	Italy	Alessandra Arioli	ITDL03
Not accredited labs	Argentina	Maria Belen Aranguren	Corteva
	Brazil	Jose B. Franca-Neto	Embrapa

Done!

Method Validation Test Plan on *Moringa oleifera*			
Test Leader	Steffi Krämer		
Participants			
ISTA accredited labs	Canada	Tauhid Parvez	CAML12
	France	Valerie Blouin	FRDL02
	Germany	Stefanie Kraemer	DEDL04
	India	Veena M Gouda	INML07
	Italy	Sergio Pasquini	ITML06
Not accredited labs	Burkina Faso	Edith Daboue	National Forestry Seed Testing Centre
	India	Sunitha BH	Sakata Seeds
	USA	Shaminder Miranpuri	University of Wisconsin

Waiting for the seeds

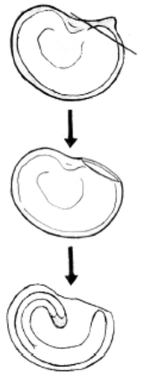


New Method Validation Test Plan made by Indian Region on *Capsicum* spp.

Tetrazolium test for evaluating seed viability

1.	Species / genus	*Capsicum annuum*, Solanaceae Pepper
	 Seed tissue (lateral view)	
2.	Instruments	Beakers (4 × 50 ml), razor blades, dissecting needle, dissecting needle (lancet tip), scalpel, surface for preparation (rubber), filter paper, forceps, surface for evaluation, binocular microscope
3.	Pretreatment	Soak 18 hours in water at 20°C
4.	Preparation before staining	Cut small piece of seed coat near base, opening embryo cavity only
5.	Staining	6 hours, 30°C, 1% TZ solution
6.	Preparation for evaluation	Cut seed on flat side into two halves and observe embryo and endosperm
7.	Evaluation (maximum area of unstained, flaccid and/or necrotic tissue permitted)	None
8.	Remarks	None

Preparation step(s)



Evaluation, examples of non-viable seeds

Capsicum annuum

Test Leader	Veena M Gouda	
Reviewers	Stefanie Kraemer	Eoin O'Connor
Participants	ISTA Accredited labs	Non accredited labs
	Indo American Hybrid seeds (IN07)	Sakata Seed India Pvt Ltd.
	Syngenta India PVT. LTD (INML1700)	Acsen Agriscience India ltd.
	East West Seeds India Pvt. Ltd. (INML4500)	
	Telangana International Seed Testing Authority (TISTA)	

Species	Pretreatment minimum time	Preparation before staining	Staining solution	Optimum staining time	Preparation for evaluation
Capsicum annuum	18 h	Cut between radicle and cotyledon 1/3 into endosperm	1% TZ	standardize with 18h or 24 h	cut the seed at flat side into two halves, and observe

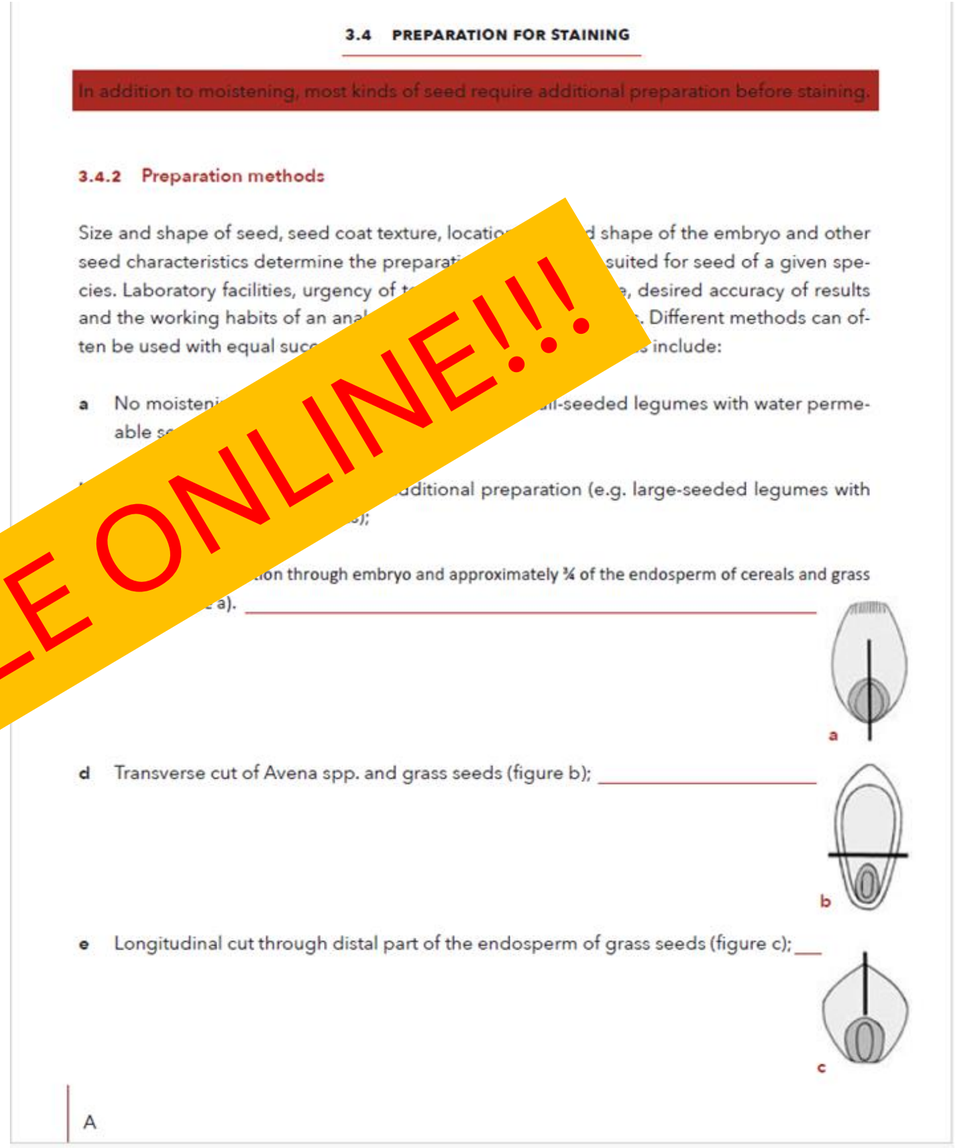
The seed lots tested will have three wide differences in germination levels.

	A3. Introduction of Rules Changes		
1	Insertion of a new paragraph 6.4.4.1		
2	Change of the names of genus *Brachiaria* spp. in *Urochloa* spp. and the staining time from 18 hours to 2 hours (editorial change)		
3	Soybean is now in Chapter 6		

6.4.1.1 Effectiveness verification of the Tetrazolium solution and salt

If the tetrazolium solution is prepared with salt of a new batch, a test of effectiveness must be done. For example, during routine testing, two replicates will be tested with the old solution and two replicates with the new one. The check tetrazolium test can also be carried out on a sample with a known quality (i.e. a sample whose quality has been ascertained either from a tetrazolium or a germination test).
If the same batch of salt is used for preparing new tetrazolium solution, no additional test of effectiveness is necessary, provided that the reagent has been stored correctly (or in accordance with the instructions).

	B2. Training publications on specific seed testing topics		
	Publication title	proposed finalisation	collaboration
1	New Tetrazolium Handbook	Available online	Secretariat
2	TEZ working sheets	to be published online, 2026	Secretariat
3	New tutorial videos (on carrot, tomato, soybean)	Available online	Secretariat



3.4 PREPARATION FOR STAINING

In addition to moistening, most kinds of seed require additional preparation before staining.

3.4.2 Preparation methods

Size and shape of seed, seed coat texture, locatio… d shape of the embryo and other seed characteristics determine the preparati… suited for seed of a given species. Laboratory facilities, urgency of t… e, desired accuracy of results and the working habits of an ana… . Different methods can often be used with equal succ… s include:

a No moisteni… ll-seeded legumes with water permeable s…

… ditional preparation (e.g. large-seeded legumes with …);

… ion through embryo and approximately ¼ of the endosperm of cereals and grass … a). _____

d Transverse cut of Avena spp. and grass seeds (figure b); _____

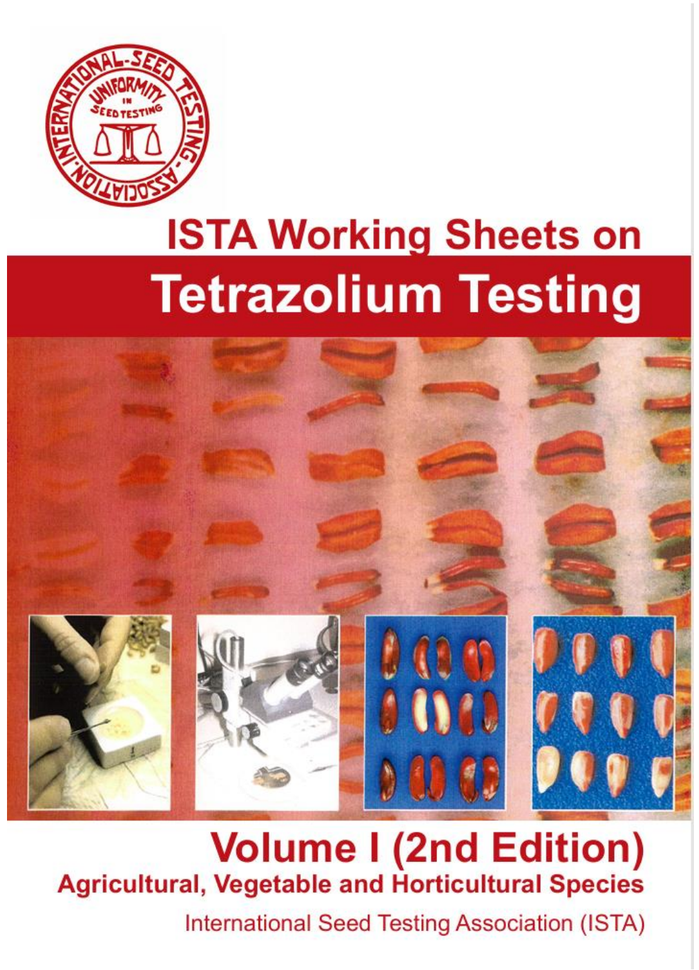
e Longitudinal cut through distal part of the endosperm of grass seeds (figure c); ___

A

ISTA Handbook on Tetrazolium Testing
3rd Edition – Vol. 1

ISTA ANNUAL MEETING 2026

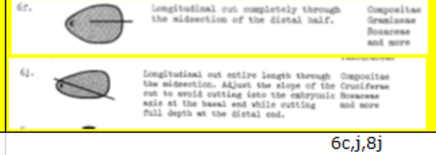
ISTA Working Sheets on Tetrazolium Testing
Volume I (2nd Edition)
Agricultural, Vegetable and Horticultural Species



New tutorial videos (on carrot, soybean and tomato)

ISTA TEZ Handbook – ANNEX 1

Da Vinci's Code

Genus	Family	Prep.of dry seeds before moistening	Moistening method and time (in hours)	Prep. After moistening	Staining time (in hours)	Preparation for evaluation	Nutritive tissues	Evaluation	
1	2	3	4	5	6	7	8	9	
Abies (T) *T=tree*	*Pinaceae*	8 h = 8 remove or server almost through: h Basal end of the seed, including a tip of the nutritive tissue, if present.	18	2,6a,i,8j = 2 Additional seed coat preparation is not usually essential, except to accelerate the uptake of water and/or the staining solution. 6 Cut the seeds longitudinally: a through the coats (and into the nutritive tissue, if present), entire length, near the midsection. i Off-center through the coats and nutritive tissues to expose the outline of the intact embryo. 8 Remove or server almost through: j Distal end of the seed, including a fragment of the nutritive tissue.	24-48	9c,d = 9 Embryo and nutritive tissue exposure for evaluation. c Expose the embryo and the adjoining nutritive tissue by tearing into the nutritive tissue, or by spreading the cut surface sufficiently to develop the desidered tear. d Expose the embryo and nutritive tissue by removal of slices of the nutritive tissue.	(+) = present	A;D = A The embryo can be observed externally either through the semitransparent seed coats, or after exposure by tearing and spreading the seed tissues that enclose the embryo. D Expose the embryo and nutritive tissue by removal of slices of the nutritive tissue.	1 = S plum existir sugges radicle,
Bellis (F) *F=flower*	*Compositae*		6 - 18	6f, j, 8k = 8 Remove or server almost through: k Distal end of the seeds, including the cotyledon tips or edges 	6 - 24	9b, j = 9 Embryo and nutritive tissue exposure for evaluation. b Expose the embryo by removal of seed coats and residual nutritive tissue, if present. j Expose the embryo by gently pressing it through the previously cut opening at the distal end. Additional cutting may be required to enlarge an incorrectly made opening in order to avoid embryo damage during removal.	0 = none, or only rudimentary tissues	C2, C17 = C Embryo completely stained, except for position and nature of necrosis listed for individual structures or conbination of structures. 2 Radicle distal 1/3. 17 Cotyledons distal 1/2, if superficial - distal 1/3 if pervading	
Abrus (T)	*Leguminosae*	3 b	18	6c,j,8j	18-20	9b,f	0	C3, C14	
Abutilon (F)	*Malvaceae*	3a	18	6e,f,n, 8b,k	18-24	9b,f	(+)	C3,C10	
Acacia (T)	*Leguminosae*	3a	18	6a,c,8j,q	18-24	9b,f	0	C3,C14	
**Acer* (T)	Aceraceae	8c	18	8b;8k,p	20-24	9b,f	0	C3,C13	
Achillea (F)	*Compositae*		18	6f,j	giu-24	9b	0	C2, C17	
Achimenea e (F)	*Gesneriacea*		18 (1b)	6a, c	18-24	9j	(+)	A; D	
Aconitum (V)	*Ranunculaceae*		18	6g, j 8m	18-24	9c, f	(+)	A; D	
Acroclinum									
Adonis (F)	*Ranunculace*		18	6g, j, 8m	18-24	9c, f	(+)	A; D	
Aeschynsnthus (F)	*Gesneriacea*		18 (1b)	6a, c	18-24	9c, d	(+)	A; D	
Agapanthus (F)	*Liliaceae*		18	6f, j, 8j	18-24	9c, d	(+)	A; D	
Agathosma (T)	*Rutaceae*		18	8j	18-24	9f,	0	A	
Ageratum (F)	*Compositae*		18	6f, j	giu-24	9b, j	0	C2, C17	
**Agropyron* (A)	Gramineae		18	6f, h, 7a	giu-24	9c	-)	C4	
Agrostema (F)	*Caryophyllaceae*		18	5b, 6g, n, 7b	giu-24	9c, f	)-(	C2, C10	
**Agrostis* (A)	Gramineae		giu-18	6f,7a	giu-24	9c	-)	C4	

C. WORKSHOPS AND SEMINARS

C1. Training and education workshops

	workshop subject location	proposed finalisation	collaboration
1	ISTA Workshop on Germination and Tetrazolium Seed Testing, Oudtshoorn, South Africa	Nov 2026	GER
2			

Agrostis capillaris
Arachis hypogaea
Glycine max
Medicago sativa
Spinacia oleracea

Contacting TEZ TCOM, within the **TEZ PT Programme** you could have the possibility to check your performance in the TEZ Test. Furthermore, you could have the possibility to collaborate in a **Working Group** participating in validation programmes regarding tetrazolium tests, which will allow you to acquire experience in this matter, demonstrate your competence on new species and get in contact with other colleagues who are also interested in sharing their knowledge.

This would allow a joint improvement with open possibilities of:

- proposing and working on a **new species** (still not included in Table 6A of the ISTA Rules) in order to validate it;

- proposing the **method** so that a new species (still not included in Table 6A of the ISTA Rules) could be studied;

- proposing that a new species already included in Table 6A of the ISTA Rules could be studied again in case you consider the **current methodology** proposed is not the most adequate.

Everybody can contact TEZ TCOM for questions, clarifications, proposals,…

	D. PROFICIENCY TESTS		
	Proficiency test subject	Scheduled in	In collaboration with
1	*PT26-3 Lolium multiflorum PUR, OSD, GER, TEZ*	2026	PTC
2	*PT27-2 Trifolium incarnatum PUR, OSD, GER, TEZ*	2027	PTC
3	*PT27-3 Helianthus annuus GER, TEZ, MIX*	2027	PTC
4	*PT28-1 Moringa oleifera GER, TEZ*	2028	PTC

Source: USDA Natural Resources Conservation Service

ISTA ANNUAL MEETING 2026

Thank you for your attention!

ISTA
ANNUAL MEETING
2026