



How to test «Wildness» according to ISTA Rules

Presenter: PhD Sergio Pasquini

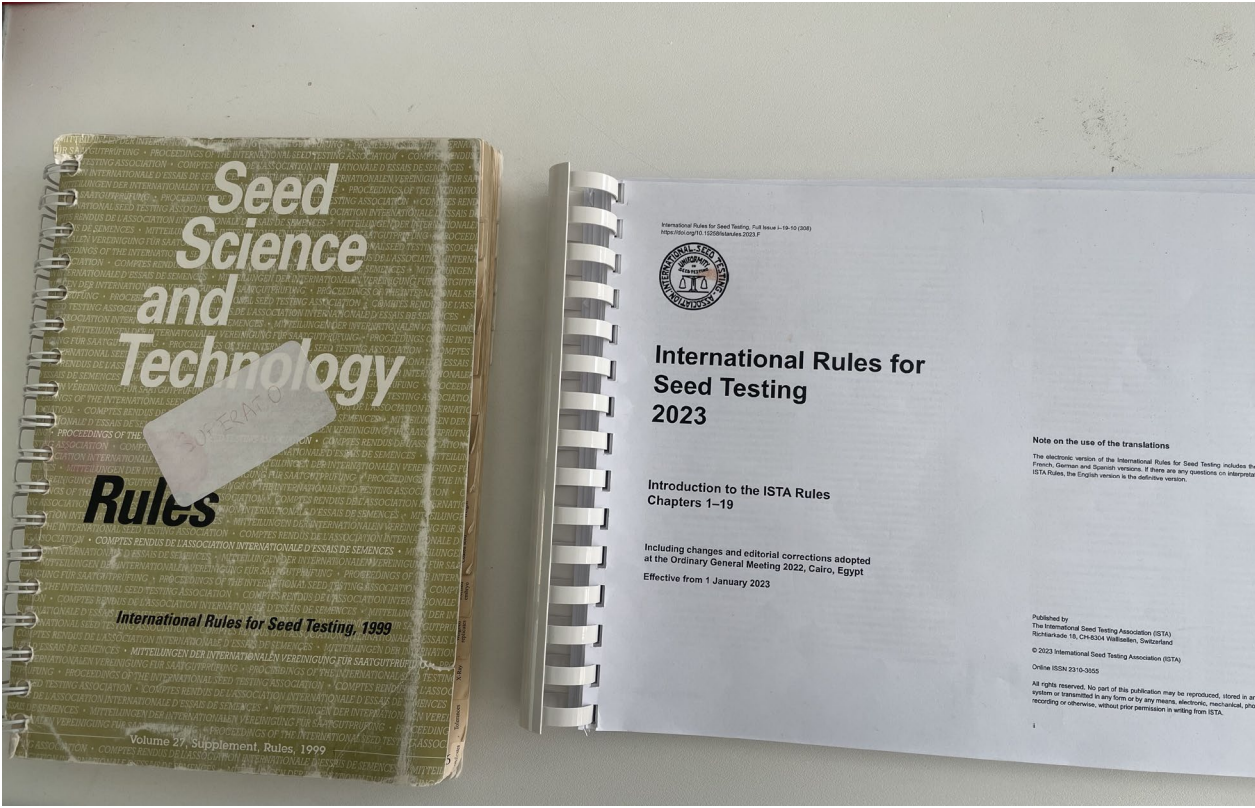
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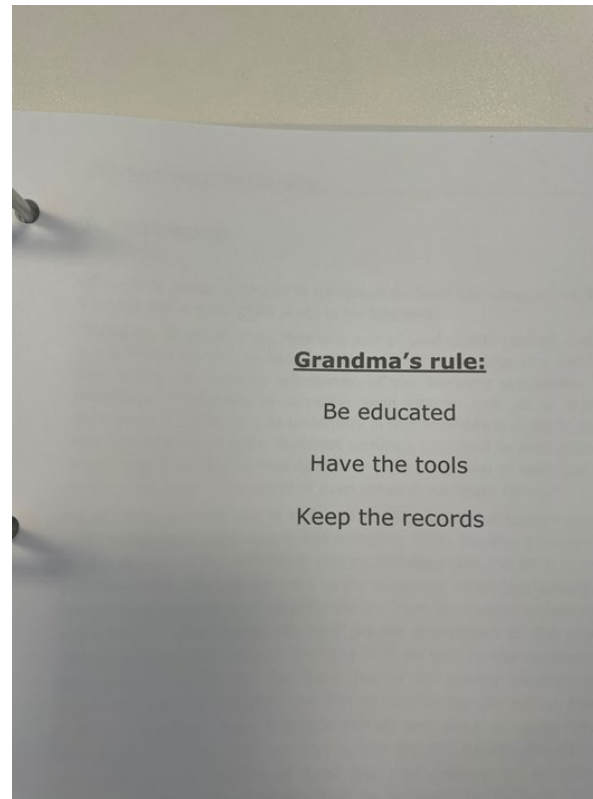
Hotel Leon d'Oro

Date:

Verona, 29 May 2023

Since 1999...





...but for wild/native species we need to consider also other two points...

This is a CAT...

...a BLACK cat...

...a FEMALE black cat...

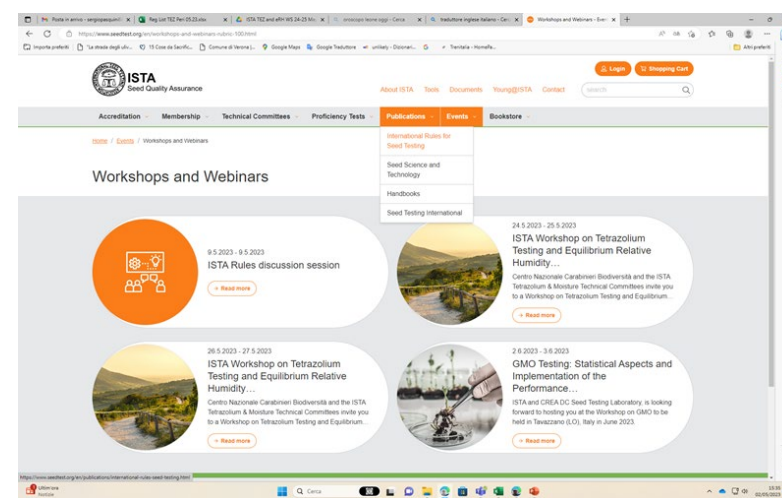
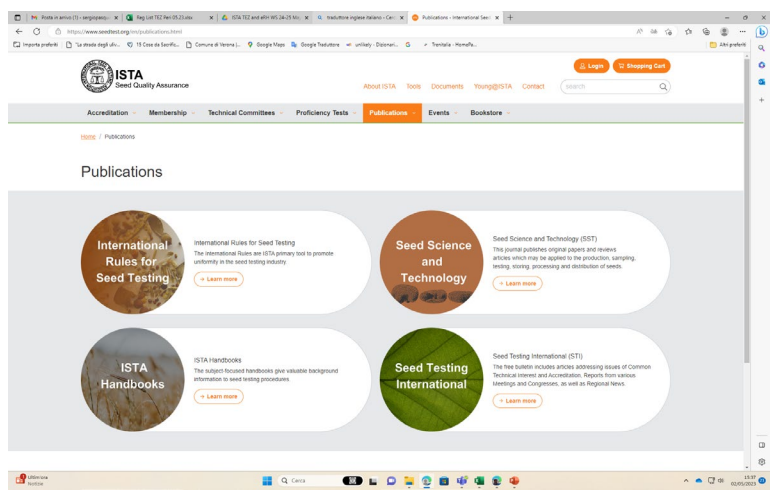
...a RELAXED female black cat...



...for testing wildness you need to be calm and confident!

...I will tell you later the name of the cat...

Be educated!



Centro Nazionale Carabinieri (Biodiversità) - Pisa (VR)
MANUALE DELLA QUALITÀ DEL LABORATORIO ANALISI SEMENTI FORESTALI

MANUALE DELLA QUALITÀ DEL LABORATORIO ANALISI SEMENTI FORESTALI

APPROVATO DA
Responsabile Qualità
Giovanni De Santis
Data: 15.05.2023

Responsabile Laboratorio
Tat. C.A. Vincenzo Di Biase
Data: 15/05/2023

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MANUALE DELLA QUALITÀ DEL LABORATORIO ANALISI SEMENTI FORESTALI

Attachment III of Quality Manual
SOP list

Identification code	Title	Personnel involved	Revision index
PO0001	Documentation control	All the staff	4
PO0002	Staff training and updating	All the staff	10
PO0003	Purchasing services and supplies management	All the staff	9
PO0004	Equipment checks	Analysts, samples, RAQ	5
PO0005	Seed reference samples	Analysts, responsible PUR	4
PO0006	Samples	Samples	10
PO0007	Samples management	All the staff	9
PO0008	Non conformities management	All the staff	6
PO0009	Corrective and preventive actions	All the staff	5
PO0010	Internal audits	Analysts, samples, RAQ	4
PO0011	Management review	RAQ, RL, DIR	5
PO0012	Design prevention control	RAQ	7
PO0013	Filing on ISTA Certificate	RAQ, RL	7
PO0014	Use of seals	Samples, RL	7
PO0015	Seedlings management	All the staff	5

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Attachment III of Quality Manual
Operative instructions list

Identification code	Title	Revision index
RO0001	Sample preparation	17
RO0002	Test methods	varie
RO0003	Seed sampling	9
RO0004	Intensities check	7
RO0005	Check of instruments for samples preparation	3
RO0006	Ovens check	4
RO0007	Germination cabinets check	7
RO0008	Certified weights and thermocouples	6
RO0009	Calibration and use of the pH meter	1
RO0010	Moisture determination	9
RO0011	Densification of Lot numbers edition	4
RO0012	Samples training and check	5
RO0013	Purchasing and check new books	2
RO0014	New species classification	2
RO0015	Bill collection	0
RO0016	Seeds calibration	0

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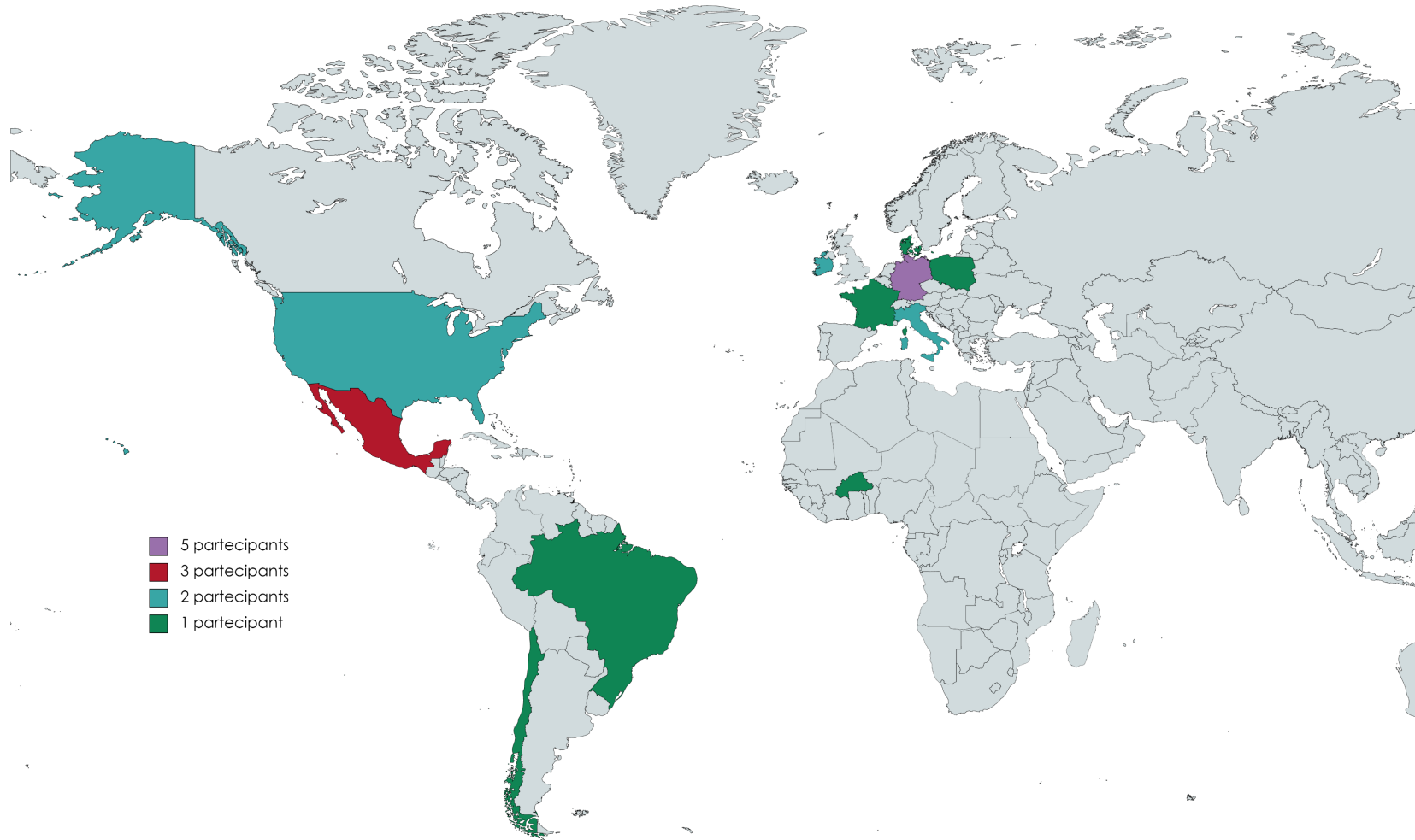
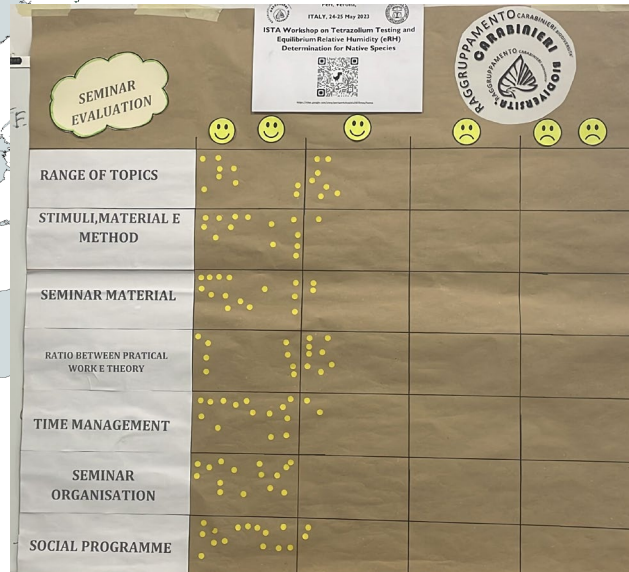
Attachment IV of Quality Manual
Quality registration list

Identification code	Title	Revision index
RO0001	End of species releases	2
RO0002	Test recording	3
RO0003	Moisture content recording	3
RO0004	Test results list	11
RO0005	Intensities list	3
RO0006	Germination test	1
RO0007	Calibration test	1
RO0008	Test preparation of keys	2
RO0009	Internal calibration	11
RO0010	Internal calibration	2
RO0011	Cut net card	3
RO0012	Report of non-conformities control	2
RO0013	Internal audit plan	2
RO0014	Report of non-conformities control	2
RO0015	Internal audit plan	2
RO0016	Internal audit plan	2
RO0017	Check for internal audit laboratory staff	0
RO0018	Distribution list of documents	0
RO0019	Check for internal audit laboratory staff	0
RO0020	Proficiency test	2
RO0021	Management review plan	2
RO0022	Management review report	3
RO0023	Handling time register	2
RO0024	Over temperature recording card	3
RO0025	Subcontract purchase to enter	2
RO0026	Procedure keeping the keys of samples count	6
RO0027	Moisture content measurement	2
RO0028	Staff performance program	14
RO0029	Germination and seeds moisture	1
RO0030	Lot's control card	2
RO0031	Samples list	2
RO0032	Samples list	0
RO0033	Samples check list	0
RO0034	Checklist	0
RO0035	Non conformities register	0
RO0036	Control review sheet	4
RO0037	Seedling Control sheet	5
RO0038	Germination schedule	1
RO0039	Price list	0

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Identification code	Title	Revision index
RO0044	Management review agenda	0
RO0045	Internal documents list	1
RO0046	Intensities check	2
RO0047	Intensities check	1
RO0048	Document reading report	1
RO0049	Seed assessment check	1
RO0050	Lot's identification	1
RO0051	Seedlings - analysis check	1
RO0052	Moisture content list	1
RO0053	Thermocouples calibration	0
RO0054	Lot's list file	0
RO0055	Internal documents list	0
RO0056	Internal audit checklist	0
RO0057	Seed and paper pH check	1
RO0058	Seed collection list	0

Workshops!





	😊	😊	😊	😊	😊	😊
RANGE OF TOPICS	1	1	1	1	1	1
STIMULI, MATERIAL E METHOD	1	1	1	1	1	1
SEMINAR MATERIAL	1	1	1	1	1	1
RATIO BETWEEN PRATICAL WORK E THEORY	1	1	1	1	1	1
TIME MANAGEMENT	1	1	1	1	1	1
SEMINAR ORGANISATION	1	1	1	1	1	1
SOCIAL PROGRAMME	1	1	1	1	1	1

Have the tools!


 INTERNATIONAL SEED TESTING ASSOCIATION
 ASSOCIATION INTERNATIONALE D'ESSAIS DE SEMENCES
 INTERNATIONALE VEREINIGUNG FÜR SAATGUTPRÜFUNG

Secretariat, Zürichstrasse 50, P.O. Box 308, 8303 Baselstadt, CH-Switzerland -
 Phone: +41-44-838 60 00 - Fax: +41-44-838 60 01 - Email: ista.office@ista.ch - <http://www.seedtest.org>



**ISTA Method Validation
for Seed Testing**

Note: Any electronic or hard copies of this document are uncontrolled copies.
 Confirm validity before use.



Glycine max L.
 «Smoothly» seed



Triers



Moringa oleifera Lam.
 «Chaffy» seed



Dividers



Ulmus glabra L.,
Populus nigra L.
 «Windy» seed



Your hands!

ISTA Method Validation test plan: the simplest case



Glycine max L.

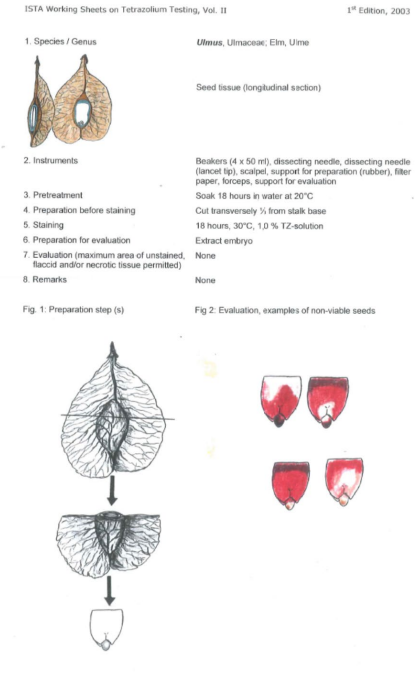
Validation test on <i>Glycine max L</i>			
Test Leader	Ignacio Aranciaga		
Reviewers	Augusto Martinelli	Didier Demilly	Jean-Louis Laffont
Participants			
ISTA Accredited labs	Argentina	Ignacio Aranciaga	ARDL01
	Canada	Tauhid Parvez	CAML12
	France	Valerie Blouin	FRDL02
	Germany	Stefanie Kraemer	DEDL04
	Italy	Alessandra Arioli	ITDL03
	United Kingdom	Asia Roberts-Yalland	GBDL01
Not accredited labs	Argentina	M. Belen Aranguren	Corteva
	Brazil	Jose B. Franca-Neto	Embrapa

ISTA Peer Method Validation test plan: a more difficult case

Participants	Country
Stefanie Kraemer (DEDL04)	Germany
Ralph Jenner (no ISTA lab)	Germany
Sergio Pasquini (ITML06)	Italy

Species	Prescription for				
	Substrate	Temp. (°C)	First count (d)	Final count (d)	Additional direction incl. recommendation from breaking dormancy
Ulmus spp.	TP	20⇔30; (20)	7	14	pericarp may be removed

Species	Pretreatment						Permitted non-viable tissue	Remarks
	type/minimum time (h)	Preparation before staining	Staining solution (%)	Optimum staining time (h)	Preparation for evaluation			
Ulmus spp.	Soak 18 hours in water at 20°C	Cut transversely 1/3 from stalk base	1	18 hours, 30°C	Extract embryo	none	none	



Ulmus glabra L.

ISTA Proposal Method for Validation test plan: the most difficult case



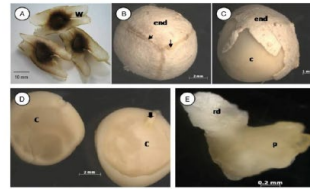
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Phone: +41 44 838 60 00 | Fax: +41 44 838 60 01
Email: ista.office@ista.ch
www.seedtest.org

1. TEZ Tetrzolium Committee

TEZ Tetrzolium Committee		
COMMITTEE MEMBERSHIP LIST		
1	Chair: Sergio Pasquini	Italy
2	Vice-Chair: Steffi Krämer	Germany
3	Ignacio Aranciaga	Argentina
4	Maria Belen Aranguren	Argentina
5	Alessandra Arioli	Italy
6	Jose de Barros Franca-Neto	Brazil
7	Sunita BH	India
8	Valerie Blouin	France
9	Augusto Martinelli	Argentina
10	Shaminder Miranpuri	USA
11	Tauhid Parvez	Canada
12	Asia Roberts-Yalland	United Kingdom
13	Veena Gouda	India
14	Edith Daboue	Burkina Faso
15	free position	from Oct. 22

Tetrazolium method for *Moringa oleifera* Lam.

Family: Moringaceae
Genus: Moringa

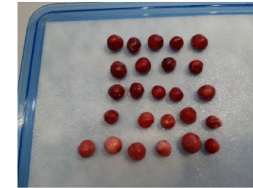


Moringa seed morphology. (A) Seed with seed coat and hilum. (B) Seed with internal seed coat and vascular bundle. (C) Seed with partially removed internal seed coat. (D) Separated cotyledon. (E) Embryo axis. W: Wing (w); vascular bundle (arrowhead); cotyledon (c); endosperm (en); embryonic axis (black arrow; radial (r); uterine (u)).

Pre-treatments: remove seed coat (first integument) from dry seeds, then soak in water 18 hours at 20°C

Preparation before staining: remove the entire second integument gently with the hands and/or with the help of a needle/scalpel without damaging the seeds.

Staining time: 18 hours, 30 °C, 1.0 % TEZ-solution



Preparation for evaluation: cut longitudinally through cotyledons, expose embryonic axis

Evaluation: cotyledons and embryonic axis have to be red coloured as in the example below. Tissues in viable seeds have to be firm and not flaccid



Note: *Moringa oleifera* is a species rapidly germinating in about a week: TEZ test is time consuming more than germination one.



Moringa oleifera Lam.

Join us!

Keep the records!

Total results - Excel

GER TEST	LAB #1				n. of seeds	LAB #2				n. of seeds	LAB #3				n. of seeds
	normal	abnormal	dead	empty		normal	abnormal	dead	n. of seeds		normal	abnormal	dead	n. of seeds	
LOT 1															
rep 1	20	1	1	3	25	rep 1	13	0	12	25	rep 1	20	2	3	25
rep 2	15	0	3	7	25	rep 2	17	1	7	25	rep 2	19	3	3	25
rep 3	20	1	1	3	25	rep 3	20	0	5	25	rep 3	19	3	3	25
rep 4	15	0	2	8	25	rep 4	15	1	9	25	rep 4	21	2	2	25
total	70	2	7	21	100	total	65	2	33	100	total	79	10	11	100
LOT 2															
rep 1	10	0	7	8	25	rep 1	12	1	12	25	rep 1	16	4	5	25
rep 2	12	0	7	6	25	rep 2	17	2	6	25	rep 2	15	2	8	25
rep 3	7	0	5	13	25	rep 3	11	1	13	25	rep 3	16	2	7	25
rep 4	14	0	2	9	25	rep 4	11	0	14	25	rep 4	17	3	5	25
total	43	0	21	36	100	total	51	4	45	100	total	64	11	25	100
LOT 3															
rep 1	18	1	0	6	25	rep 1	14	0	11	25	rep 1	17	3	5	25
rep 2	17	0	1	7	25	rep 2	13	2	10	25	rep 2	16	3	6	25
rep 3	12	3	3	7	25	rep 3	15	2	8	25	rep 3	18	3	4	25
rep 4	14	2	1	8	25	rep 4	15	2	8	25	rep 4	19	3	3	25
total	61	6	5	28	100	total	57	6	37	100	total	70	12	18	100

Total results - Excel

TEZ TEST	LAB #1			n. of seeds	LAB #2			n. of seeds	LAB #3			n. of seeds
	viable	non-viable	empty		viable	non-viable	n. of seeds		viable	non-viable	n. of seeds	
LOT 1												
rep 1	14	8	3	25	rep 1	59	41	100	rep 1	21	4	25
rep 2	21	3	1	25	rep 2	69	31	100	rep 2	22	3	25
rep 3	16	6	3	25	rep 3	62	38	100	rep 3	22	3	25
rep 4	21	4	0	25	rep 4	69	31	100	rep 4	21	4	25
total	72	21	7	100	total	65	35	100	total	86	14	100
LOT 2												
rep 1	17	3	5	25	rep 1	48	52	100	rep 1	18	7	25
rep 2	16	5	4	25	rep 2	45	55	100	rep 2	18	7	25
rep 3	16	2	7	25	rep 3	47	53	100	rep 3	20	5	25
rep 4	16	3	6	25	rep 4	51	49	100	rep 4	17	8	25
total	65	13	22	100	total	48	52	100	total	73	27	100
LOT 3												
rep 1	18	6	1	25	rep 1	61	39	100	rep 1	20	5	25
rep 2	17	5	3	25	rep 2	58	42	100	rep 2	21	4	25
rep 3	15	4	6	25	rep 3	71	29	100	rep 3	19	6	25
rep 4	18	4	3	25	rep 4	69	31	100	rep 4	21	4	25
total	68	19	13	100	total	65	35	100	total	81	19	100

Be calm and confident!

ANOVA Table of type III with Satterthwaite approximation for degrees of freedom:

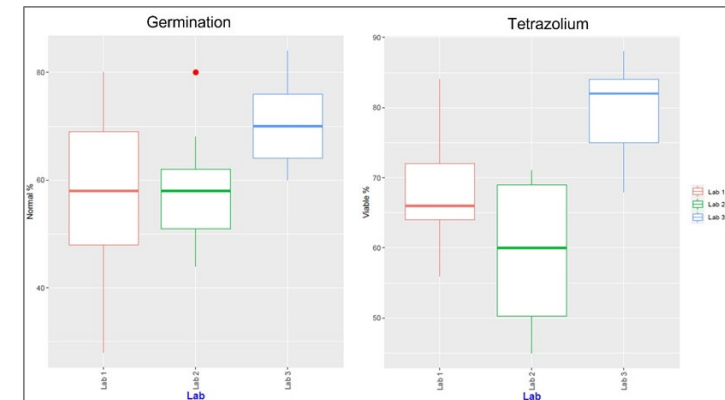
Source of variation	Sum of Squares	Mean Square	Num DF	Den DF	F value	Pr(>F)
Lot	2094.222	1047.111	2	31	13.06547	7.666548e-05

Least Squares Means Table:

	Estimate	Std. Error	lower	upper
Lot 1	71.33333	4.870724	55.89189	86.77478
Lot 2	52.66667	4.870724	37.22522	68.10811
Lot 3	62.66667	4.870724	47.22522	78.10811

Differences of Least Squares Means Table:

	Estimate	Std. Error	lower	upper
Lot 1 - Lot 2	18.66667	3.654755	11.212746	26.120588
Lot 1 - Lot 3	8.66667	3.654755	-1.212746	16.120588
Lot 2 - Lot 3	-10.00000	3.654755	-17.453921	-2.546079



...there will be always a Statistician friend of you able to maintain you **calm!**

...and he is really **confident** with all your results!

The secret «spice» of your receipt is...

the FRIENDSHIP!

Cast ...in order of appearance...



Ignacio Aranciaga, Augusto Martinelli, Didier Demilly



Steffi Kraemer, Ralph Jenner, Jean-Louis Laffont



TEZ WG: Steffi Kraemer, Valerie Blouin, Tahuid Parvez, Edith Daboue, Sergio Pasquini



ISTA Secretariat

1. Objectives

The objectives of this comparative tetrazolium test are:

1. To validate *Moringa oleifera* Lam. for introduction into Chapter 6.
2. To confirm that the protocol used will meet objective 1, and identify any amendments necessary.



New species to test

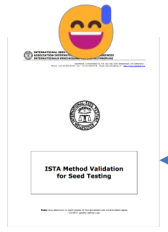
Prepare a method to test the seeds

Do you want to add this species to ISTA Rules?

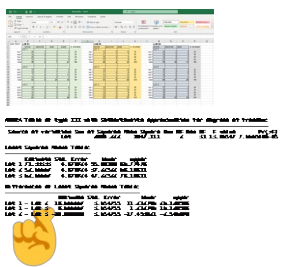
NO → Job is done! 😊

YES → Keep the seeds possibly from different seedlots and find at least two friends!

Prepare the Method Validation Test Plan and do it!



Do the tests and **KEEP THE RECORDS!**



Prepare a Rule Proposal to be voted at AM



...I will tell you the name of the cat next time, when I will be more confident... 🤔



Thank you!

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