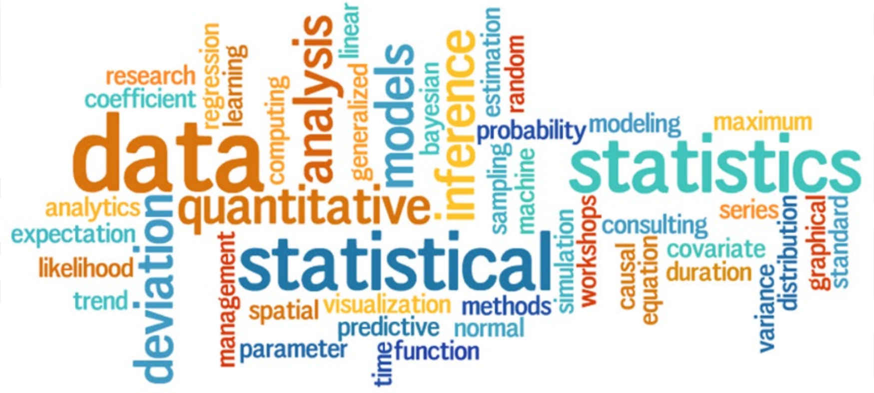
ISTA Statistics Committee 2022 – 2023 Activity Report



Presenter: Kirk Remund & Jean-Louis Laffont
Location: Verona, Italy
Date: June 2023

ISTA Statistics Committee Leadership

Kirk and Jean-Louis at face-to-face working meeting in Johnston, Iowa (US) on April 20, 2022

We have been doing training, math calculations and statistical models for ISTA for over 18 years…

ISTA Statistics Committee

Chair:	Kirk Remund	USA
Vice:	Jean-Louis Laffont	USA
Members:	Gabriel Carré	France
	Mustapha El Yakhlifi	France
	Zhou Fang	USA
	Bonnie Hong	USA
	Bo-Jein Kuo	Separate Custom Territory of Taiwan, Penghu, Kinmen and Matsu
	Ray Shillito	USA
	Thomas Michelon	Brasil
	Oluseyi Odubote	USA

ISTA ANNUAL MEETING
29 MAY-01 JUNE, 2023
VERONA, IT

ISTA Statistics Committee Activities

- Testing plan and method validation report reviews
- ISTA rules proposals
- Statistical analysis & simulation
- Seed Science & Technology reviews
- Theoretical contributions
- Seed testing tools development
- ISTA & industry workshops
- ISTA & industry collaborations
- ISTA tech. committees and member questions
- Develop next generation (Young@ISTA)

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

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

APPENDIX 5: Instructions for Reviewers: Draft Test Plan

Please review the enclosed draft test plan with reference to the evaluation criteria below, making comments on additional sheets as appropriate.

Test plan title: Additional germination method for *Papaver somniferum* – Validation study of the germination method

Author: Vladislava Gregorová

Submission date: May 19, 2021

Reviewer name: Jean-Louis Laffont

Review request date:

Review returned date: July 16, 2021

The method described in this draft test plan should be considered as a:

New Method ☐ Additional Method ☑
Replacement Method ☐ Method Modification ☐

Evaluation Criteria (not all aspects will necessarily apply):

	Yes	No	See Comments
Is the test plan presented in the correct format?	✓		
Is the nomenclature/taxonomy correct?			Not evaluated
Is the purpose of the method and need for validation adequately explained?	✓		
Is the method description clear and unambiguous?	✓		
Are parameters for accuracy, repeatability, reproducibility and uncertainty of the test method identified?	✓		
Are relevant safety precautions adequate?			Not evaluated
Are any reagents and apparatus described or defined in performance terms?			Not evaluated
Is the method described suitable for meeting the objective(s) of the test?	✓		
Are relevant critical steps/parameters identified?	✓		
Are parameters for quality control of method performance defined?			Not indicated
Are potential participating laboratories identified?	✓		
Are data analysis methods given appropriate?	✓		
Is a participant registration form included?		✓	
Are data record sheets and instructions for their completion included?	✓		
Are all tables, figures and terms sufficiently explained?			Not relevant

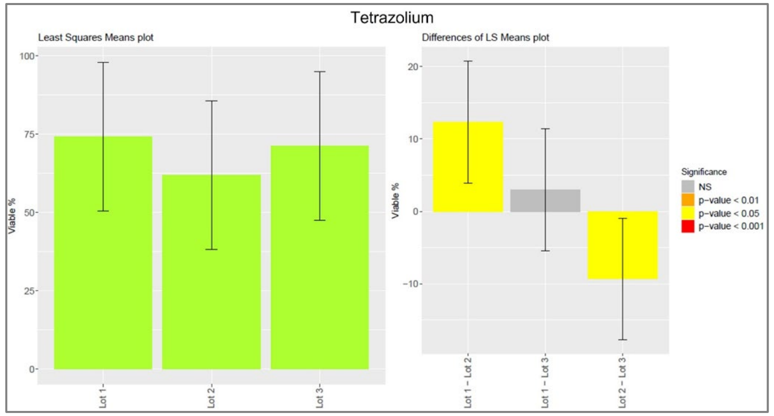
Approved by ECOM: 30.11.2006 Version: 1.0
ISTA Method Validation for Seed Testing-V1.0 Status: FINAL 29/04/2022

```
Mean s_repeatability disp s_Reproducibility s_Lab s_LotxLab
  69            6.28 0.96             12.19 10.26      1.98
```

```
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       710.1356    355.0678      2      4 9.008063 0.03300944

Least Squares Means Table:
--------------------------
      Estimate Std. Error    lower    upper
Lot 1 74.25000   6.299437 50.59267 97.90733
Lot 2 61.91667   6.299437 38.25933 85.57400
Lot 3 71.25000   6.299437 47.59267 94.90733

Differences of Least Squares Means Table:
-----------------------------------------
              Estimate Std. Error      lower      upper
Lot 1 - Lot 2 12.333333   3.030707   3.918741 20.7479255
Lot 1 - Lot 3  3.000000   3.030707  -5.414592 11.4145922
Lot 2 - Lot 3 -9.333333   3.030707 -17.747926 -0.9187411
```



Support to other TCOMs – Report reviews

- **3 test plan reviews**
- **1 validation study review**
- **1 review for SST**

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

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Is the nomenclature/taxonomy correct?			Not evaluated
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Is the method description clear and unambiguous?	✓		
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Are relevant safety precautions adequate?			Not evaluated
Are any reagents and apparatus described or defined in performance terms?			Not evaluated
Is the method described suitable for meeting the objective(s) of the test?	✓		
Are relevant critical steps/parameters identified?	✓		
Are parameters for quality control of method performance defined?			Not indicated
Are potential participating laboratories identified?	✓		
Are data analysis methods given appropriate?	✓		
Is a participant registration form included?		✓	
Are data record sheets and instructions for their completion included?	✓		
Are all tables, figures and terms sufficiently explained?			Not relevant

Approved by ECOM 30.11.2006 Version: 1.0
ISTA Method Validation for Seed Testing-V1.0 Status: FINAL 29/04/2022

Support to other TCOMs – Consulting

	Subject of Question	From	Date
1	GMO percentage estimates in presence of 2 events not stacked	Italy	June
2	AOSA validation bridging to ISTA	United States	July
3	Method Validation	Czech Republic	July
4	SeedCalc	United States	August
5	Data transformation	Italy	August
6	GMO Uncertainty	Italy	October
7	Germination Tolerance Calculator	Scotland	October
8	ISTAgermMV R package	Argentina	December
9	Use of control samples for germination	United States	February
10	Compraing moisture results across labs	Denmark	March

- Support to GMO Committee: rating of GMO PT 23 quantitative results

Support to other TCOMs – Tools

Purity/OSD minimum working sample weights calculator

for

Purity Committee

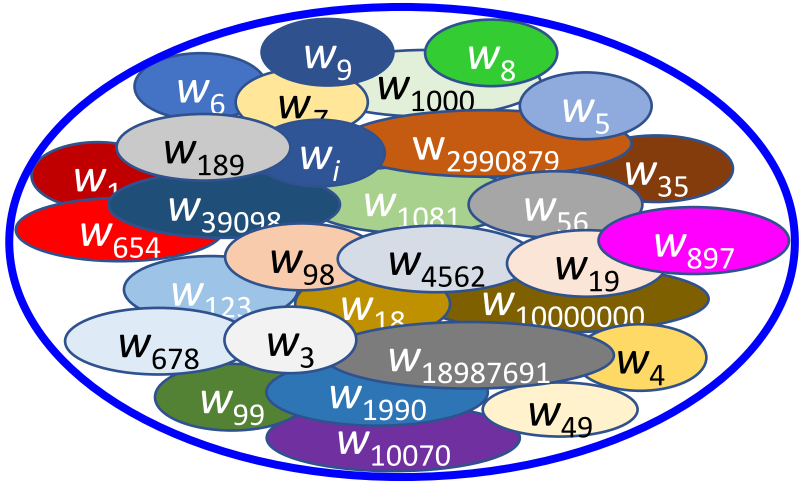
International Seed Testing Association News Bulletin No. 165 April 2023

Method Development for ISTA Rules Proposals:
Working Sample Weight Determination for a New Species

Jean-Louis Laffont[1], Kirk Remund[2] and Ruojing Wang[3]

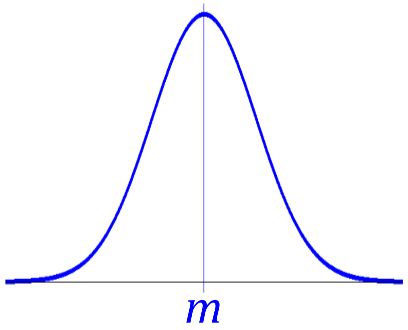
[1]ISTA Statistics Committee Vice-Chair; Grézels, France
[2]ISTA Statistics Committee Chair; Bayer Crop Science, St. Louis, Missouri, USA
[3]ISTA Purity Committee Chair; Seed Science and Technology Section, Canadian Food Inspection Agency, Saskatoon, Canada; ruojing.wang@inspection.gc.ca

Population (all possible varieties, lots, labs and 100 seeds samples) of 100 seed weights



Assumption

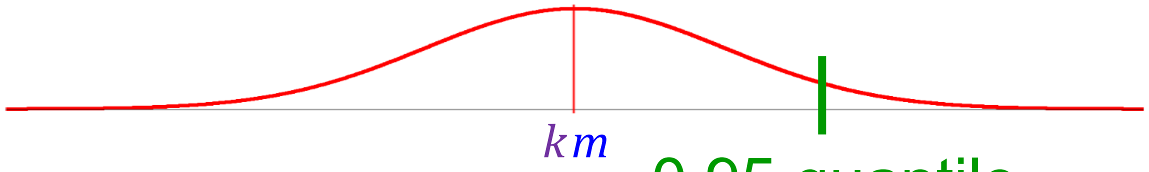
Y ~ Normal distribution with mean m and variance σ^2



X : "2500 (k = 25) or 25000 (k = 250) seeds weight"

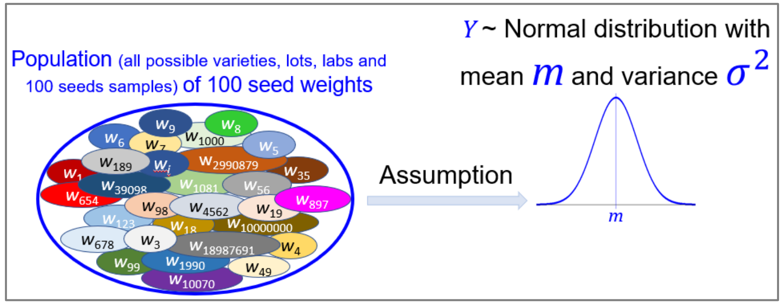
If $Y \sim N(m, \sigma^2)$, then

$X = kY$ is $\sim N(km, k^2\sigma^2)$



0.95 quantile

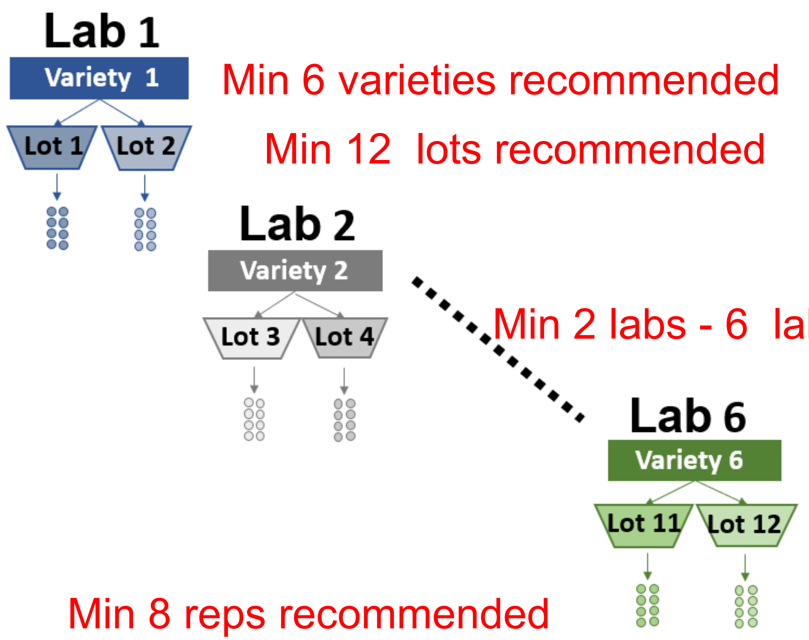
95% confident to have at least 2500 or 25000 seeds in a random sample with the 0.95 quantile weight



Estimating m and σ^2

2 experiment designs to capture all the possible sources of variation at its best

Experiment design 1: 2-way nested design



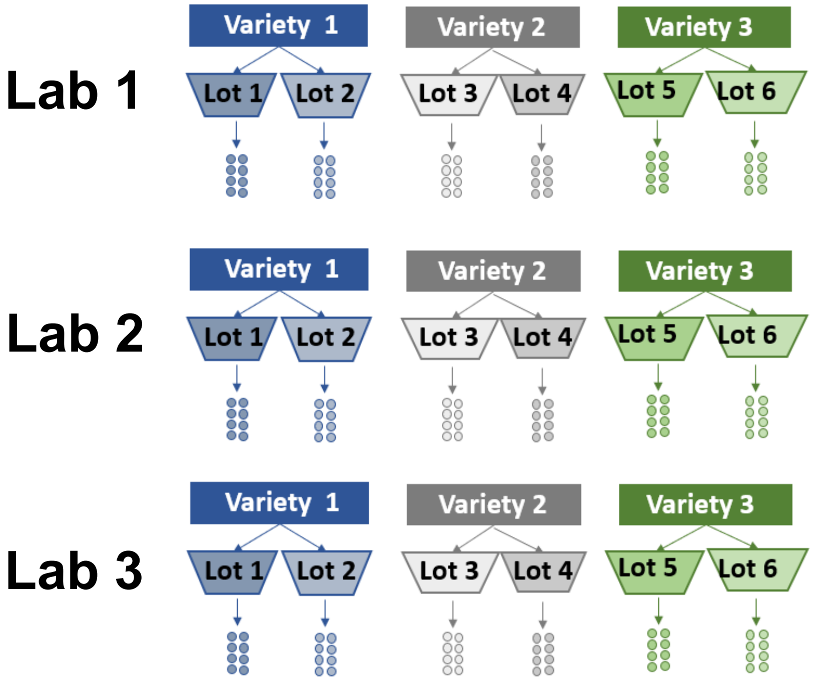
Min 6 varieties recommended

Min 12 lots recommended

Min 2 labs - 6 labs preferred

Min 8 reps recommended

Experiment design 2: 2-way crossed design



Min 3 varieties recommended

Min 6 lots recommended

Min 8 reps recommended

Min 2 labs - 6 labs preferred

Purity/OSD minimum working sample weights calculator

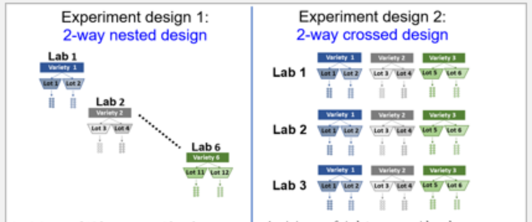
Flexibility in design and robustness of calculations within a MS Excel spreadsheet

Calculator for adding working weights to Table 2C of the ISTA Rules

THE CACULATOR IS PROVIDED "AS IS", WITHOUT WARRANTY OF ANY KIND. IN NO EVENT SHALL THE AUTHORS BE LIABLE FOR ANY CLAIM, DAMAGES OR OTHER LIABILITY ARISING IN CONNECTION WITH THE CALCULATOR.

Experiment designs

Two types of experiment designs are considered in the calculator:



A minimum of 12 lots are considered across a minimum of six varieties represented in the experiment as a general rule.
These 12 lots will be evaluated by a minimum of two labs however six labs are preferred.
A minimum of eight 100 seed reps are weighed per lot.

A minimum of six lots are considered across a minimum of three varieties represented in the experiment as a general rule.
These six lots will be evaluated each by a minimum of two labs however six labs are preferred.
A minimum of eight 100 seed reps are weighed per lot.

Calculations

The rep weights are entered into the unprotected yellow cells of the calculator. If experiment design 1 is used, data for the different lots from each lab are entered in different columns. In order to avoid conditional formatting conflicts, always copy/paste data in the calculator using Paste Special → Values.

. For each lot in a given laboratory, outliers are highlighted in red using Grubbs's method at the 5% significance level (Grubbs, 1969). These outliers are then excluded manually from the computations.

. The linear random effects models used for the analysis of the two experiment designs are:

. **Experiment design 1**:

$$y_{ijk} = \mu + \alpha_i + \beta_{ij} + e_{ijk}$$

in which:
. y_{ijk} is the observed 100-seeds weight of lot j ($j = 1, 2, ..., b_i$) in lab i ($i = 1, 2, ..., a$) and replication k ($k = 1, 2, ..., n_{ij}$);
. μ is the intercept;
. α_i is the random effect of lab i ($\alpha_i \sim$ i.i.d. $N(0, \sigma^2_{Lab})$);
. β_{ij} is the random effect of lot j within lab i ($\beta_{ij} \sim$ i.i.d. $N(0, \sigma^2_{Lot})$);
. e_{ijk} is the residual ($e_{ijk} \sim$ i.i.d. $N(0, \sigma^2_{Res})$).

. **Experiment design 2**:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

in which:
. y_{ijk} is the observed 100-seeds weight of lot j ($j = 1, 2, ..., b$) in lab i ($i = 1, 2, ..., a$) and replication k ($k = 1, 2, ..., n_{ij}$);
. μ is the intercept;
. α_i is the random effect of lab i ($\alpha_i \sim$ i.i.d. $N(0, \sigma^2_{Lab})$);
. β_j is the random effect of lot j ($\beta_j \sim$ i.i.d. $N(0, \sigma^2_{Lot})$);
. $(\alpha\beta)_{ij}$ is the random interaction effect between lab i and lot j ($(\alpha\beta)_{ij} \sim$ i.i.d. $N(0, \sigma^2_{Lab \times Lot})$);
. e_{ijk} is the residual ($e_{ijk} \sim$ i.i.d. $N(0, \sigma^2_{Res})$).

The calculator automatically selects which model to fit according to the dataset structure.

. Variance components for the two models are estimated from the data by the Henderson Method I (Searle *et al.*, 1992, Appendix F). When an estimate is negative, this estimate is reported as zero. Let $\hat{\sigma}^2_{Lab}$, $\hat{\sigma}^2_{Lot}$, $\hat{\sigma}^2_{Lab \times Lot}$ and $\hat{\sigma}^2_{Res}$ be these

Instructions | Calculator

Supporting Data of New Species Proposal to ISTA Rules Table 2C

Submitter Name:
Scientific Name of the Crop kind: Genus Species
Lab Full Name:
ISTA Member Code:
Contact Email:

Change any value in a yellow cell

Number of observations	0	
Number of labs	0	
Number of lots	0	
General mean		
Lab variance		
Lot variance		
Lab x Lot variance		
Residual variance		Decision
2500 seed weight*		
25000 seed weight*		

* 95% Confidence

Rep weights in red are identified as outliers by Grubbs's method at the 5% significance level and needs to be suppressed (removed) manually

Lab	\ Seed lot	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Lab 1	Rep1																	
	Rep2																	
	Rep3																	
	Rep4																	
	Rep5																	
	Rep6																	
	Rep7																	
	Rep8																	
	Rep9																	
	Rep10																	
	Rep11																	
	Rep12																	
	Mean																	
	St. Dev.																	
	Number of reps	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Grubbs critical values																	
Lab 2	Rep1																	
	Rep2																	
	Rep3																	
	Rep4																	
	Rep5																	
	Rep6																	
	Rep7																	
	Rep8																	
	Rep9																	
	Rep10																	
	Rep11																	

Instructions | Calculator

Purity/OSD minimum working sample weights calculator

Takes into account lab-to-lab, seed lot and within lot measurement variation to estimate conservative minimum working sample weight for high confidence of obtaining 2500 and 25000 seeds in sample

When needed, some warnings are displayed in red

Supporting Data of New Species Proposal to ISTA Rules Table 2C

Submitter Name:	XXX	Lab Full Name:	YYY
Scientific Name of the Crop kind:	Basella B. alba	ISTA Member Code:	ZZZ
		Contact Email:	AAA

Change any value in a yellow cell

Number of observations	232	
Number of labs	7	
Number of lots	5	6 lots are preferred for an accurate estimation
General mean	3.2584	
Lab variance	0.0020037	
Lot variance	0.1453077	
Lab x Lot variance	0.0963083	
Residual variance	0.0101622	Decision
2500 seed weight*	103	100
25000 seed weight*	1022	1050

* 95% Confidence

Decision value should be greater than or equal to 103

Rep weights in red are identified as outliers by Grubbs's method at the 5% significance level and needs to be suppressed (removed) manually

Lab	\ Seed lot	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Lab 2	Rep1	2.3672	3.4036	2.3585	3.1927	3.7473											
	Rep2	2.2734	3.4207	2.3530	3.0972	3.7309											
	Rep3	2.3198	3.5878	2.4268	3.2861	3.7818											
	Rep4	2.3866	3.4322	2.3827	3.2858	3.7380											
	Rep5	2.3600	3.3296	2.2917	3.2861	3.7908											
	Rep6	2.3720	3.3873	2.2663	3.1889	3.8542											
	Rep7		3.4601	2.2407	3.1103												
	Rep8			2.7779	3.2201												
	Rep9																
	Rep10																
	Rep11																
	Rep12																
	Mean	2.3465	3.4316	2.3872	3.2084	3.7738											
	St. Dev.	0.04225	0.08008	0.16963	0.07636	0.04613											
	Number of reps	6	7	8	8	6	0	0	0	0	0	0	0	0	0	0	0
	Grubbs critical values	1.89	2.02	2.13	2.13	1.89											

Outliers are automatically identified in red

Provided ISTA webinar for Purity/OSD minimum working sample weight calculator

April 24, 2023

93 participants to webinar

On Wednesday, April 26, 2023, 15:00 - 16:00 CEST, we will be holding a webinar, discussing ISTA's new statistical tool for determining working sample weight to amend Table 2C of the ISTA Rules.

Panelists:

1. Ruojing Wang, Chair of ISTA Purity Committee
2. Kirk Remund, Chair of ISTA Statistics Committee
3. Jean-Louis Laffont, Vice-Chair of ISTA Sattistics Committee

Moderator:

- Dr. Andreas Wais, ISTA Secretary General

The webinar will be interactive and questions from the audience will be gathered and discussed during the dedicated Q&A time slot.

Everyone is welcome to attend and participate, so please hurry up and register now!

Special project for ECOM and Industry

Vegetables Seed Industry Working group (VSI WG)

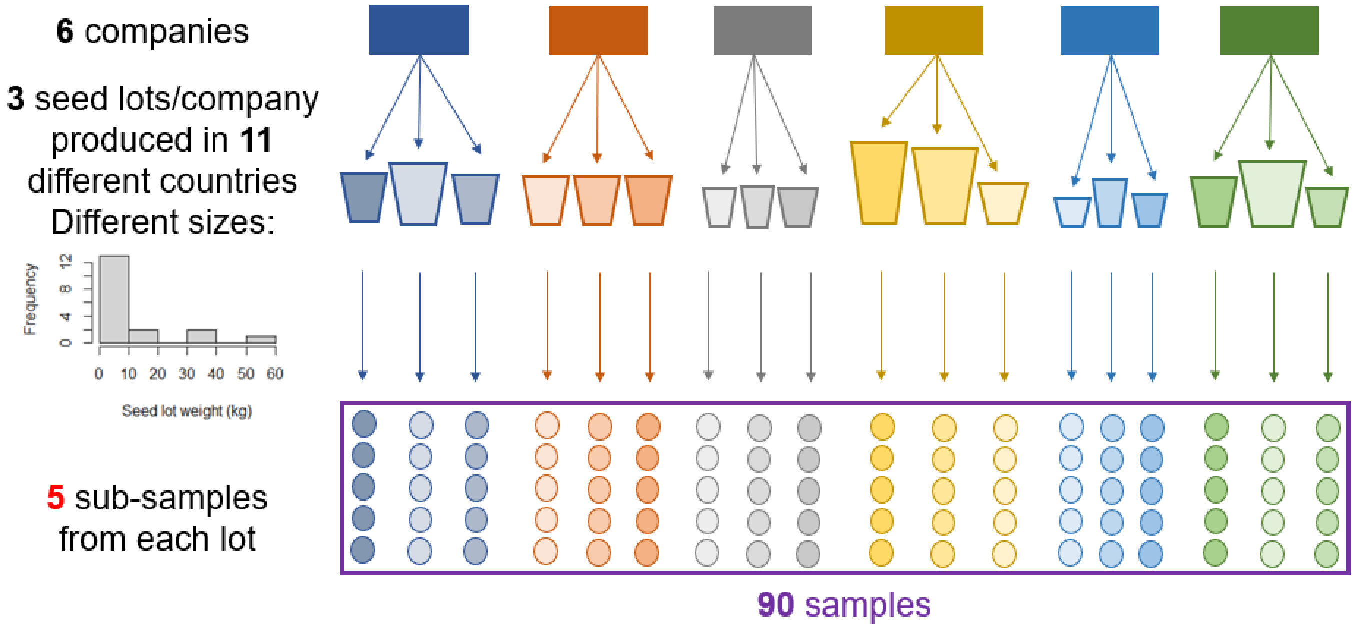
Number of sub-lots for which an OIC established for the lot is still valid

conclusion for work from previous year

Special project – Vegetables Seed Industry Working group (VSI WG)

ISTA ANNUAL MEETING 29 MAY-01 JUNE, 2023 VERONA, IT

- **Objective:** assessment of the possibility of increased number of sub-lots (increase from **5** to XXX sub-lots) from an original (mother) tomato seed lot
- Experiment design for assessing level of heterogeneity:



Conclusion from 2021 study:
Tomato seed lots are homogenous within sizes considered

Assumption: seed lot is **homogeneous**

True lot value: π

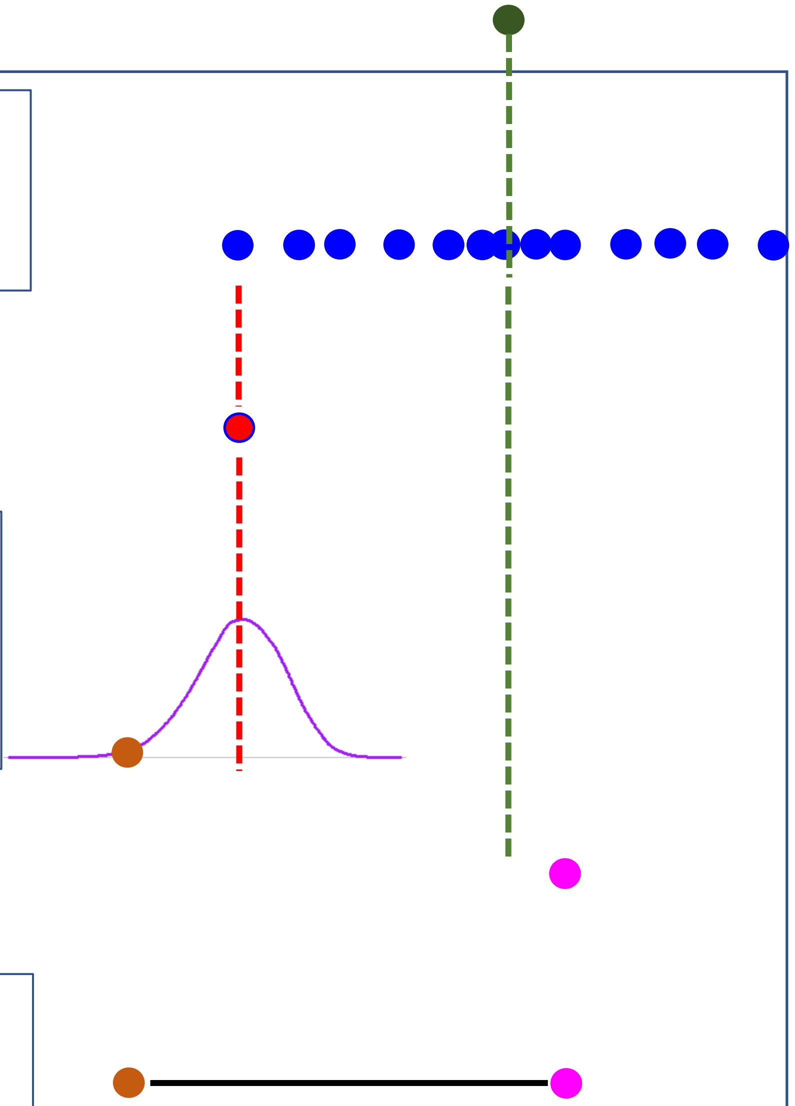
M true sub-lot values π_i from multivariate hypergeometric distribution *

Minimum of the true M sub-lot values: $\pi_m = min\left(\{\pi_i\}_{i=1}^{M}\right)$

Test value from another lab on the sub-lot with minimum value from a **Beta-binomial** distribution: p_2

Estimate of lot value: p from k seeds

Determine if p and p_2 are within tolerance according to ISTA Tolerance Table 5F



Repeated 10,000 times

Compute the probability that p is not different from p_2 as $\frac{T}{10000}$ where T is the number of times p and p_2 are within tolerance

Table 5F. Tolerances between results of two tests made in different laboratories on the same or different samples from the same seed lot (two-way test at 5 % significance level) on 400 seed tests. Updated by ISTA Statistics Technical Committee, based on Miles (1963) Table G5, column C, 400 seed tests.

Average germination percentage of 2 tests		Tolerance
51–100 %	0–50 %	
99	2	2
98	3	3
96–97	4–5	4
94–95	6–7	5
91–93	8–10	6
88–90	11–13	7
84–87	14–17	8
79–83	18–22	9
74–78	23–27	10
68–73	28–33	11
60–67	34–41	12
51–59	42–50	13

* Laffont, J-L., Hong, B., Kuo, B-J. and K.M. Remund (2019). Exact theoretical distributions around the replicate results of a germination test. *Seed Science Research* **29**, 64-72.

Special project – Vegetables Seed Industry Working group (VSI WG)

Presented results to ECOM/TCOMs and Vegetable Seed Industry Working Group

Concluded the number of sublots for tomato can be increased from 5 to 20 that can be released on same OIC (mother lot).

Sampling of other committee activities

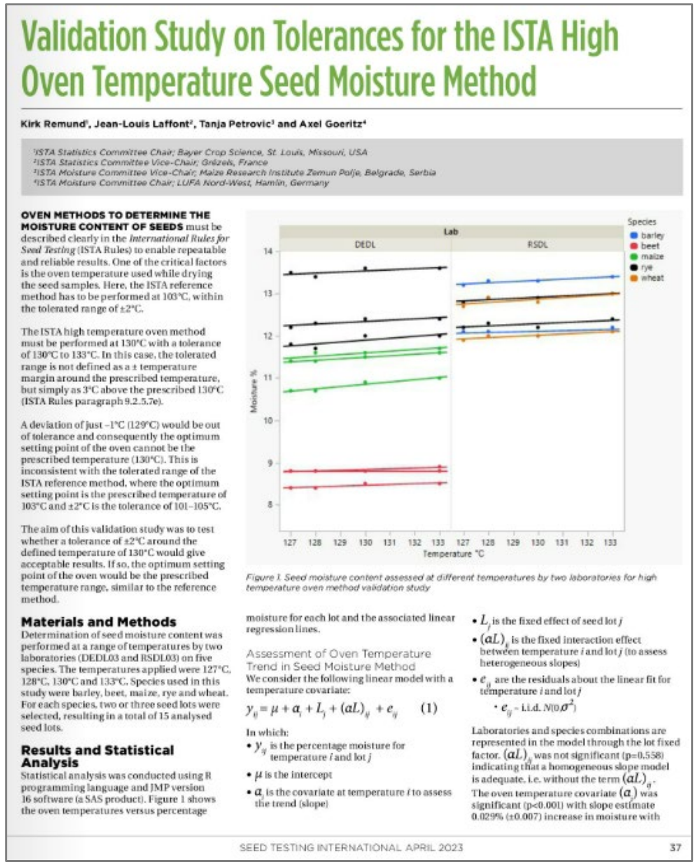
ISTA ANNUAL MEETING
29 MAY-01 JUNE, 2023
VERONA, IT

- **ISTA/ISSS/ INSR Webinar in November 2022**
  "Web-based resources for seed scientists"

- **Rebuild of ISTAGermMV for R 4.0**

- **High Oven Temperature range tolerance for seed moisture for rules change**

International Seed Testing Association News Bulletin No. 165 April 2023



Validation Study on Tolerances for the ISTA High Oven Temperature Seed Moisture Method

Kirk Remund[1], Jean-Louis Laffont[2], Tanja Petrovic[3] and Axel Goeritz[4]

[1]ISTA Statistics Committee Chair; Bayer Crop Science, St. Louis, Missouri, USA
[2]ISTA Statistics Committee Vice-Chair; Grézels, France
[3]ISTA Moisture Committee Vice-Chair; Maize Research Institute Zemun Polje, Belgrade, Serbia
[4]ISTA Moisture Committee Chair; LUFA Nord-West, Hamlin, Germany

OVEN METHODS TO DETERMINE THE MOISTURE CONTENT OF SEEDS must be described clearly in the *International Rules for Seed Testing* (ISTA Rules) to enable repeatable and reliable results. One of the critical factors is the oven temperature used while drying the seed samples. Here, the ISTA reference method has to be performed at 103°C, within the tolerated range of ±2°C.

The ISTA high temperature oven method must be performed at 130°C with a tolerance of 130°C to 133°C. In this case, the tolerated range is not defined as a ± temperature margin around the prescribed temperature, but simply as 3°C above the prescribed 130°C (ISTA Rules paragraph 9.2.5.7e).

A deviation of just –1°C (129°C) would be out of tolerance and consequently the optimum setting point of the oven cannot be the prescribed temperature (130°C). This is inconsistent with the tolerated range of the ISTA reference method, where the optimum setting point is the prescribed temperature of 103°C and ±2°C is the tolerance of 101–105°C.

The aim of this validation study was to test whether a tolerance of ±2°C around the defined temperature of 130°C would give acceptable results. If so, the optimum setting point of the oven would be the prescribed temperature range, similar to the reference method.

Figure 1. Seed moisture content assessed at different temperatures by two laboratories for high temperature oven method validation study

Materials and Methods

Determination of seed moisture content was performed at a range of temperatures by two laboratories (DEDL03 and RSDL03) on five species. The temperatures applied were 127°C, 128°C, 130°C and 133°C. Species used in this study were barley, beet, maize, rye and wheat. For each species, two or three seed lots were selected, resulting in a total of 15 analysed seed lots.

Results and Statistical Analysis

Statistical analysis was conducted using R programming language and JMP version 16 software (a SAS product). Figure 1 shows the oven temperatures versus percentage moisture for each lot and the associated linear regression lines.

Assessment of Oven Temperature Trend in Seed Moisture Method

We consider the following linear model with a temperature covariate:

$$y_{ij} = \mu + \alpha_i + L_j + (\alpha L)_{ij} + e_{ij} \qquad (1)$$

In which:

- y_{ij} is the percentage moisture for temperature i and lot j
- μ is the intercept
- α_i is the covariate at temperature i to assess the trend (slope)
- L_j is the fixed effect of seed lot j
- $(\alpha L)_{ij}$ is the fixed interaction effect between temperature i and lot j (to assess heterogeneous slopes)
- e_{ij} are the residuals about the linear fit for temperature i and lot j
  - e_{ij} ~ i.i.d. $N(0,\sigma^2)$

Laboratories and species combinations are represented in the model through the lot fixed factor. $(\alpha L)_{ij}$ was not significant (p=0.558) indicating that a homogeneous slope model is adequate, i.e. without the term $(\alpha L)_{ij}$. The oven temperature covariate (α_i) was significant (p<0.001) with slope estimate 0.029% (±0.007) increase in moisture with

Acknowledgements

- STA Committee members
- ECOM Liaison Officer, Vanessa Sosa
- ISTA Secretariat and ISTA ECOM
- TCOM members
- Users of the tools developed by the STA Committee

Thank you!

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