



Canadian Food
Inspection Agency

Agence canadienne
d'inspection des aliments

Validation Study on the Germination of *Brassica napus* var. *napus*

Dr. Ruoqing Wang

CFIA Seed Science and Technology Section

Saskatoon Laboratory

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Objectives

To promote uniformity, standardization, and precision among seed laboratories.

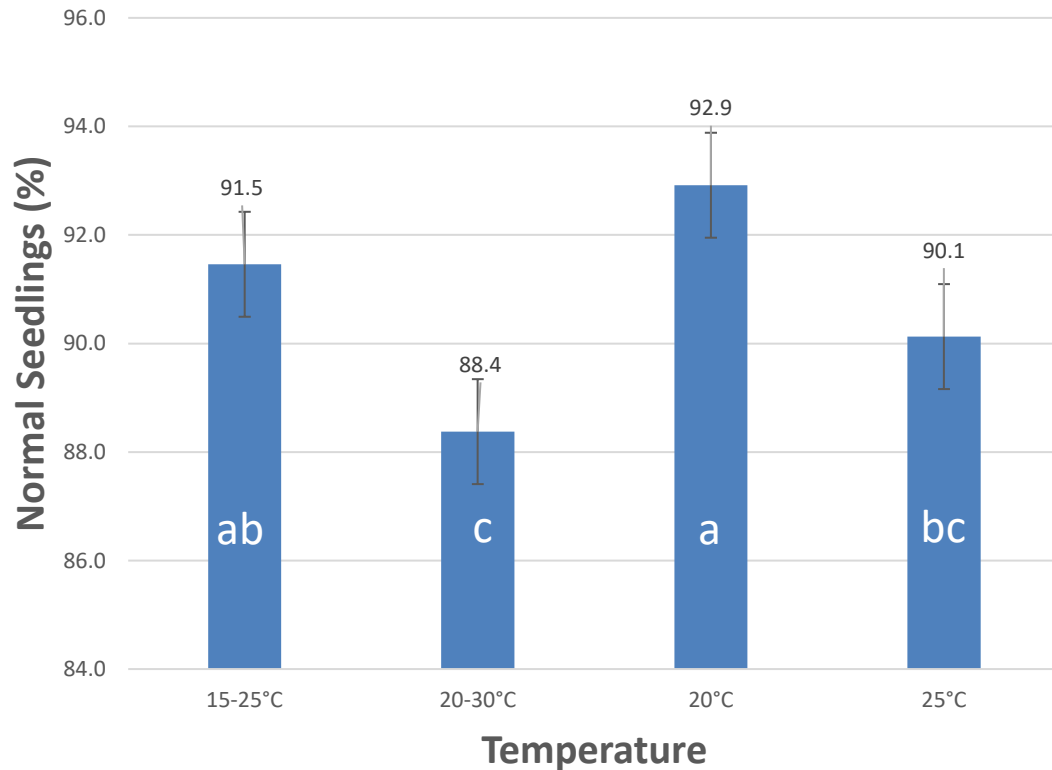
To provide supporting evidence for proposing the amendment of AOSA and ISTA rules.

Temperature and Rule Comparison

Testing Rules	Substrata	Temperatures (°C)	Other requirement	Counting days
M&P	TP	15-25; 25	Light; Prechill	4-7
ISTA	BP; TP	20-30; 20	KNO ₃ ; Prechill	5-7
AOSA	B, T;	20-30; 15-25		3-7

Temperatures difference may impact the uniformity of germination tests

Pre-trial within a lab



- Six varieties with germination range from 85 to 96
- Difference was statistically significant

ISTA Validation Study

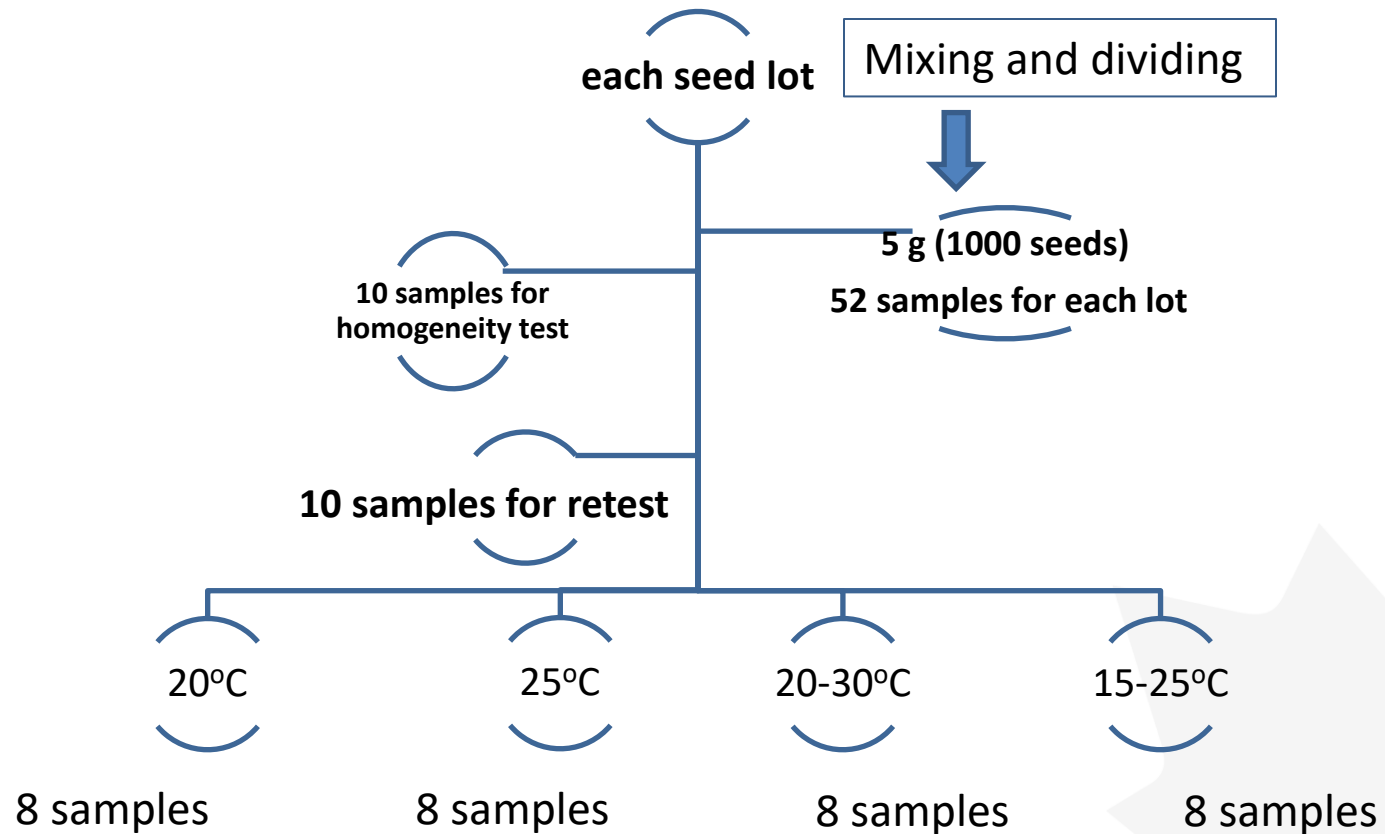
Samples & Preparation

- 6 seed lots:
 - 5 spring and 1 winter type (from Canada and Germany)
- Germination range:
 - 80-95% in pretests.
- Homogeneity test:
 - 52 samples prepared
 - 10 random samples

Participating labs

- ISTA accredited labs (8)
 - 2 from Canada
 - 2 from USA
 - 2 from France
 - 1 from Sweden
 - 1 from Scotland

Samples Preparation



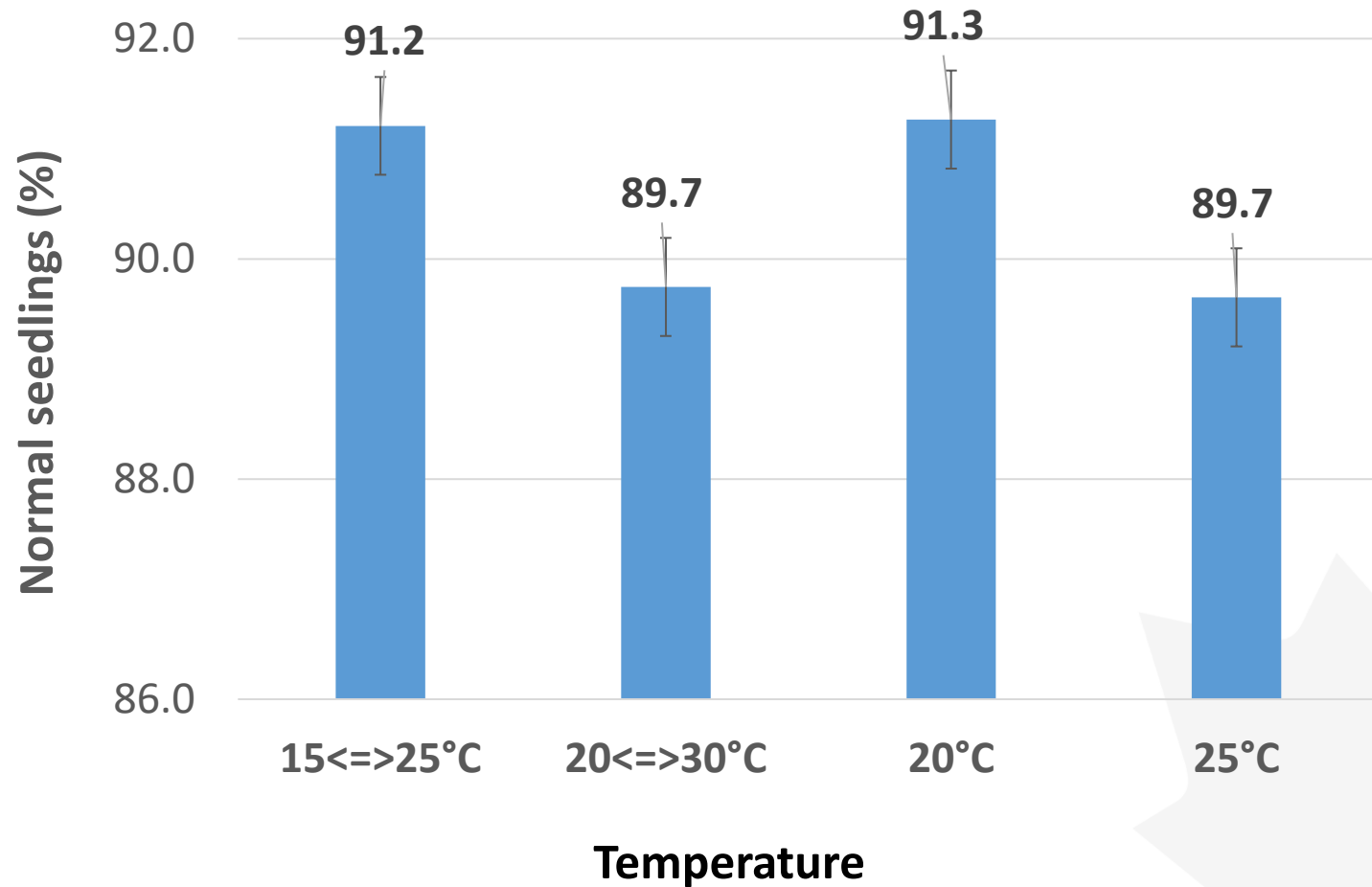
Method specification

Temperature (°C)	Number of seeds x rep	Substrate	First count (d)	Final count (d)	Breaking dormancy	Light
20<=>30	100 x 4	TP/BP	5	7	None	Light 8 hr/dark 16 hr
15<=>25	100 x4	TP/BP	5	7	None	Light 8 hr/dark 16 hr
20	100 x4	TP/BP	5	7	None	Light 8 hr/dark 16 hr
25	100 x4	TP/BP	5	7	None	Light 8 hr/dark 16 hr

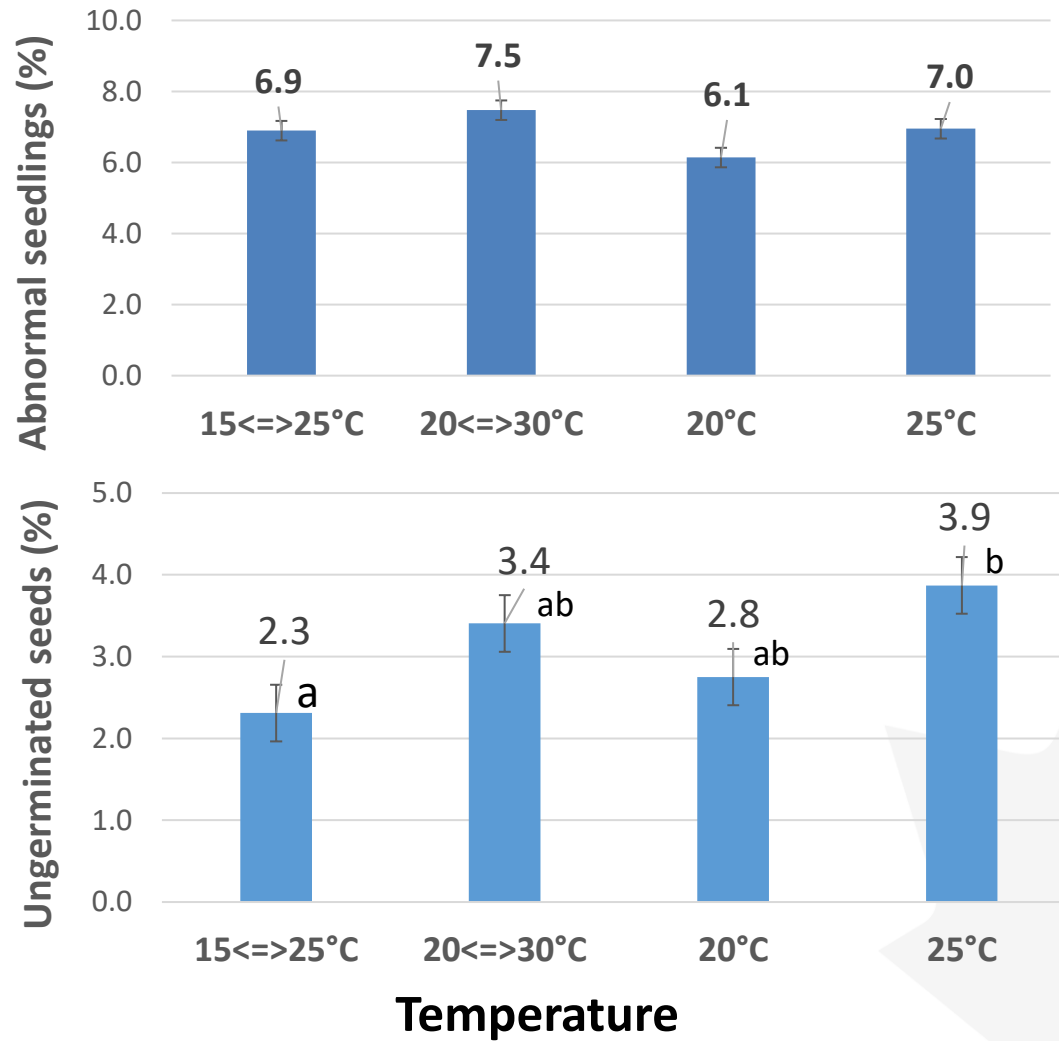
Data Analysis

- Data collection
 - % Normal seedlings
 - % Abnormal seedlings
 - % ungerminated seeds
- Data analysis (*ISTAgermMV* in R package)
 - ANOVA
 - **Repeatability**: quantifies the average variability of results within a laboratory.
 - **Reproducibility**: quantifies the average variability among laboratories.
 - **Accuracy**: a combination of trueness or bias and precision using z-scores.

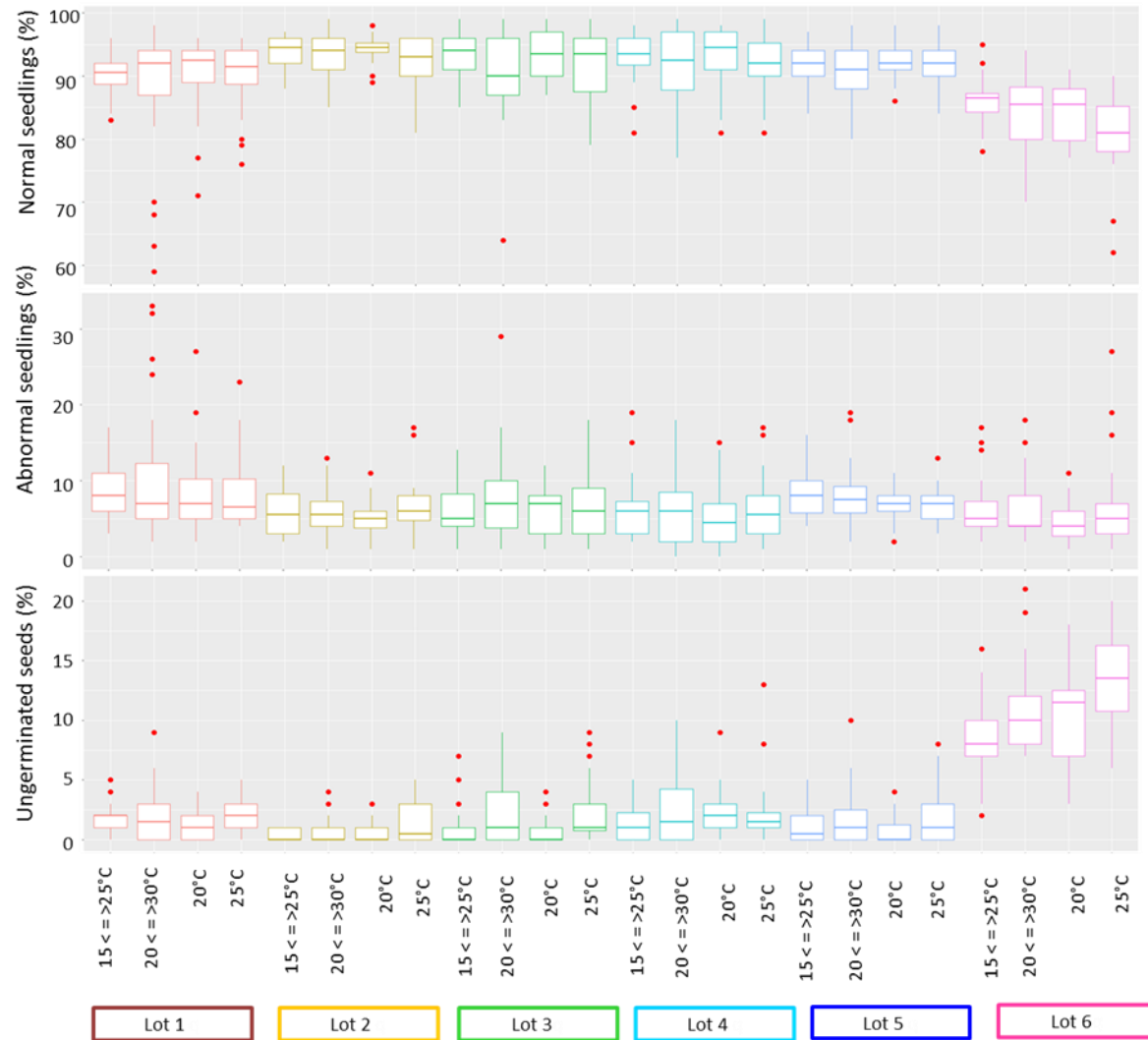
Results: Average germination%



Results: Average germination%



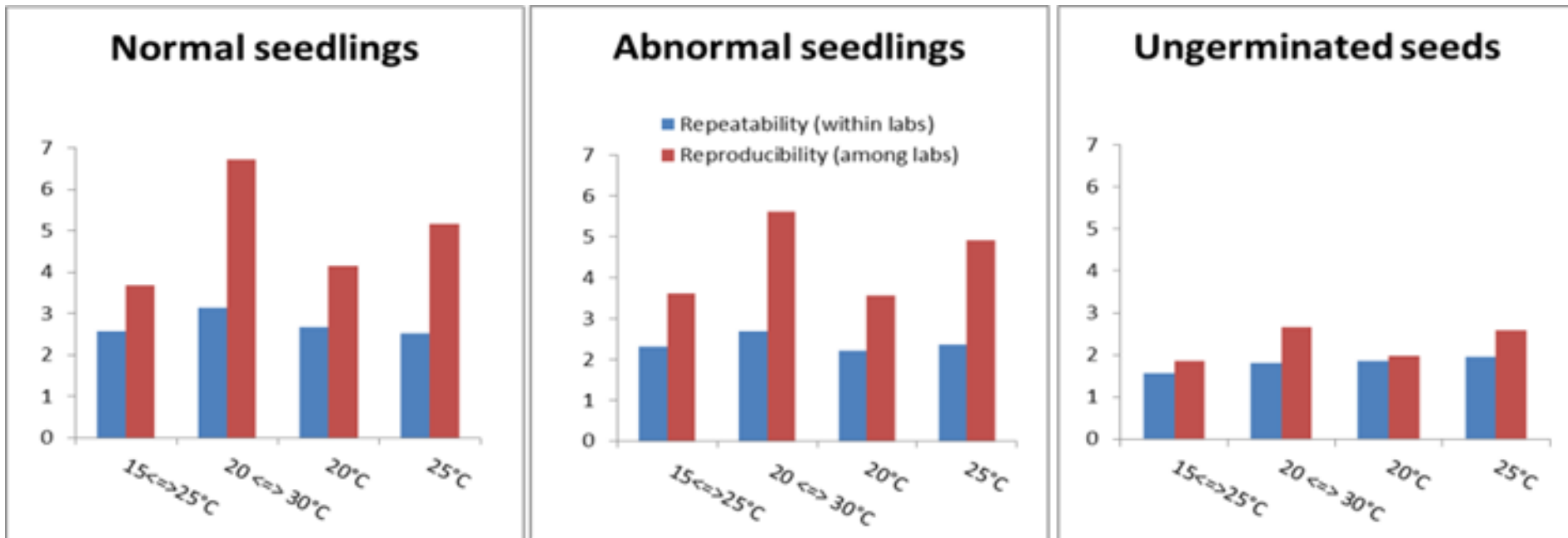
Results: Average germination%



Lot 6:

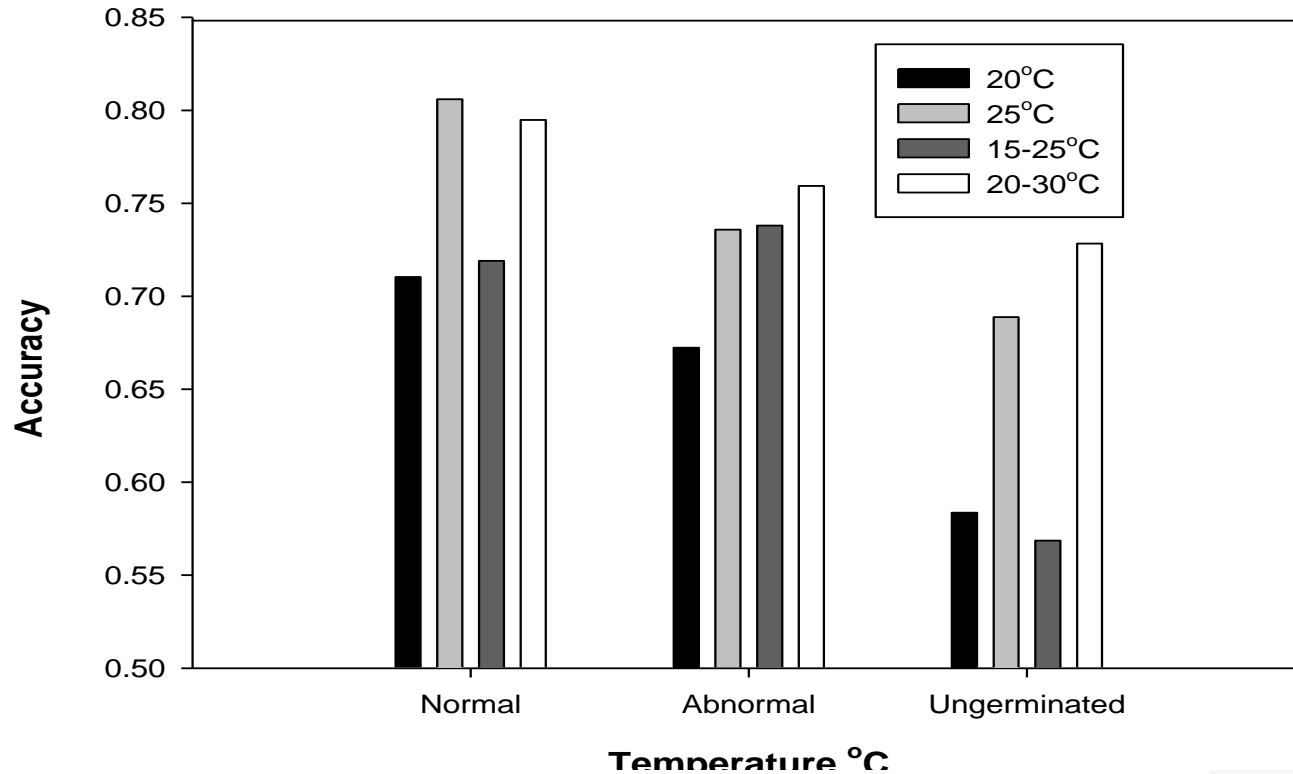
- lowest germination percentage
- 15 ≤ >= 25°C produced the highest normal seedlings, and the least ungerminated seeds
- 25°C had the least normal seedlings and the highest ungerminated seeds

Results: Repeatability and reproducibility



- The smaller the value (standard deviation) was, the better the performance of the temperatures would be
- 15<=> 25°C and 20°C had lower standard deviation among four temperatures, i.e., higher repeatability and reproducibility

Results: Average accuracy



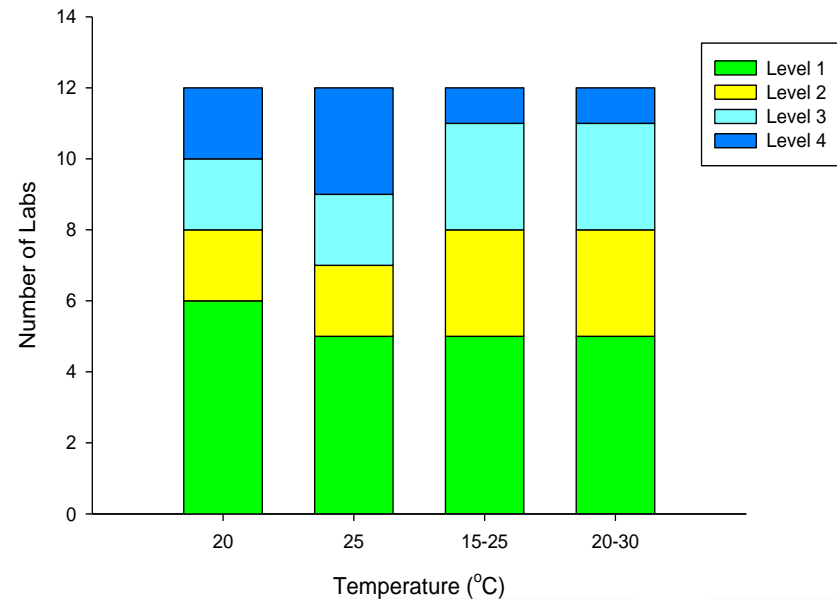
- The smaller the value is, the higher the accuracy will be
- 20°C and 15<=>25°C had higher accuracy for normal seedlings and ungerminated seeds

AOSA/SCST Referee Participation

Experience

- Level 1
0-50 Samples per year
- Level 2
50-100 samples per year
- Level 3
100-200 samples per year
- Level 4
Over 200 samples per year

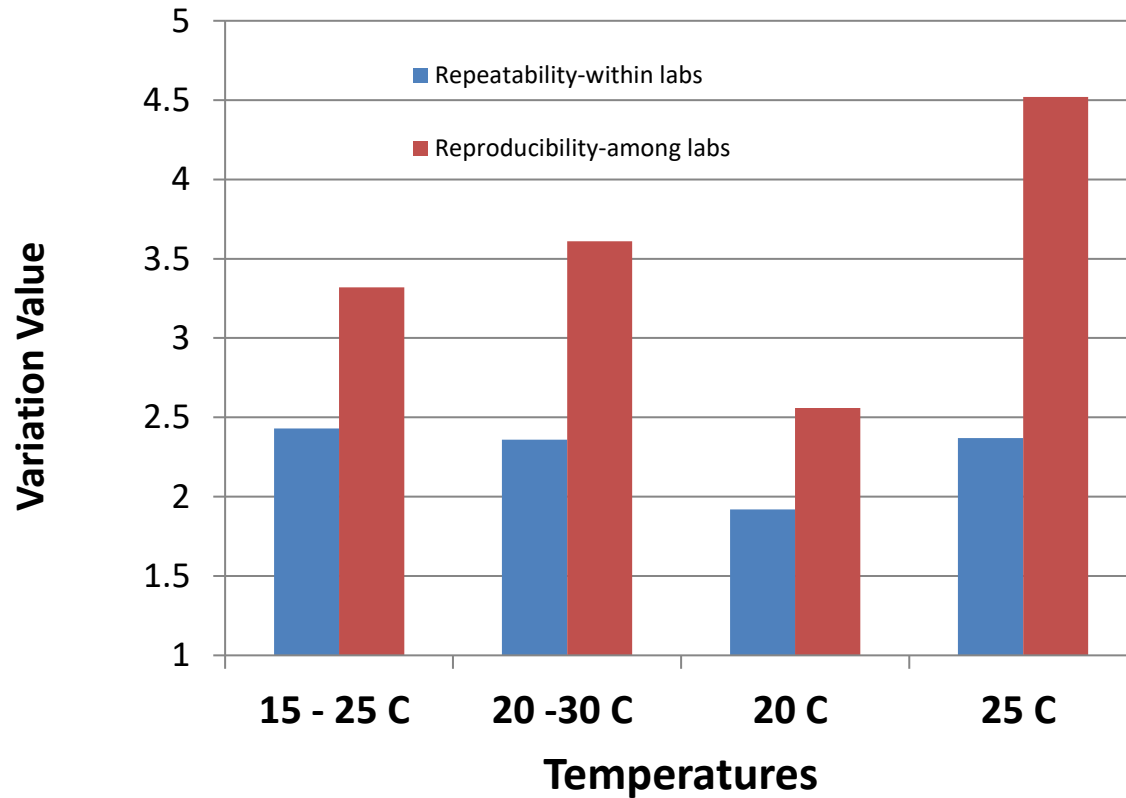
Design



12 labs with comparable level of experience in each temp.

5 labs conducted all temperatures

AOSA/SCST Referee Study



Conclusions

20°C and 15<=>25°C had:

- much lower standard deviations of repeatability and reproducibility.
- less variation among lots and labs
- higher testing accuracy
- significantly lower percentage of un-germinated seeds.

AOSA-Rule Proposal

Table 6. A. Methods of testing for laboratory germination.

Kind of seed	Substrata ^a	Temperature (C°)	First count (days)	Final count (days)	Specific requirements and notes
<i>Brassica napus</i> <i>var. napus</i> annual rape and winter rape	B, T	20-30; 20 15-25	3	7	

Acknowledgment

Germination group

- Marge Kowalchuk
- Maria Cumming
- Marlene Driedger
- Nicole Wurm

Research group

- Julie Lu
- Steve Jones



**Thank
You!!!**

ISTA validation study

- ISTA germination committee for approval of the project and testing plan
- testing plan reviewers, Simon Goertz and Zita Ripka for their suggestions and comments
- eight ISTA participating laboratories for their data contribution, and
- ISTA Statistical committee, especially Jean-Louise Laffont for data advice and software.

AOSA Referee Study:

Thanks for the 12 AOSA, SCST, and Canadian laboratories participating in the Referee



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QUESTIONS?

Canada 