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Method Validation Reports on Rules Proposals for the International Rules for Seed Testing 2020 Edition

Germination Committee Technical report: Validation of germination method on *Salvia hispanica* L. to support B.1.2.

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Summary

The study was conducted to determine a standard germination method for *Salvia hispanica* (chia). Temperatures 20°C and 20<=>30°C were compared with each other as well as with and without prechill. Seven laboratories analyzed three lots of *Salvia hispanica* using the TP substrate. Determining if prechill promotes dormancy breaking was difficult; since no dormancy was observed in the seed lots tested. Statistical analyses showed that the prechill lowered the germination percentage. The prechill method was significantly different from the no prechill method. There wasn't a significant difference between 20°C and 20<=>30°C temperatures, however 20°C produced slightly higher results.

Introduction

Salvia hispanica is not currently in the International Rules for Seed Testing. Eight other *Salvia* species are in table 5A of the International Rules for Seed Testing with the method TP, 20°C and 20<=>30°C, 4-7 day first count, 21 day final count and prechill. There is a need to introduce *Salvia hispanica* into the ISTA Rules to allow International Certificates to be issued for seed export and international trading. *Salvia hispanica*, originally from southern North America and northern South America, is expanding outward to other countries. There has been renewed interest in chia as an excellent source of ω3 fatty acids and dietary fiber for healthy diets. [1] Its demand is steadily increasing in Australia and United States, as a health food.

Materials and Methods

Seed material

Salvia hispanica seed was supplied by Naktuinbouw Laboratories, Netherlands. The seed originated from Belgium. Three seed lots of *Salvia hispanica* were procured and used for this study. The seed lots used did not have any dormancy issues.

Participant laboratories

A total of seven laboratories participated in the *Salvia hispanica* ISTA Validation Test Plan in 2018. The laboratories were in six different countries: Australia, Argentina, Chile, Israel, Netherland and USA.

Germination methods

The *Salvia hispanica* seeds were compared by germinating the seed at 20<=>30°C and 20°C; plus, use of a prechill and no prechill. A germination cabinet was used in all laboratories except one laboratory utilized a Jacobsen table. For each method and seed lot, 400 seeds were planted on top of paper moistened with water. Two laboratories planted 50 seed replicates, while the other five laboratories planted 100 seed replicates. The 50 seed replicates were due to smaller germination containers. When prechill was performed, the seed was placed in 5-10°C for 5 days method. The samples were then moved to the warm temperatures of 20<=>30°C or 20°C for the germination period. The first count was conducted at 7 days, with an additional count performed at 14 days and a final evaluation at 21 days. One laboratory did an initial count at 5 days and one lab did an initial count at 6 days with also doing the 7, 14, and 21 day counts. *Salvia hispanica* seedlings grew so quickly, that the laboratory felt the count needed to be done before 7 days.

The evaluation of the seedlings was made according to seedling type E and seedling group A-2-1-1-1 from the ISTA Handbook on seedling evaluation. In the case of 5% or more fresh seed, the seeds were evaluated as fresh or dead by using Tetrazolium. The common abnormalities found were primary infection of the seedling, primary root missing or defective and cotyledon damage.

Statistical analyses

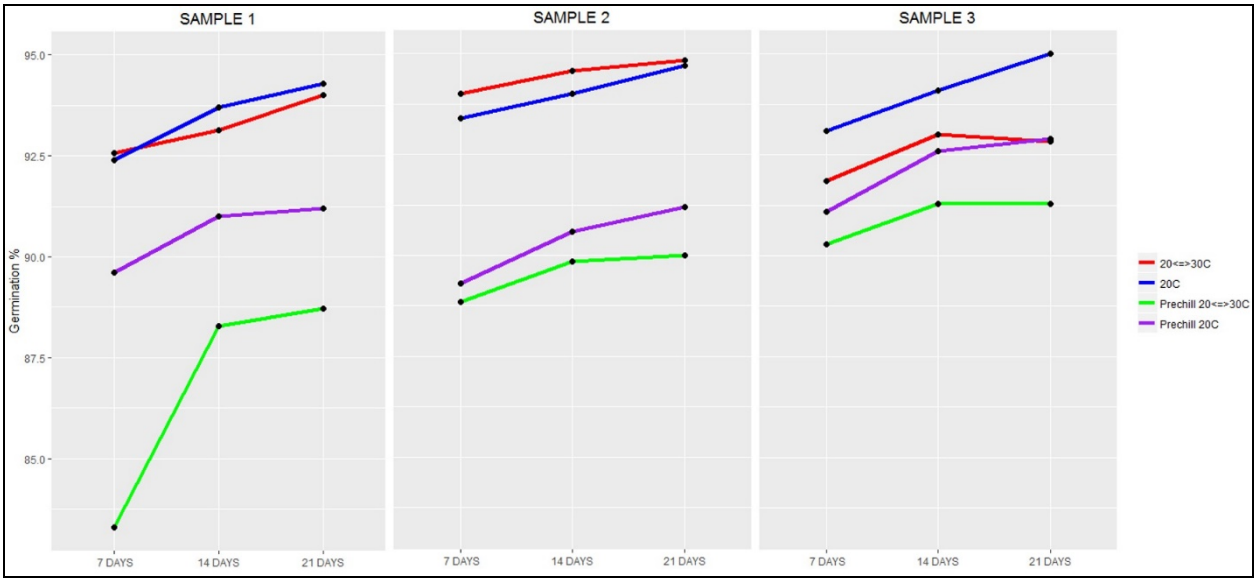
Statistical analyses were performed by Jean-Louis Laffont, chair of the ISTA Statistics Committee, by using the new R package developed by the ISTA Statistics Committee 'ISTAgermMV'. The figures with the boxplots (per lot, per method, per method x lot, and per laboratory x method) as well as the data checking, repeatability/reproducibility and the mixed model analyses were generated from this statistical tool.

Results and Discussion

Fresh seeds were not found to be present in any of the seed lots. Prechilling the samples did not promote the germination percentage. The results between 20°C and 20<=>30°C were comparable with germination results from 20°C slightly higher than 20<=>30°C. The mean result for TP 20<=>30°C is 94%, TP 20°C is 94%, TP Prechill 20<=>30°C is 90% and TP Prechill 20°C is 92%.

The speed of germination was found to be quick. Most of the *Salvia hispanica* was germinated in 7 days. Table 1 shows the average germination percentage by 7, 14, and 21 days. The difference between the 14 day and 21 day count is an average of 0.4% across all samples and methods.

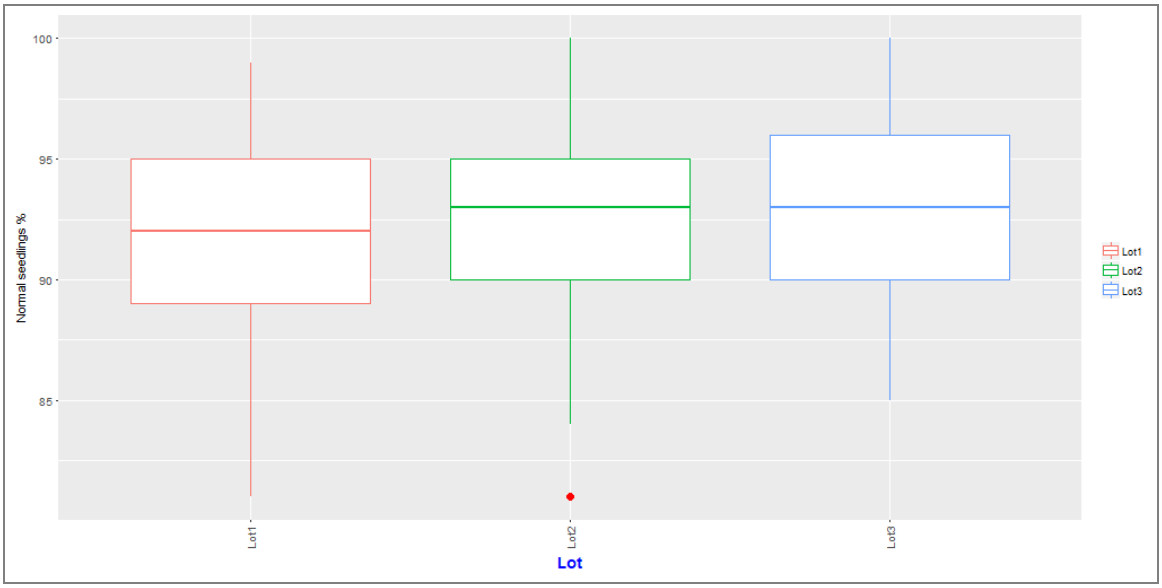
Table 1. Germination percent by 7, 14, & 21 days



Germination results by seed lot

The average germination of each seed lot across all temperatures was very similar (Table 2). The range of germination percentage was 85% to 98% overall. Low germinating lots of *Salvia hispanica* seed were not available.

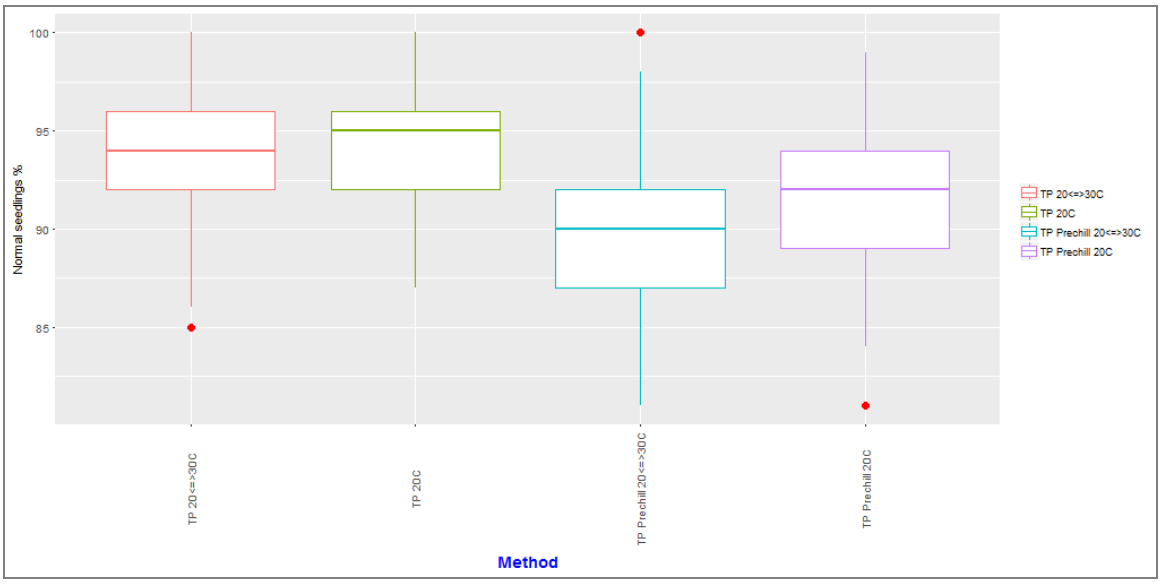
Table 2. Average germination of each seed lot



Germination results by method

The results between methods, in Table 3, showed the prechill samples having lower germination than the no prechill samples. However, the range between the prechill and no prechill is small. This shows that dormancy was not an issue with these lots.

Table 3. Average germination results by each method.



Germination results by method x lot

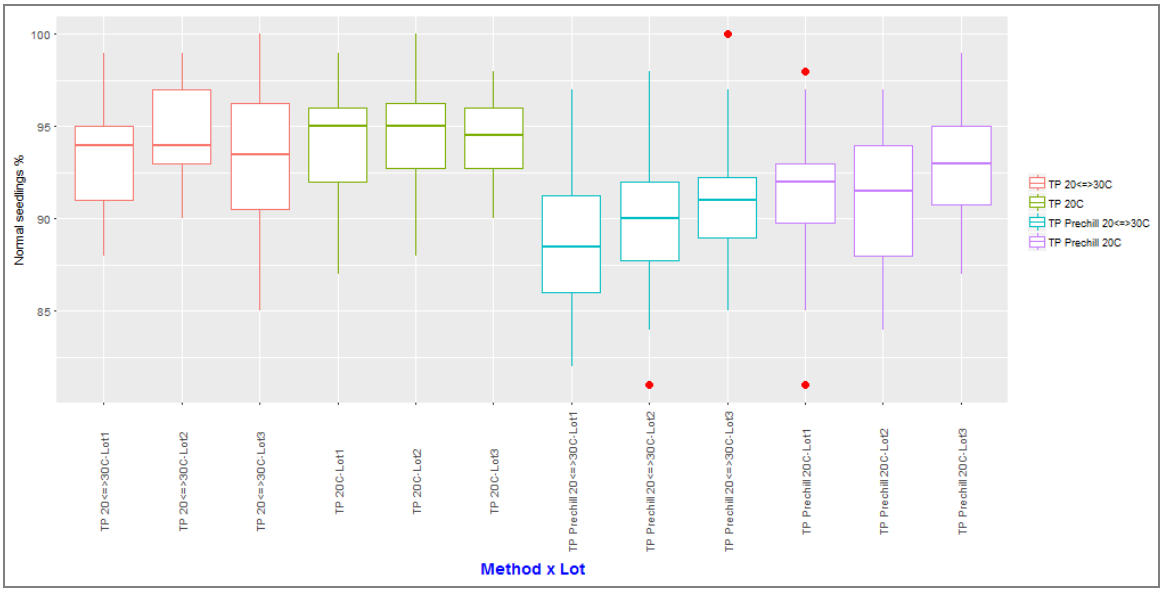
The data in Table 4 shows the mean germination percentage for each treatment. Overall the no prechill method gave higher results and the 20°C germination temperature results were slightly higher than 20<=>30°C germination temperature results. The range of mean germination for prechill samples is 89%-93% and the mean germination for no prechill samples is 93%-95% Table 5 shows the results by method x lot in a chart form.

Table 4. Germination results shown by lot and method

	Temperature	**Mean germination**
Lot 1	20<=>30°C	93%
	20°C	94%
Lot 2	20<=>30°C	94%
	20°C	95%
Lot 3	20<=>30°C	93%

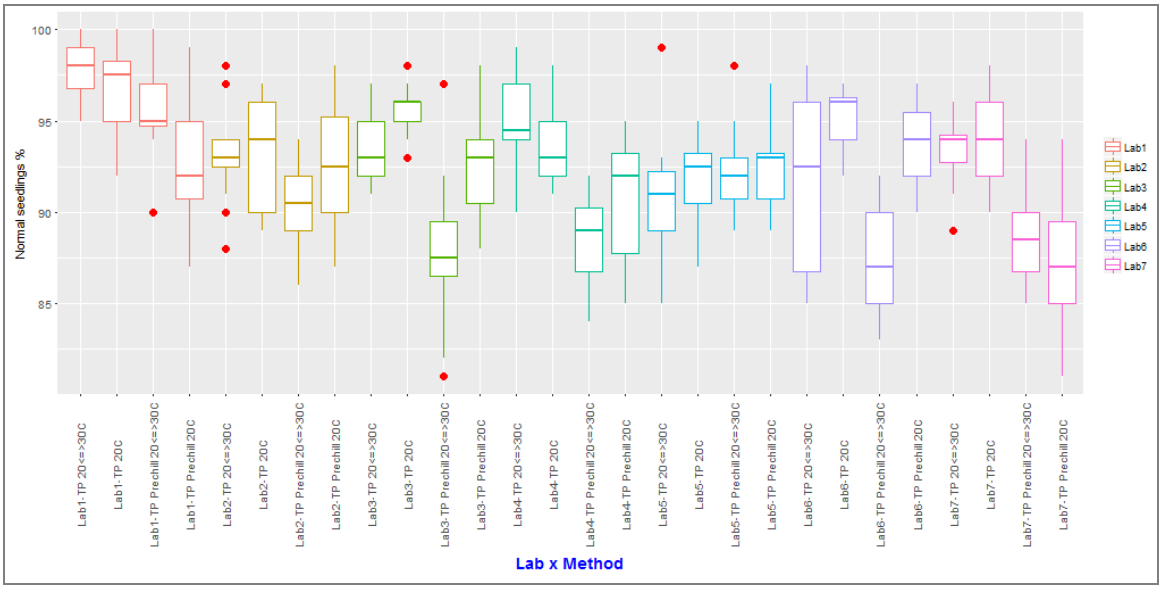
	20°C	95%
	Temperature with pre-chill	**Mean germination**
Lot 1	20<=>30°C	89%
	20°C	91%
Lot 2	20<=>30°C	90%
	20°C	91%
Lot 3	20<=>30°C	91%
	20°C	93%

Table 5. Germination results by method and lot



Germination results by laboratory and method

Table 6. Germination results across laboratories and method



Results of data checking

Data checking of the normal germination percentages has been performed according to ISTA Rules by computing tolerances for germination test replicates. The program utilized Table 5B Part 1 from the ISTA Rules.

No results are out of tolerance.

Repeatability / Reproducibility

Table 7. Repeatability/Reproducibility

Method	$\overline{p}_{...}$	S_r	f_r	S_R	$\sqrt{\hat{\sigma}^2_{Lab}}$	$\sqrt{\hat{\sigma}^2_{Lot\times Lab}}$
TP 20<=>30C	94	2.30	0.94	3.60	1.68	2.20
TP 20C	94	2.28	0.99	2.77	1.47	0.57
TP Prechill 20<=>30C	90	2.68	0.89	3.86	2.62	0.88
TP Prechill 20C	92	2.95	1.07	3.56	1.88	0.66

In Table 7, Mean corresponds to $\overline{p}_{...}$, s_repeatability to S_r , disp to f_r , s_reproducibility to S_R , s_Lab to $\hat{\sigma}_{Lab}$ and s_LotxLab to $\hat{\sigma}_{Lot\times Lab}$

The dispersion factor is calculated as $f_r = \sqrt{\frac{n\,\hat{\sigma}^2}{\overline{p}_{...}(100-\overline{p}_{...})}}$ where $\overline{p}_{...}$ is the overall average percentage of the trait analyzed and *n* is the number of seeds per Rep. If f_r > 1 one speaks of overdispersion because the data have larger variance than expected under the assumption of a binomial distribution.

The dispersion factors f_r are all right around 1, so there should not be any concerns regarding repeatability of any of the 4 methods.

The Reproducibility variance is the sum of the repeatability variance, the variance between laboratories and the interaction variance between lots and laboratories:

$$S_R = \sqrt{\hat{\sigma}^2 + \hat{\sigma}^2_{Lab} + \hat{\sigma}^2_{Lot\times Lab}}$$

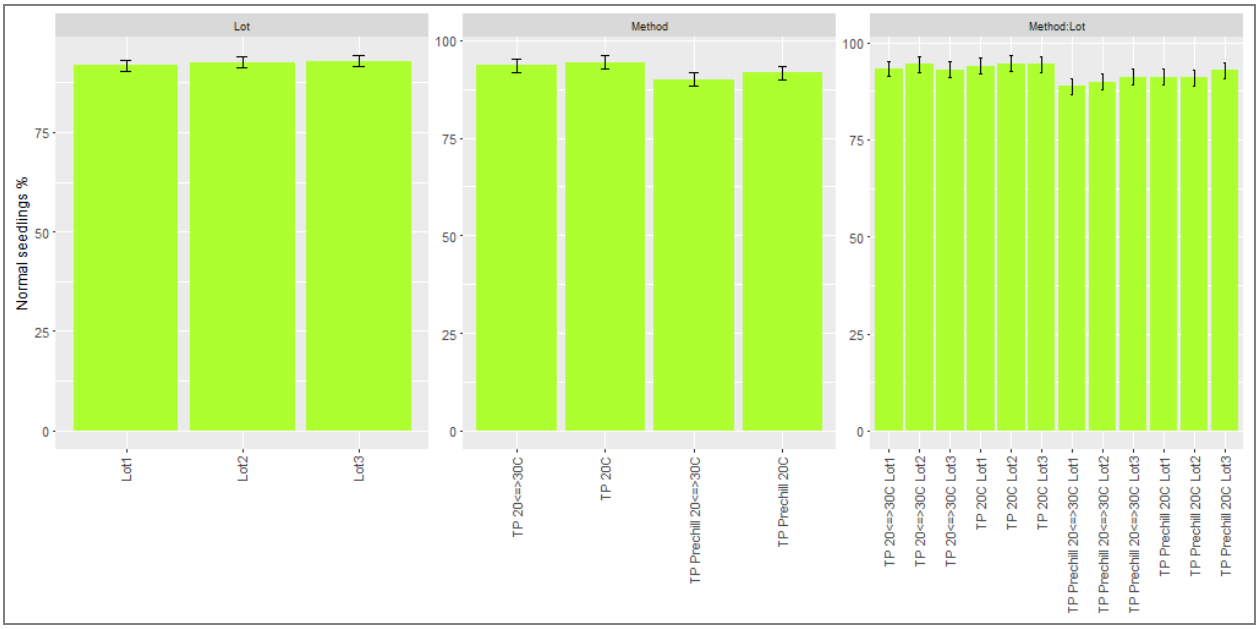
Test method TP 20<=>30C shows high values in $\hat{\sigma}_{Lab}$ and $\hat{\sigma}_{Lot\times Lab}$, which reflect low reproducibility due to big differences across the laboratories or to differences in the way laboratories are measuring lots.

Mixed model for comparing Method and Lot means

The method main effect is highly significant. The prechill method is significantly different from the no prechill method.

The Least Squares Means are displayed in Table 8 bar plots with error bars added corresponding to their standard errors:

Table 8. Bar plot for Least Square Means



General conclusion

This study shows that *Salvia hispanica* grown both at 20°C and 20<=>30°C, with Top of Paper and moistened with water, provide adequate growing environments for *Salvia hispanica* (chia). Testing without a prechill gave higher results compared to testing with a prechill at 5-10°C. The prechill method was significantly different from the no prechill method. The temperature 20°C produced slightly higher and more consistent results than 20<=>30°C. The difference between the 14 day count and the 21 day count is on average 0.4%. With this low increase, a germination duration of 14 days is suggested.

Both temperatures are suitable for germinating *Salvia hispanica*, however due to the results, a prechilling method is not suggested in the International Rules for Seed Testing. Both temperatures 20°C and 20<=>30°C with the Top of Paper method are recommended and a first count at 5-7 days with final being at 14 days.

Acknowledgements

Thanks to Naktuinbouw Laboratories of Netherland for supplying the three lots of *Salvia hispanica* for this germination validation study.

Thanks to the laboratories that participated in this study, including Department of Agriculture and Food, Western Australia; Official Seed Testing Laboratory ARO – Volcani Center, Israel; Laboratorio

Central de Análisis de Semillas del Instituto Nacional de Semillas, Argentina; Louisiana Department of Agriculture and Forestry, USA; Agricultural and Livestock Service of Chile, SAG, Chile; Naktuinbouw Laboratories, Netherland and SGS - Brookings, USA.

Thanks to the ISTA Technical reviewers Ripka Gézáné and Lea Mazor, the members of the ISTA Germination Committee, especially Sylvie Ducornau, for help and guidance during the project.

Thanks to Jean-Louis Laffont, Statistical Committee chair, for the ISTA germ MV stat program to run the data and the support he gave for interpretation.

References

1. Jamboonsri, W., Phillips, T. D, Geneve, R. L, Cahill, J. P, & Hildebrand, D. F. (2012). Extending the range of an ancient crop, *Salvia hispanica L.*—a new ω3 source. *Genetic resources and crop evolution, 59*(2), 171-178. doi: 10.1007/s10722-011-9673-x

2. International Seed Testing Association. 2018. ISTA International Rules for Seed Testing. Bassersdorf, Switzerland.

Germination Committee Technical report: Validation of germination method for *Glycine max* using Organic (O) growing media to support C.5.1.a

Test Leader: Garreau Philippe and Ducournau Sylvie
GEVES-SNES, France

Summary

The study was conducted to approve the use of Organic growing media (O) as a primary substrate for the germination of *Glycine max* in the ISTA Rules

Six laboratories analyzed four seed lots of *Glycine max*. The temperatures 25°C and 20<=>30°C were compared, combined with the Sand (S), Organic growing media (O), Between Paper (BP) and Top of Paper cover with Sand (TPS). Statistical analyses showed that the methods using Organic growing media gave good repeatability and very good reproducibility compared with the existing methods for that species in the ISTA Rules. The % normal germination results obtained with the O methods were also higher than those obtained with the other methods, leading to fewer abnormal seedlings and non-germinated seeds. It is therefore suggested that Organic growing media is added as a primary germination media for the germination of *Glycine max* in the ISTA Rules.

Introduction

The germination methods for *Glycine max* prescribed in the ISTA rules currently use Sand (S), Between Paper (BP) and Top of Paper covered with Sand (TPS).

In France, germination tests conducted for ISTA certificates are frequently retested in Organic growing media (O). Experience has shown that when parallel germination tests are conducted in sand and in Organic growing media, higher results are always obtained using Organic growing media with abnormal seedlings and non-germinated seeds often found when seeds are tested in Sand, but not in Organic growing media.

The introduction of O as a primary substrate for *Glycine max* in the ISTA Rules has been suggested by the French private laboratories and the French ISTA Station in order to reduce the workload created by routine parallel testing or retesting. The proposal was approved by the members of the Germination Committee and was included in the working programme of the committee. Therefore, a validation study was organized to compare all the current prescribed media and temperatures for the germination of *Glycine max* with Organic growing media.

Materials and Methods

Seed material

Glycine max seed lots were supplied by French seed companies. The four seed lots of *Glycine max* selected for the study were not chemically treated and were from two different varieties. Seeds lots were tested before starting the study, they did not have any dormancy issues and the quality was between 82% and 95% for normal seedlings, corresponding to commercial quality standards.

Participant laboratories

A total of six laboratories participated in the *Glycine max* validation study. The laboratories were located in five different countries: France, Germany, Israel, Argentina and the United States.

Germination methods

All the participants compared O with currently ISTA prescribed media. The participants were requested to only use the media that they had experience in using. Sand was used by all the participants, BP was used by three laboratories and TPS was used by two laboratories. All participants carried out the testing at the two temperatures (25 and 20< = > 30°C). All participants used light during testing, with the number of hours of light varying between 8 to 16 hours, except for one participant who used 24 hours of light.
The duration of the tests varied mostly between 7 and 8 days but some ended after 10 or 11 days (8 days being the prescribed duration). Some tests were extended to 10 and 13 days.

Table 1 includes the details of the germination methods used by each participant.

All combinations of media, temperature and seed lots were tested using four replicates of 100 seeds.

The seedling evaluation was based on the seedling group A-2-1-2-2 (ISTA Handbook on Seedling Evaluation, 3rd Edition). The participants provided a description of any abnormal seedlings found in the tests.

Table 1: Germination methods used by each participating laboratory.

Laboratory	Substrate	Temperature (°C)	Light (h)	Final count (days)	Comments
Lab 1	BP	25, 20<=>30	8	7	
	Sand	25, 20<=>30	8	7	
	O	25, 20<=>30	8	7	
Lab 2	Sand	20<=>30	8	8	Lot A after 13 days
	Sand	25	8	7-8	Lot A after 10 days
	O	25, 20<=>30	8	8	
Lab 3	BP	25, 20<=>30	16	8	
	Sand	25, 20<=>30	16	8	
	O	25, 20<=>30	16	8	
	TPS	25, 20<=>30	16	8	
Lab 4	Sand	20<=>30	8	8-10	
	Sand	25	8	7-8	
	O	20<=>30	8	8-10	
	O	25	8	7-8	
Lab 5	BP	25, 20<=>30	24	8	Rolled Towel
	Sand	25, 20<=>30	24	8	
	O	25, 20<=>30	24	8	
	TPS	25, 20<=>30	24	8	CCP/Sand
Lab 6	Sand	25, 20<=>30	8	11	

O	25, 20<=>30	8	11

Statistical analyses

Statistical analyses were performed using 'ISTAgermMV', the new tool developed by the ISTA Statistics Committee. Boxplots (per lot, per method, per laboratory, and per method x laboratory), checking and the repeatability/reproducibility results were generated from this statistical tool.

Results and Discussion

Results of data checking

Data checking of the normal germination percentages was performed according to the ISTA rules by computing tolerances for germination test replicates. Three results coming from lab 5, using sand and Organic growing media methods were detected as out of tolerance, in terms of results per replicates.

Germination results by seed lot

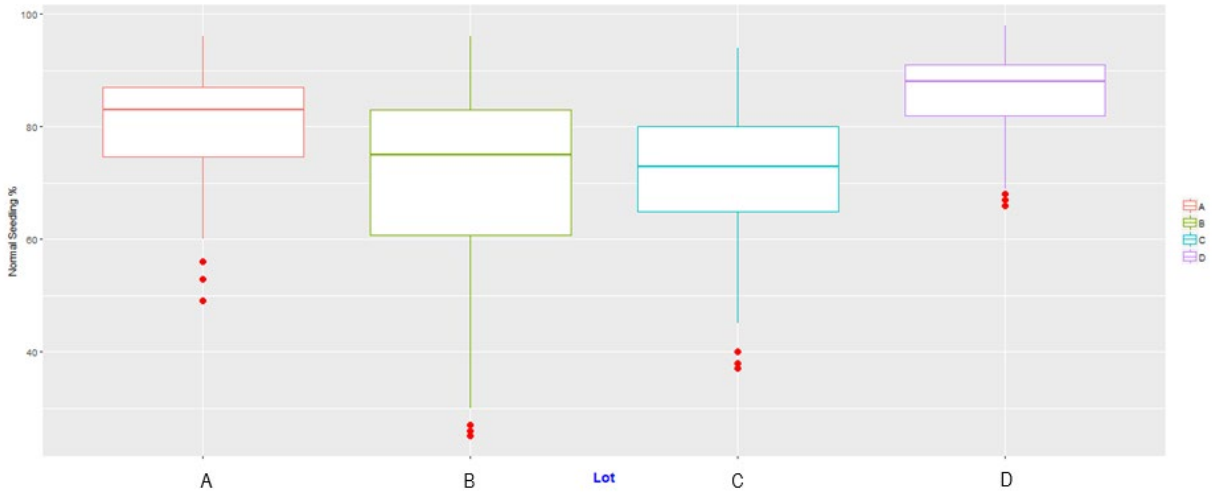


Figure 1: Boxplots for the four seed lots grouped across methods and laboratories.

Figure 1 shows the percentages of normal seedling obtained for the 4 seed lots, by all the laboratories using all the different methods. Boxplots in the figure show the distribution of the data around the median value. In terms of average results (different from the median values) in ascending order of germination %: seed lots B and C had 72% normal seedlings, lot A 80% and lot D 86%.

Germination results by laboratory

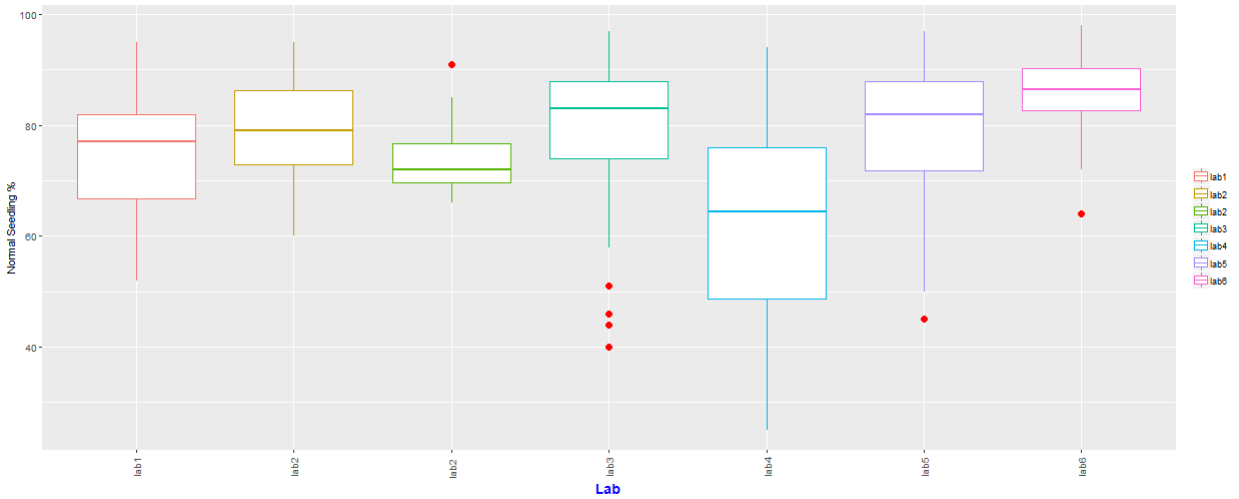


Figure 2: Boxplots for the 6 laboratories grouped across seed lots and methods.

Figure 2 shows the percentages of normal seedlings obtained by all the laboratories, on all seed lots and methods. In terms of laboratory results, lab 4 obtained the lowest germination results (63% overall average) and lab 6 obtained the best results (86% overall average).

Germination results by laboratory and method

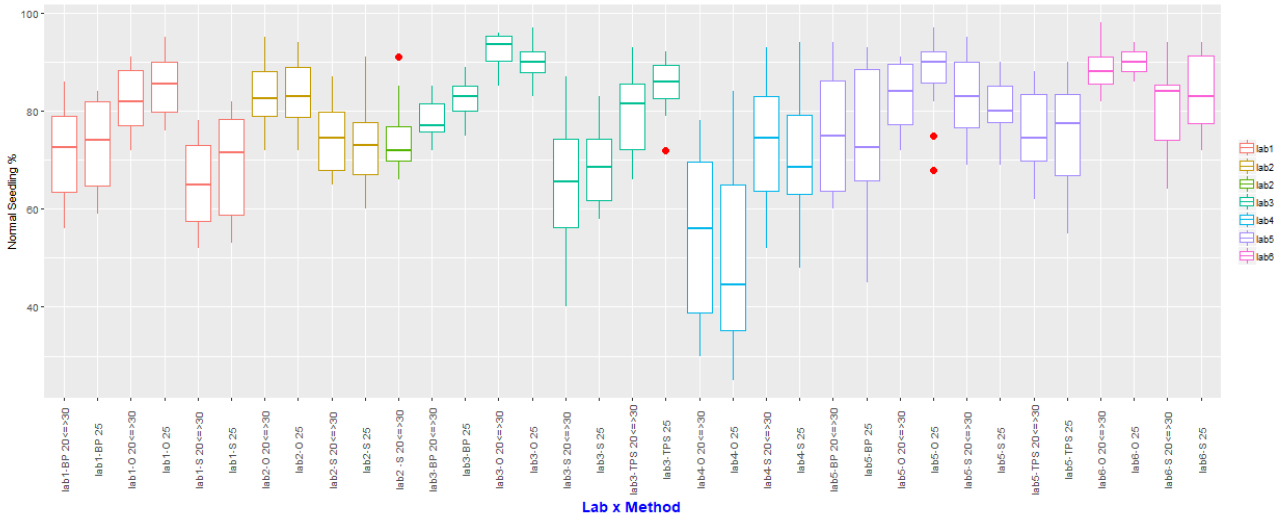


Figure 3: Boxplots for the 6 laboratories grouped across seed lots and methods (media x temperatures).

Figure 3 shows the percentages of normal seedlings obtained by the different participating laboratories using the different methods, on all the four seed lots. It shows that all the laboratories obtained better results using O compared to the other media, except for laboratory 4 that obtained very poor results using O at the two temperatures, very different from the results obtained by the other participants. It has therefore been decided to exclude this laboratory in the next analysis conducted to compare the performance of the methods used.

Germination results by method

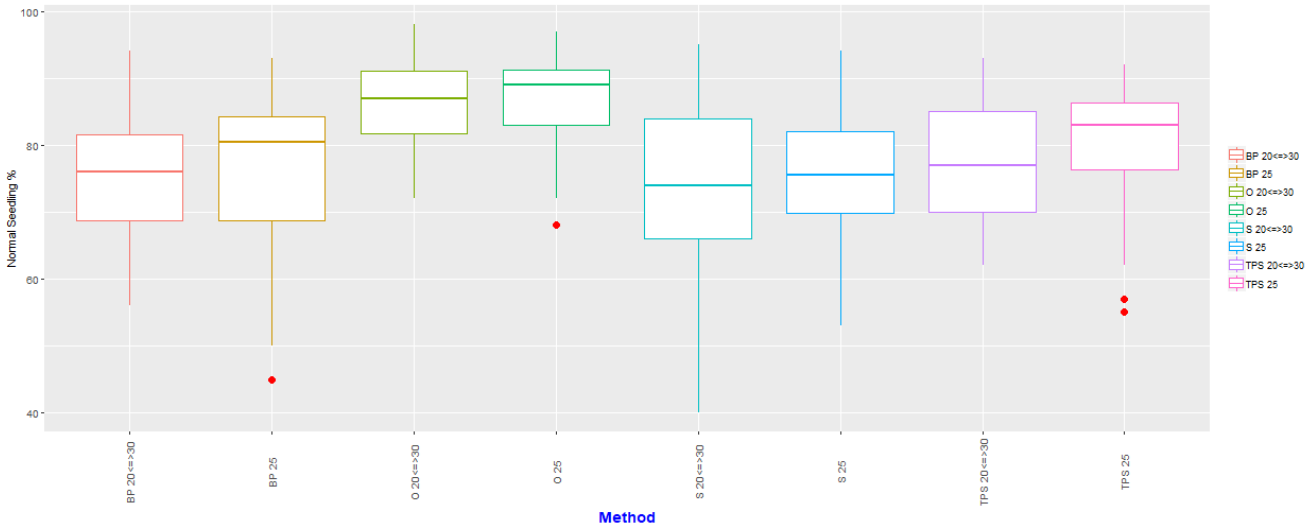


Figure 4: Boxplots of normal seedlings results for the 8 methods (media x temperatures) grouped across seed lots and laboratories.

Figure 4 shows the percentages of normal seedlings obtained with the different methods used by 5 laboratories after removing the data obtained by lab 4.

Table 2: Comparison of the methods using Student-Newman-Keuls test.
The means with the same letters are not very different.

Substrate	Temperature (°C)	Mean % Normal seedlings	SNK group
O	25	87.1	A
O	20<=>30	86.0	A
TPS	25	79.8	B
TPS	20<=>30	77.3	C
BP	25	76.6	C
S	25	75.4	CD
BP	20<=>30	75.3	CD
S	20<=>30	73.7	D

Table 2 shows the results of the SNK test results after a variance analysis performed with the results of normal seedlings.

The results of normal seedlings are higher for the two methods when using Organic (O) growing media.

In terms of average results, Sand at 20<=>30°C gave the lowest results (74% normal) and TPS at 25°C gave the best results (80% normal), apart from the O methods. The two methods using O gave on average 86% normal seedlings combined with 20<=>30°C and 87% with 25°C.

In order to know what generated the increase in normal seedlings for the O germination methods, graphs were plotted for abnormal seedlings (figure 5) and for non-germinated seeds (figure 6).

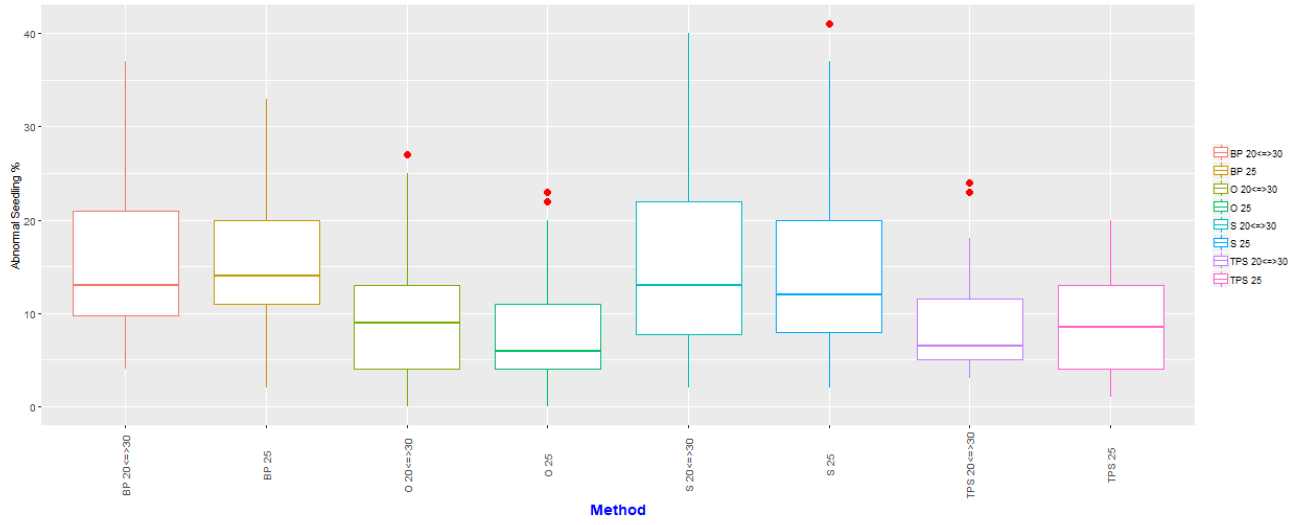


Figure 5: Boxplots of abnormal seedlings results for the 8 methods (media x temperatures) grouped across seed lots and laboratories.

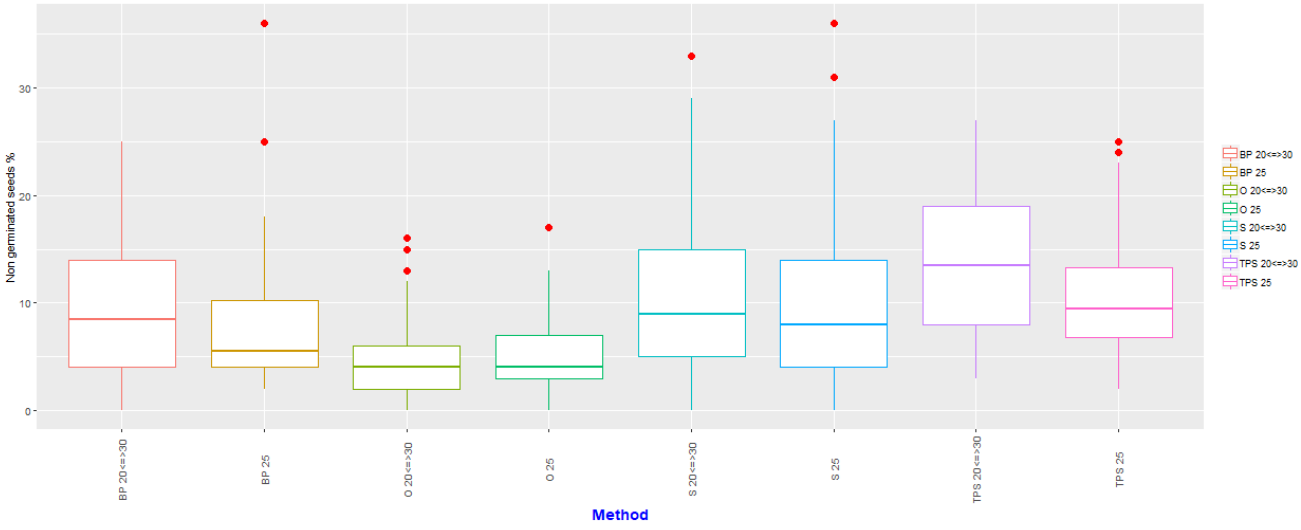


Figure 6: Boxplots of non-germinated seeds results for the 8 methods (media x temperatures) grouped across seed lots and laboratories.

The data presented in figures 5 and 6 indicate that the methods using O media give more normal seedlings because they generate fewer abnormal seedlings (in general, but also in leading to less defects on epicotyl) and fewer non-germinated seeds. Both TPS and O methods give the lowest percentages of abnormal seedlings, and in terms of non-germinated seeds, Organic growing media allows more germination than the other methods tested.

Repeatability and reproducibility of the results of the different germination methods

Table 3: Results of the repeatability and reproducibility of the different methods compared for the normal germination of *Glycine max*. The data from lab 4 are not taken into account.

Method	Mean	s_repeatability	disp	s_Reproducibility
BP 20<=>30	75.00	3.99	0.92	8.36
BP 25	77.00	4.66	1.10	11.36
O 20<=>30	86.00	3.45	0.99	5.89
O 25	87.00	4.16	1.24	5.38
S 20<=>30	74.00	4.33	0.98	9.72
S 25	75.00	4.36	1.01	8.95
TPS 20<=>30	77.00	4.48	1.07	4.79
TPS 25	80.00	4.23	1.06	10.34

Table 3 summarizes repeatability and reproducibility standard deviations, shown as s_repeatability and s_reproducibility respectively. The dispersion factor (disp.) applies to the results of repeatability and indicates an over dispersion when it is greater than 1.

The dispersion factors are all around 1, so there should not be any concerns regarding repeatability of any of the methods listed being used for the germination of *Glycine max*. Looking specifically at the dispersion factor for the O methods, the value is 0.99 for O at 20<=>30°C, indicating a repeatability comparable to the values obtained using the existing methods, and a slightly higher value of 1.24 for O at 25°C.

The reproducibility standard deviation values are very variable depending on the methods used. The lowest values and therefore the best reproducibility are for the methods using TPS at 20<=>30°C and the two O methods. The methods BP at 25°C and TPS at 25°C show the highest reproducibility standard deviations indicating that the results obtained with these methods are more variable between the participants.

The methods using organic growing media with 20<=>30°C and 25°C show comparable repeatability and better reproducibility than the methods already prescribed in the ISTA Rules for *Glycine max*.

General conclusion

This study shows clearly that germinating *Glycine max* seeds with Organic growing media (O) gives higher germination results compared to the existing germination methods prescribed in the ISTA Rules for this species, this media generating fewer abnormal seedlings and non-germinated seeds in the tests. The germination methods using Organic growing media also had good repeatability and very good reproducibility. These results therefore support the introduction of O as a primary germination substrate for *Glycine max* in Table 5A of the Germination chapter in the ISTA Rules.

This study has also shown that the results obtained with organic growing media are higher than those obtained with the prescribed ISTA methods. Nevertheless, it is difficult to propose a removal of all the existing methods for that species, as some combinations of media and temperatures give good germination results (i.e. TPS methods) and because it could be difficult for some laboratories to find and purchase acceptable organic growing media in their country.

Acknowledgements

Thanks to RAGT and La Dauphinoise companies for supplying the seed lots of *Glycine max* for this germination validation study.

Thanks to the labs that participated in this study, including LTZ Augustenberg, Germany; SGS - Brookings, USA; Monsanto Waterman SP, USA; Official Seed Testing Laboratory ARO – Volcani Center, Israel; Laboratorio Central de Análisis de Semillas del Instituto Nacional de Semillas, Argentina; GEVES – SNES, France.

Thanks to the ISTA Technical reviewers Linda Maile and Steve Jones and the members of the ISTA Germination Committee.

Thanks to Jean-Louis Laffont, Statistical Committee chair, for the ISTAgermMV stat program to run the data and for the support he gave in interpretation of the data.

Special thanks go to Sandrine Stievenard and Raphaël Suaud for their valued help in completing the files with all the data received by the participants.

References

1. International Seed Testing Association. 2018 Edition. ISTA International Rules for Seed Testing. Bassersdorf, Switzerland.
2. International Seed Testing Association. 2013 Edition. ISTA Handbook on Seedling Evaluation, Third Edition. Bassersdorf, Switzerland.

Germination Committee Technical report: Validation of germination method for *Phaseolus vulgaris* using Organic (O) growing media to support C.5.1.b

Test Leader: Garreau Philippe and Ducournau Sylvie
GEVES-SNES, France.

Summary

The study was conducted to approve the use of Organic growing media (O) as a primary substrate for the germination of *Phaseolus vulgaris* in the ISTA Rules.

Seven laboratories analyzed four lots of *Phaseolus vulgaris*. The temperatures 20°C, 25°C and 20<=>30°C were compared, combined with the Sand (S), Organic growing media (O), Between paper (BP) and Top of Paper covered with Sand (TPS). Statistical analyses showed that the methods using Organic growing media gave very good repeatability, with equal to and even better levels of reproducibility compared with the existing methods for this species in the ISTA Rules. The germination results obtained with the O methods are also equal to or greater than those obtained with the other methods. It is therefore suggested that Organic growing media is added as a primary germination media for the germination of *Phaseolus vulgaris* in the ISTA Rules.

Introduction

Germination methods currently prescribed in the ISTA Rules for *Phaseolus vulgaris* are Sand (S), Between Paper (BP) and Top of Paper covered with Sand (TPS).

In France, germination tests conducted for ISTA certificates are frequently retested in Organic growing media (O). Experience has shown that when parallel germination tests are conducted in sand and in Organic growing media, higher results are always obtained using Organic growing media with abnormal seedlings and non-germinated seeds often found when seeds are tested in Sand, but not Organic growing media.

The introduction of O as a primary substrate for *Phaseolus vulgaris* in the ISTA Rules has been suggested by the French private laboratories and the French ISTA Station, in order to reduce the workload created by routine parallel testing or retesting. The proposal was approved by the members of the Germination Committee and was included in the working program of the committee.

A validation study was organized to compare all the current prescribed media and temperatures for the germination of *Phaseolus vulgaris.* The study allowed comparison of existing prescribed substrates, at different temperatures with Organic growing media.

Materials and Methods

Seed material

Phaseolus vulgaris seed lots were supplied by French seed companies. The four seed lots of *Phaseolus vulgaris* selected for the study were not chemically treated and each was a different variety. Seeds lots were tested before starting the study, they did not have any dormancy issues and the quality was between 89% and 95% normal seedlings, corresponding to commercial quality standards.

Participant laboratories

A total of seven laboratories participated in the *Phaseolus vulgaris* ISTA validation study. The laboratories were located in six different countries: France, Germany, Israel, Scotland, Argentina and the United States.

Germination methods

All the participants compared O with current ISTA prescribed media. The participants were requested to only use the media that they had experience in using. Sand was used by all the participants, BP was used by 5 laboratories and TPS was used by two laboratories. All participants carried out the testing at three temperatures (20 °C, 25 °C and 20< = > 30 °C). All participants used light during testing, with the number of hours of light varying between 8 to 12 hours, except for one participant who used 24h of light.
The duration of the tests varied between 5 and 9 days (end of the ISTA test). Some tests were extended to 11 and 15 days.

Table 1 includes the details of the germination methods used by each participant.

All combinations of media, temperature and seed lots were tested using four replicates of 100 seeds.

The seedling evaluation was based on the seedling group A-2-1-2-2 (ISTA Handbook on Seedling Evaluation, 3rd Edition). The participants provided a description of the abnormal seedlings found in the tests.

Table 1: Germination methods used by each participating laboratory.

Laboratory	Substrate	Temperature (°C)	Light (h)	Final count (days)	Comments
1	BP	20, 25, 20<=>30	8	7	
	Sand	20, 25, 20<=>30	8	7	
	O	20, 25, 20<=>30	8	7	
2	Sand	20, 25, 20<=>30	8	7	
	O	20, 20<=>30	8	7	
	O	25	8	6	
3	BP	20, 25, 20<=>30	8	9	
	Sand	20, 25, 20<=>30	8	9	
	O	20, 25, 20<=>30	8	9	
	TPS	20, 25, 20<=>30	8	9	
4	BP	20, 25, 20<=>30	8	5	
	Sand	20, 25, 20<=>30	8	5 to 9	
	O	20, 25, 20<=>30	8	5 to 9	

5	BP	20, 25,	12	9	
	BP	20<=>30	8	9	
	Sand	20, 25,	12	9	
	Sand	20<=>30	8	9	
	O	20, 25,	12	9	
	O	20<=>30	8	9	
6	Sand	20, 25, 20<=>30	8	6 to 11	
	Sand	20<=>30	8	8 to 15	
	O	25, 20<=>30	8	5 to 8	
	O	20	8	8	
7	BP	20, 25, 20<=>30	24	9	Folded Paper
	Sand	20, 25, 20<=>30	24	9	
	O	20, 25, 20<=>30	24	9	
	TPS	20, 25, 20<=>30	24	9	CCP/Sand

Statistical analyses

Statistical analyses were performed using 'ISTAgermMV', the new tool developed by the ISTA Statistics Committee. Boxplots (per lot, per method, per method x lot, and per laboratory), data checking and the repeatability/reproducibility results were generated from this statistical tool.

Results and Discussion

Germination results by seed lot

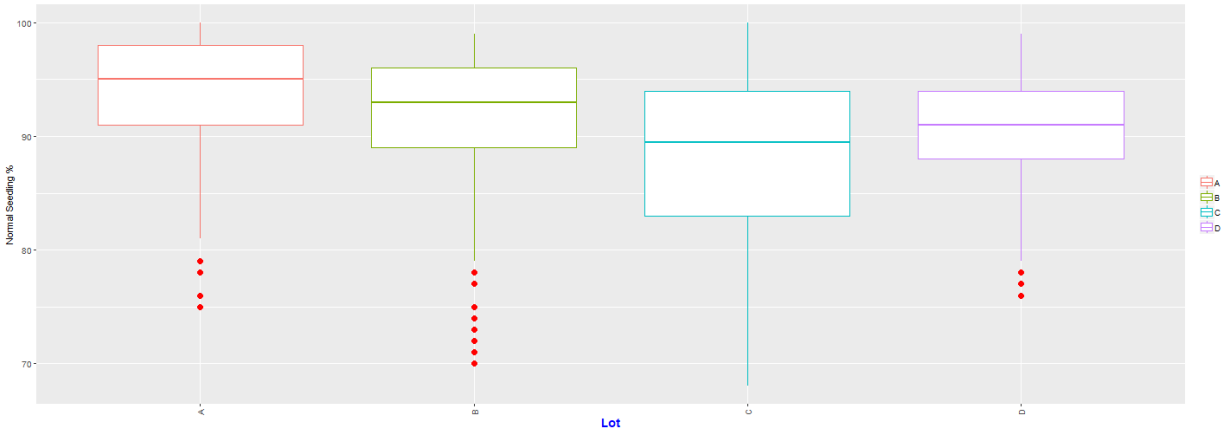


Figure 1: Boxplots for the four seed lots grouped across methods and laboratories.

Figure 1 shows the percentages of normal seedlings obtained for the 4 seed lots, by all the laboratories using all the different methods. Boxplots in the figure show the distribution of the data around the median value. In terms of average results (different from the median values) in ascending order of germination%: seed lot C had 88.0% normal seedlings, lot D 89.3%, lot B 91.4% and lot A 93.9%.

Germination results by laboratory

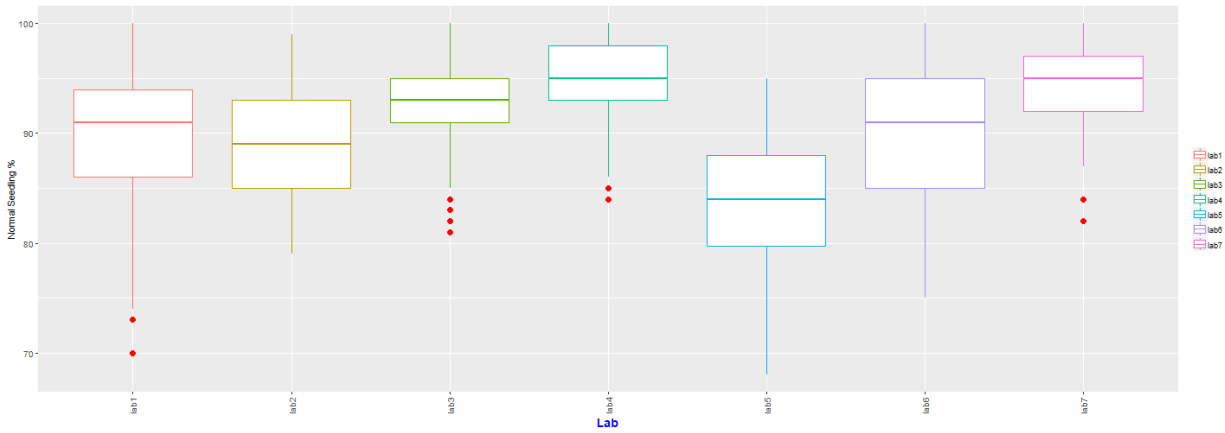


Figure 2: Boxplots for the 7 laboratories grouped across seed lots and methods.

Figure 2 shows the percentages of normal seedlings obtained by all the laboratories, on all seed lots and methods. In terms of laboratory results, lab 5 obtained the lowest germination results (83.5% overall average) and lab 4 and 7 obtained the best results (95.0% and 94.3% overall average, respectively).

Germination results by method

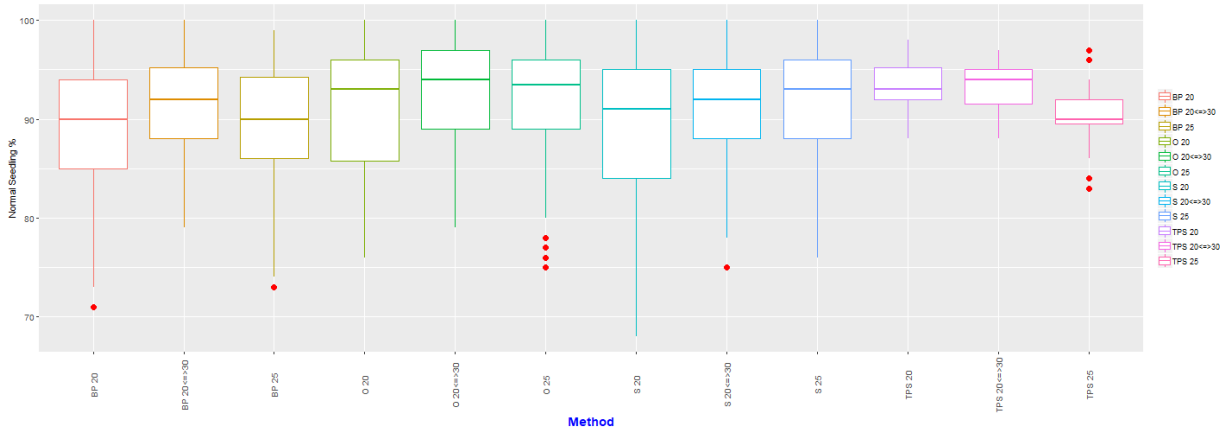


Figure 3: Boxplots for the 12 methods (media x temperatures) grouped across seed lots and laboratories.

Figure 3 shows the percentages of normal seedlings obtained with the different methods used by all the laboratories on the four seed lots. The results were very similar depending on the method used. In terms of average results, Sand at 20°C gave the lowest results (88.5% normal) and TPS at 20°C gave the best ones (93.5% normal). The methods using O gave 91.0% when combined with 20°C, 91.8% with 25°C and 92.7% with 20<=>30°C.

Germination results by laboratory and method

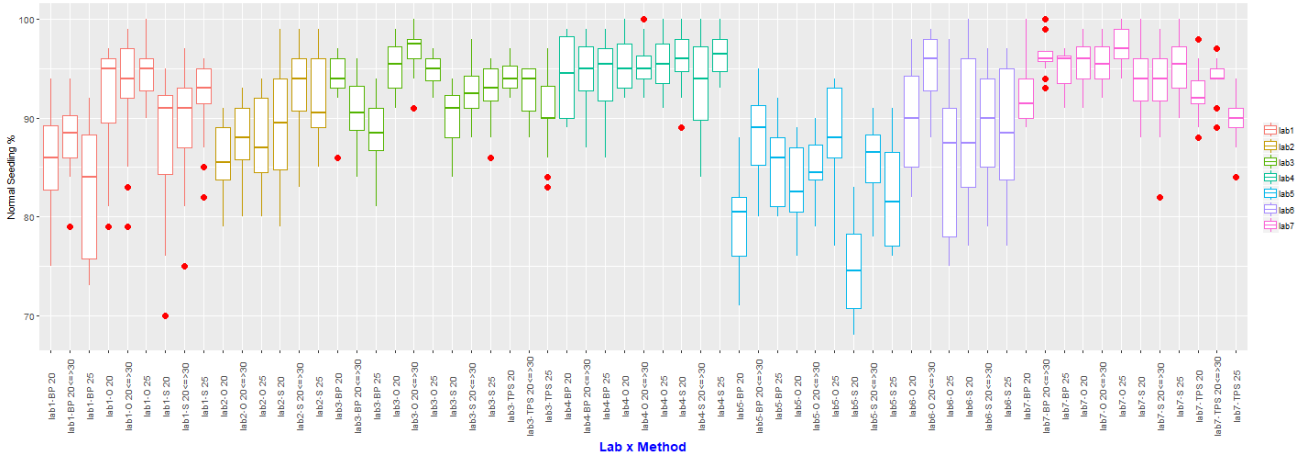


Figure 4: Boxplots for the laboratories x methods grouped across the seed lots.

Figure 4 shows the percentages of normal seedlings obtained with the different methods used by the participating laboratories on all the four seed lots. Laboratories did not obtain the same results using the different methods, but O did not lead to lower results than the other methods currently prescribed in the ISTA Rules.

Results of data checking

Data checking of the normal germination percentages was performed according to the ISTA rules by computing tolerances for germination test replicates.

No results were out of tolerance.

Repeatability and reproducibility of the results for the different germination methods

Table 2: Repeatability and reproducibility of the different methods compared for the germination of *Phaseolus vulgaris*.

Method	Mean	s_repeatability	disp	s_Reproducibility
BP 20	89.00	3.23	1.04	7.48
BP 20<=>30	92.00	2.31	0.83	4.71
BP 25	89.00	3.28	1.05	6.77
O 20	91.00	2.25	0.79	5.92
O 20<=>30	93.00	2.14	0.82	5.37
O 25	92.00	2.34	0.85	5.99
S 20	89.00	2.40	0.75	8.30
S 20<=>30	91.00	2.83	0.98	5.05
S 25	91.00	2.28	0.81	5.75
TPS 20	93.00	2.20	0.89	2.44
TPS 20<=>30	93.00	2.06	0.83	2.40
TPS 25	90.00	2.43	0.82	3.38

In Table 2, summarized by the method (media x temperature), s_repeatability and s_reproducibility correspond to the repeatability and reproducibility standard deviation. Disp. is the dispersion factor, it applies to the results of repeatability and indicates an over dispersion when its value is greater than 1.

The dispersion factors are all around 1 and mostly far below 1, so there are not any concerns regarding repeatability of any of the methods listed being used for the germination of *Phaseolus vulgaris*. Looking specifically at the dispersion factor for the O methods, the values vary from 0.79 to 0.85, indicating a good repeatability of the results obtained with these methods in the

participating laboratories. The germination results obtained with this media, whatever the temperature tested, are good compared with the existing methods in the ISTA Rules.

The reproducibility standard deviation values are very variable depending on the methods used. The lowest values and therefore the best reproducibility are for the methods using TPS media. The reproducibility standard deviation values are similar for methods using sand and O (except for sand 20°C with the lowest reproducibility with a standard deviation of 8.30). BP methods are slightly less reproducible compared to the other methods used in that comparative test, except BP 20<=>30 °C. The three germination methods BP 20°C, BP 25°C and S 20°C give the lowest performance in terms of reproducibility and mean germination results. It is difficult to suggest removing these three method combinations as it will lead to the removal of BP and S media as well as 20 or 25°C in the ISTA Rules resulting in 20 and 25°C not being available for TPS or O. Indeed, the results show also that BP 20<=>30 °C, S 20<=>30 °C and S 25°C give good germination results.

General conclusion

This study shows clearly that germinating *Phaseolus vulgaris* seeds with Organic (O) growing media gives equal or higher germination results compared to some of the existing germination methods prescribed in the ISTA Rules for this species. These results therefore support the introduction of O as a primary germination substrate in Table 5A of the Germination chapter in the ISTA Rules.

This study has also shown that some germination methods (BP 20°C, BP 25°C and S 20°C) had lower reproducibility and germination results than the other methods tested. Nevertheless, it is difficult to propose a removal of these media and temperatures, as other combinations using these media and temperatures, gave good germination results.

Acknowledgements

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Thanks to the labs that participated in this study, including LTZ Augustenberg, Germany; SGS - Brookings, USA; Monsanto Waterman SP, USA; Official Seed Testing Laboratory ARO – Volcani

Center, Israel; Laboratorio Central de Análisis de Semillas del Instituto Nacional de Semillas, Argentina; OSTS – SASA, Scotland; GEVES – SNES, France.

Thanks to the ISTA Technical reviewers Linda Maile and Steve Jones and the members of the ISTA Germination Committee.

Thanks to Jean-Louis Laffont, Statistical Committee chair, for the ISTAgermMV stat program to run the data and the support he gave for interpretation.

Special thanks go to Sandrine Stievenard and Raphaël Suaud for their valuable help in completing the files with all the data received by the participants.

References

1. 1. International Seed Testing Association. 2018 Edition. ISTA International Rules for Seed Testing. Bassersdorf, Switzerland.
2. International Seed Testing Association. 2013 Edition. ISTA Handbook on Seedling Evaluation, Third Edition. Bassersdorf, Switzerland.

Germination Committee Technical Report: Validation of germination method utilizing crepe cellulous paper (CCP) for the top of paper (TP) method for *Zea mays* to support C.5.2.

Test Leader: Sarah Dammen[1] and David M. Johnston[2]
[1]SGS, USA
[2]Louisiana, Dept. of Agriculture and Forestry, USA.

Summary

This study was conducted to determine the suitability of utilizing crepe cellulous paper (CCP) as a primary media for the top of paper (TP) method for *Zea mays* and adding this media to the ISTA Rules Table 5A Part 1. The different combinations of media and temperatures were studied. The temperatures of 20°C, 25° C, and 20⇔30° C were compared and were used in combination with the following methods of sand (S), between paper (BP), top of paper (i.e. CCP) with a sand covering (TPS), and top of paper (TP) utilizing CCP with no form of covering. Six laboratories analyzed three seed lots of *Zea mays* of varying germination and vigor levels. Statistical analyses of the data showed that the methods utilizing the CCP for the TP method produced acceptable results of repeatability when compared to the other methods used for this study. It is therefore requested to add the option of CCP for the TP method to the ISTA Rules for *Zea mays.*

Introduction

The current ISTA Rules germination media options for *Zea mays* include Sand (S), Between Paper (BP), and Top of Paper with a Sand covering (TPS) with the paper being CCP. In part, the purpose of this study is to harmonize the ISTA Rules with the Association of Official Seed Analysts (AOSA) Rules. The use of CCP for the TP method was adopted into the AOSA Rules in 1980. Since then, this highly efficient test method has been successfully utilized for accurately assessing the germination of seed lots by both governmental and non-governmental laboratories across multiple decades.

Although the use of CCP is not wide spread around the world, it is widely utilized in laboratories in North America. Several laboratories, including company laboratories that produce and test *Zea mays* seed outside of the United States, desire that the ISTA Rules also adopt the TP CCP method. These laboratories tend to have an extremely large volume of *Zea mays* samples and require an extremely fast through-put of samples and are seeking to adopt automation to meet this requirement. The TP CCP method greatly supports the implementation of technology to increase the efficiency of laboratory testing.

Materials and Methods

Seed material

Three *Zea mays* seed lots of varying germination and vigor levels were supplied by the Monsanto Seed Physiology Laboratory located in Waterman, IL USA. All three seed lots had been commercially treated prior to being used for the study. None of the seed lots exhibited any dormancy during germination testing.

Participating laboratories

A total of six laboratories participated in this ISTA validation study. All laboratories were required to be familiar with the use of the TP method utilizing CCP for this species. To allow unrestricted movement of GMO *Zea mays* samples to participates, all participating laboratories were in the USA.

Germination methods

All participating laboratories compared the TP CCP method with each of the current ISTA methods using S, BP, TPS media and using the prescribed test temperatures of 20°C, 25°C, and 20<=>30°C. All participants used 8 hours of light in every 24-hour cycle during the highest temperature setting, when an alternating temperature regime was used. All laboratories were encouraged to conduct only one seedling evaluation count at 7 days. Evaluations were conducted using ISTA Seedling Type E Seedling Group A-1-2-3-2.

Statistical analyses

Statistical analyses were performed by Jean-Louis Laffont, PhD – Chair of the ISTA Statistic Committee and Riad Baalbaki, PhD – Co-Chair of the AOSA/SCST Statistics Committee. Analysis was performed utilizing the R package developed by the ISTA Statistics Committee 'ISTAgermMV'.

Results and Discussion

1. Data checking

Data checking has been performed according to ISTA rules by computing tolerances for germination test replicates.

Complete results: see worksheet *Data checking* from the file *2018 ISTA Zea mays study - Stat analysis - 091518.xlsx*.

Four test results out of 216 were out of tolerance for replications but were included in the analysis (Table 1).

Table 1: Out of Tolerance Replicates

Method	Lot	Lab	Mean	#Reps	#Seed/Rep	Range	Tol	Out of Tol
BP 20 <=> 30 C	Lot 3	Lab 2	92	4	100	12	11	OUT
S 20 <=> 30 C	Lot 3	Lab 2	72	4	100	19	18	OUT
TPS 20 C	Lot 1	Lab 2	41	4	100	22	19	OUT
TPS 25 C	Lot 1	Lab 1	66	4	100	22	19	OUT

2. Analysis of variance

ANOVA results indicated that the choice of method, lot and their interaction all significantly affected test results (Table 2).

Table 2: ANOVA Results

Source of variation	Sum of Squares	Mean Square	Num DF	Den DF	F value	Pr(>F)
Method	921.87	83.81	11	55.00	7.38	0.00
Lot	3324.10	1662.05	2	10.00	146.40	0.00
Method x Lot	2322.98	105.59	22	110.00	9.30	0.00

3. Data exploration-comparison of boxplots

a. Lots: In addition to ANOVA indicating an overall significant effect of lots, results averaged over all methods and laboratories, verified that lots covered a wide range of quality, expressed as germination (Figure 1, Lot). The same trend was observed for the three lots over laboratories (Figure 2, Lot x Lab).

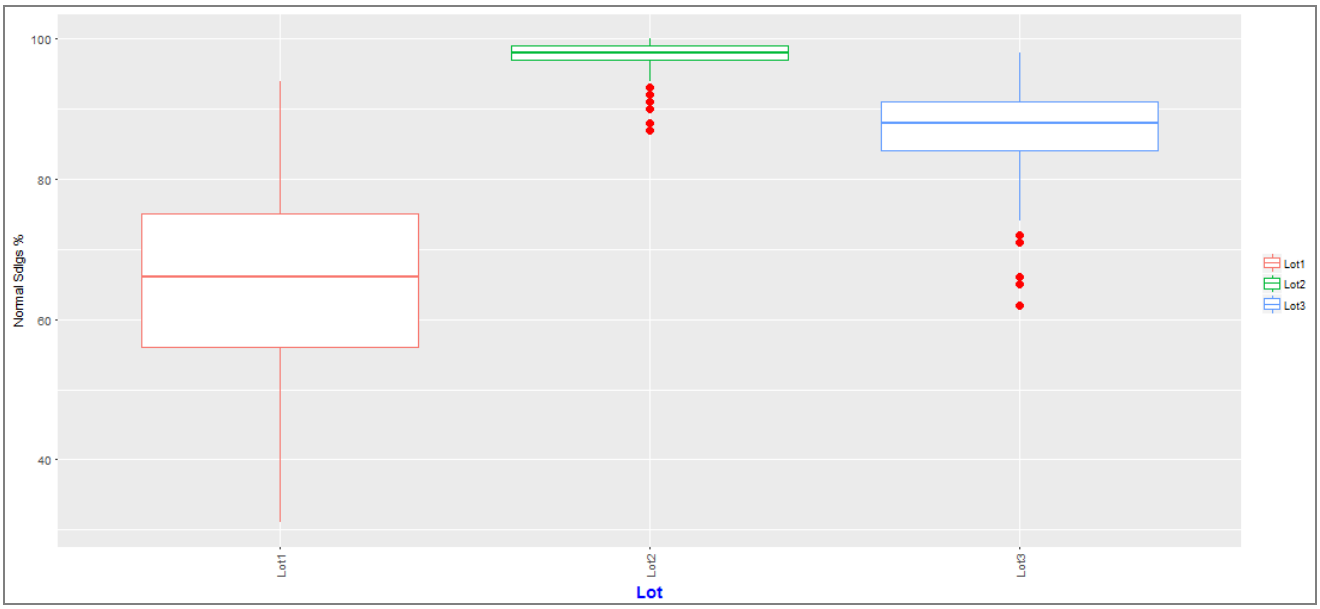

Fig. 1: Germination results of each seed lot

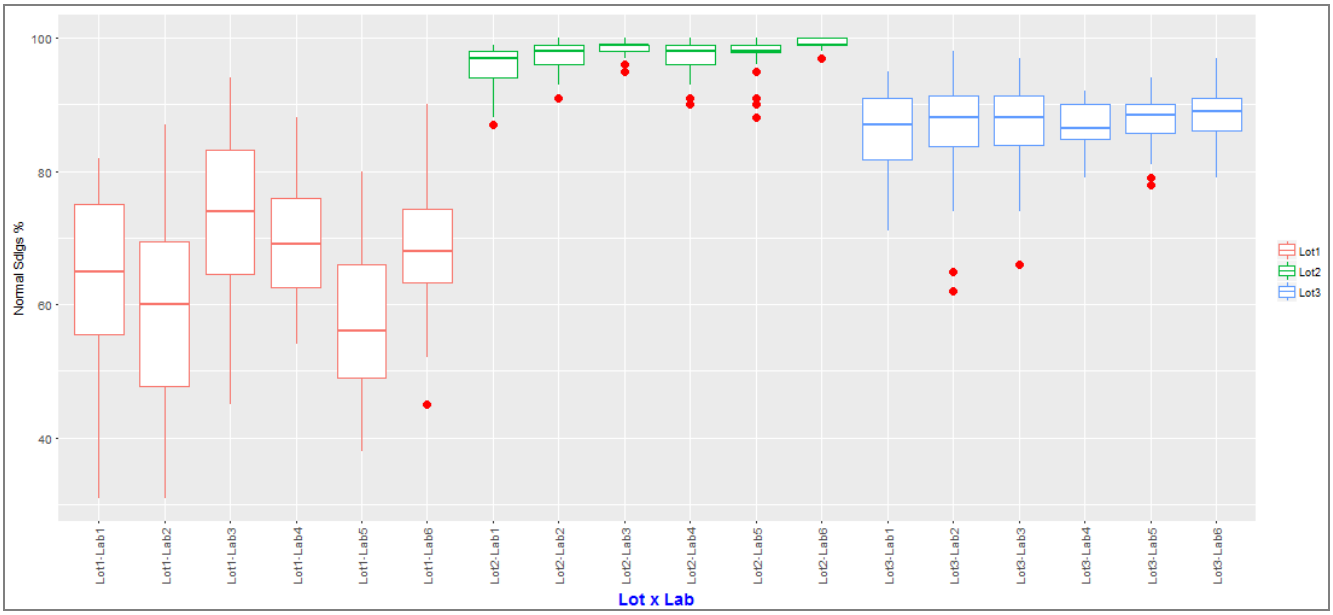

Fig. 2: Germination results by lot and laboratory

b. Methods: In addition to ANOVA indicating an overall significant effect of methods, results averaged over all lots and laboratories, indicated many significant differences. TP CCP at 20<=>30C and 25C, and to a lesser extent TP CCP 20C, showed lower variation and higher median germination percentage than most other methods, as indicated in Figure 3 be. Moreover, the higher germination percentage due to CCP was more pronounced for the lot with the lowest germination percentage, namely Lot 1 (Figure 4, Lot x Method), averaged over results from all laboratories. Specific statistical comparisons and differences among other parameters are presented in later sections.

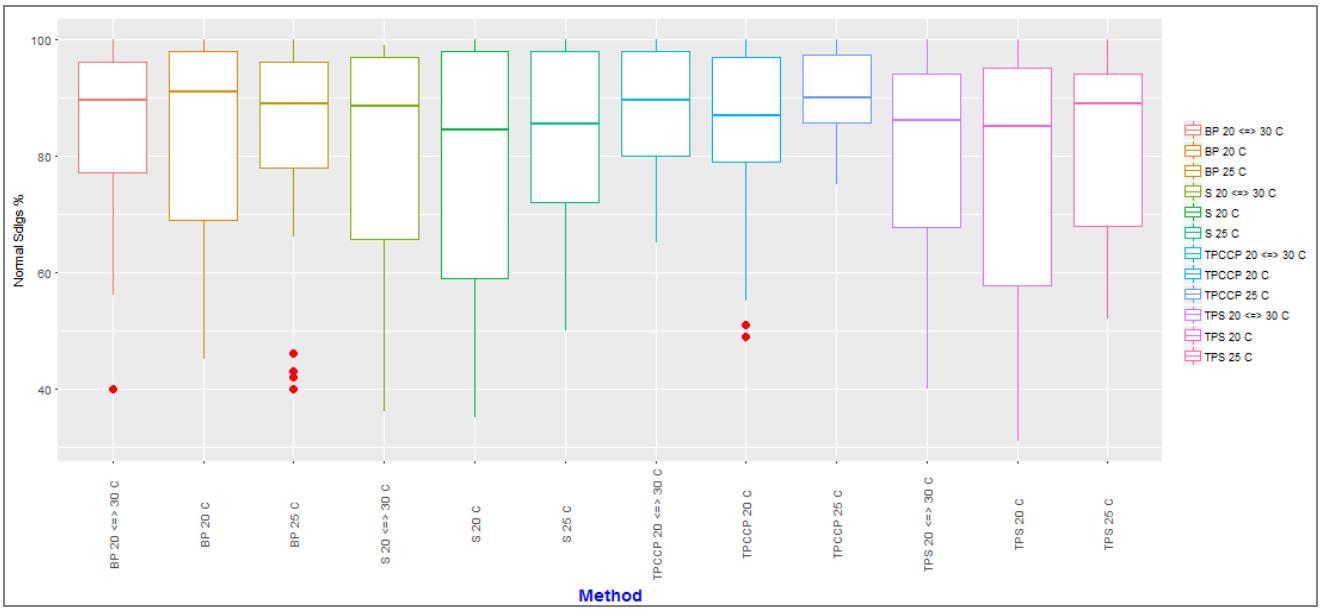

Fig. 3: Germination results by method

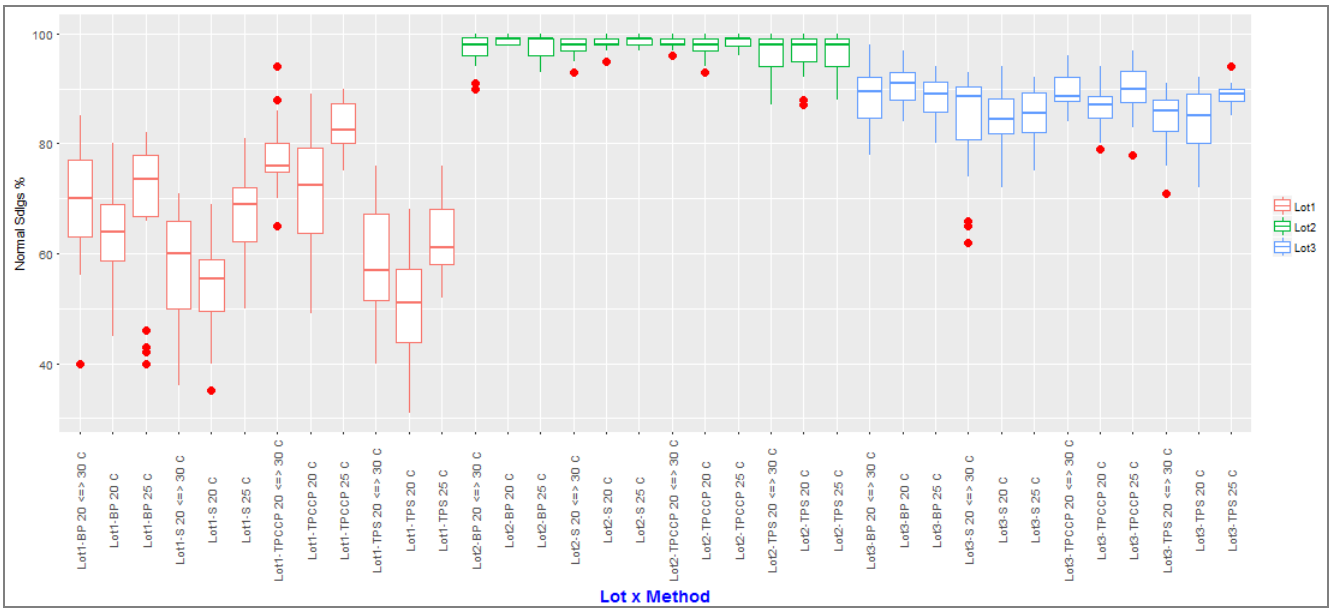

Fig. 4: Germination results by lot and method

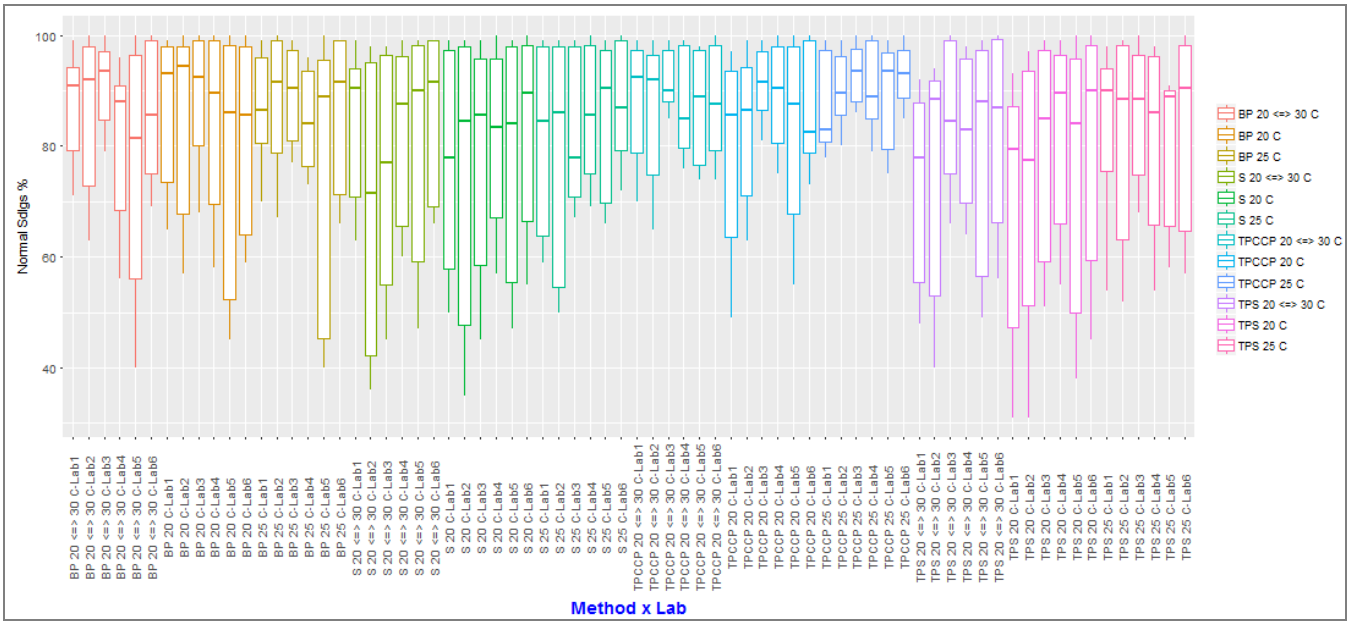

Fig. 5: Germination results by method and laboratory

3. Repeatability/Reproducibility

The dispersion factor (f_r a measure of repeatability) was less than 1 for almost all methods, and very close to 1 for BP 20<=>30C and TPS 20 C. Accordingly, proposed CCP methods did not lead to an unusual increase in variability and the Repeatability standard deviation (S_r) as well as the Reproducibility standard deviation (S_R) were in line with most other methods (Table 3). TP CCP at 20<=>30C had the lowest standard deviation for repeatability and the second lowest standard deviation for reproducibility after TP CCP 25C, across all combinations of media and temperature tested. TP CCP 25C led to the highest germination percentage averaged over all lots.

Table 3: Repeatability/Reproducibility by method

Method	$\overline{p}_{...}$	S_r	f_r	S_R	$\sqrt{\hat{\sigma}^2_{Lab}}$	$\sqrt{\hat{\sigma}^2_{Lot\times Lab}}$
BP 20 <=> 30 C	85	3.92	1.10	7.54	3.49	5.41
BP 20 C	84	3.00	0.82	5.97	2.45	4.54
BP 25 C	85	2.63	0.74	8.30	0.00	7.87
S 20 <=> 30 C	80	3.63	0.90	8.74	5.67	5.57
S 20 C	79	3.80	0.93	5.87	2.07	3.97
S 25 C	83	2.46	0.66	5.68	0.86	5.04
TPCCP 20 <=> 30 C	88	2.43	0.76	4.22	0.00	3.45
TPCCP 20 C	85	3.36	0.94	7.14	2.73	5.67
TPCCP 25 C	90	2.56	0.87	3.95	1.60	2.56
TPS 20 <=> 30 C	80	3.47	0.87	7.21	3.05	5.53
TPS 20 C	76	4.63	1.09	7.31	5.03	2.59
TPS 25 C	83	3.77	0.99	4.96	0.00	3.23

4. Comparison of Lot, Lab and Method Germination Results

Tables 4 to 6 list the means of normal seedlings percentage organized by lot, method, and lot x method. As expected, the three lots had significantly different germination percentages, ranging from 97.66% to 65.18% (Table 4). Different methods resulted in a wide range of germination results, from 76.50% to 90.49% (Table 5). TPCCP 25 C resulted in the highest germination, significantly greater than all other methods, with the exception of TPCCP 20 <=> 30 C.

Table 4: Differences between lots, averaged over all labs and methods.

Lot	Normal seedlings (%)
Lot1	65.18 a
Lot2	97.66 b
Lot3	87.06 c

Table 5: Differences between methods, averaged over all lots and laboratories.

Method		Normal seedlings (%)
Substrate	Temperature	
TPCCP	20 <=> 30 C	88.35 AB
TPCCP	25 C	90.49 A
TPCCP	20 C	85.18 BC
S	20 <=> 30 C	79.76 DEF
S	20 C	79.04 EF
S	25 C	83.44 CD
TPS	20 <=> 30 C	79.90 DEF
TPS	20 C	76.50 F
TPS	25 C	82.58 CDE
BP	20 <=> 30 C	84.96 BC
BP	20 C	84.31 BC
BP	25 C	85.10 BC

Table 6: Method: Lot

Lot	Method	Mean Germination %	
1	BP 20 <=> 30 C	83.25	A
	BP 20 C	77.46	B
	BP 25 C	70.96	C
	S 20 <=> 30 C	69.17	CD
	S 20 C	69.04	CD
	S 25 C	66.33	CDE
	TPCCP 20 <=> 30 C	63.67	DEF
	TPCCP 20 C	62.79	EFG
	TPCCP 25 C	58.62	FGH
	TPS 20 <=> 30 C	57.29	GH
	TPS 20 C	54.46	HI
	TPS 25 C	49.08	I
2	BP 20 <=> 30 C	98.96	A
	BP 20 C	98.62	A
	BP 25 C	98.42	A
	S 20 <=> 30 C	98.29	A
	S 20 C	98.25	A
	S 25 C	97.79	A
	TPCCP 20 <=> 30 C	97.67	A

	TPCCP 20 C	97.62	A
	TPCCP 25 C	97.29	A
	TPS 20 <=> 30 C	96.42	A
	TPS 20 C	96.33	A
	TPS 25 C	96.29	A
3	BP 20 <=> 30 C	90.29	A
	BP 20 C	89.79	AB
	BP 25 C	89.33	ABC
	S 20 <=> 30 C	88.67	ABC
	S 20 C	88.54	ABC
	S 25 C	88.46	ABC
	TPCCP 20 <=> 30 C	86.79	ABC
	TPCCP 20 C	85.38	ABC
	TPCCP 25 C	84.75	ABC
	TPS 20 <=> 30 C	84.38	BC
	TPS 20 C	84.38	BC
	TPS 25 C	84.00	C

Results from fitting the mixed model are in the worksheets *ANOVA table*, *LS Means* and *Diff of LS Means* from the file *2018 ISTA Zea mays study - Stat analysis - 091518.xlsx*.

All the fixed effects (Lot, Method, Lot x Method) were highly significant.

The Least Squares Means are displayed in the following bar plots (Figure 6) with error bars added corresponding to their standard errors.

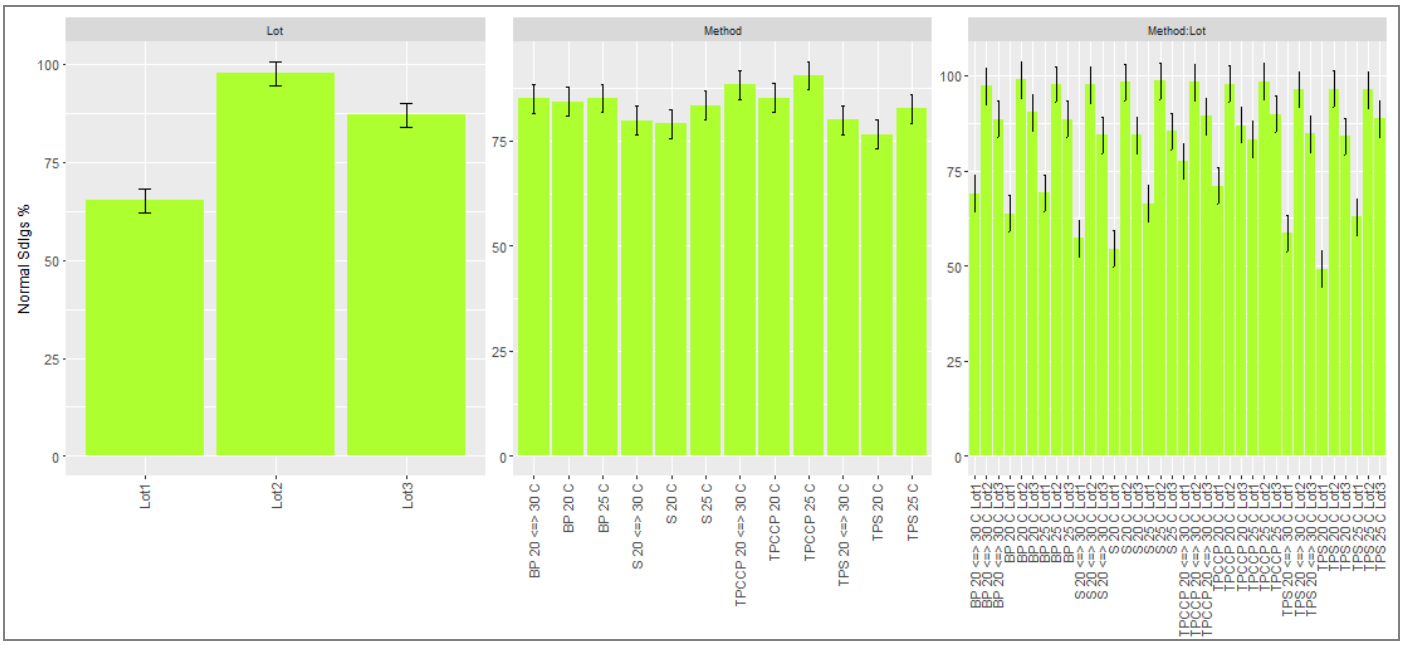

Fig. 6: Bar plot for Least Square Means

The differences of Least Squares Means table with p-values, standard-errors and 95% confidence intervals are displayed in the following bar plots (Figure 7).

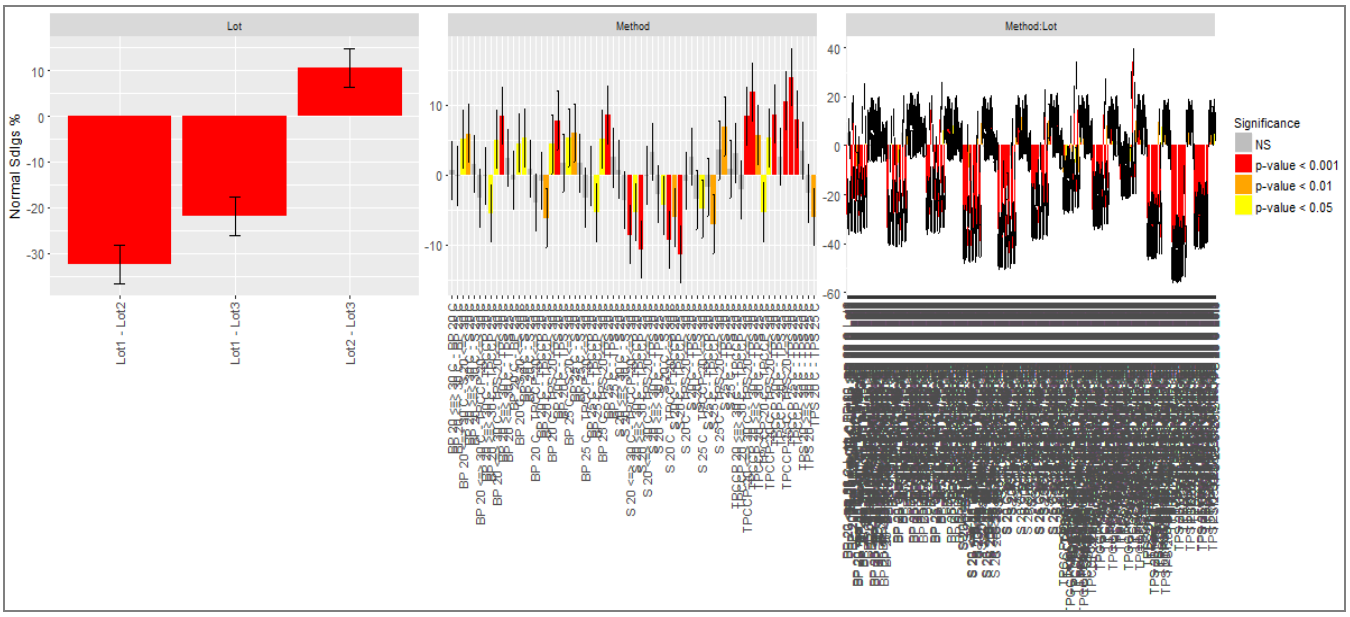

Fig. 7

General Conclusions

1. Methods using TP CPP had comparable repeatability and reproducibility when compared to other methods listed in the ISTA Rules, and in fact tended to lead to a reduced variation.
2. Methods using TP CPP led generally to higher germination results compared to currently used methods, especially compared to those with sand as the substrate.
3. Of all the methods tested, TP CCP 25 C led to the highest germination results of the *Zea mays* lots tested and showed good repeatability and reproducibility.
4. It is recommended to add TP CCP to the list of approved methods for *Zea mays*.
5. Additional studies should be initiated to investigate whether sand should be kept as a substrate for *Zea mays*, given the poor results exhibited in this study.

Acknowledgements

Thank you to the Monsanto Seed Physiology Laboratory, Waterman, IL USA staff for providing the seed for this study and to the SGS North America Brookings, SD, USA staff for packaging and shipping samples to the participating laboratories.

Thank you to the participating laboratories: Illinois Crop Improvement, Champaign, IL USA; Iowa State University, Ames, IA USA; Louisiana Dept. of Agriculture and Forestry, Baton Rouge, LA, USA; Monsanto Seed Physiology Laboratory, Waterman, IL USA; SGS North America Brookings, SD, USA; SoDak Labs, Brookings, SD USA. Their combined donated testing services exceeded a total of $5,000 USD.

Thank you to Nadine Ettel, ISTA TCOM Coordinator; ISTA Technical Reviewers Lucile Daron of Enza Zaden R&D Research Station, France and Meriam Dekalo-Keren of ARO Volcani Center Official Seed Testing Laboratory, Israel; Sylvie Ducournau and Gillian Musgrove, the very supportive ISTA Germ TCOM Chair and Vice-Chair; and the enthusiastic Germ TCOM members.

Thank you to Jean-Louis Laffont, PhD ISTA Statistical Committee Chair and Riad Baalbaki, PhD AOSA/SCST Statistical Committee Co-Chair for their much needed and appreciated statistical support.

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1. International Seed Testing Association. 2018. ISTA International Rules for Seed Testing. Bassersdorf, Switzerland

Proposal for the addition of wheat (*Triticum aestivum)* as a species to which the radicle emergence test for seed vigour can be applied to support C.15.2

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Summary

Radicle emergence (RE) was assessed for six seed lots of wheat (*Triticum aestivum)*, all having a laboratory germination of above 85%. Seeds were set to geminate between paper towels, as in a standard germination test and RE was assessed as production of a 2mm radicle after 48h at 15°C by each of four laboratories. Clear and significant differences in the RE count were observed between lots in all laboratories. All laboratories consistently identified the same significant differences in the seed lot RE and the results were repeatable within laboratories and reproducible between laboratories. This provides evidence in support of the addition of wheat (*Triticum aestivum)* to the ISTA Rules as a species for which the RE test can be applied.

Introduction

Early counts of radicle emergence are closely related to the rate of germination, as expressed by the mean germination time (MGT), and to vigour, reflected in the rate of, and final, field emergence, in a range of species (Matthews and Powell, 2011; Powell *et al.,* 2015). This has led to the development of the radicle emergence (RE) tests for *Zea mays, Brassica napus* and *Raphanus sativus*, to their validation and eventually to their publication in the ISTA Rules (ISTA, 2018).

The objective of the current work was 1) to provide evidence that the RE test can predict differences in field emergence of wheat seed lots and 2) to conduct a comparative test to determine whether the RE test applied to wheat is both sufficiently repeatable within laboratories and reproducible between laboratories, that this species could be added to those to which the RE test can be applied.

Materials & Methods

Seeds of 18 seed lots of wheat (*Triticum aestivum*) were obtained from different seed suppliers in Iran. All the seed lots were tested for laboratory germination, field emergence and radicle emergence after 48h at 15°C

Laboratory germination

Laboratory germination was tested between paper towels, as described in the ISTA Rules (2018), using four replicates of 50 seeds. All lots had standard germination above 85%.

Field emergence trials

Field emergence (FE) was carried out in a completely randomized block design with four replications of 50 seeds from each seed lot in the Research Field of the Ferdowsi University of Mashhad where seeds were sown by hand at a depth of 2 cm. The average air temperature during the experiment was 8^{o}C. Emergence was counted daily for 17 days until no further increase was observed. Mean emergence time (MET) was calculated using the formula:

$$MET = \Sigma (n.t) / \Sigma n$$

n= number of seeds newly emerged at time t;

t= days from sowing,

Σn= final emergence

Radicle emergence test

Four replicates of 50 seeds of each seed lot were set up for the RE test using paper towels pre-moistened by soaking the towels in a plastic tub of distilled water. Two towels were placed on top of each other and the fifty seeds in each replicate were arranged in rows along the length and in the centre of the towel with the embryos facing upwards. The distance between the rows was 5 cm. A third moist towel was placed on top of the seeds. The paper towels were then loosely rolled up and secured with two elastic bands at the top and bottom of the roll and were placed vertically in a plastic box. The box was covered loosely with a plastic bag to maintain moisture levels and held at 15^{o}C and darkness. If more than one box was used for the six seed lots, the position of the boxes in the incubator was changed twice within working hours (with approximately a six hour interval) during the 48h incubation. Seed lots were set up with sufficient time between them to allow for taking the radicle emergence counts after 48h ± 15 minutes.

Two hours after setting up the test, the boxes were checked to make sure there was no free water in the bottom of the plastic boxes. If there was, any water was poured out. This aimed to avoid uneven water availability among the replicates and the seed lots.

Radicle emergence for each replicate was counted after 48 hours. Radicle emergence was defined as the appearance of 2 mm radicle protrusion. The mean percentage RE was calculated for each seed lot.

Comparative test

Six lots showing high laboratory germinations and clear differences in RE were selected from the 18 lots used in the field emergence trial, so that they included two high, two medium and two low vigour lots (table 1). The lots were sealed in foil packets, coded and sent to the participating laboratories (table 2). Each laboratory completed the RE test as described above within two weeks of receipt of the samples.

The data were analysed by (a) ANOVA, (b) calculation of z-scores and (c) the statistical tool developed by S. Grégoire according to ISO 5725-2 to calculate h-values and k-values. The statistical tool is available for download at the ISTA website. Tolerances within laboratories were calculated using the Germination Tolerances calculator (V0.3) available on the Germination Committee page of the ISTA website.

http://www.seedtest.org/upload/cms/user/ISO572511.zip

Results

Establishment of a relationship between field emergence and RE

Different levels of FE were observed for seed lots with very similar germination levels. For example lots B and D, both with normal germination above 90%, gave FE of 92.5% and 13% respectively (table 1). Similarly lots C and E with normal germinations of 85% and 88% had FE of 80% and 29% respectively (table 1).

There was no significant relationship between the laboratory germination and FE (r = 0.109) of the 18 seed lots. However, a positive correlation (r = 0.792**) was observed between RE and field emergence (FE), and a negative one (r = -0.570*) was found between RE and the mean emergence time (MET). When only the six seed lots used for the comparative test were considered, an even higher significant positive correlation (r = 0.980**) was observed between RE and FE and a negative correlation (r = -0.983**) between RE with MET.

Comparative test

Box plot analysis revealed differences between the average RE for the six seed lots (figure 1A) with few outside values and there were no outliers between laboratories (figure 1B). Comparison of labs x lots (figure 1C) revealed a number of outliers, most of which were associated with lot F and E where radicle emergence was close to 100 and less than 10% respectively (Appendix 1).

There were clear and significant differences in the RE between the lots, ranging from a mean of 94% for lot F (high vigour) to 4% for E (low vigour). The differences were consistent between the laboratories, with lots D and E identified as low vigour, lots C and A as intermediate vigour and lots F and B as high vigour. There were small, although significant differences, between the means for the four laboratories.

Calculation of the z-scores (table 4) revealed that all data fell within the values that are considered acceptable within ISTA proficiency tests, i.e. +2.00 to -2.00. In addition, the replicates within the laboratories all fell within tolerance.

No significant h-values were found (figure 3), indicating that the measurements were neither over nor underestimated. There were only two significant k-values, namely for lot F in lab 3 and lot B in lab 4 (figure 2) indicating that there was greater variability between replicates.

Discussion

Field emergence trials clearly established that a single count of RE after 48 hours at 15^{o}C identifies differences in the field emergence, i.e. vigour, of seed lots of wheat that have high and similar levels of normal germination (table 1), The range of RE test results from 4 to 94%, clearly and consistently distinguished between seed lots, with lots F and B having high vigour and lots E and D with low vigour (tables 3 and 4). The test was both repeatable within four laboratories and reproducible in different laboratories. These data therefore support the proposal that the RE test could be applied as a vigour test for wheat seed lots.

Since the comparative test was completed, a paper has been published on the subject of RE in wheat (Guan *et al*., 2018). This paper supports our observations that a single RE count can predict emergence potential in wheat. However the paper differs from the work reported here in two respects. Firstly, Guan et *al*. (2018) report that the best correlations with emergence were achieved by single counts of RE after 48 hours at 20^{o}C and 72 hours at 13^{o}C. Secondly, Guan *et al.* (2018) tested emergence in trays in the laboratory, although they used soil obtained from the field. Thus our work and that of Guan *et al.* (2018) has shown that a single count of RE correlates with emergence when assessed at different times and temperatures. This observation is not unexpected since germination characteristics, such as mean germination time, have shown similar relationships with measures of seed vigour, even when they are determined at different temperatures (Khajeh Hosseini *et al*., 2009). Testing emergence in field soil in the lab provides evidence of potential differences in emergence in the field but does not expose seeds to complete field conditions. In our background research to the development of the RE test for wheat we also assessed RE at several temperatures and times, and compared these single counts to emergence in actual field conditions (paper in preparation for publication). We selected 48 hours at 15^{o}C for the comparative test as the best correlation that was achieved at a suitable time and temperature for practical use in a seed testing laboratory. The comparative test has shown that these conditions meet the requirements for the validation of this test for wheat.

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References

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Table 1. Seed lots of wheat used in the radicle emergence comparative test

Seed lot	Standard germination (%)	Field emergence (%)
F	98	97.5
B	94	92.5
A	88	86.5
C	85	80
D	92	13
E	88	29

Table 2. Participants in radicle emergence comparative test for wheat

Laboratory	Participant
Department of Crop Science, Ferdowsi University of Mashhad, Iran	Mohammad Khajeh-Hosseini
National Institute of Agricultural Research, Oliveros, Argentina	Carina Gallo
SNES, GEVES, Angers, France	Marie-Helene Wagner
OSTS, SASA, Edinburgh, UK	Gillian McLaren

Table 3. Comparison of laboratory and seed lot means of six lots of wheat tested by four laboratories using the RE test for 48 hours ± 15 minutes at 15°C

Lab	Lot						Lab means
	A	B	C	D	E	F	
Lab 1	86 ^{CD}	97 ^{A}	84 ^{CD}	25 ^{F}	11 ^{H}	99 ^{A}	66.92 ^{a}
Lab 2	85 ^{CD}	97 ^{A}	90 ^{BC}	20 ^{G}	6 ^{HJ}	97 ^{A}	65.42 ^{a}
Lab 3	69 ^{E}	94 ^{AB}	82 ^{D}	7 ^{HI}	1 ^{IJ}	89 ^{BC}	56.75 ^{b}
Lab 4	85 ^{CD}	85 ^{CD}	85 ^{CD}	0 ^{J}	0 ^{J}	94 ^{AB}	58.00 ^{b}
Lot means	81.00 ^{c}	93.13 ^{a}	85.13 ^{b}	12.75 ^{d}	4.38 ^{e}	94.25 ^{a}	

Table 4. Comparison of means, standard deviations (SD) and z-scores for six seed lots of wheat tested by four laboratories using the RE test for 48 hours ± 15 minutes at 15°C

Lot						
Lab	**A**	**B**	**C**	**D**	**E**	**F**
	a) means					
Lab 1	86	97	84	25	11	99
Lab 2	85	97	90	20	6	97
Lab 3	69	94	82	7	1	89
Lab 4	85	85	85	0	0	94
Mean	81.00	93.13	85.13	12.75	4.38	94.25
SD= S x^-	8.3566	5.5734	3.4248	11.5072	5.0229	4.3493
b) Z-scores						
Lab 1	0.598	0.695	-0.328	1.065	1.319	0.977
Lab 2	0.419	0.606	1.423	0.587	0.224	0.517
Lab 3	-1.496	0.157	-0.912	-0.543	-0.672	-1.322
Lab 4	0.479	-1.458	-0.182	-1.108	-0.871	-0.172

Table 5. Mean germinations and tolerance ranges (4 replicates x 50 seeds) for six lots of wheat tested in four laboratories using the RE test for 48 hours ± 15 minutes at 15°C

Lot		Lab 1	Lab 2	Lab 3	Lab 4
A	Mean	86	85	69	85
	Observed range	4	16	18	10
	Maximum tolerance range	20	20	26	20
B	Mean	97	97	94	85
	Observed range	2	6	6	14
	Maximum tolerance range	10	10	14	20
C	Mean	84	90	82	85
	Observed range	6	6	16	10
	Maximum tolerance range	21	17	22	20
D	Mean	25	20	7	0
	Observed range	6	8	4	0
	Maximum tolerance range	24	22	14	0
E	Mean	11	6	1	0
	Observed range	4	6	2	0
	Maximum tolerance range	17	13	4	0
F	Mean	99	97	89	94
	Observed range	2	2	12	4
	Maximum tolerance range	7	10	18	14

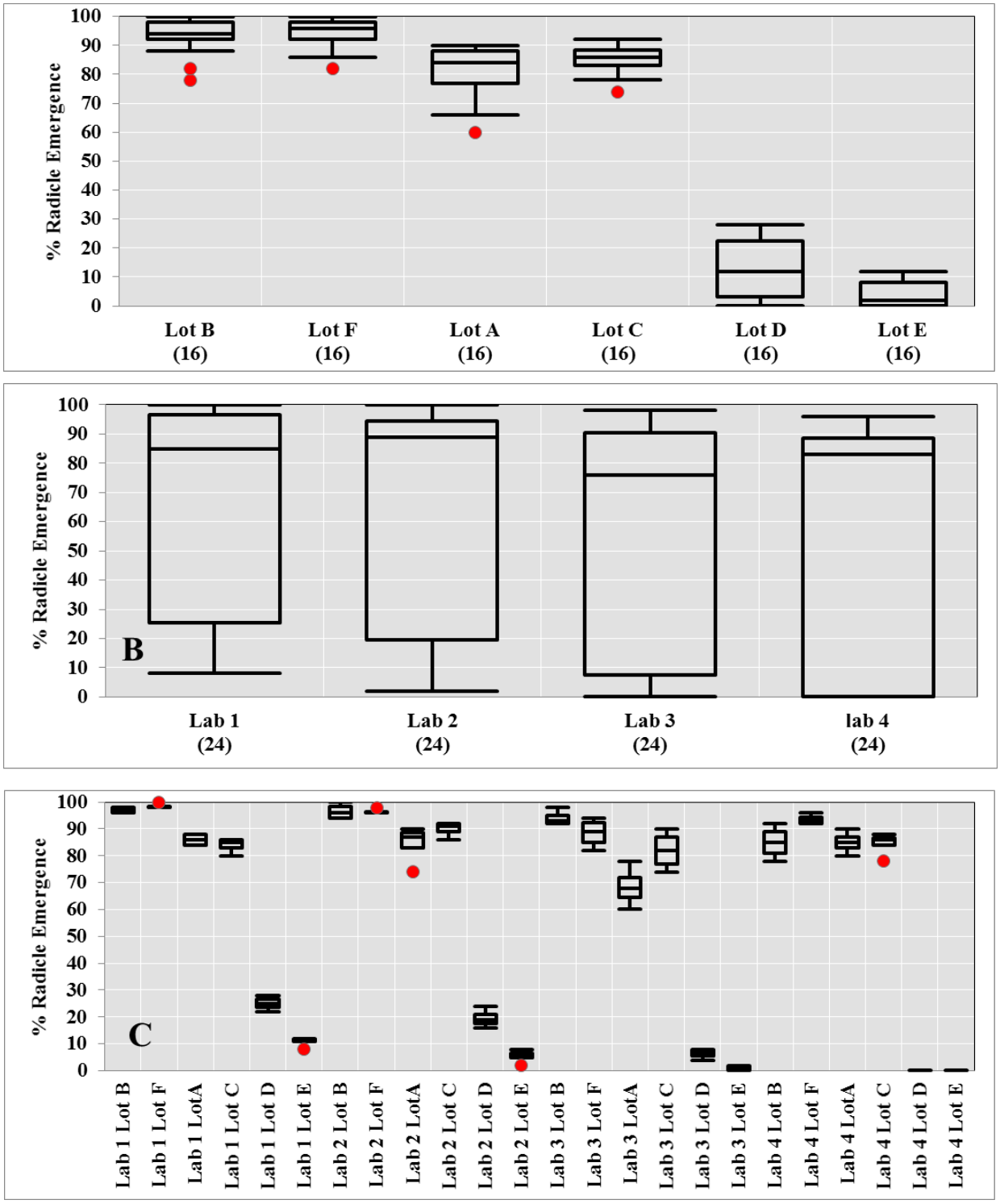

Figure 1 . Box plot comparisons of the RE data from six seed lots of Triticum aestivum (wheat): (A) seed lots, (B) laboratories and (C) seed lot x laboratory

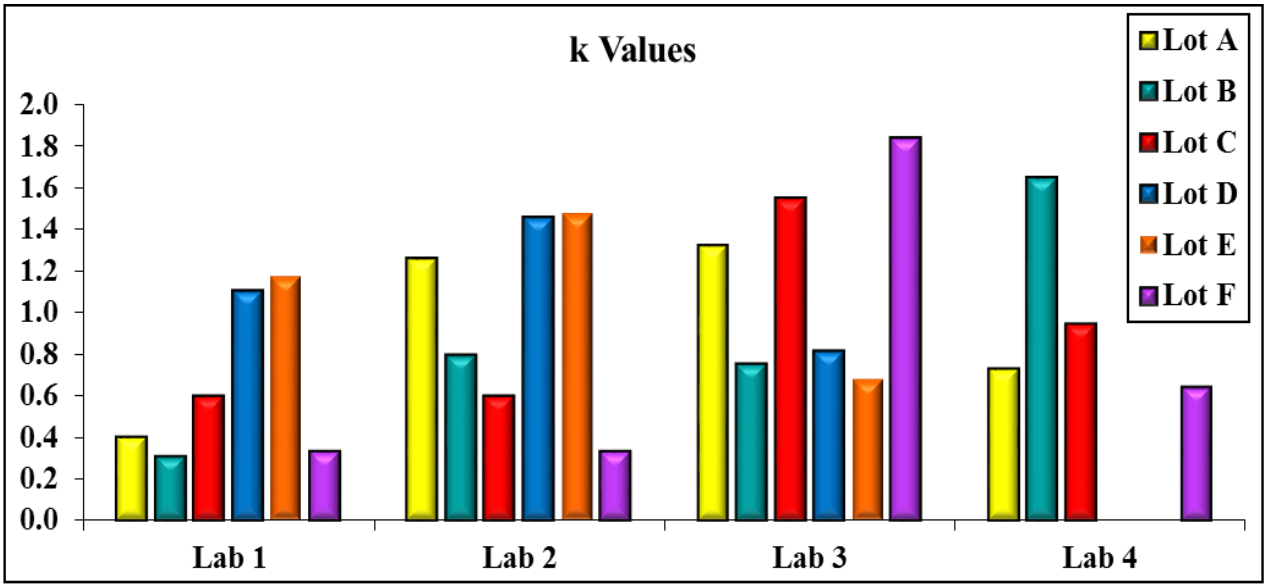

Figure 2. k-values for six seed lots of wheat following the radicle emergence test in four laboratories

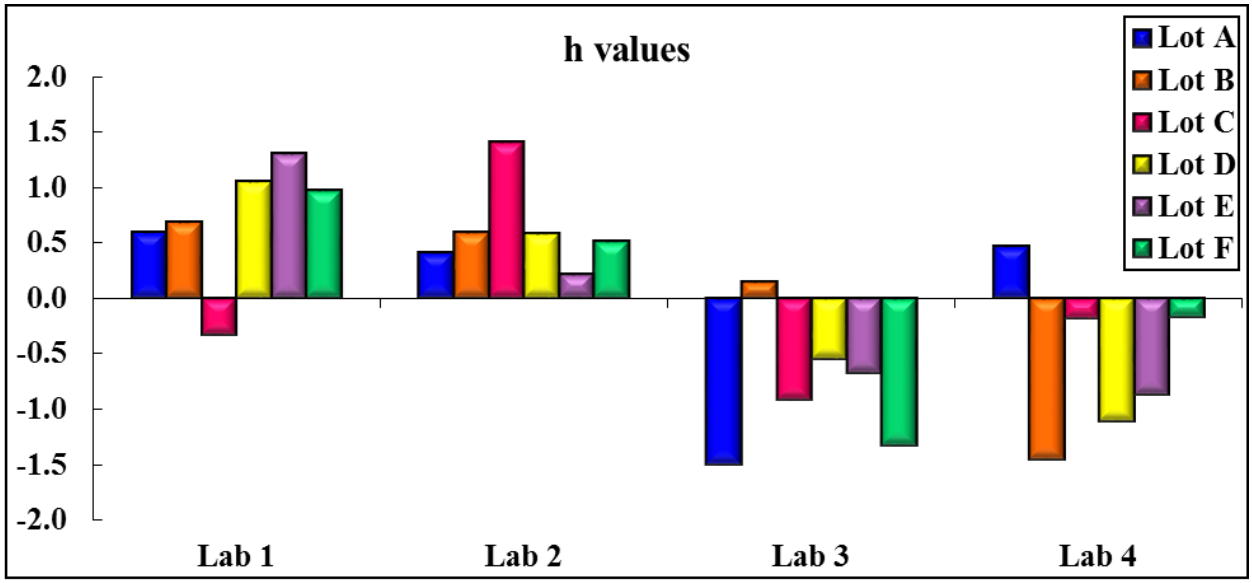

Figure 3. h-values for six seed lots of wheat following the radicle emergence test in four laboratories

Appendix 1. Summary of all raw data for wheat radicle emergence test carried out on six seed lots in four laboratories

Lot	Rep	Lab			
		1	2	3	4
A	1	88	74	66	80
	2	84	90	70	90
	3	88	86	78	84
	4	84	88	60	86
	Mean	86	84.5	68.5	85
	SD	2.3	7.2	7.5	4.2
B	1	96	100	98	92
	2	96	94	92	78
	3	98	98	92	88
	4	98	94	94	82
	Mean	97	96.5	94	85
	SD	1.2	3.0	2.8	6.2
C	1	86	90	90	88
	2	84	86	74	86
	3	86	92	78	78
	4	80	92	86	86
	Mean	84	90	82	84.5
	SD	2.8	2.8	7.3	4.4
D	1	28	16	6	0
	2	24	18	8	0
	3	26	24	8	0
	4	22	20	4	0
	Mean	25	19.5	6.5	0
	SD	2.6	3.4	1.9	0.0
E	1	12	8	2	0
	2	12	2	0	0
	3	12	6	2	0
	4	8	6	0	0
	Mean	11	5.5	1	0
	SD	2.0	2.5	1.2	0.0
F	1	98	96	94	92
	2	98	98	86	94
	3	100	96	92	92
	4	98	96	82	96

	Mean	98.5	96.5	88.5	93.5
	SD	1.0	1.0	5.5	1.9