ISTA
Seed Quality Assurance

ISTA Secretariat
Richtiarkade 18, 8304 Wallisellen, Switzerland
Phone: +41 44 838 60 00 | Fax: +41 44 838 60 01
Email: ista.office@ista.ch
www.seedtest.org

Summary results: PT 24-2 A.hyp

Species: *Arachis hypogaea* **Scope:** GER

Preparation of this PT round started one year ahead of the samples dispatch, in accordance with the procedure PT-P-02-Proficiency Test Sample Preparation. Samples were prepared from seed lots that have been processed and ready for use without further conditioning except of applying the seed treatment.

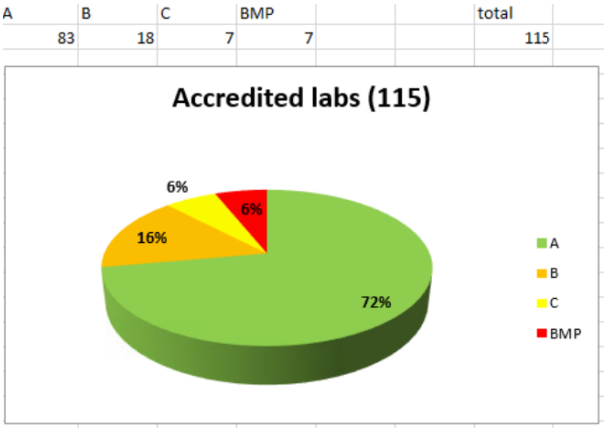
For conducting the heterogeneity test within the PT Leader laboratory, 10 randomly assigned samples were treated with chemicals according to the ISTA Rule 5.6.3.4 “Disinfection of the seed: for samples of *Arachis hypogaea* and *Beta vulgaris* only, a fungicide treatment may be applied by the laboratory before planting the seed for germination.” The heterogeneity test results were very good, without indication of high fungi contamination.

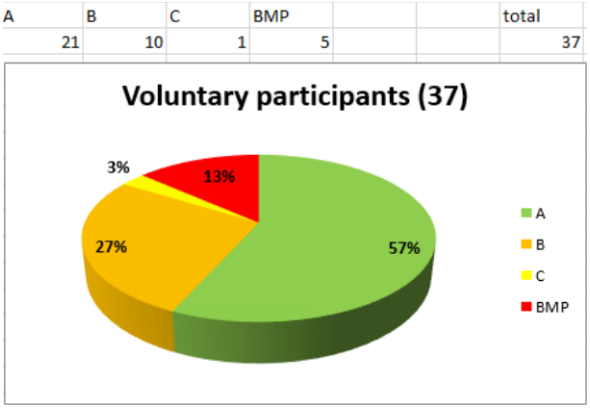
Samples were dispatched to the ISTA Secretariat from the country of origin as non-treated seed, in accordance with the Swiss import regulations for this species. Therefore, the non-treated seed samples were distributed to the participants from the ISTA Secretariat in July 2024.

The deadline for reporting test results to the ISTA Secretariat was 1 November 2024, which was postponed to 15 December 2024. By this date only **86%** out of 176 participants submitted their test results. This is a total number of participants of 152, with 115 obligatory participants and 37 voluntary participants, and a **very low reporting rate in comparison with other PT Rounds.**

<u>Evaluation of the preliminary results and rankings</u>

During the testing period participants faced many challenges with this PT round, from the samples’ delivery restrictions to the high presence of pathogens in the samples. In addition, most of the EU countries were legally restricted with the availability of effective fungicides required for pre-treatment of the seed lots.
When calculating the preliminary results, unusually high percentage of C and BMP ratings is observed both for the accredited and for the voluntary participants:





There were 12% C and BMP ratings among accredited obligatory participants and 16% C and BMP ratings among voluntary participants.

Mean and Standard Deviation

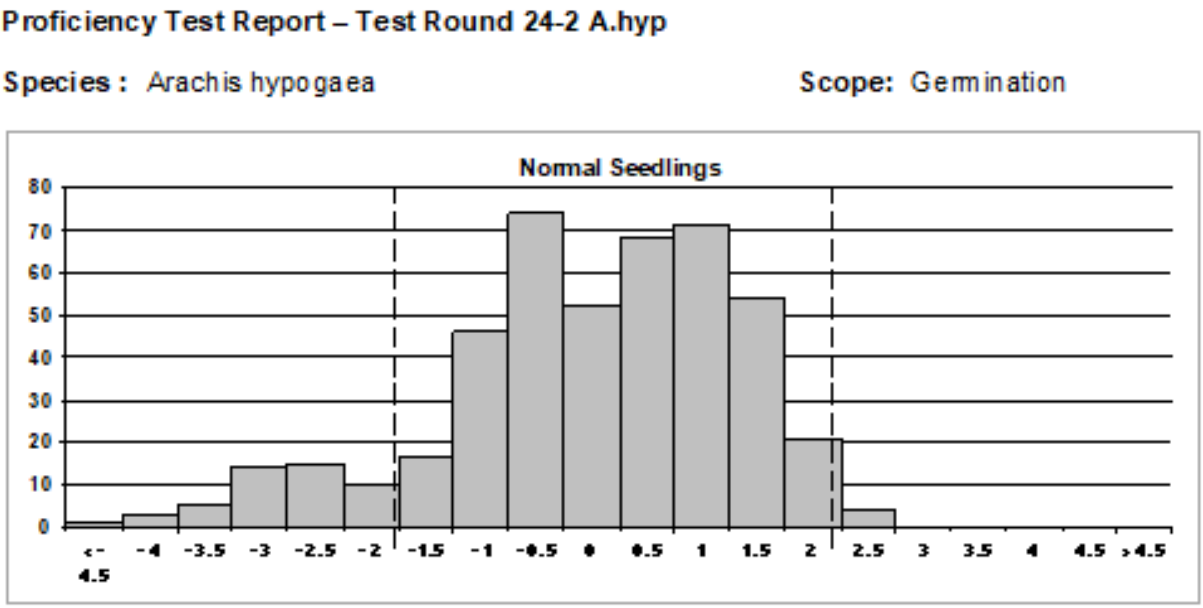
The calculation is based on the results provided by obligatory accredited participants, and it is calculated for the category of normal seedlings. Calculated means significantly differ from the mean that was obtained during the samples' preparation:

Categories	Calculated Mean Values %			Starting seed germination % (when the seed lots were selected)		
	Lot 1	Lot 2	Lot3	Lot 1	Lot 2	Lot 3
Normal seedlings	63	62	60	81	84	68

This PT is also characterized by very high Standard Deviations (calculated on laboratories with compulsory participation and excluding outliers) compared with previous germination PT rounds.

Categories	Standard Deviation (sd)		
	Lot 1	Lot 2	Lot 3
Normal seedlings	13.46	16.96	14.41

The Z-score Distribution confirms dispersity of the results



Despite of the large standard deviation and potentially many A and B ratings (due to the easier evaluation criteria), there were

- 12 outliers in the test and
- 14 obligatory participants would be potentially rated C or BMP

As a result, the preliminary results were NOT sent to the participants, and the Statistical Committee was asked to provide expert advice. They received the anonymous results and the ranking of the laboratories for their validation.

Conclusion of the ISTA Statistical Committee

The estimate of the reproducibility standard deviation (**sd**) is large compared to the theoretical value defined by Miles using the formula σ_R=sqrt(p(100-p)/400)*(2.38-0.008321*p):

Observed			Theoretical (Miles)		
sd n1	sd n2	sd n3	sd n1 th	sd n2 th	sd n3 th
13.46	16.96	14.41	4.49	4.54	4.59

- If we use the **theoretical sd** in the denominator, the number of labs achieving a BMP or a C rating will be very high: 113 out of 152 participants would receive BMP or C ratings, which equals **74%** of participants.
- If we use the **observed sd** instead, this percentage drops to **13%** (including outlier labs).
- **This data clearly indicates a reproducibility issue for this species.**

Out of the 152 accredited laboratories, 12 have at least one outlier across the three lots. The total number of outliers among the 3 × 152 = 456 results is 24, representing 5.3%, which aligns with the 5% level of the outlier test used. However, the outlier test is designed to provide a robust estimate of the mean and variance used to compute the z-scores, rather than to determine the validity of the test round.

Method effects

Complementary to the statistical analysis the PTC chair and PTC Observer investigated the methods effect.

Presented for the temperature, substrate and pre-treatment, overview for all participants:

Temperature °C	# participants		# participants TOTAL
	Accredited labs	Voluntary labs	
20⇔30	67	22	**89**
25	45	15	**60**
15⇔20*	1		**1**
20*	1		**1**
Not reported	1		**1**

* temperature not in accordance with the ISTA Rules

Substrate	# participants		# participants TOTAL
	Accredited labs	Voluntary labs	
S	73	16	**89**
BP	33	19	**52**
O	6	2	**8**
PP	1		**1**
Soil	2		**2**

It was difficult to clearly capture the exact pre-treatment method reported by the participants, as sometimes several methods were used in combination. The possible overview of the pretreatment used is as follows:

Pre-treatment	# participants		# participants TOTAL
	Accredited labs	Voluntary labs	
fungicide	55	13	**68**
sodium hypochlorite	31	6	**37**
preheat / prewash	12	11	**23**
not treated	17	7	**24**

Study of the applied pretreatment

We tried to relate the obtained germination results with the pretreatment laboratories used, in order to verify if any specific pretreatment led to a higher germination percentage, having the original germination of the seed lots in mind.

	Obligatory				
		Germination %			Average of 3 lots
Treatment type	**# of labs**	Lot 1	Lot 2	Lot 3	
fungicide	52	**64**	**63**	**60**	**62**
desinfection (**sodium hypochlorite)**	33	58	58	58	58
preheat/prewash	12	56	54	53	54
no treatment	18	55	51	48	51
Total obligatory	**115**				

	Voluntary				
		Germination %			Average of 3 lots
Treatment type	**# of labs**	Lot 1	Lot 2	Lot 3	
fungicide	13	58	58	60	59
desinfection (**sodium hypochlorite)**	6	**73**	**77**	**71**	**74**
preheat/prewash	11	47	50	47	48
no treatment	7	57	49	48	51
Total voluntary	**37**				
Grand total	**152**				

Data shows that fungicide used as a pre-treatment by majority of the accredited laboratories resulted in the average of 62% germination, which is significantly lower from the starting germination percentage where the fungicide was also applied. ISTA Rules do not give any precision about the way fungicide may be used or its concentration, as each fungicide must be applied as described by the manufacturer's instructions on application. This was also an obstacle for the participants who do not regularly test this species.

Voluntary participants obtained better results with seed disinfection.

Conclusions:

- PT Leader confirmed that the quality of *Arachis* seed deteriorates fast, which makes this species very unstable for the purpose of the ISTA Proficiency Test Programme
- Many participants did not have testing experience with this species and the experience was not gained in preparation of testing this species within the PT Programme Plan 2023-2025
- In testing this species many participants could not apply an effective fungicide due to the legislation restrictions, which makes ISTA Rule 5.6.3.4 “Disinfection of the seed” no longer fully applicable
- Even if the treatment with the fungicide or other disinfection was possible, guidelines are not available on the concentration of the chemical use and for possible duration of the pre-treatment as each individual fungicide must be applied as specified by the manufacturer
- Obtained results are not reliable due to the significant difference of particular results from the calculated mean (for example, for the germination difference of even 20% a participant would be rated with an “A”)
- Based on these results no guarantee can be given to participants regarding the objectivity of the ratings and there will be no certainty of the laboratory technical competence in case of a potential request for the extension of the laboratory scope of accreditation for the crop group of pulses (crop group 4)
- The results cannot be included in the final calculation of the overall ratings as the high percentage of C and BMP ratings will negatively influence laboratories’ accreditations
- It will be difficult for the laboratories with C and BMP ratings to take corrective actions in this case.

Consequently, **the PT round will be cancelled** but the individual ranking (A, B, C, BMP ratings) may be calculated by each of the participants as information only, using the formula for the Z score calculation:

$Zi = (xi - x)/s$

- **Zi** score of individual laboratory
- **x**i mean of individual laboratory
- **x** overall mean
- **s** standard deviation

For the calculation explanation please refer to the online available procedure ISTA Standard Proficiency Test, related pages are 6 and 7.

Questions that this PT round generated

The outcome of this PT round rose several questions to be considered:

- Should there be any actions taken by ISTA to see how to continue with *Arachis* species in the ISTA Rules?
- Should *Arachis* be considered as a very sensitive species that can deteriorate fast due to the high content in oil?
- Should the fungicide pre-treatment method for this species be considered by the Germination Committee in the ISTA Rules?
- Considering the species instability, can the results of *Arachis* tests be reported on Orange ISTA Certificates or on the Blue ISTA Certificates only?

The PT Committee was in close contact during this PT round with the Germination Committee. Therefore, the issues are acknowledged, and the Committee will discuss the issues with *Arachis* species in the near future, to see if any improvements could be considered.

Report prepared by the PTC Chair and PTC Observer, 17.01.2025