

## DETERMINATION OF GERMINATION PERCENTAGE AND GERMINATION INDEX—COLLABORATIVE TRIAL AND RUGGEDNESS TESTING

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**A method for the determination of Germination Percentage and Germination Index in malting barley has been tested by the EBC Analysis Committee and approved for inclusion in Analytica-EBC. In the collaborative trial three pairs of barley samples with high, medium and low vigour were analysed by 16 laboratories. The  $r_{95}$  values for the Germination Percentage ranged from 0.5% to 6.3% and the  $R_{95}$  values ranged from 0.5% to 8.7% increasing with lower level of mean germination percentage. The  $r_{95}$  values for the Germination Index ranged from 0.29 to 0.74 and the  $R_{95}$  values ranged from 0.97 to 1.20. The ruggedness testing of the Germination Index shows that the factor temperature has a minor, but significant effect on the result, the factors time for the first count together with the volume of water, have major significant effects. The tested factors have no significant effect on Germination Percentage.**

**Key Words:** *Barley germination, vigour, ruggedness testing, collaborative trial, germination percentage, germination index.*

### INTRODUCTION

The EBC Analysis Committee decided to evaluate a new method for germination with emphasis on seed vigour, based on the results presented by Riis and Bang-Olsen at the EBC congress in Lisbon 1991<sup>1</sup>. The method is based on an extension of the BRF method for Germination Energy<sup>2</sup>. The result of the method is expressed as two values, one for the Germination Percentage and one for Germination Index. In non-dormant seed samples the Germination Index is a good measure of seed vigour.

The main differences between the new germination method and the BRF method are as follows:

- The petri dishes have been changed in size to 85 mm standard petri dishes;
- 400 grains are analysed in the test instead of 200 grains;
- Germinated grains are recorded every 24 hours instead of only after 72 hours;
- Improved control of temperature.

### COLLABORATIVE TRIAL

#### EXPERIMENTAL

The organisation of the collaborative trial and the statistical treatment of the data were carried out according to the guidelines given in the International Standard ISO 5725 (3). A uniform design was employed and three pairs of barley samples with low, medium and high vigour, were distributed to 16 laboratories, representing nine European countries.

### RESULTS AND DISCUSSION

The raw data of the collaborative trial are presented in Tables I and II for Germination Percentage and Germination Index respectively.

Based on Cochran's test on cell ranges and Dixon's outlier test on cell means, the results from laboratory 3 showed an outlier at sample A for the Germination Percentage.

Based on Dixon's outlier test a straggler was observed at sample B for laboratory 8 for Germination Percentage.

TABLE I. Raw data for Germination Percentage

Lab	Sample A		Sample B		Sample C	
	1	2	1	2	1	2
1	100	100	98	96	84	85
2	100	100	97	96	79	81
3	99	100	97	96	83	80
4	100	100	98	97	85	89
5	100	100	96	97	86	84
6	100	100	97	98	83	81
7	100	100	98	97	87	84
8	100	100	100	100	91	85
9	100	100	98	97	85	86
10	100	100	97	97	88	88
11	100	100	97	96	83	79
12	100	100	98	97	82	85
13	100	100	98	99	77	83
14	100	100	98	98	85	84
15	100	100	97	99	84	82
16	100	100	96	99	85	88

TABLE II. Raw data for Germination Index

Lab	Sample A		Sample B		Sample C	
	1	2	1	2	1	2
1	9.7	9.7	7.8	7.7	5.1	5.1
2	9.7	9.4	8.0	7.8	5.9	6.0
3	9.7	9.7	7.2	7.3	5.8	5.6
4	9.1	9.3	7.2	6.9	5.1	5.2
5	9.7	9.6	7.0	7.5	5.3	5.3
6	9.3	8.7	7.6	7.4	5.3	5.1
7	9.5	9.5	8.3	8.0	6.2	6.2
8	9.2	9.4	7.2	7.3	5.7	5.6
9	9.1	9.6	7.9	7.7	5.7	5.7
10	9.3	9.4	7.0	7.9	5.5	5.4
11	8.9	9.5	7.1	7.9	5.7	5.3
12	9.2	8.7	7.1	6.7	6.0	5.8
13	9.4	9.2	7.0	7.0	4.9	5.0
14	9.5	9.5	8.1	8.1	6.1	6.1
15	9.4	8.7	7.3	7.3	5.9	5.8
16	9.6	9.8	7.2	7.2	5.1	5.1

TABLE III. Summary of precision values for Germination Percentage

	Sample A	Sample B	Sample C
No. of laboratories	16	16	16
Mean (%)	100.0	97.5	84.1
r <sub>95</sub> (%)	0.5	2.6	6.3
CV <sub>r</sub> (%)	0.2	0.9	2.7
R <sub>95</sub> (%)	0.5	3.2	8.7
CV <sub>R</sub> (%)	0.2	1.1	3.7

TABLE IV. Summary of precision values for Germination Index

	Sample A	Sample B	Sample C
No. of laboratories	16	16	16
Mean	9.38	7.46	5.55
r <sub>95</sub>	0.70	0.74	0.29
CV <sub>r</sub> (%)	2.65	3.51	1.86
R <sub>95</sub>	0.87	1.20	1.12
CV <sub>R</sub> (%)	3.28	5.70	7.14

The outlier at sample A for laboratory 3 was not rejected from the computations of r and R as it was due to a minimum difference of 1% between 100 and 99% germination. Such a small difference will often appear in practical samples.

For the Germination Index a straggler at sample C from laboratory 11 was found based on Cochran's test on cell ranges. No outliers or other stragglers were observed concerning ranges or means.

A summary of the results for the statistical treatment of the data is given in Tables III and IV for Germination Percentage and Germination Index respectively.

As expected due to the binormal nature of the analysis, the repeatability and reproducibility values increased with decreasing level of Germination Percentage.

No correlation between repeatability and reproducibility values was found.

RUGGEDNESS TEST

TESTING PROCEDURE

Ruggedness testing was performed according to method 13.1 in EBC-Analytica<sup>4</sup>.

In the measurement of the germination rate, the analysis is considered to be sensitive to the germination conditions. The following incubation conditions found in the description of the method for Germination Index must be observed: specified size of petri dishes; specified number of filter papers; a growth temperature of 20±0.5°C in a growth cabinet with ventilation; addition of 4.0 ml of water; a record of germinated seeds at specified times, 24±0.5 hours, 48±1 hours and 72±1 hours after the addition of water.

The following three factors were included in the ruggedness testing:

- A. The temperature
- B. The time for the first count (which has a decisive influence on the final test results)
- C. The volume of water.

The chosen levels are listed in Table V. A medium vigour barley sample was chosen as test sample.

Other factors like type and number of filter papers, size and type of petri dishes may also influence the test results, but these factors, which are well defined in the description of the analysis, were not tested in the ruggedness testing, as they are considered as general faults.

TABLE V. Factors used in the ruggedness testing of Germination Percentage and Germination Index

Factor	Name	-	+
A	Temperature	19.4°C	20.6°C
B	First count	22.5 hours	25.5 hours
C	Volume of water	3.5 ml	4.5 ml

The importance of using the correct number of filter papers is dealt with separately, as this type of fault easily appears during the performance of many analyses.

RESULTS AND DISCUSSION

The results from the 3-level full factorial experiment with 2 replicates per cell are found in Table VI (Germination Percentage) and Table VII (Germination Index).

TABLE VI. Tabulation Chart for Germination Percentage

Trial Number	Factor			Test Results Replicates	Totals T	Signed Differences D	
	A	B	C				
1	-	-	-	96	97	193	-1
2	+	-	-	96	97	193	-1
3	-	+	-	96	97	193	-1
4	+	+	-	96	96	192	0
5	-	-	+	96	97	193	-1
6	+	-	+	96	95	191	1
7	-	+	+	96	95	191	1
8	+	+	+	95	96	191	-1

TABLE VII. Tabulation Chart for Germination Index

Trial Number	Factor			Test Results Replicates	Totals T	Signed Differences D	
	A	B	C				
1	-	-	-	6.0	6.3	12.3	-0.3
2	+	-	-	6.5	6.5	13.0	0
3	-	+	-	6.8	7.0	13.8	-0.2
4	+	+	-	7.2	7.3	14.5	-0.1
5	-	-	+	6.9	6.9	13.8	0
6	+	-	+	7.3	7.2	14.5	0.1
7	-	+	+	7.8	7.8	15.6	0
8	+	+	+	7.4	7.6	15.0	-0.2

TABLE VIII. Summary Table for Germination Percentage

Factor	Name	Effect	Confidence Limits	Confidence Interval
A	Temperature	-0.38	±0.76	-1.14 to 0.38
B	First count	-0.38	±0.76	-1.14 to 0.38
C	Volume of water	-0.63	±0.76	-1.39 to 0.13

TABLE IX. Summary Table for Germination Index

Factor	Name	Effect	Confidence Limits	Confidence Interval
A	Temperature	0.19	±0.13	0.06 to 0.31
B	First count	0.66	±0.13	0.53 to 0.79
C	Volume of water	0.66	±0.13	0.53 to 0.79

