2016 AOSA Rules Change Proposal 15

Purpose of Proposal: To clarify use of tables 14I and 14J for all germination test components.

I. Present Rule

14.5 Germination tolerances.

a. **Tolerances between replicates. –** Table 14I lists the maximum tolerance between two and four replicates of 100 seeds in a single germination test. The tolerance values were computed to allow for only random-sampling variation at the 0.025 probability level. Table 14I is adapted from Miles (1963; Table G1, columns A, B, D and L).

b. **Procedure for finding the maximum tolerance between replicates in a germination test. –** Calculate the average germination of the two or four replicates to the nearest whole number. Calculate the difference between the highest and the lowest germinating replicates. Locate the average germination in column A or B in Table 14I. The maximum tolerance between four replicates appears in the same row in column C. For two replicates, it appears in the same row in column D. Compare the calculated difference between replicates with the maximum tolerance found in the table. If the difference between the replicates is equal to or less than the value found in Table 14I, retesting is not necessary but a retest must be performed if it is greater.

Table 14I. Maximum tolerance values between two and four replicates of 100 seeds in a single germination or tetrazolium test (2-way test at *P = 0.025*).

Average percent germination		**No. replicates of 100 seeds**		**Average percent germination**		**No. replicates of 100 seeds**	
		4	2			**4**	2
A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
99	2	5	---	75	26	17	14
98	3	6	---	74	27	17	14
97	4	7	6	73	28	17	14
96	5	8	6	72	29	18	14
95	6	9	7	71	30	18	14
94	7	10	8	70	31	18	14
93	8	10	8	69	32	18	14
92	9	11	9	68	33	18	15
91	10	11	9	67	34	18	15
90	11	12	9	66	35	19	15
89	12	12	10	65	36	19	15
88	13	13	10	64	37	19	15
87	14	13	11	63	38	19	15
86	15	14	11	62	39	19	15
85	16	14	11	61	40	19	15
84	17	14	11	60	41	19	15
83	18	15	12	59	42	19	15
82	19	15	12	58	43	19	15
81	20	15	12	57	44	19	15
80	21	16	13	56	45	19	15
79	22	16	13	55	46	20	15
78	23	16	13	54	47	20	16
77	24	17	13	53	48	20	16
76	25	17	13	52	49	20	16
				51	50	20	16

Example 1

Use of Table 14I:
1) **Test Results:** Replicate 1 = 92%; Replicate 2 = 88%; Replicate 3 = 79%; Replicate 4 = 86%
2) **Tolerance Calculation:**
Average: (92 + 88 + 79 + 86) ÷ 4 = 86.25% (rounded to nearest whole number = 86%)
Difference between the highest and lowest germinating replicates: 92 - 79 = 13%
3) **Application of Tolerance:** In Table 14I, for 86% average germination (Column A), the maximum tolerance for four 100-seed replicates is 14 (Column C). Since the difference between the highest and lowest replicates (13%) is less than the maximum tolerance value (14%), a retest is not necessary. The differences in germination among replicates are assumed to be due to random sampling variation.

Example 2
Use of Table 14I:
1) **Test Results:** Replicate 1 = 92%; Replicate 2 = 79%
2) **Tolerance Calculation:**
Average: (92 + 79) ÷ 2 = 85.5 (rounded to nearest whole number = 86%)
Difference between the two replicates: 92 - 79 = 13%
3) **Application of Tolerance:** In Table 14I, for 86% average germination (Column A), the maximum tolerance for two 100-seed replicates is 11 (Column D). Since the difference between the highest and lowest replicates (13%) is more than the maximum tolerance value (11%), a retest is necessary. The difference in germination between replicates exceeds the maximum that can be assumed due to random-sampling variation.

c. **Tolerances between germination tests.** Table 14J lists the maximum tolerance between two germination tests on the same seed lot. The values are to be used to compare germination percentages (or the sum of germination and hard seed) determined from 400-seed tests. The tolerances are applicable for comparison of tests of the same seed lot, whether from the same or different submitted samples of that lot, and whether performed in one laboratory or different laboratories. Table 14J is adapted from Miles (1963; p. 646, Table G3, columns A, B and C).

d. **Procedure for determining whether two tests are within tolerance.** Calculate the average of the two germination test results to the nearest whole number. Calculate the difference between the two germination results. Locate the average in Table 14J (column A or B) and the tolerance for that average (column C). When the difference between the two germination test results is equal to or less than the tolerance values, the difference is due only to random-sampling variation.

Example 1
Use of Table 14J:
The germination for a 400-seed test of wheat is 87%. The germination of a sub-sample of the same seed lot submitted to another laboratory was reported to be 76%. Are these two results within tolerance?
1) **Test Results:** Analysis 1: 87%; Analysis 2: 76%.
2) **Tolerance Calculation:**
Average: (87 + 76) ÷ 2 = 81.5 (rounded to nearest whole number = 82%)
Difference between the two germination tests: 87 - 76 = 11%
3) **Application of Tolerance:** In Table 14J, for 82% average germination, the tolerance is 7. Since the difference between the two test results (11%) is greater than 7%, the difference is significant and not due to random-sampling variation. Differences among results might be due to sampling method, test conditions, seedling evaluation, or reporting error.

Example 2
Use of Table 14J:
A 200-seed germination test of orchardgrass from a forage mixture resulted in 79% germination. The label on the mixture indicated a germination of 87%. Are these two results within tolerance?
1) **Test Results:** Analysis 1 (Label): 87%; Analysis 2 (test): 79%
2) **Tolerance Calculation:**
Average: (79 + 87) ÷ 2 = 83%
Difference between the two germination tests: 87 - 79 = 8%
3) **Application of Tolerance:** In Table 14J, for 83% average germination, the tolerance is 7%. Since the tests being compared are 200-seed tests, 2% is added to the tolerance, for a total of 9%. Since the difference between the two test results (8%) is less the 9%, it can be assumed to be due to random sample variation, thus the two test results are within tolerance.

Table 14J. Maximum tolerance values for comparing two 400-seed germination tests of the same or different submitted samples tested in the same or different laboratories (one-way test at *P=0.05*).

Average Percent Germination		Tolerance*
A	**B**	**C**
99	2	2
97 - 98	3 - 4	3
94 - 96	5 - 7	4
91 - 93	8 - 10	5
87 - 90	11 - 14	6
82 - 86	15 - 19	7
76 - 81	20 - 25	8
70 - 75	26 - 31	9
60 - 69	32 - 41	10
51 - 59	42 - 50	11

*When only 200 seeds of mixtures are tested, 2% shall be added to the above germination tolerances.

II. Proposed Rule

14.5 Germination tolerances.

Germination tolerances are used to compare single components of germination analysis, i.e., normal seedlings, abnormal seedlings, dormant seed, hard seed and dead seed, or any combination of two or more of those components.

a. **Tolerances between replicates. –** Table 14I lists the maximum tolerance between two and four replicates of 100 seeds in a single germination test. The tolerance values were computed to allow for only random-sampling variation at the 0.025 probability level. Table 14I is adapted from Miles (1963; Table G1, columns A, B, D and L).

b. **Procedure for finding the maximum tolerance between replicates in a germination test. –**

To check if replicates are within tolerance for percentage germination, **c**alculate the average germination of the two or four replicates to the nearest whole number. Calculate the difference between the highest and the lowest germinating replicates. Locate the average germination in column A or B in Table 14I. The maximum tolerance between four replicates appears in the same row in column C. For two replicates, it appears in the same row in column D. Compare the calculated difference between replicates with the maximum tolerance found in the table. If the difference between the replicates is equal to or less than the value found in Table 14I, retesting is not necessary but a retest must be performed if it is greater. Follow the same procedure to

check whether replications are within tolerance for other single components or combinations of two or more components.

Table 14I. Maximum tolerance values between two and four replicates of 100 seeds in a single germination or tetrazolium test (2-way test at *P = 0.025*).

Average percent germination*		No. replicates of 100 seeds		Average percent germination*		No. replicates of 100 seeds	
		4	2			4	2
A	B	C	D	A	B	C	D
99	2	5	---	75	26	17	14
98	3	6	---	74	27	17	14
97	4	7	6	73	28	17	14
96	5	8	6	72	29	18	14
95	6	9	7	71	30	18	14
94	7	10	8	70	31	18	14
93	8	10	8	69	32	18	14
92	9	11	9	68	33	18	15
91	10	11	9	67	34	18	15
90	11	12	9	66	35	19	15
89	12	12	10	65	36	19	15
88	13	13	10	64	37	19	15
87	14	13	11	63	38	19	15
86	15	14	11	62	39	19	15
85	16	14	11	61	40	19	15
84	17	14	11	60	41	19	15
83	18	15	12	59	42	19	15
82	19	15	12	58	43	19	15
81	20	15	12	57	44	19	15
80	21	16	13	56	45	19	15
79	22	16	13	55	46	20	15
78	23	16	13	54	47	20	16
77	24	17	13	53	48	20	16
76	25	17	13	52	49	20	16
				51	50	20	16

* The same tabulated values are used to find the maximum tolerance between replicates for any other test component, or a combination of two or more test components.

Example 1
Use of Table 14I:
1) **Percentage Germination Test Results:** Replicate 1 = 92%; Replicate 2 = 88%; Replicate 3 = 79%; Replicate 4 = 86%
2) **Tolerance Calculation:**
Average: (92 + 88 + 79 + 86) ÷ 4 = 86.25% (rounded to nearest whole number = 86%)
Difference between the highest and lowest germinating replicates: 92 - 79 = 13%
3) **Application of Tolerance:** In Table 14I, for 86% average germination (Column A), the maximum tolerance for four 100-seed replicates is 14 (Column C). Since the difference between the highest and lowest replicates (13%) is less than the maximum tolerance value (14%), a retest is not necessary. The differences in germination among replicates are assumed to be due to random sampling variation.

Example 2
Use of Table 14I:
1) **Percentage Germination Test Results:** Replicate 1 = 92%; Replicate 2 = 79%
2) **Tolerance Calculation:**
Average: (92 + 79) ÷ 2 = 85.5 (rounded to nearest whole number = 86%)
Difference between the two replicates: 92 - 79 = 13%
3) **Application of Tolerance:** In Table 14I, for 86% average germination (Column A), the maximum tolerance for two 100-seed replicates is 11 (Column D). Since the difference between the highest and lowest replicates (13%) is more than the maximum tolerance value (11%), a retest is necessary. The difference in germination between replicates exceeds the maximum that can be assumed due to random-sampling variation.

Example 3
Use of Table 14I:
1) **Test Results:**
Germination: Replicate 1 = 86%; Replicate 2 = 88%; Replicate 3 = 74%; Replicate 4 = 82%
Hard seed: Replicate 1 = 1%; Replicate 2 = 0%; Replicate 3 = 4%; Replicate 4 = 2%
Viability: Calculate viability by adding germination and hard seeds results of each replicate: Replicate 1 = (86 +1) = 87%; Replicate 2 = (88+0) = 88%; Replicate 3 = (74+4) =78%; Replicate 4 = (82+2) = 84%
2) **Tolerance Calculation:**
Average: (87 + 88 + 78 + 84) ÷ 4 = 84.25% (rounded to nearest whole number = 84%)
Difference between the highest and lowest replicates: 88- 78 = 10%
3) **Application of Tolerance:** In Table 14I, for 84% average germination (Column A), the maximum tolerance for four 100-seed replicates is 14 (Column C). Since the difference between the highest and lowest replicates (10%) is less than the maximum tolerance value (14%), a retest is not necessary. The differences in viability among replicates are assumed to be due to random sampling variation.

c. **Tolerances between germination tests.** Table 14J lists the maximum tolerance between two germination tests on the same seed lot. The values are to be used to compare germination percentages, other single components, or any combination of components (e.g., the sum of germination and hard seed) determined from 400-seed tests. The tolerances are applicable for comparison of tests of the same seed lot, whether from the same or different submitted samples of that lot, and whether performed in one laboratory or different laboratories. Table 14J is adapted from Miles (1963; p. 646, Table G3, columns A, B and C).

d. **Procedure for determining whether two tests are within tolerance.** To check if two tests are within tolerance for percentage germination, calculate the average of the two germination test results to the nearest whole number. Calculate the difference between the two germination results. Locate the average in Table 14J (column A or B) and the tolerance for that average (column C). When the difference between the two germination test results is equal to or less than the tolerance values, the difference is due only to random-sampling variation. Follow the same procedure to check whether two tests are within tolerance for other single components or combinations of two or more components.

Example 1
Use of Table 14J:
The germination for a 400-seed test of wheat is 87%. The germination of a sub-sample of the same seed lot submitted to another laboratory was reported to be 76%. Are these two results within tolerance?
1) **Percentage Germination Test Results:** Analysis 1: 87%; Analysis 2: 76%.
2) **Tolerance Calculation:**
Average: (87 + 76) ÷ 2 = 81.5 (rounded to nearest whole number = 82%)

Difference between the two germination tests: 87 - 76 = 11%
3) **Application of Tolerance:** In Table 14J, for 82% average germination, the tolerance is 7. Since the difference between the two test results (11%) is greater than 7%, the difference is significant and not due to random-sampling variation. Differences among results might be due to sampling method, test conditions, seedling evaluation, or reporting error.

Example 2
Use of Table 14J:
A 200-seed germination test of orchardgrass from a forage mixture resulted in 79% germination. The label on the mixture indicated a germination of 87%. Are these two results within tolerance?
1) **Percentage Germination Test Results:** Analysis 1 (Label): 87%; Analysis 2 (test): 79%
2) **Tolerance Calculation:**
Average: (79 + 87) ÷ 2 = 83%
Difference between the two germination tests: 87 - 79 = 8%
3) **Application of Tolerance:** In Table 14J, for 83% average germination, the tolerance is 7%. Since the tests being compared are 200-seed tests, 2% is added to the tolerance, for a total of 9%. Since the difference between the two test results (8%) is less the 9%, it can be assumed to be due to random sample variation, thus the two test results are within tolerance.

Example 3
Use of Table 14J:
Results of a 400-seed test of tall fescue were: Germination: 69%; Dormant seed: 14%; Abnormal seedlings: 4%, Dead seeds: 13%. The test results of a sub-sample of the same seed lot submitted to another laboratory were reported to be: Germination: 81%; Dormant seed: 6%; Abnormal seedlings: 5%, Dead seeds: 8%. Are the two test results within tolerance for viability?
1) Test Results:
Analysis 1: Viability = germination + dormant = 69 + 14 = 83%
Analysis 2: Viability = germination + dormant = 81 + 6 = 87%
2) **Tolerance Calculation:**
Average: (83 + 87) ÷ 2 = 85%
Difference between the two germination tests: 87 - 83= 4%
3) **Application of Tolerance:** In Table 14J, for 85% average germination, the tolerance is 7. Since the difference between the two test results (4%) is less than 7%, the two tests are within tolerance for viability.

Table 14J. Maximum tolerance values for comparing two 400-seed germination tests of the same or different submitted samples tested in the same or different laboratories (one-way test at *P=0.05*).

Average Percent Germination*		Tolerance**
A	**B**	**C**
99	2	2
97 - 98	3 - 4	3
94 - 96	5 - 7	4
91 - 93	8 - 10	5
87 - 90	11 - 14	6
82 - 86	15 - 19	7
76 - 81	20 - 25	8
70 - 75	26 - 31	9
60 - 69	32 - 41	10
51 - 59	42 - 50	11

* The same tabulated values are used to find the maximum tolerance between test results of other components, or a combination of two or more test components.
**When only 200 seeds of mixtures are tested, 2% shall be added to the above germination tolerances.

III. Harmonization and Impact Statement

AOSA and ISTA rules imply that Tables 14I and 14J can be used to determine whether a single germination test component or a combination thereof, are in tolerance with the same component or combination of another test or label. The AOSA statement to that effect is as follows:

"Table 14J lists the maximum tolerance between two germination tests on the same seed lot. The values are to be used to compare germination percentages (or the sum of germination and hard seed) determined from 400-seed tests." (Section 14.5.c)

ISTA includes the following statement under section 5.8.1 of their rules: "Tolerances are applied to at least the category of normal seedlings."

This proposal aims to clearly state that germination test components, singly or in combination, can be checked for tolerance among replicates and tests using tables 14I and 14 J. The most common use of germination tolerances, other than a direct check of percentage germination (normal seedlings) among tests, is to determine whether viability results, calculated as the sum of normal seedlings, hard seed and dormant seed, of one test are within tolerance of a labeled value or of another test. For example, the Federal Seed Act mandates that labeled viability, in addition to germination, must be within tolerance of tested viability.

IV. Supporting evidence

Tolerance tables are based on experimentally determined variances. The variance of percentage components is assumed to be homogenous, and therefore tables 14I and 14J are applicable to single components and their combinations derived from germination tests.

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