A newsletter for members of AOSA/SCST

Seed Technologists Newsletter

Volume 90, Issue 1

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"If you can look into the seeds of time, and say which grain will grow and which will not, speak then unto me."
- William Shakespeare

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Connecting Virtually

Quinn Gillespie

At last year's meeting we were able to experience some of the benefits of adding a virtual element to our in-person meetings. While virtual meetings don't fully replace the connections we make in person, the ability to present and participate in discussions virtually can help to broaden our reach. This year the planning committee and executive director have worked to bring some elements of the meeting online, including some presentations which will be presented in a hybrid model, and some which will be conducted entirely virtually before the in-person meeting in Saskatoon, SK.

Last year the Referee Presentations were conducted in a hybrid model, which allowed members who were not in attendance in Skokie to present their research, and this will be continued this year and likely for years to come.

Several committee meetings will take place virtually in May, preceding the conference. This will save on time and scheduling conflicts during the annual meeting and allow for the co-chairs to connect before the annual meeting, even if both are not able to attend.

The primary change to our meeting agenda is that the **Open Rules** discussion will take place entirely virtually. The Open Rules meeting in Saskatoon will not lead to significant edits, but only serve as a brief presentation of the 2023 rule proposals in their updated form after any edits made during the **Virtual Open Rules** discussion.

Time zone	Meeting time
Eastern	12:00 pm
Central	11:00 am
Mountain	10:00 am
Pacific	9:00 am

The **Virtual Open Rules Discussion** will take place on Tuesday, May 16th, 2023, 10am, MT. See inset for your time zone. A preliminary schedule of other virtual and hybrid meetings is posted on the next Page. Be sure to check the annual meeting website for updates to this schedule.

Members who have already registered for the in-person annual meeting are automatically registered for all virtual components as well. Virtual-only registrations are available for a reduced rate for those who cannot attend in person but would like to participate in the virtual components of the annual meeting. Agenda updates will be sent out to the membership by the executive director, and updated regularly on the website for the Saskatoon meeting.

Check for Annual Meeting Updates

Virtual Meeting Schedule - **All times are MT**

Check the analyzeseeds.com website for continuing updates, Saskatchewan local time is CST.

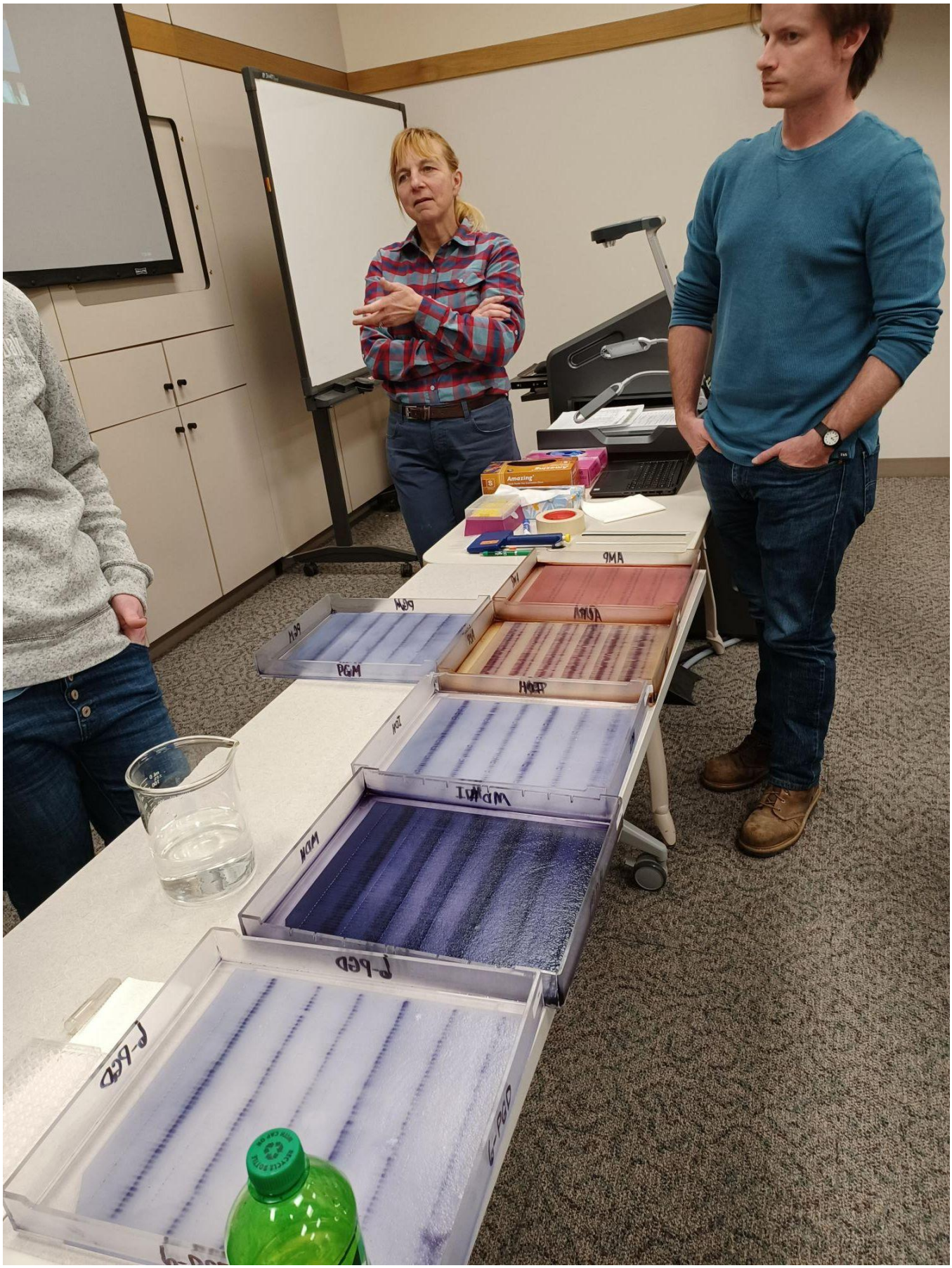
Monday	Tuesday	Wednesday	Thursday	Friday
				May 12 11:00 am - Conservation & Reclamation/Tree & Shrub
May 15	**May 16** 10:00 am - Open Rules 11:00 am Teaching and Training	**May 17** 10:00 am - Flower Seed 1:00 pm - Vigor	**May 18** 9:00 am - Cultivar purity	**May 19**
May 22	**May 23** 9:00 am - Continuing Education	**May 24** 10:00 am - handbook	**May 25** 10:00 am - RGT BOE	**May 26**
May 29	**May 30**	**May 31**	**June 1** 10:00 am - Statistics 11:00 am - Moisture	**June 2**
June 5	**June 6** 9:00 am - Referee (Closed)	**June 7**	**June 8**	**June 9**
June 12	**June 13** 8:00am - Open rules Update	**June 14** 9:30am - Referee Presentations 1:00 pm - Research seminar 3:00pm - Long Range planning	**June 15**	**June 16**

Hybrid meetings taking place during the annual meeting require virtual registration to participate for those not present at the annual meeting.

2022 Genetic Technology Super Workshop

By Molly Richeson

The 2022 Genetics Super Workshop was held at the Seed Science lab of Iowa State University from March 7th – March 10th. The workshop is designed to assist with studying for the Registered Genetic Technologist (RGT) exams. The first day was spent going over molecular genetics with an introduction to herbicide bioassay. Lectures were given by Dr. Shui-zhang Fei, Dr. Siddique Muhammad-Aboobucker, Dr. Kan Want, and Tyler Tunning. The second was spent on ELISA with a significant amount of hands-on training from Dave Rambow from AgDia, Amanda Patin from SGS, Mike Stahr, from ISU, and Brian Beal from Agreliant, as well as the remainder of the herbicide bioassay material. Electrophoresis was covered with some hands-on training by Kevil Balvin, SGS, Molly Richeson, AgReliant, Kelyn Brix, SoDak Labs, and Brian Beal on the third day. The fourth day of the workshop attendees studied adventitious presence and PCR troubleshooting taught by Emily Whiston, Envirologix, Brian Beal, Don Mittanck, Bayer, Sherry Whitt, BASF, and Ray Shillito, BASF. The closing of the workshop included a tour of the Molecular Biology building at ISU. We had a good turnout of 13 attendees with a cap placed at 15 attendees maximum due to COVID. The evaluations of the superworkshop received were overwhelmingly positive. A huge thanks goes out to all of our speakers and organizers that made this year's workshop not only possible but successful.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Effect of Temperature and Prechilling Treatment on Hemp (*Cannabis sativa)* Germination and Dormancy

Sabry Elias and Yeaching Wu

INTRODUCTION

Since the 2018 Farm Bill legislation, hemp has become an important cash crop, which opened the door for conducting a wide range of scientific research and crop improvement (1). The total licensed hemp acres across 34 states in 2019 was 511,442. However, this number dropped by 9% in 2020 (2). In November 2020, there were 27,434 acres of outdoor hemp registered in Oregon (3).

Using high quality seeds is the cornerstone of any successful production program (4). Oregon State University has seed certification program for hemp in place, which requires inspecting the crop at different stages of development in the greenhouse and in the field. In addition, seed quality is tested after harvest, for purity, viability, and PCR gender testing before issuing the blue tags (5).

New hemp varieties have been continuously developed since the Farm Bill Legislation in 2018. The current germination recommendation needs to be validated and/or updated to make sure that it achieves maximum potential germination for a wide range of varieties. Furthermore, measuring the repeatability of the germination test results among labs is important to attain uniformity in seed testing.

There are two main problems in germinating hemp seeds, firstly, hemp seeds have various levels of dormancy, some are short-lived (1), and others are deeper. Recently, several new varieties have been developed by some seed companies and were sent to OSU Seed Lab for testing. It was observed that some of which possess deep dormancy, not only in freshly harvested seeds, but also in stored seeds. Newly developed triploid varieties that has been stored for 3-6 months or more were found to possess dormancy ranging from 26 to 41% at the end of the standard germination test (Personal communication). Such seeds were found to be viable when tested by the TZ test. The current AOSA Rules for germinating hemp seeds do not require prechilling treatment (AOSA Rules vol. 1, Table 6A) for breaking dormancy. Secondly, during germination at 20-30°C, medium to low quality hemp samples develop extensive fungal infection. In some cases, many seedlings were dead at the final count (7 days). It was therefore hypothesized that germination temperature at 20-30°C provides an optimal warm environment for fungal growth; and at 15-25°C, the growth of fungus may slow down.

The overall objective of the study was to evaluate and establish conditions that achieve maximum germination potential in hemp seeds. The specific objectives were to: 1) Measure the effectiveness of prechilling treatment on breaking the dormancy of six hemp varieties and the possibility of adding it to the AOSA Rules, if proven effective; 2) Compare germination temperature of 15-25°C to the current AOSA 20-30°C with and without prechilling, and the possibility of adding 15-25°C as an alternating temperature regime to the AOSA Rules; and 3) Measure the variability among six labs when the same study protocol was followed.

Materials and Methods

Seed Materials

Six hemp samples that represent different qualities and levels of dormancy were used in the study. Samples were produced in summer of 2020.

National Referee

A national referee was conducted to measure the effectiveness of prechilling treatment on breaking the dormancy and the alternating 15-25°C effect on germination compared to 20-30°C. Six private and state seed labs from USA and Canada that have experiences in testing hemp seeds were selected to participate in the national referee to ensure the reliability of the results and the objectivity of the conclusions attained.

Study Protocol

Six blind samples including 1600 seeds each were sent to the participating labs. Four replicates of 100 seeds each were planted according to the following four germination methods:

1. **Control method:** The **current AOSA standard germination method** (AOSA Rules Table 6A) was used as a baseline for comparison. No prechill treatment, seeds were germinated at 20-30°C. The 1st count was in day 3 and the final count in day 7.
2. **Current AOSA method with 7d prechill at 10°C**. The objective was to determine whether the prechilling treatment might overcome any dormancy that may be exist.
3. **Germination at 15-25°C without prechill**.
4. **Germinated at 15-25°C with 7d prechill at 10°C**.

Media: Paper toweling (T), moistened with water, either as folded or rolled towel test in horizontal or vertical position was used.

Tetrazolium Test: In all methods, at the end of the germination tests, the ungerminated seeds were tested by TZ to determine whether the seeds were dead or dormant.

Summary of study protocol: Six varieties, six participating labs, and four combinations of temperatures and prechill treatments.

Temperature	Pre-chill (10°C, 7d)
20-30°C (AOSA)	No (baseline method/control)
	Yes
15-25°C	No
	Yes

Germination Procedures

- Four-100 seed replications were planted from each of the six samples for each of the above four treatments.
- The first count was done at day 3 to minimize the secondary infection, if any, and were discarded. Final count at day 7. Normal seedlings was counted and recorded.
- Data sheets were prepared for each lab for uniformity in recording.

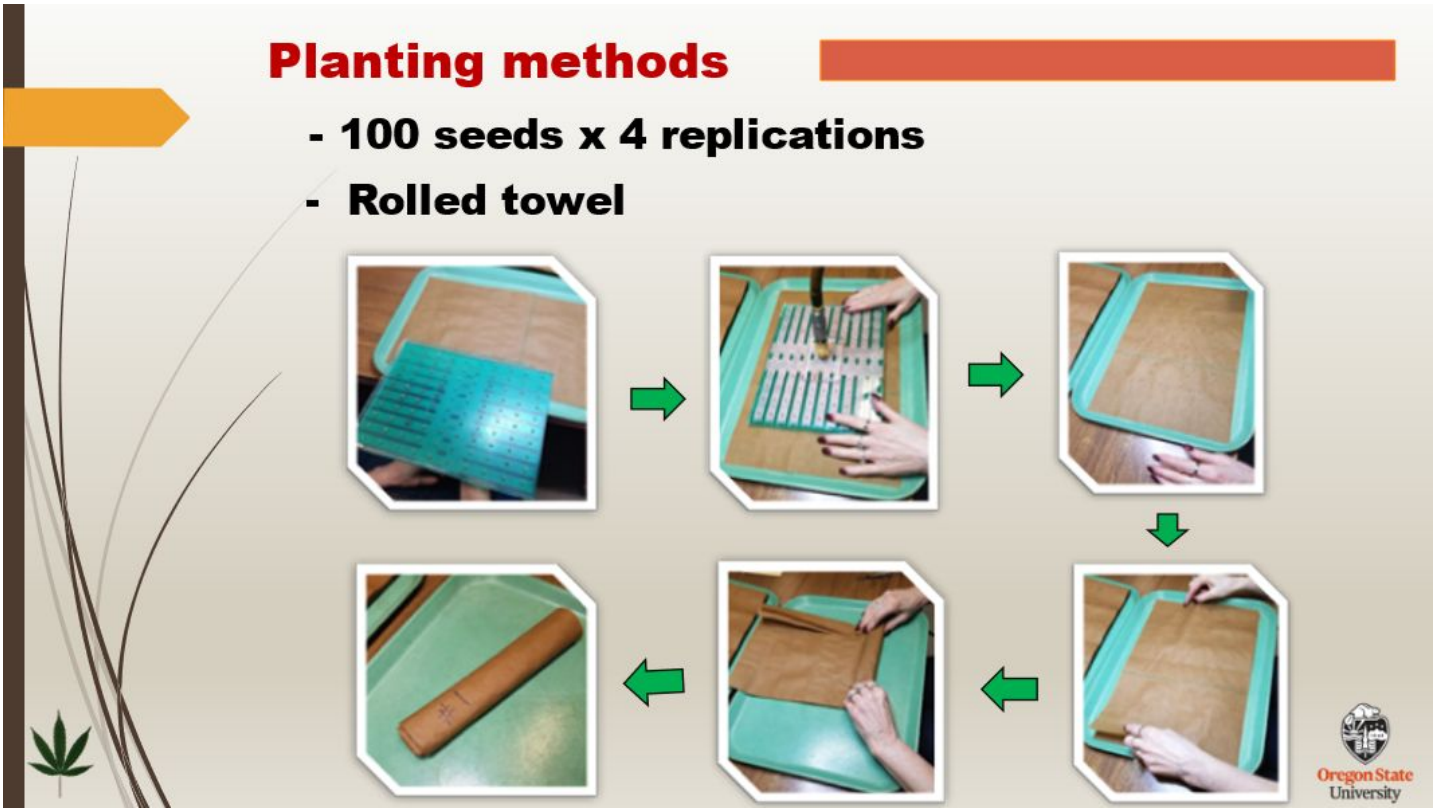

Figure 1. Planting method of hemp seeds. Four replications of 100 seeds each on moistened germination paper using rolled towels.

- At the end of the germination test, TZ tests were conducted on all firm ungerminated seeds. Seeds were soaked overnight (16 hours) in water, and then were cut longitudinally so that the TZ solution stains the internal tissues of seeds. Seeds were then soaked in 1% tetrazolium solution at 30°C overnight, and then evaluated and classified into viable or nonviable seeds. Viable seeds were evenly stained red or ¼ or less of the tip of the radicle unstained. The embryo may need to be separated from the seed coat to see the staining pattern clearly.

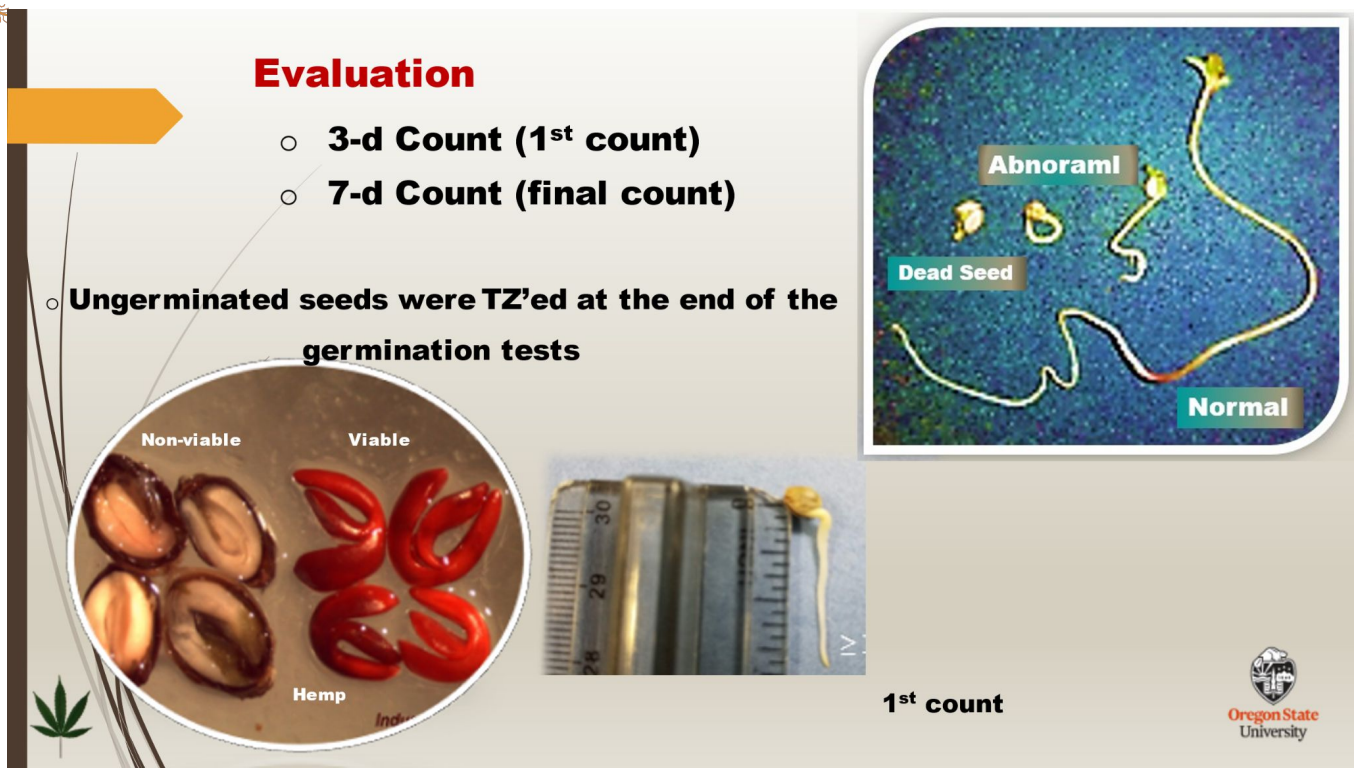

Figure 2. Evaluation of germination and TZ tests. At 3-day count, seedling developed a normal root system approximately 1.5 cm with root hair. Normal seedlings have well developed shoot and root systems. Abnormal seedlings have deformed shoot and/or root systems. For TZ test, seeds with embryonic axes uniformly stained red were viable; unstained, non-viable.

Experimental Design and Data Analysis

The experimental design used was three-factor completely randomized design (CRD), with four replications of each treatment. The factors were varieties, method of germination (temperature/prechilling), and participating labs. The data were collected and were subjected to analysis of variance (ANOVA) to measure the effect of the different treatments on the final germination/viability and dormancy results. Mean separation test (LSD) performed at 0.05 probability level whenever the effect of the treatments was significant. Standard error was calculated to estimate the variability across samples and treatments. The statistical package MSTAT was used in the statistical analysis.

Results and Discussion

The ANOVA indicated that varieties, method of germinations (germination and prechilling treatments), and laboratories affected the germination and dormancy of the six hemp varieties significantly at $P \leq 0.05$ (Table 1). Some hemp varieties had higher germination (var. 5 and 6) than others (var. 2 and 3); some varieties had deeper dormancy (var 2 and 3) than others (5 and 6); some varieties responded to the prechilling better than others, and different patterns of germination at 20°C-30°C and 15-25°C among varieties were recorded (Figs. 3 and 6). In addition, the interactions among these factors were significantly different (at $P \leq 0.001$), indicating that the responses of varieties to the germination regimes were not similar among labs (Table 1).

Table 1. Analysis of variance (ANOVA) for the effects of four temperature and prechilling combinations on germination of six hemp varieties tested in six different laboratories.

Source of variation	df	3-d count	7-d count	Dormant seeds	Viable by TZ
		Mean Square (Prob0.05)			
Varieties (V)	5	37480.2***	17084.1***	24441.4***	22933.1***
Method of germination (M)	3	76240.9***	9515.2***	9935.7***	9974.0***
Labs (L)	5	18883.0***	3411.7***	1291.5***	142.1***
Interaction					
V x M	15	3932.9***	2644.8***	2649.1***	196.3***
V x L	25	1146.3***	302.6***	399.7***	30.9***
M x L	15	2176.6***	794.5***	844.9***	53.1***
V x M x L	75	805.1***	232.1***	210.9***	16.0***

*** Highly significant at 0.001 level of probability.

First Germination Count at Day 3

Prechilling treatment improved the germination of first count at day 3 in both 20-30°C and 15-25°C at 60 and 56%, respectively, compared to non-chilled seeds at 39% and 10%, respectively (Fig. 3A). The prechilling at 10°C for 7 days affected seed germination in two ways, 1) break dormancy, and 2) work as hydropriming, which activate the germination enzymes, so that once moved to the warmer temperature, seedlings grow faster and more uniform than non-chilled seeds. The non-chilled seeds germinated better at 20-30°C than 15-25°C (Fig. 3A), probably because the warmer temperature helped seedlings to grow faster.

Varieties 2 and 3 have deeper dormancy than varieties 1, 4, 5, and 6 (Fig. 3B). Varieties 4, 5 and 6 had the least dormant seeds and the highest first germination count at 53%, 55% and 62%, respectively, whereas varieties 2 and 3 had only 17% (Fig. 3B). The average first count of germination over varieties, germination regimes and labs was 41%, with LSD $^{(0.05)}$ = 7.6.

Significant variation in the first germination count among laboratories at $P \leq 0.05$ were reported. Lab two was conservative in evaluating first count seedlings, whereas labs 5 and 6 were generous (Fig. 3C). This is probably because the criteria of classifying young seedlings into normal and abnormal in the first count, based on the morphological evaluation of the roots, was not consistent in all labs. Roots that are not stubby or deformed, with length of approximately 1.5 cm and root hairs developed are classified as normal. Such seedlings were removed at 1st count to reduce the secondary infection. This is particularly important in medium to low quality seeds, where the fungal infection can be high, and the seedling growth is slow. In a preliminary study by the authors, it was found that young seedlings with root length of approximately 1.5 cm and root hairs developed (without shoot system developed) produced normal seedlings.

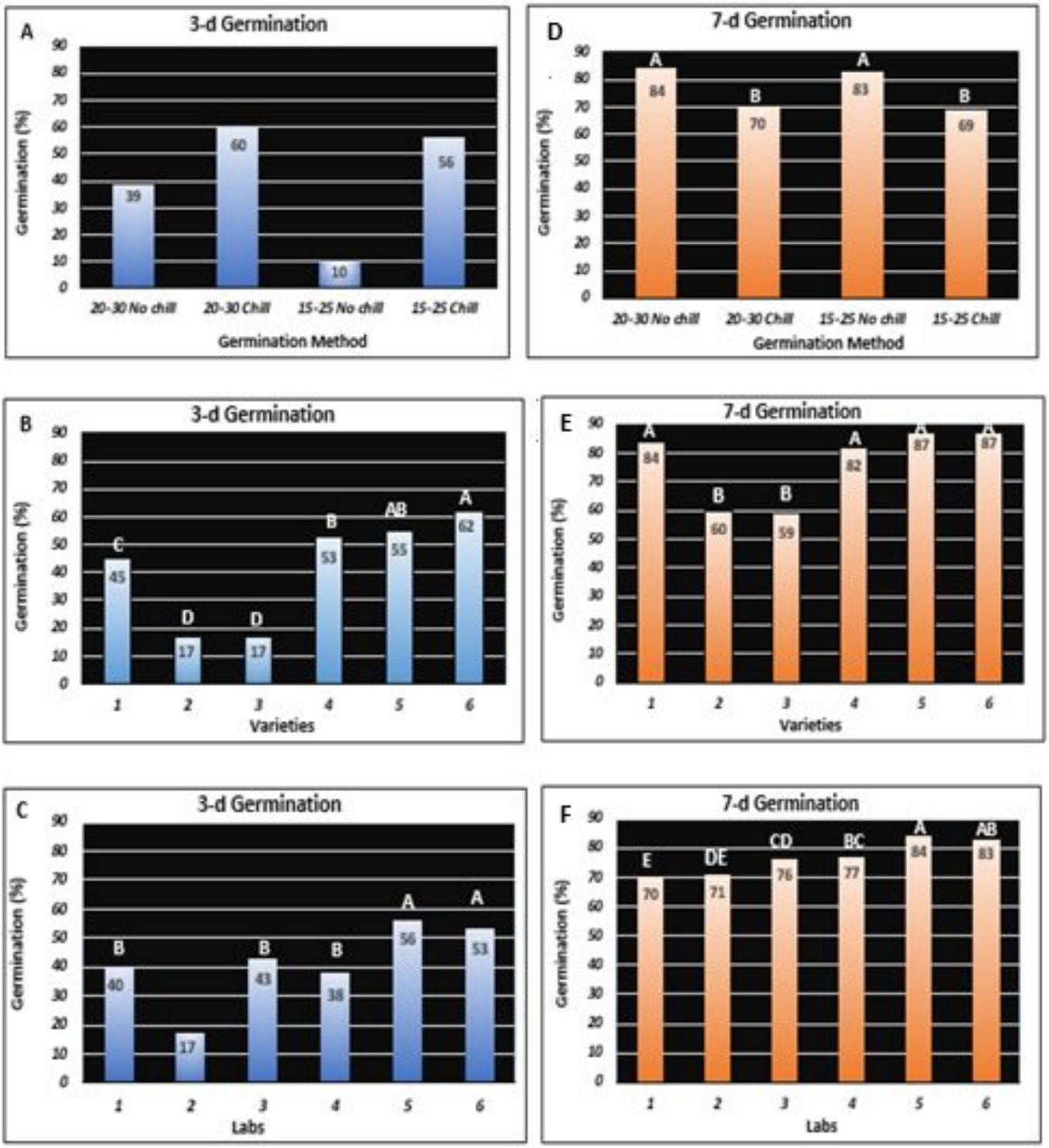

Figure 3. Average 3- day germination (first count) and 7- day germination (final count) of six hemp varieties tested using four combinations of temperature and prechilling treatments at six different laboratories. Means with different letters are significantly different from each other at $P \leq 0.05\%$.

Figure 4. Example of first count (day 3) germination with and without prechilling treatment of hemp variety No. 5 (*low dormancy*) at 20-30°C.

Figure 5. Example of first count (day 7) germination with and without prechilling treatment of hemp variety No. 2 (*deep dormancy*) at 15-25°C.

Final Germination Count at Day 7

Final germination counts at day 7 increased significantly compared to the first count at day 3 in both chilled and non-chilled seeds of all varieties (Figs. 3D & 3E). Four out of the six varieties (1, 4, 5, and 6) had slight or no dormancy, and therefore had significantly higher final germination percentage than varieties 2 and 3, which had deep dormancy and less final germination counts. This is probably because the prechilling treatment did not completely break the dormancy of varieties 2 and 3. Both treatments 20-30°C

and 15-25°C with no prechill had similar average final germination of 84% and 83%, respectively. Likewise, both treatments 20-30°C and 15-25°C with chill had similar average final germination of 70% and 69%, respectively (Fig. 3D). This suggested that the prechilling treatment, along with the varieties, not the temperatures, made the difference in the final germination count (Figs. 3D and 3E).

Varieties 2 and 3 had deeper dormancy than varieties 1 & 4 & 5 & 6, which have light dormancy. Prechilling treatment was not completely effective in breaking the dormancy of varieties 2 and 3. The final germination of varieties 2 and 3 was 60% and 59%, respectively. The final germination of varieties 1, 4, 5 and 6 was 84%, 82%, 87%, and 87%, respectively (Fig. 3E). The average final germination count over varieties, germination regimes and labs was 77%, with $LSD_{(0.05)} = 5.9$.

Significant variation among **laboratories** at $P \leq 0.05$ were reported. Laboratories 1 and 2 were conservative in evaluation and Labs 5 and 6 were generous (Lab 3F).

Sample 1. The average final germination of non-chilled seeds ranged between 94% for 20-30°C to 91% for 15-25°C; whereas ranged between 79% for 20-30°C to 63% for 15-25°C in chilled seeds (Fig. 6). The variation in the final germination count among the six labs ranged between 73% and 92%. Clearly, sample 1 has some level of dormancy that was not broken by the prechilling treatment used in this study. That may indicated that the dormancy may be due to the seed coat or combination between physical and physiological dormancy.

Samples 2 and 3 had deep dormancy. The average final germination of non-chilled seeds of sample 2 ranged between 77% for 20-30°C to 81% for 15-25°C; whereas ranged between 40% for 20-30°C to 42% for 15-25°C in chilled seeds (Fig. 6). Similarly, the average final germination of non-chilled seeds of sample 3 ranged between 79% for 20-30°C to 71% for 15-25°C; whereas ranged between 46% for 20-30°C to 41% for 15-25°C in chilled seeds (Fig. 6). The variation in the final germination count among the six labs ranged between 46% and 75% in sample 2; and 45% and 72% in sample 3.

Sample 4 and 5 had low level of dormancy. The average final germination of non-chilled seeds of sample 4 ranged between 84% for 20-30°C to 83% for 15-25°C; whereas ranged between 81% for 20-30°C to 82% for 15-25°C in chilled seeds (Fig. 6). Similarly, the average final germination of non-chilled seeds of sample 5 ranged between 86% for 20-30°C to 88% for 15-25°C; whereas it was 88% in both 20-30°C and 15-25°C in chilled seeds (Fig. 6). The variation in the final germination count among the six labs ranged between 71% and 88% in sample 4; and 83% and 91% in sample 5.

Sample 6 does not have dormancy, the average final germination of non-chilled seeds of sample 6 ranged between 84% for 20-30°C to 87% for 15-25°C; whereas it was 88% in both 20-30°C and 15-25°C in chilled seeds (Fig. 6). The variation in the final germination count among the six labs ranged between 78% and 92%.

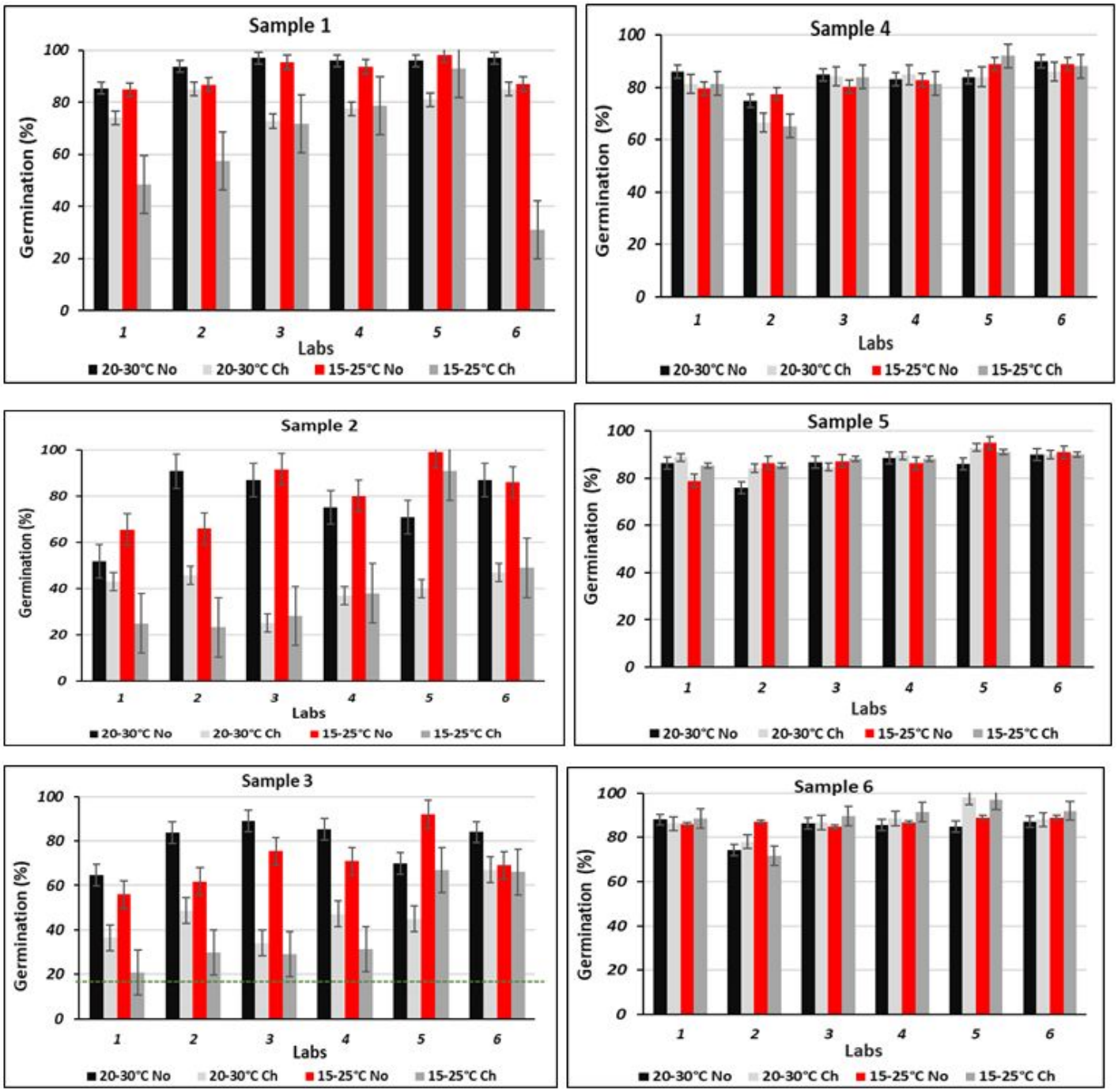

Figure 6. Final count (7 days) of six hemp varieties tested using four combinations of temperature and prechilling treatments at six different laboratories. Means with different letters are significantly different from each other at $P \leq 0.05\%$.

Number of Dormant Seeds

More dormant seeds were found in varieties 2 and 3 (average, 38 and 37 seeds, respectively), compared to varieties 1, 4, 5, and 6 which had averages of 14, 8, 7, and 1 seeds, respectively (Fig. 7). These results showed that the prechilling treatments did not break the dormancy in varieties 2 and 3 completely, however, it may help in breaking the dormancy in the other varieties. The overall average of the dormant seeds over varieties, germination regimes, and labs was 17%, with LSD (0.05) = 4.92.

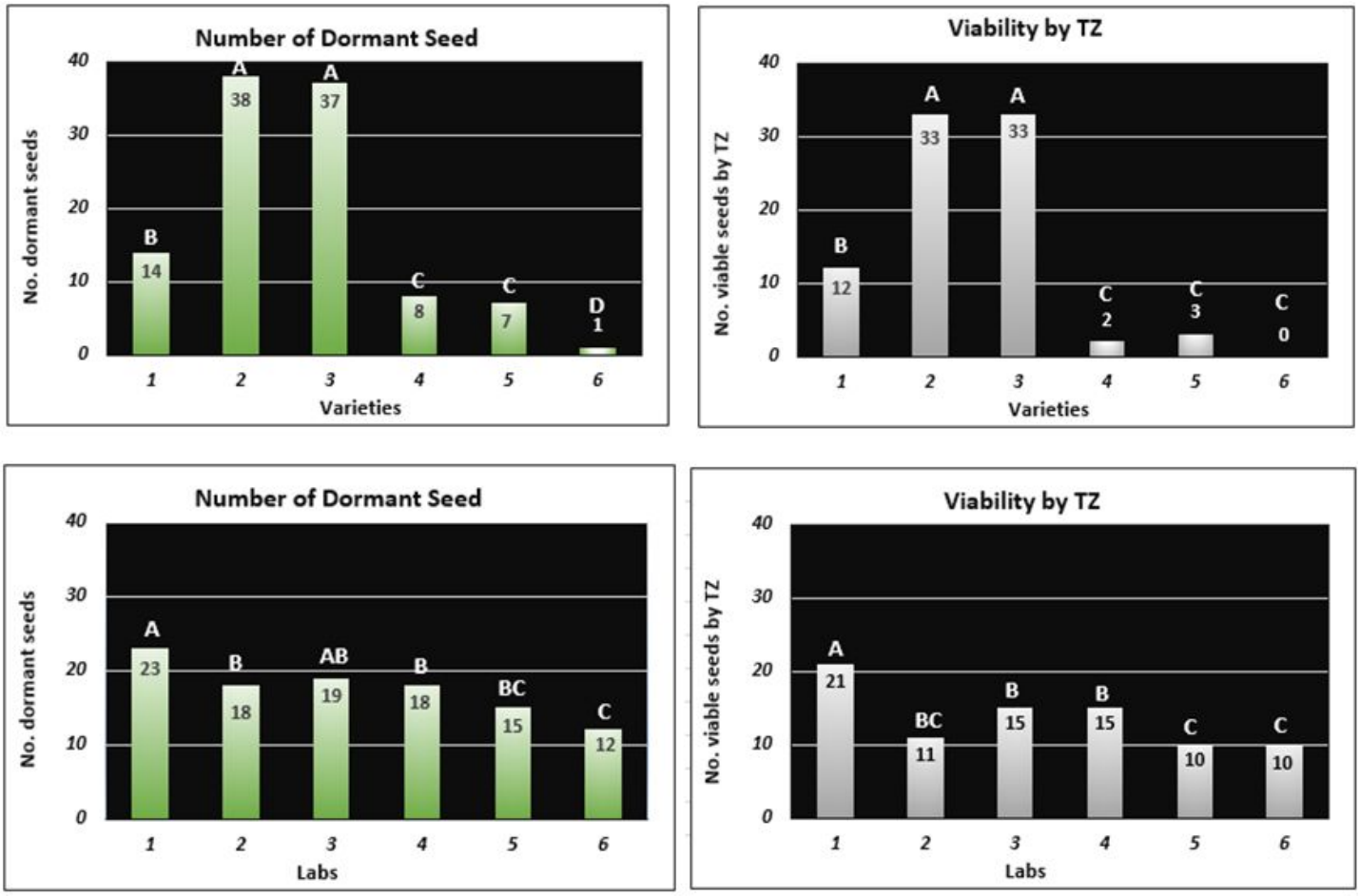

Figure 7. Average number of dormant seeds and number of viable seeds by TZ test of ungerminated seeds at the end of germination tests of six hemp varieties tested using four combinations of temperature and prechilling treatments at six different laboratories. Means with different letters are significantly different from each other at $P \leq 0.05\%$.

Significant variation among labs in number of dormant seeds (Fig. 7), with average ranging from 12 to 23 seed, with an average of 14 seeds over all other treatments, with LSD $^{(0.05)}$ = 4.85.

Number of Viable Seed by TZ for Ungerminated Seeds after the Standard Germination Test

Most of the dormant seed were found to be viable when tested by TZ. This shows the importance of testing non-germinated seeds at the end of the standard germination test. In order to confirm whether ungerminated seeds were dead or dormant, it is crucial to conduct TZ test on ungerminated seeds to reflect the actual viability of the sample. This is particularly essential for the seeds that possess deep dormancy that is difficult to break with prechilling treatment. Varieties 2 and 3 had the deepest dormancy followed by variety 1. Varieties 4 and 5 had shallow dormancy, whereas variety 6 had no dormancy (Fig. 7 and 8).

These results indicated that further studies are needed to develop more effective dormancy breaking methods for hemp varieties with deep dormancy issue, such as varieties 2 and 3 used in this study (Fig. 7 and 8). However, the prechilling method has value in breaking shallower dormancy and producing fast uniform seedlings as it acts as hydropriming treatment.

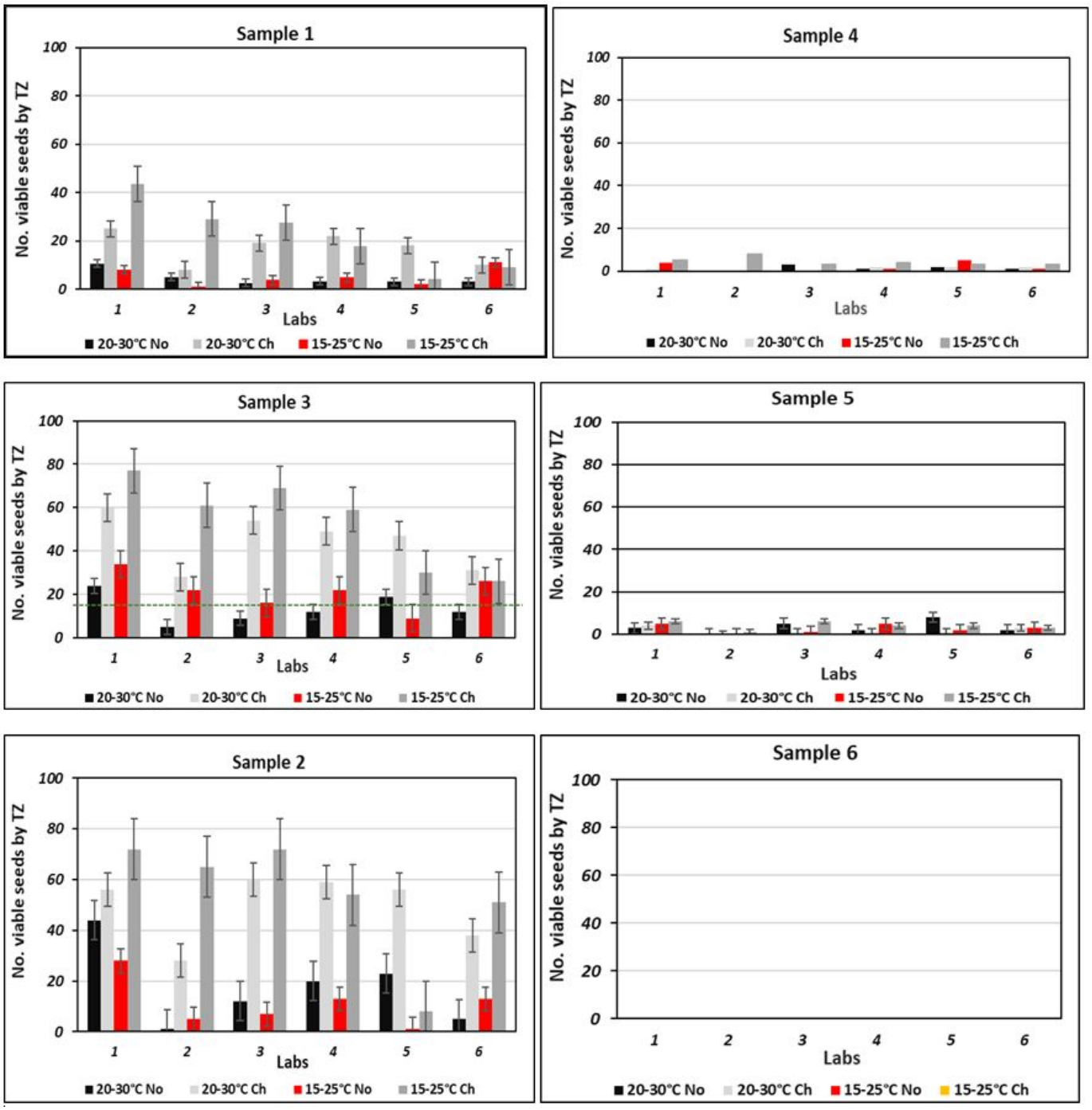

Figure 8. Average number of viable seeds by TZ test of ungerminated seeds at the end of germination tests of six hemp varieties tested using four combinations of temperature and prechilling treatments at six different laboratories. Means with different letters are significantly different from each other at $P \leq 0.05\%$. (Sample 6 did not have dormancy).

Sample 1. The average viable seed by TZ test of ungerminated seeds at the end of germination tests of non-chilled seeds was 5% for both 20-30°C and 15-25°C; whereas ranged between 17% for 20-30°C to 22% for 15-25°C in chilled seeds (Fig. 8). The variation in the viability by TZ among the six labs ranged between 7% and 22% in sample 1.

Samples 2 and 3 had deep dormancy. The average viable seed by TZ test of ungerminated seeds at the end of germination tests of non-chilled seeds of sample 2 ranged between 18% for 20-30°C to 11% for 15-25°C; whereas ranged between 50% for 20-30°C and 54% for 15-25°C in chilled seeds (Fig. 8). Similarly, the average viability of non-chilled seeds of sample 3 ranged between 14% for 20-30°C to 22% for 15-25° C; whereas ranged between 45% for 20-30°C to 54% for 15-25°C in chilled seeds (Fig. 8). The variation in the viability by TZ among of the six labs ranged between 22% and 50% in sample 2; and 24% and 49% in sample 3.

Sample 4 and 5 had low level of dormancy. The average viable seed by TZ test of ungerminated seeds at the end of germination tests of non-chilled seeds of sample 4 ranged between 1% for 20-30°C to 2% for 15-25°C; whereas ranged between 1% for 20-30°C to 4% for 15-25°C in chilled seeds (Fig. 8). Similarly, the average viability of non-chilled seeds of sample 5 was 3% for both 20-30°C and 15-25°C; whereas it was 2% and 4% for 20-30°C and 15-25°C, respectively in chilled seeds (Fig. 8). The variation in the viability by TZ among of the six labs ranged between 2% and 3% in sample 4; and 0% to 5% in sample 5.

Sample 6 did not have dormancy, no viable seeds by TZ of chilled or non-chilled seeds for both 20-30°C and 15-25°C (Fig. 8). The variation in viability by TZ among the six labs is 0%.

Total Viable Seeds

Sample 1. The average total viability (final germination count + viability by TZ of ungerminated seeds at the end of germination test) of non-chilled seeds ranged between 84% for 20-30°C to 96% for 15-25°C; whereas ranged between 96% for 20-30°C to 85% for 15-25°C in chilled seeds (Fig. 9). The variation in the total viability among the six labs ranged between 80% and 95%.

Samples 2 and 3. They had deep dormancy. The average total viability of non-chilled seeds of sample 2 ranged between 95% for 20-30°C to 92% for 15-25°C; whereas ranged between 89% for 20-30°C to 97% for 15-25°C in chilled seeds (Fig. 9). Similarly, the average total viability of non-chilled seeds of sample 3 ranged between 93% for 20-30°C to 92% for 15-25°C; whereas ranged between 91% for 20-30°C to 94% for 15-25°C in chilled seeds (Fig. 9). The variation in the total viability among the six labs ranged between 81% and 97% in sample 2; and 85% and 95% in sample 3.

Sample 4 and 5. They had low level of dormancy. The average total viability of non-chilled seeds of sample 4 was 85% for both 20-30°C and 15-25°C; whereas ranged between 82% for 20-30°C to 86% for 15-25°C in chilled seeds (Fig. 9). Similarly, the average final germination of non-chilled seeds of sample 5 ranged between 89% for 20-30°C to 90% for 15-25°C; whereas it was 90% to 92% in 20-30°C and 15-25°C, respectively in chilled seeds (Fig. 9). The variation in the total viability among the six labs ranged between 73% and 90% in sample 4; and 83% and 95% in sample 5.

Sample 6 does not have dormancy, the average total viability of non-chilled seeds of sample 6 ranged between 84% for 20-30°C to 87% for 15-25°C; whereas it was 88% in both 20-30°C and 15-25°C in chilled seeds (Fig. 9). The variation in the total viability among the six labs ranged between 78% and 92%.

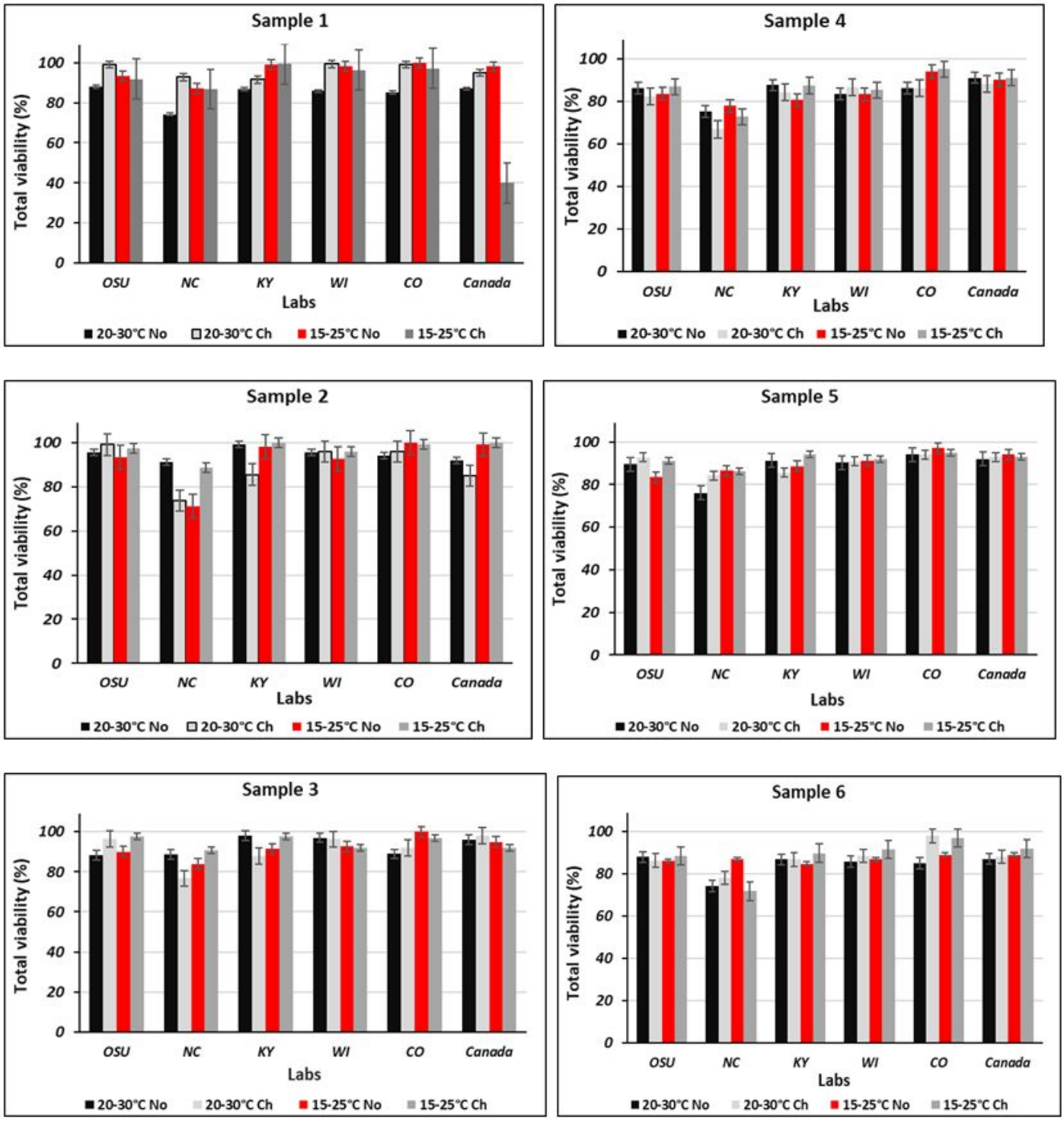

Figure 9. Total viable (normal seedlings after 7 days + viable seeds by TZ test of ungerminated seeds at the end of germination tests) of six hemp varieties tested using four combinations of temperature and prechilling treatments at six different laboratories. Means with different letters are significantly different from each other at $P \leq 0.05\%$.

Conclusions

First Count (3-day)

- Prechilling treatment helped, achieving better 3-day count (average 62%) than non-chilled seeds (average 17 %) but did not break deep dormant seeds (var. 2 & 3).
- First count ranged from 62% to 44% for samples 6, 5, 4 & 1; the lowest was in samples 2 & 3 at 17%.
- Germinating at 20-30°C gave similar results to 15-25°C for chilled seeds.
- Variability among labs in 3-day count ranged from 17% to 56%. This may be due to subjectivity of criteria in the 3-day evaluations (1.5 cm with root hair).
- First count is important to control secondary infection.

Final Count (7-day) – Prechilling Treatment

- Prechilling seeds at 10°C for 7d were not enough to break deep dormant seed.
- Final germination ranged from 87 to 82% for samples 6, 5, 4 & 1; the lowest germination was in samples 2 & 3 at 59-60% for deep dormant varieties.
- Germination at 15-25°C gave similar results to 20-30°C for chilled or non-chilled seeds.
- Average germination of non-chilled samples was 84% and for chill was 70%. The two dormant samples may cause the average chilled seeds to drop.
- Variability among labs ranged from 84 to 70%.
- TZ of the ungerminated seeds at the end of the germination test is essential to determine the total viability of deep dormant hemp varieties.

RECOMMENDATIONS

- First count is important to control secondary infection.
- Prechilling achieves better 1st count and helps in breaking short-lived light dormancy.
- TZ of the ungerminated seeds, at the end of the germination test, is essential to determine the total viability of deep dormant hemp varieties.
- Germination at 15-25°C gave similar results to 20-30°C for chilled or non-chilled seeds.
- Low-viable seed is needed in order to investigate potential advantage of 15-25°C by reducing the mold growth.

ACKNOWLEDGEMENT

- This study was funded in part by the Seed Testing Research Foundation. Final report was submitted to the Seed Testing Research Foundation in June 2022.
- Thanks to the Labs who participated in the Referee study.
- Thanks to the seed companies that donated the seeds for the study.

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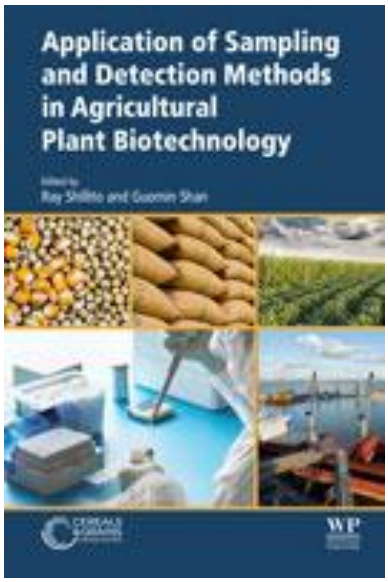
Resource Review: Application of Sampling and Detection Methods in Agricultural Plant Biotechnology

Doug Miller, RGT



The Cereals and Grains Association (formerly AACCI), in cooperation with its partner Elsiver Books, has recently published "Application of Sampling and Detection Methods in Agricultural Plant Biotechnology." The book is a natural extension of the training workshops sponsored by the International Life Sciences Institute and Cereals and Grains Association (formerly AACCI).

Of interest to SCST members, the book references our own Seed Technologist Training Manual, and several members and friends of the society were contributors. Contributors include Ray Shillito, BASF, Sherry Whitt, BASF, Farhad Ghavami, EBDI, and Doug Miller, ICIA. Edited by Ray Shillito, BASF, and Guomin Shan, Corteva Agriscience, the book introduces genetically modified crops and their detection with a chapter dedicated to "Seed Purity Testing and Low-Level Presence." A primary driver for the workshops, and the resulting book, are reflected in the chapter "Grain Supply Chain Stewardship and Testing," This chapter looks at both import and export perspectives. There are several Chapters of interest to laboratories, such as; Principles of Nucleic Acid-Based Detection Methods, Method validation: DNA-based detection methods, Qualitative and Quantitative PCR Method Validation, Protein-Based Detection Methods, Protein Methods: Antibody-Based Protein Method Validation and Assay Verification, Reference Materials and Working Standards." Following the ILSI/AACCI sponsored workshops, the book addresses "Testing Laboratory Design and Management" and several chapters on topics such as International Standards and Guidelines, Analytical Strategy and Interpretation of Results, Detection Methods for Genome-Edited Crops, and Future Perspectives and Challenges. The book also includes chapters on "Seed and Grain Sampling," a significant emphasis for the workshops, and the typically neglected subject of tissue sampling in the "Plant and Field Sampling" chapter.

If you want to expand your library and are interested in the subject of "reliable sampling, detection, and data interpretation," consider buying a copy. The book would be an excellent resource for "agricultural biotechnology companies, GM test kit manufacturers, research and testing laboratories, government agencies, and students and academics."

Application of Sampling and Detection Methods in Agricultural Plant Biotechnology is an invaluable resource for developing, validating, and using GM detection methods.

Link:

Product Detail - Application of Sampling and Detection Methods (cerealsgrains.org)

url:

https://my.cerealsgrains.org/AACCStore/Product-Detail.aspx?WebsiteKey=DCA5C7D5-F454-4D2D-AE5F-B545A01C8D57&iProductCode=92930

2023 AOSA Rule Proposals

AOSA Rules Committee

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4	Hard seeds	David Johnston, Riad Baalbaki	6
5	Missing seeds in germ test	Todd Erickson	6
6	PLS Rounding	Heidi Jo Larson	6
7	Remove raised blotter method	Heidi Jo Larson	6
8	# of seeds tested for TZ	Heidi Jo Larson	6
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10	Deviation statement	Kathy Mathiason	6,8,15, and Vol, 2,3,4
11	Mechanical seed counts	David Johnston	12
12	Reporting abnormals and dead	David Johnston, Riad Baalbaki	15
13	Marram grass common name	Debbie Meyer, Nishit Patel	Vol. 3
14	Corn seedling drawings	David Johnston, Riad Baalbaki	Vol. 4
15	Lettuce necrosis	David Johnston, Riad Baalbaki	Vol. 4
16	Lettuce necrosis (Supplemental guide)	David Johnston, Riad Baalbaki	Vol. 4
17	Organic media	David Johnston	Vol. 4
18	Primary/secondary roots	David Johnston, Riad Baalbaki	Vol. 4
Don't forget to attend the virtual Open Rules Discussion on May 16th, 2023!			

Proposal #1

Debbie Meyer and Nishit Patel

Purpose: Restructure section 3.8 pelleted, coated or encrusted seed purity procedures and provide further clarification of the procedures within the section. The two purity methods for testing coated seed will not be changed by this proposal.

Current Rule:

3.8 Pelleted, coated or encrusted seed purity procedures

a. Where reference is made to coated units, the rules apply to pelleted, coated and encrusted seed. Refer to section 2.1 d.

b. **Size of working sample:** refer to section 2.3 b (5).

c. **Obtaining the working sample:** Methods described in section 2.2 shall be used.

d. **Purity analysis of coated units** Refer to section 3.8g for verification of kind or cultivar of seed under consideration. When the percentage of coating material must be determined for purposes of labeling or regulatory label compliance testing, use the method in section 3.8 e. The method under 3.8 e must be followed for all mixtures of kinds, single component seed samples of Poaceae, or upon customer request.

(1) Separation of component parts: The working sample shall be weighed in grams to the appropriate number of decimal places (refer to section 2.3) and shall be separated into four parts:

(a) Pure coated units as defined in section 3.8 d (2).

(b) Uncoated crop seed as defined in section 3.8 d (3) (including the kind under consideration).

(c) Inert matter as defined in section 3.8 d (4).

(d) Uncoated weed seed as defined in section 3.8 d (5)

(2) Pure coated units shall include:

(a) Entire coated units regardless of whether or not they contain a seed.

(b) Broken and damaged coated units in which more than half the surface of the seed is covered by coating material, except when it can be seen that, either the seed is not of the species stated by the sender, or there is no seed present.

(3) Uncoated crop seed shall include:

(a) Free seeds of any crop species; refer to sections 3.2 and 3.3

(b) Broken coated units containing a crop seed that is recognizably not of the species stated by the sender.
(c) Broken coated units of the species stated when the coating material covers half or less of the surface of the seed.

(4) Inert matter shall include:
(a) Loose coating material.
(b) Broken coated units in which it is obvious there is no seed.
(c) Any other material defined as inert matter in section 3.5.

(5) Uncoated weed seed shall include:
(a) Free seeds of any weed species; refer to section 3.4.
(b) Broken coated units containing a weed seed.

e. **Purity analysis of de-coated units** (This section shall apply to all mixtures of kinds, single component seed samples of Poaceae, or upon request for other kinds)**:**

(1) Determine the working sample size as in section 2.3 b (5), and weigh the working sample in grams to the appropriate number of decimal places (refer to section 2.3 a).

(2) Remove the coating material from the seed by washing with water or other solvents such as, but not limited to, dilute sodium hydroxide. Use of fine mesh sieves is recommended for this procedure and stirring or shaking the coated units may be necessary to obtain de-coated seed.

(3) Spread on blotters or filter paper in a shallow container. Air dry overnight at room temperature.

(4) Separation of component parts:
(a) Kind or cultivar considered pure seed as defined in section 3.2 and Table 3A.
(b) Other crop seed.
(c) Inert matter.
(d) Weed seed.
(e) Coating material.

The de-coated working sample shall be separated into the first four components in accordance with sections 3.2 through 3.5. Sections 3.6 and 3.7 shall not be followed. The weight of the coating material component is determined by subtracting the sum of the weights of the other four components from the original weight of the working sample. Calculate percentages of all five components based on the original weight of the working sample.

f. **Noxious weed seed examination or bulk examination:** The working sample size shall be approximately 25,000 coated units or a maximum of 1,000 grams of kinds listed in Table 2A for which the working sample weight of raw seed is 500 grams. A noxious weed seed examination shall be made by examining the working sample after it has been de-coated.

g. **Identification and cultivar determination when method under 3.8 d is applied:** Verification of the kind of seed under consideration shall be made on 100 coated units taken from the pure coated unit component of the purity separation. Before examination, the coating material shall be removed by washing or other appropriate method. The name and number of each kind found shall be reported under other determinations on the report of analysis. If requested for cultivar determination, a minimum of 400 coated units shall be examined as above and results reported under other determinations on the report of analysis.

Identification and cultivar determination for seed tapes and seed mats: Verification of the kind of seed under consideration shall be made on 100 seed units taken from the working sample of seed tape or seed mat. Before examination, the seed units are removed from the seed tape or seed mat. Methods of removal may include striping away the tape or mat material to free the seed units, or if necessary, moistening or washing off the tape or mat material with an appropriate solvent [refer to 3.8.e (2)]. Moistened seed units should be allowed to dry prior to examination. The name and number of each kind found shall be reported under other determinations on the report of analysis. If requested for cultivar determination, a minimum of 400 seed units shall be examined as above and results reported under other determinations on the report of analysis.

Proposed Rule: (Substantive changes noted in red text. Changes in subsection numbers are made throughout the proposal to accommodate the addition of new subsections.)

3.8 Pelleted, coated or encrusted seed purity procedures

a. Where reference is made to coated seed units, the rules apply to pelleted, coated and encrusted seed. Refer to section 2.1 d.

b. When the percentage of coating material must be determined for purposes of labeling or regulatory label compliance testing, the procedure in section 3.8 g must be used.

c. The procedure under 3.8 g must be followed for all single component seed samples of the grass family (Poaceae), all mixtures of kinds (whether or not all components of the mixture are coated), or upon customer request.

d. **Size of working sample:** refer to section 2.3 b (5).

e. **Obtaining the working sample:** Methods described in section 2.2 shall be used.

f. **Procedure for purity analysis of coated seed units.** This section shall only apply to single component kinds where coating material is not required to be removed (refer to sections 3.8 b and 3.8 c)**.** Refer to section 3.8 i for required verification of kind or cultivar of seed under consideration.

(1) Separation of component parts: The working sample shall be weighed in grams to the appropriate number of decimal places (refer to section 2.3) and shall be separated into four parts:

(a) Pure coated seed units as defined in section 3.8 f (2).
(b) Uncoated crop seed as defined in section 3.8 f (3) (including the kind under consideration).
(c) Inert matter as defined in section 3.8 f (4).
(d) Uncoated weed seed as defined in section 3.8 f (5)

(2) Pure coated seed units shall include:

(a) Entire coated seed units regardless of whether or not they contain a seed.
(b) Broken and damaged coated seed units in which more than half the surface of the seed is covered by coating material, except when it can be seen that, either the seed is not of the species stated by the sender, or there is no seed present.

(3) Uncoated crop seed shall include:
(a) Free seeds of any crop species; refer to sections 3.2 and 3.3
(b) Broken coated seed units containing a crop seed that is recognizably not of the species stated by the sender.
(c) Broken coated seed units of the species stated when the coating material covers half or less of the surface of the seed.

(4) Inert matter shall include:
(a) Loose coating material.
(b) Broken coated seed units in which it is obvious there is no seed.
(c) Any other material defined as inert matter in section 3.5.

(5) Uncoated weed seed shall include:
(a) Free seeds of any weed species; refer to section 3.4.
(b) Broken coated seed units containing a weed seed.

g. **Procedure for purity analysis of de-coated seed units.** This section shall apply to all purity analyses where coating material is required to be removed. Refer to sections 3.8 b and 3.8 c.

(1) Determine the working sample size as in section 2.3 b (5) and weigh the working sample in grams to the appropriate number of decimal places (refer to section 2.3 a).

(2) Remove the coating material from the seed by washing with water or other solvents such as, but not limited to, dilute sodium hydroxide. Use of fine mesh sieves is recommended for this procedure and stirring or shaking the coated seed units may be necessary to obtain de-coated seed.

(3) Spread on blotters or filter paper in a shallow container. Air dry overnight at room temperature.

(4) Separation of component parts:
  (a) Kind or cultivar considered pure seed as defined in section 3.2 and Table 3A.
  (b) Other crop seed.
  (c) Inert matter.
  (d) Weed seed.
  (e) Coating material.

The de-coated working sample shall be separated into the first four components in accordance with sections 3.2 through 3.5. Sections 3.6 and 3.7 shall not be followed. The weight of the coating material component is determined by subtracting the sum of the weights of the other four components from the original weight of the working sample. Calculate percentages of all five components based on the original weight of the working sample.

h. **Noxious weed seed examination or bulk examination:** The working sample size shall be approximately 25,000 coated seed units or a maximum of 1,000 grams of kinds listed in Table 2A for which the working sample weight of raw seed is 500 grams. A noxious weed seed examination shall be made by examining the working sample after it has been de-coated. Refer to 3.8 g (2) for appropriate method for removing coating material.

i. **Identification and cultivar determination when method under 3.8 f is applied:** Verification of the kind of seed under consideration shall be made on 100 coated seed units taken from the pure coated seed unit component of the purity separation. Before examination, the coating material shall be removed by washing or other appropriate method. The name and number of each kind found shall be reported under other determinations on the report of analysis. If requested for cultivar determination, a minimum of 400 coated seed units shall be examined as above and results reported under other determinations on the report of analysis.

Identification and cultivar determination for seed tapes and seed mats: Verification of the kind of seed under consideration shall be made on 100 seed units taken from the working sample of seed tape or seed mat. Before examination, the seed units are removed from the seed tape or seed mat. Methods of removal may include striping away the tape or mat material to free the seed units, or if necessary, moistening or washing off the tape or mat material with an appropriate solvent [refer to 3.8.g (2)]. Moistened seed units should be allowed to dry prior to examination. The name and number of each kind found shall be reported under other determinations on the report of analysis. If requested for cultivar determination, a minimum of 400 seed units shall be examined as above and results reported under other determinations on the report of analysis.

Harmonization Statement:

Under the Federal Seed Act (FSA), testing of coated seed is similar to the AOSA method for purity analysis of de-coated seed units. The FSA requires coating material to be removed for all kinds of seeds (i.e., not only for members of the Poaceae or for mixtures of kinds). The final purity analysis results under the FSA differs from those under the AOSA Rules with regard to the percentage of coating material. Under the FSA, the coating material is included as part of the percentage of inert matter. This proposal will not address the current differences in final purity analysis results obtained under the FSA verses the AOSA Rules. It should be noted that the FSA only requires purity analyses of agricultural seed kinds (as defined by the FSA).

The definitions of coated, pelleted, and encrusted seed in the ISTA Rules, as well as the definitions of pure coated seed units and testing methods, are essentially the same as the AOSA Rules. The ISTA Purity Committee is considering some modifications to the ISTA Rules regarding testing of coated seed; however, none of the proposed changes to the ISTA Rules are in conflict with this AOSA rule change proposal.

The Canadian Methods and Procedures for Testing Seed (M&P) refers to the AOSA Rules for determining the percentages by weight of coated seed.

Supporting Evidence:

This proposal does not change the two current methods for testing coated seed, but it provides clarification on when to apply each of the methods.

Submitted by: Deborah J. Lionakis Meyer and Nishit Patel AOSA-SCST Purity Subcommittee Co-chairs.

Date Submitted: October 11, 2022. Revised December 9, 2022.

Proposal #2

Sarah Dammen

1. **PURPOSE:** To add *Salvia hispanica* germination methods to table 6A.

2. **PRESENT RULE:**
None
PROPOSED RULE:
Table 6A. Methods of testing for laboratory germination.

Kind of Seed	Substrata [a]	Temperature (°C)	First count (days)	Final count (days)	Specific requirements and notes	Fresh and dormant seed
Salvia hispanica chia	P	20; 20-30	7	14		

4. **HARMONIZATION AND IMPACT STATEMENT:**
ISTA Rules for Seed Testing has Salvia hispanica methods in the purity and germination chapters. Canada Methods and Procedures do not mention Salvia hispanica and neither does AOSA Rules. 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.

5. **SUPPORTING EVIDENCE:**

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. When prechill was performed, the seed was placed in 5-10°C for 5 days method. The samples were then moved to 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 laboratory 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 using the new R package developed by the ISTA Statistics Committee 'ISTAgermMV'.

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. 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

Proposal #3

Sue Alvarez

Purpose of Proposal: To clarify guidelines for the evaluation of borderline seedlings in germination testing.

Present Rule: New Rule

Proposed Rule: AOSA Rules Volume 1

6.5 Evaluation of seedlings

. . .

d. **Borderline seedlings.** --- These are seedlings which are on the borderline between normal and abnormal. If there is only one such seedling, count it as normal. If there is an even number, count half of them as normal. If there is an odd number, count 2 out of 3, 3 out of 5, etc., as normal. As a guideline, the percentage of doubtful seedlings evaluated should be no more than 5%.

Harmonization and Impact Statement: The Canadian M & P includes the following statement:

Canadian Methods and Procedures for Testing Seed (M&P) 2020

4.10.5 Borderline Seedlings

These are seedlings which are on the borderline between normal and abnormal. If there is only one such seedling, count it as normal. If there is an even number, count half of them as normal. If there is an odd number, count 2 out of 3, 3 out of 5, etc., as normal.

The ISTA Handbook on Seedling Evaluation (4th Ed. 2018) includes the following statement:

8.3 Stage of development for seedling evaluation and evaluation guidelines

(Footnote 9) As a guideline, the percentage of doubtful seedlings evaluated should be no more than 5%.

Supporting Evidence: This proposal is meant to be a *guideline* for evaluating "borderline seedlings" found in many germination tests. It was put together as a part of the effort to reduce some of the variation when borderline seedlings are found in a germination test. In 2022, a similar proposal was narrowly defeated (128.90, less than 134.0 needed).This new proposal would incorporate into the AOSA Rules the *exact wording* found in the Canadian M & P and the ISTA Handbook on Seedling Evaluation.

Submitted by: Sue Alvarez, R.S.T., Lab manager, Ransom Seed Lab, Carpinteria, CA
sue.alvarez@ransomseedlab.com

Date submitted: July 26, 2022

Proposal #4

David Johnston and Riad Baalbaki

Purpose of the Proposal: Revise information in the Rules (Vols. 1 and 4) of species belonging to families known to have hard seeds, and revise Table 6A instructions and requirements for testing and reporting hard seed test results.

Present and Proposed Rules:

Present Rule: Vol. 1, Sec. 6.2. d.

Hard seeds. — Seeds that remain hard at the end of the prescribed test period because they have not absorbed water due to an impermeable seed coat. Seeds known and recognized to contain hard seed are indicated in either the "Specific Requirements and notes" column or "Dormant Seed" column of Table 6A. The percentage hard seed is to be reported in addition to the percentage germination.

Proposed Rule: Vol. 1, Sec. 6.2. d.

Hard seeds. — Seeds that remain hard at the end of the prescribed test period because they have not absorbed water due to an impermeable seed coat. ~~Seeds~~ Species of Bixaceae, Cannaceae, Cistaceae, Fabaceae, Geraniaceae, and Malvaceae, are known and recognized to contain hard seed. Other families reported with few hard-seeded species include Anacardiaceae, Cochlospermaceae, Convolvulacea, Dipterocarpaceae, Nelumbonaceae, Rhamnaceae, Sapindaceae, and Sarcolaenaceae. For species of all families listed above, indicated in either the "Specific Requirements and notes" column or "Dormant Seed" column of Table 6A, the percentage hard seed is to be reported in addition to the percentage germination. Families for which there is no evidence of hard seeds include Amaryllidaceae, Brassicaseae, Chenopodiaceae, Cucurbitaceae [(footnote X)], Poaceae, Rosaceae, and Solanaceae. In addition, seeds of Gymnosperms do not exhibit hard seed dormancy. For species of the preceding seven families and gymnosperms, report hard seed as N/A.

Footnote X: Very few wild species of this family have been reported to have hard seeds. However, none of the cultivated species of this family listed in Table 6A produce hard seeds. Seeds of those species that appear 'hard' are rigid due to physical and physiological constraints, but the seed coat is permeable to water.

Present Rule: Vol. 4, Sec. 3.5.4: 3.5.4 Hard, swollen, dormant and dead seeds. Hard seeds are seeds that remain hard at the end of the prescribed test period because they have not absorbed water due to an impermeable seed coat. Species known to produce hard seeds are indicated by footnotes in Table 6A of the AOSA Rules for Testing Seeds Vol. 1. The percentage of hard seeds occurring in the germination test will vary with the age, kind, variety and

moisture content of the seed. The hard seed content of some recently harvested legumes such as red clover, lespedeza and field peas may decrease rapidly within the first few weeks or months of dry laboratory storage. Conversely, seeds of okra, vetch and certain other legumes may increase in hard seed content during dry laboratory storage. The hard seededness in beans is increased as the beans become desiccated. The relative humidity of the air in the storage area may cause moisture changes within the seeds and hence changes in the number of hard seeds. These changes are reversible. In reporting the test results, the percentage of hard seeds is reported in addition to the percentage germination.

Proposed Rule: Vol. 4, Sec. 3.5.4: 3.5.4 Hard, swollen, dormant and dead seeds. Hard seeds are seeds that remain hard at the end of the prescribed test period because they have not absorbed water due to an impermeable seed coat. Species known to produce hard seeds are indicated ~~by footnotes~~ in Table 6A of the AOSA Rules for Testing Seeds Vol. 1, under "Specific requirements and notes" and "Dormant seed." Families known and recognized to contain hard seed include Bixaceae, Cannaceae, Cistaceae, Fabaceae, Geraniaceae, and Malvaceae. Families reported to include only a few species with hard seeds include Anacardiaceae, Cochlospermaceae, Convolvulacea, Dipterocarpaceae, Nelumbonaceae, Rhamnaceae, Sapindaceae, and Sarcolaenaceae. Families for which there is no evidence of hard seeds include Amaryllidaceae, Brassicaseae, Chenopodiaceae, Cucurbitaceae[(footnote X)], Poaceae, Rosaceae, and Solanaceae. In addition, seeds of Gymnosperms do not exhibit hard seed dormancy. The percentage of hard seeds occurring in the germination test will vary with the age, kind, variety and moisture content of the seed. The hard seed content of some recently harvested legumes such as red clover, lespedeza and field peas may decrease rapidly within the first few weeks or months of dry laboratory storage. Conversely, seeds of okra, vetch and certain other legumes may increase in hard seed content during dry laboratory storage. The hard seededness in beans is increased as the beans become desiccated. The relative humidity of the air in the storage area may cause moisture changes within the seeds and hence changes in the number of hard seeds. These changes are reversible. In reporting the test results, the percentage of hard seeds is reported in addition to the percentage germination.

……

Footnote X: Very few wild species of this family have been reported to have hard seeds. However, none of the cultivated species of this family listed in Table 6A produce hard seeds. Seeds of those species that appear 'hard' are rigid due to physical and physiological constraints, but the seed coat is permeable to water.

Proposed changes to Vol. 1, Table 6A:

-For each current and future species listed in Table 6A of the Rules (Vol. 1), belonging to Anacardiaceae, Bixaceae, Cannaceae, Cistaceae, Cochlospermaceae, Convolvulacea, Dipterocarpaceae, Fabaceae, Geraniaceae, Malvaceae, Nelumbonaceae, Rhamnaceae, Sapindaceae and Sarcolaenaceae, include the hard seed statement [**Hard seeds: see sec. 6.2d**

and 6.9m (6)] to the "Specific requirements and notes" column of Table 6A. For the very few occurrences where that statement appears in the "Dormant seed" column of the current Table 6A, it should be moved to the "Specific requirements and notes" column.

Harmonization and Impact Statement:

The proposed changes reduce the differences and improve harmonization with ISTA Rules, although some differences in hard seed reporting requirements will still exist.

Supporting Evidence:

Studies in the past 25 years have produced a wealth of new information on the taxonomy, anatomy and physiology of hard seed dormancy. Hard seed dormancy, or physical dormancy, is common in some plant families, absent in others, and exhibited in only one or few species in some families. Physical dormancy was defined by Baskin and Baskin (1) and Baskin et al. (3) as "Seed dormancy caused by a water-impermeable seed (or fruit) coat," essentially the same definition as the one cited in the AOSA Rules. While many classification systems of dormancy have been proposed, that of Baskin and Baskin (2) is the most widely used by seed scientists and technologists, and has been since refined by other researchers (4, 5). It is proposed that their classification and identification of families with hard seeds be used to update the Rules and clarify testing and reporting requirements for hard seeds.

- Based on Baskin and Baskin's (2) classification, as well as subsequent research, each of the following families include many species with hard seeds: Bixaceae, Cannaceae (the only monocot), Cistaceae, Fabaceae, Geraniaceae, and Malvaceae.
- Families within which only few species were reported to have hard seeds include Anacardiaceae, Cochlospermaceae, Convolvulacea, Dipterocarpaceae, Nelumbonaceae, Rhamnaceae, Sapindaceae, and Sarcolaenaceae.
- Families for which there is no evidence of physical dormancy (hard seeds) include Amaryllidaceae, Brassicaseae, Chenopodiaceae, Cucurbitaceae (cultivated species), Poaceae, Rosaceae, and Solanaceae.
- In addition, Gymnosperms do not have hard seeds.

References

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5. Leubner, G. 2021. Phylogenetic table: Seed dormancy classification with examples. The seed biology place. Retrieved from http://www.seedbiology.de/dormancy2.asp (verified 10 March 2021).

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Proposal #5

Ernest Allen and Todd Erickson

Purpose of Proposal: To harmonize AOSA with ISTA, giving analysts the option to adjust final percentages rather than retest if there are +/- 5 or less seeds at the end of a germination test.

Present Rule: none

Proposed Rule:

6.7 e If the total number of evaluated seeds and seedlings is more than 5 above or below the minimum required number (400 or 200 seeds), a retest must be conducted. If the number of extra or missing seedlings is 5 or less, the final percentages are adjusted by dividing by the total number of seeds tested, rather than 400 or 200. Alternately, a retest may be conducted.

Example:

Category	Rep 1	Rep 2	Rep 3	Rep 4	Total	Calculation	Final %	Adjusted Final %
Normal	95	93	96	94	378	378/397	95.2	95%
Abnormal	1	3	2	2	8	8/397	2.0	2%
Dead	4	4	2	1	11	11/397	2.8	3%
Total	100	100	100	97	397			100%

In calculating the adjusted final percentage, the same rounding rules described in 6.7a shall apply. Table 14J is still used to calculate tolerance between replicates.

This method applies to coated and uncoated samples. Refer to 11.5.6.5 for coated seed containing no seeds or other seeds.

Harmonization Statement: This method is already in the ISTA Rules. The Federal Seed Act Regulations do not address what to do with extra or missing seeds/seedlings.

Supporting Evidence: This is a procedural change to more accurately reflect final germination percentages when small counting errors have been made. The Germination Committee was consulted in developing this proposal.

Submitted by: Ernest Allen and Todd Erickson, USDA, AMS, SRTD, 801 Summit Crossing Place, Suite C, Gastonia NC 28054.

Date: 10/14/22

Proposal #6 - Amended*

Heidi Jo Larson

Purpose: The purpose of this proposal is to change the reporting percentage of pure live seed (PLS) from a whole number to two decimal places.

Present Rule:

6.2.k. Pure live seed. —The percentage of pure seeds in a lot that are viable.

The basic formula to calculate Pure Live Seed (PLS) is:

Percent (%) Pure x Percent (%) Total Viable / 100 = % PLS

where

% Total Viable = % Germination + %Hard Seed + %Dormant Seed

Example:

Pure seed = 98.55%; Germination = 67%, Hard seed = 5%, Dormant seed = 17%

Step 1: % Total Viable = 67% + 5% + 17% = 89% Total Viable

Step 2: % Pure Live Seed $= \frac{\%\ \text{Pure} \times \%\ \text{Total Viable}}{100}$

$= \frac{98.55\% \times 89\%}{100} = 87.7095\%$

Step 3: Round to nearest whole number = 88% PLS

Note: In all cases % total viable and % pure seed used to calculate the % pure live seed must be from the same working sample. The correct procedure according to the AOSA Rules for Testing Seeds is to complete the purity analysis, germinate the pure seed, and determine the % of hard and/or % dormant seed, as applicable according to methods in Table 6A, at the conclusion of the germination test.

**Amended text is blue and highlighted*

Proposed Rule:

6.2.k. Pure live seed. —The percentage of pure seeds in a lot that are viable.

The basic formula to calculate Pure Live Seed (PLS) is:

Percent (%) Pure x Percent (%) Total Viable / 100 = % PLS

where

% Total Viable = % Germination + %Hard Seed + %Dormant Seed

Example:

Pure seed = 98.55%; Germination = 67.25%, Hard seed = 5.5%, Dormant seed = 17.5%

Step 1: % Total Viable = 67.25% + 5.5% + 17.5% = 90.25% Total Viable

Step 2: % Pure Live Seed = % Pure x % Total Viable

100

= 98.55% x 90.25% = 88.9414%

100

Step 3: Round to ~~nearest whole number~~ two decimal places = ~~88%~~ 88.94% PLS

Note: **The % germination, % hard seed, and % dormant seed are to be calculated using the raw data to two decimal places and not the rounded numbers.** In all cases % total viable and % pure seed used to calculate the % pure live seed must be from the same working sample. The correct procedure according to the AOSA Rules for Testing Seeds is to complete the purity analysis, germinate the pure seed, and determine the % of hard and/or % dormant seed, as applicable according to methods in Table 6A, at the conclusion of the germination test.

Harmonization and Impact Statement:

The Federal Seed Act does not state how to report the final number. It only includes the formula to be used:

201.64 Pure live seed.

The tolerance for pure live seed shall be determined by applying the respective tolerances to the germination plus the hard seed and dormant seed, and the pure seed.

$$PLS = \frac{[\text{Germination \%} + \text{Hard Seed \%} + \text{Dormant Seed \%}]\text{x Pure Seed \%}}{100}$$

ISTA does not define pure live seed, so this rule proposal would not impact harmonization with ISTA.

This rule proposal would cause the AOSA Rules to not harmonize with the Canada M&P. The Canada M&P under section 4.11.5.b.(xii) requires the PLS to be reported as a whole number.

(xii). Pure Living Seed is to be reported as a percentage calculated to the nearest whole number.

(See Section 4.11.4.b)

Supporting evidence:

When the definition of PLS was placed into the AOSA Rules for Testing seeds, according to the authors it was reported to a whole number because that is how the germination percentage is reported. When seed is being sold off a percentage and monetary basis, rounding to a whole number can greatly impact the buyer or seller depending on the rounding. If PLS is rounded down to a whole number, the seller is not getting the accurate dollar value of their seed and the customer is getting more than what they are paying for. If PLS is rounded up to a whole number, the buyer is getting less than what they are paying for, and the seller is getting paid for seed that isn't there.

The agronomy/agriculture field is going with a focus more on precision agriculture. PLS is being used to calculate how many pounds of corn, soybeans, small grains, etc. of a seed lot will need to be planted to obtain a desired plant population in the field. Seed testing should provide the best and most accurate information for producers in calculating planting rates for desired field populations.

Reviewing native company's websites, the price of seed per pound of PLS ranges from $10.20 to $720.00. At $720/lb. of PLS a difference of 88.00% PLS versus 88.48% PLS makes a difference of $3.46/lb. of PLS. In this situation the seller is being shorted $3.46 per pound of PLS and the buyer is getting 0.48% more seed than what they are paying for. On the other side selling at 89.00% PLS versus 88.55% PLS, the seller is getting $3.24/lb. of PLS more than what they are selling. The buyer is believing they are paying for 89%/lb. of PLS when they are being shorted 0.45%/lb. of PLS.

As seed analysts it is our job to provide truthful and accurate information to the customers. By rounding instead of reporting to two decimal places it appears that we are not providing truthful and accurate information to either the buyer or the seller of the seed.

Respectfully Submitted: Heidi Jo Larson, RST, SGS, Brookings, SD. Heidi.larson@sgs.com

Date Submitted: August 5, 2022 Amended December 13, 2022

Proposal #7

Germination Uniformity Working group

Purpose of the Rule: The purpose of this rule proposal is to remove raised blotters (RB) as a media option from the AOSA Rules for Testing Seeds.
Present Rule:

Section 6.9.a

Substrata. — Any medium listed for a particular species in the substrata column of Table 6A may be used. The order listed does not indicate preference. Symbols for substrata in column 2, Table 6A are:

A: top of agar, polysaccharide powder solidifier made from red algae (without any additional nutrients, vitamins or hormones). Agar powder should be approximately 99% pure. Agar media must be free of extra salts that may inhibit plant growth.

B: between blotters
C: creped cellulose paper wadding (0.3-inch thick Kimpak or equivalent) covered with a single thickness of blotter through which holes are punched for the seed that are pressed for about one-half their thickness into the paper wadding
O: organic growing media
OT: organic growing media covering seed planted on top of paper toweling (T)
P: covered petri dishes or other rigid transparent containers, with appropriate layers of blotters, filter paper, paper toweling, creped cellulose paper, pleated paper or sand that provide adequate moisture to the seeds during the test period
PP: pleated filter paper (see footnote a in Table 6A)
PT: substrata listed for P with the following substrata also allowed: sponge rok, vermiculite, terralite, or a mixture of 50 percent sand and vermiculite, sand and perlite, etc.
RB: blotters and raised covers, prepared by folding up the edges of the blotter to form a good support for the upper fold which serves as a cover, preventing the top from making direct contact with the seeds
S: sand
T: paper toweling, used either as folded towel tests or as rolled towel tests in horizontal or vertical position
TB: top of blotters
TS: top of sand
TC: on top of creped cellulose paper without a blotter
TCS: on top of creped cellulose paper without a blotter and covered with ½ to ¾ inch layer of sand.

Table 6A

Kind of Seed	Substrate[a]	Temperature (C°)	First Count (days)	Final Count (days)	Specific requirements	Fresh and Dormant
Capsicum chinense Jacq. habanero pepper	T, B, TB, RB, P	20-30	10	21		Light and GA3(500 ppm)
Solanum lycopersicum var. lycopersicum tomato	T, B, P, RB, A	20-30	5	14		Light; KNO3
Capsicum spp. vegetable and ornamental pepper	T, B, TB, RB, P	20-30	10	14		Light and KNO3. See footnotes b and c for ornamental varieties
Solanum melongena eggplant	P, TB, RB, T	20-30	7	14		Light; KNO3

Proposed Rule:

Section 6.9.a

Substrata. — Any medium listed for a particular species in the substrata column of Table 6A may be used. The order listed does not indicate preference. Symbols for substrata in column 2, Table 6A are:
A: top of agar, polysaccharide powder solidifier made from red algae (without any additional nutrients, vitamins or hormones). Agar powder should be approximately 99% pure. Agar media must be free of extra salts that may inhibit plant growth.
B: between blotters
C: creped cellulose paper wadding (0.3-inch thick Kimpak or equivalent) covered with a single thickness of blotter through which holes are punched for the seed that are pressed for about one-half their thickness into the paper wadding
O: organic growing media
OT: organic growing media covering seed planted on top of paper toweling (T)
P: covered petri dishes or other rigid transparent containers, with appropriate layers of blotters, filter paper, paper toweling, creped cellulose paper, pleated paper or sand that provide adequate moisture to the seeds during the test period
PP: pleated filter paper (see footnote a in Table 6A)
PT: substrata listed for P with the following substrata also allowed: sponge rok, vermiculite, terralite, or a mixture of 50 percent sand and vermiculite, sand and perlite, etc.
~~**RB:** blotters and raised covers, prepared by folding up the edges of the blotter to form a good support for the upper fold which serves as a cover, preventing the top from making direct contact with the seeds~~
S: sand
T: paper toweling, used either as folded towel tests or as rolled towel tests in horizontal or vertical position
TB: top of blotters
TS: top of sand
TC: on top of creped cellulose paper without a blotter
TCS: on top of creped cellulose paper without a blotter and covered with ½ to ¾ inch layer of sand.

Table 6A

Kind of Seed	Substrate[a]	Temperature (C°)	First Count (days)	Final Count (days)	Specific requirements	Fresh and Dormant
Capsicum chinense Jacq. habanero pepper	T, B, TB, ~~RB~~, RB, P	20-30	10	21		Light and GA3(500 ppm)
Capsicum spp. vegetable and ornamental pepper	T, B, TB, ~~RB~~, RB, P	20-30	10	14		Light and KNO3. See footnotes b and c for ornamental varieties
Solanum lycopersicum var. lycopersicum tomato	T, B, P, ~~RB~~, RB, A	20-30	5	14		Light; KNO3
Solanum melongena eggplant	P, TB, ~~RB~~, RB, T	20-30	7	14		Light; KNO3

Harmonization and Impact Statement: This rule proposal would not affect harmonization with ISTA as raised blotter is not a media option for eggplant, peppers, or tomato.

This proposal would not harmonize with FSA or Canada M&P because raised blotter is a recognized media option in both sets of germination methods.

Supporting Evidence: In April of 2021, the germination working group submitted a survey to the AOSA and SCST membership for 33 crop species that have either multiple temperatures and/or multiple media options. The survey listed all the current temperature and substrate options that are currently listed in Table 6A along with the option to list "other" if the laboratory uses a temperature and/or substrate that is not listed in the AOSA Rules. The goal of this survey was to clean up Table 6A and increase uniformity among laboratories by removing any media or temperature options that are not being used by any laboratories.

There are approximately 100 AOSA and SCST labs. The survey had a respondent rate of 68 individuals.

Of the 68 respondents, 32 laboratories test eggplant with 95% testing less than 250 samples per year.

The media options listed in Table 6A for pepper are covered petri dish (P), top of blotter (TB), raised blotter (RB), and paper towelling (T). 35% of laboratories use covered petri dish (P), 26% use top of blotter (TB), 0% use raised blotter (RB), and 48% use paper towelling (T). One laboratory uses top of creped cellulose with sand, which is not an approved method. These numbers add up to over 100% because some labs reported the use of multiple methods.

Of the 68 respondents, 36 laboratories test tomato with 84% testing 11 samples or more per year.

The media options listed in Table 6A for tomato are paper towelling (T), between blotters (B), covered petri dish (P), raised blotter (RB), and agar (A). 73% of labs use paper towelling (T), 5% use between blotters (B), 30% use covered petri dish (P), 0% use raised blotter (RB), and 0% use agar (A). One laboratory uses top of creped cellulose with sand and two laboratories use top of blotter, neither of which are approved methods. These numbers add up to over 100% because some labs reported the use of multiple methods.

Of the 68 respondents, 25 laboratories test habanero pepper with 62% testing 11 samples or more per year.

The media options listed in Table 6A for habanero pepper are paper towelling (T), between blotters (B), top of blotter (TB), raised blotter (RB), and covered petri dish (P). 58% of laboratories use paper towelling (T), 12% use between blotters (B), 23% use top of blotter (TB), 0% use raised blotter (RB), and 15% use covered petri dish (P). These numbers add up to over 100% because some labs reported the use of multiple methods.

Of the 68 respondents, 36 laboratories test pepper with 84% testing 11 samples or more per year.

The media options listed in Table 6A for pepper are paper towelling (T), between blotters (B), top of blotter (TB), raised blotter (RB), and covered petri dish (P). 73% of labs use paper towelling (T), 18% use between blotters (B), 16% use top of blotter (TB), 0% use raised blotter (RB), and 14% use covered petri dish (P). One laboratory uses top of creped cellulose with sand which is not an approved method. These numbers add up to over 100% because some of the labs reported the use of multiple methods.

In the 1937 AOSA Annual meeting proceedings it was discussed that raised blotters were recommended for small seeded species, those with a mucilaginous coat, or those that would not otherwise receive sufficient aeration. Almost twenty years prior to 1954, raised blotters were listed under remarks for eggplant, peppers, and tomatoes as an alternative method. In 1954, the raised blotters were moved into an approved media option for these four species. There is no documentation or scientific evidence that can be found as to why this decision was made. Raised blotters were also recommended for laboratories that used water jacket chambers to ensure adequate moisture be maintained. With the advances in technologies, very few labs, if any, still use the water jacketed germination chambers.

Raised blotter is a more time-consuming process for planting eggplant, pepper, and tomatoes. The average time for planting eggplant with raised blotters is 4.39 minutes versus 3.70 minutes for planting with towel or top of blotter (Table 1). The average time for planting peppers with raised blotters is 4.66 minutes versus 3.75 minutes for planting with towel or top of blotter (Table 2). The average time for planting tomato with raised blotters is 4.41 minutes versus 3.69 minutes for planting with towel or top of blotter (Table 3). These numbers may not seem like a significant time difference for one sample. However, if a lab were to be planting hundreds of samples, the time difference would be significant.

Table 1. Time comparison of ten individuals (with varying experience levels) planting eggplant with raised blotter versus top of blotter or towel.

Person	RB	T/TB
1	3.62	2.85
2	4.48	3.73
3	3.97	3.28
4	3.65	3.14
5	4.87	4.02
6	4.68	4.20
7	5.13	4.38
8	5.00	4.29
9	4.46	4.13
10	4.01	2.97
Average	4.39	3.70

Table 2. Time comparison of ten individuals (with varying experience levels) planting pepper with raised blotter versus top of blotter or towel.

Person	RB	T/TB
1	3.87	3.12
2	4.72	3.97
3	4.13	3.38
4	4.90	3.73
5	5.13	4.38
6	4.55	3.82
7	5.24	3.73
8	5.67	4.69
9	4.28	3.46
10	4.13	3.21
Average	4.66	3.75

Table 3. Time comparison of ten individuals (with varying experience levels) planting tomato with raised blotter versus top of blotter or towel.

Person	RB	T/TB
1	3.62	2.90
2	4.57	3.82
3	4.38	3.41
4	3.43	2.97
5	4.40	3.75
6	4.50	3.80
7	5.00	4.47
8	5.38	4.70
9	4.57	3.85
10	4.25	3.20
Average	4.41	3.69

Respectfully submitted: Germination Uniformity Working Group: Sue Alvarez, Riad Baalbaki, Matthew Conway, Laura Donaldson, David Johnston, Sari Kopinksy, Kathy Mathiason, Raymond Shillito, Marija Topic, Bridget Westfall, and Heidi Jo Larson

Date Submitted: August 18, 2022

Proposal #8

TZ Uniformity Working Group

PURPOSE OF RULE PROPOSAL: The purpose of this proposal is to require the number of seeds tested during the Tetrazolium test to be reported on the report of analysis if less than the required 200 seeds.

PRESENT RULE:
l. When a stand-alone tetrazolium test is conducted the following information must be

reported under Tetrazolium Test:

(1) Percentage of viable seed as a whole number. (Refer to sec. 8.6a.)

(2) Percentage of hard seed, if applicable, as a whole number. (Refer to sec. 8.6a.)

PROPOSED RULE:

SECTION 15: REPORT OF ANALYSIS (ROA)

l. When a stand-alone tetrazolium test is conducted the following information must be

reported under Tetrazolium Test:

(1) Percentage of viable seed as a whole number. (Refer to sec. 8.6a.)

(2) Percentage of hard seed, if applicable, as a whole number. (Refer to sec. 8.6a.)

(3) Number of seeds tested if less than the required 200 seeds.

HARMONIZATION AND IMPACT STATEMENT:

Canada M&P and the FSA do not have any requirements for reporting TZ results.

ISTA only requires that the statement: Tetrazolium test: …..% of seeds were viable must be reported under other determinations. Any deviations from the procedures in Section 6A must also be identified.

SUPPORTING EVIDENCE: The rules require 200 seeds be tested for a TZ test. If testing less than the required 200 seeds the results may not be representative of the entire seed lot. The tolerance tables for comparing TZ tests do not exist for testing less than 200 seeds. If 200 seeds are not being tested this information needs to be known so the tolerance tables are not being used to determine if results are within or out of tolerance.

SUBMITTED BY:

The TZ Uniformity Working group: Michael Aberle, Heidi Arneson, Riad Baalbaki, Neal Foster, Terry Freeman, Hannah Gillen, Shaminder Miranpuri, Mike Stahr, Vic Vankus, Diandra Viner, Heidi Larson

Submitted: August 19, 2022

Proposal #9

Sue Alvarez, Linda Barbosa

Purpose of Proposal: To add Quinoa (*Chenopodium quinoa*) purity weights and germination methods to the AOSA Rules Volume 1.

Present Rule: New Rule

Proposed Rule:

Table 2A. Weights for working samples.

Pure Seed Unit #	Chaffy Seed [a]	Kind of seed	Minimum weight for purity analysis [b]	Minimum weight for noxious-weed seed or bulk examination	Approximate number of seeds per gram [c]	Approximate number of seeds per ounce [d]
			Grams	Grams	Number	Number
38	C	*Chenopodium quinoa* Willd. quinoa	7	70	367	10,805

Table 6A. Methods of testing for laboratory germination.

Kind of seed	Substrata	Temperature (°C)	First count (days)	Final count (days)	Specific requirements and notes	Dormant seed
Chenopodium quinoa quinoa	TB	20; 15	4	7		

Harmonization and Impact Statement:

In 2021 the testing procedures for *Chenopodium quinoa* were added to the ISTA Rules.

ISTA methods (effective 2022) are as follows:

Minimum weights – purity: 10 g; Other seeds: 100g

PSD 2 (achene)

Germination – TP, BP 20°C, 4 day first count, 7 day final count

This species is not included in the Canadian M&P or the Federal Seed Act.

Supporting Evidence:

Quinoa (*Chenopodium quinoa*) is an edible grain or pseudocereal which is increasing in use and therefore being tested in seed laboratories. ISTA added this species to their Rules in 2021. Analysts in the US were interested in adding this species to the AOSA Rules, and in 2021 a referee was conducted in order to gather data with the purpose of adding germination methods to Table 6A. The referee organizers were encouraged to compare **15°C** to **20°C** as part of this study, as some labs reported having success using the lower temperature. The referee design was approved by the co-chairs of the Germination subcommittee. Four lots of varying quality were sent to 12 laboratories, along with the germination media to be used (white blotters) in order to reduce a source of variability. Data from 11 labs completing the referee was compiled and analyzed. The following is a summary of the results:

Average germination results for quinoa from 11 laboratories:

Sample	Tested at 20°C	Tested at 15°C
1	45.23	47.52
2	47.75	49.98
3	71.82	72.00
4	59.14	55.41
Overall Average	**55.99%**	**56.23%**

The raw data was analyzed by Dr. Riad Baalbaki of the California Department of Agriculture and AOSA co-chair of the Germination subcommittee. – his analysis is included as supporting evidence. The main findings from the data analysis indicate that 1) Samples had a range in quality; 2) Using either 15 or 20C as test temperature had no effect on final germination percent when averaged over all other factors; 3) With a single exception (lab 9 sample 4), no lab produced significantly different results when germination was evaluated at 15 and 20C; and 4) The extent of variability among labs depended on the sample being tested and was not effected by temperature.

Referee results were presented at the 2022 AOSA/SCST meeting in Skokie, IL. This PowerPoint presentation is also included as supporting evidence.

In addition, seed count data was collected in order to add purity weights to Table 2A. Thirteen lots of quinoa seeds (seven commercial samples and six germplasm samples from the USDA) were obtained and sampled for seed count information. The following table is a summary of the seed count data:

Seed count data for quinoa:

Sample Number	Origin	**1000 seeds (g)**	**Number of seeds per gram**	**Number of seeds per ounce**
1	Pacific Grain	2.999	333	9,453
2	Pacific Grain	2.782	360	10,192
3	Pacific Grain	3.029	330	9,359
4	Pacific Grain	2.798	357	10,133
5	Country Creek Acres	2.420	413	11,716
6	Botanical Interests	2.952	339	9,602
7	Botanical Interests	3.072	326	9,230
8	Chile	1.960	510	14,464
9	Colorado, USA	2.373	421	11,946
10	Chile, Maule	2.660	376	10,659
11	Chile, Pichilemu	2.371	422	11,955
12	Chile, Faro	3.953	253	12,360
13	Chile	3.015	332	9,402
Average		**2.799**	**367**	**10,805**

Using this data, the average seed counts weights are as follows:

2,500 seeds = 6.998 grams

25,000 seeds = 69.98 grams

Minimum purity weights to add to the AOSA Rules should be 7 grams for purity and 70 grams for the noxious weed /bulk exam. (Note that this is somewhat lower than the weights added to the ISTA Rules in 2021.) The seed unit for this species is described as a utricle by Hortus Third (Bailey 1976), so the proper Pure Seed Unit for this species is PSU 38; the seed is chaffy.

Submitted by:

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Linda Barbosa, RST, Sakata Seed, Morgan Hill, CA lbarbosa@sakata.com

Date submitted: July 26, 2022

View Referee Results

View original Referee

View Raw Data

Proposal #10 - *Amended

Kathy Mathiason, Mike Stahr

PURPOSE OF PROPOSAL: **AMENDED**

The purpose of this proposal is to add statements regarding deviating from the Rules into all volumes of the AOSA Rules for Testing Seeds and into additional areas of Volume 1. Currently there is only one such statement, and it is in the Introduction of Volume 1. Principles and Procedures:

When individual samples appear to require special treatment resulting in deviations from the Rules, the following statement must be made in the remarks section of the report of analysis: "(*insert name of test*) test was not conducted in accordance with the AOSA Rules for Testing Seeds." This statement must then be followed by a citation of the AOSA rule and a description/explanation of the deviation. The allowance for deviation should not be construed as an authorization to indiscriminately conduct and report testing not in accordance with these rules.

PRESENT RULE

AOSA Rules Volume 1 Section 6.2 k.:

Note: In all cases % total viable and % pure seed used to calculate the % pure live seed must be from the same working sample. The correct procedure according to the AOSA Rules for Testing Seeds is to complete the purity analysis, germinate the pure seed, and determine the % of hard and/or % dormant seed, as applicable according to methods in Table 6A, at the conclusion of the germination test.

AOSA Rules Volume 1 Section 6.9 e.:

e. **Specific requirements, dormant seed.** — The 'Specific requirements and notes' column lists additional requirements prescribed for certain species. These requirements, including dormancy breaking measures, shall be applied to all tested lots, regardless of origin or condition. In contrast, the 'Dormant seed' column provides supplemental measures applicable only under certain conditions, namely when testing recently harvested lots or those with observed or expected dormancy. To illustrate, consider the two species, *Abies grandis* and *Agrostis capillaris*. According to Table 6A, prechilling is an integral part of the germination test for *Abies grandis*, applied to every lot. In contrast, there is no prechilling requirement for *Agrostis capillaris*. However, in the special case of recently harvested *Agrostis capillaris* lots, or when dormancy is observed during the germination test, prechilling is prescribed as a dormancy-breaking measure. When 6.9 p is opted to test a species which could have dormancy, the use of the dormancy breaking methodologies with the exception of light and alternating temperatures shall not be required.

AOSA Rules Volume 1 Section 6.9 m.:

m. **Viability testing of ungerminated seed.** — Any of the following methods or combination of methods, unless otherwise specified, may be used to determine the viability of ungerminated seed that remain at the end of the prescribed test period. The results are to be reported as percentage dormant or hard seed as determined by the specified method.

AOSA Rules Volume 1 Section 8: currently no statement

AOSA Rules Volume 1 Section 15: m.:

m. Any report of analysis containing tests not conducted in accordance with the AOSA Rules, when such rule exists, must contain this statement in the remarks section of the report: "(*Insert name of test*) test was not conducted in accordance with the AOSA Rules for Testing Seeds." This statement must then be followed by a citation of the AOSA rule and a description/explanation of the deviation.

AOSA Rules Volume 2: currently no statement

AOSA Rules Volume 3: currently no statement

AOSA Rules Volume 4 Part 1. Section 1: Introduction

Laboratory test results are used as a basis for buying and selling seed, for the labelling of seed as required by federal and state laws, and for monitoring the labelling to ensure it is current and truthful. Since it is essential that accurate test information be provided, all commercial and official seed testing laboratories must follow accepted rules for testing. These rules must be fully explained and standardized.

PROPOSED RULE

AOSA Rules Volume 1 Section 6.2 k.:

Note: In all cases % total viable and % pure seed used to calculate the % pure live seed must be from the same working sample. The correct procedure according to the AOSA Rules for Testing Seeds is to complete the purity analysis, germinate the pure seed, and determine the % of hard and/or % dormant seed, as applicable according to methods in Table 6A, at the conclusion of the germination test. When individual samples appear to require special treatment resulting in a deviation from this procedure, a statement must be made in the remarks section of the report of analysis. The allowance for deviation should not be construed as an authorization to indiscriminately conduct and report testing not in accordance with the Rules.

AOSA Rules Volume 1 Section 6.9 e.:

e. **Specific requirements, dormant seed.** — The 'Specific requirements and notes' column lists additional requirements . . . recently harvested *Agrostis capillaris* lots, or when dormancy is observed during the germination test, prechilling is prescribed as a dormancy-breaking measure. When individual samples appear to require special treatment resulting in a deviation from these procedures, a statement must be made in the remarks section of the report of analysis. The allowance for deviation should not be construed as an authorization to indiscriminately conduct and report testing not in accordance with the Rules. When 6.9 p is opted to test a species which could have dormancy, the use of the dormancy breaking methodologies with the exception of light and alternating temperatures shall not be required.

AOSA Rules Volume 1 Section 6.9 m.:

m. **Viability testing of ungerminated seed.** — Any of the following methods or combination of methods, unless otherwise specified, may be used to determine the viability of ungerminated seed that remain at the end of the prescribed test period. The results are to be reported as percentage dormant or hard seed as determined by the specified method. When individual samples appear to require special treatment resulting in a deviation from these procedures, a statement must be made in the remarks section of the report of analysis. The allowance for deviation should not be construed as an authorization to indiscriminately conduct and report testing not in accordance with the Rules.

AOSA Rules Volume 1 Section 8:

8.7 Deviations

When individual samples appear to require special treatment resulting in a deviation from the Rules, a statement must be made in the remarks section of the report of analysis. The allowance for deviation should not be construed as an authorization to indiscriminately conduct and report testing not in accordance with the Rules.

AOSA Rules Volume 1 Section 15: m.:

m. Any report of analysis containing tests not conducted in accordance with the AOSA Rules, when such rule exists, must contain this statement in the remarks section of the report: "(*Insert name of test*) test was not conducted in accordance with the AOSA Rules for Testing Seeds." This statement must then be followed by a citation of the AOSA rule and a description/explanation of the deviation. The allowance for deviation should not be construed as an authorization to indiscriminately conduct and report testing not in accordance with the Rules.

AOSA Rules Volume 2 Section 1: at the end of Introduction:

When individual samples appear to require special treatment resulting in a deviation from the Rules, a statement must be made in the remarks section of the report of analysis. The allowance for deviation should not be construed as an authorization to indiscriminately conduct and report testing not in accordance with the Rules.

AOSA Rules Volume 3 at the end of Introduction:
When individual samples appear to require special treatment resulting in a deviation from the Rules, a statement must be made in the remarks section of the report of analysis. The allowance for deviation should not be construed as an authorization to indiscriminately conduct and report testing not in accordance with the Rules.

AOSA Rules Volume 4 Part 1. Section 1: Introduction:
Laboratory test results are used as a basis for buying and selling seed, for the labelling of seed as required by federal and state laws, and for monitoring the labelling to ensure it is current and truthful. Since it is essential that accurate test information be provided, all commercial and official seed testing laboratories must follow accepted rules for testing. These rules must be fully explained and standardized.
When individual samples appear to require special treatment resulting in a deviation from the Rules, a statement must be made in the remarks section of the report of analysis. The allowance for deviation should not be construed as an authorization to indiscriminately conduct and report testing not in accordance with the Rules.

HARMONIZATION AND IMPACT STATEMENT
The Federal Seed Act, the Canadian M&P, and the ISTA Rules do not contain statements regarding deviating from the rules, remarking about deviations on the report of analysis, and indiscriminately repeating the deviation. The impact of placing the proposed statements in multiple sections of the AOSA Rules is increased recognition of the importance of consistently following the Rules.

SUPPORTING EVIDENCE
NA

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DATE SUBMITTED
October 14, 2022
DATE AMENDED
January 5, 2023

Proposal #11

David Johnston

1. PURPOSE OF PROPOSAL:

The primary purpose of this proposal is to clarify that the mechanical seed counting process outlined in AOSA Rules Vol. 1 Section 12, may be used to determine the number of seeds contained in a sample of additional crop kinds not listed. The mechanical seed counter must be proven it is fit for purpose for seed kinds not listed, by using a 1,000 seed calibration sample of the seed kind under consideration.

2. PRESENT RULE:

SECTION 12: MECHANICAL SEED COUNT

The following method shall be employed when using a mechanical seed counter to determine the number of seeds contained in a sample of soybean (Glycine max), corn (Zea mays), wheat (Triticum aestivum) field bean (Phaseolus vulgaris.

12.1 Samples

Samples for testing shall be of at least 500 grams for soybean, corn and field beans, and 100 grams for wheat and received in moisture proof containers. Samples shall be retained in moisture proof containers until the weight of the sample prepared for purity analysis is recorded.

12.2 Seed counter calibration

…

(b) Carefully pour the 1,000 seed calibration sample into the seed counter. Start the counter and run it until all the seeds have been counted. The seeds should not touch as they run through the counter. Record the number of seeds as displayed on the counter read out. The seed count should not vary more than ±2 seeds from 1,000. If the count is not within this tolerance, clean the mirrors, adjust the feed rate and/or reading sensitivity. Rerun the calibration sample until it is within the ±2 seed tolerance. If the seed counter continues to fail the calibration procedure and the calibration sample has been checked to ensure that it contains 1,000 seeds, do not use the counter until it has been repaired.

3. PROPOSED RULE:

SECTION 12: MECHANICAL SEED COUNT

The following method shall be employed when using a mechanical seed counter to determine the number of seeds contained in a sample of soybean (Glycine max), corn (Zea mays), wheat (Triticum aestivum), field bean (Phaseolus vulgaris) and other seed kinds. CAUTION: A mechanical seed counter may not be appropriate to use for counting all seed kinds.

12.1 Samples

Samples for testing shall be of at least 500 grams for soybean, corn, and field beans, and 100 grams for wheat. The sample weight for other seed kinds being tested shall be the weight of the purity exam listed in AOSA Rules Volume 1 Table 2A. ~~and~~ All samples shall be received in moisture proof containers. Samples shall be retained in moisture proof containers until the weight of the sample prepared for purity analysis is recorded.

12.2 Seed counter calibration

…

(b) Carefully pour the 1,000 seed calibration sample into the seed counter. Start the counter and run it until all the seeds have been counted. The seeds should not touch as they run through the counter. Record the number of seeds as displayed on the counter read out. The seed count should not vary more than ±2 seeds from 1,000. If the count is not within this tolerance, clean the mirrors, adjust the feed rate and/or reading sensitivity. Rerun the calibration sample until it is within the ±2 seed tolerance. If the seed counter continues to fail the calibration procedure and the calibration sample has been checked to ensure that it contains 1,000 seeds, do not use the counter until it has been repaired~~.~~ and then verified using the 1,000 seed calibration sample.

CAUTION: If the 1,000 seed calibration sample for a non-listed seed kind being counted always varies more than the permitted ±2 seeds from 1,000, then the use of the mechanical seed counter is not appropriate for that seed kind and must not be used for counting.

4. HARMONIZATION AND IMPACT STATEMENT: (ISTA/FSA/Canadian Methods & Procedures)

N/A

5. SUPPORTING EVIDENCE:

The author of this proposal has tested rice, cotton, and hemp seed following the procedures outlined in AOSA Rules Volume 1 Section 12. The sample weight for the seed kinds tested was the weight of the purity exam listed in AOSA Rules Volume 1 Table 2A. A 1,000 seed calibration sample was created for each seed kind tested [ref. section 12.2(a)]. The calibration samples were used to successfully determine the proper settings for the mechanical seed counter for each seed kind and meet the required ±2 seeds from 1,000 [ref. section 12.2(b)].

6. SUBMITTED BY:

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7. DATE SUBMITTED:

July 14, 2022

View Supporting Evidence

Proposal #12

David Johnston, Riad Baalbaki

1. PURPOSE OF PROPOSAL:

The purpose of this proposal is to revise reporting requirements of germination test results on the Report of Analysis (ROA). For the germination test, current Rules state that the percentage of normal seedlings, hard seed (if applicable), and dormant seed (if applicable) must be reported (AOSA Rules Vol. 1 Section 15.k). For non-applicable components, N/A is included on the report. This proposal adds the requirement of including the percentage abnormal seedlings and dead seeds to the ROA, so that all applicable germination components are reported.

Reporting all germination test components is recommended for transparency and ease of interpretation by ROA recipients. Excluding both abnormal seedling and dead seed results can lead to misinterpretation of the germination test results, frequently obscuring important information to seed producers and consumers. Revising reporting requirements is necessary for a complete picture of germination behavior of the seed lot, improving germination test reporting uniformity among labs, and enhancing test transparency to the Seed Industry and the Seed Testing Industry.

2. PRESENT RULE:

SECTION 15: REPORT OF ANALYSIS (ROA)

Laboratory reports of analysis that indicate laboratory testing was performed in accordance to the AOSA Rules for Testing Seeds are required to include, but not be limited to, the following information:

…

k. When a germination test is conducted the following information must be reported under Germination Test:

(1) Percentage of normal seedlings as a whole number (refer to section 6.7).

(2) Percentage of hard seed, if applicable, as a whole number (refer to section 6.7).

(3) Percentage of dormant seeds, if applicable, as a whole number (refer to section 6.7).

…

3. PROPOSED RULE[1]:

SECTION 15: REPORT OF ANALYSIS (ROA)…

k. When a germination test is conducted the following information must be reported under Germination Test:

(1) Percentage of normal seedlings as a whole number (refer to section 6.7).
(2) Percentage of hard seeds, if applicable, as a whole number (refer to section 6.7).
(3) Percentage of dormant seeds, if applicable, as a whole number (refer to section 6.7).
(4) Percentage of abnormal seedlings as a whole number (refer to section 6.7).
(5) Percentage of dead seeds as a whole number (refer to section 6.7).

…

4. HARMONIZATION AND IMPACT STATEMENT:

A. If adopted, this proposal increases harmonization with ISTA:

"ISTA Rules Section 5. The Germination Test; 5.9 Reporting results:
The result of a germination test must be reported in the spaces provided as follows:
…
· the percentages, calculated to the nearest whole number (5.8.2), of normal seedlings, hard seeds, fresh seeds, abnormal seedlings and dead seeds. If the result for any of these categories is found to be zero, it must be reported as '0'.
…"

B. If adopted, this proposal may increase harmonization with Canadian Methods & Procedures (M&P). Although M&P rules do not include a reporting requirement for percentage abnormal seedlings, M&P rules include a requirement that reported percentages must equal 100%:

"**Canadian Methods & Procedures section 4.0 Germination; 4.11 Calculation and reporting of germination results; 4.11.5 Reporting of results:**

c. The germination result, as a percentage germination or for kinds listed in Section 4.10.7, percent germination plus hard seeds calculated to the nearest whole number (0.5 is taken to the higher figure) except for values between 99.5% and 99.9% which should be dropped to 99%. The sum of the percentages reported must be 100. (See Section 4.10.7, 4.11.1, 4.11.2, 4.11.3 and 4.11.4.a)."

C. Officially, if adopted, this proposal will not improve harmonization with the Federal Seed Act; reporting requirements already differ between FSA and AOSA Rules. But as a matter of current practice, the USDA SRTD Seed Laboratory routinely reports the percentage of abnormal seedlings and the percentage of dead seeds on their ROA voluntarily.

[1] Sub-section numbering and sequence may change if other proposed ROA requirements are adopted.

5. SUPPORTING EVIDENCE:

Incomplete reporting of germination test results can lead to inappropriate assessments and interpretation of germination performance of the seed lot, and sound decisions based on results may not be possible. While percentage normal seedlings results convey all necessary information for high-germination seed lots (95-100%), the same is not true for seed lots with lower germination. The examples below demonstrate only some consequences of incomplete reporting, i.e., when neither abnormal nor dead results are included.

Editor's note: Evidence table on next page

		Germination component (%)				
		Normal	**Hard**	**Dormant**	**Abnormal**	**Dead**
Example 1 Different versions of ROA	Incomplete results	82	2	N/A	-	-
	Complete results	82	2	N/A	15	1
	Acceptable results†	82	2	N/A	-	1
Comments	Sample tested following seed treatment. In most cases, improper seed treatment produces abnormal rather than dead seeds. Recipient of incomplete ROA would not have enough information to suggest possible effects of seed treatment.					
Example 2 Different versions of ROA	Incomplete results	82	N/A	N/A	-	-
	Complete results	82	N/A	N/A	2	16
	Acceptable results	82	N/A	N/A	2	-
Comments	Same germination percentage as Example 1, but tests on an inventory sample (2-3 years old) after seed treatment. Incomplete ROA does not allow the recipient to judge whether the decrease in germination was more likely due to age (usually manifested as a high number of dead seeds) or seed treatment.					
Example 4 Different versions of ROA	Incomplete results	99	N/A	N/A	-	-
	Complete results	99	N/A	N/A	0	1
	Acceptable results	99	N/A	N/A	-	1
Comments	High germination sample; both complete and incomplete ROA convey the same information.					
Example 3 Same sample tested in 2 labs	Lab 1	84	N/A	2	-	-
	Lab 2	94	N/A	1	5	0
Comments	Incomplete ROA from Lab 1 indicated 84% germination; sample retested in Lab 2 resulted in 94% germination and 5% abnormals. The 10% germination difference between labs could be due to either abnormal, dead or both results. Because ROA of Lab I is incomplete, determining which germination components differ and possible reasons for lack of uniformity are not possible.					

†Acceptable: one component (abnormal or dead) is not reported but can be determined by subtraction.

6. **SUBMITTED BY:**

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7. **DATE SUBMITTED:**

October 14, 2022

Proposal #13

Deborah Meyer, Nishit Patel

Purpose: To correct the common name for *Calamagrostis arenaria* (L.) Roth, formerly known as *Ammophila arenaria* (L.) Link, in Volume 3 of the AOSA Rules.

Current Rule:

Volume 3. Uniform Classification of Weed and Crop Seeds (if adopted, changes will be made to all sections of Vol. 3)

Nomen #	Scientific name	Common name	Family	Spp class	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
497334	*Calamagrostis arenaria* (L.) Roth	beachgrass	Poaceae	W	W	W	W	W	W	W	W

Proposed Rule:

Volume 3. Uniform Classification of Weed and Crop Seeds (if adopted, changes will be made to all sections of Vol. 3)

Nomen #	Scientific name	Common name	Family	Spp class	CONTAMINATING CLASSIFICATION						
					A	F	H	R	S	T	V
497334	*Calamagrostis arenaria* (L.) Roth	European beachgrass; marram grass	Poaceae	W	W	W	W	W	W	W	W

Harmonization Statement

Calamagrostis arenaria (L.) Roth [*Ammophila arenaria* (L.) Link] is not listed in the Federal Seed Act, ISTA Rules, or CFIA M&P. This species is found in the Flora of North America (Barkworth et al. 2007) and in the GRIN database (USDA-ARS-NPGS 2022) where the two English common names recognized in both these references are European beachgrass and marram grass. This species is referred to as European beachgrass by the California Invasive Plant Council website (CAL-IPC 2022) and the Jepson Manual (Baldwin et al. 2012).

Supporting Evidence

Calamagrostis arenaria [*Ammophila arenaria*] is a native European species that has become naturalized along the Pacific coast (California to British Columbia) and the interior of western North America where it was introduced as a sand binder. It is also reported to occur in a sand dune located in Erie County, Pennsylvania (Barkworth et al. 2007). The species has been introduced and naturalized in other temperate regions of the world (Barkworth et al. 2007). In some areas it is considered an invasive species as it tends to out compete native species and interferes with native sand dune ecology (CABI 2022; CAL-IPC 2022). This species is commonly referred to by the English common names of European beachgrass and marram grass (Barkworth et al. 2007; Baldwin et al. 2012; CABI 2022; CAL-IPC 2022; Florabase 2022; USDA-ARS-NPGS 2022).

References

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CAL-IPC. 2022. *Ammophila arenaria* https://www.cal-ipc.org/plants/profile/ammophila-arenaria-profile/ [Accessed 10 October 2022]

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Submitted by: Deborah J. Lionakis Meyer and Nishit Patel, AOSA-SCST Purity Subcommittee.

Date Submitted: October 11, 2022

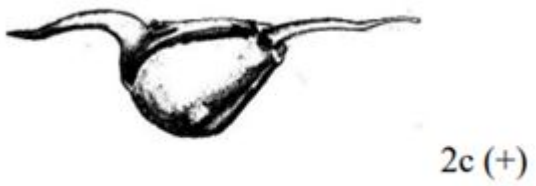
Proposal #14

David Johnston, Riad Baalbaki

1. **PURPOSE OF PROPOSAL:**

The purpose of this proposal is to improve seedling drawing 2c in POACEAE GRASS FAMILY III - Corn section in AOSA Rules Volume 4. In the current drawing, the coleoptile is not properly depicted and has raised questions from analysts. The description and evaluation of drawing 2c we not changed.

2. **PRESENT RULE:**



2c (+)

2c. Shoot and root the length of the kernel.

3. **PROPOSED RULE:**

2c (+)

2c. Shoot and root the length of the kernel.

4. HARMONIZATION AND IMPACT STATEMENT: (ISTA/FSA/Canadian Methods & Procedures)

N/A

5. SUPPORTING EVIDENCE:

With the current drawing in AOSA Rules Volume 4, the coleoptile is not very well depicted. This issue has brought questions and concerns from AOSA and SCST analysts to the Co-Chairs of the Germination and Dormancy Subcommittee. To assist with addressing these concerns, the coleoptile has been amended to better depict an appropriate coleoptile for this type of seedling.

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7. **DATE SUBMITTED:**

August 8, 2022

Proposal #15

David Johnston and Riad Baalbaki

1. PURPOSE OF PROPOSAL:

The primary purpose of this proposal is to revise AOSA Rules Volume 4 ASTERACEAE, SUNFLOWER FAMILY I – Lettuce Fig. 3 'Physiological necrosis of lettuce cotyledons' drawings, so that they reflect the recent changes of evaluation criteria described in AOSA Rules Volume 4 under Part I SECTION 3 section 3.5.10 (see excerpt below), and to add additional drawings for necrosis evaluation clarification.

"3.5.10 Decay at the point of attachment of the cotyledons and terminal bud decay. Seedlings exhibiting decay at the point of attachment of the cotyledons to the seedling and/or decay (that was not caused by test conditions) in and around the terminal bud, causes the seedling to be classified as abnormal. The 50% Rule (see section 3.5.8) does not apply when either of these conditions is present."

Also, the recommendation to use magnification and light to evaluate seedlings exhibiting necrosis to assist with more precise necrosis evaluations has been proposed.

2. PRESENT RULE:

…

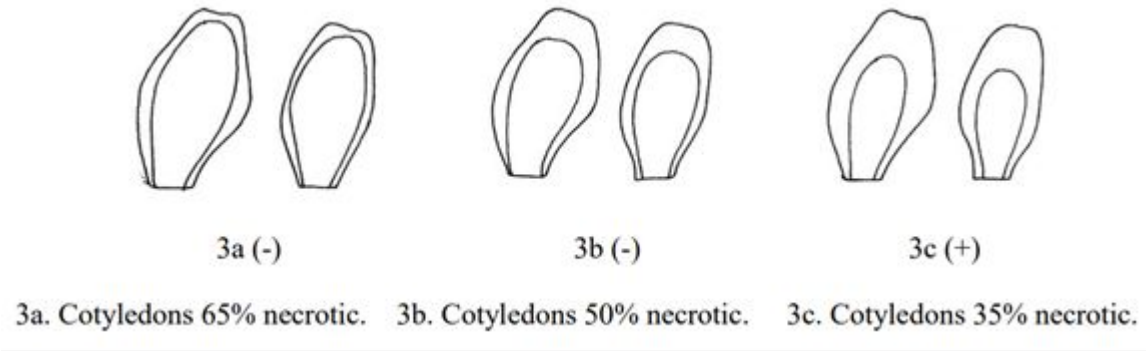
NOTES

…

8. The 50% rule must be followed to classify seedlings with damaged cotyledons (dark areas of discoloration or decay) as either normal or abnormal.

…

Fig. 3 Physiological necrosis of lettuce cotyledons.



3a. Cotyledons 65% necrotic. 3b. Cotyledons 50% necrotic. 3c. Cotyledons 35% necrotic.

3. PROPOSED RULE:

…

NOTES

…

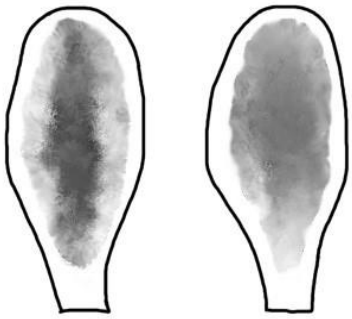
8. The 50% rule must be followed to classify seedlings with damaged cotyledons (dark areas of discoloration or decay) as either normal or abnormal.

9. Necrosis at point of attachment of a cotyledon causes that cotyledon to be classified as completely defective. A seedling is classified as abnormal if a) both cotyledons show necrosis at point of attachment, or b) one cotyledon shows necrosis at point of attachment and more than 50% of total cotyledonary tissue is defective.

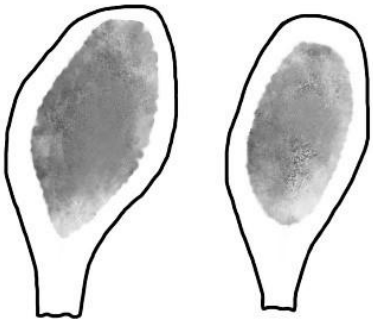
10. The use of light and moderate magnification (e.g., 2X, 3X) are highly recommended to make the evaluation of seedlings exhibiting necrosis less difficult and more accurate.

…

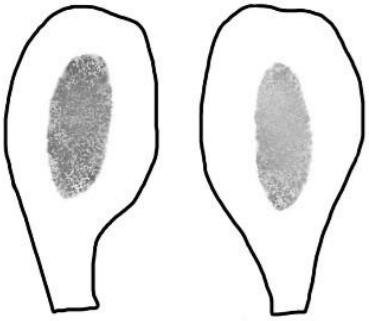
Fig. 3 Physiological necrosis of lettuce cotyledons.



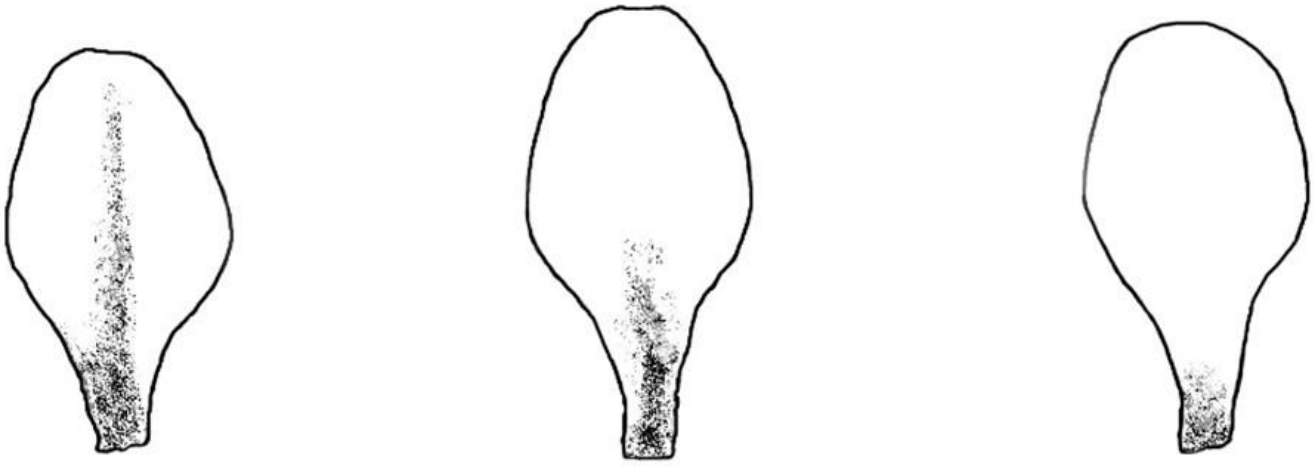
3a (-)
3a. Cotyledons ≈60% necrotic



3b (+)
3b. Cotyledons ≈48% necrotic



3c (+)
3c. Cotyledons ≈20% necrotic



3d (-) 3e (-) 3f (-)

3d-f Necrosis at point of cotyledon attachment

4. HARMONIZATION AND IMPACT STATEMENT: (ISTA/FSA/Canadian Methods & Procedures)

This proposal validates the previous change relating to decay at the point of attachment, which harmonized AOSA Rules evaluation criteria with those in the ISTA Rules. The proposed change will improve uniformity and consistency of evaluations among analysts by using drawings to assist analysts with correctly evaluating lettuce cotyledons based on the degree and pattern of necrosis, which was had not been previously illustrated.

5. SUPPORTING EVIDENCE:

The current Fig. 3 drawings were not updated when evaluation criteria specific to point of attachment of cotyledons to hypocotyls was updated. The current Fig. 3 drawings do not show complete lettuce cotyledons, especially the point of attachment to the hypocotyl, a critical evaluation area.

Therefore, the current figures do not illustrate how patterns of necrosis, particularly when the necrosis is at the point of attachment, should be evaluated. Revised Fig. 3 a-c drawings basically illustrate the same concept as those used in the current figure but add the 'missing' part of each cotyledon. New additional Fig. 3 d-f, as well as Note 9, illustrate proper evaluation of necrosis at the point of attachment, even when less than 50% of the cotyledon is affected. These changes are necessary to align Fig. 3 with the revised evaluation criteria previously adopted.

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7. **DATE SUBMITTED:**

October 5, 2022

Proposal #16

David Johnston, Riad Baalbaki

1. PURPOSE OF PROPOSAL:

Improve uniformity of evaluating cotyledons of lettuce seedlings with various degrees and patterns of necrotic tissue damage. Color pictorial examples, with a range of symptoms, are proposed as supplementary material to the 'ASTERACEAE, SUNFLOWER FAMILY I – Lettuce' section of Vol. 4 of the AOSA Rules. Black and white seedling drawings depicting necrosis are inadequate due to their lack of coloration and detail. No changes in evaluation rules are proposed.

2. PRESENT RULE:

None. Currently there are no photos in AOSA Rules Vol. 4

3. PROPOSED RULE:

Proposed supplementary material for addition to the 'ASTERACEAE, SUNFLOWER FAMILY I – Lettuce' section

Supplemental Evaluation Guide

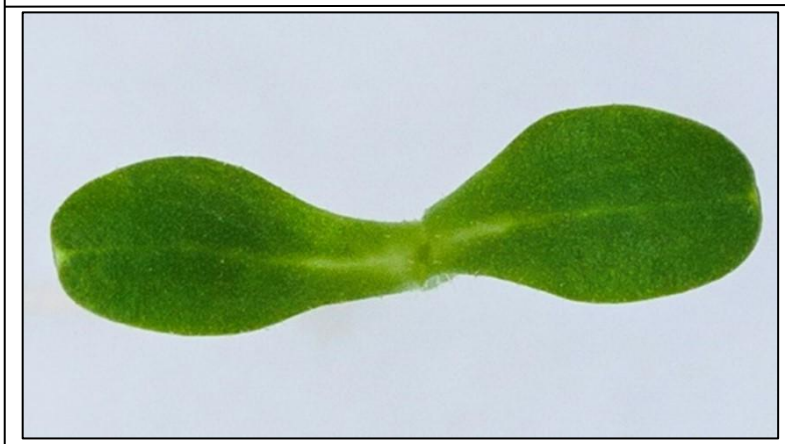
	**Figure 1** **Normal** **Healthy cotyledons. Lighter coloration is normal for lettuce at point of attachment to hypocotyl. Intact epicotyl**
	**Figure 2** **Normal** **Lighter areas (sometimes tissue appears transparent) at the point of attachment to hypocotyl and cotyledon tps are normal for lettuce.**

Figure 3

Normal

MInor necrosis on one cotyledon. Apparent blotchiness on surface is outer epidermal cells of cotyledons.

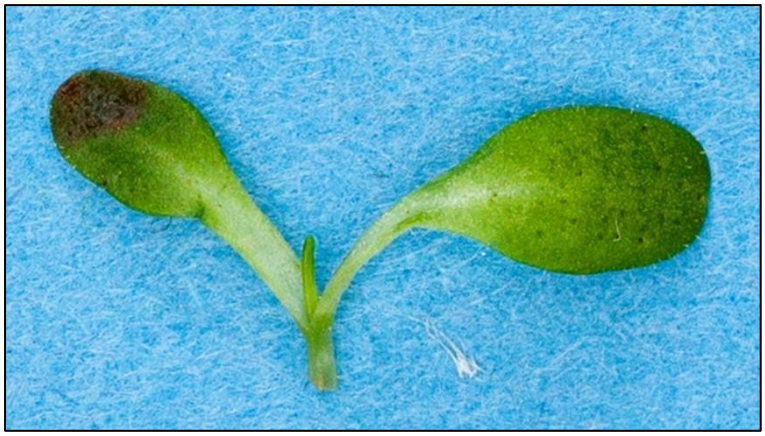


Figure 4

Normal

Necrosis or decay affects less than 50% of cotyledon tissue. No necrosis at point of attachment to hypocotyl. Healthy epicotyl.

Figure 5

Normal

Physical necrosis affecting less than 50% of cotyledon tissue; no necrosis at point of attachment to hypocotyl.

Figure 6

Abnormal

Necrosis at the point of cotyledon attachment to the hypocotyl.

Figure 7

Abnormal

Severe necrosis at the point of cotyledon attachment to the hypocotyl as well as other pre-necrotic (light green or brown) cotyledonary tissue.

Figure 8

Abnormal

Necrosis or decay at point of attachment, extending into the hypocotyl and the epicotyl.

Figure 9

Abnormal

Necrosis at the point of cotyledon attachment to the hypocotyl. Same seedling is depicted from two angles.

Figure 10

Abnormal

Necrosis at the point of cotyledon attachment to the hypocotyl and extending to the rest of the cotyledons.

Figure 11

Abnormal

Necrosis affecting more than 50% of cotyledonary tissue. Light-green tissue surrounding dark necrotic area is an example of early stages of necrosis.

Figure 12

Abnormal

Necrosis and pre-necrosis affecting most cotyledonary tissue. 'Transparent' tissue of the cotyledon-hypocotyl juncture is normal for lettuce.

Figure 13

Normal

Physical necrosis, usually due to insect or mechanical damage. Epicotyl is healthy. 'Transparent' tissue at the hypocotyl juncture is normal.

Figure 14

Normal

Example of physical rather than physiological necrosis due to insect or mechanical damage, affecting less than 50% of cotyledon tissue.

	**Figure 15** **Normal** **Another example of physical necrosis due to insect or mechanical damage, affecting less than 50% of cotyledon tissue.**
	**Figure 16** **Abnormal** **Decayed epicotyl. Without the aid of magnification epicotyl deterioration might not be apparent. Healthy cotyledons with normal semi-transparent tissue at the hypocotyl juncture- normal for lettuce.**

4. HARMONIZATION AND IMPACT STATEMENT:

No changes in evaluation rules or criteria are proposed, so this proposal has no impact on harmonization. However, the pictorial illustrations enhance uniformity among AOSA, ISTA, and Canadian M&P by demonstrating similarities in evaluating necrosis at the cotyledon-hypocotyl juncture.

5. SUPPORTING EVIDENCE:

Authors reviewed three lettuce referee results which, to our knowledge, were the only lettuce referees in the past 10 years (attached). Review results indicated that necrosis evaluation remains a major area of concern and lack of uniformity. Some referee responses specific to necrosis sometimes diverged by more than 40% among respondents. The reviewed referees were:

· 2013 Virtual Lettuce Referee; Seedling Evaluation Handbook Committee (henceforth referred to as VLR)

· 2016 Southwest Region Referee (henceforth referred to as SWRR)

· 2022 CFIA Referee Study on Lettuce Seedling Evaluation (henceforth referred to as CFIA).

Our review identified four common themes:

1. General agreement when evaluating obvious cases of either intact or severely defective seedlings. Examples include VLR images 2, 4 and 5; and CFIA images 2, 6, 7 and 8.

2. A moderate to high degree on variation among analysts when evaluating necrosis at the point of attachment of cotyledons to the hypocotyl. Examples include VLR images 1 and 25; SWRR images 2, 5 and 9; and CFIA images 9, 12 and 13. In the case of CFIA images 9 and 12, it was apparent that many AOSA analysts do not consider necrosis at point of attachment as an abnormality if less than 50% of the cotyledonary tissue is defective,

3. Difficulty in sometimes distinguishing pre-necrotic tissue, usually apparent around darker necrotic areas, from light (low chlorophyl) coloration of normal tissue, which in the case of lettuce is highly dependent on test conditions. Examples include VLR images 18 and 28; SWRR images 2, 4, 7 and 10; and CFIA images 1, 5 11 and 14.

4. It was not possible to draw any reliable conclusions regarding pigmentation evaluations, but it seems likely that pigmentation differences influenced cotyledon evaluations. In most cases, this tissue appears lighter in color or transparent (lacks pigmentation), which is a normal condition for lettuce and is light- and variety-dependent. Based on some referee results, this lack or reduced pigmentation could have been regarded as a defect. Examples include VLR images 3, 15, 18 and 26.

The proposed supplementary material provides examples and descriptions of cotyledon evaluations to address and clarify the above issues. No modifications to evaluation criteria are proposed.

NOTE: THIS PROPOSAL IS CONTINGENT ON PROPOSAL #15 PASSING

6. SUBMITTED BY:

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7. DATE SUBMITTED: October 14, 2022

View Supporting Evidence

Proposal #17

David Johnston and Riad Baalbaki

1. **PURPOSE OF PROPOSAL:**

The purpose of this proposal is to list organic growing media substrate in the appropriate locations within AOSA Rules Vol. 4, where sand, soil, and/or sand/soil are also listed. Organic growing media is considered equivalent to soil, sand, and a soil/sand as an approved substrate. This proposal seeks to harmonize the references and uses of organic growing media in AOSA Rules Vol. 4 with those currently listed in AOSA Rules Vol. 1 (e.g., 6.5.b.(1), 6.6.d., 6.8.l.(3)).

2. **PRESENT RULE/PROPOSED RULE:**
(*Proposed changes listed as "a – m"; Present Rule listed first with Proposed Rule listed underneath with proposed changes in red text.*)

a) 3.4 Causes of seedling abnormalities
"…it is advisable to retest the seed in sand, soil or a sand/soil mixture."

3.4 Causes of seedling abnormalities
"…it is advisable to retest the seed in sand, soil, ~~or a~~ sand/soil mixture or organic growing media."

b) 3.4.6 Chemical treatment injury.
"Retesting in sand or soil is recommended when damage due to chemical exposure is suspected."

3.4.6 Chemical treatment injury.
"Retesting in sand, ~~or~~ soil or organic growing media is recommended when damage due to chemical exposure is suspected."

c) 3.4.8 Pathogenic infections.
"Retests in sand or soil are recommended when evaluation of seedlings is difficult."

3.4.8 Pathogenic infections.
"Retests in sand, ~~or~~ soil or organic growing media are recommended when evaluation of seedlings is difficult."

d) 3.5.2 Seedling response to moisture, temperature light
a. Moisture
…
"For information on preparation of sand and soil for use as germination substrates,…"

3.5.2 Seedling response to moisture, temperature light
a. Moisture
…
"For information on preparation of sand, ~~and~~ soil and organic growing media for use as germination substrates,…"

e) 3.5.3 Counting seedlings of multiple seed units and coated seeds.

"…a retest should be conducted in sand or soil…."

3.5.3 Counting seedlings of multiple seed units and coated seeds.

"…a retest should be conducted in sand, ~~or~~ soil or organic growing media…."

f) 3.5.5 Diseased and decayed seedlings.

"Retesting in sand or soil will usually reduce the level of secondary infection."

3.5.5 Diseased and decayed seedlings.

"Retesting in sand, ~~or~~ soil or organic growing media will usually reduce the level of secondary infection."

g) 3.5.6 Negative geotropism.

"…the sample should be retested under favorable conditions, including retests made in sand or soil."

3.5.6 Negative geotropism.

"…the sample should be retested under favorable conditions, including retests made in sand, ~~or~~ soil or organic growing media."

h) 3.5.7 "Use of sand or soil. Sand, soil or a sand/soil mixture should be used in a retest whenever difficulty is experienced in judging essential seedling structures…"

…

"b. Sand and soil are less favorable environments for the growth of saprophytic fungi…"

…

"d. The ability of roots to anchor seedlings in sand or soil makes it possible to grow…"

"Germination analysts should become familiar with the appearance of seedlings of all species when grown in sand or soil so they can evaluate seedlings correctly when they are grown on artificial substrata. Simultaneous tests on artificial substrata and in sand or soil are particularly helpful."

3.5.7 "Use of sand, ~~or~~ soil or organic growing media. Sand, soil, ~~or a~~ sand/soil mixture or organic growing media should be used in a retest whenever difficulty is experienced in judging essential seedling structures…"

…

"b. Sand, ~~and~~ soil and organic growing media are less favorable environments for the growth of saprophytic fungi…"

…

"d. The ability of roots to anchor seedlings in sand, ~~or~~ soil or organic growing media makes it possible to grow…"
"Germination analysts should become familiar with the appearance of seedlings of all species when grown in sand, ~~or~~ soil or organic growing media so they can evaluate seedlings correctly when they are grown on artificial substrata. Simultaneous tests on artificial substrata and in sand, ~~or~~ soil or organic growing media are particularly helpful."

i) CUCURBITACEAE, CUCURBIT FAMILY (page 42)
…
NOTES
…
2. "Samples should be retested in sand or soil if there is evidence of chemical injury…"

CUCURBITACEAE, CUCURBIT FAMILY (page 42)
…
NOTES
…
2. "Samples should be retested in sand, ~~or~~ soil or organic growing media if there is evidence of chemical injury…"

j) FABACEAE, LEGUME FAMILY I – (page 47)
…
NOTES
…
8. "…Retests, preferably in soil or sand, will aid in interpretation of such seedlings."

FABACEAE, LEGUME FAMILY I – (page 47)
…
NOTES
…
8. "…Retests, preferably in soil, ~~or~~ sand or organic growing media, will aid in interpretation of such seedlings."

k) FABACEAE, LEGUME FAMILY II – (page 53-54)

…

NOTES

…

3. "Secondary infection is common in towel and blotter tests. "…A retest in sand or soil may be advisable."

4. "If a few seedlings with a partial decay of the epicotyl are found, …A retest, preferably in soil or sand, will aid in interpretation of such seedlings."

5. "Hypocotyl development is slow until the roots start functioning and reach sufficient size; …A retest, preferably in soil or sand, will aid in interpretation of such seedlings."

FABACEAE, LEGUME FAMILY II – (page 53-54)
…
NOTES
…
3. "Secondary infection is common in towel and blotter tests. "…A retest in sand, ~~or~~ soil or organic growing media may be advisable."
4. "If a few seedlings with a partial decay of the epicotyl are found, …A retest, preferably in soil, ~~or~~ sand or organic growing media, will aid in interpretation of such seedlings."

5. "Hypocotyl development is slow until the roots start functioning and reach sufficient size;…A retest, preferably in soil, ~~or~~ sand or organic growing media, will aid in interpretation of such seedlings."

l) POACEAE, GRASS FAMILY I – Cereals (page 86)
…
NOTES
…
3. "Seedlings with badly thickened and shortened roots and shoots due to injury from chemical treatment are to be classified as abnormal. If such seedlings are difficult to evaluate on paper substrata, the interpretation should be based on the seedling performance in sand or soil."

POACEAE, GRASS FAMILY I – Cereals (page 86)
…
NOTES
…
3. "Seedlings with badly thickened and shortened roots and shoots due to injury from chemical treatment are to be classified as abnormal. If such seedlings are difficult to evaluate on paper substrata, the interpretation should be based on the seedling performance in sand, ~~or~~ soil or organic growing media."

m) POACEAE, GRASS FAMILY II – Rice (page 91)
…
NOTES
1. "Fungal development may cause variation in test results; more uniform results will be obtained if seeds are well spaced or grown in sand or soil…."
POACEAE, GRASS FAMILY II – Rice (page 91)
…
NOTES
1. "Fungal development may cause variation in test results; more uniform results will be obtained if seeds are well spaced or grown in sand, ~~or~~ soil or organic growing media…."

4. HARMONIZATION AND IMPACT STATEMENT: (ISTA/FSA/Canadian Methods & Procedures)

N/A

5. SUPPORTING EVIDENCE:

Since organic growing media is already listed in AOSA Rules Vol. 1 as an approved substrate and guide for classifying questionable seedlings [sec. 6.5.b(1)], it must also be referenced in AOSA Rules Vol. 4 to harmonize with Vol. 1.

6. SUBMITTED BY:

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7. **DATE SUBMITTED:**

July 12, 2022

Proposal #18

David Johnston and Riad Baalbaki

1. **PURPOSE OF PROPOSAL:**

The primary purpose of this proposal is to clarify in AOSA Rules Volume 4, for which Families sufficient secondary, seminal, and/or adventitious roots are permitted to compensate for a missing or defective primary root. These proposed changes will lead to more uniformity with root evaluations amongst analysts and increase laboratory testing uniformity. The established root evaluation criteria in Volume 4 are not changed with this proposal, only clarified.

2. **PRESENT RULE/PROPOSED RULE:**

Proposed changes listed as "a" through "x". Present Rule is listed first with the Proposed Rule listed underneath with proposed changes in red text.

a) AIZOACEAE, CARPETWEED FAMILY (p. 19)

…

Root system: A primary root; secondary roots may develop within the test period.

AIZOACEAE, CARPETWEED FAMILY

…

Root system: A primary root; weak, stubby, or missing primary root with sufficient secondary or adventitious roots; secondary roots may develop within the test period.

b) ASTERACEAE, SUNFLOWER FAMILY II - Kinds other than lettuce (p. 27)

…

Root system: A long primary root with secondary roots usually developing within the test period.

ASTERACEAE, SUNFLOWER FAMILY II - Kinds other than lettuce

…

Root system: A long primary root with secondary roots usually developing within the test period. Weak, stubby, or missing primary root with sufficient secondary or adventitious roots.

c) BALSAMINACEAE, BALSAM FAMILY (p. 30)

…

Root system: A primary root, with one to many secondary roots, which usually develop within the test period. The primary root is not always readily distinguishable from the secondary roots.

BALSAMINACEAE, BALSAM FAMILY

…

Root system: A primary root, with one ~~to many~~ or more secondary roots, which usually develop within the test period. Weak, stubby, or missing primary root with two or more sufficient secondary roots. The primary root is not always readily distinguishable from the secondary roots.

d) CHENOPODIACEAE, GOOSEFOOT FAMILY (p. 38)

…

Root system: A primary root; secondary roots may develop within the test period.

CHENOPODIACEAE, GOOSEFOOT FAMILY

…

Root system: A primary root; weak, stubby, or missing primary root with sufficient secondary or adventitious roots; secondary roots may develop within the test period.

e) CUCURBITACEAE, CUCURBIT FAMILY (p. 41)

…

Root system: A long primary root with numerous secondary roots.

CUCURBITACEAE, CUCURBIT FAMILY

…

Root system: A long primary root with numerous secondary roots; weak, stubby, or missing primary root with two or more sufficient secondary or adventitious roots.

f) FABACEAE, LEGUME FAMILY I - Large-seeded epigeal, except soybean, peanut, lupine (p. 45)

…

Root system: A long primary root with secondary roots.

FABACEAE, LEGUME FAMILY I - Large-seeded epigeal, except soybean, peanut, lupine

…

Root system: A long primary root with secondary roots; weak, stubby, or missing primary root with sufficient secondary or adventitious roots.

g) FABACEAE, LEGUME FAMILY II - Soybean and lupine (p. 52)

…

Root system: A long primary root with secondary roots.

FABACEAE, LEGUME FAMILY II - Soybean and lupine

…

Root system: A long primary root with secondary roots; weak, stubby, or missing primary root with sufficient secondary or adventitious roots.

h) FABACEAE, LEGUME FAMILY III - Peanut (p. 57)

…

Root system: A long primary root with secondary roots. Adventitious roots develop from the base of the hypocotyl if the primary root is damaged.

FABACEAE, LEGUME FAMILY III - Peanut

…

Root system: A long primary root with secondary roots. Weak, stubby, or missing primary root with sufficient secondary or adventitious roots. Adventitious roots develop from the base of the hypocotyl if the primary root is damaged

i) FABACEAE, LEGUME FAMILY IV - Large-seeded hypogeal (p. 61)

…

Root system: A long primary root with secondary roots.

FABACEAE, LEGUME FAMILY IV - Large-seeded hypogeal

…

Root system: A long primary root with secondary roots; weak, stubby, or missing primary root with sufficient secondary roots.

j) FABACEAE, LEGUME FAMILY V (p. 67)

…

ABNORMAL SEEDLING DESCRIPTION

…

Root:

· none.

· primary root stubby (for sweetclover and crownvetch, or for roots bound by the seed coat see note 1). · split extending into the hypocotyl

FABACEAE, LEGUME FAMILY V

…

ABNORMAL SEEDLING DESCRIPTION

…

Root:

· none.

· primary root stubby (for sweetclover and crownvetch, or for roots bound by the seed coat see note 1).
· split extending into the hypocotyl.
· secondary roots will not compensate for a defective primary root.

k) LILIACEAE, LILY FAMILY I – Asparagus (p. 74)
…
Root system: A long slender primary root.

LILIACEAE, LILY FAMILY I – Asparagus
…
Root system: A long slender primary root; stubby primary root with sufficient secondary roots.

l) LINACEAE, FLAX FAMILY (p. 79)
…
Root system: A primary root, with secondary roots usually developing within the test period.

LINACEAE, FLAX FAMILY
…
Root system: A primary root, with secondary roots usually developing within the test period; weak, stubby, or missing primary root with sufficient secondary roots.

m) MALVACEAE, MALLOW FAMILY (p. 82)
…
Root system: A primary root, with secondary roots usually developing within the test period. Areas of yellowish pigmentation may develop on the root in cotton.

MALVACEAE, MALLOW FAMILY
…

Root system: A primary root, with secondary roots usually developing within the test period. Weak, stubby, or missing primary root with sufficient secondary or adventitious roots. Areas of yellowish pigmentation may develop on the root in cotton.

n) POACEAE, GRASS FAMILY I – Cereals (p. 85)
…
Root system: A primary root and seminal roots. The primary root is not readily distinguishable from the seminal roots, therefore all roots arising from the seed are referred to as seminal roots.

POACEAE, GRASS FAMILY I – Cereals
…
Root system: A primary root and seminal roots. The primary root is not readily distinguishable from the seminal roots, therefore all roots rising from the seed are referred to as seminal roots; one or more sufficient seminal roots.

o) POACEAE, GRASS FAMILY II – Rice (p. 90)

…

Root system: Strong primary root and seminal roots. Adventitious roots may start to develop from the mesocotyl or coleoptilar node within the test period. If the mesocotyl elongates the adventitious roots will be carried above the grain.

POACEAE, GRASS FAMILY II – Rice

…

Root system: Strong primary root and seminal roots. Adventitious roots may start to develop from the mesocotyl or coleoptilar node within the test period. Weak primary root with sufficient seminal or adventitious roots. If the mesocotyl elongates the adventitious roots will be carried above the grain.

p) POACEAE, GRASS FAMILY III – Corn (p. 93)
…
Root system: Strong primary root and seminal roots. Adventitious roots may start to develop from the mesocotyl or coleoptilar node within the test period.

POACEAE, GRASS FAMILY III – Corn
…
Root system: Strong primary root and seminal roots. Weak, stubby, or missing primary root with sufficient seminal roots. Adventitious roots may start to develop from the mesocotyl or coleoptilar node within the test period

q) POACEAE, GRASS FAMILY IV – Sorghum (p. 100)
…
Root system: A long primary root, usually with secondary roots developing within the test period. Adventitious roots arising from the mesocotyl and coleoptilar node may start development within the test period. Areas of natural, reddish pigmentation may develop on the root.

POACEAE, GRASS FAMILY IV – Sorghum
…
Root system: Strong primary root and seminal roots. Damaged or weak primary root with two or more sufficient secondary roots. Adventitious roots may start to develop from the mesocotyl or coleoptilar node within the test period. Areas of natural, reddish pigmentation may develop on the root.

r) POLYGONACEAE, KNOTWEED FAMILY (p. 107)
…
Root system: A primary root with secondary roots developing within the test period for some species.

POLYGONACEAE, KNOTWEED FAMILY

…

Root system: A primary roots with secondary roots developing within the test period for some species. Weak, stubby, or missing primary root with sufficient secondary or adventitious roots.

s) PRIMULACEAE, PRIMROSE FAMILY I – Cyclamen (p. 110)

…

Root system: Several seminal roots, developing more or less simultaneously at the distal end of the hypocotyl.

PRIMULACEAE, PRIMROSE FAMILY I – Cyclamen

…

Root system: Several seminal roots, developing more or less simultaneously at the distal end of the hypocotyl. More than one sufficient seminal root is required.

`

t) SOLANACEAE, NIGHTSHADE FAMILY I – Pepper, tomato, and husk tomato (p. 114)

…

Root system: A long primary root, usually with root hairs. Secondary or adventitious roots usually do not develop within the test period unless the primary root has been damaged.

SOLANACEAE, NIGHTSHADE FAMILY I – Pepper, tomato, and husk tomato

…

Root system: A long primary root, usually with root hairs. Weak, stubby, or missing primary root with sufficient secondary or adventitious roots. Secondary or adventitious roots usually do not develop within the test period unless primary root has been damaged.

u) TROPAEOLACEAE, TROPAEOLUM FAMILY (p. 120)

…

Root system: The root system consists of a primary root and secondary roots.

TROPAEOLACEAE, TROPAEOLUM FAMILY

…

Root system: The root system consists of a primary root and secondary roots. Weak, stubby, or missing primary root with two or more sufficient secondary roots.

v) MISCELLANEOUS AGRICULTURAL AND HORTICULTURAL (p. 126)

…

GENERAL DESCRIPTION

Seedlings are considered normal if they possess those essential structures that are indicative of its ability to produce a plant under favorable conditions.

MISCELLANEOUS AGRICULTURAL AND HORTICULTURAL

…

GENERAL DESCRIPTION

Seedlings are considered normal if they possess those essential structures that are indicative of its ability to produce a plant under favorable conditions. Sufficient secondary or adventitious roots can compensate for a missing or stubby primary root.

w) TREES AND SHRUBS II - Angiosperms with hypogeal germination (p. 129)

…

Root system: A long primary root with secondary roots.

TREES AND SHRUBS II - Angiosperms with hypogeal germination

…

Root system: A long primary root with secondary roots. A weak, stubby, or missing primary root with sufficient secondary roots.

x) TREES AND SHRUBS III - Angiosperms with epigeal germination (p. 135)
…
Root system: A primary root; secondary roots may develop within the test period.

TREES AND SHRUBS III - Angiosperms with epigeal germination

…
Root system: A primary root; missing or stubby primary root with sufficient secondary or adventitious roots; secondary roots may develop within the test period.

4. HARMONIZATION AND IMPACT STATEMENT: (ISTA/FSA/Canadian Methods & Procedures)

N/A

5. SUPPORTING EVIDENCE:

AOSA Rules for Testing Seeds - Volume 4

6. SUBMITTED BY:

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7. **DATE SUBMITTED:**

August 2, 2022

2022 AOSA/SCST Annual Meeting Memories

Compiled by Scottie Pouliot

Angie Croft, RST, Growmark, Illinois. 20 years of experience

Why do you like coming to meetings? The comradery with people who do the same thing that you do.

What is your favorite memory from attending meetings? The site seeing in the different cities that I normally wouldn't get to experience if I wasn't at the meeting.

What has been your favorite part of the meeting this year? Catching up with everybody since it's been two years since we last seen each other.

Jean Tolliver RST, Syngenta, Washington 36 years of experience

Why do you like coming to meetings? I like coming to meetings because there's a lot to learn no matter how many years of experience you have, you get to meet and talk with friends and fellow seed nerds.

What is your favorite memory from attending meetings? Passing my RST exam in Oklahoma city in 1992.

What has been your favorite part of the meeting this year? This year my favorite part is preparing for retirement and saying goodbye to longtime friends and colleges. It's been a fun ride; you can't like what you do you have to love it, and I love seed testing.

One important thing for upcoming test takers: it's a lot of information but it gives you more opportunities than you'd normally have in the seed industry.

Donna Grubisic, RST, MD Seed Analysis Inc, 42 years of experience, 25 meetings attended

Why do you like coming to meetings? It's nice to have connections with people and talking more on a one to one basis with people in the industry.

What is your favorite memory from attending meetings? Savanna Georgia in 1997, because it was in the city and we could walk out and experience the city, and go sightseeing. There was a movie the Midnight in the Garden of Good and Evil that was filmed there and it had just came out in theaters. We got to see the house where it was filmed.

Second favorite memory: Albuquerque, New Mexico. It was in the south western hotel that let us walk out into Albuquerque and experience the local culture of the old town.

What has been your favorite part of the meeting this year? Meeting new people and seeing the people you know and haven't seen before COVID.

Tia Tyler, USDA FS National Seed Lab, Georgia 8 years of experience

What has been your favorite part of the meeting this year? Learning about the organization and opportunities. Meeting people who do the same things and me.

Favorite thing about seed testing: It is interesting and there is a lot to be curious about.

Sue Alvarez,RST, Ransom Seed Lab, California 37 years of experience

Why do you like coming to meetings? I like to network with people, I like to see old friends that I've had for years because I've been coming to these meetings for so long. I like to go different parts of the country and see what's there. I feel passionate about seed testing and AOSA rules, and I like to be part of the discussion and help find solutions to problems.

What is your favorite memory from attending meetings? The Savanna Georgia meeting in 1997, it was a neat location with a really nice tour afterwards. I took photos with Donna Grubisic for the 75th STSC photo album.

What has been your favorite part of the meeting this year? The best thing about this meeting is seeing all these people I love after three years.

Jason Perrault, RST, Seedway, New York, 16 years of experience

Why do you like coming to meetings? To hang out with fun people, to network, meet new people and learn the new laws. I get to meet the faces behind all of the emails.

What is your favorite memory from attending meetings? The dinner cruise in lake Tahoe at the Spark's conference in 2019. It was awesome.

What has been your favorite part of the meeting this year? The White Sox game, it was amazing. SoDak took great care of us.

AOSA & SCST Award Recipients

Roger Burton, AOSA Honorary Member

Seed Marketing Specialist Supervisor Roger Burton retired from the USDA Seed Regulatory and Testing Division in the Summer of 2022. Roger worked with the Maryland Department of Agriculture for 27 years serving as an Agricultural Field Inspector and as a Supervising Inspector in charge of the Seed Regulatory Program. He started working with SRTD in March 2004 as a Seed Marketing Specialist, in 2016 he became the first Regulatory Supervisor. Roger's knowledge and years of expertise was invaluable to SRTD and the seed industry as he has a wealth of knowledge and loved what he did. He will be missed!

Photo credit, CDFA.ca.gov.

Robert Price, PhD, SCST Honorary Member

Bob Price has worked in seed technology, phylogeny, molecular biology, and botany for over 35 years including defining a rare new species of Brassicaceae, *Draba streptobrachia,* Twisted draba in 1980.

Bob received his PhD from University of California, Berkeley in 1987. From 1987-1993 he worked as a postdoctoral fellow at the New York State Museum-Albany, Harvard University, and the Department of Biology at Indiana University. Bob was appointed as Assistant Professor (1993-2000) at the University of Georgia's Department of Botany. From 2000-2009 he served as Technical Editor, Biological Consultant and Scientific Editor for the Conifers Around the World project. Bob joined CDFA's Seed Laboratory at the Plant Pest Diagnostic Branch in 2009 as Associate Seed Botanist and was later promoted to Senior Seed Botanist in 2013. His many responsibilities at the Seed Laboratory were focused on purity analysis and seed identification. Bob also established a DNA sequencing program for seed identification at the CDFA lab. Bob became a Certified Seed Analyst-Purity in 2013. In addition to his work as Senior Seed Botanist, he served as Senior Plant Taxonomist (2016-2017) and was appointed as Primary State Botanist in 2019, assessing potential risks of introducing plant species to California's agriculture and the environment.

Bob has been an active member of the Association of Official Seed Analysts/Society of Commercial Seed Technologists, and has assisted with revisions of seed identification manuals, as well as with publication of the Seed Technology journal as associate editor. Those who have had the pleasure to work with Bob on committees and in working groups appreciate his attention to detail, kindness, good humor, and willingness to step in where his help is needed. SCST is very pleased to have him as an honorary member. We wish him all the best and many years of continued investigation of conifers and Brassicaceae at a more leisurely pace during his well-earned retirement.

AOSA & SCST Award Recipients

Heidi Jo Larson, SCST Meritorious Service Award

This year's SCST Meritorious Award winner grew up in rural South Dakota on a family farm. According to local rumor, this person has been involved in seed testing since they were six years old. They graduated from South Dakota State University in 1999 with a Bachelor of Science in Agronomy. They went on to receive a Master of Science in Agronomy in 2002 – with a focus on breaking seed dormancy in three Poaceae Species. During their time at SDSU, they became an RST in 2001.

While attending and after graduating at SDSU, they worked at the SDSU Seed Testing Lab until moving to Wisconsin to work at Wisconsin Crop Improvement. This individual worked there for 10 years, successfully managing the laboratory and effectively developing and training staff. In 2012 they moved back to South Dakota to be closer to family and has worked at SGS in the purity lab for the past 10 years where they continue to deliver superior quality and timely results to clients, develop and train fellow employees, and be an invaluable source of knowledge to staff and clients.

Everyone who knows this individual knows that they can never sit still or work on one thing at a time! They are committed to the success of the family farm and business, along with being committed to their career and the success of SCST. This person puts in countless hours and effort towards the success of the SCST Association.

When not involved in the seed testing business, SCST projects and duties or the family farm, they can usually be found with a fishing rod, shotgun, rifle or machete in hand stalking the elusive Walleye, Perch, goose, white tail deer or feral hog. They also make time to train their oldest niece in the fun and excitement of catching fish and enjoying nature.

After serving as President from 2019-2021, they continue to serve SCST through many roles. Their continued focus on uniformity and devotion to many different working groups has moved AOSA and SCST into future collaboration and success. This person researched countless hours for information to compile the amazing SCST 100th Anniversary Commemorative book for us to enjoy. I believe that this may be the first time we have ever had a repeat winner of this award.

Bio submitted by Steve Beals

AOSA & SCST Award Recipients

Photo credit: Seed World

Chet Boruff, AOSA/SCST Distinguished Service Award

Mr. Chet Boruff began his career as Chief Executive Officer of the Association of Official Seed Certifying Agencies (AOSCA) in October 2004. AOSCA represents seed certifying agencies across the U.S. and seven other countries. AOSCA is responsible for applying uniform standards to maintain varietal purity for over 60 major agricultural crops.

In his role as AOSCA's very first CEO, Chet has been responsible for serving as its spokesman, managing the Association, providing assistance to its members as they administer AOSCA seed certifying standards, and developing related services for the seed and ag industry. He has been an active liaison with other seed industry stakeholder groups, such as AOSA and SCST.

Earlier in his career, Chet served for seven years as Deputy Director at the Illinois Department of Agriculture. In this role, he was responsible for the Department's natural resource and regulatory programs, including administering Illinois' state seed law. He also worked for the Farm Credit System and organizations promoting value-added agricultural commodities.

Throughout his career, Chet has operated his family's cash grain farm in Rock Island County, Illinois.

Chet is an alumnus of Iowa State University and has served on its College of Agriculture Endowment Board. He is also a graduate of the Illinois Ag Leadership Program and serves on its Board of Directors.

Chet and his wife, Joy, have two married children who live and work in Chicago. We wish Chet the very best in this next phase of life!!!

Thank you Chet for all your years of service to the Seed Industry and to AOSA and SCST!!!

Info source: ASTA website

Lost Resources

Don Ogawa

Donald Ogawa, 70, of Caldwell, ID, passed away at his home on September 27, 2022 following a multi-year battle with cancer.

Don was born April 14, 1952, in Nampa, Idaho. He graduated from Middleton High School in 1970. He attended Boise College for a time. Early in his career he managed the Karcher Ranch Market, worked for Idaho Sand & Gravel, Max Lewellin Insurance and several years for Iseri Insurance in Ontario, OR. Most of his career was spent as a registered seed technologist employed by The Crookham Company from where he retired in 2017.

Don's lifetime interests were fishing, hunting, bowling, and community service. He treasured the countless fishing trips with his close friends and family. He enjoyed bird hunting and traveled to participate in countless regional bowling tournaments. His community involvement was extensive. He donated considerable effort and resources to the creation of the Caldwell Japanese Densho Garden. He was extremely dedicated to the Caldwell Exchange Club for roughly four decades. Over the years, he also participated in the Caldwell Jaycees, Caldwell Independent Insurance Agents Association, Coalition for Agricultures' Future, and the Caldwell Chamber of Congress helping coordinate the annual agricultural tour.

Don is survived by his mother, Lilly Yamaki; stepmother Judi Ogawa; brothers Ed Ogawa and Jeff Zmuda (Cheryl); sisters June Ogawa, Kapri Zmuda, and Wendi Zmuda-Scott; daughter Melissa McGladrie (Randy); grandchildren Tanner (Brooke), Kyle, Ryan, and Hannah McGladrie. Also, Cecilia Hiatt; Anthony Ogawa; Alexander McMains (Kuemei); Kristi Hendry (Rolando); Trina Reyna (Robert); Marcus Ogawa. As well as Aunt Ida Ogawa, Uncle Tom Ogawa, and Uncle Ken Ogawa (Yuki); beloved friends and dozens of cousins.

Don was preceded in death by his father, Mos Ogawa; stepfather Bill Yamaki; Uncles and Aunts: Todd Ogawa, Yosie Ogawa, Fumi Ogawa, Mary and Tommy Miyasaki, Tak and Emmy Ogawa, May Ogawa, Short Fujikawa, Shiz and Irene Fujikawa, and June and Nancy Fujikawa; Cousin Wendy Fujikawa; and close friend Richard "Dick" Jones.

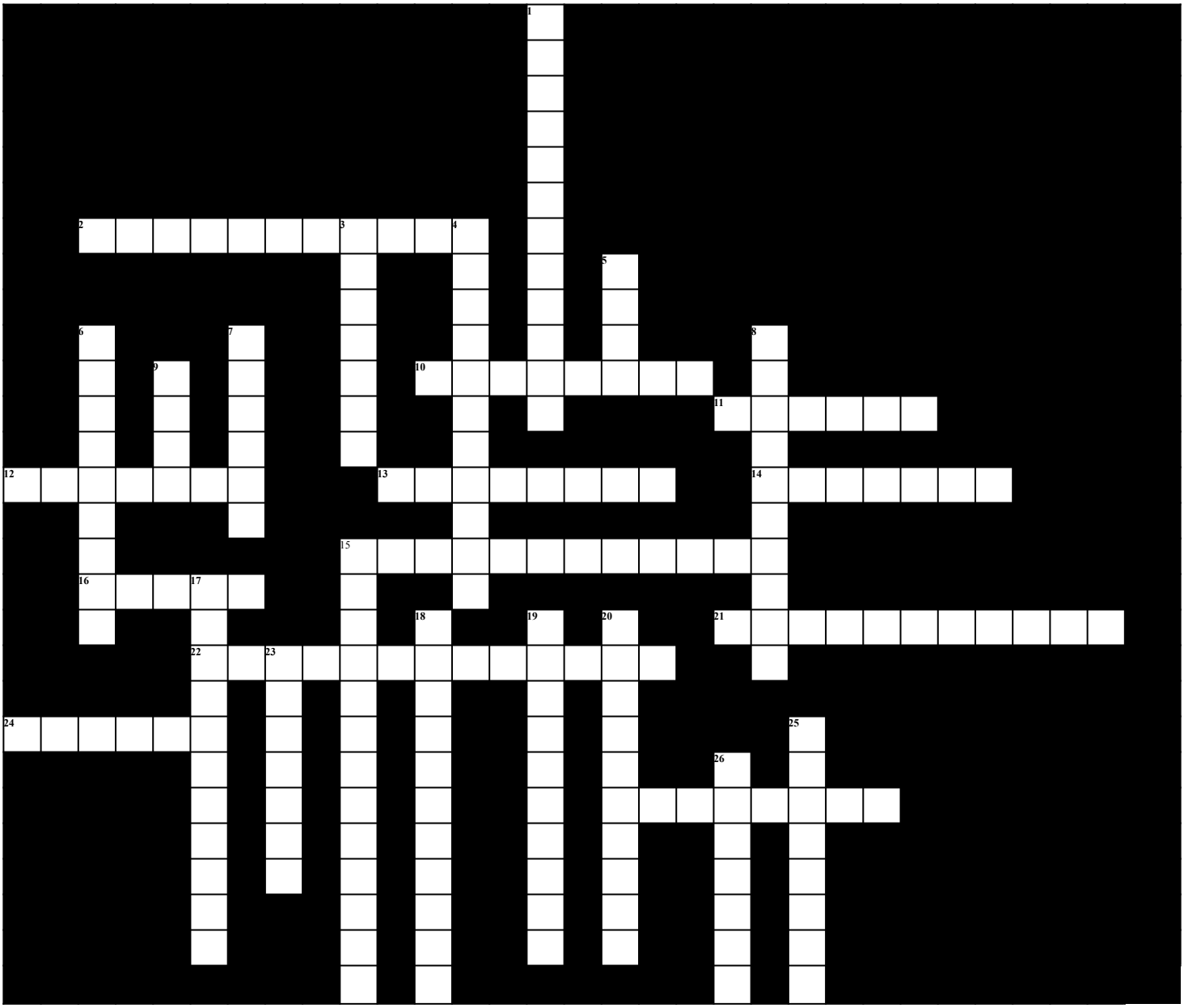
He embraced the concept that Families are Forever. His generosity and willingness to serve his family and the community will be greatly missed.

No funeral service will be held. A memorial gathering will be held at a later date. Condolences may be left at www.dakanfuneralchapel.com

Study Guide: RGT Vocabulary Review

Submitted by RGT Board of Examiners

Questions are based on some of the most missed questions from previous RGT exams.



Down:

1. A class of herbicides also known as ALS herbicides that inhibit the acetolactase synthase enzyme.
3. Where the conventional/end point PCR product is detected in a graph

Across:

2. To detect hybrid seed molecularly the parents must be this for a particular marker.
10. Another name for Uracil DNA Glycosylase (UDG) or Uracil N-Glycosylase (UNG)

4. Discrete units of the genome carrying many genes and consisting of proteins and a very long molecule of DNA.
5. The USDA and this organization established policies that provide information on appropriate sampling for AP/LLP testing.
6. A PCR reaction in which more than one amplification product can be produce because two or more sets of primers are added to the reaction.
7. RNA contains these types of sugars.
8. An organism whose gamete cells contain genetic material originally derived from an organism other than the parents or in addition to the parental genetic material.
9. EDTA in DNA extraction solvents chelates these that are needed by nucleases for activity and membranes to maintain integrity.
15. The pairing of two complementary single strands of RNA and/or DNA to give a double stranded molecule.
17. A large chain-like molecule containing phosphate groups, sugar groups, and purine and pyrimidine bases
18. A naturally occurring bacterium that is capable of inserting its dNA into plants is called Agrobacterium _____
19. Multi-celled organisms with organelles
20. To detect hybrid seed molecularly the marker must be _____
23. The herbicide bioassay test ignite herbicide commonly used in _____
25. The purpose of testing for adventitious or low level presence of GMO events is to determine the presence and this of GMO in products where the absence of GMO is desired
26. A high absorbance at 280 nm by a DNA extract most likely indicates contamination by this:
11. Along with Crick, he proposed that the structure for DNA is in the form of a double helix.
12. The cellular process of genetic material replication and division to daughter cells. .
13. The most specific type of GMO PCR assay for identifying traits for a particular GMO
14. Mutations that occur in an organism but are not passed on to the offspring.
15. An individual organism with different alleles at one or more particular loci.
16. The least specific type of GMO PCR assay for identifying traits for a particular GMO
21. 2 or more genes that are inserted within the same construct as well as into the same chromosome location and are always present together.
22. The active ingredient of Glean herbicide.
24. One complete set of genetic information from a genetic system.
27. In a typical PCR situation when you increase the annealing temperature, the specificity of the reaction will do this_____

Who are these cousins?

1 Sulfonylurea, 2. Polymorphic, 3. Plateau, 4. Chromosomes, 5. ISTA, 6. Multiplex, 7. Ribose, 8. Transgenic, 9. Ions, 10. Amperase, 11. Watson, 12. Meiosis, 13. Promotor, 14. Somatic, 15. Heterozygote, 16. Event, 17. Nucleic acid, 18. Tumefaciens, 19. Eukaryotes, 20. Codominant, 21. Lined Genes, 22. Chlorsulfuron, 23. Liberty, 24. Genome, 25. Quantity, 26. Protein. 27. Increase.

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Don't forget your passport!

Visit the Annual Meeting Website