2020 Rule Change Proposal 13

Purpose of Proposal: To change the germination temperature for *Brassica napus* var. *napus*.

Present 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	Dormant seed[f]
…						
Brassica napus var. *napus* annual rape and winter rape	B, T	20-30; 15-25	3	7		

Proposed Rule:

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Harmonization and Impact Statement:

The testing rules, such as AOSA and ISTA (International Seed Testing Association) seed testing rules, aim to achieve testing uniformity; i.e., reproducible and repeatable results. With a comparison of current seed testing rules or methods described by ISTA, AOSA, and Canadian Methods and Procedures for Testing Seeds (Canadian M&P), all three testing rules provide different temperatures for the germination test of *Brassica napus* var. *napus*. The three sets of rules specify two alternating temperatures: 15<=>25°C (Canadian M&P, AOSA Rules) and 20<=>30°C (ISTA Rules & AOSA Rules), and two constant temperatures: 25°C (Canadian M&P) and 20°C (ISTA Rules). Will these temperatures generate a comparable results?

An ISTA validation study on the germination temperatures for *Brassica napus* var. *napus* was carried out to provide scientific data and evidence for rule harmonization and the optimal temperatures for laboratory testing.

Supporting Evidence:

Eight ISTA-accredited laboratories from five countries, including laboratories from the USA and Canada, participated in the study. Six seed lots varying in varieties, spring and winter types, and germination levels were used for the study. Testing temperatures are in two alternating temperatures: 15<=>25°C (Canadian M&P, AOSA Rules) and 20<=>30°C (ISTA Rules & AOSA Rules), and two constant temperatures: 25°C (Canadian M&P) and 20°C (ISTA Rules). The germination tests were conducted on either top of papers or between papers with 8 hrs light during the high-temperature phase without dormancy break treatments. Counts of normal and abnormal seedlings and ungerminated seeds were collected at 7 days. Collected data were analyzed using statistical programs of SAS, ISTAgermMV, and z-scores.

The study showed the largest variation source was among laboratories excluding seed lot as a designed factor. It was significantly different on ungerminated seeds among temperatures, and the percentage of ungerminated seeds was lower at 15<=>25°C and 20°C. The repeatability and reproducibility had a lower standard deviation at 20°C and 15<=>25°C. The Z-scores showed that 20°C and 15<=>25°C had less variation from means on the percentages of normal seedlings, abnormal seedlings, and ungerminated seeds. 20°C and 15<=>25°C also had higher accuracy for the evaluation of seedlings and ungerminated seeds. In summary, the data supported that 20°C and 15<=>25°C result in higher normal seedlings and lower ungerminated seeds.

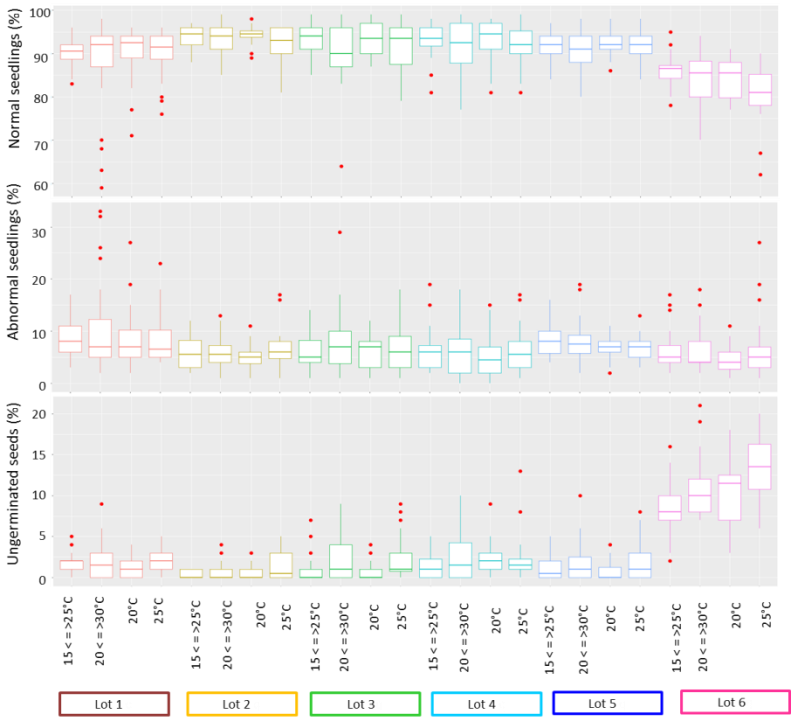

Figure 2. Box plots of normal seedlings, abnormal seedlings, and ungerminated seeds as affected by germination temperatures and seed lots.

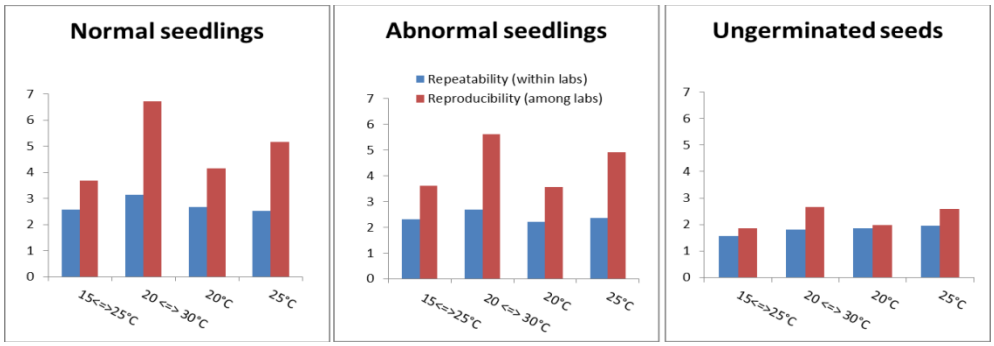

Figure 3. *Statistical values of repeatability and reproducibility of normal seedlings, abnormal seedlings, and ungerminated seeds in each of the four germination temperatures.
*Please note the value was calculated using *ISTAGermMV* developed by ISTA Statistical committee, the smaller the value was, the better the performance of the temperatures would be.

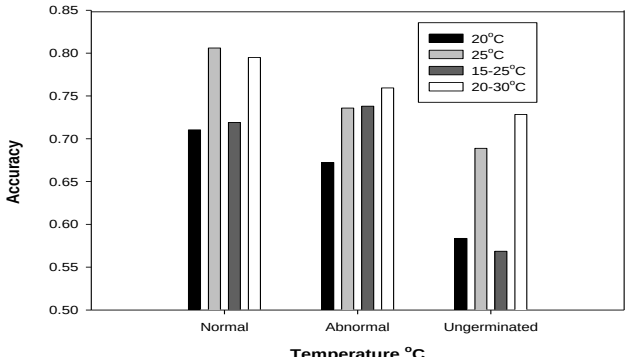

Figure 5. Average accuracy* among four temperatures measured for percentages of normal, abnormal seedlings and ungerminated seeds with six seed lots.

*Note: The smaller the value was, the higher the accuracy would be.

Conclusion:

To promote harmonization among testing rules, we recommend ISTA and AOSA revise testing temperatures for *B. napus* var. *napus* as 20°C and 15<=>25°C, which will result in higher normal seedlings and lower ungerminated seeds, more important to increase the reproducibility among laboratories.

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