Validation study on germination method for hybrid bromegrass (*Bromus riparius* X *B. inermis*)

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Summary

Hybrid bromegrass is a breeding cross between meadow bromegrass (*Bromus riparius*) and smooth bromegrass (*B. inermis*). Two varieties, 'Knowles' and 'Success', were released by Canadian breeders in 2000 and 2003, and one variety 'BigFoot' was released from USDA as a new forage crop. Because of the superior quality of hybrid bromegrass in fast growth and high yield to their parental species, it was widely used as dual purposes forage as hay or pasture in Canada and the USA. However, the hybrid bromegrass has not been included in any of the seed-testing rules, e.g. *Canadian Methods and Procedures for Testing Seeds*, the *AOSA (Association of Official Seed Analysts) Rules for Testing Seeds*, and *International Rules for Seed Testing*. We conducted in-house studies and organized an inter-laboratory study to test the germination difference with dormancy treatments, counting days, and temperatures. The germination dormancy treatment and final counting days were determined with the in-house studies using six seeds lots of two varieties and a breeding lines that germination ranged from 79% to 89%. The inter-laboratory study validated the germination temperatures with the statistical parameters of repeatability and reproducibility with four seed lots that germination at 82-91% in pre-tests. The results of the in-house and inter-laboratory studies showed that germination of the hybrid bromegrass has no requirement for dormancy breaking measures and 15-25°C with the final count at 14 days was an optimum methods with accurate, consistent, and satisfactory performance comparing to other temperatures.

Background

Hybrid bromegrass is generated by crossing meadow bromegrass (*Bromus riparius*) and smooth bromegrass (*B. inermis*), which is a slightly creeping, winter hardy, long-lived perennial , dual purpose forage grass for both hay and pasture systems in Canada. Two varieties, Knowles and Success, were released in 2000 (Coulman, 2004) and 2003 (Coulman, 2006), and one variety 'BigFoot' was released from USDA as a new forage crop. Since the superior quality of hybrid bromegrass in fast growth and high yield than their parental species (Coulman, 2004 and 2006), it was widely used as forage in Canada and the USA.

As a newly developed species, few documents were found about its seed germination method. Meanwhile, it has not had a published testing rule or method for the hybrid bromegrass, in Canadian Methods and Procedures for Testing Seeds (referred to as the M&P hereafter) (2021), the AOSA (Association of Official Seed Analysts) Rules for Testing Seeds (2021), and International Rules for Seed Testing (referred to as ISTA Rules) (2021).

The germination methods for hybrid bromegrass could be similar to their parental species, *B. riparius* and *B. inermis*, which were summarized in Table 1. Treating seeds with KNO3 before germination is only recommended by ISTA method. This could be the starting points for obtaining the germination method for hybrid bromegrass.

Table 1. Germination methods for bromegrass (*Bromus riparius* and *B. inermis*) in different seed-testing rules.

	Seed Testing Rules		
Species Name	**ISTA**	**ASOA**	**M&P**
Bromus inermis (Smooth brome)	Prechill, KNO3, 15-25 or 20-30°C	Prechill, light (optional), 20-30°C	Prechill, 15-25°C
Bromus riparius (Meadow brome)	Prechill, KNO3, 15-25 or 20-30°C	NA	Prechill, 15-25 or 20-30°C

To provide a standard germination method in testing hybrid bromegrass seeds, two separate studies, in-house studies and an inter-laboratories study were conducted. The specific objectives were 1) determine the optimum germination conditions for the germination of

hybrid bormegrass; 2) evaluate method performance using statistical parameters according to ISO standards; 3) conclude a method with data support for proposing to the seed-testing rules.

Materials and Methods

In-house studies

Six seed lots of two varieties, AC Knowles and AC Success, and a breeding line (S-9556M) were used in the in-house studies, obtained from the breeders of University of Saskatchewan. Germination tests with dormancy treatments of pre-chilling 3 days at 8°C in darkness and 0.2% KNO3 were conducted and compared with no dormancy treatment. Germination temperatures of 15-25°C, 20-30°C, and 20°C were used. All germination tests applied for 8 hours of light at higher temperature and /16 hours of dark at lower temperatures. The accumulative germination were counted up to 21 days.

An inter-laboratory study

Testing samples selection and germination methods

Four seed lots in total, two lots of each from 'AC Knowles' and 'AC Success', were selected with germination ranging from 82-91% based on pre-tests. Each selected seed lot was mixed and divided according to ISTA Rules for sample reduction to obtain approximately 5 g (for approximately 1000 seeds) (ISTA, 2021). Total 44 samples were prepared. A homogeneity test of 10 random samples of each lot was conducted and all seed lots passed the homogeneity test with a pre-determined tolerance. When the seed lot passed the homogeneity test, samples from four seed lots were randomly assigned and sent to seven participating laboratories.

Four temperatures including two alternating temperatures, 15-25^{o}C and 20-30^{o}C and two constant temperature 20^{o}C and 25^{o}C were used for the inter-laboratory study. The specified germination method is showed in Table 2.

Table 2. Germination methods for the inter-laboratory study in hybrid bromegrass.

Temperature (°C)	Number of seeds / reps	Substrate	First count (d)	Final count (d)	Breaking dormancy	Additional directions
20-30	100 x 4	TP	7	14	None	16 hr dark (20°C)/ 8 hr light (30°C)
25	100 x4	TP	7	14	None	16 hr dark / 8 hr light
15-25	100 x4	TP	7	14	None	16 hr dark (15°C) / 8 hr light (25°C)
20	100 x4	TP	7	14	None	16 hr dark / 8 hr light

Participating laboratories

Seven laboratories participated in this inter-laboratory study. Participating laboratories selected and met the pre-determined qualifications, such as testing experience in bromegrass and willingness to provide testing data.

Data Analysis

The study data from in-house studies or the inter-laboratory study was checked and the sum of the normal and abnormal seedlings and dead seeds at the final counts, which was verified or re-calculated to 100 percent. The Analysis of Variance (ANOVA) of normal and abnormal seedlings and dead seeds was conducted using SAS software (SAS, 2013) using a GLM model. Repeatability and reproducibility for each temperature were assessed using the ISTAgermMV software developed by ISTA statistical committee, where, the calculation followed ISO 5725-2 definitions (ISTA Method Validation for Seed Testing, 2006). Repeatability quantifies the average variability of results within each laboratory, when repeats are made on samples from the same given lots. Reproducibility is repeatability, increased by the variability of results from laboratory to laboratory.

Results and Discussion

In-house study for optimum germination methods

The in-house studies compared the effect of pre-chilling treatments of 3 days at 8°C in darkness and 0.2% KNO3 showed no significant difference from the germination without dormancy treatments (Figure 1). The results suggested the dormancy treatments are not required in the germination test. Compared the germination responses as affected by three germination temperatures, 20°C, 15-25°C and 20-30°C, alternating temperatures was not superior to constant temperature.

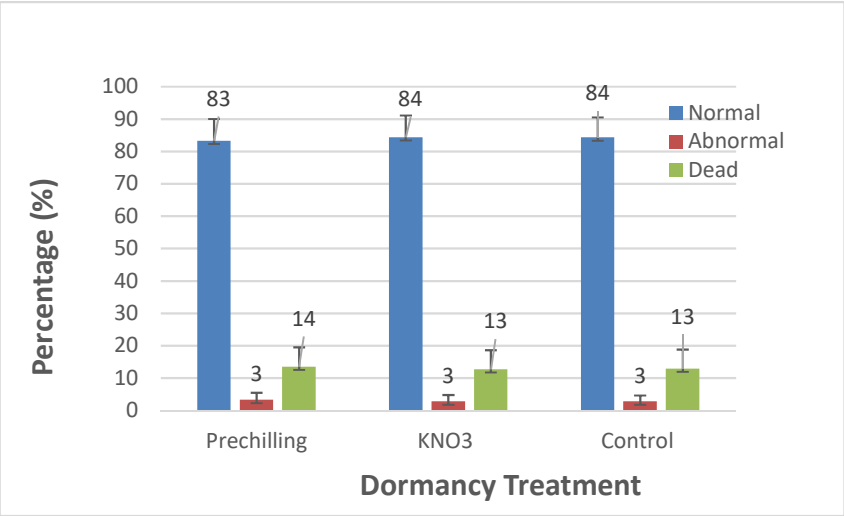

Figure 1. Average germination percentage (%), abnormal seedlings and dead seeds in 6 lots of hybrid bromegrass with dormancy treatment of pre-chilling (3 days at 8°C) and 0.2% KNO_3, at 15-25°C and 20-30°C. The germination of the 6 seed lots used had average germination ranges from 79-89% at 21 days.

The germination percentage was level off at 14 days, and had not much increase at 21 days compared to 14 days (Figure 2). The 14 days germination was determined as the final counting day.

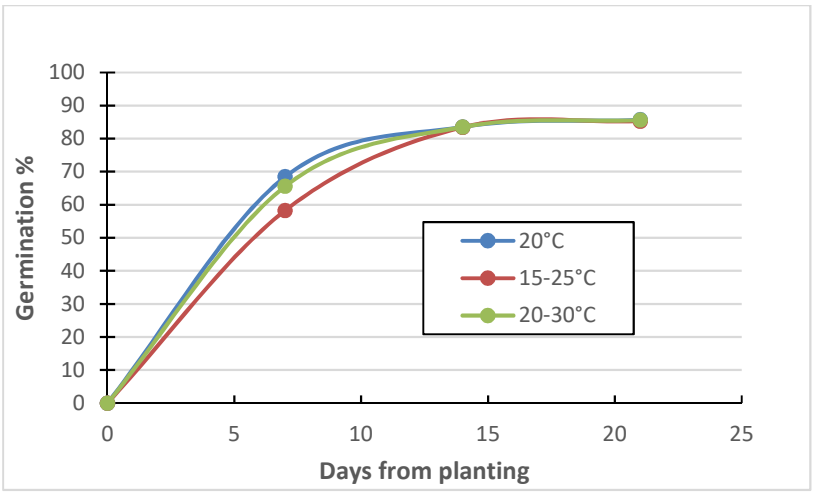

Figure 2. The accumulative germination percentage at 20°C, 15-25°C, 20-30°C with 3 different counting days (7th days; 14th days; 21 days after planting) in hybrid bromegrass. Note: six seed lots used had average germination ranges from 79-89% at 21 days.

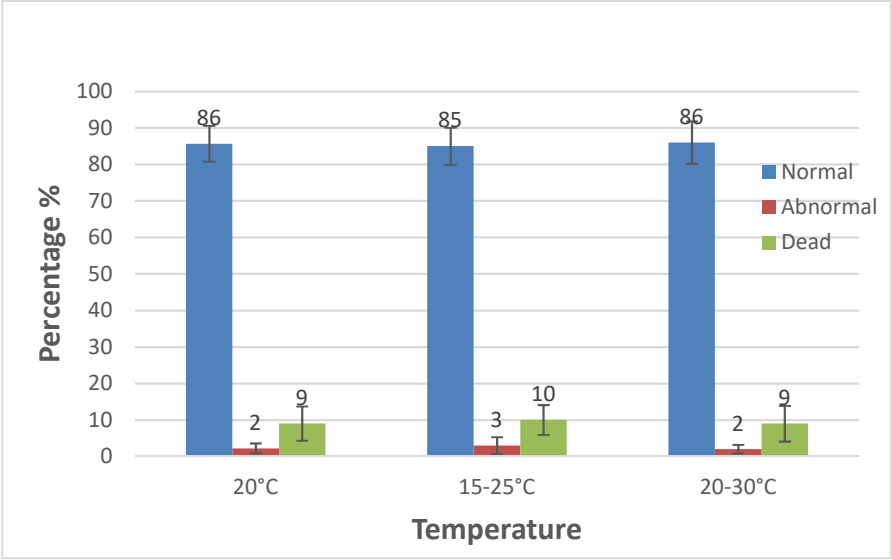

Figure 3. The final average percentage of normal, abnormal seedlings and dead seeds at 20°C, 15-25°C, 20-30°C at 21 days after planting in hybrid bromegrass. Note: six seed lots used had average germination ranges from 79-89% at 21 days.

Figure 3 showed the germination results were not impacted by different germination temperatures. This may also indicate the hybrid bromegrass can germinate at a wide range of

temperature at optimum germination conditions, e.g., sufficient moisture. Temperature may result a slightly difference in germination rate from the germination curved showed in Figure 2.

Inter laboratory study on method reliability

Source of variation for testing results from the inter-laboratory study

The Figure 4A showed testing results variation among laboratories at four germination temperatures. The testing result from the same lab, there were different variation ranges in four temperatures. Consistent temperatures, 20 and 25°C seems had a trend of higher variation within a laboratory or among laboratories. The results of Lab 3 was significant lower than other laboratories due to late submission, and the data not used for further analysis.

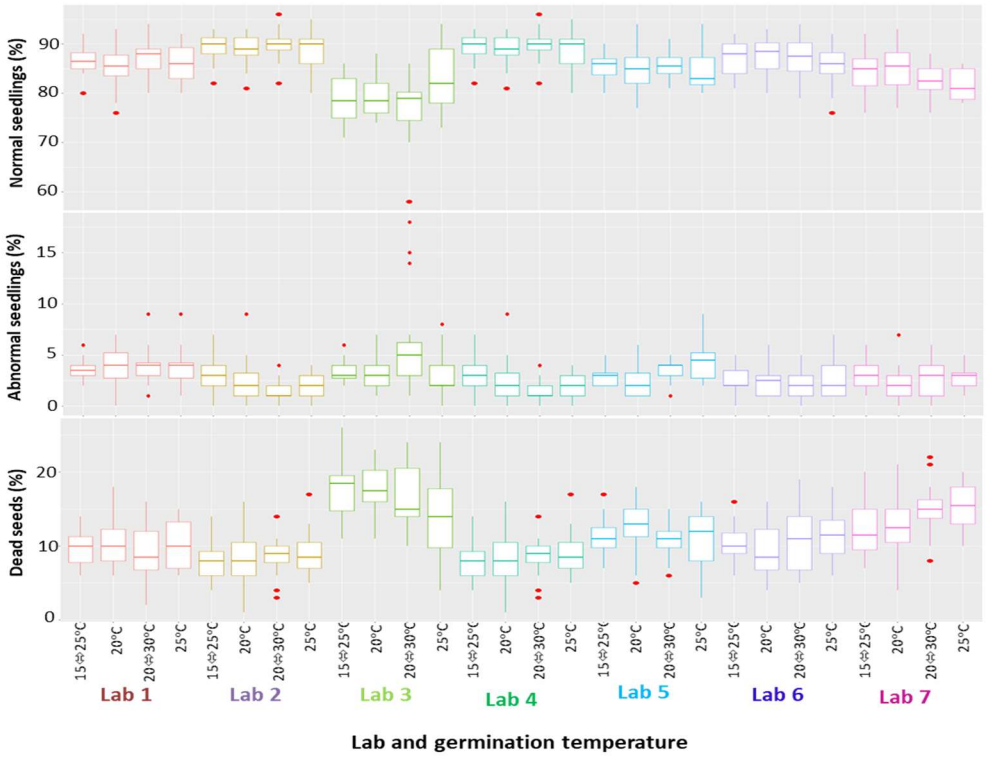

Figure 4 A. Plot box of the percentage results of normal seedlings, abnormal seedlings and dead seeds from seven participating laboratories in four germination temperatures with four seed lots of hybrid bromegrass.

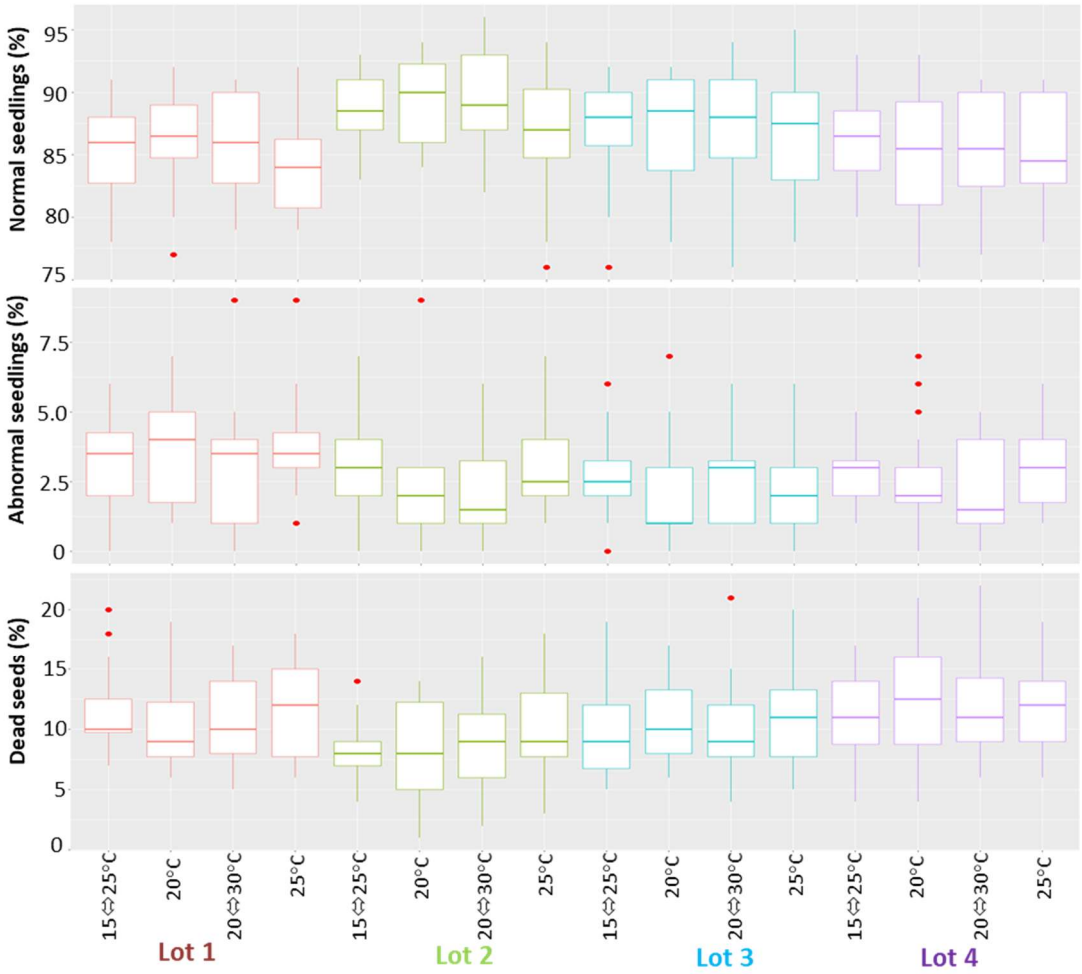

Figure 4 B. Plot box of the percentage results of normal seedlings, abnormal seedlings and dead seeds among four seed lots in four germination temperatures with six participating laboratories in hybrid bromegrass.

Figure 4B showed that the test results of germination have high variations with germination temperatures at 20°C, 20-30°C and 25°C in four lots. Testing results of germination are less variable and abnormal seedlings and dead seeds at 15-25°C in the four lots.

Table 3 showed that the "Lab" caused the largest variation among other factors besides the "seed lot" that was selected to be different for the study.

Table 3. Analysis of variance of normal and abnormal seedlings and dead seeds results of four different seed lots of hybrid bromegrass subjected to four germination temperatures at six different laboratories.

Source of variance	df	Mean Square		
		Normal	Abnormal	Dead seeds
temperature	3	26.91	5.95	19.58
Seed lot	3	190.41**	22.43**	142.13**
temperature*seed lot	9	6.49	3.33	8.27
lab	5	324.67***	23.84*	269.26***
lab*temperature	15	10.25	3.43	11.43
lab*seed lot	15	23.74*	3.37	17.50**
lab*temperature*seed lot	45	11.55	3.73**	7.36

*significant at p =0.05, ** significant at P= 0.01, *** significant at P =0.001.

The accuracy of germination at different temperatures

Fig 5 showed that the average germination were not significantly different, similar to the results of in-house studies (see Figure 3).

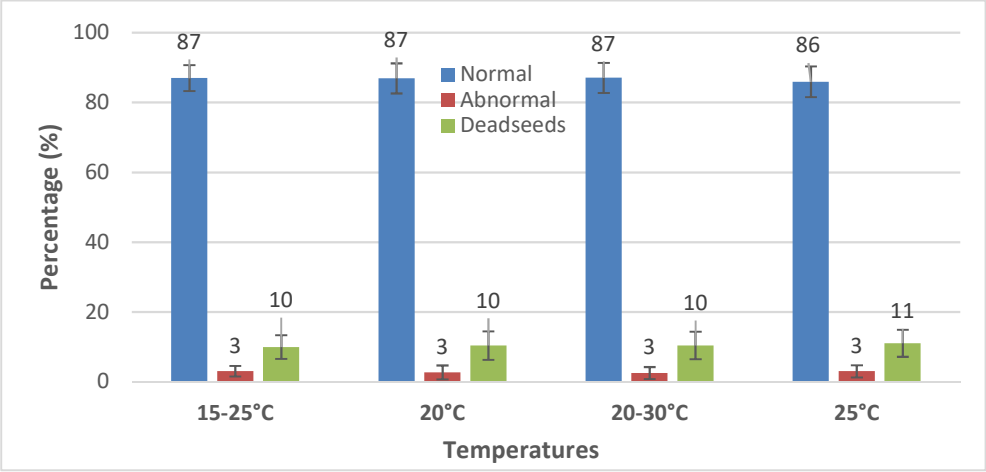

Figure 5. Average results from six participating laboratories in the percentage of normal and abnormal seedlings and dead seeds of hybrid bromegrass in four germination temperatures with four seed lots.

The repeatability and reproducibility were consistently performed better with lower variations in 15-25°C comparing to other temperatures in normal and abnormal seedlings and dead seeds (Figure 6). The reproducibility is particularly important for a method uniformity among different

testing laboratories since it is a largest factor for the testing variation (Table 3).

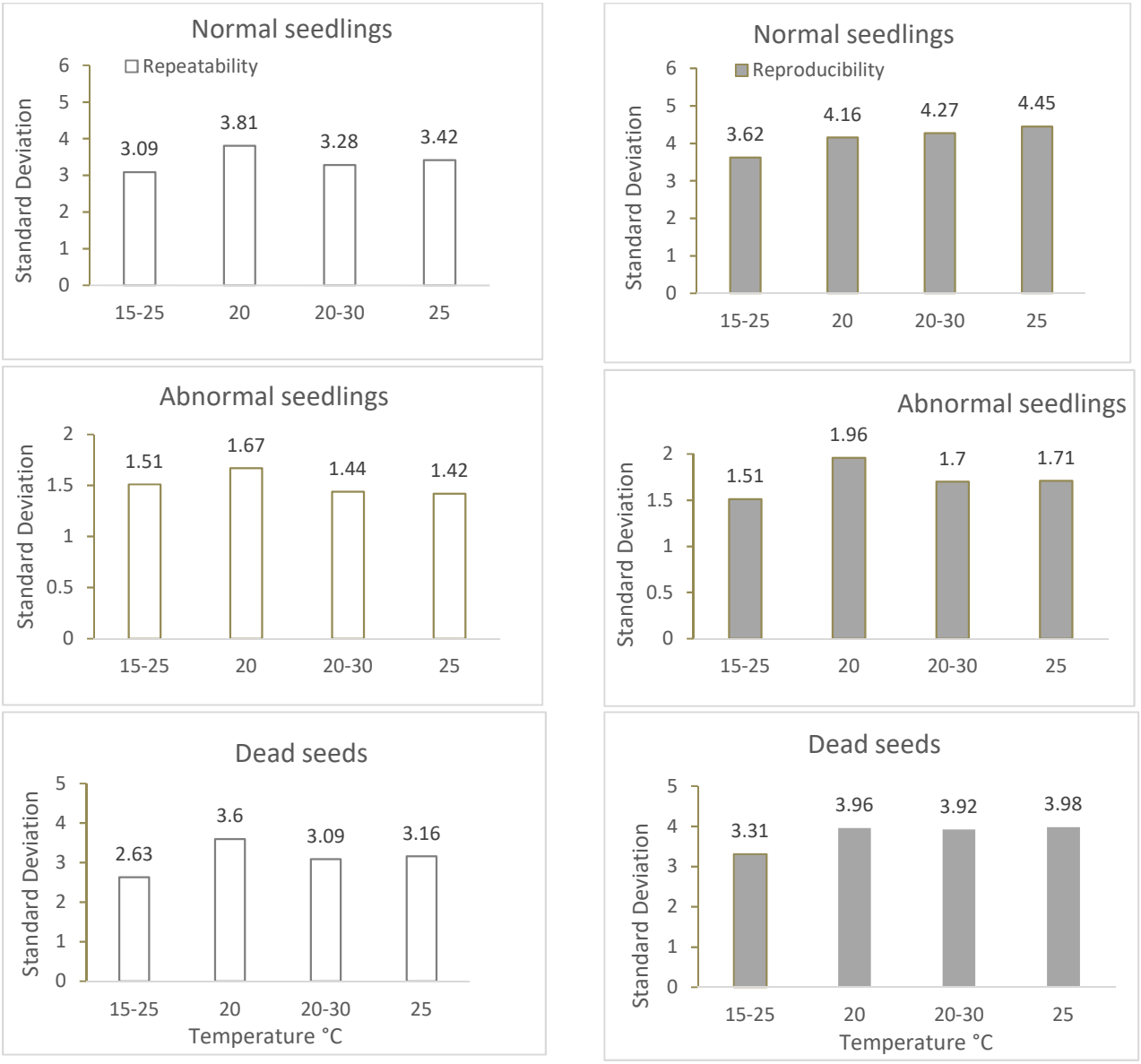

Figure 6. The repeatability □ and reproducibility■ measured by the standard deviations of testing results under four temperatures from six participating laboratories in hybrid bromegrass. Four seed lots were used with germination ranges from 82-91% based on pre-tests.

Conclusion

The results from in-house study and inter-laboratory studies showed that hybrid bromegrass can have equivalent germination at a wider germination range with no requirement of dormancy breaking measures. The reproducibility and repeatability of the inter-laboratory study showed that tests under 20°C, 20-30°C and 25°C had higher variation among laboratories in normal and abnormal seedlings and dead seeds than that under 15-25°C. Therefore 15-25°C with the final count at 14 days was determined as the optimum and reliable method for the hybrid bromegrass, which is different from one of their parental species in the AOSA rules.

Reference:

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