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Method Validation Reports on Rules Proposals for the International Rules for Seed Testing 2021 Edition

ISTA Validation study for moisture content test of *Carica papaya* L.

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Summary

The objective of this validation test is to introduce a moisture content determination method for *Carica papaya L.* into Chapter 9 of ISTA Rules. The experiment was carried out by 10 ISTA accredited laboratories and 1 seed company (Known-You Seed co., LTD) using 2 seed lots (A and B) with 2 moisture levels (L and H). The Statistical analysis showed that the moisture content determined by both ground seed and whole seed methods have comparable variance and are not significantly different, which suggested that both methods are acceptable for moisture determination in papaya seeds. In addition, as both methods show high repeatability and reproducibility in moisture determination and all participating laboratories had high repeatability, the accuracy of the validation study is reliable. The proportion of results with a difference of 0.3% or greater between the test results for ground seed and whole seed were 54.5%, 81.8%, 81.8% and 90.9% for AL, AH, BL, and BH seeds respectively.

The standard deviations obtained for ground seeds were larger than those obtained when the whole seed method was used. We suggest that this is because *C. papaya* seeds are very oily and after grinding particles tend to stick together and are then retained in the grinding mill. We therefore recommend that the whole seed method is the most appropriate method for *C. papaya*.

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Introduction

Papaya is an important tropical fruit crop, and the seeds classified as tree/shrub species. Rules for moisture testing in ISTA relevant to papaya seeds have not yet been developed. Papaya seeds have been classified into the category of tree and shrub species (ISTA rule Table 9A Part 2). The seeds of papaya have intermediate storage behaviour, and the moisture content of papaya seeds is higher than 65% after removing the sarcotesta at harvest. Such high moisture content prevents grinding. Commercially, papaya seed moisture content is dried to approximately 10 %. As a result, the texture of the papaya seed coat becomes loose and brittle after drying, which means that it becomes impossible to meet the standards required for coarse grinding. The ground seed does meet the fine grinding standard; however, due to the high oil content of the seeds (approximately 30%) the ground particles tend to stick to and are retained within the grinder. In addition, the oil content of dry papaya seeds is approximately 30% which might influence the quality of grinding (Syed *et al.*, 2011). For the reasons mentioned above, papaya seeds probably belong to the group of seeds for which the grinding method should not be applied. However, testing the necessity for grinding is compulsory for introducing *Carica papaya* L. into the ISTA rules. This validation study therefore tests the necessity for fine grinding against the whole seed method.

Material and methods

Seed material

Two lots (A and B) of *Carica papaya* L. seeds were obtained from fruits of 'Tainung no. 2', and the moisture content was 67% to 70% at harvest. For each seed lot two moisture levels were assessed, high level (10%-12%) and low level (5%-7%). Each seed lot was placed in a dehumidifier at 40°C to decrease the moisture content. For each seed lot, a total of 1800g of seeds were used, which was then split into two sub lots for each moisture level. At the end of the moisture adjustment each sub lot was further subsampled into 45 smaller packets and placed into sealed foil bags (11*19 cm). Among the 45 packets, 10 were for homogeneity testing, 33 for the participating labs, and 2 for after-all check.

A. 2 seed lots x 2 moisture x 10 packets = 40 for verification of moisture homogeneity
B. 2 seed lots x 11 labs x 2 moistures x 3 (whole seed, grinding and 1 extra) = 132 for distributing to the participating labs
C. 2 seed lots x 2 moisture x 2 packets = 8 for after-all check

The homogeneity of the seed lots was assessed by determining the moisture of 10 randomly selected packets for each seed lot at each moisture level. Only the whole seed method was used to determine the moisture content.

Participating laboratories

Samples were sent to 11 laboratories as listed in Table 1. All laboratories returned data sheets by the end of 2018 with the exception of the laboratory in Zambia (ZM01) due to a delivery problem.

Table 1. The participants of the validation test.

Participant	Country
Gerarda de Boer	The Netherlands
Baymolo Goma	Zambia
Axel Göeritz	Germany
Craig McGill	New Zealand
Laura Bowden	United Kingdom
Céline Herbert	France
Papassorn Wattanakulpakin	Thailand
Tapanee Attamangkune	Thailand
Junaidi	Indonesia
Greg Lozano/Czarina Mae Realubit	Philippines
Yu-Ling Li	Separate Custom Territory of Taiwan
Wen Ju Yang/TSIPS	Separate Custom Territory of Taiwan

Testing the necessity for grinding

The whole seed method was compared with the reference method, fine grinding method. Total time of grinding must not exceed 2 min. The moisture contents of whole seeds and ground seeds was determined by low constant temperature at 103˚C for 17 hours. Comparative test participants were asked to check that their grinding mill and oven met the qualifications of ISTA rules 9.1.4.1 and 9.1.4.2. Since the moisture content in this study was less than 12% and the thousand seed weight of *C. papaya* is less than 200g, the tolerance between two replicates is 0.3% according to ISTA rules Table 9B.

Statistical analysis of the results

The moisture data submitted by the participating laboratories was analysed to assess the repeatability and reproducibility of the method. Statistical analyses were supported by Bo-Jein Kuo, PhD -- member of the ISTA Statistics Committee.

Results

Moisture content determined for confirmation of homogeneity

The moisture content was determined using whole seeds. The average moisture contents of Lot A seeds were 6.5±0.05% and 11.6±0.08% for the low (AL) and high (AH) seeds respectively, and the largest differences between samples was within the tolerance range of

0.3% (Table 1). Therefore, the homogeneity of Lot A seeds was confirmed. Lot B seeds were also confirmed to be homogeneous. The average moisture contents of Lot B seeds were 6.1±0.05% and 10.6±0.07 % for the low (BL) and high (BH) seeds, respectively. The difference between the low and high moisture levels were 5.2% and 4.5% for lot A and B seeds, respectively. A t test showed the high and low moisture levels to be significantly different ($p < 0.01$) in both seed lots. The homogeneity and moisture levels of both seed lots was sufficient to proceed with the validation study.

Table 1. Homogeneity test of papaya seed moisture content by low constant temperature oven method. There are two moisture levels, high (H) and low (L) for Lot A and Lot B seeds. Whole seed method was used to determine the moisture content, and the individual data averaged over the duplicate working sample according to ISTA rules 9.1.6.2.

Lot	Moisture (%)	Sample number										Mean ± s.d.
		1	2	3	4	5	6	7	8	9	10	(%)
Lot A	High	11.7	11.7	11.6	11.5	11.6	11.7	11.6	11.5	11.7	11.6	11.6±0.08
	Low	6.5	6.4	6.5	6.4	6.5	6.5	6.4	6.5	6.5	6.6	6.5±0.06
Lot B	High	10.5	10.6	10.5	10.6	10.6	10.6	10.5	10.6	10.6	10.4	10.6±0.07
	Low	6.0	6.1	6.1	6.1	6.0	6.1	6.2	6.1	6.1	6.0	6.1±0.06

Moisture content determined by the participating laboratories

There were differences among the participating laboratories in the moisture content determined using ground seed versus whole seed for the two moisture levels of both seed lot, but the differences were not significant (Table 2). The analysis of the pooled data was not significantly different between methods ($p=0.94$) (Table 3). In addition, a paired sample t-test showed that the moisture determined by ground seed and whole seed methods of each laboratory was not significantly different ($p= 0.39$) (Table 4).

Table 2 T-test for difference between means of each moisture level of both seed lots obtained by ground seed and whole seed methods.

Seed	Method	Mean	Variance	t-test	p value
AL	Ground seed	7.04	0.09	1.86	0.08
	Whole seed	6.84	0.04		
AH	Ground seed	11.68	0.15	-0.94	0.36
	Whole seed	11.80	0.03		
BL	Ground seed	6.45	0.06	2.11	0.05
	Whole seed	6.26	0.03		
BH	Ground seed	10.49	0.12	-1.05	0.31
	Whole seed	10.61	0.03		

Table 3 T-test for difference between means of the pooled data obtained by ground seed and whole seed methods.

Method	Mean	Variance	t-test	p value
Ground seed	8.92	5.14	0.08	0.94
Whole seed	8.88	5.81		

Note: The variances determined by ground seed and whole seed methods were not significant different by F-test.

Table 4 Paired sample t-test for difference of moisture content determined between ground seed and whole seed methods. Each laboratory contained 4 pairs of data, and the 11 laboratories generated 44 pairs in total.

	df	Mean	t-test	p value
Difference	43	0.038	0.87	0.39

Tables 5 and 6 summarize the results of both seed lots. In the whole seed method, the standard deviation was within the tolerance range (0.3%) and the BoxPlot graph showed no outliers in either seed lot (Figure 1). The standard deviation obtained by grinding method was larger than by whole seed method and only the standard deviation of BL seeds was smaller than tolerance range. In addition, the only outlier was also determined by grinding method. These data revealed that moisture content obtained by grinding method was not as stable as for the whole seed method.

The proportion of results with a difference of 0.3% or greater between the ground seed and whole seed methods were 54.5%, 81.8%, 81.8% and 90.9% for AL, AH, BL, and BH seeds respectively (Tables 5 and 6).

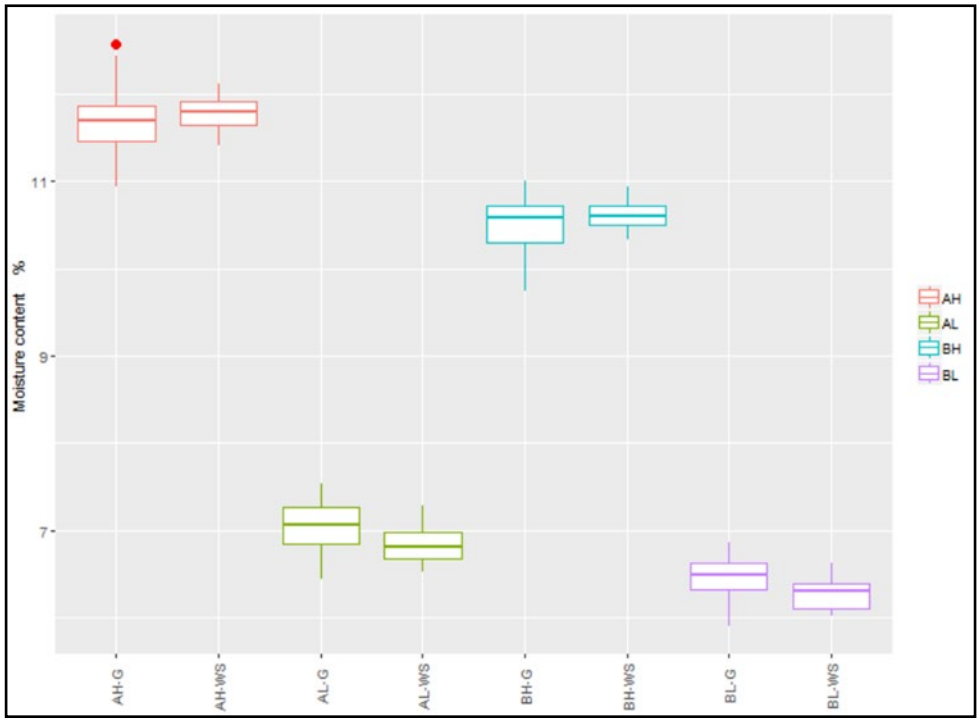

Figure 1 BoxPlot of the tested data. The moisture content was determined by low constant temperature oven method. Each moisture level using whole (WS) and finely ground (G) seeds.

Table 5 The moisture content of Lot A seeds determined by low constant temperature oven method. Each moisture level using whole and fine grinded seeds. **Data presented was the average of the duplicate working sample. The bold numbers represent results where the difference between whole and finely ground seeds was out of tolerance (> 0.3%).**

Seed Lot A Moisture (%)	Treatment	Lab 1	2	3	4	5	6	7	8	9	10	TW01 (AF)	Mean±s.d. (%)
Low (AL)	Whole seed	**6.8**	7.3	6.8	7.0	**7.0**	**6.7**	**6.8**	6.7	6.9	6.5	**6.6**	6.8±0.22
	Ground seed	**7.2**	7.2	6.5	7.0	**7.5**	**7.1**	**7.4**	6.7	7.1	6.7	**7.0**	7.0±0.30
High (AH)	Whole seed	11.8	12.1	11.7	11.9	**12.0**	**11.7**	11.9	11.7	11.9	11.5	11.7	11.8±0.17
	Ground seed	11.5	11.8	11.8	11.7	**12.5**	**11.0**	11.9	11.8	11.8	11.2	11.5	11.7±0.30

TW01(AF) was the result of afterward test.

Table 6 The moisture content of Lot B seeds determined by low constant temperature oven method. Each moisture level using whole and fine grinded seeds. **Data presented was the average of the duplicate working sample. The bold numbers represent results where the difference between whole and finely ground seeds was out of tolerance (> 0.3%).**

Seed Lot B Moisture (%)	Treatment	Lab 1	2	3	4	5	6	7	8	9	10	TW01 (AF)	Mean±s.d. (%)
Low (BL)	Whole seed	**6.3**	6.6	6.1	6.4	6.5	**6.0**	6.3	6.0	6.4	6.0	6.2	6.3±0.21
	Ground seed	**6.7**	6.8	6.0	6.3	6.6	**6.5**	6.5	6.3	6.6	6.2	6.4	6.5±0.23
High (BH)	Whole seed	10.7	10.7	10.5	10.8	10.9	**10.5**	10.6	10.4	10.7	10.3	10.5	10.6±0.18
	Ground seed	10.7	10.8	10.2	10.7	10.9	**9.8**	10.5	10.5	10.6	10.0	10.5	10.5±0.34

TW01(AF) was the result of afterward test.

Analysis of the Repeatability and Reproducibility of the methods

Table 7 contains the estimates of variance composition, repeatability and reproducibility standard-deviations for each method. The estimates were calculated by using Linear Mixed model. The results indicated that the repeatability and reproducibility was very high in both methods. Table 8 gives the repeatability estimates for each laboratory. The results also revealed that repeatability and reproducibility was higher when the moisture content was determined by the whole seed method.

Table 7 Estimates of the repeatability and reproducibility standard-deviations by using ground seed and whole seed methods.

Method	Random Effect	Variance Composition	% of Total	Repeatability standard-deviation	Reproducibility standard-deviation
Ground seed	Lab	0.0535	49	0.0901	0.3300
	Lab*Sample	0.0473	43		
	Residual	0.0081	7		
Whole seed	Lab	0.0302	86	0.0502	0.1880
	Lab*Sample	0.0026	7		
	Residual	0.0025	7		

Table 8 Estimate of the repeatability standard-deviation for each laboratory by using ground seed and whole seed methods.

Laboratory	Repeatability standard-deviation	
	Ground Seed	Whole Seed
01	0.1066	0.0357
02	0.0881	0.0414
03	0.0902	0.0803
04	0.0385	0.0490
05	0.0863	0.0464
06	0.0524	0.0356
07	0.0993	0.0247
08	0.1053	0.0539
09	0.0933	0.0518
10	0.0928	0.0599
11	0.1096	0.0522

Discussion and recommendations

The results of this validation study demonstrate that moisture contents determined by the ground seed and whole seed methods were not significantly different. The results show that there is no need to grind papaya seeds prior to moisture determination. Since both methods show high repeatability and reproducibility in moisture determination and all participating laboratories have high repeatability (Tables 7 and 8), the accuracy of the validation study is reliable.

The moisture content of papaya seeds is higher than 65% at harvest, and the moisture content of commercially sold papaya seed is approximately 10%. The drying process causes the seed coat to become loose and brittle, which prevents coarse grinding as particles fail to meet the standards for coarse grinding (ISTA Rule 9.1.5.4). Therefore only fine grinding was tested against the whole seed method in this study.

The statistical analysis showed that both ground seed and whole seed methods for moisture content obtained comparable variance, suggesting that both methods are acceptable for moisture determination in papaya seeds. However, the difference of the paired data within tolerance range (0.3%) was 54.5%, 81.8%, 81.8% and 90.9% (Tables 5 and 6). Low moisture level seeds of Lot A did not exceed 75%. The two methods could not be treated as identical methods.

The oil content of dry papaya seeds is approximately 30% (Syed et al, 2011), which might influence the quality of grinding (Nijènstein, 2008). In the current test, we observed that following grinding particles remained stuck to the grinder surface. This might explain why the variance obtained when using ground seeds is larger. In addition, the only outlier observed was obtained by the ground seed method (Figure 1). The results suggested that the whole seed method is much more stable than the ground seed method.

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References
Syed, H.M, S P Kunte, B.A. Jadhav, and R. V. Salve, 2011. Extraction and characterization of papaya seed oil. Elixir Agriculture 37A 4102-1405.
Nijènstein Harry. 2008. Grinding in ISTA moisture testing. <https://www.seedtest.org/upload/cms/user/GRINDINGINISTAMOISTURETESTING2008-09-14.pdf>

Validation of temperatures used in germination test of Brassica napus

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Summary
An ISTA validation study on the germination temperatures for *Brassica napus* was carried out to provide scientific data and evidence for rule proposals and harmonization. Eight ISTA-accredited laboratories in five countries participated in the study. Six seed lots varying in varieties, spring and winter types, and germination levels were used for the study. The germination tested at 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 conducted either on top of papers or between papers with 8 hr light during the high-temperature phase without dormancy break treatments. Counts of normal and abnormal seedlings and ungerminated seeds were collected according to ISTA rules at 7 days. The data were analyzed using statistical programs of SAS, ISTAgermMV, and z-scores to compare the germination results in four temperatures. The study showed the largest variation source was among laboratories excluding seed lot, which was a designed factor. It was significantly different on ungerminated seeds among temperatures. The percentages of ungerminated seeds were lower at 15<=>25°C and 20°C. The testing repeatability and reproducibility at 20°C and 15<=>25°C had a lower standard deviation, therefore better performance. The Z-scores, the variation of the germination results from means, at 20°C and 15<=>25°C were lower in the percentages of normal seedlings, abnormal seedlings, and ungerminated seeds. Testing results at 20°C and 15<=>25°C also had higher accuracy for normal seedlings and ungerminated seeds. In summary, the data supported that the tests at 20°C and 15<=>25°C result in higher normal seedlings and lower ungerminated seeds. More importantly, these two temperature regimes will enhance testing reproducibility among labs and the harmonization among testing rules.

Introduction

Seed germination and seedling development is influenced by the environmental factors such as seed-bed, besides internal factors, e.g., seed quality under the test. Temperature, moisture and air exchange (i.e., O2) are the main external factors that will determine seed germination and seedling development. Different species and cultivars will respond to germination temperatures differently, that is, base temperatures and optimum temperatures are characteristics of a given species or cultivar (Kamkar et al., 2012; Gilbertson et al., 2014; Andreucci et al., 2016; Derakhshan et al., 2018). In a germination test, when moisture is adequate, both the rate and percentage of germination of viable seeds are controlled by germination temperature (Kamkar et al., 2012; Derakhshan et al., 2018). And alternating temperature could reduce dormancy or enhance germination (Qiu et al., 2006).
The testing rules, such as ISTA (International Seed Testing Association) International Seed Testing Rules, aim to achieve testing uniformity; i.e., reproducible and repeatable results will be generated from different labs or analysts for the same seed lots. Seed testing using standardized methods facilitates seed trade domestically and internationally, as well as being used for seed certification and regulatory enforcement. With a comparison of current seed testing rules or methods described by ISTA, AOSA and Canadian M&P, all three testing rules provide different environment conditions for germination test of canola, *Brassica napus* var. *napus.* The different 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).
The objectives of this validation study on the four germination temperatures in *Brassica napus* was: 1) to provide data as supporting evidence for proposals of testing rule amendment in *Brassica napus*; 2) to promote testing rule harmonization; and 3)to ensure the accuracy and equivalency of the germination methods .

Materials and Methods

Testing samples selection and preparation
Multiple seed lots with significantly different quality were used to evaluate the temperature impact. Six seed lots in six varieties of *B. napus* (rapeseed) were sourced from Canada and Germany, including both spring and winter type. Three levels of germination ranged from 80-95% were selected based on pre-tests. The low

germination level was about 80-85%, the medium level was about 86-90%, and the high level was about 91-95%.
Each selected seed lot was mixed and divided according to ISTA procedures into approximately 5 g sample quantity. The total 56 samples were prepared, including samples for homogeneity test (10), extra samples (10) for potential retest requests and testing samples for 4 temperatures with eight participating labs (36). Prepared samples were randomly assigned through Microsoft Excel function for homogeneity test, four temperatures and participating laboratories. The tolerance was calculated using ISTA PT program.

Testing methods

Four temperatures including two alternating temperatures, 20<=>30°C and 15<=>25°C and two constant temperatures 20°C and 25°C were compared for germination following ISTA rule for seedling evaluation. From our pre-test and multiple-laboratory referee study, we found the variation among laboratories was larger than the variation among temperatures. As TP and BP are two substrata allowed in the three testing rules for canola and each participant has their own preference during routine test. With a request, we allowed TP or BP to be used to accommodate their available substrate, which was a modification from the testing plan. To minimize testing variation, the method for conducting the validation study was standardized (Table 1).

Table 1. Germination method of the validation study of temperature comparison in *B. napus*

Temperature (°C)	Number of seeds x rep	Substrate	First count (d)	Final count (d)	Breaking dormancy	Light
20<=>30	100 x 4	TP/BP	5	7	None	Light 8 hr/dark 16 hr
15<=>25	100 x4	TP/BP	5	7	None	Light 8 hr/dark 16 hr
20	100 x4	TP/BP	5	7	None	Light 8 hr/dark 16 hr
25	100 x4	TP/BP	5	7	None	Light 8 hr/dark 16 hr

Participating laboratories

Participating laboratories in the validation study were confirmed that met the following qualification:

- ISTA accredited laboratories with the accreditation scope of the germination in *Brassica napus.*
- Expressed willingness and accept the obligation to conduct the tests required and to provide data for the validation study.
- Have the skilled personnel, appropriate facilities and equipment recommended for performing the test.

Eight laboratories from multiple locations in Canada, USA, France, Sweden, and Scotland participated in the validation study. The participating laboratories tested samples between February 1 and March 30, 2019 following the provided instruction.

Data analysis

A standard data collecting sheet was provided to each participant in order to obtain the same information in the same format. All participants submitted data with normal, abnormal, dead seeds and ungerminated seeds, except for lab 4 which submitted data for normal seedlings only. Since “ungerminated (fresh) seeds” were reported as “0” for all submitted data, here all data presented as sum of dead and fresh as “ungerminated seeds”. The data received from participants was checked and the sum of the normal and abnormal seedlings and ungerminated seeds should be 100 percent; if not, the percentages were calculated. Tolerance of difference among the four replicates for each test was checked using the R package *ISTAgermMV* and only one test in 20< =>30°C out of 192 tests was out of tolerance. This one test was not removed as this was an additional indication of poor repeatability/reproducibility of the method. The outliers were detected using Hampel’s method to identify xi as an outlier if $|Xi - X| > 5.2$ MAD, where MAD = median, but not removed for final analysis, because they were true data from each participant and they were within tolerance.

Significant difference of the four germination temperatures in normal seedlings, abnormal seedlings and ungerminated seeds was evaluated using Generalized Linear Model (GLM) in SAS software at 95% confidence level, with seed lot and temperature as fixed effects and laboratory and replicate as random effects. Analysis of Variance (ANOVA) was generated to detect the variation sources.
Data of normal and abnormal seedlings and ungerminated seeds were analyzed using softwere *ISTAgermMV* in R package following statistical tools “Inter laboratory tests using ISO 5725-2” developed by ISTA statistical committee. Comparison of means among germination temperatures and means among germination temperatures interacted with seed lots were generated. Repeatability and reproducibility were calculated with *ISTAgermMV* program, where, Repeatability quantifies the average variability of results within a laboratory, and
Reproducibility quantifies the average variability among laboratories.
Testing result variation in each participating laboratory was also analyzed using z-scores, which compare the distance of the participants’ results from the overall sample mean of all participants under each temperature for each seed lot. Accuracy is a combination of trueness or bias and precision using z-scores. Average accuracy of the eight participating laboratories over the six seed lots was used to indicate testing accuracy under each temperature. The calculation formula for accuracy was submitted in data analysis sheets.

Results

Variation sources on the testing results of the percentage of normal seedlings, abnormal seedlings and ungerminated seeds

The analysis of variance on the percentage of normal seedlings, abnormal seedlings and ungerminated seeds showed the lab is the biggest variation source for normal and abnormal seedlings(Table 2 and 3). And the temperature was the biggest source significantly (p=0.0094) impacted on the percentage of ungerminated seeds, excluding the designed factor of seed lots (Table 4).

Table 2. Analysis of variance (ANOVA) on the percentage of normal seedlings in four germination temperatures and six seed lots among eight testing laboratories.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
temp	3	455.61	151.87	**2.48**	**0.0889**
lot	5	8155.84	1631.17	21.16	<.0001
temp*lot	15	455.94	30.40	1.66	0.0701
lab	7	7553.88	1079.13	**8.99**	**<.0001**
lab*temp	21	1284.83	61.18	3.35	<.0001
lab*lot	35	2698.13	77.09	4.22	<.0001
lab*temp*lot	105	1920.32	18.29	2.44	<.0001

* significant at P= 0.05 level.

Table 3. Analysis of variance (ANOVA) on the percentage of abnormal seedlings in four germination temperatures and six seed lots among eight testing laboratories.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
temp	3	151.83	50.60	**1.36**	**0.2857**
lot	5	834.34	166.87	2.90	0.0298
temp*lot	15	143.47	9.56	0.69	0.7923
lab	6	5550.61	925.10	**11.46**	**<.0001**
lab*temp	18	668.13	37.12	2.66	0.0012
lab*lot	30	1726.36	57.55	4.12	<.0001
lab*temp*lot	90	1255.94	13.96	2.42	<.0001

* significant at P= 0.05 level.

Table 4. Analysis of variance (ANOVA) on the percentage of ungerminated seeds in four germination temperatures and six seed lots among eight testing laboratories.

Source	DF	Type III SS	Mean Square	F Value	Pr > F
temp	3	236.37	78.79	**5.17**	**0.0094**
lot	5	8155.69	1631.14	93.37	<.0001
temp*lot	15	249.63	16.64	4.07	<.0001
lab	6	490.52	81.75	**2.86**	**0.0226**
lab*temp	18	274.36	15.24	3.72	<.0001
lab*lot	30	524.41	17.48	4.27	<.0001
lab*temp*lot	90	368.53	4.09	1.26	0.0634

* significant at P= 0.05 level.

Temperatures regimes on testing results of germination

Temperatures at 20^{o}C and 15<=> 25^{o}C were tended to have higher normal seedlings (Figure 1) although the difference among temperatures did not have statistically significance. There was significant difference on ungerminated seeds among temperatures, 15-25°C and 20°C had the lower ungerminated seeds (Figure 1). Ungerminated seeds were generally influenced by the germination conditions, and not by the analysts' evaluation.

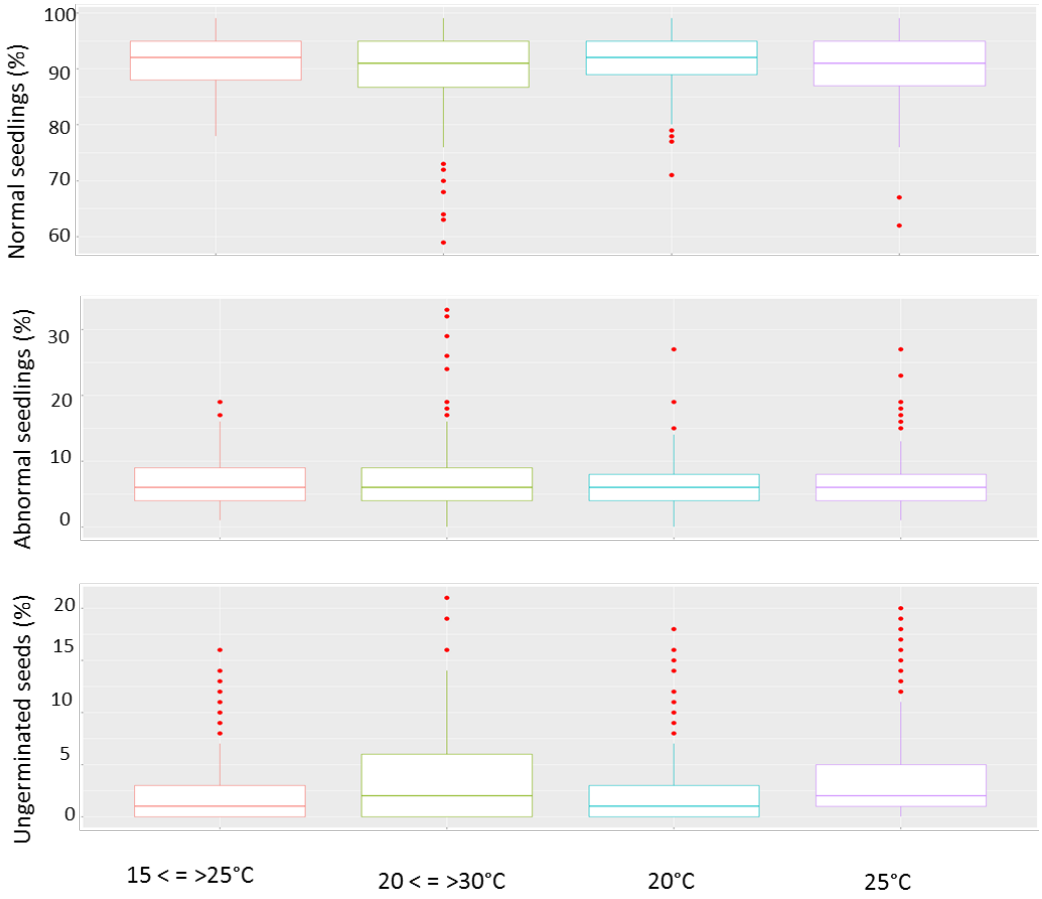

Figure 1. Box plots of normal seedlings, abnormal seedlings and ungerminated seeds as affected by germination temperatures.

Temperature affected normal seedlings, abnormal seedlings, and ungerminated seeds interacting with seed lots (Figure 2). Alternating temperatures had lower ungerminated seeds. For seed lot 6 with the lowest germination percentage among the six seed lots, 15<=> 25°C produced the highest normal seedlings, and the least ungerminated seeds, while 25°C had the opposite results. Germination results were generally consistently superior at temperatures of 15<=> 25°C and 20°C across the six seed lots. The germination results had consistently larger variation in 20<=> 30°C among seed lots.

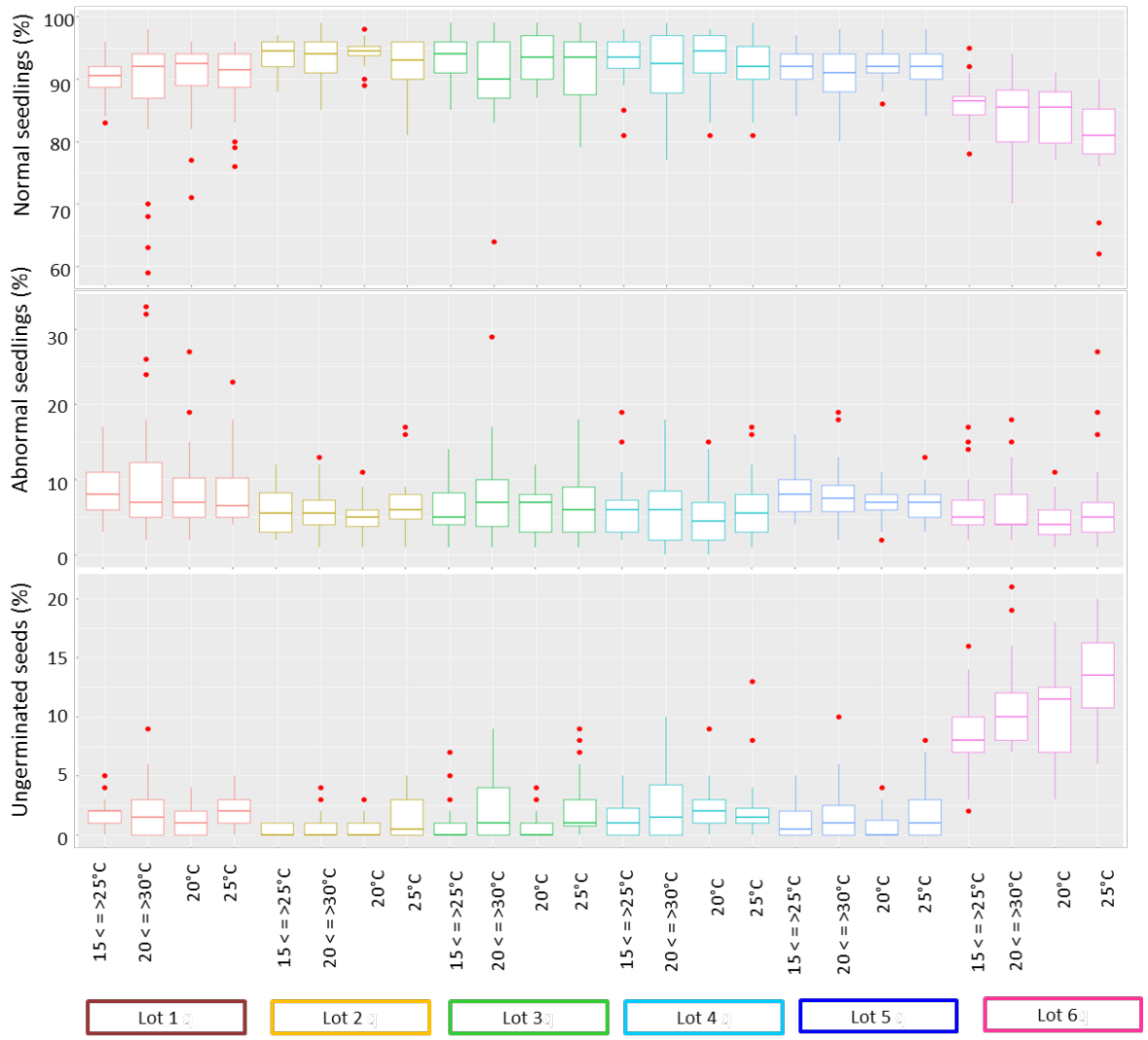

Figure 2. Box plots of normal seedlings, abnormal seedlings, and ungerminated seeds as affected by germination temperatures and seed lots.

Testing uniformity among participating laboratories as affected by the germination temperature

The testing uniformity of participating laboratories was assessed with repeatability, which is an indicator for variation within a lab; and reproducibility, which is an indicator for variation among labs. The statistical values of repeatability and reproducibility were calculated using *ISTAgermMV*.

The repeatability was acceptable for all four temperatures, as the dispersion factors of repeatability on normal and abnormal seedlings and ungerminated seeds were all around 1.00. (Table 5). However, the standard deviation of repeatability is consistently lower in 15<=> 25°C , better performance with in a lab.

The standard deviation of reproducibility among laboratories with all data sets was much lower in 15<=>25°C and 20°C than the other two temperatures for normal seedlings, abnormal seedlings, and ungerminated seeds. Other standard deviations of 15<=> 25°C and 20°C under lab and under lot*lab were also all consistently lower than the other two temperatures (Table 5).

Table 5. Comparison of repeatability and reproducibility results of percentages of normal seedlings, abnormal seedlings, and ungerminated seeds among four germination temperatures over six seed lots

*Please note there are missing data from one lab for abnormal seedlings and ungerminated seeds.

Temperature	Mean	Repeatability SD	Dispersion factor	Reproducibility SD	Lab SD	Lot x Lab SD
			Normal seedlings			
15<=>25°C	91	2.58	0.91	3.69	1.99	1.73
20 <=> 30°C	90	3.13	1.03	6.71	4.79	3.50
20°C	91	2.67	0.95	4.15	2.28	2.21
25°C	90	2.51	0.83	5.17	3.89	2.31
			Abnormal seedlings			
15<=>25°C	7	2.30	0.91	3.62	2.13	1.82
20 <=> 30°C	7	2.69	1.02	5.61	4.10	2.74
20°C	6	2.20	0.92	3.55	2.15	1.77
25°C	7	2.37	0.93	4.90	3.63	2.28
			Ungerminated seeds			
15<=>25°C	2	1.56	1.04	1.86	0.37	0.94
20 <=> 30°C	3	1.80	0.99	2.67	1.31	1.48
20°C	3	1.85	1.13	1.97	0.49	0.46
25°C	4	1.96	1.02	2.59	1.40	0.95

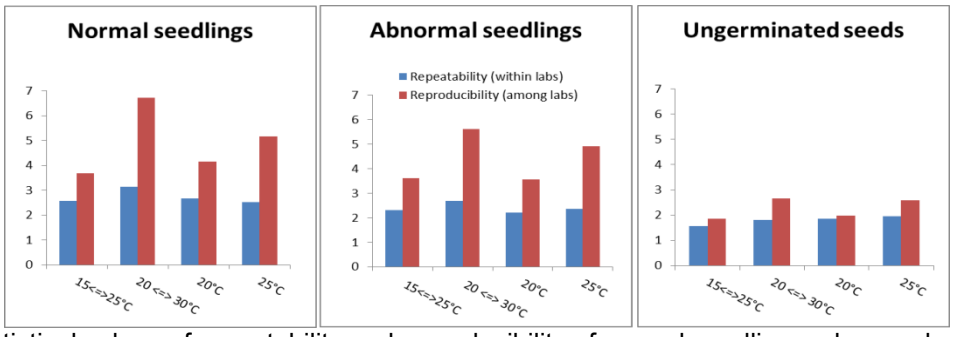

Figure 3. *Statistical values of repeatability and reproducibility of normal seedlings, abnormal seedlings, and ungerminated seeds in each of the four germination temperatures.

*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.
Among four temperatures in Figure 3, the statistical values of reproducibility were the highest at 20<=>30°C for normal and abnormal seedlings and ungerminated seeds, the least reproducible temperature for the germination. The statistical values of 15<=> 25°C and 20°C were lower among four temperatures, i.e., higher repeatability and reproducibility.
Testing result variation among participating laboratories was also analyzed using z-scores, the distance from the means of each lot, under four germination temperatures. Usually the variation of testing results within one standardized deviation, z-score =1, is an acceptable tolerance. Figure 4.1 -3 showed the least variation among participating labs in % normal and abnormal seedlings at 20°C and in %ungerminated seeds at 15°C <=> 25°C.

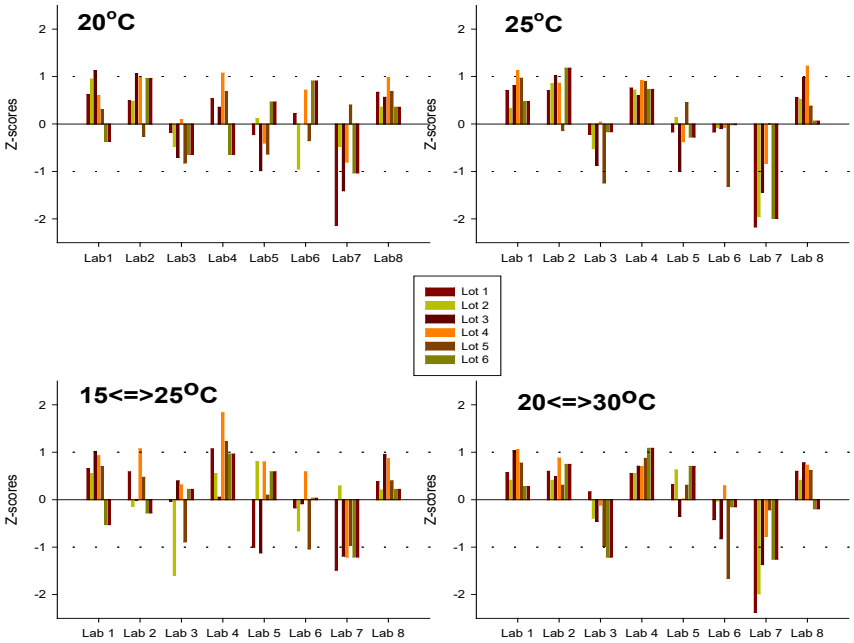

Figure 4.1. Laboratory Z-scores, the variation of each lab from the means, measured for % normal seedlings under each temperature with six seed lots.

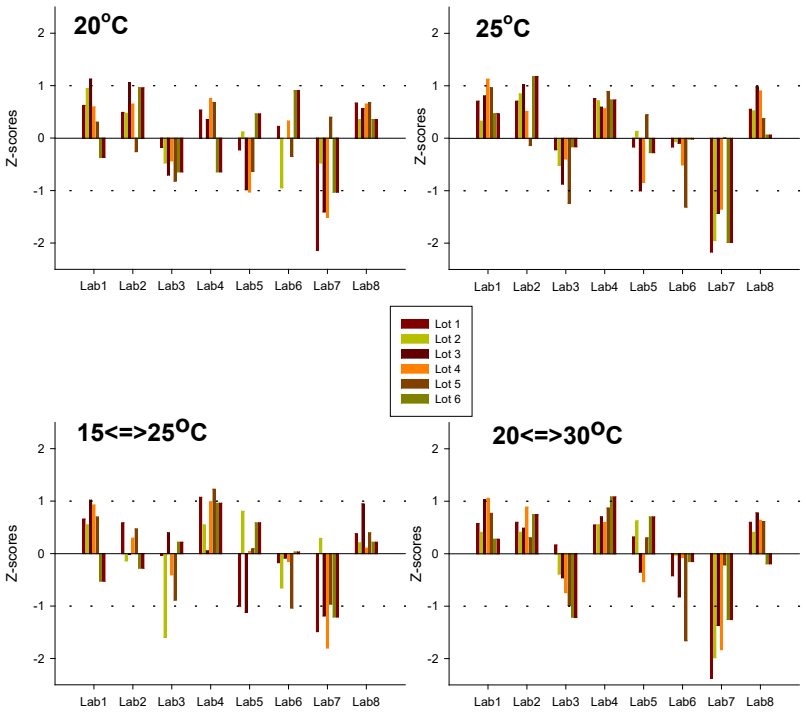

Figure 4.2. Laboratory Z-scores, the variation of each lab from the means, measured for % abnormal seedlings under each temperature with six seed lots

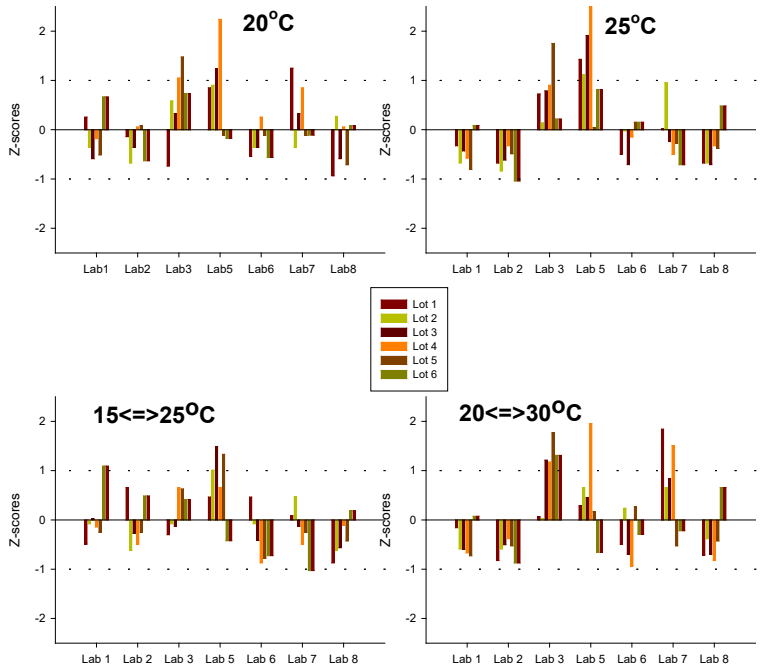

Figure 4.3. Laboratory Z-scores, the variation of each lab from the means, measured for %ungerminated seeds under each temperature with six seed lots.

Testing Accuracy:

The average accuracy values among the participants showed that 20°C and 15<=>25°C had the higher accuracy for the evaluation of normal seedlings, and 20°C had the highest accuracy for abnormal seedlings, and 20°C and 15<=>25°C had much higher accuracy for ungerminated seeds (Figure 5).

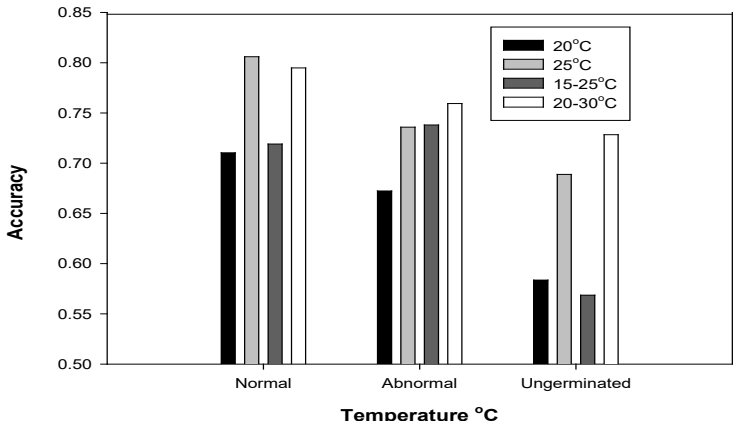

Figure 5. Average accuracy* among four temperatures measured for percentages of normal, abnormal seedlings and ungerminated seeds with six seed lots.

*Note: The smaller the value is, the higher the accuracy will be.

Conclusions and recommendations

The study showed the largest variation source was among laboratories, excluding seed lots as a designed factor. The percentage of normal seedlings tended to be higher at 20°C and 15<=>25°C, although no significant difference was detected among the four temperatures. 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 testing repeatability and reproducibility at 20°C and 15<=>25°C had much low standard deviations than the other two temperatures. Z-score showed that 20°C and 15<=>25°C had less variation from means on the percentages of normal seedlings, abnormal seedlings and ungerminated seeds among seed lots and laboratories. These two germination temperatures also had higher accuracy for the evaluation of seedlings and ungerminated seeds. To promote harmonization among testing rules, we recommend ISTA and AOSA revise testing temperatures for B. napus as 20°C and 15<=>25°C, which will result in higher normal seedlings and lower ungerminated seeds. More importantly, these two temperature

regimes will enhance testing repeatability within a lab, reproducibility among labs, and the harmonization among testing rules, which will ensure the accuracy and equivalency of the germination methods.

Acknowledgements

Organizers sincerely thank ISTA germination committee for approval of the project, testing plan reviewers, Simon Goertz and Zita Ripka for their suggestions and comments, eight participating laboratories for their data contribution, and ISTA Statistical committee, especially Jean-Louise Laffont for data advice and software.

References:

1. Andreucci, M.P., Moot, D.J., Black, A.D. and Sedcole, R. (2016). A comparison of cardinal temperatures estimated by linear and nonlinear models for germination and bulb growth of forage brassicas. European Journal of Agronomy, **81**, 52-63.
2. Association of Seed Analysts (AOSA) Rules for testing seeds, (2019), AOSA
3. Canadian Methods and Procedures for Testing Seed, (2019), Canadian Food Inspection Agency.
4. Derakhshan, A., Bakhshandeh, A., Siadat, S.A., Moradi-Telavat, M-R. and Andarzian, S.B. (2018). Quantifying the germination response of spring canola (Brassica napus L.) to temperature. Industrial Crops & Products, **122**, 195-201.
5. Gilberston, P.K., Berti, M.T. and Johnson, B.L. (2014). Borage cardinal germination temperatures and seed development. Industrial Crops and Products, **59**, 202-209.
6. International Rules for Seed Testing, (2019), International Seed Testing Association (ISTA)
7. ISTA Method Validation for Seed Testing (V.1.0), (2006), ISTA
8. Kamkar, B., Al-Aladhmadi, M.J., Mahdavi-Damghani, A. and Villalobos, F.J., (2012). Quantification of the cardinal temperatures and thermal time requirement of opium poppy (Papaver somniferum L.) seeds to germinate using non-liner regression models. Industrial Crops and Products, **35**, 192-198.
9. Qiu, J., Bai, Y., Coulman, B., and Romo, J.T. (2006). Using thermal time models to predict seedling emergence of orchardgrass (*Dactylis glomerata* L.) under alternating temperature regimes. Seed Science Research **16**, 261–271.

Appendix 1. ISTA Proposal

CURRENT VERSION: Table 5A Part 1. Detailed methods for germination tests: Agriculture and Vegetable seeds

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Brassica napus	BP; TP	20<=>30; 20	5	7	KNO_3 ; Prechill	-	-

PROPOSED REVISION: Table 5A Part 1. Detailed methods for germination tests: Agriculture and Vegetable seeds

Species	Substrate	Temperature* (°C)	First count (d)	Final count (d)	Recommendations for breaking dormancy	Additional directions	Additional advice
1	2	3	4	5	6	7	8
Brassica napus var. *napus*	BP; TP	~~20<=>30;~~ 20 15<=> 25	5	7	KNO_3 ; Prechill	-	-

Validation study to support a modification to the evaluation criteria for normal seedlings of Helianthus annuus L.

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Summary
The objective of this validation study is to modify the root criteria of *Helianthus* seedling evaluation.
The validation study showed no significant differences between seedlings with no defect and with defective primary root with well-developed secondary roots, in latter growth traits such as height of plant, total leaf number, diameter of disk and diameter of flower. A slight difference for only one variety was observed in days to flower.
The results of this validation study indicate that secondary roots of *Helianthus* may compensate for the damage of the primary root and support the modification for the seedling evaluation criteria of *Helianthus* root system from “A-2-1-1-1: the primary root is essential” to “A-2-1-1-2: the primary root may be replaced by secondary roots”.

Introduction
It is prescribed in the ISTA rule that germination substrates of *Helianthus annuus* are BP, TPS, S and O. In the germination test of *Helianthus annuus*, abnormal seedlings with damage to the primary root but with sufficiently well-developed secondary roots are often found, especially in the paper towel test. While on the other hand, few abnormal seedlings are found in the usable plant test. It might be considered that the seedlings with a defective primary root but with well-developed secondary roots can grow sufficiently.
Preliminary experiments conducted in Takii’s laboratory in autumn 2017 and spring 2018 confirmed that the seedlings for which the primary root cut off artificially, but with well-developed secondary roots, grow as well as the intact seedlings. However, this study was not sufficient evidence to modify the criteria as the number of seedlings initially tested was low.
The Germination Committee members recommended to conduct an additional test with a higher number of seedlings tested compared to the preliminary experiment, and with seedlings which have naturally defective primary root, instead of artificially creating the defect. Indeed, a seedling with naturally defective primary root might not have the same physiological capacities as normal seedlings with artificial damaged do. Also, four different types of Sunflower seeds, including edible ones, were tested during the validation study. This additional test was therefore carried out in order to provide sufficient evidence to support the proposed change of group regarding the root criteria of *Helianthus annuus* seedlings.

Material and methods

Seed material
Four samples of *Helianthus annuus* were used in this study. Two of them (1 and 2) are ornamental varieties which are edible, and another two (3 and 4) are oil sunflower varieties.

Lot	Variety name	Type	Production year
1	Sunrich fresh Orange	Cut flower variety	2016
2	Good smile	Bedding and pot variety; Dwarf type	2011
3	Green manure sunflower	Oleic variety	2017
4	Kids smile	Mid-oleic variety; Dwarf type	2016

Methods
Ten replicates of 100 seed samples per each variety were germinated at 20⇔30℃ (16 h / 8 h respectively) using paper towel. Seven days after sowing, normal seedlings and seedlings with defective primary root were selected as follows:
Normal seedling: 16 seedlings
Primary root defected with well-developed secondary root: 8 seedlings

Primary root defected with poorly-developed secondary root: 8 seedlings

For samples of Lots 1 and 3, the seedlings were planted on big planter (inner dimension; 65cm x 35cm x 25cm) with sterilized field soil. For samples of Lots 2 and 4 (dwarf types), the seedlings were planted in pots (diameter; 15cm) with field soil. When fully bloomed, basic traits such as height of plant, total leaf number, days to flower, diameter of disk, and diameter of flower were recorded or measured individually (**Figure 1**).

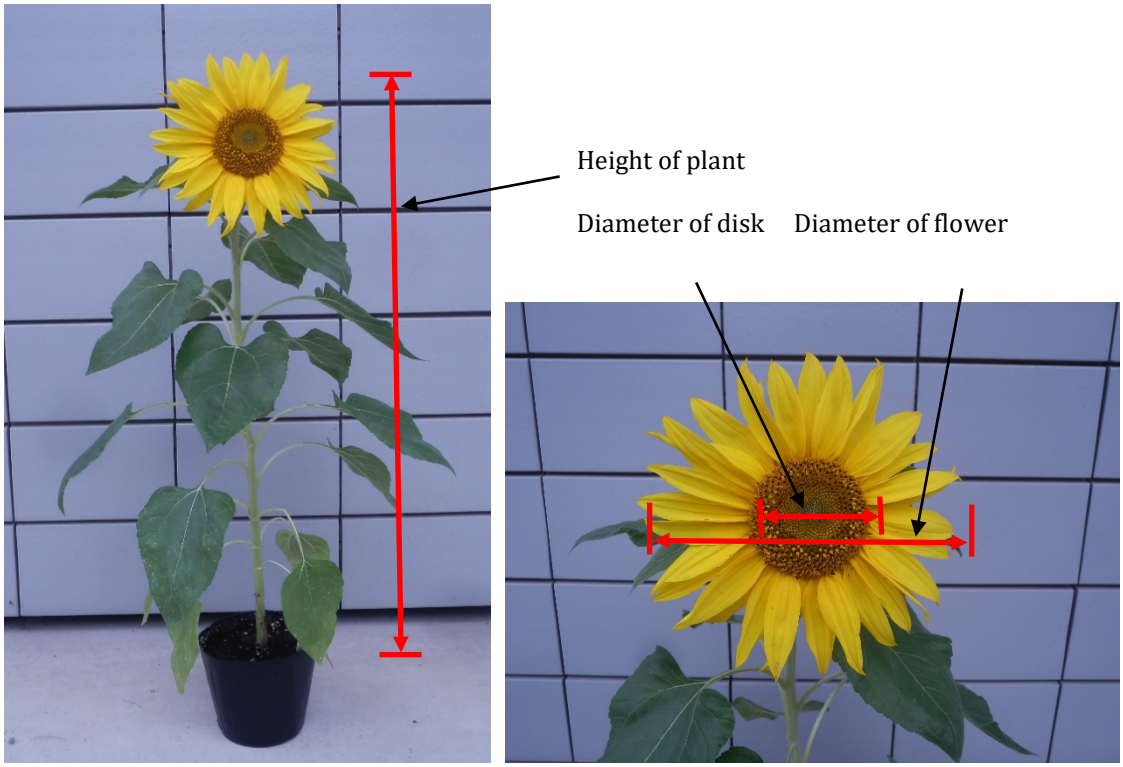


Figure 1. Measurement basis of height of plant, diameter of disk and diameter of flower (Kids smile).

Outline of cultivation

Sowing day: 2018-08-22
Planting day: 2018-08-29
Research finished: 2018-11-13

After planting, plants were cultivated in the greenhouse. Cultural environment was as follows:

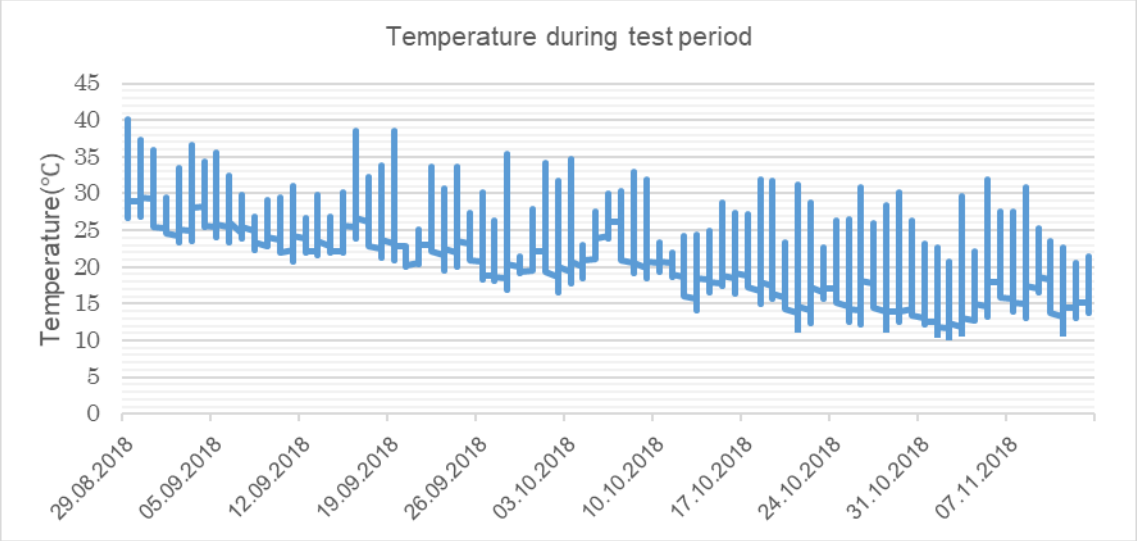


Figure 2. Temperature during test period

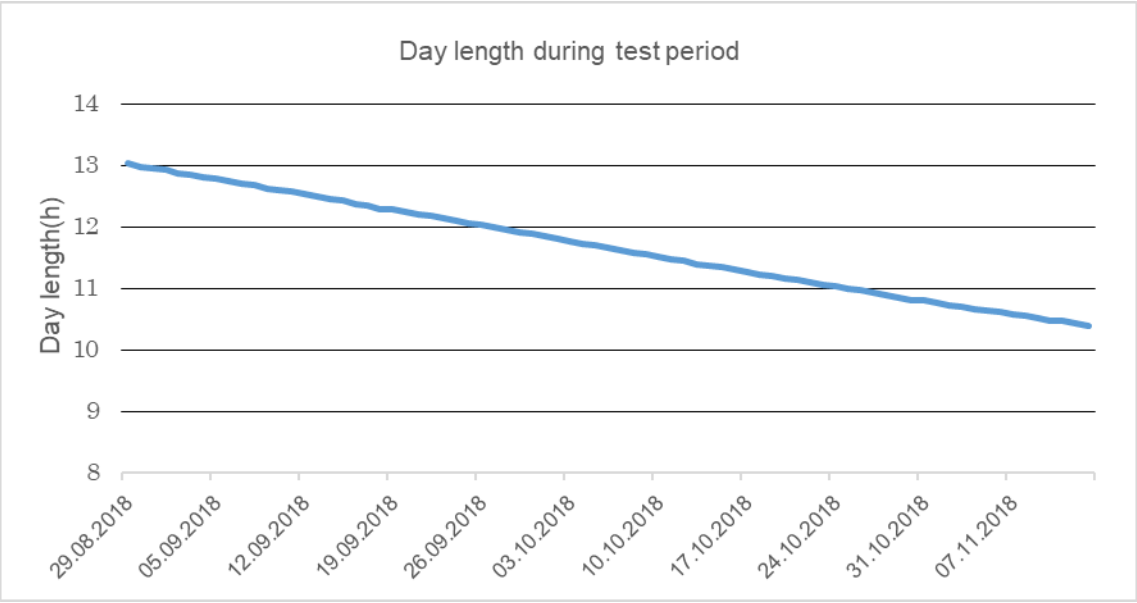


Figure 3. Day length during test period

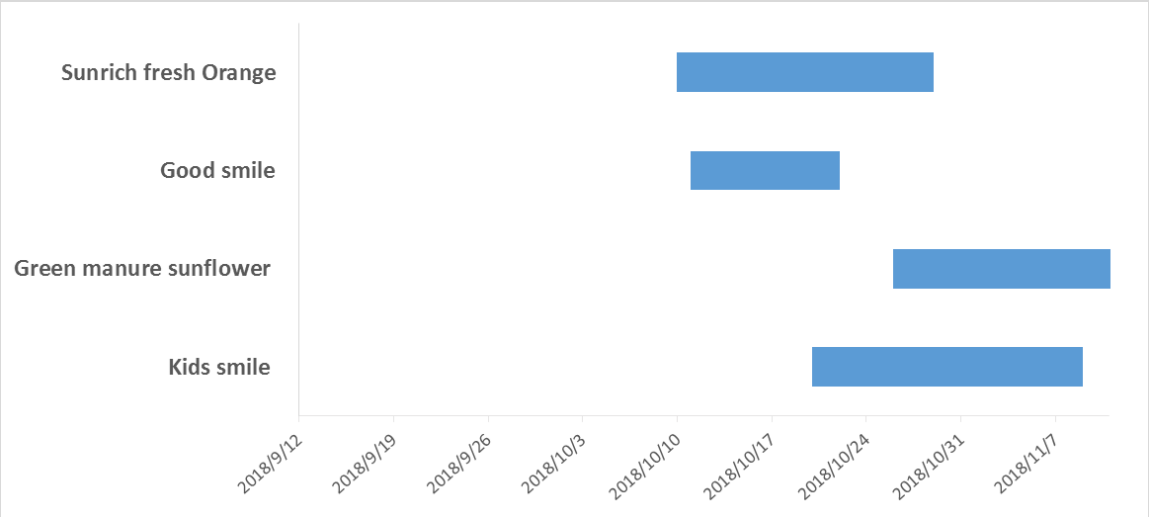


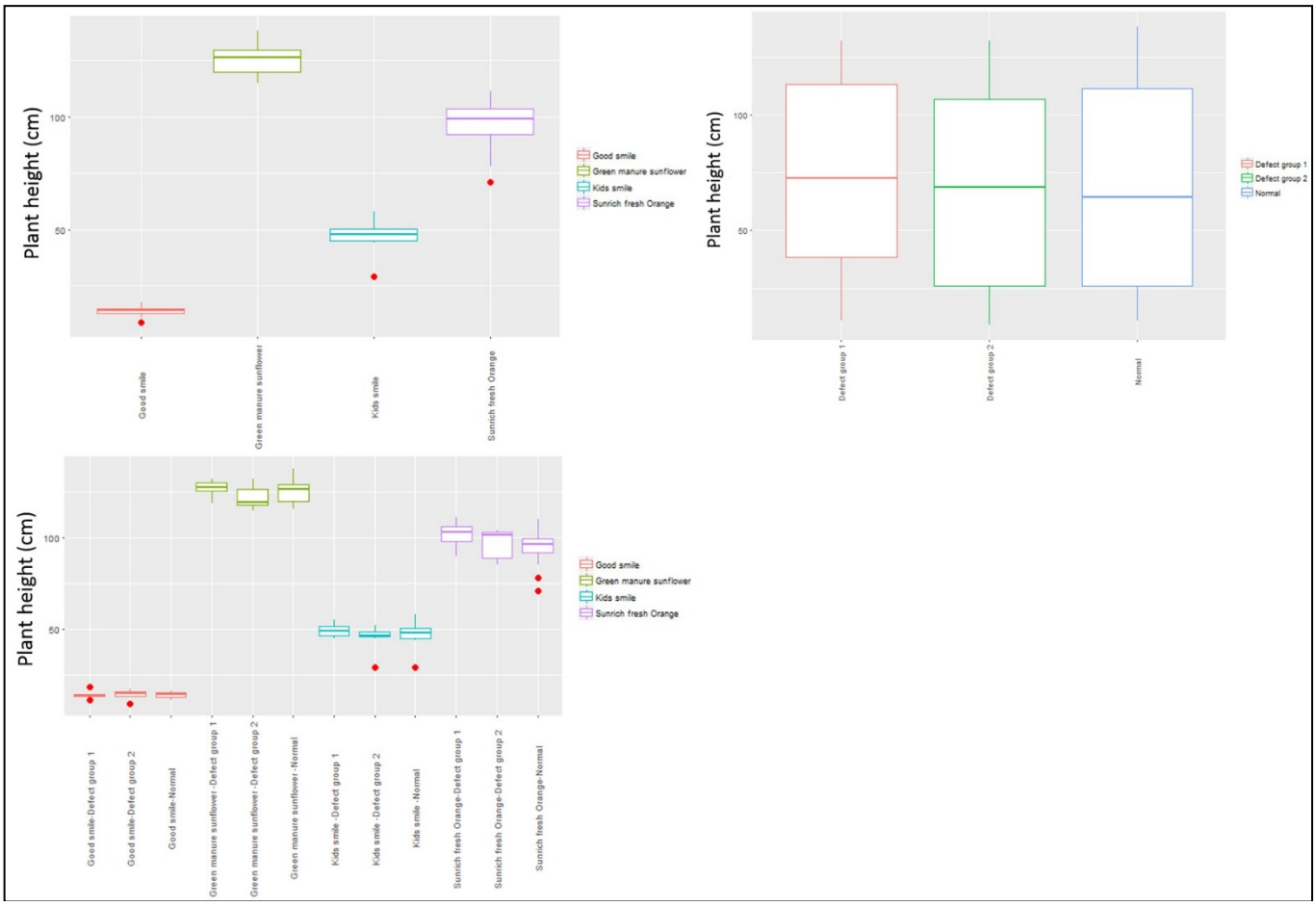
Figure 4. Period of days to flower per varieties

Statistical analysis

Measured data was checked to see whether the traits measured showed a significant difference between normal seedlings and seedlings which primary root defected with well-developed secondary roots, and between normal seedlings and seedlings which primary root defected with poorly-developed secondary roots.
The analysis was performed by the ISTA Statistics Committee.

1. Data exploration with side-by-side box plots

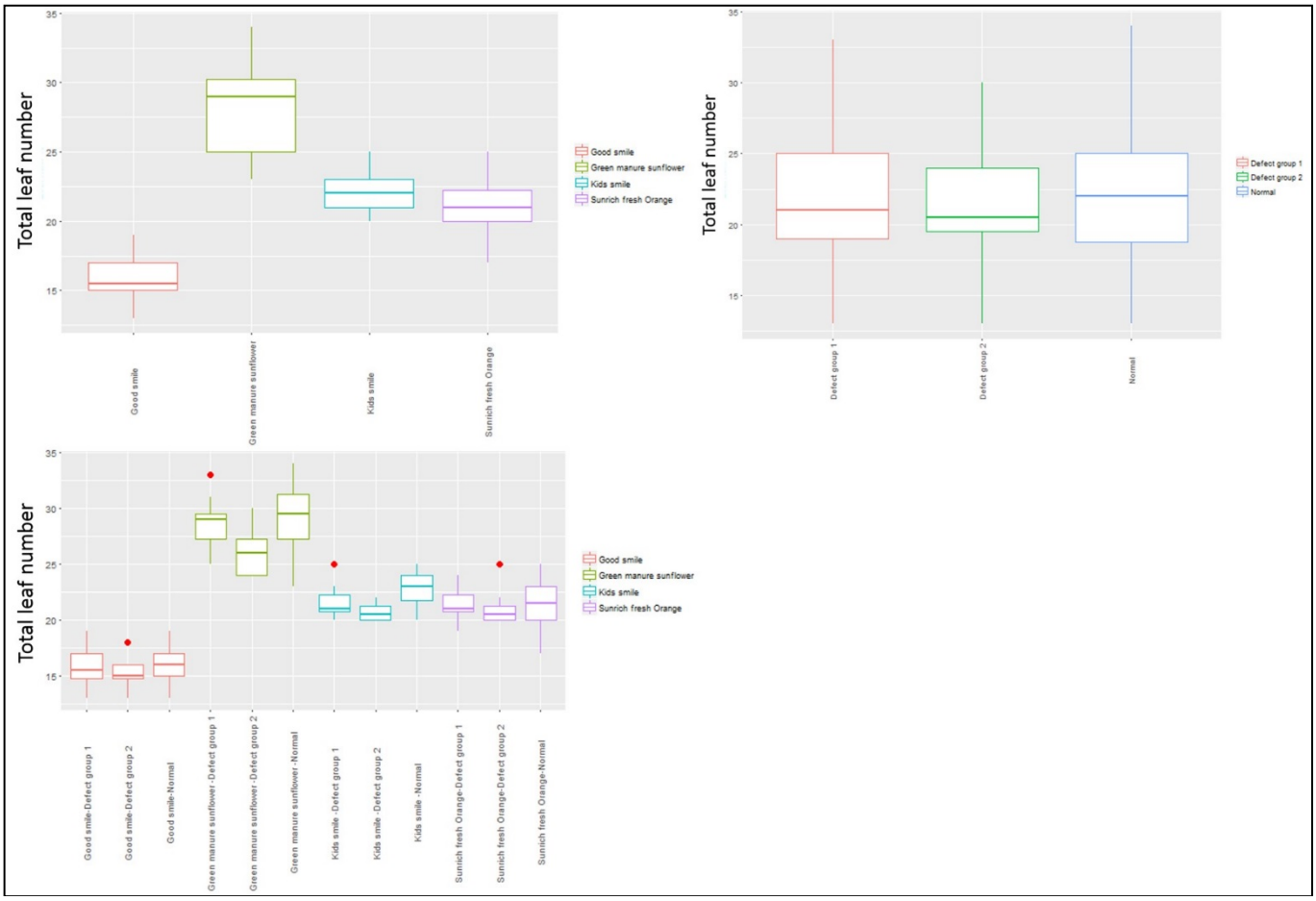
- Plant height (cm):



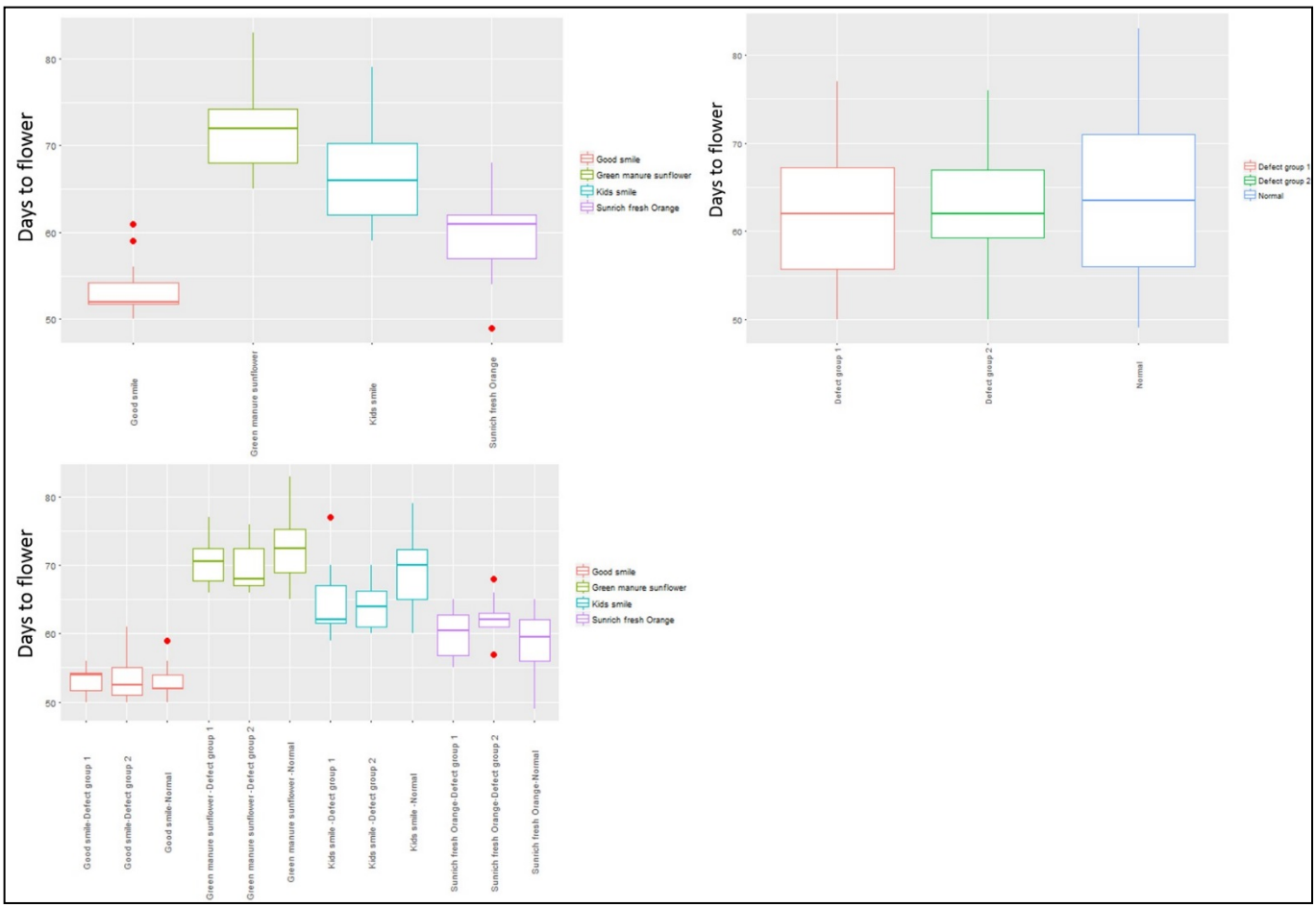
＊Remark

Normal: Normal seedling
Defect group 1: Primary root defected with well-developed secondary root
Defect group 2: Primary root defected with poorly-developed secondary root

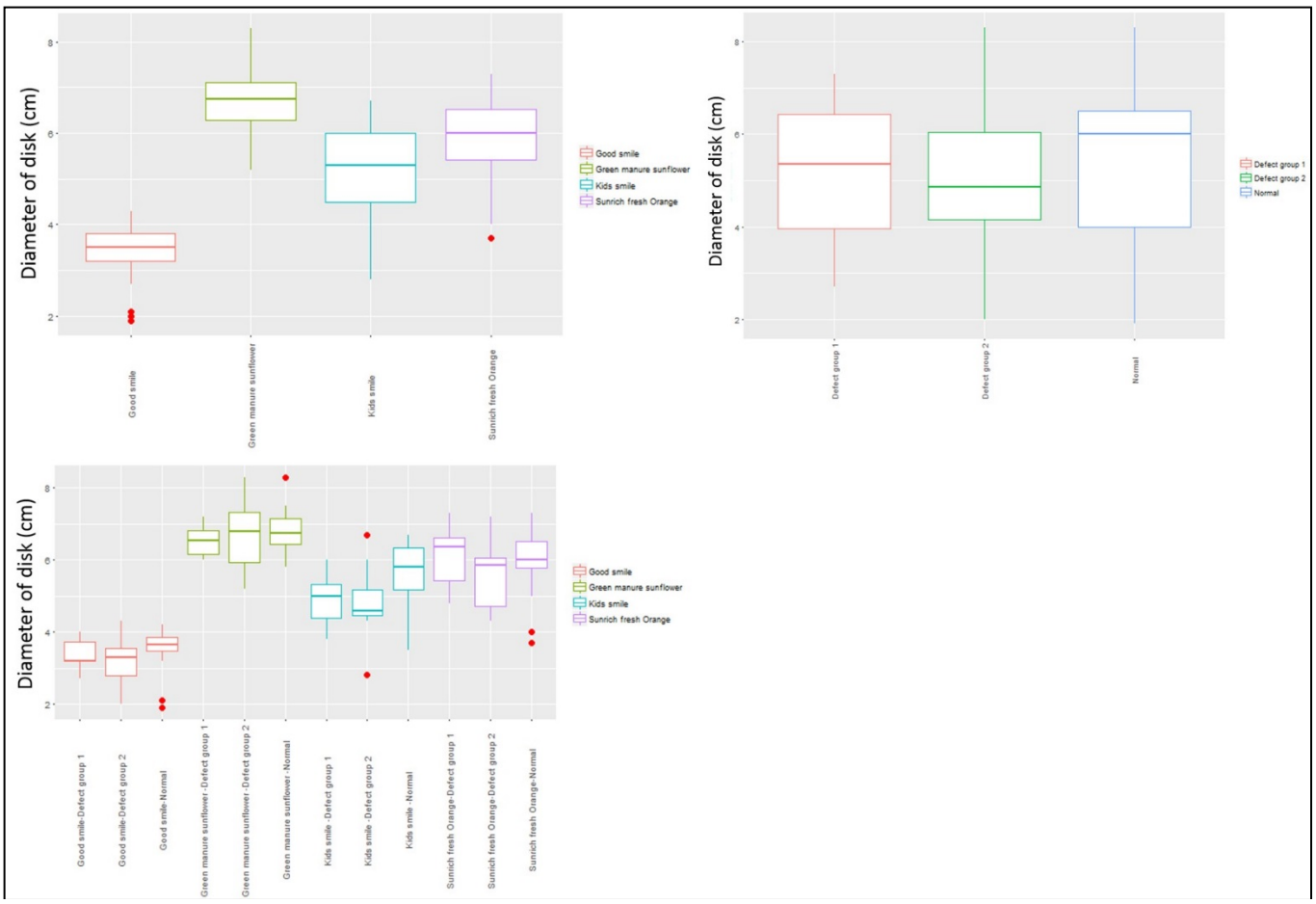
- Total leaf number:



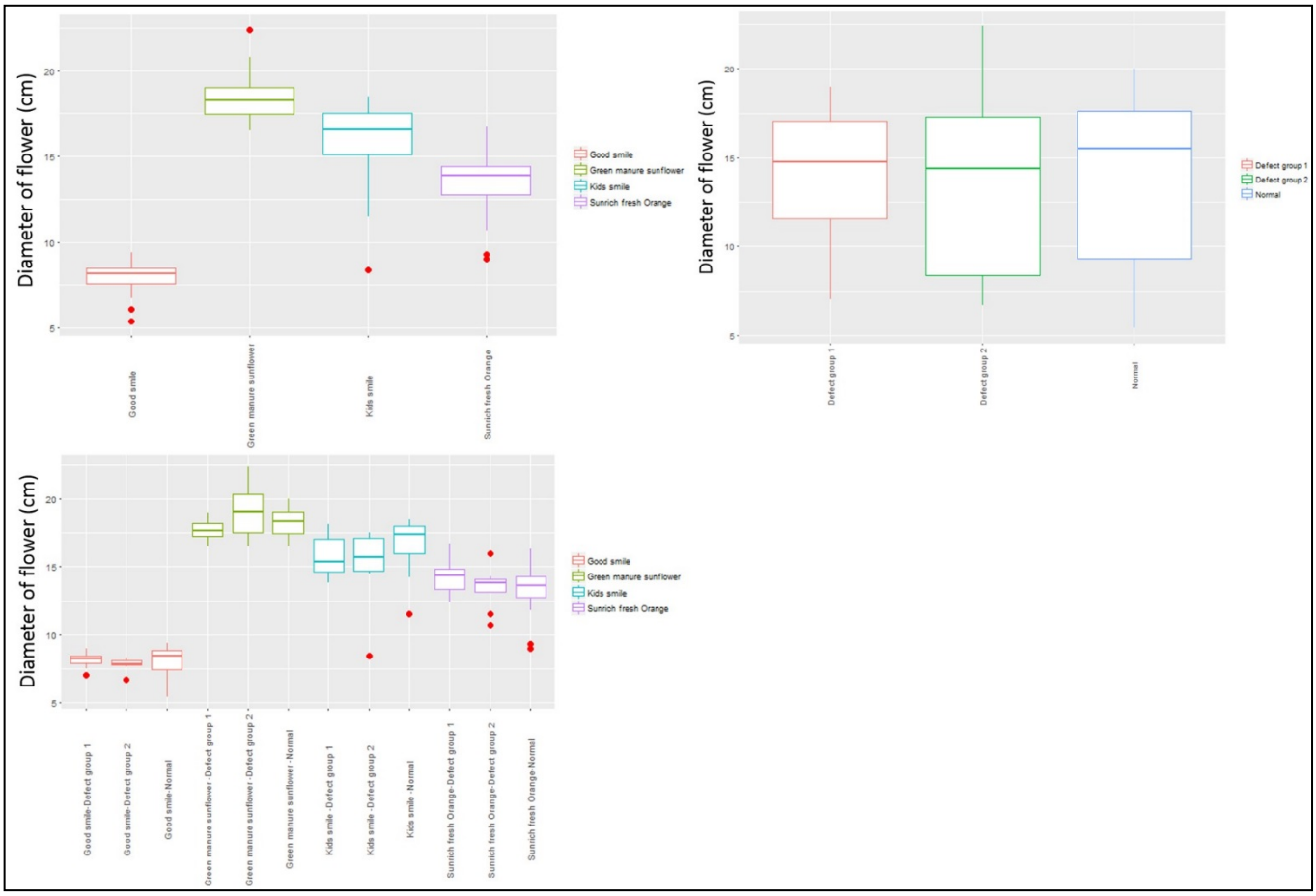
- Days to flower:



- Diameter of disk (cm):



- Diameter of flower (cm):



The box plots don't reveal any particular problems with the data. The ISTA Statistics committee note that the variation within varieties is different across varieties.

2. Statistical model

The following linear model has been fitted to each trait:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

in which:

. y_{ijk} is the value of the trait for Category *i* (Normal, Defect gp 1, Defect gp 2) observed in plant *k* from Variety *j*.

. μ is the intercept.

. α_i is the fixed effect of Category *i*.

. β_j is the fixed effect of Variety *j*.

. $(\alpha\beta)_{ij}$ is the interaction effect between Category *i* and Variety *j*.

. e_{ijk} are the residuals. As the box plots exhibit different variations across varieties, a heteroscedastic model is considered (residual variances are assumed to be different for each variety):

$$\begin{cases} e_{i1k} \sim \text{i.i.d. } N(0, \sigma^2_{\text{Variety 1}}) \\ e_{i2k} \sim \text{i.i.d. } N(0, \sigma^2_{\text{Variety 2}}) \\ e_{i3k} \sim \text{i.i.d. } N(0, \sigma^2_{\text{Variety 3}}) \\ e_{i4k} \sim \text{i.i.d. } N(0, \sigma^2_{\text{Variety 4}}) \end{cases}$$

This model has been fitted with SAS.

3. Results

Residual variance estimates, ANOVA tables and Least Square Means are provided below.
For all the traits, the Variety effect is highly significant.
The Category effect and its interaction with Variety are not significant for Plant Height, Diameter of Disk and Diameter of Flower.
For Total Leaf number, the Category effect is significant overall (Defect group 2 significantly different from Normal).
For Days to Flower, Defect group 1 and 2 are significantly different from Normal for Variety Kids smile, and Defect group 2 is significantly different from Normal for Variety Sunrich Fresh Orange.

Plant height (cm)

Residual variance estimates

Variety	Variance
Good smile	3.89
Green manure sunflower	35.34
Kids smile	35.56
Sunrich fresh Orange	84.31

Tests of fixed effects

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	116	3693.80	<.0001
Category	2	116	2.60	0.0785
Category x Variety	6	116	1.37	0.2310

Variety Least Squares Means

Variety	Estimate	Standard-error	
Good smile	13.90	0.37	A
Kids smile	47.38	1.11	B
Sunrich fresh Orange	97.81	1.71	C
Green manure sunflower	124.96	1.11	D

Category Least Squares Means

Category	Estimate	Standard-error
Defect group 2	69.50	1.11
Normal	70.56	0.79
Defect group 1	72.97	1.11

Category x Variety Least Squares Means

Variety	Category	Estimate	Standard-error
Good smile	Defect group 1	13.75	0.70
Good smile	Normal	13.81	0.49
Good smile	Defect group 2	14.13	0.70
Green manure sunflower	Defect group 2	121.87	2.10
Green manure sunflower	Normal	126.00	1.49
Green manure sunflower	Defect group 1	127.00	2.10
Kids smile	Defect group 2	45.25	2.11
Kids smile	Normal	47.75	1.49
Kids smile	Defect group 1	49.13	2.11
Sunrich fresh Orange	Normal	94.69	2.30
Sunrich fresh Orange	Defect group 2	96.75	3.25
Sunrich fresh Orange	Defect group 1	102.00	3.25

Total leaf number

Residual variance estimates

Variety	Variance
Good smile	3.21
Green manure sunflower	9.70
Kids smile	2.18
Sunrich fresh Orange	3.83

Tests of fixed effects

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	116	133.53	**<.0001**
Category	2	116	5.08	**0.0077**
Category x Variety	6	116	1.15	0.3399

Variety Least Squares Means

Variety	Estimate	Standard-error	
Good smile	15.67	0.33	A
Sunrich fresh Orange	21.23	0.36	B
Kids smile	21.75	0.27	B
Green manure sunflower	27.98	0.58	C

Category Least Squares Means

Category	Estimate	Standard-error	
Defect group 2	20.81	0.38	A
Defect group 1	21.84	0.38	AB
Normal	22.31	0.27	B

Category x Variety Least Squares Means

Variety	Category	Estimate	Standard-error
Good smile	Defect group 2	15.25	0.63
Good smile	Defect group 1	15.75	0.63
Good smile	Normal	16.00	0.45
Green manure sunflower	Defect group 2	26.13	1.10
Green manure sunflower	Defect group 1	28.63	1.10
Green manure sunflower	Normal	29.19	0.78
Kids smile	Defect group 2	20.75	0.52
Kids smile	Defect group 1	21.63	0.52
Kids smile	Normal	22.88	0.37
Sunrich fresh Orange	Defect group 2	21.13	0.69
Sunrich fresh Orange	Normal	21.19	0.49
Sunrich fresh Orange	Defect group 1	21.38	0.69

Days to flower

Residual variance estimates

Variety	Variance
Good smile	7.15
Green manure sunflower	19.08
Kids smile	26.12
Sunrich fresh Orange	15.71

Tests of fixed effects

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	116	137.42	**<.0001**
Category	2	116	1.39	0.2530
Category x Variety	6	116	2.41	**0.0315**

Variety Least Squares Means

Variety	Estimate	Standard-error	
Good smile	53.21	0.50	A
Sunrich fresh Orange	60.48	0.74	B
Kids smile	66.04	0.95	C
Green manure sunflower	71.13	0.81	D

Category Least Squares Means

Category	Estimate	Standard-error
Defect group 1	62.19	0.73
Defect group 2	62.44	0.73
Normal	63.52	0.52

Category x Variety Least Squares Means

Variety	Category	Estimate	Standard-error	
Good smile	Normal	52.88	0.67	A
Good smile	Defect group 1	53.25	0.95	A
Good smile	Defect group 2	53.50	0.95	A
Green manure sunflower	Defect group 2	69.75	1.54	B
Green manure sunflower	Defect group 1	70.63	1.54	B
Green manure sunflower	Normal	73.00	1.09	B
Kids smile	Defect group 2	64.13	1.81	C
Kids smile	Defect group 1	64.75	1.81	C
Kids smile	Normal	69.25	1.28	D
Sunrich fresh Orange	Normal	58.94	0.99	E
Sunrich fresh Orange	Defect group 1	60.13	1.40	EF
Sunrich fresh Orange	Defect group 2	62.38	1.40	F

Disk diameter (cm)

Residual variance estimates

Variety	Variance
Good smile	0.38
Green manure sunflower	0.53
Kids smile	0.87
Sunrich fresh Orange	0.90

Tests of fixed effects

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	116	127.84	**<.0001**
Category	2	116	2.56	0.0815
Category x Variety	6	116	0.70	0.6490

Variety Least Squares Means

Variety	Estimate	Standard-error	
Good smile	3.36	0.12	A
Kids smile	5.10	0.17	B
Sunrich fresh Orange	5.87	0.18	C
Green manure sunflower	6.69	0.14	D

Category Least Squares Means

Category	Estimate	Standard-error
Defect group 2	5.08	0.14
Defect group 1	5.23	0.14
Normal	5.46	0.10

Category x Variety Least Squares Means

Variety	Category	Estimate	Standard-error
Good smile	Defect group 2	3.20	0.22
Good smile	Defect group 1	3.38	0.22
Good smile	Normal	3.51	0.15
Green manure sunflower	Defect group 1	6.53	0.26
Green manure sunflower	Defect group 2	6.74	0.26
Green manure sunflower	Normal	6.81	0.18
Kids smile	Defect group 2	4.80	0.33
Kids smile	Defect group 1	4.90	0.33
Kids smile	Normal	5.61	0.23
Sunrich fresh Orange	Defect group 2	5.59	0.33
Sunrich fresh Orange	Normal	5.93	0.24
Sunrich fresh Orange	Defect group 1	6.10	0.33

Flower diameter (cm)

Residual variance estimates

Variety	Variance
Good smile	0.83
Green manure sunflower	1.67
Kids smile	4.33
Sunrich fresh Orange	3.23

Tests of fixed effects

Effect	Num DF	Den DF	F Value	Pr > F
Variety	3	116	463.00	**<.0001**
Category	2	116	0.17	0.8438
Category x Variety	6	116	1.67	0.1357

Variety Least Squares Means

Variety	Estimate	Standard-error	
Good smile	7.97	0.17	A
Sunrich fresh Orange	13.69	0.34	B
Kids smile	15.86	0.39	C
Green manure sunflower	18.38	0.24	D

Category Least Squares Means

Category	Estimate	Standard-error
Defect group 2	13.88	0.28
Defect group 1	13.96	0.28
Normal	14.08	0.20

Category x Variety Least Squares Means

Variety	Category	Estimate	Standard-error
Good smile	Defect group 2	7.79	0.32
Good smile	Normal	8.00	0.23
Good smile	Defect group 1	8.11	0.32
Green manure sunflower	Defect group 1	17.73	0.46
Green manure sunflower	Normal	18.28	0.32
Green manure sunflower	Defect group 2	19.14	0.46
Kids smile	Defect group 2	15.11	0.74
Kids smile	Defect group 1	15.78	0.74
Kids smile	Normal	16.68	0.52
Sunrich fresh Orange	Normal	13.34	0.45
Sunrich fresh Orange	Defect group 2	13.49	0.64
Sunrich fresh Orange	Defect group 1	14.24	0.64

Conclusions

The seedlings with a defective primary root and with non-sufficiently developed secondary roots have the tendency to an early flowering and a decreased number of leaves compared to normal seedlings.
As to variety Kids smile, defect group 1; primary root defected with well-developed secondary root is significantly different from normal seedlings for days to flower. However except for this variety and this trait, all other characteristics measured show no significant difference between normal seedlings and seedlings with defective primary root but with well-developed secondary roots.
The results of this validation study indicate that secondary roots of *Helianthus* may compensate for the damage of the primary root and support the modification for the seedling evaluation criteria of *Helianthus* root system from “A-2-1-1-1: the primary root is essential” to “A-2-1-1-2: the primary root may be replaced by secondary roots”.