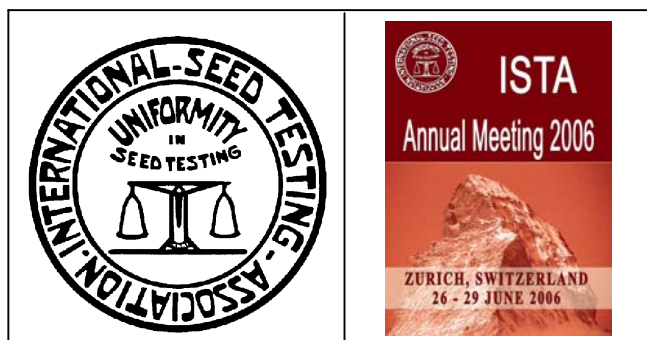


PROPOSED RULES CHANGES 2006

**TO BE VOTED ON AT THE
ORDINARY MEETING IN ZURICH**





Introduction by the Chairman of the ISTA Rules Committee

The loose-leaf edition of the ISTA Rules has been updated. The current version is the 2006 edition. Single copies of replacement pages and front covers for the 2006 edition have been sent free to all ISTA Member Laboratories. Extra copies are available for purchase from the ISTA Publications section. As the Rules are an evolving document it is worth remembering that pages could be headed with different 'effective from' dates. The preface for each edition includes details of changes and when replacement pages have been issued. Previous prefaces as a 'history of changes' are available on the ISTA Website.

The following proposals will be discussed at the ISTA meeting in Zurich, Switzerland during June 2006. In appendices 1, 2, 3 and 4 of this document you will find the full text of the proposed method 7-021: Detection of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* on *Phaseolus vulgaris* (bean) (item 5a) and the supporting data reports for the items 4a, 6a and 6c. If the proposals are accepted by the membership amendments will be issued and they will become the 2007 edition of the ISTA Rules. This year sees the next set of editorially merged Chapters where the rules and annexes are combined into a continuous text. This process has two stages: firstly the rules and annexes were combined section by section without any changes, they were then read through and editorial changes like renumbering sections and removal of redundant text was suggested. This version was sent to the ISTA scrutiny team (Doug Ashton and Simon Cooper), both ex-members of the ISTA Executive Committee. Once the scrutiny team has agreed that the editorial changes were indeed editorial these can be made and do not need to be voted on at the voting meeting. Anything other than editorial changes are presented as rules proposals at the ordinary meeting for a vote in the normal way. This year there are only editorially merged chapters.

Any subsequent editorial changes to any of the Rules remain the responsibility of the Chair of the Rules Committee.

Please let me know of any problems with these proposals.

Many thanks to John Hampton and Bettina Kahlert in helping me to collate and review the proposals.

Steve

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3	Proposals for Chapter 3 and Annexe, Purity	Chapter 3	4-8
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	Appendix 1: Supporting Data and Evidence for the Proposed Rules Changes 2006 – Item 4A - Modification to Dormancy Breakage for <i>Hordeum vulgare</i>	Chapter 5	
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	Appendix 3: Supporting Data and Evidence for the Proposed Rules Changes 2006 – Item 6A – Addition of <i>Triticum dicoccum</i>	Chapter 2, 3 and 5	
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~~Text proposed for deletion~~

Proposed new text

ITEM 1. GENERAL EDITING ISSUES.

Item 1a. Editorial corrections.

The following are editorial changes/errors that have been noticed in the 2006 edition of the Rules.

List of edits

Chapter 2: reference in 2.5.4.3 to 2.5.1.4.1 should be 2.5.1

Chapter 2: Table 2B. Purity analysis should be 2 500 not 2 000.

Chapter 3: reference in 3.5.1 to 2.7 should be 2.5.2

Chapter 3: reference in 3.5.1 to 2.6 should be 2.5.1

Chapter 8. section 8.5.4., second paragraph, first line; it should be 8.9 instead of 8.8.2

Chapter 8: reference in 8.8.4.3.2 not paragraph 8.8.4.3.1.1 must be 8.8.4.3.1

Chapter 8: page 8-22: it must be 8.8.5.5 and not 8.5.5

Chapter 8: references under 8.8.5.4 need also to be corrected to 8.5.5

Chapter 17: reference in 17.4.1 to 2.4 should be 2.5.4

Chapter 17: reference in 17.4.1 to 2.6 should be 2.5.1

Chapter 17: reference in 17.5.1 to 2.6.7 should be 2.5.1.6

Item 1b. Editorial merger of Chapters 6, 7, 9 and 15.

For Chapters 6, 7, 9 and 15 the rules and annexes have been editorially merged. This has been confirmed by Doug Ashton and Simon Cooper. Therefore, these chapters do not require a vote. Chapters 6, 7 and 15 can be reprinted for edition 2007. For Chapter 9 the merger is the first part of a major revision that will require a vote in 2007 so Chapter 9 will not be reprinted until after a successful vote in 2007.

Any cross references will be checked and amended if necessary.

As for the procedure last year the only vote for editorially merged chapters will be to accept the report of the scrutiny team.



ITEM 2. PROPOSALS FOR CHAPTER 2 AND ANNEXE, SAMPLING.

Item 2a. Amendment to section 2.5.4.3.

The Bulking and Sampling Committee have concerns about the new rule 2.5.4.3 with regard to the necessity to completely seal seed lots in large containers while they are waiting to be packed into the smaller containers in which they will be sold. This situation often arises with vegetable seed lots and in grass seed companies.

For example, with the new rule 2.5.4.3 a grass seed lot can be sampled when filling the large paper boxes and the results are still valid when the lot is repacked into the final size 25 kg bags. However rule 2.5.4.3 would require that the first set of paper boxes to be completely sealed. This is not practicable and the same holds true for some types of repacking operations from silos/bulk bins. If you take an ISTA sample and the boxes are not permanently under the control of the seed sampler, they would need to be completely sealed. So the idea was, to have a paper cover for these boxes to prevent cross contamination.

The following rules proposal aims to solve the difficulty.

New sentence following at the end of the second paragraph of section 2.5.4.3:

[Seed lots in large containers \(> 100 kg\) need not be sealed if the seed is to be repacked and sealed into other containers at the same facility and the large containers are covered appropriately to avoid cross contamination.](#)

**ITEM 3. PROPOSALS FOR CHAPTER 3, PURITY.****Item 3a. Move *Cannabis* from PSD 1 to PSD 4.**

Some samples of *Cannabis sativa* present bracts attached to the seed and removing the bracts is very time consuming. *Cannabis* is the only Cannabaceae genus taken into account in the PSD 1 and no other Cannabaceae are included in other PSD. Other genera taken into account in PSD 1 are not concerned by bracts.

Therefore PSD 4 is considered to better apply to *Cannabis*. PSD 1 and PSD 4 have the same seed descriptions except that PSD 4 has the additional possibility of bracts attached to the seed.

3.2.1. A.1 Part 1 Pure Seed Definition numbers by genus and family.

...

Cannabis Cannabaceae + 4

Item 3b. Move *Consolida* from PSD 4 to PSD 10.

This proposal comes from the Flower Seeds Committee that is working on the new Handbook for Flower Seeds. PSDs of *Consolida* and *Delphinium* are currently PSD 4 and PSD 10 respectively but these genera have almost identical seeds. Therefore it is proposed to move *Consolida* to PSD 10.

3.2.1. A.1 Part 1 Pure Seed Definition numbers by genus and family.

...

Consolida Ranunculaceae + 10

Item 3c. Modify PSD 22.

This modification is necessary to adapt it to allow the addition of *Crambe* to the ISTA Rules by adding the term siliqua.

22. Pod or siliqua, with or without calyx or bracts, with one seed.

Seed, provided a portion of the testa is attached.

Piece of seed larger than one-half the original size, provided a portion of the testa is attached.

~~For *Fabaceae* (*Leguminosae*) only:~~ Seeds and pieces of seed without testa are regarded as inert matter. For *Fabaceae* (*Leguminosae*) only: Separated cotyledons are regarded as inert matter irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

**Item 3d. Reporting results of Determination of Other Seeds by Number.**

When the weight of 25 000 seeds is below the weight as fixed in Table 2A, 'Complete test' still applies but this creates an ambiguity. This is because the weight used will be not be the weight stated in Table 2A, but the weight of at least 25 000 seeds. When this situation occurs adding a statement to the ISTA Seed Analysis Certificate stating that the complete test is based on at least 25 000 seeds will help clarify the size of the working sample used.

4.7 Reporting results

...

When a sample is tested to determine 'other seeds by number' using at least 25 000 seeds and this sample weight is below the required weight stated in Table 2A, then the weight of seed examined and the statement 'Complete test based on at least 25 000 seeds' must be stated on the certificate.

Item 3e. Reporting results of purity.

Erase, move and add sentences to "17.5.A.2.1 Purity" for harmonisation with the statement in 3.7 Reporting result: "When on the request of the applicant, the content of a particular kind of inert matter, species of other seed, Multiple Seed Unit (MSU) or seeds with appendages attached as specified in 3.5.2.A.8 has been determined, the percentage of such material must be shown on the ISTA International Seed Analysis Certificate."

17.5.A.2.1 Purity

....

~~The percentage by weight of a species be reported if it is 1.0% or more. On the request of the applicant, the percentage by weight for a specified species must be reported.~~ If required by the applicant, the percentage by weight for a specified species must be reported. The percentage shall be stated immediately after the name of the species to the nearest 0.1%. Species for which the percentage by weight shall be stated are listed first.

...

If required by the applicant multiple seed units shall be reported as ...% MSU.
If required by the applicant seeds with appendages attached shall be reported as ...% seeds with appendages attached.

...

If required by the applicant, ~~the~~ the kinds of inert matter shall be reported, together with the percentage by weight of any particular kind ~~found to the extent of 1.0% or more~~ (to one decimal place).

**Item 3f. Modify PSD 47.**

This modification is necessary as PSD 47 includes seeds with and without wings.

47. Seed, with or without wing or integument, provided a portion of the testa is attached. Piece of seed larger than one-half the original size, with or without wing or integument, provided a portion of the testa is attached.

N.B. 'Integument' refers to the tissue attaching the wing to the seed. In *Pinaceae* with this definition, the integument is not intimately associated with the seed and is usually removed in processing thus removing the wing. However, if an integument (with or without wing) is still attached to any seed during the purity analysis such seed will be regarded as 'winged seed' and must be left intact - neither the integument nor wing should be deliberately removed. Winged seed (i.e. seed with an attached integument with or without a wing of any size) must be weighed and reported as a separate percentage from 'pure seed' according to paragraphs 3.5.2.A.9 and 3.7. After weighing, the winged seed and pure seed fractions should be recombined and used in representative proportions for counting out the germination replicates

Item 3g. Modify PSD 51.

This modification is necessary as PSD 51 includes seeds with and without wings. It also improves PSD 51 and adapts it to genera with an integument fused to the seed (*Pseudotsuga* and *Larix*) and also to genera with integument intimately associated with the seed (*Abies*, *Cedrus* and *Tsuga*).

51. Seed, with or without wing, with (but occasionally without) integument, provided a part of the testa is attached. Piece of seed larger than one-half the original size, with or without wing, with (but occasionally without) integument, provided a portion of the testa is attached. Seed with integument fused or attached is considered to be 'pure seed'.

N.B. 'Integument' refers to the tissue attaching the wing to the seed. ~~In *Pinaceae* with this definition;~~

Only for *Pseudotsuga* and *Larix*: the integument is fused to the seed, ~~is rarely removed in processing~~, and is impossible to ~~consistently~~ remove in processing ~~without causing damage~~. Only the wing can be removed without causing damage. Hence, seed with fused integument attached is considered to be 'pure seed'.

Only for *Abies*, *Cedrus* and *Tsuga*: the integument is intimately associated with, but is not fused to, the seed. The integument is difficult to consistently remove in processing without causing damage. Hence, seed with integument attached is considered to be 'pure seed'.

Winged seed - i.e. seed with an attached integument ~~plus~~ with or without wing ~~still attached~~ of any size must be weighed and reported as a separate percentage from 'pure seed' according to paragraphs 3.5.2.A.9 and 3.7. After weighing, the winged seed and pure seed fractions should be recombined and used in representative proportions for counting out the germination replicates



Item 3h. Modify Rule 3.5.2.A.9.

If item 3g is accepted, rule 3.5.2.A.9 needs to be harmonized with the new PSD 51.

3.5.2.A.9 Winged seed

For seeds with PSD 47 and PSD 51, winged seeds are those which retain an integument either with or without wing. ~~For seeds with PSD 51, winged seeds are only those which retain an integument plus wing.~~ Whenever present such appendages must be left attached to the seed and the content of ‘winged’ seed reported on the certificate according to 3.7.

Item 3i. Move *Pinus* I (*P. palustris*, *P. rigida*) from PSD 51 to PSD 47.

This allows all Pinaceae to be covered by PSD 47.

Pinus Pinaceae ~~51~~ 47 C

Item 3j. Move *Calocedrus* from PSD 51 to PSD 49.

Calocedrus decurrens bears two wings not one so PSD 49 applies rather than PSD 51.

Calocedrus Cupressaceae ~~51~~ 49 C

Item 3k. Move *Sequoiadendron* from PSD 50 to PSD 49.

Sequoiadendron seeds do not form within arils so PSD 49 applies rather than PSD 50.

Sequoiadendron Taxodiaceae ~~50~~ 49

Item 3l. Move *Taxus* from PSD 10 to PSD 50.

Taxus seeds develop within arils so PSD 50 applies rather than PSD 10. If this item is voted through Taxaceae needs to be removed from PSD 10 as there are no longer any members of Taxaceae family covered by PSD 10.

Taxus Taxaceae ~~10~~ 50

- 10. Seed, with or without testa.
- Piece of seed larger than one-half the original size, with or without testa.
- Fabaceae* (*Leguminosae*), *Brassicaceae* (*Cruciferae*), *Cupressaceae*, ~~*Taxaceae*~~,
- Taxodiaceae*:

**Item 3m. Modify Rules 3.2.1 and 3.2.3 and PSD 10.**

The mention of Pinaceae has already been deleted in PSD 10 (voted during OM 2005), but not yet changed in the paragraphs 3.2.1 and 3.3.3 of ISTA Rules. If item 3l is voted through Taxaceae also needs to be removed from 3.2.1 and 3.2.3.

3.2.1 Pure seed

...

2. From the above main principles certain exceptions are made for particular genera or species (3.2.1.A).

1. Seed units of the families *Fabaceae* (*Leguminosae*), *Brassicaceae* (*Cruciferae*), *Cupressaceae*, ~~*Pinaceae*~~, ~~*Taxaceae*~~ and *Taxodiaceae* with seed coats entirely removed shall be regarded as inert matter (3.2.3 (5)).

Separated cotyledons of *Fabaceae* (*Leguminosae*) are regarded as inert matter, irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

...

3.2.3 Inert matter

...

5. Seeds of *Fabaceae* (*Leguminosae*), *Brassicaceae* (*Cruciferae*), *Cupressaceae*, ~~*Pinaceae*~~, ~~*Taxaceae*~~ and *Taxodiaceae* with the seed coat entirely removed.

Fabaceae (*Leguminosae*): separated cotyledons are regarded as inert matter, irrespective of whether or not the radicle-plumule axis and/or more than half of the testa may be attached.

...

ITEM 4. PROPOSALS FOR CHAPTER 5, GERMINATION.**Item 4a. Modification to dormancy breakage for *Hordeum vulgare*.**

Proposal from the Germination Committee.

Data has been provided to show KNO_3 successfully breaks dormancy in *Hordeum vulgare*. The supporting data report can be found as appendix 1.

Table 5A, modified entry for *Hordeum vulgare*

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature °C	First count (days)	Final count (days)	
1	2	3	4	5	6
<i>Hordeum vulgare</i>	BP; S	20	4	7	Preheat (30-35°C); Prechill; GA ₃ ; KNO₃



Item 4b. To allow the possibility of testing *Betula pendula* and *B. pubescens* germination by counted (4 x 100 seeds) replicates as well as by weighed replicate.

Proposal from Forest Tree and Shrub Seed Committee.

Based on the requirements of the Council Directive 1999/105/EC the seed lots marketed must be accompanied by a label or other document from the supplier ('the supplier's label or document') giving, beside others, information about seed quality. This information includes purity, germination or viability, weight of 1000 seeds and the number of germinable (viable) seeds per kg (see below). In many, if not most cases, laboratories perform purity test, then count 8 x 100 seed replicates for determination of the weight of 1000 pure seeds. Four, one hundred seed replicates are then used for germination or viability tests. In Annex I List of Tree Species and Artificial Hybrids, two birch species are listed: *Betula pendula* and *Betula pubescens*.

According to the 2006 ISTA Rules the seed germination of those two species can only be tested by the Weighed Replicates Method (Chapter 13). The Forest Tree and Shrub Committee therefore recommend the following change to allow the possibility of testing germination by counted (4 x 100 seeds) replicates as well as by weighed replicate. This proposal is also supported by the Germination Committee.

Amend entries in Table 5A. Part 2. Tree and shrub seeds

Species	Prescriptions for:		First count (days)	Final count (days)	Additional directions including recommendations for breaking dormancy
	Substrate	Temperature °C			
1	2	3	4	5	6
<i>Betula pendula</i>	<u>TP</u>	<u>20-30</u>	<u>7</u>	<u>21</u>	<u>No prechill and prechill for 21 days at 4 °C.</u> <u>Double tests.</u>
<i>Betula pubescens</i>	<u>TP</u>	<u>20-30</u>	<u>7</u>	<u>21</u>	



ITEM 5. PROPOSALS FOR ANNEXE TO CHAPTER 7, SEED HEALTH TESTING METHODS.
Item 5a: Modifications and additions to the validated seed health testing methods.

The following method sponsored by ISTA has been validated by the Seed Health Committee, and is submitted for acceptance by the membership to be included in Annexe to Chapter 7 of the International Rules for Seed Testing as Method 7-021. Any problems or errors should be directed to the Chairpersons of the Seed Health and Rules Committees. The full text of the proposed method can be found as appendix 2. The method validation report will be available prior the meeting.

New Method:

7-021: Detection of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* on *Phaseolus vulgaris* (Bean)

Crop: *Phaseolus vulgaris*

Pathogen: *Xanthomonas axonopodis* pv. *phaseoli*

Background:

Xanthomonas axonopodis pv. *phaseoli* (Xap) and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* (Xapf) (previously known as *Xanthomonas campestris* pv. *phaseoli*) (Vauterin *et al.*, 1995) are the causal organisms of common bacterial blight and fuscous blight of bean, respectively. In the past these organisms were considered as a single pathogen. However Xapf produces a diffusible brown pigment in culture that is enhanced by tyrosine (Goodwin and Sopher, 1994) and Xapf isolates are considered in general more pathogenic than Xap isolates. In addition several genetic studies based on repPCR, PFGE and RFLP revealed that this pathovar should be segregated into two subgroups and should obtain distinct taxonomic status (Mkandawire *et al.*, 2004).

Currently there is no standard seed health testing procedure for the detection of Xap or Xapf in bean seed lots. In comparative tests (Sheppard, 1998a) several semi-selective media were evaluated for the detection of Xap in bean seed lots. As a result two media, XCP1 and MT, have been chosen for their ease of use and selectivity for Xap. The media rely on the ability of Xap to hydrolyse casein and Tween (MT medium) or starch (XCP1 medium) (Sheppard, 1998a). In addition both media can be used to detect both Xap and Xapf as initially the morphology of fuscans and non-fuscans strains of Xap appear to be similar on medium. However after a longer incubation the fuscans colonies are distinguished by a distinct brown pigmentation. Especially on a tyrosine medium (MT) the production of the diffusible extra cellular brown, melanin-like pigment is prominent (Chan and Goodwin, 1999). A further advantage of MT medium is that it can also be used for identifying other bacterial seed-borne pathogens of beans, e.g. *Pseudomonas savastanoi* pv. *phaseolicola* and *Pseudomonas syringae* pv. *syringae*.

This method is derived from the validation studies carried out by ISTA in 2003, in collaboration with the International Seed Health Initiative for Vegetables (ISHI-Veg) (Sheppard and Remeus, 2005). For routine testing of bean seed a combination of two complementary semi-selective media, MT and XCP1, is recommended with a pathogenicity test to confirm suspect isolates.

Method Abstract:

Seeds are suspended in saline plus Tween 20 in polythene bags or containers, and soaked overnight at 5°C (+/- 2°C). The diluted extracts are plated on MT and XCP1 media. Plates are incubated at 27-28°C for 3-5 d and then examined for the presence of suspect colonies of *Xanthomonas axonopodis* pv. *phaseoli*. Suspect colonies are sub-cultured to plates of YDC medium and their identity confirmed by a pathogenicity test on susceptible bean seedlings.



ITEM 6. PROPOSALS FOR INCLUSION OF NEW SPECIES AND A CHANGE IN NOMENCLATURE.

Item 6a. Addition of *Triticum dicoccum* to the ISTA Rules

An application was received from one of the Italian ISTA accredited laboratories to add *Triticum dicoccum* Schrank ex Schübl. to the list of species for ISTA testing. A supporting data report has been provided and can be found as appendix 3. The relevant ISTA Technical Committees have been consulted and agree to the following proposal.

Table 2A, new entry for *Triticum dicoccum* Schrank ex Schübl.

Species	Maximum weight of lot	Minimum sample weights		
		Submitted sample	Working sample for purity analysis	Working sample for count of other species
	kg	g	g	g
<i>Triticum dicoccum</i> Schrank ex Schübl.	30 000	1000	270	1000

Table 3.2.1.A.1, modify entry for PSD 40 and 33

Genus	Family	Pure seed Definition (PSD) number	Chaffyness
<i>Triticum</i> (excluding <i>T. spelta</i> and <i>T.dicoccum</i>)	Poaceae (Gramineae)	40	
<i>Triticum</i> (only <i>T. spelta</i> and <i>T.dicoccum</i>)	Poaceae (Gramineae)	33	C

Table 5A, new entry for *Triticum dicoccum* Schrank ex Schübl.

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature °C	First count (days)	Final count (days)	
1	2	3	4	5	6
<i>Triticum dicoccum</i>	BP;S	20	4	8	Preheat (30-35°C); Prechill; GA₃;



Item 6b. Inclusion of *Avena nuda* L.

Avena sativa is already included in the ISTA Rules and *Avena nuda* included in the ISTA Stabilised List and listed as an OECD species. To facilitate international trade it is suggested by the Executive Committee of ISTA that *Avena nuda* should be included in the ISTA Rules with the same details as for *Avena sativa*. *Avena nuda* is currently treated as a variety of *Avena sativa* within the European Union.

Table 2A, new entry for *Avena nuda*

Species	Maximum weight of lot	Minimum sample weights		
		Submitted sample	Working sample for purity analysis	Working sample for count of other species
	kg	g	g	g
<u>Avena nuda L.</u>	<u>30 000</u>	<u>1000</u>	<u>120</u>	<u>1000</u>

Table 5A, new entry for *Avena nuda*

Species	Prescriptions for:		Additional directions including recommendations for breaking dormancy		
	Substrate	Temperature °C	First count (days)	Final count (days)	
1	2	3	4	5	6
<u>Avena nuda L.</u>	<u>BP;S</u>	<u>20</u>	<u>5</u>	<u>10</u>	<u>Preheat (30-35°C); Prechill</u>



Item 6c. Addition of *Crambe abyssinica* Hochst. ex R. E. Fr. into the ISTA Rules

An application was received from the Germination Committee to add *Crambe abyssinica* Hochst. ex R. E. Fr. to the list of species for ISTA testing. A supporting data report has been provided and can be found as appendix 4. The relevant ISTA Technical Committees have been consulted and agree to the following proposal.

Table 2A, new entry for *Crambe abyssinica*

Species	Maximum weight of lot	Minimum sample weights		
		Submitted sample	Working sample for purity analysis	Working sample for count of other species
	kg	g	g	g
<i>Crambe abyssinica</i> Hochst. ex R. E. Fr.	10 000	200	20	200

Table 3.2.1.A.1, include entry for *Crambe* in PSD table

Genus	Family	Pure seed Definition (PSD) number	Chaffyness
<i>Crambe</i>	Brassicaceae (Cruciferae)	22	

Table 5A, new entry for *Crambe abyssinica*

Species	Prescriptions for:				Additional directions including recommendations for breaking dormancy
	Substrate	Temperature °C	First count (days)	Final count (days)	
1	2	3	4	5	6
<i>Crambe abyssinica</i> Hochst. ex R. E. Fr.	TP; BP	20-30; 20	4	7	KNO₃

Item 6d. Wider interpretation of *xFestulolium* as a species

There is the need to allow the wider interpretation of *xFestulolium* as a species because more than just *xFestulolium braunii* crosses are available in commercial trade.

Amendment in Table 2A

Change the species name *xFestulolium braunii* (K.Richt) A. Camus to *xFestulolium* spp. Asch. & Graebn.

Amendment to Table 5A

Change the species name *xFestulolium braunii* to *xFestulolium* spp.

**ITEM 7. PROPOSALS FOR MODIFICATION TO CHAPTER 8.**

The following proposals have all come from the Variety Committee.

Item 7a. *Zea mays* method. Distilled water as an optional extraction buffer.

Including distilled water as an optional extraction buffer.

8.8.5.1 Principle

The alcohol soluble proteins (zeins) [or water soluble proteins](#) are extracted.....

8.8.5.2.3 Solutions

a) Extraction solution - 30 % (v/v) 2 Chloroethanol: 30 ml 2-Chloroethanol made up to 100 ml with distilled water. This solution can be stored for at least two weeks at room temperature.

[Optional extraction solution - distilled water.](#)

Item 7b. *Zea mays* method. Inclusion of new gel solution.

Including an optional gel solution.

8.8.5.3.2 Gel preparation

[Optional gel solution:](#)

Stock gel solution (8.8.5.2.3d)	50 ml
Urea	16 g
Taurin	1.5 g
Ampholytes pH 5-8	2.90 ml
pH 2-11	1.50 ml

[\(Note: dissolve Taurin **first into the** Stock gel solution because of the slow solubility\)](#)

Item 7c. *Helianthus annuus*: Inclusion of new gel solution.

Including an optional gel solution.

8.8.7.3.2 Gel preparation

[Optional gel solution:](#)

Stock gel solution (8.8.7.2.3.d)	50 ml
Urea	16 g
Ampholytes pH 2-4	0.55 ml
pH 4-6	0.55 ml
pH 5-8	1.40 ml
pH 4-9	1.90 ml

**Item 7d. Clarification and corrections to several sections.**

Several improvements or additions to the text have been grouped together for one vote.

8.8.4.3.3.1 Main (resolving) gel

.....75 ~~ml~~ μl TEMED (full strength, straight out of the bottle).

8.8.4.3.3.2 Stacking gel

.....80 ~~ml~~ μl TEMED (full strength, straight out of the bottle).

8.8.4.3.4 Electrophoresis

....., between 5 and 15 ~~ml~~ μl is appropriate in most cases.

8.8.5.3.2 Gel Preparation

.....Gels not immediately used can be stored ~~in the gel cassettes~~ wrapped at 4°C for at least one week.

8.8.5.3.3 Electrophoresis

.....

Samples (approx ~~15ml~~ 22 μl) are loaded in the applicator strip 0.5 cm below the bufferwick of the anode and focusing carried out at 2500 V, 15 mA, 40 W for about 1750 volt/hours (70 minutes) until completion (for one gel).

.....

8.8.5.3.4 Fixing and staining

... with slow shaking for at least ~~30~~ 20 minutes. . .

The gel dried overnight at room temperature or in an oven at 70°C for 20 minutes and can then. . .

8.8.7.3.2 Gel preparation

.....carrier sheet held on glass and a cover glass plate.

8.8.7.3.3 Electrophoresis

Samples (approx. ~~0.004 ml~~ 4 μl) are loaded ~~at the anode~~ in the applicator strip 0.5 cm below the bufferwick of the anode and focusing carried out at 2500 V, 15 mA, 40 W for about 1750 volt/hours (70 minutes) until completion (for one gel).



Item 8a. Removal of the requirement to use a Green International Seed Lot Certificate (GIC) instead of an Orange International Seed Lot Certificate

Several cases have been either discussed by auditors with laboratories, or resulted from direct enquiries from laboratories, about when and how to use the ISTA Green International Seed Lot Certificate (GIC). The main criteria for using the GIC surrounded the need to separate sampling and testing, then to consider whether the sampling and testing laboratories were within the same country. All ISTA accredited testing laboratories are able to issue the full range of ISTA Certificates, i.e. Orange, Green and Blue. The Head of the Laboratory is always responsible for establishing that the sampling is to ISTA standards when issuing results on an Orange or Green certificate. Therefore the need to distinguish between an Orange and Green Seed Lot Certificate on the basis of sampling in one country and testing in another is now considered unnecessary. In an attempt to simplify the process the Executive Committee set up a Working Group to look at Certificates. The Working Group recommends the deletion of the need for the Green International Seed Lot Certificate in Chapter 17 and supplies the following Rules proposal for a vote. If this proposal is accepted it will come into force on the 1st January 2007 but the Working Group also recommends that laboratories have until the 1st January 2008 to use up existing stocks of Green Certificates. If a positive vote is achieved this option will be explained in the preface of the 2007 edition of the ISTA Rules.

Selected text from Chapter 17 where revisions are necessary

Chapter 17: Certificates ISTA International Seed Analysis Certificates

17.2 Definitions

ISTA International Seed Analysis Certificates ~~a form of certificate produced only by the~~ are only available from ISTA, and only provided to authorised accredited member laboratories (see 17.2.5) and used for reporting the results of tests. These Certificates are the property of the ISTA and may only be issued under the authority of the Association.

17.2.1 *ISTA Orange International Seed Lot Certificate*

The ~~form~~ type of *ISTA International Seed Analysis Certificate* issued when the sample is drawn ~~officially~~ from the seed lot under the authority of an authorised accredited member laboratory. The procedure followed identifies the Certificate with the seed lot. It is coloured orange.

~~There are two types:~~

1. ~~Orange International Seed Lot Certificate~~

~~The Certificate is issued when the sample is drawn officially from the lot and tests carried out under the authority of the same member laboratory and the procedure followed identifies the Certificate with the seed lot. It is coloured orange.~~

2. ~~Green International Seed Lot Certificate~~



~~—The Certificate is issued when the sample is drawn officially from the lot under the authority of one authorised member laboratory but the tests are carried out at another authorised member laboratory in a different country and the procedure followed identifies the Certificate with the seed lot. It is coloured green.~~

17.2.2 *ISTA Blue International Seed Sample Certificate*

The ~~form~~ type of *ISTA International Seed Analysis Certificate* issued when sampling from the lot is not under the responsibility of an authorised accredited member laboratory. The authorised accredited member laboratory is responsible only for testing the sample as submitted. It is not responsible for the relationship between the sample and any seed lot from which it may have been derived. The Certificate is coloured blue.

~~1. Blue International Seed Sample Certificate~~

~~—The authorised member laboratory is responsible only for testing the sample as submitted. It is not responsible for the relationship between the sample and any seed lot from which it may have been derived. The Certificate is coloured blue.~~

17.2.3 *Duplicate Certificate*

An exact copy of a completed ISTA International Seed Analysis Certificate, marked DUPLICATE.

17.2.4 *Provisional Certificate*

A *Certificate* issued before the completion of a test or tests which includes a statement that a final *Certificate* will be issued upon completion.

17.2.5 *Authorised Seed Testing Member Laboratory.*

An authorised seed testing member laboratory is an accredited member laboratory authorised by the ISTA Executive Committee under Article VII of the ISTA Constitution to issue *ISTA International Seed Analysis Certificates*.

17.3 Principles

In case of any doubt as to the precise meaning of the sections that follow, they shall be interpreted in a sense that is compatible with the following principles.

- (1) An *ISTA International Seed Analysis Certificate* shall be issued only if sampling and testing were in accordance with the rules pertaining to the type of *Certificate* issued.
- (2) For the ISTA Orange International ~~Seed Lot~~ *Certificate*, each container in the lot must be sealed or be self-sealing and bear an identifying mark or number which also appears on the *Certificate* so that it is clear to which containers the *Certificate* refers.
- (3) A *Certificate* shall be valid until it is superseded by a *Certificate* subsequently issued on the same lot.

Not more than one ISTA Orange International *Seed Lot Certificate* shall be valid for a lot at one time for any particular attribute- (see also 17.6).

- (4) The assessment of any attribute reported on a *Certificate* shall be calculated from tests of one submitted sample from the lot and shall represent its true quality insofar as this can be deduced from a consideration of all the tests of that attribute performed on that sample.



17.4 Conditions for Issuance of Certificates

17.4.1 General conditions for issuance

ISTA International Seed Analysis Certificates shall be issued only on forms obtained from the ISTA Secretariat and approved by the ISTA Executive Committee. The *Certificates* shall be multilingual and shall be of two kinds: *ISTA Orange International Seed Lot Certificates* and *ISTA Blue International Seed Sample Certificates* as defined in 17.2.

Duplicate and provisional *Certificates* as defined in 17.2 may be issued.

An *ISTA International Seed Analysis Certificate* may be issued only by the ~~seed testing~~ authorised accredited member laboratory which carried out tests to be reported, and provided that:

- (1) The issuing laboratory is currently authorised to do so by the Executive Committee.
- (2) The seed tested is of a species listed in Table 2A, lot and sample weights, of these *ISTA Rules*. Where in other Tables, such as Table 5A and Table 6A, methods are prescribed as being for a group of species, only those species specifically listed in Table 2A shall be considered to be covered.
- (3) The tests are carried out in accordance with these Rules. However, additionally and on request, results of tests not covered by these Rules may be reported on an *ISTA International Seed Analysis Certificate* (see 17.5.5).
- (4) In the case of *ISTA Orange ~~or Green~~ International Seed Lot Certificates*
 - (a) The seed lot complies with the requirements prescribed in ~~2.4~~ 2.5.4;
 - and
 - (b) The submitted sample is drawn and dealt with in accordance with ~~2.5.1,6~~

ISTA International Seed Analysis Certificates are issued on the understanding that:

- (a) In the case of *an ISTA International Orange Seed Lot Certificate* the results reported refer strictly to the lot as a whole at the time of sampling, and
- (b) in the case of *an ISTA Blue International Seed Sample Certificate*, to the sample at the time of receipt.

17.4.2 Orange International Seed Lot Certificate

For an *ISTA Orange International Seed Lot Certificate*, the submitted sample must be tested at an authorised ~~seed testing~~ member laboratory ~~of the country in which the lot is located~~. The authorised testing laboratory shall be responsible for sampling, sealing, labelling, testing and issuance of the *Certificate*; unless the sample has been received from another authorised laboratory. If the testing laboratory was not responsible for the sampling then there must be confirmation that the sampling, sealing and labelling were according to the ISTA Rules.

The completed *Certificate* shall show the following information:

- (a) Name of issuing laboratory,
- (b) Name of sampling and sealing agency,
- (c) ~~Official m~~ Mark and seal of the lot,
- (d) Number of containers in the lot,
- (e) Date of sampling,
- (f) Date sample received by the testing laboratory,



- (g) Date Certificate issued,
- (h) Testing ~~laboratories~~ laboratory's test or sample number,
- (i) Results of tests,
- (j) The following statement signed by the director of the issuing laboratory, or his assignee:
“I certify that sampling, sealing and testing have been carried out in accordance with the International Rules for Seed Testing of the ISTA and that the tests have been made at a laboratory accredited by the International Seed Testing Association to issue International Seed Analysis Certificates.”

~~17.4.3 Green International Seed Lot Certificate~~

~~For a Green Seed Lot Certificate an accredited member laboratory in the country where the lot is located shall be responsible for the sampling and labelling and for the despatch of the submitted sample with pertinent information to the accredited member laboratory designated to carry out the tests (see 17.4.A.3). The testing laboratory shall be responsible for testing the sample and issuance of the Certificate.~~

~~— The completed Certificate shall show the following information:~~

- ~~(a) Name of issuing laboratory,~~
- ~~(b) Name of sampling and sealing agency,~~
- ~~(c) Official mark and seal of the lot,~~
- ~~(d) Number of containers in the lot,~~
- ~~(e) Date of sampling,~~
- ~~(f) Date sample received by the testing laboratory,~~
- ~~(g) Date Certificate issued,~~
- ~~(h) Testing laboratory's test or sample number,~~
- ~~(i) Results of tests,~~
- ~~(j) The following statement signed by the director of the issuing laboratory, or his assignee:
— “I certify that sampling, sealing and testing have been carried out in accordance with the International Rules for Seed Testing of the ISTA and that the tests have been made at the Official Station authorised by the International Seed Testing Association to issue International Seed Analysis Certificates.”~~

17.4.43 Blue International Seed Sample Certificate

The Blue International Seed Sample Certificate refers only to the sample submitted for testing.

The completed Certificate shall show the following information:

- (a) Name of issuing laboratory,
- (b) Date sample received by the testing laboratory,
- (c) Date Certificate issued,
- (d) Testing laboratory's test or sample number,
- (e) Results of tests,
- (f) The following statement signed by the director of the issuing laboratory, or his assignee:
“I certify that testing has been carried out in accordance with the International Rules for Seed Testing of the ISTA and that the tests have been made at a laboratory accredited by the International Seed Testing Association to issue International Certificates.”



17.4.5 ~~4~~ Duplicate Certificate

A Duplicate Certificate may be issued on request to the owner of the seed lot. It shall be identical to the original Certificate in all respects, including the original date of issue, except it shall be marked DUPLICATE.

17.4.6 ~~5~~ Provisional Certificate

A provisional *Certificate* may be issued to the owner of the seed lot on request. It shall contain all the pertinent information required by 17.4.2, or 17.4.3 ~~or 17.4.4~~, plus a statement that a final *Certificate* will be issued on completion of all the tests requested. It shall be clearly marked PROVISIONAL.

17.5 Reporting results

17.5.1 Sampling and testing

From one sampling operation, only one sample may be submitted for testing. The sample may be subjected to one or more of the tests described in these *ISTA Rules* as requested by the applicant. However, in certain situations (see ~~2.6.7~~ 2.5.1.6) the submission of a separate moisture proof packed sample from the same sampling operation attached to the submitted sample is required....

Annexe to Chapter 17: Certificates

...

17.4.A Conditions for Issuance of Certificates

17.4.A.2-4 Certificates

The principles for completing *ISTA Blue International Seed Sample Certificates* are the same as for *ISTA Orange* ~~or Green~~ *International Seed Lot Certificates*.

....

- (4) All dates must be written in the ~~International Organization for Standardization (ISO)~~ ISO 8601 Calendar date representation format: year in full-month-day, with two figures for both month and day. (Example: ~~1996~~ 2006-07-01)...

17.4.A.3 Provision of seed lot and sampling information ~~for the Green International Seed Lot Certificate~~

If a sample is taken by one authorised accredited laboratory for submission to and testing at another authorised accredited laboratory, the ~~The director of the accredited~~ laboratory responsible for sampling, sealing and marking the seed lot shall supply information and a statement to the testing laboratory as follows:

- (a) The name of the sampling and sealing agency.
- (b) The ~~official~~ mark and seal of the lot.
- (c) Date of sampling.
- (d) Number of containers in the lot.



- (e) Information supplied by the applicant necessary to complete the Certificate, including the tests required.
- (f) Information concerning previously issued Certificates for the lot.

The director [of the authorised accredited laboratory](#) shall [sign the following statement](#):

“I certify that sampling and sealing have been carried out in accordance with the current International Rules for Seed Testing of the International Seed Testing Association.”

...

Item 8b. Clarification of the restrictions for issuing ISTA certificates

One case provided by the ISTA auditors has resulted in the following rules proposal to clarify the restrictions on issuing ISTA certificates and re-sampling ISTA seed lots. To keep item 8a (the removal of the need for GICs) self-contained proposal this is a separate proposal.

17.6 Validity of Certificates

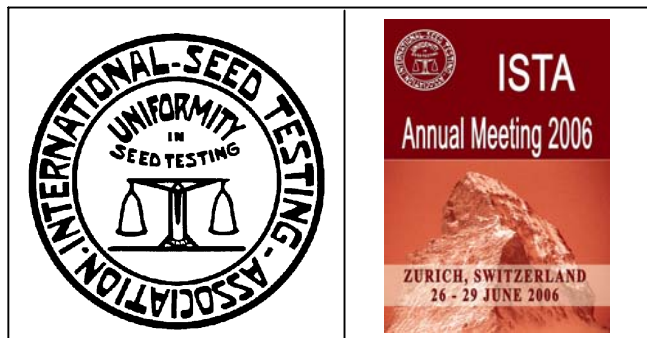
~~It is not permissible for the director of the same seed testing laboratory to issue another Certificate of the same colour (see 17.5.2, 17.5.3 and 17.5.4) as a result of a further sampling from the same seed lot within one month of the previous sampling or to approve such sampling. Exceptions can be made for chemically treated or coated seed lots which have been previously tested in the untreated state.~~

An authorised accredited laboratory must not issue a new *ISTA Orange International Seed Lot Certificate* or *ISTA Blue International Seed Sample Certificate* as the result of resampling the same seed lot within 28 days. If a *Certificate* of a different ~~category type~~ is required, or a *Certificate* is to be issued by a different [accredited seed testing](#) laboratory, or the ~~28 day one month~~ time limit is exceeded, another *Certificate* may be issued provided that the reference number, colour of the previous *Certificate* and the date of previous sampling are indicated ~~or the lot has been re-labelled and re-sealed. If the identity of the lot has not been changed, t.~~ There ~~should~~ must be a statement that the previous *Certificate* has been cancelled in respect of the tests reported in the later *Certificate*.

Appendix 1

SUPPORTING DATA AND EVIDENCE FOR THE PROPOSED RULES CHANGES 2006 – ITEM 4A – MODIFICATION TO DORMANCY BREAKAGE FOR *HORDEUM VULGARE*

**FOR INFORMATION AND CONSIDERATION AT THE
ORDINARY MEETING 2006**





Potassium Nitrate for Dormancy Breaking on Cereal Seeds

Results of a ISTA Comparative Test
on *Hordeum vulgare*

Sample Preparation

- Germination testing of 2 different winter barley varieties from Germany
- Germination testing of 2 different winter barley varieties from Scotland
- Germination testing of 2 different summer barley varieties from Norway



Participants of the Comparative Test

- FR 02
- DK 01
- GB 01
- GB 04
- IE 01
- SE 02
- NO 01
- DE 04
- DE 10
- DE 15
- DE 18

Methods to Breaking Dormancy

- Water only (without dormancy breaking)
- Water and prechill (3 and 5 days)
- Gibberellic acid and prechill (3 and 5 days)
- Potassium nitrate and prechill (3 and 5 days)
- Gibberellic acid
- Potassium nitrate
- Count after seven and fourteen days



Supporting Data and Evidence for Proposed Rules Changes - Item 4a:
Modification to Dormancy Breakage for *Hordeum Vulgare*

Normal Seedlings, Count after 7 days

	Prechilling			KNO ₃ /Prechilling			GA ₃ / Prchilling		
	0	3 d	5 d	0	3 d	5 d	0	3 d	5 d
1	33	40	51	31	43	57	55	70	72
2	30	44	62	32	61	82	66	77	81
3	33	81	93	49	95	98	78	93	97
4	67	95	97	82	95	96	93	97	97
5	59	84	89	73	89	92	82	92	93
6	52	86	89	66	93	92	85	93	93
x	46	72	80	55	79	86	76	87	89

Abnormal Seedlings, Count after 7 days

	Prechilling			KNO ₃ /Prechilling			GA ₃ / Prchilling		
	0	3 d	5 d	0	3 d	5 d	0	3 d	5 d
1	6.3	7.5	5.8	6,6	4.6	6.3	7.9	7.5	7.5
2	3.5	3.1	2.7	2,8	3.5	2.8	4.6	4.1	4.3
3	0.8	0.7	1.0	0,7	0.9	0.8	1.6	1.5	1.0
4	1.5	1.2	1.4	1.0	1.3	2.0	1.4	1.2	1.6
5	3.3	3.0	4.3	3.5	3.6	4.5	4.3	3.6	4.4
6	1.5	3.6	4.3	3.3	3.6	4.4	2.8	3.7	4.4
x	2.8	3.2	3.3	3.0	2.9	3.5	3.7	3.6	3.9



Supporting Data and Evidence for Proposed Rules Changes - Item 4a:
Modification to Dormancy Breakage for *Hordeum Vulgare*

Fresh ungerminated Seeds, Count after 7 days

	Prechilling			KNO ₃ /Prechilling			GA ₃ /Prechilling		
	0	3 d	5 d	0	3 d	5 d	0	3 d	5 d
1	52	43	35	52	42	28	26	15	11
2	63	49	32	61	31	12	26	15	9
3	65	17	6	50	3	1	19	4	1
4	30	3	1	16	2	0	4	1	0
5	36	9	4	21	3	1	12	1	1
6	44	8	5	29	2	1	11	1	1
x	48	22	14	38	14	7	16	6	4

Dead Seeds, Count after 7 days

	Prechilling			KNO ₃ /Prechilling			GA ₃ /Prechilling		
	0	3 d	5 d	0	3 d	5 d	0	3 d	5 d
1	8,9	9,0	8,1	11,1	9,8	8,9	11,1	7,6	10,1
2	3,6	3,8	3,3	4,1	4,3	3,6	3,3	3,8	5,1
3	0,8	1,0	0,3	0,9	1,1	0,7	1,0	0,9	0,6
4	1,5	1,5	1,1	1,6	1,7	1,3	1,6	1,1	1,4
5	1,9	2,8	2,5	2,4	4,0	2,7	2,0	3,5	1,8
6	2,4	2,1	2,1	1,8	2,2	1,3	1,1	2,2	1,7
x	3,2	3,4	2,9	3,6	3,9	3,1	3,4	3,2	3,5



Supporting Data and Evidence for Proposed Rules Changes - Item 4a:
Modification to Dormancy Breakage for *Hordeum Vulgare*

Normal Seedlings, Count after 14 days

	Prechilling			KNO ₃ /Prechilling			GA ₃ / Prechilling		
	0	3 d	5 d	0	3 d	5 d	0	3 d	5 d
1	42	49	58	43	50	61	66	73	75
2	43	52	66	46	66	82	78	84	85
3	47	80	92	69	94	97	95	96	97
4	84	95	93	90	96	96	96	97	96
5	74	86	89	77	89	92	90	92	93
6	72	88	90	76	92	92	93	94	93
x	60	75	81	67	81	87	86	89	90

Abnormal Seeds, Count after 14 days

	Prechilling			KNO ₃ /Prechilling			GA ₃ / Prechilling		
	0	3 d	5 d	0	3 d	5 d	0	3 d	5 d
1	10.2	8.5	7.5	9.8	7.0	8.0	13.2	11.4	10.7
2	6.3	4.1	4.3	5.1	4.9	4.1	9.6	6.6	5.9
3	1.4	1.1	1.0	1.8	1.3	1.0	1.9	1.7	1.4
4	2.0	1.4	1.7	1.7	1.8	2.5	1.6	1.6	1.9
5	4.3	3.8	5.0	5.4	4.5	4.9	6.4	3.8	4.6
6	3.7	4.0	4.8	5.9	4.2	5.3	4.5	3.8	4.7
x	4.7	3.8	4.0	4.9	3.9	4.3	6.2	4.8	4.8



Supporting Data and Evidence for Proposed Rules Changes - Item 4a:
Modification to Dormancy Breakage for *Hordeum Vulgare*

Fresh ungerminated Seeds, Count after 14 days

	Prechilling			KNO ₃ /Prechilling			GA ₃ / Prechilling		
	0	3 d	5 d	0	3 d	5 d	0	3 d	5 d
1	32	28	23	30	27	17	3	2	2
2	44	38	24	38	22	7	3	2	2
3	49	14	6	26	3	0	1	1	1
4	11	1	0	5	0	0	0	0	0
5	19	6	3	12	2	1	2	0	0
6	20	4	2	12	1	1	1	0	0
x	29	15	10	21	9	4	2	1	1

Dead Seeds, Count after 14 days

	Prechilling			KNO ₃ /Prechilling			GA ₃ / Prechilling		
	0	3 d	5 d	0	3 d	5 d	0	3 d	5 d
1	13.7	13.4	11.5	17.3	16.3	14.3	17.3	13.7	13.0
2	5.5	6.1	4.7	10.5	7.1	6.5	9.0	7.2	6.5
3	1.8	1.0	0.9	2.0	1.8	1.5	1.6	1.3	1.0
4	2.8	1.9	1.6	2.5	2.1	1.9	2.0	1.7	1.8
5	3.7	3.8	3.2	4.9	4.7	3.0	3.5	3.5	2.1
6	4.0	3.3	2.9	4.0	3.1	2.2	1.9	2.4	2.0
x	5.3	4.9	4.1	6.9	5.8	4.9	5.9	5.0	4.4



Supporting Data and Evidence for Proposed Rules Changes - Item 4a:
Modification to Dormancy Breakage for *Hordeum Vulgare*

Results of all the Methods to Breaking Dormancy after 7 days

Methode	Normal seedlings		Fresh ungerminated seeds)	
	%	SD	%	SD
Water only	45.8	8.3	48.3	7.9
Water and 3 days prechilling	71.8	4.0	21.5	3.7
GA ₃ and 3 days prechilling	87.2	4.1	6.1	4.3
KNO ₃ and 3 days prechilling	79.4	2.8	13.9	2.6
GA ₃	76.4	9.4	16.5	9.5
KNO ₃	55.4	8.9	38.0	8.4

Results of all the Methods to Breaking Dormancy after 7 days

Methode	Normal seedlings		Fresh ungerminated seeds)	
	%	SD	%	SD
Water only	45.8	8.3	48.3	7,9.
Water and 5 days prechilling	80.2	3.8	13.7	4.1
GA ₃ and 5 days prechilling	88.8	3.5	4.1	2.9
KNO ₃ and 5 days prechilling	86.2	2.9	7.2	3.0
GA ₃	76.4	9.4	16.5	9.5
KNO ₃	55.4	8.9	38.0	8.4



Supporting Data and Evidence for Proposed Rules Changes - Item 4a:
Modification to Dormancy Breakage for *Hordeum Vulgare*

Results of all the Methods to Breaking Dormancy after 14 days

Methode	Normal seedlings		Fresh ungerminated seeds)	
	%	SD	%	SD
Water only	60.4	6.0	29.1	6.8
Water and 3 days prechilling	75.0	3.7	15.3	3.6
GA ₃ and 3 days prechilling	89.4	2.2	0.8	1.2
KNO ₃ and 3 days prechilling	81.0	2.3	9.1	2.9
GA ₃	86.4	3.4	1.8	1.6
KNO ₃	66.8	4.1	26.6	5.8

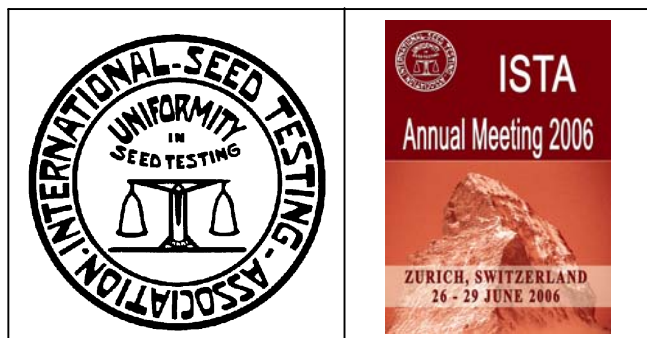
Results of all the Methods to Breaking Dormancy after 14 days

Methode	Normal seedlings		Fresh ungerminated seeds)	
	%	SD	%	SD
Water only	60.4	6.0	29.1	6.8
Water and 5 days prechilling	81.1	4.6	9.7	3.6
GA ₃ and 5 days prechilling	89.9	2.3	0.9	1.1
KNO ₃ and 5 days prechilling	86.5	2.9	4.3	2.2
GA ₃	86.4	3.4	1.8	1.6
KNO ₃	66.8	4.1	26.6	5.8

APPENDIX 2

FULL TEXT OF THE PROPOSED METHOD 7-021 - RULES CHANGES 2006 - ITEM 5A MODIFICATIONS AND ADDITIONS TO THE VALIDATED SEED HEALTH METHODS

**FOR INFORMATION AND CONSIDERATION AT THE
ORDINARY MEETING 2006**





International Rules for Seed Testing
Annexe to Chapter 7: Seed Health Testing Methods



7-021: Detection of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans* on *Phaseolus vulgaris* (Bean)

Published by: International Seed Testing Association (ISTA), Bassersdorf, Switzerland
2007

DISCLAIMER: whilst ISTA has taken care to ensure the accuracy of the methods and information described in this method description ISTA shall not be liable for any loss damage etc., resulting from the use of this method.

- Crop:** *Phaseolus vulgaris* (bean)
- Pathogen:** *Xanthomonas axonopodis* pv. *phaseoli* (common blight) and *Xanthomonas axonopodis* pv. *phaseoli* var. *fuscans*
- Prepared by:** Sheppard, J.¹, Kurowski, C.² and Remeeus, P.M.³
¹Canadian Food Inspection Agency, P.O. Box 960 Carling Avenue, Ottawa, Ontario, Canada
²Harris Moran Seed Company, 9142 Mace Blvd, Davis, CA 95616, US.
E-mail: c.kurowski@harrismoran.com
³Naktuinbouw, P.O. Box 40, 2370 AA Roelofarendsveen, The Netherlands.
E-mail: p.remeeus@naktuinbouw.nl

Revision History: Version 1.0, 22 February 2006.

Background

This method is derived from the validation studies carried out by ISTA in 2003, in collaboration with the International Seed Health Initiative for Vegetables (ISHI-Veg) (Sheppard and Remeeus, 2005). For routine testing of bean seed a combination of two complementary semi-selective media, MT and XCP1, is recommended with a pathogenicity test to confirm suspect isolates.

The two media, XCP1 and MT, have been chosen for their ease of use and selectivity for *X. axonopodis* pv. *phaseoli*. In addition both media can be used to detect both *X. axonopodis* pv. *phaseoli* and *X. axonopodis* pv. *phaseoli* var. *fuscans*. Although initially the morphology of fuscans and non-fuscans strains of *X. axonopodis* pv. *phaseoli* appear to be similar on the media after a longer incubation the fuscans colonies are distinguished by a distinct brown pigmentation. A further advantage of MT medium is that it can also be used for identifying other bacterial seed-borne pathogens of beans, e.g. *Pseudomonas savastanoi* pv. *phaseolicola* and *Pseudomonas syringae* pv. *syringae*.

Validation Studies

Sheppard, J.W. and Remeeus, P.M. (2005)

Copies are available: by E-mail from ista.office@ista.ch; by mail from the ISTA Secretariat, Zürichstrasse 50, 8303 Bassersdorf, Switzerland.

Please send comments, suggestions or reports of problems relating to this method to the
ISTA Seed Health Committee, c/o ISTA Secretariat

Annexe to Chapter 7: Seed Health Methods: 7-021-2

Safety Precautions

Ensure you are familiar with hazard data and take appropriate safety precautions, especially during preparation of media, autoclaving, and weighing out of ingredients. It is assumed that this procedure is being carried out in a microbiological laboratory by persons familiar with the principles of Good Laboratory Practice, Good Microbiological Practice, and aseptic technique. Dispose of all waste materials in an appropriate way (e.g. autoclave, disinfect) and in accordance with local health, environmental and safety regulations.

Treated Seed

Chemical seed treatments may affect the performance of this test. It should only be performed on untreated seed.

Sample and sub-sample size

The sample (total number of seeds tested) and sub-sample size to be tested depends on the desired tolerance standard (maximum acceptable percentage of seeds infested) and detection limit (theoretical minimum number of pathogen propagules per seed which can be detected); in any case the maximum sub-sample size should be 1 000 seeds.

Materials

Reference material	- A known strain for both fuscans and non fuscans types of <i>X. axonopodisi</i> pv. <i>phaseoli</i> or standardised reference material.
Plates of MT medium	- 9.0 cm Petri dishes (3 plates of each medium per sub-sample + controls)
Plates of XCP1 medium	- 9.0 cm Petri dishes (3 plates of each medium per sub-sample + controls)
Plates of YDC	- for sub-culture (at least 1 per sub-sample).
Polythene bags or containers	- of sterile saline (0.85% NaCl) plus Tween 20 (0.02% - 20ml per 100 ml) for soaking of seeds (1 000 seeds in 600 ml or determined according to the Thousand Seed Weight).
Dilution bottles	- containing 4.5 ml of sterile saline (2 per sub-sample). Other volumes may be acceptable, see General Methods.
70% ethanol	- for disinfection of surfaces, equipment
Incubator	- operating at 27-28°C
Balance	- capable of weighing to the nearest 0.001 mg
pH meter	- capable of being read to the nearest 0.01 pH unit
Automatic pipettes	- check accuracy and precision regularly
Bean seedlings	- susceptible to all races of the pathogen for pathogenicity test (e.g. Michelet, Contender)
Orbital shaker	
Sterile pipette tips	
Sterile bent glass rods	

Sample Preparation

1. This can be done in advance of the assay.
2. It is vital to exclude any possibility of cross-contamination between seed samples. It is therefore essential to disinfect all surfaces, containers, hands, etc. both before and after handling each sample. This can be achieved by swabbing/spraying equipment and gloved hands with 70% ethanol.
3. If the submitted sample is received in several packets, these should be combined by emptying into a new, clean polythene bag and mixing by hand to give a composite sample.
4. Count the number of seeds in a known weight. Estimate the Thousand Seed Weight (TSW) as:

$$TSW = \frac{\text{Weight of seed}}{\text{Number of seed}} \times 1000$$

5. Based on the TSW, weigh out sub-samples of the required size into new, clean polythene bags or containers.

Method

[Critical control points are indicated by **CCP**]

1. *Extraction*
 - 1.1. Suspend seeds in sterile saline plus Tween 20 (0.02% v/v) in a polythene bag or container. The volume of saline should be adjusted according to the number of seeds used (600 ml per 1 000 seeds) (Olivier and Remeus, 2004).
 - 1.2. Soak sub samples overnight (16-18 h) at 5°C (± 2°C).
2. *Dilution and plating*
 - 2.1. Shake the flasks to mix just before dilution.
 - 2.2. Prepare a tenfold dilution series from the seed extract. Pipette 0.5 ml of the extract into 4.5 ml of sterile saline and vortex to mix (10¹ dilution). Pipette 0.5 ml of the 10¹ dilution into another 4.5 ml of sterile saline and vortex to mix (10² dilution) (see General Methods).
 - 2.3. Pipette 100 µl of each dilution and the un-diluted seed extract onto plates of each of the selective media (MT and XCP1) and spread over the surface with a sterile bent glass rod (see General Methods).
 - 2.4. Incubate plates at 27-28°C up side down and examine after 3-5 d.
3. *Positive control (culture or reference material)*
 - 3.1. Prepare a suspension of a known strain of *X. axonopodis* pv. *phaseoli*, fuscans and non-fuscans, in sterile saline or reconstitute standardised reference material according to the supplier's instructions.
 - 3.2. Dilute sufficiently to obtain dilutions containing approximately 10² to 10⁴ cfu/ml. This may require up to seven ten-fold dilutions from a turbid suspension.
 - 3.3. Pipette 100 µl of appropriate countable dilutions onto plates of each of the selective media (MT and XCP1) and non-selective media (YDC) and spread over the surface with a sterile bent glass rod.
 - 3.4. Incubate plates with the sample plates.
4. *Sterility check*
 - 4.1. Prepare a dilution series from a sample of the extraction medium (i.e., saline plus Tween 20), containing no seeds, and plate on each of the media as for samples.

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5. *Examination of the plates*
 - 5.1. Examine sterility check and recovery of positive control on semi-selective medium (**CCP**).
 - 5.2. Examine the sample plates for the presence of typical *X. axonopodis* pv. *phaseoli* colonies by comparison with the positive control plates.
 - 5.3. After 3-4 d on MT, *X. axonopodis* pv. *phaseoli* colonies are yellow distinguished by two zones of hydrolysis; a large clear zone of casein hydrolysis and a smaller milky zone of Tween 80 lysis (Fig. 1a and b). The fuscans of *X. axonopodis* pv. *phaseoli* colonies produce a brown diffusible pigment after 5 d of incubation. Often the fuscans type colonies show Tween 80 lysis.
 - 5.4. After 3-4 d on XCP1, *X. axonopodis* pv. *phaseoli* colonies are yellow, glistening and surrounded by a clear zone of starch hydrolysis (Fig. 2). The fuscans of *X. axonopodis* pv. *phaseoli* colonies produce a brown diffusible pigment after 5 d of incubation. Often the fuscans type colonies show Tween 80 lysis.
 - 5.5. The colony size and colour can differ within a sample.
 - 5.6. Record the number of suspect and other colonies (see General Methods).
 6. *Confirmation/identification of suspect colonies*
 - 6.1. Sub-culture suspect colonies to sector plates of YDC. To avoid the potential for cross-contamination of isolates, use a new sector plate for each sub-sample. The precise numbers of colonies sub-cultured will depend on the number and variability of suspect colonies on the plate: if present, at least six colonies should be sub-cultured per sub-sample (**CCP**).
 - 6.2. Sub-culture the positive control isolate to a sector plate for comparison (Fig. 3).
 - 6.3. Incubate sector plates for 24-48 h at 27°C ($\pm 1^\circ\text{C}$).
 - 6.4. Compare appearance of growth with positive control. On YDC *X. axonopodis* pv. *phaseoli* colonies are yellow and mucoid in appearance (**CCP**).
 - 6.5. Confirm the identity of isolates by pathogenicity on bean seedlings of known susceptibility (**CCP**).
 - 6.6. Record results for each colony sub-cultured.
 7. *Pathogenicity* (Saettler, 1971)
 - 7.1. Grow seedlings of a bean cultivar known to be susceptible to all races of *X. axonopodis* pv. *phaseoli* (e.g. Michelet or Contender at 21°C ($\pm 2^\circ\text{C}$) in small pots or modules until the first leaf is just visible (usually about 10 days after sowing).
 - 7.2. Water plants 1-3 h before inoculation to provide better conditions for infection.
 - 7.3. Dip a sterile toothpick or needle into the bacterial culture of a 2-4 d old YDC culture (e.g. sector plate).
 - 7.4. Inoculate the seedling by stabbing the toothpick or needle through the primary node at an angle of about 45°. Stop stabbing as the needle emerges from the opposite side of the node. Turn the toothpick or needle slightly while drawing to release bacteria. Support the seedling with one hand during the process.
 - 7.5. The number of plants which should be inoculated will depend on the variability of the cultivar and experience of the operator, but 1-3 plants per isolate should usually be sufficient. It is better to inoculate more isolates with 1 plant per isolate than one isolate with 3 plants.
 - 7.6. Inoculate seedlings with the two positive control isolates (following 7.3-7.5) (**CCP**) and a negative control with only a sterile toothpick or needle (**CCP**).
 - 7.7. Grow on plants at 20-25°C.
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- 7.8. Record symptoms after 4-5 d, 8-10 d and again at 14 d. Compare with positive control (**CCP**). Typical *X. axonopodis* pv. *phaseoli* symptoms include dark green water-soaked lesions at the point of entry of the toothpick or needle. Lesions can become red-brown, elongate extending into the stem causing slight to severe stem cracking. Wilting and flagging of the top foliage followed by necrosis can occur 14-18 d after inoculation (Fig. 4).

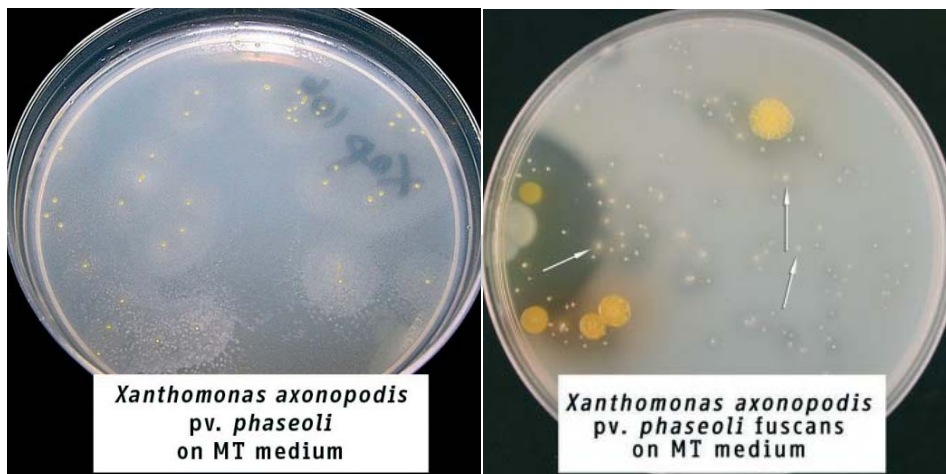


Fig. 1a

Fig. 1b

Fig. 1. *X. axonopodis* pv. *phaseoli* colonies on MT plates after 4 d indicated by a large clear zone of casein hydrolysis (a) and a smaller milky zone of Tween 80 lysis (b).



Fig. 2a

Fig. 2b

Fig. 2. *X. axonopodis* pv. *phaseoli* colonies, non-fuscans (a) and fuscans (b), on XCP1 plates showing a clear zone of starch hydrolysis and fuscans on XCP1 showing a milky zone, after 4 d

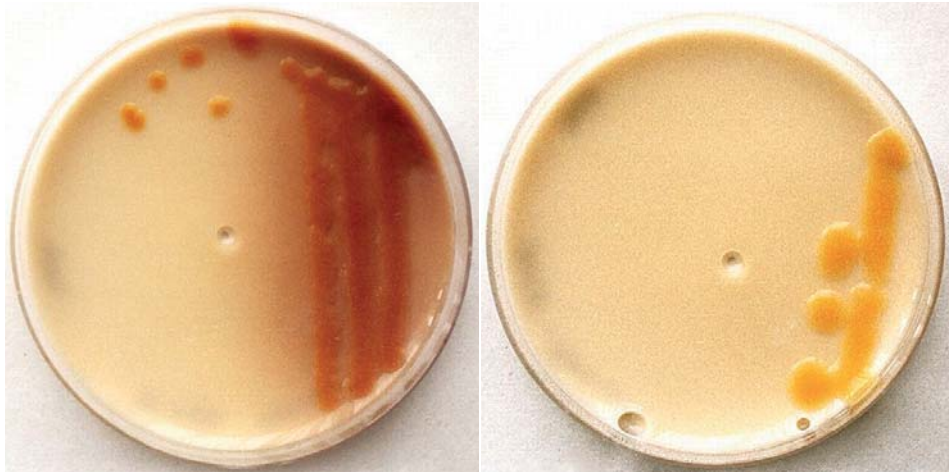


Fig. 3a

Fig. 3b

Fig. 3. *X. axonopodisi* pv. *phaseoli* colonies, fuscans (a) and non-fuscans (b), on YDC plates after 2 d are brown and yellow in appearance.



Fig. 4a



Fig. 4b



Fig. 4c

Fig. 4. Typical *X. axonopodisi* pv. *phaseoli* symptoms in a pathogenicity test are red-brown, elongate lesions extending into the stem causing slight to severe stem cracking (a). A negative control compared to fuscans (b) and non-fuscans (c).

General Methods (common to many test procedures)

1. Preparation of ten-fold dilution series

Each dilution should be prepared by pipetting 0.5 ml (\pm 5%) from a well-mixed seed extract or previous dilution into a universal bottle (screw-capped) or similar container containing 4.5 ml (\pm 2%) of sterile diluent and then vortexing to mix prior to the next dilution step. A new sterile pipette tip should be used for each dilution step. Pipettes should be checked regularly for accuracy and precision and re-calibrated as necessary. It is acceptable to prepare ten-fold dilutions using other volumes provided that the laboratory can demonstrate that the required accuracy and precision can be achieved.

2. Plating of dilutions.

This should be done as soon as possible after dilutions have been prepared and certainly within 30 min. Working from the highest (most dilute) dilution to the undiluted extract, 0.1 ml is pipetted onto the centre of a surface-dry, labelled agar plate. The liquid should then be spread evenly over the entire surface of the medium with a bent glass rod. If care is taken to work from the highest to the lowest dilution (or undiluted extract) a single pipette tip and a single bent glass rod can be used for each sample. Ensure that all liquid has been absorbed by the agar before inverting and incubating plates. If necessary allow plates to dry under a sterile air-flow in a microbiological safety cabinet or laminar flow hood.

3. Recording of dilution plates

Record the results for all dilution plates. The most accurate estimate of bacterial numbers should be obtained from spread plates with total number between 30 and 300 colonies. However this may be further complicated depending on the relative numbers of suspect pathogen and other colonies. In order to minimise effort, start recording with the highest dilution (most dilute) and count the number of suspect and the number of other colonies. If the total number of colonies on a plate greatly exceeds 300 there is little value in trying to make a precise count if a more reliable count has already been obtained from a more dilute plate, in which case it is sufficient to record the number of colonies as 'm' (many) if they are still separate or 'c' (confluent) if they have run together.

4. Sectored Plates

Using a laboratory marker pen draw lines on the base of a standard 9 cm plate (Petri dish) to divide it into six equal sectors. Sub-culture single colonies from dilution plates and make a single zigzagged streak within a single sector on the plate. Take care to leave sufficient space between each isolate to ensure the growth does not coalesce. Thus six suspect colonies can be sub-cultured to each sectored plate. Separate plates should be used for each sample/sub-sample. If the purity of sub-cultured isolates is doubtful, they should be further streaked out on whole plates.

5. Reporting Results

The result of a seed health test should indicate the scientific name of the pathogen and the test method used. When reported on an ISTA Certificate, results are entered under *Other Determinations*.

In the case of a negative result (pathogen not detected in any sub-samples), the results should be reported in terms of the tolerance standard and detection limit. The tolerance standard depends on the total number of seeds tested, n , and is approximately $3/n$ ($P=0.95$) (see Roberts *et al.*, 1993); the detection limit per sub-sample is equal to the detection limit per ml multiplied by the volume of extract.

In the case of a positive result, the report should indicate the mean number of pathogen propagules (cfu) per seed and either the number of positive sub-samples out of the total number tested and the sample size or the maximum likelihood estimate of the proportion of infested seeds (Most Probable Number (Cochran, 1950)).

Quality Assurance

General

A record should be kept of the date and results of pipette calibration checks.

It is essential that operators have received appropriate training and use automatic pipettes correctly.

Critical Control Points

[Identified by **CCP** in the methods]

Dilution plates prepared from positive control isolate(s) or reference material, should give single colonies with typical morphology (Step 5.1 and 6.4).

The numbers of colonies on dilution plates prepared from the positive control isolate(s) or reference material should be similar on both media (Step 5.1).

Numbers of bacteria on dilution plates should be consistent with the dilution (i.e. should decrease approximately ten-fold with each dilution) (Step 5.1). Note that recovery of the fuscans type *X. axonopodis* pv. *phaseoli* is in general lower on MT than on XCP1.

There should be no growth on dilution plates prepared as a sterility check (Step 5.1).

Due to the potential for non-pathogenic isolates to be present in seed lots together with pathogenic isolates, it is essential to sub-culture (Step 6.1), if present, at least the minimum number of suspect colonies specified (six per sub-sample) and to test all *Xanthomonas*-like sub-cultured isolates for pathogenicity (Step 6.5).

Positive control isolates should be included in every pathogenicity test (Step 7.6).

The positive control isolate should give typical symptoms in a pathogenicity test (Step 7.8).

The activity (g) of some antibiotics may vary between batches. It may be necessary to adjust the weight or volume added to ensure that the final number of units per litre of medium is consistent (MT and XCP1 media).

Preparation of sterile saline (van Vuurde *et al.*, 1989)

Compound	g/l	g/500 ml
Sodium chloride (NaCl)	8.5	4.25
Distilled/de-ionised water	1 000 ml	500 ml

Preparation

1. Weigh out all ingredients into a suitable container.
2. Add 1 000 ml (or 500 ml) of distilled/de-ionised water.
3. Dissolve and dispense into final containers.
4. Autoclave at 121°C, 115 psi for 15 min.
5. For extraction of seeds, add 20 µl of sterile Tween 20 per 1 000 ml.

Storage

Provided containers are tightly closed, may be stored for several months before use.

Preparation of MT (Milk Tween) agar medium
(adapted from Goszczynska and Serfontein, 1998)

Compound	g/l	g/500 ml
Proteose peptone #3	10.0	5.0
CaCl ₂	0.25	0.125
Tyrosine	0.5	0.25
Agar	15.0	7.5
Distilled/de-ionised water	1 000 ml	500 ml
Skim milk powder (Oxoid, Sigma) ^a	10.0	5.0
Tween 80 ^b	10.0 ml	5.0 ml
Nystatin ^c	40 mg (1 ml)	20 mg (0.5 ml)
Cephalexin ^d	80 mg (1 ml)	40 mg (0.5 ml)
Vancomycin ^e	10 mg (1 ml)	5 mg (0.5 ml)

a, b, c, d, e Added after autoclaving

Preparation

1. Weigh out all ingredients except agar, antibiotics, skim milk powder and Tween 80 into a suitable container.
2. Add 1 000 ml (or 500 ml) of distilled/de-ionised water.
3. Dissolve and check pH which should be 6.5, adjust if necessary.
4. Add agar then steam to dissolve.
5. Autoclave at 121°C, 115 psi for 15 min.
6. Prepare antibiotic solutions if necessary and filter sterilise as appropriate.
7. Allow medium to cool to approximately 50°C and add antibiotic solutions, skim milk powder and Tween 80.
8. Mix thoroughly but gently by inversion/swirling to avoid air bubbles and pour plates (22 ml per 9.0 cm plate).
9. Leave plates to dry in a laminar flow bench or similar before use.

Antibiotics (amounts for guidance only, CCP)

^c Dissolve 400 mg nystatin in 10 ml 70% ethanol. Add 1 ml/l (0.5 ml/500 ml).

^d Dissolve 800 mg cephalexin in 10 ml 70% ethanol. Add 1 ml/l (0.5 ml/500 ml).

^e Dissolve 100 mg vancomycin in 10 ml 70% ethanol. Add 1 ml/l (0.5 ml/500 ml).

Storage

Store prepared plates inverted in polythene bags at 4°C and use within two weeks of preparation to ensure activity of antibiotics.

Preparation of XCP1 agar medium(adapted from McGuire *et al.*, 1986)

Compound	g/l	g/500 ml
KBr	10.0	5.0
CaCl ₂	0.25	0.125
Soluble Potato Starch	10.0	5.0
Peptone	10.0	5.0
Agar	15.0	7.5
Distilled/de-ionised water	1 000 ml	500 ml
Tween 80 ^a	10.0 ml	5.0 ml
Crystal violet ^b (1% aqueous)	0.15 ml	0.075 ml
Nystatin ^c	40 mg (1 ml)	20 mg (0.5 ml)
Cephalexin ^d	10 mg (1 ml)	5 mg (0.5 ml)
Fluorouracil ^e	3 mg (1 ml)	1.5 mg (0.5 ml)
Tobramycin ^f	0.16 mg (1 ml)	0.08 mg (0.5 ml)

^{a, b, c, d, e, f} Added after autoclaving**Preparation**

1. Weigh out all ingredients except antibiotics, Tween 80, fluorouracil, tobramycin and crystal violet into a suitable container.
2. Add 1 000 ml (or 500 ml) of distilled/de-ionised water.
3. Steam to dissolve.
4. Autoclave at 121°C, 115 psi for 15 min.
5. Prepare antibiotic solutions, Tween 80, fluorouracil, tobramycin and crystal violet solutions and filter sterilise as appropriate.
6. Allow medium to cool to approximately 50°C before adding antibiotic, Tween 80, fluorouracil, tobramycin and crystal violet solutions.
7. Mix the molten medium gently to avoid air bubbles and pour plates (22 ml per 9.0 cm plate).
8. Leave plates to dry in a laminar flow bench or similar before use.

Antibiotics (amounts for guidance only, CCP)^c Dissolve 400 mg nystatin in 10 ml 70% ethanol. Add 1 ml/l (0.5 ml/500 ml).^d Dissolve 100 mg cephalexin in 10 ml 70% ethanol. Add 1 ml/l (0.5 ml/500 ml).^e Dissolve 30 mg fluorouracil in 100 ml 70% ethanol. Add 1 ml/l (0.5 ml/500 ml).^f Dissolve 16 mg tobramycin in 100 ml 70% ethanol. Add 1 ml/l (0.5 ml/500 ml).**Storage**

Store prepared plates inverted in polythene bags at 4°C and use within two weeks of preparation to ensure activity of antibiotics.

Depending on the source of starch, pre-storage in the refrigerator for several days before use may result in more easily visible zones of starch hydrolysis.

Preparation of Yeast Dextrose Chalk (YDC) agar medium

(Wilson et al., 1967)

Compound	g/l	g/500 ml
Bacto Agar	15.0	7.5
Yeast Extract	10.0	5.0
CaCO ₃ (light powder)	20.0	10.0
D-Glucose (Dextrose)	20.0	10.0
Distilled/de-ionised water	1 000 ml	500 ml

Preparation

1. Weigh out all ingredients into a suitable oversize container (i.e. 250 ml of medium in a 500 ml bottle/flask) to allow swirling of medium just before pouring.
2. Add 1 000 ml (or 500 ml) of distilled/de-ionised water.
3. Steam to dissolve.
4. Autoclave at 121°C, 115 psi for 15 min.
5. Allow medium to cool to approximately 50°C.
6. Swirl to ensure even distribution of CaCO₃ and avoid air bubbles, and pour plates (22 ml per 9.0 cm plate).
7. Leave plates to dry in a laminar flow bench or similar before use.

Storage

Store prepared plates inverted in polythene bags at room temperature.

Prepared plates can be stored for several months provided they do not dry out.

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APPENDIX 3

SUPPORTING DATA AND EVIDENCE FOR THE PROPOSED RULES CHANGES 2006 – ITEM 6A – ADDITION OF *TRITICUM DICOCCUM*

FOR INFORMATION AND CONSIDERATION AT THE
ORDINARY MEETING 2006





**SUPPORTING DATA AND EVIDENCE FOR PROPOSED RULES CHANGES - ITEM 6A:
ADDITION OF *TRITICUM DICOCCUM* TO THE ISTA RULES**

DATA OF AN INTERNATIONAL RING TEST ORGANIZED IN 2004

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In this ring test four laboratories from two countries, Italy and France, participated. The results from the germination test of *Triticum dicoccum* on paper substrates: TP (Top of Paper) and BP (Between Paper) and in sand (S): are reported in the table below.

Sample 1	TP				BP				S			
	TAV	BO	PI	SNES	TAV	BO	PI	SNES	TAV	BO	PI	SNES
A	92	92	85	82	96	98	92	95	96	98	94	99
B	93	93	86	86	92	97	96	98	92	98	98	99
C	91	91	88	87	93	97	95	96	91	97	92	95
D	93	92	90	93	95	96	93	99	97	99	93	98
Mean	92.25	92.00	87.25	87.00	94.00	97.00	94.00	97.00	94.00	98.00	94.25	97.75

Sample 2	TP				BP				S			
	TAV	BO	PI	SNES	TAV	BO	PI	SNES	TAV	BO	PI	SNES
A	88	81	90	84	93	90	92	97	91	90	91	95
B	89	83	86	82	92	89	89	94	93	93	90	89
C	89	85	88	91	93	88	91	98	89	91	93	95
D	90	83	89	93	94	90	88	94	95	94	90	92
Mean	89.00	83.00	88.25	87.50	93.00	89.25	90.00	95.75	92.00	92.00	91.00	92.75

Sample 3	TP				BP				S			
	TAV	BO	PI	SNES	TAV	BO	PI	SNES	TAV	BO	PI	SNES
A	92	86	93	81	91	90	98	96	92	93	91	91
B	89	87	90	89	95	92	94	94	93	92	89	86
C	88	85	89	93	92	91	94	90	89	95	84	85
D	91	86	92	92	94	89	94	87	94	92	85	93
Mean	90.00	86.00	91.00	88.75	93.00	90.50	95.00	91.75	92.00	93.00	87.25	88.75



**Supporting Data and Evidence for the
Proposed Rules Changes 2006 - Item 6A**

Sample 4	TP				BP				S			
	TAV	BO	PI	SNES	TAV	BO	PI	SNES	TAV	BO	PI	SNES
A	82	82	85	78	88	91	88	95	92	89	89	88
B	85	84	86	75	88	91	89	92	88	88	85	86
C	83	84	81	81	93	92	89	93	90	87	87	81
D	82	82	84	82	87	86	90	93	91	88	88	87
Mean	83.00	83.00	84.00	79.00	89.00	90.00	89.00	93.25	90.25	88.00	87.25	85.50

Legenda

TAV ENSE - Laboratorio Analisi Sementi, Tavazzano (LO) Italy

BO LaRAS - DISTA, Università di Bologna (BO) Italy

PI Laboratorio Ricerca Analisi Sementi, Dip. Agronomia, Università di Pisa (PI) Italy

SNES GEVES - SNES Beaucouzé, France

TP Top Paper

BP Between Paper (Plated Paper suggested)

S Sand (Top Sand suggested)

Comments: opinion of the applicant is that only BP and S are suitable as substrates for germination of Triticum diccocom seeds, while TP doesn't give reliable results. BP and S are the substrates prescribed also for the similar species Triticum spelta.

Inclusion of *Triticum dicoccum* in the ISTA Rules

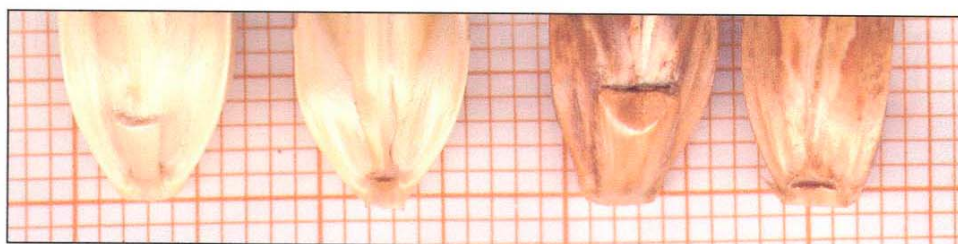
Purity Analysis: differences between *Triticum dicoccum* and *Triticum spelta*

<i>T. dicoccum</i> (spikelet)		<i>T. spelta</i> (spikelet)	
Colour:	yellow-grey	Colour:	reddish
Size:	13-18 mm (length) 5-9 mm (width)	Size:	10-15 mm (length) 4-8 mm (width)
Rachis segment scar:	small	Rachis segment scar:	large
Glume shoulder width:	very narrow	Glume shoulder width:	large



Triticum dicoccum

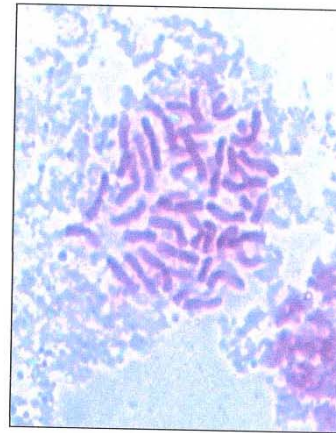
Triticum spelta



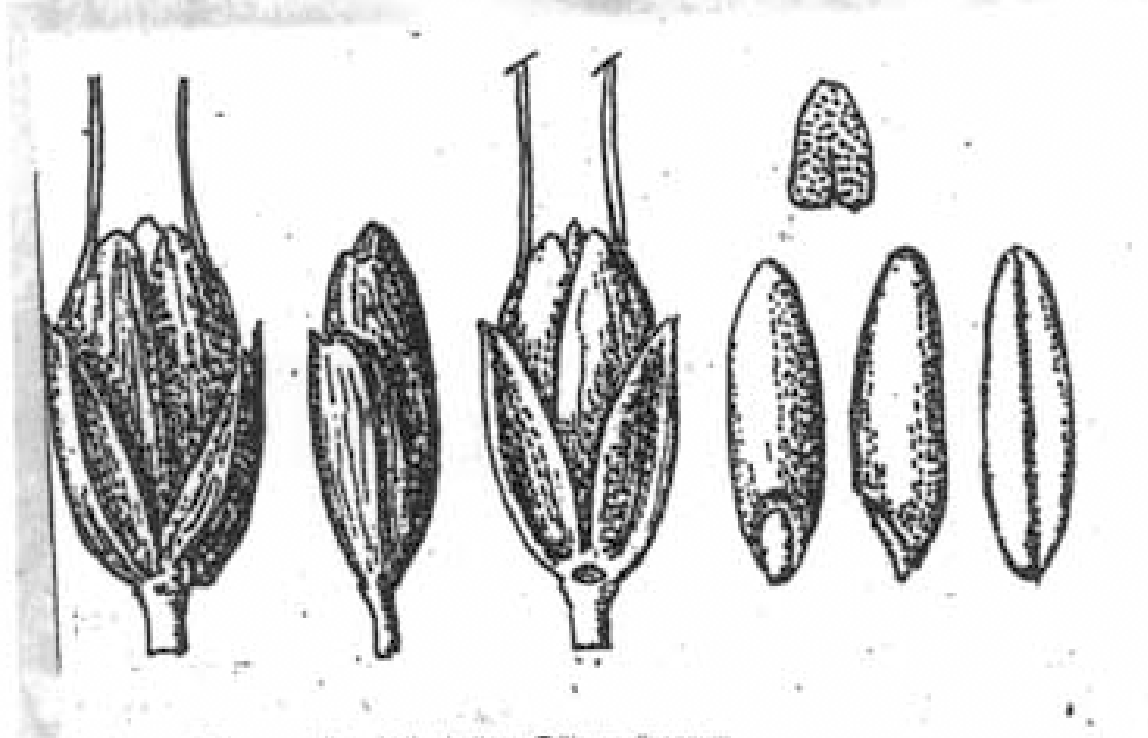
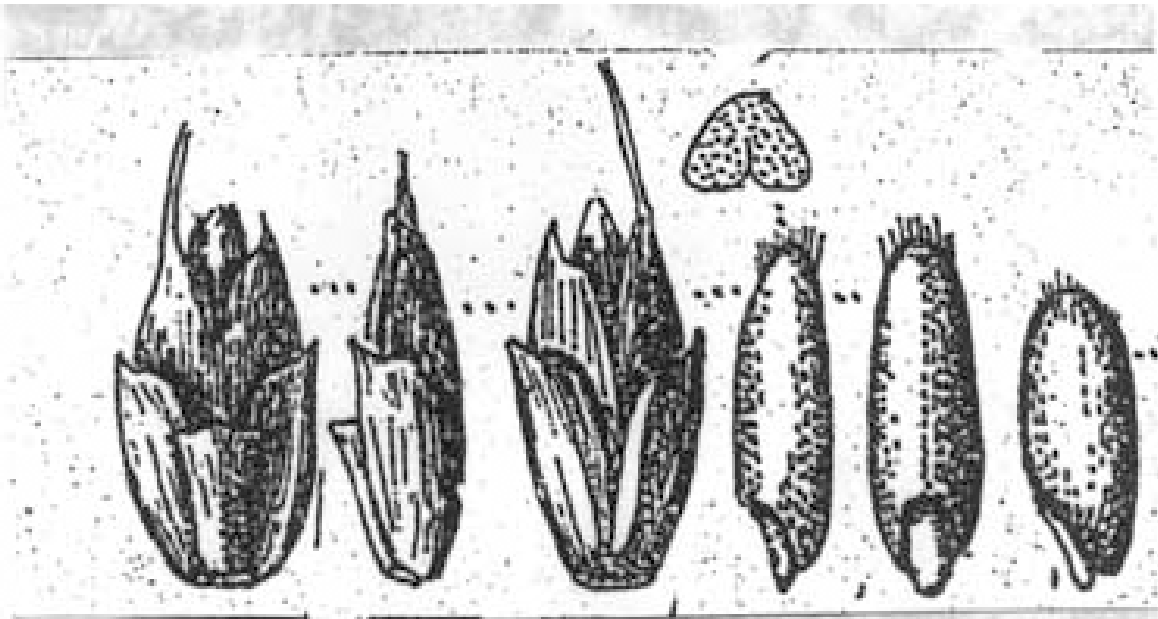
Rachis segment and its scar: small in *T. dicoccum* (left), large in *T. spelta* (right)



Glume shoulder: narrow in *T. dicoccum* (left), large in *T. spelta* (right)



T. dicoccum: tetraploid $2n=28$ (left), *T. spelta*: hexaploid $2n=42$ (right)



On top: *Triticum spelta* – At the bottom: *Triticum dicoccum*
(From: Magismeret II – Dr. Schemmann Szilard – Akadémiai Kiadó, Budapest 1967)

Appendix 4

SUPPORTING DATA AND EVIDENCE FOR THE PROPOSED RULES CHANGES 2006 – ITEM 6C – ADDITION OF *CRAMBE ABYSSINICA*

FOR INFORMATION AND CONSIDERATION AT THE
ORDINARY MEETING 2006





Introduction of *Crambe abyssinica* in the ISTA Rules

Results of an ISTA Comparative
Test on *Crambe abyssinica*

Material

- 4 seed samples of *Crambe abyssinica* with different germination
- Paper substrate



Participants of the Comparative Test

- DE 04
- DE 13
- DE 15
- DE 18
- DK 01
- FR 02
- GB 04

Methods

- **Treatments for promoting germination**
- Without dormancy breaking
- KNO_3
- 5 days prechilling
- **Germination test**
- Paper substrates TP
- Constant temperature 20°C
- Alternating temperatures 20-30°C
- Counts after 4 and 7 days



Supporting Data and Evidence for Proposed Rules Changes - Item 6c:
Addition of *Crambe abyssinica* to the ISTA Rules

Test results (Normal Seedlings)

Pretreatment	Temperature			
	20°C		20-30°C	
	Mean value (%)	SD	Mean value (%)	SD
0	72.64	7.82	85.47	3.49
KNO ₃	88.22	3.79	87.05	2.46
Prechill 5 days	21.09	16.62	45.34	19.02

Test results (Abnormal Seedlings)

Pretreatment	Temperature			
	20°C		20-30°C	
	Mean value (%)	SD	Mean value (%)	SD
0	4.44	2.71	5.44	4.05
KNO ₃	6.12	1.92	4.31	2.01
Prechill 5 days	2.43	1.62	3.52	2.13



Supporting Data and Evidence for Proposed Rules Changes - Item 6c:
Addition of *Crambe abyssinica* to the ISTA Rules

Test results (Dead Seeds)

Pretreatment	Temperature			
	20°C		20-30°C	
	Mean value (%)	SD	Mean value (%)	SD
0	10.39	4.35	7.05	2.05
KNO3	8.73	2.27	7.18	1.87
Prechill 5 days	13.53	11.54	12.96	8.50

Test results (Fresh Seeds)

Pretreatment	Temperature			
	20°C		20-30°C	
	Mean value (%)	SD	Mean value (%)	SD
0	12.53	10.43	2.00	2.52
KNO3	2.93	2.89	1.18	1.67
Prechill 5 days	62.96	26.68	38.19	24.14



AOSA Rules for *Crambe abyssinica*

- Substrate T (paper towels) or B (between blotters)
- Temperature 20 or 25°C
- First count after 4 days
- Final count after 7 days
- Additional directions for fresh and dormant seeds KNO_3