2016 AOSA Rules Change Proposal 12

Purpose of Proposal:

It is proposed that Agar (plant culturing media) be defined and added as a substratum. The proposed change will affect sections 6.2, 6.3 and 6.9 of the rules.

Present Rules:
AOSA Rules for Testing Seeds Volume 1. Principles and Procedures:

6.2 f. Dormant seed. — Viable seeds, other than hard seeds, that fail to germinate when provided the specified germination conditions for the kind of seed in question. Viability of firm, ungerminated seeds of all species (see note below for clarification) must be determined by any appropriate method or combination of methods. Refer to section 6.9 m. The percentage of dormant seeds, if present, must be reported in addition to the percentage germination. If germination is over 90%, dormancy determination is not mandatory, unless it is a species sold on a pure live seed basis.
Note: Refer to "specific requirements and notes" and/or "fresh and dormant seed" in Table 6A. Reference to prechill, paired test and chemicals which promote germination such as: KNO_3, GA_3, ethephon and ethylene are indicators of dormancy in that species. Any reference to 6.2 f, 6.9 m, and Section 8: Tetrazolium Testing, are indicators of dormancy. Due to the short-lived nature of dormancy in some agronomic species such as: grain crops, peanuts, and vegetables (See Federal Seed Act 201.2 (i) for kinds of vegetable seeds), these species shall be exempt from dormancy criteria. However, this exemption does not preclude dormancy determination at the analyst's discretion.

6.3 Moisture and Aeration
— The substratum must be moist enough to supply the needed moisture to the seeds at all times. Avoid supplying excessive moisture that will restrict aeration of the seeds. Except as provided for those kinds of seeds requiring high moisture levels in the germination media, the substrata should never be so wet that a film of water is formed around the seeds. For most kinds of seeds, blotters or other paper substrata should not be so wet that by pressing, a film of water forms around the finger. See section 6.9b.
The addition of water subsequent to placing the seeds in test will depend on the evaporation from the substrata in the germination chambers. Since the rate of evaporation will depend upon the relative humidity of the air, it is desirable to keep water in the germination chambers or to provide other means of supplying a relative humidity of approximately 95 percent. Germination tests should be inspected at frequent intervals to insure that an adequate moisture supply is available at all times.
The amount of water provided by this formula is satisfactory for seeds the size of clovers and will have to be modified slightly, depending on the kind of seed being tested and the kind of sand used. For example, slightly more moisture should be added when the larger seeds are to be tested.
In preparing soil or organic growing media tests, water should be added to the soil or organic media until it can be formed into a ball when squeezed in the palm of the hand but will break freely when pressed between two fingers. After the soil or organic media has been moistened, it should be rubbed through a sieve and put in the containers for the test, without packing.

6.9 Explanation of Table 6A

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

B: between blotters

C: creped cellulose paper wadding (0.3-inch thick Kimpak or equivalent) covered with a single thickness of blotter through which holes are punched for the seed that are pressed for about one-half their thickness into the paper wadding

P: covered petri dishes with (a) two layers of blotters, or (b) three thicknesses of filter paper, or (c) top of sand

PP: pleated filter paper (see footnote a in Table 6A)

PT: substrata listed for P with the following substrata also allowed: sponge rok, vermiculite, terralite, or a mixture of 50 percent sand and vermiculite, sand and perlite, etc.

RB: blotters and raised covers, prepared by folding up the edges of the blotter to form a good support for the upper fold which serves as a cover, preventing the top from making direct contact with the seeds

S: sand

T: paper toweling, used either as folded towel tests or as rolled towel tests in horizontal or vertical position

TB: top of blotters

TS: top of sand

TC: on top of creped cellulose paper without a blotter

TCS: on top of creped cellulose paper without a blotter and covered with ½ to ¾ inch layer of sand.

O: organic growing media

OT: organic growing media covering seed planted on top of paper toweling (T)

Proposed Rules:

6.2 f. Dormant seed. — Viable seeds, other than hard seeds, that fail to germinate when provided the specified germination conditions for the kind of seed in question. Viability of firm, ungerminated seeds of all species (see note below for clarification) must be determined by any appropriate method or combination of methods. Refer to section 6.9 m. The percentage of dormant seeds, if present, must be reported in addition to the percentage germination. If germination is over 90%, dormancy determination is not mandatory, unless it is a species sold on a pure live seed basis.

Note: Refer to "specific requirements and notes" and/or "fresh and dormant seed" in Table 6A. Reference to prechill, paired test and chemicals which promote germination such as: KNO_3, GA_3, ethephon and ethylene are indicators of dormancy in that species. Any reference to 6.2 f, 6.9 m, and Section 8: Tetrazolium Testing, are indicators of dormancy. When using agar as a planting media and table 6A indicates the use of an additive such as KNO3 or GA3, the analyst can either directly add the appropriate concentration of the additive to the molten agar or spray it over the top of seeds after planting.

Due to the short-lived nature of dormancy in some agronomic species such as: grain crops, peanuts, and vegetables (See Federal Seed Act 201.2 (i) for kinds of vegetable seeds), these species shall be exempt from dormancy criteria. However, this exemption does not preclude dormancy determination at the analyst's discretion.

6.3 Moisture and Aeration:
— The substratum must be moist enough to supply the needed moisture to the seeds at all times. Avoid supplying excessive moisture that will restrict aeration of the seeds. Except as provided for those kinds of seeds requiring high moisture levels in the germination media, the substrata should never be so wet that a film of water is formed around the seeds. For most kinds of seeds, blotters or other paper substrata should not be so wet that by pressing, a film of water forms around the finger. See section 6.9b.
The addition of water subsequent to placing the seeds in test will depend on the evaporation from the substrata in the germination chambers. Since the rate of evaporation will depend upon the relative humidity of the air, it is desirable to keep water in the germination chambers or to provide other means of supplying a relative humidity of approximately 95 percent. Germination tests should be inspected at frequent intervals to insure that an adequate moisture supply is available at all times.

The amount of water provided by this formula is satisfactory for seeds the size of clovers and will have to be modified slightly, depending on the kind of seed being tested and the kind of sand used. For example, slightly more moisture should be added when the larger seeds are to be tested.
In preparing soil or organic growing media tests, water should be added to the soil or organic media until it can be formed into a ball when squeezed in the palm of the hand but will break freely when pressed between two fingers. After the soil or organic media has been moistened, it should be rubbed through a sieve and put in the containers for the test, without packing. The depth of the agar should be sufficient to supply adequate moisture throughout the testing period. As with all other media, agar will dry out if left out in the open in the germinator. It is critical agar samples are sealed in a manner that will limit the amount of evaporation that can occur. The use of a closed container, germination cart, bag or lid is required to avoid excessive evaporation during the testing period.

6.9
a**. Substrata.** — Any medium listed for a particular species in the substrata column of Table 6A may be used. The order listed does not indicate preference. Symbols for substrata in column 2, Table 6A is:

B: between blotters
C: creped cellulose paper wadding (0.3-inch thick Kimpak or equivalent) covered with a single thickness of blotter through which holes are punched for the seed that are pressed for about one-half their thickness into the paper wadding
P: covered petri dishes with (a) two layers of blotters, or (b) three thicknesses of filter paper, or (c) top of sand
PP: pleated filter paper (see footnote a in Table 6A)
PT: substrata listed for P with the following substrata also allowed: sponge rok, vermiculite, terralite, or a mixture of 50 percent sand and vermiculite, sand and perlite, etc.

RB: blotters and raised covers, prepared by folding up the edges of the blotter to form a good support for the upper fold which serves as a cover, preventing the top from making direct contact with the seeds
S: sand
T: paper toweling, used either as folded towel tests or as rolled towel tests in horizontal or vertical position
TB: top of blotters
TS: top of sand
TC: on top of creped cellulose paper without a blotter
TCS: on top of creped cellulose paper without a blotter and covered with ½ to ¾ inch layer of sand.
O: organic growing media
OT: organic growing media covering seed planted on top of paper toweling (T)
A: top of agar, polysaccharide powder solidifier made from red algae (without any additional nutrients, vitamins or hormones). Agar powder should be approximately 99% pure. Agar media must be free of extra salts that may inhibit plant growth.

Harmonization and Impact Statement:
Canadian M&P rules currently do not address the use of agar. ISTA rules currently do not address the use of agar.

ISTA is also pursuing the validation of agar as a germination media using *Pinus sylvestris.* This species was selected by the ISTA Germination Technical Committee for validation because the ISTA Forrest, Tree and Shrub Technical Committee had already proposed the use of agar as a substrate. The ISTA required validation study is under way comparing the current ISTA Rules method using top of paper (TP) and the proposed method of using agar as the substrates. This study should be concluded in late 2016. Due to this agar validation study and multiple agar presentations at ISTA meetings, ISTA labs are experimenting with the use of agar as a germination media in their own labs.

Supporting Evidence:
Agar is a paper media alternative with the ability to support the needs for seed testing. Current medias must; deliver adequate moisture for the test duration, withstand long germination durations, pre-chilling, not promote nor prohibit microbial growth, and allow for the inclusion of additives to meet the needs of fresh and dormant seeds. Agar has been used for germination media for many years by Kew Gardens and other laboratories worldwide.

See Soybean on Agar, Appendix 12.1; Tef on Agar, Appendix 12.;, and Germination of Multi-species on Agar, Appendix 12.3

References:
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