PRAIRIE RECOMMENDING COMMITTEE FOR OILSEEDS (PRCO)

APPENDIX B

APPENDIX B - Operating Procedures for the Registration of a Condiment Mustard Cultivar for Production in Western Canada

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B1. Background

Three types of condiment mustard are grown in western Canada, representing two species.

1. Yellow mustard, *Sinapis alba* L. (yellow seeded)
2. Oriental mustard, *Brassica juncea* (L.) Czern. (yellow seeded)
3. Brown mustard, *Brassica juncea* (L.) Czern. (brown seeded)

Western Canada is the world's leading producer of condiment mustard, with up to 800,000 acres seeded annually, usually on a producer contract basis. The majority of production in western Canada is of the yellow mustard type, and the remaining production is split between the oriental and brown types. Market supply and demand for a given year dictates the percentage split in production among the three types, with yellow mustard typically commanding 40 to 60 percent of the total acreage.

B2. The Prairie Recommending Committee for Oilseeds (PRCO)

This document outlines the evaluation and testing system operated by the Prairie Recommending Committee for Oilseeds (PRCO). The PRCO makes recommendations to the Variety Registration Office (VRO) of the Field Crops Division, Canadian Food Inspection Agency (CFIA) regarding the suitability of candidate cultivars of flax and condiment mustard for registration in western Canada. The PRCO operates under the umbrella of the Prairie Grain Development Committee (PGDC) (www.pgdc.ca).

The procedural framework outlined in this document exists to generate relevant, unbiased and representative data for candidate cultivars. A recommendation to "support" or "object to" an application for registration of a condiment mustard cultivar will be based on data generated by the Co-operative Mustard Test. The candidate cultivar must demonstrate acceptability and merit for agronomic, seed quality and disease resistance traits, and meet the minimum standards outlined in *Sub-Appendix B5*.

The PRCO may suspend a particular guideline to allow consideration of a candidate by a two-thirds majority vote. The rationale for such action and the record of the empowering vote will form part of the recorded decision. The voting is valid only when a quorum is present. The quorum for the PRCO is a simple majority of the membership. The PRCO votes are cast in three categories: "Support", "Object to", or "Abstain". Abstentions are only expected in the case of an openly declared conflict of interest, or in the absence of information on which to base a decision. Recommendations in support of registration by the PRCO remain in effect for two (2) years.

B3. The Co-operative Mustard Test

B3.1 Requirements for Entry

Candidate cultivars may be entered into the Co-operative Mustard Test by Canadian public institutions or through a private sector Canadian sponsor or breeder's agent. There are no restrictions on candidate cultivars entering into first year (private data) trials as part of the Co-operative Mustard Test. For advancement into second year (public data) Co-op trials, the sponsor must obtain approval from the Co-ordinator of the Co-operative Mustard Test. Requests for entry of a candidate cultivar into public trials must be received by the Test Co-ordinator by March 15 of each year and a two (2) year supply of seed for planting the trials must be provided to the Test Co-ordinator by April 15.

A minimum of three (3) station-years of data collected from valid replicated field trials in the mustard growing areas of western Canada is required for entry into second year (public data) Co-op trials. The data must indicate that the candidate cultivar has merit for agronomic performance and seed quality in comparison to the designated check cultivars indicated in *Sub-Appendix B4*.

A minimum of one (1) station-year of valid data for blackleg and white rust reaction is also required for entry into second year (public data) Co-op trials. The data must indicate that the candidate cultivar has merit for disease resistance in comparison to the designated check cultivars indicated in *Sub-Appendix B4*. In the event that first year private data for disease reaction is not available, but where due diligence was demonstrated, the candidate cultivar will be allowed to advance to second year public trials and the required disease reaction data must be provided from either private or public trials in the second year of Co-op testing.

The maximum number of entries in the Co-operative Mustard Test public trials is 30 entries of *S. alba* including checks, and 30 entries of *B. juncea* including checks. If the maximum number of entries exceeds this limit, then supporting data submitted for each candidate cultivar will be reviewed by the Test Co-ordinator. Merit will be considered as well as date of submission of the supporting data.

B3.2 Testing Period

Evaluation of candidate cultivars in the Co-operative Mustard Test normally takes two (2) calendar years. First year trials are private data trials and second year trials are public data trials. An additional year of testing in public trials may be required if the minimum data requirements are not met after two (2) calendar years of testing. Candidate cultivars having achieved merit as defined by meeting or surpassing the minimum standards listed in *Sub-Appendix B5* after two (2) or more calendar years of testing are eligible to be considered for recommendation for registration by the PRCO. Candidate cultivars eligible to be considered for recommendation for registration may remain in public trials at the discretion of the Test Co-ordinator.

B3.3 Test Co-ordinator and Co-operators

The Co-ordinator of the Co-operative Mustard Test is responsible for co-ordinating the movement of seed for test entries, generation of randomizations and field books, trial site inspection, data analysis, and production of a report for the PRCO. The Test Co-ordinator retains the prerogative to delegate these responsibilities to designated alternates. The current Test Co-ordinator and the designated alternates are listed in *Sub-Appendix B6*. Experimental protocols and the size of trials are communicated to sponsors and/or the breeder of candidate cultivars. Co-operators are then contacted and trial sites are arranged throughout the mustard growing areas of Saskatchewan, Alberta and Manitoba (*Sub-Appendix B1*).

Trial site management is the responsibility of each of the Co-operators. All trials are managed and harvested according to sound agronomic and scientific practices appropriate for each trial site. This includes using appropriate fertilizers and pesticides applied according to label recommendations.

All Co-operators are expected to conduct the trials in an ethical manner, allowing the Test Co-ordinator approved visitations to trial sites and making efforts to ensure the security and confidentiality of trials.

Trial sites are planted according to planting plans provided by the Test Co-ordinator. No additional lines may be added to the Co-operative Mustard Test and no additional testing may be done at the trial site(s) (*e.g.*, disease evaluation) without the approval of the Test Co-ordinator.

After seeding, the Co-operators will label the trial site and the first plot. Trial site information is supplied to the Test Co-ordinator or designated alternate(s) indicating trial site location, seeding date and any other relevant information specific to the trial site.

Currently, no "special management" practices will be performed for any candidate cultivar. If the breeder or sponsor can demonstrate the need for special management of a candidate cultivar, a new testing procedure may be considered.

B3.4 Data Collected

Information is collected on days to flower, plant height, resistance to lodging and days to maturity. Detailed instructions are available in *Sub-Appendix B1*. Problems associated with specific plots (*e.g.,* poor emergence, flooding) must be noted in the field books and relayed to the Test Co-ordinator.

Seed yields are obtained at all trial sites and are recorded to represent yields on a dry weight basis. Adjustments for moisture content should be indicated with the data collected. Sub-samples of 50 to 60 grams from each plot are sent to the Test Co-ordinator for determination of seed quality traits. Measurements of seed quality traits are determined using the methodologies outlined in *Sub-Appendix B2*. Disease reactions are determined by plant pathologists following the procedures outlined in *Sub-Appendix B3*.

B3.5 Check Cultivars

Check cultivars are included in the Co-operative Mustard Test as comparisons for agronomic and seed quality traits and disease reaction purposes. Check cultivars are determined by the Co-ordinator of the Co-operative Mustard Test based on recommendations by stakeholders. Changes in check cultivars must be approved by the PRCO. The current check cultivars are listed in *Sub-Appendix B4*.

Widely grown cultivars may remain in the Co-operative Mustard Test to provide additional information for provincial variety recommendation committees. Additional or alternate checks may be suggested when deemed appropriate. These changes would appear as amendments to the sub-appendices after acceptance by the Test Co-ordinator and the PRCO.

B3.6 Experimental Design

A four (4) replicate randomized complete block design experiment is used to evaluate agronomic and seed quality traits. However, a lattice design may be utilized if an appropriate number of entries are tested. Experimental design details for disease resistance testing are given in *Sub-Appendix B3*.

Plot size may vary from four (4) to six (6) rows in width and five (5) to seven (7) metres in length. The entire plot or only the centre rows may be harvested. Other modifications may be made as necessary at each location.

The seeding rate for *B. juncea* is 6 lbs/acre (1.1 g/plot or 200 seeds/6 m or 20 ft row); for *S. alba*, the appropriate seeding rate is 12 lbs/acre (2.1 g/plot or 200 seeds/6 m or 20 ft row). Adjustments for germination percentage and seed weight may be made if necessary.

B3.7 Seed Requirement and Seed Treatment

On the first year of entry into public trials (second year Co-op), a two (2) kg quantity of untreated seed must be provided to the Test Co-ordinator for each of the candidate cultivars being tested in the Co-operative Mustard Test. This allows the same seed source to be used for two (2) calendar years of testing in public trials in the event that an additional year of testing is required.

Co-operators retain the prerogative to treat seed for pest management purposes if it is deemed necessary at the trial site. If seed treatment is used, this information must be relayed to the Test Co-ordinator.

B3.8 Trial Inspection and Validation

It is the intent of the Co-ordinator of the Co-operative Mustard Test to inspect trials on an annual basis.

In order for a trial to be valid, the inspector must approve a minimum of three (3) replicates. For yield trials, plots must be a minimum of four (4) rows in width, and five (5) metres in length. A trial is considered non valid if visual inspection by the Test Co-ordinator or a Co-operator reveals unacceptable planting, emergence, soil gradient, pest or environmental problems. If a check cultivar has only two (2) acceptable plots in a trial, the entire trial shall be considered non valid and the data will not be included in the Co-operative Mustard report.

If a candidate cultivar has only two (2) acceptable plots in a trial, the data for that line or cultivar will not be used from that trial; however, data from the remaining entries that have a minimum of three (3) good replicates may be considered acceptable.

Data collected from a trial site will be considered valid if the overall coefficient of variation (C.V.) for plot and/or kg/ha yield is less than 16%. If the C.V. for yield is equal to or exceeds 16%, then data collected from the trial site will not be used in the Co-operative Mustard Test report. No quality analyses will be conducted on seed from such trials. If there were unusual circumstances (*e.g.*, wind blown swaths, hail damage) that contributed to the C.V. for yield exceeding 16%, and if the Co-operator is confident that agronomic observations are valid, then these agronomic observations may be included in the Co-operative Mustard Test report.

Blackleg disease evaluation trials will be considered valid when the mean severity rating for Westar is greater than or equal to 2.60 (scale: 0–5 where 0 = healthy, 5 = dead); a minimum of three (3) replicates must be approved. Westar is a *B. napus* canola cultivar highly susceptible to blackleg, and is included in the trials as a check to monitor disease pressure. In years where there is poor disease development in western Canada the use of data from trials with a rating for Westar exceeding 2.0 may be used. A white rust trial will be considered valid when the susceptible check has a disease incidence >90% for the appropriate race(s) of white rust.

B3.9 Fees

Currently, there is no fee associated with the testing of entries in the Co-operative Mustard Test. This Test is financially supported by the Saskatoon Research Centre of Agriculture and Agri-Food Canada, and the Canadian Mustard Association and its members. If there is a substantial increase in the number of entries in the Co-operative Mustard Test, then fees may be charged for seed quality testing of entries.

B3.10 Security of Entries

The Test Co-ordinator and Co-operators will take reasonable precautions to ensure the security of entries and will not distribute seed for purposes other than registration testing without the consent of the owner(s).

B4. Submission of Data for Support for Registration

B4.1 Years and Checks

The data submitted for consideration for support for registration must include all the valid data from all the years in which the entry was included in private and public trials as part of the Co-operative Mustard Test. Candidate cultivars must be compared to the designated check cultivar of similar type.

During all years of private and public testing in the Co-operative Mustard Test, a candidate cultivar shall be compared to the same check cultivar(s) that were in place in the year of initial entry into the Co-operative Mustard Test.

B4.2 Relevant Data

All relevant data, including screening and laboratory data judged to be acceptable and useful by the PRCO, may be used in support of registration in addition to official test data generated from the Co-operative Mustard Test. Data provided for Request for Support for Registration other than official Co-operative Mustard Test data must be peer approved. Subsets of data from the Co-operative Mustard Test must be accurately represented and meet with the approval of the Test Co-ordinator, pathologists and chemist. Agronomic and seed quality data from trials grown outside the western Canadian mustard growing areas is not acceptable.

B4.3 Analyses of Data

Analyses of data to generate means over all locations and years of testing must utilize recognized statistical models that use the appropriate error term to generate standard error or least significant differences (LSD). Generally a two-tailed test is used.

B4.4 Performance and Definition of Merit

To receive support for registration, a candidate cultivar must show merit for agronomy, seed quality and disease reaction. The minimum standards are described in *Sub-Appendix B5*.

A candidate cultivar that shows merit is “equal to”, “better than”, or “superior to” current check cultivars. The category “equal to” is defined as the arithmetic equality to the check or mean of the checks. The category “better than” is defined as the arithmetic superiority to the check or mean of the checks with or without statistical significance. The category “superior to” is defined as statistically superior by a two-tailed test at a 5% level of significance.

If a candidate cultivar fails to meet the minimum standards for the traits normally considered for recommendation for registration of a condiment mustard variety, but shows a collection of other strengths in relation to the check(s) that are deemed to be of value to the Industry (*e.g.*, to develop new technologies, to respond to new pathogen threats, to create new market opportunities for specific oil and meal qualities, etc.), then this collection of traits must be considered by the PRCO when the sponsor of the candidate cultivar presents the support for registration data.

B4.5 Deadline

The breeder or sponsor of a candidate cultivar must provide a “Request for Support for Registration” and a written summary of the data to all voting members of the PRCO no later than seven (7) working days prior to the annual meeting of the PRCO. This time should allow PRCO members to consider the merit of the candidate cultivar proposed for registration prior to the PRCO meeting. The PRCO may refuse to consider a request on the grounds of late circulation, illegibility or inaccuracy of data.

In addition, a copy of the "Request for Support for Registration" and a written summary of the data should be sent to the Variety Registration Office (VRO) of the CFIA. The address is listed in Section B6. This is not a requirement, but rather a request from the VRO to allow efficient processing of documentation if a candidate cultivar has been supported for registration.

B4.6 Appeal of PRCO Recommendation

Appeals of PRCO recommendations on the suitability of candidate cultivars for registration are directed to the PGDC executive under the procedures set out in the PGDC Operating Procedures.

B5. Review of Procedures

The Operating Procedures for Registration of a Condiment Mustard Cultivar For Production in Western Canada (*Appendix B*) shall be reviewed and amended at least once every five (5) years or when necessary, at the discretion of the Co-ordinator of the Co-operative Mustard Test, based on recommendations made by stakeholders.

Check cultivar(s) and minimum standards for agronomic and seed quality traits, and disease reaction may be reviewed each year at the annual meeting of the PRCO and any of the other relevant sub-appendices may be modified accordingly.

B6. Registration Office

Based on information provided by the Co-operative Mustard Test and interpretation by the Evaluation Teams, a recommendation to "support" or "object to" the application for registration of a candidate cultivar shall be made.

The PRCO Secretary shall inform the Registrar of the VRO in writing, of the decision of the PRCO. Copies of the decision will be sent to the breeder or sponsor and the PRCO Chair. Copies of the statements from the Evaluation Teams shall be provided by the Secretary and sent to the breeder or sponsor and to the VRO.

Applications for registration of the recommended candidate should be submitted on the Variety Registration Application Form available from the VRO, or from the CFIA web site (www.inspection.gc.ca). The application, along with other required supporting documentation, reference samples and prescribed fees, must be sent to the address indicated below. For further information, refer to the most recent publication of the "Procedures for the Registration of Crop Varieties in Canada".

Variety Registration Office
Field Crops Division
Canadian Food Inspection Agency
59 Camelot Drive
Ottawa, Ontario K1A 0Y9
Telephone: (613) 773-7146
Fax: (613) 773-7144

B7. Oilseed Mustards

The PRCO will not test oilseed *B. juncea* (*e.g.*, canola quality) lines and cultivars. This is the responsibility of the Western Canada Canola/Rapeseed Recommending Committee (WCC/RRC).

B8. Contract Registration

Contract registration is not to be used as a substitute for traditional forms of registration (full or interim) when the PRCO has objected to the registration of a candidate cultivar based on deficiency in merit. However, the PRCO may suggest that the candidate cultivar be considered for contract registration when there is rationale to do so. The Contract Registration Committee (CRC) may be required to consider the case and determine if the required conditions for contract registration have been met.

Contract registration is available for candidate cultivars where biochemical or biophysical characteristics distinguish them from the majority of registered cultivars of the same kind or species. It must be shown that these characteristics could compromise the end-use suitability of cultivars registered for traditional commodity markets. The sponsor of the candidate cultivar must demonstrate the possibility of industry harm if granted an unrestricted registration.

For more information regarding Contract Registration, refer to the VRO Contract Registration procedures.

SUB-APPENDIX B1 - Agronomy Evaluation Co-operative Mustard Test

SB1.1 Trials

At each trial site there are two trials, one for each species, *Sinapis alba* and *Brassica juncea*.

Each of the two trials is replicated and randomized, and the two trials must be treated separately. Species borders on the sides of each trial must be the same as the species in the trial.

SB1.2 Trial Sites

In western Canada, mustard production is best suited to the Brown and Dark Brown Soil Zones; however, there is limited acreage in the Black and Grey Zones. Saskatchewan is the primary production area, followed by Alberta and Manitoba. Trial sites are established to reflect the areas of production. Specific locations are selected on an annual basis, depending on the Co-operator and land availability.

SB1.3 Measurement and Rating Scales

SB1.3.1 Height

A minimum of two measurements of plant height is taken in each plot; the average is determined and expressed in centimetres (cm).

SB1.3.2 Lodging

A visual rating scale is used to measure resistance to lodging:

1 to 5 (1 = completely erect, 5 = completely lodged, flat on ground)

SB1.3.3 Maturity

Maturity is the number of days from date of seeding to swathing maturity. Swathing maturity is reached when the seeds are firm when pressed between the fingers, which represents a moisture content of about 25%. The lower pods will have changed colour, but the upper pods may be green. At this time, 60% of the seeds in the pod have started to change colour to reddish brown in brown mustard and 75% of the seeds will have turned yellow in oriental mustard. Yellow mustard is fully mature when the plot is free of green seed.

N.B. The use of a desiccant is an undesirable method for the Co-operative Mustard Test, but if it is necessary, then the desiccant must be applied to the yellow mustard when at least 60–70% of the seeds within the pod have reached their mature colour.

SB1.3.4 Yield

Plot yield at each site is measured, adjusted to dry weight and harvest area if different from seeded area, and expressed as kg/ha and yield relative to the designated check(s).

SB1.3.5 Seed Weight

Seed weight is determined on a randomly selected sample of 500 counted seeds dried to <5% moisture content, measured as grams per 500 seeds, and then adjusted to 1,000 kernel weight and expressed as grams per 1,000 seeds.

Height, Lodging (when differences occur among entries), Maturity and Yield are determined on all four replicates from valid trials.

Seed weight is determined on a minimum of two (2) replicates from trials with acceptable C.V. for yield. If the number of trials throughout western Canada exceeds the required number for a given year, then seed weight will be determined on trials with the lowest C.V. for yield.

SB1.4 Trial Sites for Evaluation of Agronomic Traits

Agronomic traits are measured only on valid trials.

A minimum of sixteen (16) station-years of valid agronomic data collected over two (2) or more calendar years, including one (1) year private trial and a minimum of one (1) year public Co-op Test, is required.

All reasonable attempts will be made to collect agronomic data from a minimum of eight (8) valid trials per year. If the number of valid trials in any given year is fewer than eight (8), then the deficient trials may be made up in another year of the Co-operative Mustard Test.

SUB-APPENDIX B2 - Seed Quality Evaluation Co-operative Mustard Test

SB2.1 Seed Quality Traits to be Measured

SB2.1.1 Oil Content

Oil content is determined by near infrared spectroscopy (NIRS) using measurements calibrated against the nuclear magnetic resonance method adapted for use in mustard from the American Oil Chemist Society (A.O.C.S.) recommended practice (Ak 3-94) - Oil Content of Rapeseed by Nuclear Magnetic Resonance. Results are reported as percent whole seed, calculated on a dry weight basis.

SB2.1.2 Protein Content

Protein content is determined by near infrared spectroscopy (NIRS) using measurements calibrated against the Dumas Combustion method adapted for mustard from the A.O.C.S. Official Method Ba 4e-93. Total nitrogen content of the seed is measured and the value is multiplied by a correction factor of 6.25 (N × 6.25) to give a value for protein. Results for protein are reported as percent whole seed on a dry weight basis.

SB2.1.3 Glucosinolates

The glucosinolate content is determined by capillary gas chromatography of the trimethylsilyl derivatives of extracted and purified desulfoglucosinolates (Sosulski and Dabrowski 1984). Intact glucosinolates are extracted from the seed using 67% methanol and purified via the ion-exchange chromatography and “on-column” enzymatic method of Thies (1980). Preparation of trimethylsilyl derivatives utilizes the acetone and 1-methylimidiazole based method of Landerouin et al. (1987). Benzyl glucosinolate is used as the internal standard. Potential allyl isothiocyanate evolution (synonyms: AITC, volatile oil) is reported for oriental and brown mustard as mg/g on a seed (8.5% moisture) basis. It is based on a direct conversion of the measured allyl glucosinolate content to an equivalent value for allyl isothiocyanate.

References:

Landerouin, A., Quinsac, A. and Ribailler, D. (1987) Optimization of silation reactions of desulfoglucosinolates before gas chromatography. World Crops: Production, Utilization, Description 13:26–37.

Sosulski, F. and Dabrowski, K.J. (1984) Determination of glucosinolates in canola meal and protein products by desulfation and capillary gas-liquid chromatography. J. Agric. Food Chem. 32:1172–1175.

Thies, W. (1980) Analysis of Glucosinolates via “on-column” desulfation. Proc. Symposium “Analytical Chemistry of Rapeseed and its Products”, Winnipeg, Canada. pp. 66–71.

SB2.1.4 Seed Colour

Seed colour is determined by near infrared spectroscopy (NIRS) using measurements calibrated against the HunterLab colour system. The calibrated index of reflectance that best describes the darkness and purity of the brown colour of the seed coat and/or meal and the brightness or intensity of the yellow colour is used.

SB2.1.5 Mucilage Content

Mucilage content is determined by measurement of the viscosity of the mucilage released from the seed. Mucilage content is reported in Centistokes(Cs) × ml/g seed.

SB2.1.6 Green Seed

The number of green seeds is determined by a subjective measurement of the number of distinctly green seeds after a random sample of 1,000 seeds has been crushed with a roller. Results are reported on a percentage basis.

SB2.1.7 Chlorophyll

Chlorophyll content is determined by a spectrophotometric method adapted for mustard seed from the A.O.C.S. official method Ak 2-92 - Determination of chlorophyll content in rapeseed (Colza) by spectrophotometry. The results are reported as mg/kg on a seed (tel que) basis.

The above methods of measuring the required seed quality traits may be modified, or new methods utilized, as they are developed and accepted as providing equivalent or better data to the above described methods.

Other seed quality traits that may be viewed as important characteristics for condiment mustard will be added to the list of required seed quality analyses when appropriate testing methods have been developed and accepted. These modified and additional measurements of seed quality will then be presented as an amendment to *Sub-Appendix B2*.

Seed quality data will be collected from a minimum of two (2) separate replicates for each of the traits, using the same two replicates whenever possible.

SB2.2 Trial Sites for Evaluation of Seed Quality Traits

Measurement of seed quality traits is done only for valid trials with an acceptable C.V. for yield.

A minimum of sixteen (16) station-years of seed quality data collected over two (2) or more calendar years, including one (1) year private trial and a minimum of one (1) year public Co-op Test, is required. Refer to *Sub-Appendix B1*, Section B1.4 (Trial Sites for Evaluation of Agronomic Traits) for more detail.

SUB-APPENDIX B3 - Disease Evaluation Co-operative Mustard Test

SB3.1 Procedures for Blackleg (*Leptosphaeria maculans*; anamorph: *Phoma lingam*) Evaluation

Candidate cultivars of *Brassica juncea* (brown and oriental mustard) and *Sinapis alba* (yellow mustard) are evaluated for resistance to blackleg of crucifers in comparison to designated check cultivars in field nurseries naturally infested with *L. maculans*. Separate trials are conducted for the *B. juncea* and *S. alba* entries. Westar, a *B. napus* canola cultivar highly susceptible to blackleg, is also included in each trial as a check to monitor disease pressure.

For each trial, single (1) or two (2) row plots of each entry are sown in a randomized complete block with four (4) replicates; rows are at least three (3) metres long and contain 100 seeds/3 m. Planting is normally done during mid to late May. Additional rows of Westar may also be sown in each trial as indicators of disease pressure and uniformity of inoculum, and to provide a source of secondary (pycnidiospore) inoculum. Additional pycnidiospore inoculum from agar cultures of *P. lingam* may be sprayed on the plants at the 2–3 leaf stage. Plots may be irrigated with overhead sprinklers as required to maintain moisture conditions conducive to blackleg.

The severity of blackleg infection is evaluated on a minimum of 25 plants per entry in each replicate after the plants are fully podded and just beginning to ripen. In trials having only three (3) good replicates, 35 plants per replicate must be evaluated for each entry. Plants are uprooted, cut through the hypocotyl and/or tap root, and blackleg severity is scored for each plant using the following scale based on the area of diseased tissue in the cross-section:

0 = No diseased tissue visible in the cross-section.
1 = Diseased tissue occupies less than or equal to 25% of cross-section.
2 = Diseased tissue occupies 26% to 50% of cross-section.
3 = Diseased tissue occupies 51% to 75% of cross-section.
4 = Diseased tissue occupies more than 75% of cross-section with little or no constriction of affected tissues.
5 = Diseased tissue occupies 100% of cross-section with significant constriction of affected tissues; tissue dry and brittle; plant dead.

Mean blackleg severity is calculated for each combination of replicate and entry as follows:

$$\text{Blackleg severity} = \frac{\sum [(\text{Numerical value of disease category}) \times (\text{Number of plants in disease category})]}{[\text{Total number of plants in all disease categories}]}$$

Mean blackleg severity values over all replicates are reported for each entry.

The minimum requirement for blackleg reaction to support a recommendation for registration of a *B. juncea* (brown and oriental mustard) or a *S. alba* (yellow mustard) candidate cultivar is disease severity less than or equal to the designated check for each type of mustard (*Sub-Appendix B4* and *Sub-Appendix B5*). All designated checks are highly resistant to blackleg.

SB3.2 Procedures for White Rust (*Albugo candida*) Evaluation

Candidate cultivars of *B. juncea* (brown and oriental mustard) are evaluated for resistance to race 2a of *A. candida* in comparison to designated check cultivars; additional testing with race 2v may also be conducted.

Candidate cultivars of *S. alba* (yellow mustard) are evaluated for resistance to races 2 and 7 of *A. candida* in comparison to designated check cultivars. Evaluations are conducted with races 2v and 7v since these are currently the most prevalent races in western Canada; additional testing with races 2a and 7a may also be conducted.

All trials are conducted in controlled environment chambers. Separate trials are conducted for each race of white rust, and each trial includes appropriate checks to monitor race purity.

The severity of white rust infection is evaluated on a minimum of 100 plants per entry for each race of *A. candida*. A suspension of zoospores (10^4 zoospores/mL) from germinated sporangia is applied to the plants using one of the following methods:

a) A 10 μl droplet of the zoospore suspension is applied to each cotyledon 6–7 days after planting.
b) The zoospore suspension is sprayed onto the seedlings 12–14 days after planting, when the first true leaf is expanding.

Immediately following inoculation, plants are incubated in a misting chamber (100% RH) in the dark for 24–48 hours. White rust severity is scored for each plant 7–8 days (cotyledon) or 10 days (leaf) after inoculation using the following scale:

0 = No visible symptoms on either leaf surface.
1 = Small, pinpoint to larger brown necrotic flecks under the point of inoculation on upper surface, occasionally necrosis extending to lower epidermis. No sporulation.
3 = Very sparse, one to few, minute scattered pustules on upper surface. None to very few pustules on lower surface. Lesions usually have chlorosis and/or necrosis.
5 = Few to many scattered pustules on upper surface. None to few scattered pustules on lower surface. Pustules sometimes have a chlorotic halo.
7 = Many to few pustules on upper surface. Many scattered small to larger pustules on lower surface.
9 = Very few to no pustules on upper surface. Many large coalescing pustules on lower surface.

Mean white rust severity is calculated for each entry as follows:

$$\text{White rust severity} = \frac{\sum [(\text{Numerical value of disease category}) \times (\text{Number of plants in disease category})]}{[\text{Total number of plants in all disease categories}]}$$

Mean white rust severity values are reported for each entry. The mean percentage of infected plants (disease incidence; includes plants with disease scores of 3 or more) may also be provided.

The minimum requirement for white rust reaction to support a recommendation for registration of a *B. juncea* (brown mustard) candidate cultivar is disease severity less than or equal to the designated check for race 2a (*Sub-Appendix B4* and *Sub-Appendix B5*). The designated check is susceptible (2011) or resistant (from 2012 onwards) to race 2a. Resistance requirements for race 2v will be established when cultivars resistant to this race are commercialized.

The minimum requirement for white rust reaction to support a recommendation for registration of a *B. juncea* (oriental mustard) candidate cultivar is disease severity less than or equal to the designated check for race 2a (*Sub-Appendix B4* and *Sub-Appendix B5*). The designated check is resistant to race 2a. Resistance requirements for race 2v will be established when cultivars resistant to this race are commercialized.

The minimum requirement for white rust reaction to support a recommendation for registration of a *S. alba* (yellow mustard) candidate cultivar is disease severity less than or equal to the designated check for races 2 and 7 (*Sub-Appendix B4* and *Sub-Appendix B5*). The designated check is moderately resistant to races 2a, 2v, 7a, and 7v.

SB3.3 Other Diseases

Alternaria black spot: This disease is prevalent on *B. juncea* mustard. All currently registered cultivars of brown and oriental mustard are susceptible to this disease. No minimum standards can be established until reliable sources of resistance/tolerance are identified and become available to breeding programs. All currently registered cultivars of yellow mustard are moderately resistant to this disease.

Sclerotinia stem rot: This disease attacks all members of the Brassicaceae. All currently registered cultivars of brown, oriental, and yellow mustard are susceptible to this disease. No minimum standards can be established until reliable sources of resistance/tolerance are identified and become available to breeding programs.

Other diseases such as seedling blight, root and foot rot, aster yellows, and downy mildew are of minor importance. There are currently no guidelines or standard requirements for these diseases in the evaluation of candidate cultivars.

SB3.4 Trial Sites for Evaluation of Disease Resistance

A minimum of one (1) station-year of valid blackleg and white rust resistance data, collected from private or public trials, is required for recommendation for registration of a condiment mustard variety.

SUB-APPENDIX B4 - Designated Check Cultivars for the Co-operative Mustard Test

Cultivars that have significant commercial production in the condiment mustard growing areas of western Canada are generally chosen as checks. Checks will include established cultivars, special purpose cultivars or recent cultivars of superior merit. Changes in check cultivars must be approved by the PRCO. A candidate cultivar will be compared to the appropriate check cultivar(s) in place at the time the candidate cultivar is first entered into the Co-operative Mustard Test; assigned check(s) will remain with the candidate cultivar for the duration of testing.

SB4.1 Yellow Mustard (*Sinapis alba*)

The current check cultivar for yellow mustard is AC Pennant. The check cultivar for yellow mustard candidate cultivars first entering the Co-operative Mustard Test in 2014 is Andante.

SB4.2 Oriental Mustard (*Brassica juncea*)

The current check cultivar for oriental mustard is Cutlass.

SB4.3 Brown Mustard (*Brassica juncea*)

The current check cultivar for agronomy and seed quality for brown mustard is Duchess. The check cultivar for agronomy and seed quality for brown mustard candidate cultivars first entering the Co-operative Mustard Test in 2014 is Centennial Brown.

The current check cultivar for blackleg and white rust resistance for brown mustard is Duchess. The check cultivar for blackleg and white rust resistance for brown mustard candidate cultivars first entering the Co-operative Mustard Test in 2012 is Amigo.

SUB-APPENDIX B5 - Minimum Standards for Condiment Mustard Cultivar Registration

To receive support for registration, a candidate must show merit. A candidate that shows merit is "equal to", "better than" or "superior to" current check cultivars.

If a candidate cultivar fails to meet the minimum standards for the traits normally considered for recommendation for registration of a condiment mustard variety, but shows a collection of other strengths in relation to the check(s) that are deemed to be of value to the Industry (*e.g.*, to develop new technologies, to respond to new pathogen threats, to create new market opportunities for specific oil and meal qualities, etc.), then this collection of traits must be considered by the PRCO when the sponsor of the candidate cultivar presents the support for registration data.

Agronomic and seed quality data collected over a minimum of two (2) calendar years and sixteen (16) station-years from valid trials, blackleg resistance data collected from a minimum of one (1) valid trial, and white rust resistance data collected from a minimum of one (1) valid trial, are required for consideration for recommendation for registration of a candidate cultivar.

Valid trial sites for evaluation of agronomic and seed quality traits shall be representative of the mustard growing areas of western Canada and have a C.V. for yield less than 16%.

Agronomic and seed quality data from a minimum of eight (8) valid trials per year shall be collected, whenever possible, to ensure that candidate cultivars have enough data to meet this minimum standard. If the number of valid trials in any year is lower than eight (8), then the deficient trials may be made up in another year of public Co-op testing.

Agronomic traits considered for merit evaluation include: Yield.

Seed quality traits considered for merit evaluation include: Oil content (fixed oil for *B. juncea* only), protein content, glucosinolate content (for *B. juncea* only), mucilage content (for *S. alba* only), chlorophyll and/or green seed content (*B. juncea* only). Seed weight and seed uniformity are traits that may be measured and reported in the Co-operative Mustard Test, but merit consideration is not required for these traits.

Resistance to blackleg and white rust diseases is considered in the evaluation for merit. Refer to *Sub-Appendix B3* for the different pathogen races for the different mustard species.

a) Brown mustard (*Brassica juncea*)
   Blackleg - highly resistant; equivalent to but not more susceptible than the designated check (*Sub-Appendix B4*)
   White rust - susceptible (2011) or resistant (from 2012 onwards) to race 2a; equivalent to but not more susceptible than the designated check (*Sub-Appendix B4*)

b) Oriental mustard (*Brassica juncea*)
   Blackleg - highly resistant; equivalent to but not more susceptible than the designated check (*Sub-Appendix B4*)
   White rust - resistant to race 2a; equivalent to but not more susceptible than the designated check (*Sub-Appendix B4*)

c) Yellow mustard (*Sinapis alba*)
   Blackleg - highly resistant; equivalent to but not more susceptible than the designated check (*Sub-Appendix B4*)
   White rust - moderately resistant to races 2 and 7; equivalent to but not more susceptible than the designated check (*Sub-Appendix B4*)

SUB-APPENDIX B6 - Current Co-operative Mustard Test Co-ordinator, Chemist and Pathologists

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