

AUSTRALIAN CROP ACCREDITATION SYSTEM

OILSEEDS

CANOLA/MUSTARD PROTOCOLS

SECTION 1

AGRONOMIC CHARACTERISTICS

DESCRIPTORS

These are characteristics which are virtually independent of the environment

- Seed colour
- Maturity (early, mid, late)
- Quality type (high oleic acid, low linolenic acid)
- Herbicide resistance type (conventional, Triazine resistant, Roundup resistant, Liberty resistant)
- specific genetic traits
- transformations

GENERAL PRINCIPLES FOR EVALUATION OF OTHER CHARACTERS

1. Each character will be evaluated relative to agreed control cultivars. Control/check varieties must be chosen to fairly evaluate the new variety in the nominated environment(s). Data from the new variety and controls must come from the same experiments and must come from the target area for the new variety. As variations in seed size and nutrient content can bias results, seed should come from the same source each year, wherever possible
2. For all characters which are measured objectively, as by weight, length or weight per unit area, the data should be presented in the original metric units (g, mm, t ha⁻¹) and not transformed into percentages or non-continuous scores.
3. For all characters which can be measured objectively, data must be obtained from randomised replicated experiments and be analysed by analysis of variance, or other method of equal rigour. Standard errors must be presented.
4. All data collected from the nominated target area shall be included so as to present an accurate picture of the new cultivar's performance. The exclusion of any data from the analysis shall be justified.
5. Records must be maintained in a manner which can be audited. Trial data must be made available for auditing as required by the accreditation committee. Raw data from all trials, sample and data analysis shall be maintained by the breeder for a period of at least five years.

1. PROTOCOLS FOR INDIVIDUAL AGRONOMIC CHARACTERS

1.1 Seed size

Assessed as weight (grams) of 1,000 grains.
Comparisons must be made with controls of similar maturity.
From a minimum of 5 experiments grown over 2 or more years.

1.2 Early vigour

Assessed as a visual rating on a 1 to 9 scale (1 poor vigour, 9 high vigour).
From a minimum of 5 experiments grown over 2 or more years.

1.3 Flowering date

Assessed as 50% of plants with one or more flowers. Presented relative to the control varieties as days after sowing.

Flowering time only to be assessed from trials sown at the normal sowing time for the region.

From a minimum of 5 experiments grown over 2 or more years.

1.4 Plant height

Measured as the average height (cm) of plants in each plot at maturity. Presented as a mean height and range relative to the control varieties.

Height measurement should reflect majority of plot, not a small number of taller individuals.

From a minimum of 5 experiments grown over 2 or more years.

1.5 Lodging resistance

Assessed as a visual rating on a 1 to 9 scale (1 on the ground, 9 no lodging). Each score to represent approximately 10 degrees of lean.

From a minimum of 3 experiments with the trait expressed.

1.6 Maturity

Assessed as the time when the plot has reached windrowing maturity (40% - 60% seeds changing colour). Presented relative to the control varieties as days after sowing or flowering.

From a minimum of 5 experiments grown over 2 or more years.

1.7 Shattering resistance

Assessed as a visual rating on a 1 to 9 scale (1 high shattering, 9 low shattering), measured prior to harvest. Plots of different maturity shall not be compared. Can only be measured on plots left standing.

From a minimum of 3 experiments with the trait expressed.

1.8 Yield

Measured as the grain yield in kg/ha or t/ha. Yield is to be assessed relative to the controls in a target environment. There have been 3 agro-ecological environments recognised in the Northern Region, 6 in the Southern Region (including an irrigation environment) and 5 in the Western Region. A target environment, as defined by the breeder submitting data for accreditation, would generally consist of one or more of these agro-ecological environments. However, other target environments may be nominated.

Any relevant GXE analysis should be referenced.

Data should be presented from a minimum of 15 sites grown in 3 or more years.

The number of sites may be unevenly distributed across years. The coefficient of variation after spatial or blocking adjustment should be rated to determine whether a trial should be excluded for excessive variability.

Data should be presented for all sites where the new variety was evaluated in the target environment. Specific sites can be eliminated from the analysis by argument, such as damage by an uncontrollable factor, for example mice or uneven waterlogging, or trials which are exceptionally low yielding for that environment.

The minimum plot size should be 1m x 5m (measured centre to centre). Variations from this must be justified. Data should only be obtained from plots with other plots grown along their long axes (bordered plots).

SITE CHARACTERISATION

Site data is not accredited information, but is an important contributor to explaining GXE and other effects. The minimum desirable characterisation is given below. Other soil measurements would be highly desirable. The following site characterisation information should be kept for each experiment.

1. Location of the trial. This can be given as a grid reference, but in addition should be given by the nearest town.
2. Paddock history. For the previous 3 seasons, including herbicide applications and disease status. Longer histories may be desirable in some cases.
3. Soil type. May be given with an appropriate reference, such as Stephens, C.G. (1962), 'A Manual of Australian Soils'.
4. pH. 0-10 cm minimum depth - additional depths may be required for certain regions.
5. Soil P. 0-10 cm minimum depth.
6. Soil Nitrogen 0-10 cm minimum depth - additional depths may be required for some regions
7. Soil moisture at seeding using an appropriate indicator.
8. Monthly rainfall. At each site, or nearby. This should be recorded for the growing season, and with pre-season rainfall and/or soil stored water, where appropriate.
9. Sowing date.
10. Seeding rates and dressings, fertilisers, herbicides, insecticide and fungicide rates and dates.
11. Harvest date.
12. Plot dimensions and statistical design of the experiments.
13. Seasonal observations for the site and crop.

SECTION 2 QUALITY CHARACTERISTICS

GENERAL PRINCIPLES FOR SAMPLE COLLECTION AND ANALYSIS

2.1 SAMPLE COLLECTION

- 2.1.1.** Prior to analysis, samples are to be stored under conditions which maintain the integrity and the quality of the sample:
- Without pesticide treatment or pickling.
 - In conditions free from moulds, fungi, insects or rodent infestation.
 - Not in contact with treated surfaces.
 - Controls are to be stored under the same conditions.
 - Below 8% moisture for canola.
- 2.1.2** Grain samples should be cleaned of admixture including broken seed, weed seeds or other contaminants using suitable devices.

2.2 SAMPLE ANALYSIS

2.2.1 Analysis conducted by laboratories which have either NATA or ISO 9000 accreditation is preferred. A minimum requirement is that the laboratory conducting sample analysis must be actively participating in an interlaboratory testing program with demonstrated satisfactory performance for all relevant tests. Such programs include:

The American Oil Chemists Society (AOCS) "Smalley Program"

or

The Australian Oilseed Federation (AOF) "Test Check Program"

2.2.2 Sufficient sampling should be carried out such that a statistically valid comparison of the variety from each trial with the control varieties is possible.

2.2.3 Analytical methods must be as approved by the accreditation committee and will normally be accepted international reference methods. Alternative methods must be calibrated against official reference methods. The method used must be specified.

2.2.4 Samples of the control cultivars within each trial must meet canola quality standards.

- Erucic acid must be less than 1% of the total of all fatty acids.

- Total glucosinolates must be less than 18 umoles/g of whole seed at 8.5% moisture.

2.2.5 Results must be reported at a set moisture content or on a dry basis. For canola and mustard, seed characteristics (eg oil and glucosinolate content) are reported at 8.5% and oil-free meal components (eg protein in meal) at 13% moisture.

2.2.6 Moisture content should be calculated using official 'oven dry' method (AOCS - Ai2-75) or alternatively with NIR.

2.3 Oil content

Official test method: Soxhlet or Goldfisch (Reference AOCS - Ai 3-75)

Alternative test methods: NIR, NMR, critical flow analysis.

From a minimum of 15 experiments grown over 2 or more years.

2.4 Protein content

Official test method: Kjeldahl or Dumas (Reference AOCS - Ai 4-91 or Ba4e-93)

Alternative test methods: NIR.

Protein results should be presented both on a whole seed and a meal basis.

From a minimum of 15 experiments grown over 2 or more years.

2.5 Glucosinolate content

Official test method: HPLC. (Reference AOCS - Ak 1-92) or glucose hydrolysis (AOF 15-90)

Alternative test methods: NIR, X-Ray fluorescence

From a minimum of 15 experiments grown over 2 or more years.

2.6 Fatty acids

Official test method: Gas chromatography. (Reference AOCS - Ce 1-62)

Alternative test methods: -

From a minimum of 5 experiments grown over 2 or more years.

SECTION 3 DISEASE CHARACTERISTICS

GENERAL PRINCIPLES

3.1 Screening Conditions

The data should reflect field reactions as likely to be experienced in crops. Seedling, greenhouse or other new methods of assessment can be used as supporting evidence providing they can be shown to reflect field circumstances. For some diseases identification of the presence of a known effective resistance gene can be used as evidence for resistance.

3.2 Check Varieties

Data must be compared with well known check varieties that support the classification being claimed. Evidence for the inheritance of a known resistance, from a known parent variety, will also be useful.

3.3 Disease Levels

There must be a sufficient level of disease in the susceptible check varieties to provide confidence in the data.

3.4 Replication

Data must be replicated over years and/or sites. The level of replication will depend on the disease and will vary depending on the uniformity of the data. Statistical evidence using analysis of variance will be required for assessment of data where there can be any doubt about claims being made.

3.5 Scoring Scales

The scoring scale used should reflect crop damage or else a close correlation with crop damage must be evident. For leaf diseases, percentage leaf area infected is recommended rather than reaction type scales although the latter can be used as supporting evidence.

Data can be presented using a numerical scale but for farmer extension it will be converted to the preferred rating scale as of :

R	Resistant
MR	Moderately resistant
MS	Moderately Susceptible
S	Susceptible
VS	Very Susceptible

Where necessary intermediate ratings can be used.

A similar scale will be used for tolerance ratings for nematode and BYDV reactions.

VT	Very Tolerant
T	Tolerant
MT	Moderately Tolerant
MI	Moderately Intolerant
I	Intolerant
VI	Very Intolerant

As a general guide:

The rating scale is based on the principle that for fungal diseases an:

R signifies that the disease, although it may be observed on the variety, will not cause a yield loss whilst the resistance is operating. For nematodes it signifies that very few nematodes will be produced on the variety and that the variety can be relied upon as a disease break.

MR signifies that whilst disease may be observed on a variety under high inoculum pressure no significant yield losses can be expected and certainly no losses greater than 5%. For nematodes an MR will be expected to provide a disease break under most conditions but that nematodes will be seen on roots more readily.

MS yield losses for plants under disease pressure will rarely exceed 15%.

S losses can be expected to exceed 15%.

VS is reserved for varieties that should not be grown in areas where the disease has a regular risk of occurring.

It is not expected that yield loss data would be provided. The above guide is provided as a conceptual framework and is not relevant for diseases that rarely cause significant yield loss eg. in an environment such as for wheat rust in WA.

3.6 Pathogen Variation

Pathogen variation must be addressed when making claims. For variable pathogens, an appropriate or sufficiently wide range of isolates should be used in the generation of disease data. In making claims advice should be sought on such variation. For leaf rust, stripe rust and stem rust the pathotypes/isolate(s) present in the trials should be identified.

3.7 Regional variation

The resistance rating claim must take into account any regional variation in the pathogen populations and/or environments. The claims being made for a variety must match the environments in which it has been tested for disease resistance. It is the responsibility of those seeking accreditation to seek advice on such issues.

3.8 Diseases

The above principles apply to the following diseases, but should not be seen as exclusive of further diseases such as stripe rust, Russian wheat aphid etc, should a breeder wish to include them:

Leaf rust, stem rust, scald, net blotch (spot and net forms), spot blotch, powdery mildew, BYDV, common root rot, covered smut, CCN, *Pratylenchus neglectus*, *P. thornei*

3.9 Disease resistance/tolerance breakdown

ACAS should be advised immediately of any known breakdown in the disease resistance/tolerance of a variety that would affect the accuracy of previously supplied data. Breakdown may be on a local, regional or national basis. ACAS will accept information on changes in disease status from the breeder of the variety or other reliable sources.

The only significant disease of Canola which requires assessment at this time is blackleg.

BLACKLEG RESISTANCE

GENERAL PRINCIPLES FOR EVALUATION OF BLACKLEG RESISTANCE

1. Field rows (easier) or plots to be used.
2. Minimum of one susceptible control as nominated annually by the ACAS oilseeds committee, to be included (eg. Tower, Niklas, Westar).
3. High disease pressure field site to be used. This is best achieved by growing on or directly alongside stubbles or by spreading stubbles over trial area.

4. Minimum two replications.
5. Either the Variety Blackleg Survival Test or the Variety Blackleg Canker Test on Surviving Plants can be used.
6. Varietal Resistance Reaction equivalence to either the Variety Blackleg Survival Rating or to the Variety Blackleg Canker Rating are 1 = highly susceptible, 3 = susceptible, 5 = moderately resistant, 7 = resistant, and 9 = highly resistant

BLACKLEG SURVIVAL TEST

Test method: Survival % is determined from seedling establishment and maturity counts. To be effective at least 75% plant death is required in a nominated susceptible control.

Varietal scores are then expressed using the following:

Blackleg survival (unadjusted score)	% survival
1	0-15
2	16-30
3	31-40
4	41-50
5	51-60
6	61-70
7	71-80
8	81-90
9	91-100

To eliminate variability in varietal scores between sites and years, a Variety Blackleg Survival Rating must be calculated for each variety in each test.

$$\text{Variety Blackleg Survival Rating} = \frac{(\text{varietal score} - \text{minimum score})}{(\text{maximum score} - \text{minimum score})} \times 9$$

(where the maximum and minimum score refer to the highest and lowest *B. napus* scoring variety at that site).

For each set of trial data, the Variety Blackleg Survival Ratings to be adjusted such that the ranking for the variety Dunkeld equals score 7. Adjusted Variety Blackleg Survival Ratings that exceed 7 must not exceed 9; with ratings of 9 to be accompanied by an indication of the relative resistance compared with Dunkeld.

Based on results from field trials with a minimum of 3 site years over at least two years.

BLACKLEG CANKER TEST ON SURVIVING PLANTS

Test method: After the end of flowering, but before maturity, assess the blackleg on crowns of surviving plants. To be effective at least 20% plant deaths/lodging required in a nominated susceptible control.

Take a total of 35 plants/plot or row from a minimum seven positions within each plot or row (i.e. 5 plants each from seven widely spaced positions within each plot or row, and any lodged or dead plants to be included). Data from <10 plants/plot will not be accredited.

Cut the tops off plants and retain 20 cm of lower stem portion with the roots intact. After collection keep samples refrigerated until rated.

Thoroughly wash crowns including roots. Assess varietal scores, visually without sectioning, using the following scoring systems :

Variety blackleg	Severity of crown cankering
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canker score	
0	Plant completely healthy
1	Up to 1/3 rd of crown circumference cankered
2	> 1/3 rd to 1/2 of crown circumference cankered
3	> 1/2 of crown circumference cankered
4	Plant dead or lodged

Percent disease index to be calculated for each variety as follows:

Percent disease index = sum of all scores x 100/ total no. of plants sampled x highest disease category

$$\text{ie. Percent disease index} = \frac{(0 \times N_0) + (1 \times N_1) + (2 \times N_2) + (3 \times N_3) + (4 \times N_4) \times 100}{(N_0 + N_1 + N_2 + N_3 + N_4) \times 4}$$

Where

N₀ = number of plants with score 0

N₁ = number of plants with score 1

N₂ = number of plants with score 2

N₃ = number of plants with score 3

Variety Blackleg Canker Rating to be expressed using the following:

Variety Blackleg Canker Rating	Percent canker disease index
1	86-100
2	71-85
3	61-70
4	51-60
5	41-50
6	31-40
7	21-30
8	11-20
9	1-10

Based on results from field trials with a minimum of 3 site years over at least two years.