

# AUSTRALIAN CROP ACCREDITATION SYSTEM

## BARLEY PROTOCOLS

### SECTION 1

#### AGRONOMIC CHARACTERISTICS

##### DESCRIPTORS

These are characteristics which are virtually independent of the environment

- grain aleurone (white, blue aleurone)
- husk retention (hulled/covered)
- head type (2 or 6 row)
- maturity classification
- early growth habit (prostrate, intermediate, erect)
- specific genetic traits
- transformations
- ease of skinning

##### PRINCIPLES FOR THE EVALUATION OF OTHER CHARACTERS

1. Each character will be evaluated relative to an agreed set of standards. Standard/check varieties must be chosen to fairly evaluate the new variety in the target environment(s). Data from the new variety and checks must come from the same experiments. As variation in seed size and nutrient content can bias results, seed should come from the same source wherever possible.
2. The data for all characters which are measured objectively, e.g. by weight, length or weight per unit area, must be presented in the original metric units (g, mm, t ha<sup>-1</sup>) 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 should be included so that an accurate picture of the new cultivar's performance is presented. The exclusion of any data from the analysis must be justified.
5. Records must be maintained in a manner which can be audited. Trial data must be made available for auditing if required by the accreditation committee. Raw data from all trials, sample and data analysis must be maintained for at least 5 years.

#### 1. PROTOCOLS FOR INDIVIDUAL AGRONOMIC CHARACTERS

Where relevant the following characteristics are assessed in relation to check varieties which will usually be represented in the yield trials.

##### 1.1 Seed Weight

Assessed as weight (grams) of 1000 grains.

Comparisons must be made with checks of similar maturity.

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

- 1.2 Hectolitre weight (test weight)**  
Assessed as the weight (grams) of one hectolitre of grain using standard equipment as used at grain receival sites.  
Comparisons must be made with checks of similar maturity.  
From a minimum of 5 experiments over 2 or more years.
- 1.3 Screenings and grain size**  
Screenings must be expressed as % < 2.2 mm.  
Plump grain must be expressed as % > 2.5mm.  
Comparisons must be made with checks of similar maturity.  
From a minimum of 5 experiments grown over 2 or more years.
- 1.4 Coleoptile length**  
Assessed in mm.  
Test lines are surface sterilised using 0.5% hypochlorite for 30 seconds, or equivalent, and rinsed. A check cultivar with long coleoptiles and one with short coleoptiles must be included. The control filter papers (Ekwip 32\*46 cm grade R6) are soaked in a solution of 0.0025mM ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.5mM Ca(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O 0.015mM H<sub>3</sub>B<sub>3</sub>O<sub>3</sub> and drained for 2-3 minutes. Approximately 8 seeds each of 2 test lines and 3 seeds of each check are included per paper (use an 8:3:3:8 design). The design can be varied, but the checks must be included in each roll. Seeds are placed embryo downwards at a spacing of approximately 2 cm across the middle of the paper. The papers are rolled and covered in aluminium foil. They are then stored upright at 15°C for 12-14 days. The lengths of the coleoptiles are then measured.  
All seeds tested must have been grown at the same site.  
Other published methods are acceptable, but must be referenced.  
From a minimum of 3 experiments grown over 2 or more years.
- 1.5 Early Vigour**  
A visual rating on a 1 to 9 scale (1 poor vigour, 9 high vigour).  
The check should be in the mid range for each experiment.  
Care must be taken to distinguish vigour from growth habit.  
From a minimum of 5 experiments grown over 2 or more years.
- 1.6 Maturity Classification**  
Maturity should be classified as awn emergence relative to appropriate standard cultivars for a given sowing date range for a given environment or region. Days + or - the standard.  
From 10 experiments over three years.
- 1.7 Zinc efficiency**  
Assessed as the ratio of yield in a low zinc soil to yield in the same soil with zinc fertiliser added. A check known to be efficient and one known to be inefficient must be included.  
Comparisons must be from the same site.  
From a minimum of 3 experiments grown over 2 or more years.
- 1.8 Manganese efficiency**  
Assessed as the ratio of yield in a low manganese soil to yield in the same soil with manganese fertiliser and foliar sprays applied to eliminate the deficiency. A check known to be efficient and one known to be inefficient must be included. The manganese level in the soil should be too low for a standard cultivar.  
Comparisons must be from the same site.  
From a minimum of 3 experiments grown over 2 or more years.
- 1.9 Copper efficiency**  
Assessed as the ratio of yield in a low copper soil to yield in the same soil with copper fertiliser added. A check known to be efficient and one known to be inefficient must be included. The copper level in the soil should be too low for a standard cultivar.  
Comparisons must be from the same site

From a minimum of 3 experiments grown over 2 or more years.

**1.10 Iron efficiency**

Assessed as the ratio of yield in a low iron soil to yield in the same soil with iron fertiliser and foliar sprays applied to eliminate the deficiency. A check known to be efficient and one known to be inefficient must be included. The iron level in the soil should be too low for a standard cultivar. Comparisons must be from the same site.

From a minimum of 3 experiments grown over 2 year.

**1.11 Boron tolerance**

Following method of Chantachume *et al* (1995).

Seeds of each line are surface sterilised with 5.0% sodium hypochlorite and pre germinated for eight days at 4°C. Approximately eight seeds of each test line and three of each check should be included in each roll. A check known to be tolerant to boron and one known to be intolerant to boron must be included in each roll. Seeds are placed embryo downwards spaced across the middle of a filter paper (Ekwip 32\*46 cm grade R6 ) which has been soaked in a base solution of 0.0025mM ZnSO<sub>4</sub>.7H<sub>2</sub>O, 0.5mM Ca(NO<sub>3</sub>)<sub>2</sub>.4H<sub>2</sub>O 0.0015mM H<sub>3</sub>BO<sub>3</sub> plus 100 mg/litre of boron as boric acid and drained for 2-3 minutes. The papers are rolled and covered in aluminium foil. They are then stored upright at 15°C for 12-14 days. The lengths of the longest roots are then measured. More definitive tests for boron toxicity may become available.

All test seed should be from the same source.

From a minimum of 3 experiments over 2 or more years.

**1.12 Herbicide tolerance**

Measured as the ratio of the grain yield of a weed-free crop sprayed with the recommended rate of a herbicide to the yield of an unsprayed, weed-free crop. A second measure is the ratio of the grain yield of a weed-free crop sprayed with twice the recommended rate of a herbicide to the yield of an unsprayed, weed-free crop.

From a minimum of 2 experiments grown over 2 years.

**1.13 Tillering**

A visual rating on a 1 to 9 scale (1 low tillering, 9 high tillering). The check should be in the mid range for each experiment and plant populations (plants per unit area) must be presented.

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

**1.14 Plant height**

Height to base of the ear at maturity.

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

**1.15 Lodging Resistance**

A visual rating on a 1 to 9 scale (1 all on the ground; 9 all erect).

From a minimum of 5 experiments where lodging occurs.

**1.16 Shattering Resistance**

A visual rating on a 1 to 9 scale (1 high shattering, 9 no shattering), measured prior to harvest

From a minimum of 5 experiments where shattering occurs.

**1.17 Head Loss**

Counts of heads per unit area on the ground, after harvest.

From a minimum of 5 experiments where head loss occurs.

**1.18 Tolerance to sprouting**

No protocol is available at this time.

**1.19 Yield**

This needs to be assessed relative to the checks in a target environment(s), nominated by the breeder, which may not be a recognised agro-ecological zone. 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. These are subject to continuing research. A target environment, as defined by the breeder submitting data for accreditation, would generally consist of one or more of these agro-ecological environments, but other target environments may be nominated.

The results of any relevant GXE analysis should be referenced.

Data should be presented from a minimum of 30 sites grown over 3 or more years. The number of sites may be unevenly distributed across years. The coefficients 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 sites which are exceptionally low yielding.

The minimum plot size (measured centre to centre) should be 1m x 5 m. Reasons for variation 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, disease status. Longer histories may be valuable in some cases.
3. Soil type. Can 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 of status.
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 site and crop.

## SECTION 2 MALT QUALITY CHARACTERISTICS

### 2.1 SAMPLE COLLECTION

- 2.1.1 Samples must come from the same trials used previously for collection of agronomic and yield data.
- 2.1.2 Trials should be representative of the target area nominated by the breeder.
- 2.1.3 Grain samples including the controls must have the following grain quality parameters measured:
- Plump grains (% > 2.5 mm) and screenings (% < 2.2 mm)
  - Moisture (%)
  - Protein (% db)

**Additional parameters may include:**

- Sprouting
- Grain Colour (brightness/ colour)
- Germination
- Grain germ-end colour (black point/germ-end staining)

Variations to the above may be necessary to meet the specifications used by marketing authorities across Australia.

Visual assessment of grain must ensure that samples meet local market authority specifications.

- 2.1.4 Trials must include control varieties that are relevant for the purpose of comparison.
- 2.1.5 A list of control varieties is to be submitted to the accreditation committee at the beginning of each season.
- 2.1.6 Analyses may be carried out on individual replicates, bulk replicates, or composites of sites.
- 2.1.7 Samples are to be stored, as soon as is practical, under conditions which will ensure the integrity and quality of the grain. A temperature within the range 10 - 15°C is preferred. Extremes of temperature are to be avoided.

### 2.2 MALTING

If samples are being assessed for malting quality then grain size and protein specifications should meet those outlined by the Malting and Brewing Industry Barley Technical Committee (MBIBTC) (Appendix 1, MBIBTC Industry Guidelines, 1998). If samples are being tested for a specific market or to meet regional marketing requirements then deviations from these grain specifications should be noted.

Where possible, micromalting is to be carried out using the protocol recommended by the MBIBTC (Appendix 1, MBIBTC Industry Guidelines, 1998). Variations to the MBIBTC protocol may be necessary in order to accommodate the quality requirements of a number of potential markets.

The malt should be in the range of 36 to 48 Kolbach Index and have a minimal friability of 70. Micromalting processes should be reviewed if analyses are consistently outside this range.

Samples are to be stored, as soon as is practical, in air tight containers under conditions which will ensure the integrity and quality of the malt. A temperature within the range 18-22°C is preferred. Extremes of temperature are to be avoided.

## **2.3 MALT ANALYSES**

- 2.3.1** Samples must be analysed by accredited laboratories (NATA, ISO) or approved laboratories (regularly participating in interlaboratory testing program(s) with satisfactory performance).
- 2.3.2** Analytical methods must be as approved by the accreditation committee and will normally be accepted industry procedures. (Appendix 1).
- 2.3.3** The same methodologies must be employed over the duration of testing.
- 2.3.4** The accreditation committee will specify parameters for which it will receive data.

These include the following parameters used in the MBIBTC rating scheme:

- Extract
- Diastatic Power
- Modification (Kolbach Index)
- Viscosity
- Wort  $\beta$ -glucan
- Apparent Attenuation Limit

Other malt quality parameters will be accepted. Data provided by malting industry laboratories will also be accepted.

## **2.4 ANALYSIS OF DATA (GRAIN AND MALT)**

- 2.4.1** Data should be analysed to achieve a comparison with the approved control variety (varieties).
- 2.4.2** All available data should be included in the analysis in order to achieve a true indication of the variety's quality. This should also include data on any controls used during micromalting and analysis. Data not included must be justified.
- 2.4.3** Data submitted should include a summary of the analysis and a brief statement of the quality of the variety. Expression of parameters as raw data and as a percentage of the control variety taken as 100% is recommended.
- 2.4.4** Records must be maintained in a format which can be audited.

## **2.5 FEED QUALITY**

Where quality attributes are claimed the conventional industry/laboratory standards must be used, with appropriate statistically valid sample collection. More explicit protocols may be developed after further consultation with industry.

## 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.

**APPENDIX I. QUALITY ASSESSMENT METHODOLOGIES\***

<b>GRAIN</b>	<b>UNITS</b>	<b>METHOD</b>
Grain Size - Screenings (SCR) - Plump Grain (PG)	% < 2.2 mm % > 2.5 mm	Grain size was measured by screening samples through a Sortimat for 1 minute. Four grain size fractions were recorded (< 2.2mm, 2.2 - 2.5 mm, 2.5-2.8 mm, > 2.8 mm). Plump grain was recorded as a percentage for the combined 2.5 - 2.8 mm and > 2.8 mm fractions.
Grain Moisture	%	As per EBC method 3.1
Grain protein	% db % db	Kjeldahl method - As per EBC method 3.2 Dumas method - As per IOB method 2.11
Falling Number (FN)	seconds	As per AACC method
Germination	%	As per EBC method 3.6.2
<b>MALT</b>		
Friability (FRI)	%	As per IOB method 2.17
Moisture	%	As per EBC method 4.1
Diastatic power (DP)	WK	As per EBC method 4.12.1
β-Glucanase (MBG)	U/kg	As per modified McCleary method
Full-scale EBC extract coarse/fine	%	As per EBC method 4.4
Soluble protein - (Kolbach Index (KI))	% ( )	As per EBC method 4.9
Wort colour (WC)	EBC Units	As per EBC method 4.7
Wort Haze (WH)		No published method available for wort haze
Wort viscosity (WV)	cP	As per EBC method 4.8
Free amino nitrogen (FAN)	mg/L	As per EBC method 4.10
Wort Beta-glucan (mg/L)	mg/L	Modified McCleary method
AAL	%	As per EBC method 4.11

\*The committee will accept modifications to these methods provided details are outlined when results are submitted.