Protocol for the examination of Value for Cultivation and Use (VCU) of white mustard and fodder radish

2025

Raad voor plantenrassen **(Rvp: Board for Plant Varieties)**
Commissie Samenstelling Aanbevelende Rassenlijst **(CSAR: Recommended List Committee)**

April 2025

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1. Introduction

The examination of the Value for Cultivation and Use (VCU) of white mustard and fodder radish consists of a two-year official test period for inclusion in the National List, supplemented by a third year of testing for varieties to be included in the Recommended List.

The examination of the VCU of white mustard and fodder radish concerns:

- Use as a stubble crop

In addition to the agricultural characteristics, the examination also includes testing the resistance to sugar beet cyst nematodes – SBCN – *(Heterodera schachtii)* and root-knot nematodes *(Meloidogyne chitwoodi).*

The most recent data of the SBCN test are adopted from the official tests for the Federal Plant Variety Office (*Bundessortenamt*) in Germany (see annexes 1 and 2).

The tests for *Meloidogyne chitwoodi* may be performed voluntarily in accordance with annex 3.

All varieties applicated for inclusion in the National Variety Register (for Plant Breeders' Rights and/or listing), as well as varieties that are listed elsewhere in the EU, are eligible for testing.

2. Trials of white mustard and fodder radish

This protocol is based on the assumption of sufficient basic knowledge of the executive organisation of the husbandry of the crop. Commonly used methods and treatments and techniques are not explicitly described. The agronomy should follow the best local practice of an average Dutch arable farm.

2.1. Seed for the testing

Before 1 June, agreements are made between the executive organisation and the breeding companies regarding the varieties to be tested. Withdrawing a variety from the trial is only possible if the executive organisation has been informed before 1 June. From 1 June onwards, the breeder must accept the conditions for the trial. Every year, the seed must be submitted to the executive organisation before 15 June. The executive organisation will then distribute the seed over the various different trial locations.

Quality of the seed

The seed submitted must comply with the following criteria:
- Minimum germination power in accordance with NAK requirements;
- Untreated.

For the standard varieties, a unit of certified seed must be submitted to the executive organisation.

Quantity of seed
The quantity of seed depends on the thousand grain weight (TGW).
The following quantities must be submitted per variety for each test year:
White mustard: 2 field and 1 flowering trial: minimum 80 x TGW (provided germination power is at least 86 %).
Fodder radish: 2 trials: 40 x TGW.

Residual seed (from the sample originally submitted) must be saved for identity inspections. All residual seed is saved for one growing season. Subsequently, 10 grams of residual seed is saved for three years per variety/number.

2.2. Trial design

The trials are performed at two sites for fodder radish and at two sites for white mustard with three replicates at each site. The sites must spread as far as possible over the main growing regions. The sites must be roughly distributed as follows:

	Fodder radish	White mustard
Central marine clay	1 trial	1 trial
South east sandy soil	1 trial - sown early	1 trial
Central marine clay		1 flowering trial – early sown – ad hoc

Fodder radish:
- one stubble trial in the central marine clay region;
- one early sown stubble trial in the south east sandy soil region.

White mustard:
- one stubble trial in the central marine clay region;
- one stubble trial in the south east sandy soil region;
- one ad hoc stubble trial in the central marine clay region- early sown (flowering trial).

Due to the late sowing date of the white mustard trials, it is not always possible to perform (usable) observations of flowering. For a reliable determination of the time of flowering, an early-sown flowering trial should be sown after a year in which there were no observations of flowering. In this trial, all A and N varieties of the Recommended Variety List are tested in addition to all new varieties. Depending on the reliability of the data set, the VCU Crop Working Group will decide whether to resow a flowering trial the following year, so that at least two reliable flowering observations of the varieties being trialled have been performed after three years.

At least four successful trials (excluding flowering trials) are necessary for listing on the National List.

The plot size is 1.5 - 2 metres by net 9 metres. All trials are carried out in complete replicates. If there is a sufficient number of varieties to create sub-blocks, these replicates are split into sub-blocks of 5 or 6 plots.
The varieties of each replicate should preferably be grown in one lane. In case replicates need to be split, varieties must not be removed from their original sub-block.

2.3. Trial layout and husbandry

The trials will be sown and husbanded as follows:

Sowing date:	Fodder radish sown as a stubble crop is sown from 20 July (until no later than 10 August). The latest sowing date for early sown trials on sandy soil is 1 August and for the trials on clay soil 10 August. If, due to force majeure, fodder radish is sown later, it must be irrigated. White mustard is sown in the 2nd half of August (if possible slightly earlier, but no later than 1 September).
Preceding crop:	Fallow land, except for trials on sandy soil. In consultation with the Technical Committee at Plantum, phacelia or short-lived grass may be planted as a preceding crop.
Row distance:	10-20 cm.
Seed rate	Based on the thousand grain weight and germination power, the target plant population is 300 and 170 plants per m^2 for white mustard and fodder radish, respectively;
Fertilisation:	Fodder radish gets a standard application of 50-60 kg N/ha and white mustard 40-50kg N/ha corrected for any effects of the preceding crop. In the trials of stubble crops no additional nitrogen is applied.
Weeds:	No weed control; the weeds must be suppressed by both species.
Insects:	Caterpillar damage may occur occasionally. If crop damage occurs, the executive organisation can treat the crops with insecticide. If necessary, Decis can be sprayed as a preventive measure against cabbage fly.
Cutting:	For stubble crop trials of fodder radish, the 9 m long fields are cut 5 m across the direction of sowing once to prevent seed formation in the earliest varieties. Cutting takes place as soon as the earliest variety starts to show sign of seed formation. Cutting height 20 cm. In case of extreme seed formation, in consultation with the executive organisations, a decision may be made to cut the tops of the plants.

End of trial: On sandy soil, the trial is left until 1 March to enable winter hardiness to be observed. On clay soil the trials ends in April, unless the trial host wants end the trial earlier.

2.4. Varieties to be tested

In addition to the new varieties applicated for listing in the variety lists for the relevant target, five A and N varieties are also sown as permanent standard varieties at each trial site. With white mustard, these varieties are Accent, Architect, Carline, Venice, Victoria and Panter. With fodder radish, these varieties are Angus, Colonel, Dracula, Final and Toledo. The other A and N varieties are tested in alternate years.
If an A variety to be tested is withdrawn, the Board for Plant Varieties and the executive organisation must be informed in writing. The variety concerned will then automatically be categorised as a B variety.
Varieties in the trials are examined for a maximum of three years. Varieties, of which not all resistances and/or recorded results are known after three years of VCU examination, will be placed on a pending list. The data is maintained in the data set and processed pending the remaining data being obtained.
There is no restriction on the number of new varieties to be tested – depending on agreements between the financiers and the executive organisation.

3. Characteristics to be observed

The following characteristics must be observed in the VCU of white mustard and fodder radish. All replicates must be assessed. When scores are given, a high score indicates a favourable assessment of the characteristic involved. In principle, the variety characteristics are scored on a scale of 1 to 9.
All operations, plus any irregularities or unforeseen matters that may affect the trial results must be recorded in a log.

3.1. Speed of ground cover

For the highest possible yields and good weed suppression, a crop that establishes quickly is required. Good ground cover is also very important in preventing structural damage caused by rain, dehydration and dust.
The growing conditions have a significant influence on the rate of establishment. However, the variety itself also plays a clear role. Varieties that establish quickly achieve full ground cover sooner than varieties that establish slower.
The first observation is performed as soon as the earliest variety has reached ground coverage of 75 %, followed by a second observation a few days later and, if necessary, a third observation.

3.2. Flowering

When growing white mustard or fodder radish as a stubble crop, excessive or early flowering is undesirable, because flowering inhibits vegetative development. Early flowering is also undesirable due to the risk of volunteer plants in the following crop.
The extent of flowering is observed 2-3 times and recorded as a percentage of flowering.

The percentages observed are then converted to a score.

3.3. Tuber formation

After a mild winter, any tubers of fodder radish not completely incorporated into the soil can start to grow shoots again. A high proportion of tubers is undesirable when fodder radish is used as a stubble crop.
The extent of the tuber formation with fodder radish as a stubble crop is estimated visually at the end of the growing season and expressed as a score.

3.4. Length of the plant

A crop that is long is more difficult to incorporate into the soil than a short crop.
The length of the plant (including the flowering stem) is measured at the end of the growing season.

3.5. Susceptibility to lodging

Crops that have lodged often need pre-treatment such as disc harrowing or rolling so they can be incorporated into the soil properly.
The susceptibility of white mustard to lodging is observed 1-2 times (end of October) and recorded as a score.

3.6. Disease resistance

If any diseases occur, such as Alternaria, mildew and/or Albugo (white rust), differences in the extent of infection must be observed and recorded as a score.

3.7. Re-growth after cutting

Stubble crops are sometimes cut to prevent seed formation. The plants regrows after cutting. Part of one of the fodder radish trials is mown. The rate of re-growth is observed twice and recorded as a score.

3.8. Winter hardiness

It is important that a green manure crop dies in winter, especially on sandy soil where the green manure crop is only incorporated into the soil in spring. The winter hardness of the varieties of fodder radish in the trial on sandy soil is observed at the end of February and recorded as a score. Both the plants as the roots are observed. This is done for the varieties in the trial on clay soil at the end of April.

4. Nematode resistance

4.1 Resistance to sugar beet cyst nematodes

Resistant varieties of fodder radish and white mustard attract sugar beet cyst nematodes (SBCN), but no or practically no multiplication occurs. Sowing these crops in spring or as a stubble crop sown before 15 August, reduces the extent of infection. If the crops are sown after 15 August, fewer nematodes are attracted, so the reduction of the extent of infection is lower.

Resistance to sugar beet cyst nematodes is established based on determination of the pf/pi (i.e. final population number/initial population number based on eggs and larvae). This determination is performed within the framework of the examination of VCU (*Wertprüfung*) in Germany. This SBCN test is not repeated in the Netherlands. Data from the German SBCN test is adopted if testing was performed in accordance with the method described in annex 1. A variety is considered to be susceptible if no data from the German VCU is available on a tested variety. The breeder is responsible for re-testing the variety for SBCN resistance and submitting the results if data from the official German test is no longer available.

4.2 Resistance to root-knot nematodes (*Meloidogyne chitwoodi)*

Root-knot nematodes, including *Meloidogyne chitwoodi*, are endo-parasitic. The nematodes penetrate the roots of the host plant and move intracellularly. In spring, when soil temperatures rise above 5 ^{o}C, ripe Meloidogyne eggs hatch spontaneously. The infective larvae (second stage juveniles J2) penetrate the roots of the host plant. J2 larvae, and adult male nematodes, are the only mobile stages. Female nematodes complete their life cycle inside the roots and produce new eggs.
Meloidogyne chitwoodi infections do not generally cause yield reductions. However, the financial damage caused by *Meloidogyne chitwoodi* can be considerable due to a poor quality end product and a low market value.

Well-chosen crop rotation, appropriate to the local nematode infestation levels, is an important measure to control damage caused by nematodes. Knowledge of host suitability for the parasite and the associated damage thresholds of crops for nematodes are indispensable to design a crop rotation plan The choice of green manure crops is also of significant importance within sound crop rotation, as nematodes can multiply considerably in green manure crops. Most green manure crops are good host plants for *M. chitwoodi.* Resistant varieties of fodder radish can reduce the nematode population and limit any damage.

The method for determining resistance is described in annex 3.

A new test to determine resistance to *M. chitwoodi.* is currently available based on determination of the pf/pi (i.e. final population number/initial population number based on eggs and larvae). The varieties being examined are included in the validation test. If the validation gives positive results, the varieties being examined will be assessed based on this test. The test is described in annex 3.

Annexe 1 Determination of resistance to sugar beet cyst nematodes

Resistance to sugar beet cyst nematodes is determined by the Julius Kuhn Institut (JKI) in Brunswick, Germany, based on the protocol below:

Müller, Joachim and Hans Jürgen Rumpenhorst (Eds.), 2000.
Die Prüfung von Pflanzen auf ihre Widerstandsfähigkeit gegen Schadorganismen in der Biologische Bundesanstalt – Teil 1: Prüfung von Kulturpflanzen auf Resistenz gegen pflanzenparasitäre Nematoden. Mitteilungen aus der Biologische Bundesanstalt für Land- und Forstwirtschaft, Berlin-Dahlem. Heft 372: 38 pp.

Annexe 2 Application for testing for resistance to sugar beet cyst nematodes

Resistance to sugar beet cyst nematodes is determined by the Julius Kuhn Institut in Brunswick, Germany. The seed used for the SBCN test originates from the identity sample submitted for the DUS testing to Federal Plant Variety Office (Bundessortenamt (BSA)), DE or the Tystoffe Foundation, DK.
An application to perform the SBCN test on behalf of the Board for Plant Varieties (RvP) must be submitted on German (FPVO) application forms.
Application can be done in various ways (RMB= fodder radish; MSG= white mustard).
BSA charges a fee for the SBCN test.
The results of the SBCN tests for stubble crops must be submitted to the RvP and CSAR before 15 March.

Crop:	RMB/MSG	RMB	RMB	MSG
1st application VCU	BSA	NL	NL	NL
2nd application VCU	NL	-	-	-
1st application DUS testing	BSA	NL	FR	NL
DUS testing outsourced to:	-	BSA	BSA	DK
Adoption DUS testing by:	NL	-	NL	-
Appl. SBCN test with:	BSA (incl)	RvP and BSA	RvP and BSA	RvP and BSA
SBCN test outsourced to:	-	BSA	BSA	BSA
Verification sample JKI	-	-	-	-
Latest date for appl.	-	15.12	15.12	15.12 (SBCN)
Submission date seed	-	01.02 (BSA)	01.02 (BSA)	01.02 (SBCN)
Payment by breeder to:	none: direct adoption breeder	BSA	BSA	BSA

First column: Application for the variety (RMB or MSG) has already been submitted for the VCU in Germany. The SBCN test is performed in the framework of the VCU (included). The official data is adopted directly from the breeder for listing in the Dutch (NL) National Variety List.

Second column: Application for the variety (RMB) for the first time in the Netherlands (NL). The registration test (DUS testing) is outsourced to Germany (BSA).
A separate SBCN application must be submitted to the BSA before 15 December. The latest date for submitting the identity sample is 1 February.
The SBCN test is outsourced (and payment due) to the BSA.

Third column: Application for the variety (RMB) for the first time in the Netherlands (NL).
Application for the variety for DUS testing submitted in France, this is outsourced to the BSA.
A separate SBCN application must be submitted to the BSA before 15 December. The latest date for submitting the identity sample is 1 February.
The SBCN test is outsourced (and payment is due) to the BSA.

Fourth column: Application for the variety (MSG) for the first time in the Netherlands (NL).
DUS testing is outsourced to Denmark. An SBCN test can be applied for in DK or a separate SBCN test application must be submitted to the BSA. The latest date for application in Denmark is 20 January, but a separate SBCN test application must be submitted to the BSA before 15 December. The latest date for submitting the identity sample in DK is 10 February, but the seed

for the SBCN test originates from the DK identity sample, this partial sample must be submitted to the BSA before 1 February.
If the SBCN test is requested via NL, the test is outsourced (and payment due) to the BSA.

Annexe 3 Determination of resistance to root-knot nematodes *(Meloidogyne chitwoodi)*

1. Methods of determining resistance to *M. chitwood*.

The resistance to root-knot nematodes (*Melodoigyne chitwoodi*) is carried out by the Hilbrands Laboratory (HLB) based on pf/pi (i.e. final population number/initial population number based on eggs and larvae). The trial contains beside the tomato variety Money maker, the susceptible varieties Radical and Siletina and the resistance varieties Angus and Doublet. The susceptible varieties are intended to check if the infection is sufficient.

2. Application and origin of plant material

The application for the test must be submitted using the TQ-VCU application form: www.raadvoorplantenrassen.nl) to the Board for Plant Varieties (please send a copy to the Trials Coordinator Delphy) before 1 June. The seed (approximately 10 grams) originates from the identity material submitted for Registration (DUS testing) at BSA. A partial sample is requested by Naktuinbouw from the Registration authority, with a consent form of the breeder. If possible, the control varieties must also be obtained from a Registration authority.
Delphy will assign a code to the varieties and will submit a partial sample to the Hilbrands Laboratory.

3. Population selection and inoculum

Meloidogyne chitwoodi is a quarantine organism. It may only be used for testing if an exemption has been granted by the Netherlands Plant Protection Service (PD). The standard population used as the inoculum (currently the Smakt population) is supplied by WUR, which has been granted an exemption for this purpose by the PD.
The identity and purity of the population is tested using molecular techniques and microscopically before distribution and is accompanied by a written declaration from the supplier.

4. Determining resistance to *M. chitwoodi*

The HLB has developed a pot test in a conditioned environment. The first validation test was performed in 2022 and 2023 and produced good results. This test was inoculated with the standard population of *Meloidogyne chitwoodi* (the Smakt population). The resistance is determined based on counting the number of larvae. The test is performed in pots of 1.5 litres. There are five plants in each pot. To achieve this number in all the pots, 20 seeds are sown per pot. For inoculation, the plants are thinned to 15 plants per pot. Each variety is tested in 8 pots. To check growth above soil level, a pot planted with a reference of each variety was included in the test. This pot is not inoculated. At the 2-3 leaf stage, the pots were inoculated with a suspension of 470 nematodes/plant.
The time the test is terminated is determined by the stage of the juveniles found in the egg masses. At least half of the eggs must have reached the second juvenile stage. This is with a T-sum of = 550°Cd (Base-T = 8°C) after inoculation (at 20°C after ~46 days or 6.5 weeks). From 500°Cd, regular inspections are made to see whether this point has been reached. This is done in additional pots included for this purpose in the test (8 pots planted with the sensitive variety Siletina and 8 pots planted with the tomato variety Moneymaker).
When the test terminates, the number of plants is counted in each pot. A distinction is made between plants showing constriction at the base of stem. Per pot, the plant development above the soil is visually recorded compared with the reference pots of non-inoculated plants (0 –

100%). All the roots are then carefully removed from each pot, weighed and placed on a sieve for incubation. Removing the roots from the soil and placing the roots on a sieve for incubation must be done on no more than two consecutive days (For this reason, the number of varieties that can be included in each test is limited to no more than 12). The incubation period is three weeks. After the incubation period, the nematodes are collected. The solution containing the nematodes is stored in chilled conditions. This enables the nematodes to be counted over a number of days.

5. Test reliability

The results of the test, as well as the raw data (of the coded varieties), are submitted to Delphy. Based on the validation tests, standards are established for the reliability of the test. Especially the infection of Siletina and Radical are good indicators, The final result for the new varieties is determined based on two trials of testing.

6. Determining resistance

The resistant varieties Angus and Doublet and the susceptible varieties Radical, Siletina and Iris are included as standard varieties in the trials. Based on the results of the earlier trials provisional standards are established.
Varieties are considered to be very resistance if the level of resistance is equal or better than the standard varieties Angus and Doublet. Whereby the maximum pf/pi (in a pot) never may exceed the highest level (of a pot) of Angus or Doublet.
The test population (Smakt) must be stated as a footnote with the resistance.

ANNEX 4 CONTACT DETAILS

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