PROTOCOL FOR THE EXAMINATION OF VALUE FOR CULTIVATION AND USE OF

SUGAR BEET VARIETIES

In The Netherlands

2025

Raad voor plantenrassen **(Rvp)**
Plant Variety Board

Commissie Samenstelling Aanbevelende Rassenlijst **(CSAR)**
Recommended List Committee

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Contents

1. Introduction

This protocol sets out the procedures to be used for the examination of the Value for Cultivation and Use (VCU) of new sugar beet varieties in the Netherlands and has been compiled under the responsibility of the Working Group on Variety Testing of Sugar Beet.

This protocol is based on the assumption of sufficient basic knowledge of the agronomy of sugar beet. Commonly used methods and treatments and techniques are not explicitly described. For certain methods, reference is made to the Standard Operation Procedures (SOPs), established in the ISO-quality assurance system of the IRS (Institute of Sugar Beet Research in the Netherlands). Unless otherwise indicated it is assumed that the agronomy should follow the best local practice of an average Dutch arable farm.

VCU testing of sugar beet varieties comprises testing for:

- resistance to rhizomania
- resistance to rhizomania and resistance to rhizoctonia
- resistance to rhizomania and resistance to beet cyst nematodes (BCN)
- triple resistance to: rhizomania, rhizoctonia and BCN
- one or more of the resistances above and additional resistance to the AYPR-variant of the rhizomania virus
- one or more of the resistances above and additional resistance to herbicides based on ALS inhibitors (Conviso One)
- one or more of the resistances above and additional resistance to virus yellows
- one or more of the resistances above and additional resistance to *Meloidogyne chitwoodi*

NOTE:

In this protocol, resistance in many cases is taken to understand partial (i.e. incomplete) resistance.

2. Trials

2.1 Trial seed

Applications of new varieties for both the National List and the Recommended List must be filed with the Plant Variety Board and the Trials Organiser before 31 January. Special variety characteristics must be claimed in the first year of application.

In the Netherlands, only pelleted, monogerm seed is used. Pelleted seed is required for all varieties. This seed must be suitable for average sowing conditions using the standard D-drilling discs normally used in the Netherlands.

The pelleted seed must be treated with a legally permitted systemic fungicide and insecticide. The seed must also satisfy the minimum standards for germination rate (> 90%) and monogermity (>95%). The required quantity of seed is established by the Trials Operator.

For the testing of DUS (Distinctness, Uniformity and Stability) and VCU, the same sample is used as that supplied by the applicant (or representative) of the variety to the Trials Operator. If commercial seed is used, the seed is obtained directly from the breeder concerned, originating from certified lots (the lot number must be stated and the seed must be accompanied by a GM test report). A GM test report must also be available for non-commercial seed samples. The report must show that the seed satisfies the set standards.

The samples must be received by the Trials Operator before 20 February. If samples are received after this date, the variety concerned may be removed from the VCU test

2.2 Trial design

Trial plots consist of 6 rows with a minimum length of 10 metres and a distance between the rows of 50 cm, limited by a cross-drilled pathway at the end of the plots (cross-lanes).
Discard pathways can also be planted in the lengthwise direction of the plots to enable crop treatments. These crop treatment pathways can also be cross-drilled (through the cross-lanes) where there is no need for pathways in the plot direction.
At the time of harvest cross-drilled pathways (either discard or treatment pathways) must be free from beet roots to avoid carry-over of discard roots in the net harvest plots.
All trials are performed with complete replicates divided into sub-blocks (randomised, incomplete block design), if possible. All 6 rows are used for observations and measurements.

2.2.1 Examination of varieties with rhizomania resistance

In the rhizomania trials, resistant varieties, including 4 varieties with resistance to rhizoctonia, are sown on 4 sites that are representative for the growing regions. The trial is sown in three replicates. The 4 varieties with resistance to rhizoctonia are used as “long-term linkage standards” to demonstrate the yield difference between the rhizomania and rhizoctonia segment of varieties.

2.2.2 Examination of varieties with rhizomania resistance and rhizoctonia resistance

In the rhizoctonia trials, potentially resistant varieties are tested in 4 trials. The trials are sown in regions where problems caused by rhizoctonia may be expected. When selecting trial sites, the intention is to avoid the risk of a severe rhizoctonia infection.
The trial plan is a randomised complete block design in 4 replicates. A minimum of 2, rhizoctonia susceptible reference varieties are sown in the trials. Plant losses during the growing season should be observed and recorded.
In addition to the naturally infected trials, the varieties are also tested for their level of resistance in one artificially infected trial using two different isolates. The plants are infected in compliance with the method developed by the IRS (described in SOP 8.4). In this trial, a plot consists of just a single row of 0.5 metres wide and a minimum (net) length of 5 metres. The plot is lifted manually in order to assess the degree of infection of the roots (SOP 8.4). No yield determination is performed. The trial plan is a randomised complete block design in 6 replicates. A minimum of 2 rhizoctonia susceptible reference varieties are sown in the trials.

2.2.3 Examination of varieties with rhizomania resistance and beet cyst nematode (BCN) resistance

In the BCN trials, potentially resistant varieties are tested in 4 trials under high or medium levels of infection (150-1500 eggs and larvae per 100 ml soil with a maximum of 2 trials with levels of infection in the range of 150-300 eggs and larvae per 100 ml soil) and in 4 trials with no, or very low levels of infection (less than 10 eggs and larvae per 100 ml soil). The varieties with rhizomania resistance only are also tested in these last 4 trials (see 2.2.1).
The trials are sown in 4 and 3 replicates (for trials with and without (or with very low levels of) infection respectively). The trials are performed with complete replicates, where possible divided into sub-blocks (randomised incomplete block design) or with a randomised complete block design. A minimum of 2, BCN susceptible reference varieties are included in the trials.

2.2.4 Examination of varieties with triple resistance: rhizomania, rhizoctonia and beet cyst nematode (BCN)

The varieties with triple resistance to rhizomania, rhizoctonia and BCN are tested in the 4 rhizoctonia

trials (see section 2.2.2) and in the 4 infected BCN trials (see section 2.2.3). The varieties are also tested for their levels of resistance in the trial which is artificially infected with rhizoctonia (see section 2.2.2).

2.2.5 Examination of varieties with additional rhizomania resistance

Varieties with additional resistance to resistance breaking strains (e.g. AYPR) of the rhizomania virus will be tested in the same way as varieties without this additional resistance in each segment (rhizomania, BCN, rhizoctonia and triple). In addition, multiplication of the variant virus in the plant will be determined under controlled conditions in a climate chamber trial. Varieties with additional resistance will be compared with a standard rhizomania variety and a variety without rhizomania resistance. Only those varieties from the first year of testing which are promoted to the second year of testing are included in the climate chamber trial, using both seed of the first and seed of the second year of testing of the varieties concerned.

2.2.6 Examination of varieties with resistance to the Conviso One herbicide

Varieties with resistance to Conviso One are tested in the same way as the other varieties in the category for which these varieties have been applied for (rhizomania, BCN, rhizoctonia and triple resistance). In addition, the Conviso One resistance will be tested once every two years in a field trial.. This is done by spraying the plants with a dose of the herbicide corresponding to a dose used in practice. A minimum of 95% of the plants of the resistant variety must survive, compared with 0% of the non-resistant control variety.

2.2.7 Examination of varieties with resistance to virus yellows

Varieties with resistance to virus yellows are tested in the same way as varieties without resistance to virus yellows in each segment (rhizomania, BCN, rhizoctonia and triple). The level of resistance to virus yellows is tested in a separate trial. In this trial varieties are tested in 4 replicates. In May part of the plots is inoculated with aphids which are infected with one of the three virus strains (BMYV, BChV or BYV). There are separate plots for each virus strain. After inoculation there should be periodic observations on the appearance of virus symptoms in the plots. Yield losses between inoculated and non-inoculated plots should be determined.

2.2.8 Examination of varieties with resistance to *Meloidogyne chitwoodi*

Varieties with resistance to *M. chitwoodi* are tested in the same way as varieties without resistance to *M. chitwoodi* in each segment (rhizomania, BCN, rhizoctonia and triple). The level of resistance is tested in a greenhouse test once in two years, by comparing the multiplication of *M. chitwoodi* of the resistant varieties with the multiplication on a reference variety, starting from different levels of infection with *M. chitwoodi*. In addition, the level of resistance to *M. fallax* is tested once in two years in a similar way. The frequency of testing on the level of resistance to *M. chitwoodi* or *M. fallax* can be adjusted in consultation with the Working Group on Variety Testing of Sugar Beet

2.3 Varieties to be tested

Each year, the Working Group on Variety Testing of Sugar Beet establishes the optimal number of varieties to be included in VCU testing. The number of entries in the first year are divided among the applicants based on consultation between the applicants mutually and consultation between the applicant and the Trials Organiser, on the mandate of the Working Group.
The promotion of varieties to the second or third year of testing is also based on consultation between the applicant and the Trials Organiser (on the mandate of the Working Group).

The Trials Organiser drafts a proposal on which varieties should continue in the trials each year, taking the following into consideration:

- the total number of entries in the trial.
- the chance a variety stands of becoming recommended after the trial period (based on the valid criteria at that moment).
- the best possible distribution of the number of entries per trial year and per resistance group.
- the distribution of the number of new entries among the applicants as agreed in the Working Group.

Applicants are free to replace a variety in a trial which they are entitled to participate in under the terms of the aforementioned consultation, with a different variety to the one that resulted in the accepted entry. When varieties are promoted to the next trialling phase, the same criteria as those used for recommendation of varieties on the Recommended List are leading.

In addition to new varieties being tested, all A (generally recommended) and N (newly recommended) varieties of the Recommended List are included in the trials, with the possible addition of:

- varieties in a trial phase with a new characteristic of importance to practical applications, or
- B (declassified) varieties with an exceptional characteristic and/or a considerable market share.

Trialling these additional varieties can be requested by:

- the joint financiers of the variety trial, whereby the trial then falls under collective funding;
- one of the breeders, whereby the extra trial place as well as the funding then falls under the responsibility of, and will be invoiced to, the breeder concerned;
- the Trials Organiser, if the total number of applications is less than the number of varieties required to reach a reliable, orthogonal trial plan.

2.4 Trial layout, trial operations, crop treatments and husbandry

Trial fields used for testing must be as regular as possible.
The fields must be inspected in advance for the presence of beet cyst nematodes. The maximum acceptable number of beet cyst nematodes on a trial site is 10 eggs and larvae per 100 ml soil. This does not apply to the 4 separate trial sites used to test BCN resistant varieties under infected conditions. The number of eggs and larvae at these trial sites should be between 150 and 1500 per 100 ml soil.

The trial sites on sandy and peat soil must be tested in advance on pH $CaCl_2$ which needs to be 5.0 as a minimum.

A minimum crop and trial rotation of 3 years should be applied (unless a shorter rotation is necessary to realise a certain infection pressure). If (practically) possible the plots should be drilled across the direction of cultivation. The trials must be surrounded by edging strips and discard plots.

In order to avoid resistance-breaking variants of the rhizomania virus (e.g. AYPR) influencing trial results, trial sites known to be risky for symptoms of resistance-breakthroughs should be avoided. As these variants are increasingly present in farmers' fields in The Netherlands whereas testing in advance will not be feasible, it may be inevitable that resistance-breakthroughs of rhizomania will occur in trial fields in future.

The agronomy should follow best local practice of an average Dutch arable farm. Seed bed preparation, sowing depth, fertiliser applications and treatments using herbicides, insecticides should be in compliance with this practice. Agreements will be made with the Trial Operator in advance regarding the expected agronomy on the trial, using a "Focus Points form". In case of doubt about the level of mineral Nitrogen a soil sample should be analysed on $N_{min.}$

To avoid (localised patches of) infection by foliar diseases, the trials are treated with a fungicide according to the principles of IPM (Integrated Pest Management).

Crop treatments should be performed as fully as possible in compliance with the generally applicable advice (see information including The Crop Protection Update of the IRS in sugar beet industry journals and/or the Delphy manual on controlling pests and diseases).
As soon as any sign of significant manganese deficiency is visible in any of the varieties, the entire trial should be treated with a formulation containing manganese. The treatment is repeated if necessary. Irrigation can be applied, if symptoms of drought are likely to occur.

Sowing times must comply with local practice (approx. mid-March to end of April).
The aim is to achieve a target plant population of 80,000 to 90,000 plants per hectare that is as uniform as possible. To achieve this, the trials are drilled directly at a final planting distance of 17-20 centimetres in the row.

3. Observations and measurements during the growing season

In variety trials of sugar beet, the following observations and measurements are performed. All the plots in the trial must be assessed. When scores are given, a high score indicates a favourable assessment of the characteristic involved.

3.1 Number of plants

The number of plants is only counted on trials with irregular emergence. At the 4-6 true leaf stage a minimum of 9 square metres is counted per plot. If the plant population is very irregular, a decision can be made to count the entire plot, in compliance with SOP 5.1.

3.2 Earliness of ground cover

Early ground cover is estimated per plot just before the leaf canopy is almost closed over the entire trial (usually second half of June). This characteristic is expressed as a score. Plots on which the crop cover within rows is almost complete are scored 9. Plots on which the rows are most open are scored 1. This characteristic is assessed on 3 of the trials of each of the categories (rhizomania, rhizoctonia and BCN) on uninfected trials.

3.3 Sensitivity to bolting

From June until harvest, the number of bolters per plot is counted at least monthly. These bolters are also removed after each count until a few weeks before harvest. Bolters are understood to be all beets that, at that moment, have formed one or more clearly recognisable flowering stems. If small, lateral bolters occur on the sugar beet, they should be removed by being torn off leaving the beet intact. If lateral bolters reappear on the beet at a later stage, they should be recounted again.
Late bolters, flowering stems that are formed within the last few weeks before harvest, are counted just before harvest. To determine the final score, the counted number of bolters is added up.
The percentage of bolters is based on the established number of plants (see 3.1)

3.4 Height of root top

The height of the top of the root is measured of the untopped (or non-defoliated) beets in the field just before harvest with a measuring system on the trialharvester. The height of about 50 plants is measured in the 3th and 4th row except the plants of the first and last meters. If sufficient homogeneous trials are available two rhizomania/BCN trials and two rhizoctonia trials will be measured.

3.5 Pests and diseases

Any infections are reported as a score per plot whereby 10 indicates no infection and 1 indicates severe infection. The most commonly occurring diseases of sugar beets can generally best be observed in the period from early September until harvest.

The following are assessed:

Rhizomania (*Beta-virus BNYVV* / vector *Polymyxa betae*)
When the symptoms on the rhizomania trials are sufficiently noticeable, the number of infected

plants (%) per plot can be established based on the colour of the foliage (so-called blinkers).

Rhizoctonia
In the specific trials with artificial infection, the level of infection of the manually lifted beets is assessed according to SOP no. 8.4. The degree of rotting is scored on a scale from 1-7 whereby 1 indicates no visible infection and 7 indicates complete rotting. In the trials with a potentially natural infection, the degree of rotting is assessed on the inspection belt in the central tare house.

Beet Cyst Nematodes
The level of infection in the trials is determined by sampling according to protocol (SOP no. 5.8).

If necessary, other pests and diseases can be recorded if they have an influence on the results of the trial (e.g. infections of virus yellows or Aphanomyces).

3.6 Foliar diseases

Sensitivity of varieties for foliar diseases is observed in three separate trials including all varieties from the first year of testing. Trials consist of 3-row plots in 4 replicates. There should be a trial in the northeastern part of the country (for reasons of a high disease pressure of Stemphylium) and a trial in the southeastern part of the country (for reasons of a high disease pressure of Cercospora). A third trial is sown if this trial is considered fit for purpose beforehand. During the growing season there will be no fungicide treatments. Two trials showing the most uniform infection will be scored on foliar diseases.

3.7 Aphanomyces

Susceptibility of varieties for Aphanomyces is observed in a trial on sandy- or peat soil in the northeastern part of the country. The trial consists of single row plots in 4 replicates. All varieties in the second and third year of testing will be included. Plants will be counted at the 4-6 true leaf stage and from then onwards the trial will be kept wet by regular (sprinkler) irrigation until the closure of the leaf canopy. Plants will be counted again just before harvest in order to establish the number of plants disappeared. Manually lifted beets are spread out on the ground and visually assessed for infection by Aphanomyces. No yield determination is performed.

3.8 Other observations

If virus yellows is noticed, the site and the extent of the patch(es) must be recorded.
Infections caused by cercospora leaf spot *(Cercospora beticola),* ramularia (*Ramularia beticola*)*,* powdery mildew (*Erysiphe betae*), downy mildew (*Peronospora schachtii*), stemphylium (*Stemphylium* sp. nov.) and rust (*Uromyces betae*) are assessed using a specially developed scale such as the Agronomica scale (SOP 5.6) for foliar diseases. Infections by nematodes (visual assessment of % of drooping beet leaves not only caused by drought) are recorded on a scale of 1 (100% drooping leaves) to 10 (no drooping leaves) (SOP no. 5.11) and rhizoctonia infections on a scale of 1 (0% infection) to 7 (completely rotten) (SOP no. 8.4).

In addition to the observations above, any other incidences that may be of importance in examining the variety and/or trial must be systemically recorded and the plots/trials in question must be validated for their suitability for VCU testing.

4. Harvest

4.1 Time

Harvesting of the variety trials starts in September. In relation to data processing for the new edition of the Recommended List, the aim is to harvest the last trial no later than the middle of November.

4.2 Harvesting method

The trials are to be harvested using a 6-row trial plot harvester (PASSI X6) developed by IRS featuring a weighing unit and sampling system. The trial sites should be chosen to ensure minimum transportation of the harvester. This requires a certain extent of clustering the trial sites.

The lifting method greatly influences the relative differences in root yield between the varieties. In practice, the harvester can be optimally set to suit the crop (variety) being lifted. However, variety trials involve a wide range of different types of sugar beets. The varieties exhibit considerable differences in root length, profusion of branching, height of the top and in variations of root top height. It is therefore impossible to optimally lift all the varieties using a single, uniform setting on the harvester. For that reason the harvester should be set to ensure there are no extra lifting losses even when extreme variety types are lifted. The underlying principle is that the actually realised yield must be harvested of each variety. The beets must be harvested fairly deeply, so that long rooted or deep growing varieties can be harvested without any extra root tip breakage.

Careful attention must be paid to the setting of the flail defoliator. In line with the principle of ‘top indeed but no leaves’ beets must be defoliated as much as possible while minimising top losses.

The artificially infected, single row rhizoctonia trials are lifted manually and then assessed for infection.

4.3 Yield determination and sampling

All the lifted beets from each plot are weighed on the PASSI X6 (gross yield, including sub-sample), and a sub-sample is taken (approx. 80 kg) that is transported to a container. All sub-samples are delivered to the central tare house at Dinteloord for further analysis and divided there into two sub-samples per plot.

5. Laboratory analyses and determinations

Samples from all the trials are processed and analysed at one central tare house at Dinteloord (CTS). All determinations and analyses performed in this laboratory comply with the "Uniform method for weight determination, sampling and sample analysis of sugar beet in the Netherlands". These regulations are published annually by the Dutch Sugar Industry.

The following determinations and analyses (including calculations) are performed in the laboratory:

- gross weight samples

  This is the weight of the samples delivered, including soil adhering to beets and top tare.

- adherence of soil

  This is the difference between the gross weight and the weight of the beets after washing expressed as the soil delivered in kg.

- top tare

  No top tare percentage is determined and beets will not be retopped. Green parts are removed.

- net weight

  This is the gross weight of the samples minus the amount of soil tare. The gross weight determined in the field is converted into a net weight based on the amount of soil tare measured on the samples.

- quality testing

  The pulp (brei sample) obtained after washing, weighing and cutting of the two plot sub-samples is then analysed to establish the quality of the following:

  - sugar content as a percentage
  - potassium content in mmol per kg of beet
  - sodium content in mmol per kg of beet
  - amino-nitrogen content in mmol per kg of beet
  - glucose content (as a measure of invert sugar) in mmol per kg of beet

If a severe rhizoctonia infection is observed in the trials with a potentially natural rhizoctonia infection, the beets should be assessed individually on the inspection belt for the level of infection in compliance with SOP no. 8.4. Results from plots of resistant varieties with more than 10% of rotten beets will be excluded from the calculations of the final results.

6. Data handling procedures

6.1 Calculation of the multi-year averages

The results of observations and analyses are averaged per variety for each trial separately. These averages are then converted into a ratio whereby the averages of the A- and N-varieties from the previous Recommended List, provided they are actually tested in the current year, is set at 100. The single-year averages are then calculated using the ratios of each trial. Finally, the yearly average ratios obtained in the three previous years, are converted to the same standard (A and N-varieties from the previous Recommended List) and together with the single-year average of the current year are averaged to create a multi-year average.
The calculation of the compound characteristics is based on this multi-year average.
The ratio for earliness is converted into a score.
The ratio for top height, adherence of soil and resistance to the foliar diseases cercospora and stemphylium is converted into a classification.
Varieties with triple resistance are tested together with varieties with BCN resistance in trials which are infected with beet cyst nematodes. Moreover, they are also tested together with the rhizoctonia resistant varieties. Multi-year averages are calculated separately for both categories.

6.2 Calculation of compound characteristics

With sugar beet, various characteristics are combined using certain formulae to create new "characteristics". The data used to perform these calculations, and the relevant characteristics are shown below.

6.2.1 Extractability Index Netherlands (WIN)

To achieve the greatest extraction of sugar when processing beets, it is vital to cultivate sugar beet of good, internal quality. Internal quality is related to the sugar content and extractability index (WIN). The WIN is calculated using the formula applied by the Dutch Sugar Industry to calculate payments made to beet growers.

6.2.2 Financial yield

Each year, the Working Group on Variety Testing of Sugar Beet, via the IRS, agrees the principles to be applied when calculating the WIN and the financial yield based on the multi-year averages of the harvesting campaign of the sugar industry.

Any amendments to the principles are proposed by the Trials Organiser. This proposal is communicated with the Working Group via the “Draft Recommended List.”

By subsequently offsetting the agreed reference values against the multi-year variety averages in ratios of sugar content, K + Na, amino nitrogen, soil tare and root yield, respectively, the multi-year average figures are obtained per variety which are offset according to the WIN-formula, and the applicable payment scheme. The WIN and financial yields are indicated as ratios in the Recommended List. The ratios are rounded to a whole number.

7. Publication of data

The IRS includes the calculated three and four year averages of the trials in three tables:

1. A table of information on varieties for cultivation on fields free from rhizoctonia or beet cyst nematodes. Varieties with BCN resistance which are tested on fields without or with very low levels of infestation (less than 10 eggs and larvae per 100 ml soil) are also included in this table.

2. A table of information on varieties for cultivation on fields with rhizoctonia. Information obtained from trials in the rhizoctonia region. This table also states the results of the triple resistance varieties trialled on fields with a risk of rhizoctonia infection. The resistance to rhizoctonia is indicated in three classes: very good (disease-index ≤ 2.7), good (disease-index 2.8-3.0) or moderate (disease-index ≥ 3.1)

3. A table of information on varieties with BCN resistance. Information obtained from trials with BCN infestation.

The varieties with additional resistance are classified as very good, good or moderate in all three tables. Proposals for acceptance and classification in the Recommended List are also stated in the tables. This is proposed to the CSAR. After it has approved the results, CSAR will issue a press release containing the results and its decisions. Based on the press release, the IRS publishes the results in the Seed Brochure on behalf of the sugar industry.

The above results, supplemented with the results of varieties trialled for two years and the financial yields of the BCN varieties trialled on uninfected fields, are published in the Seed Brochure.

A table is also created showing the resistance level of rhizoctonia resistant varieties trialled on artificially infected fields. This table is only published in the Variety Bulletin and is made available to the Working Group on Variety Testing of Sugar Beet.

One-year figures are made available to members of the Working Group, the Plant Variety Board and other stakeholding breeders. These figures are confidential and for personal use only.

Two documents for financiers can be used externally: (1) the press release and annex published by CSAR and (2) the Seed Brochure. Reference must be made to the sources when publishing this information externally. Reproduction of, and references to, figures and variety names in the press release or the Seed Brochure must be complete and accurate.

Annex Contact details

IRS (Institute of Sugar Beet Research) - Trials Organiser

Post address:
Postbus 20
4670 AA Dinteloord, NL

Visitors address:
Kreekweg 1
4671 VA Dinteloord, NL

leijdekkers@irs.nl
dezinger@irs.nl
www.irs.nl

Raad voor plantenrassen (Rvp) / Naktuinbouw
Plant Variety Board / Naktuinbouw

Post address:
Postbus 40
2370 AA Roelofarendsveen, NL

Visitors address:
Sotaweg 22
2371 GD Roelofarendsveen, NL

J.r.vd.schoot@naktuinbouw.nl
www.naktuinbouw.nl
/www.raadvoorplantenrassen.nl

Commissie Samenstelling Aanbevelende Rassenlijst (CSAR)
Recommended List Committee

CSAR
Frans Verwer

c/o Brancheorganisatie Akkerbouw
Louis Braillelaan 80
2719 EK Zoetermeer, NL

Telephone: · +31 (0) 6 5272 3623

mailto:verwer@bo-akkerbouw.nl
www.bo-akkerbouw.nl
www.rassenlijst.info