PROTOCOL FOR THE EXAMINATION OF VALUE FOR CULTIVATION AND USE OF

FORAGE AND GRAIN MAIZE VARIETIES

In The Netherlands

2026

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 forage maize, grain maize and CCM in the Netherlands.

The VCU consists of:

- two years of official trials for varieties to be included in the National List (NL1 and NL2)
- followed by a third year of trials for varieties to be included in the Recommended List. (RL3)

The VCU also includes trials of genetically modified varieties and other derived varieties.
The trials are coordinated and/or executed by an independent Trials Coordinator/Trials Organiser.

This protocol is based on the assumption of sufficient basic knowledge of the husbandry of maize; commonly used methods and treatments are not explicitly described. Unless otherwise indicated it is assumed that the agronomy should follow the best local practice of an average Dutch farm.

After NL2 the *Raad voor plantenrassen* (Rvp; Plant Variety Board) decides whether or not the variety can be included in the National List based on the VCU results. Varieties included in the National List are approved for marketing.

After RL3, the *Commissie Samenstelling Aanbevelende Rassenlijst* (CSAR; Recommended List Committee) decides whether or not the variety can be included and classified in the Recommended List.

See annex 1 for contact details.

2. Examination of varieties of forage maize

2.1. Trial seed

All seed submitted for the trials must be disinfected with a fungicide (Redigo M). The seed used in the trials is distributed by the Trials Organiser.

First year trials
The applicant must submit 17 bags each containing 800 seeds of each variety admitted to the trials to the Trials Organiser no later than 15 March. The number of bags equals the number of plots plus three spare bags (1 bag for storage).

Second/third year trials and subsequent trials
The applicant must submit 19 bags each containing 800 seeds of the second and third year variety to the Trials Organiser no later than 15 March. Two bags are possibly used for the Maize Head Smut trials.
For the standard varieties, (A and N varieties in the latest Recommended List) the breeder must also submit 19 bags each containing 800 seeds of each variety admitted to the trials to the Trials Organiser.

Common standard varieties
The breeder must submit in total 33 bags each containing 800 seeds of these varieties, included both in the early and in the late trials, to the Trials Organiser no later than 15 March.

If (due to circumstances) no seed can be submitted on 15 March, the closing date for submission may be delayed until 1 April, provided this request for postponement is announced at the breeders' meeting.

2.2. Trial design

2.2.1. Classification according to earliness

The varieties in the official trials are tested in two groups according to the degree of earliness. At application, the breeder indicates whether a variety is to be tested in the very early/early or medium-early/medium-late group.

The delineation between both groups is the limit between early and medium-early (i.e. 34.0 % dry matter (absolute) on the Recommended List 2011, this is 34.23% dry matter in RL2013 for the very early/early group and 34.60% dry matter in RL2013 for the medium-early/medium-late group). Varieties in the transitional zone may apply for trials in both groups. The transitional zone is defined as 0.75 % dry matter (absolute) above and below the limit value.

The limit value will be recalculated every year *(see annex 2)*

In exceptional cases, a variety may:
- be tested in both groups in the first year, after which time the breeder/applicant must choose one of the two groups.
- be tested in both groups in the second year, after which time the breeder/applicant must choose one of the two groups.

After the second year of testing, a variety can:
- only be tested in one of the two groups.
- can possibly be included in the National List (without indicating the earliness group).
- can possibly proceed to the third year trials for the Recommended List.
- a variety is never recommended for a different group than that in which it was tested.

2.2.2. Official variety examination

The official testing consists of first year trials and second/third year trials and subsequent trials. The testing is performed in two earliness groups on seven sites per earliness group and with two replicates per site. The sites should be spread as far as possible over the main growing regions. The sites should be generally spread as follows:

	Very early to early	Medium-early to Medium-late
Region	Number of sites	Number of sites
Sandy soil	5	6
Southern sandy soil	1	3
Eastern sandy soil	2	3
Northern sandy soil	2	0
River clay Central Netherlands	1	1
Southwestern marine clay	1	0

The number of rows per plot is 6 with a distance between the rows of 75 cm. The length of the plots is at least 7.5 metres. Only the two central rows of a plot are used for observations and determinations.
All trials are carried out in complete replicates. These replicates (blocks) are sub-divided into sub-blocks of 5 or 6 plots.
The Trials Organiser sets up the trial plans and sends them to the Trial Operators. The varieties of each replicate should preferably be grown in a single lane. In case replicates need to be split, due to the local farming conditions, varieties must not be removed from their original sub-block.

2.2.3. Examining genetically modified (GM) varieties of forage maize

VCU testing of genetically modified varieties (GMs) depends on the way in which the GM variety was created. Refer to EU-directive 2001/18 for a definition of derived and independent genetically modified organisms. At the moment, a decision has been made not to test (derived) GM varieties in the regular trials. If a modified variety has not (yet) been granted commercial authorisation in the EU, trials may take place under an authorisation for the deliberate release of GMOs for any other purpose than for placing on the market (Part B release), if necessary with a permit of one or more of the applicants.

<u>Derived GM varieties</u>
If a GM variety is derived from an existing hybrid variety, which has completed at least the second year of trials in the Netherlands - and whereby a new characteristic has been added to the original variety through genetic modification - testing can be limited to the extent to which the GM variety is comparable to the original variety in terms of its VCU characteristics. It is assumed that year interactions do not play a role in this comparison, meaning testing can be completed in one year.
This comparative test examines whether the derived variety differs from the original variety, assuming the null hypothesis that both varieties are identical. As these derived GMs cannot be included in the regular trials, separate trials must be sown for the purpose of this comparison.

Independent GM varieties

These GMs (created independently to an existing hybrid variety) are tested in the same way as conventional (unmodified) varieties. Derived GMs of varieties that are not in the Dutch National List are also included in this category. However, similar to varieties included in the EU Common Catalogue, these varieties can enter the official trials directly. Testing is performed in accordance with the standard protocol and generally lasts 2 years for the National List and 3 years for the Recommended List. As these independent GM varieties cannot be included in the regular trials, separate trials must be sown for this purpose. Further details will be provided when applications are received for these GM varieties. This chapter of the VCU protocol for maize therefore only discusses the examination of derived GM varieties.

VCU testing of the characteristic added through modification depends on the **type of modification**. Details of these tests will be established on an *ad hoc* basis at such time as an application for a new type of modification is received. A DUS report in which the modification in question has been tested and included (performed by an organisation such as GEVES) can demonstrate the modification concerned.

Admission of derived GM varieties to the trials

Applications for GM varieties derived of varieties that have completed at least 2 years of VCU trials can be considered.

To limit the size of these trials, admission to the trials may be limited in the following way:

- GMs based on existing varieties from the Recommended List will be given priority
- GMs based on varieties which are in the third year of testing can enter the trials as long as the maximum trial capacity is not exceeded
- In the case of varieties that are only included in the National List, admission to the trials is only possible if places are still available.

For inclusion in the National List, the GM variety is tested for one year. The null hypothesis is tested based on the results after one year (with the exception of the added characteristic). After one year of testing, the GM variety can be included in the National List if the GM is identical to the original variety. The derived GM variety can be re-tested if there is a reason for the deviation from the null hypothesis which is not caused by the GM itself (e.g. germination power / emergence). Insufficient reliability of the trials or doubt concerning the validity of the null hypothesis for one or more characteristics may be a reason to test a derived variety for a second year. If a positive decision on VCU is arrived at, but Plant Breeders' Rights have not yet been granted (due to unfinished DUS test, for example), the derived variety does not need to be re-tested. When Plant Breeders' Rights are granted, the derived GM variety will then be included in the National List.

The examination

The distinctness of a variety is primarily determined using the UPOV characteristics that are established during the DUS testing (DUS - Distinctness, Uniformity and Stability). These characteristics are mainly morphological. For derived GM varieties, the modification (e.g. herbicide tolerance) is often added as the distinctive characteristic. The DUS report shows whether the derived GM is distinct for this characteristic and that all the other characteristics are identical to those of the original variety.
The purpose of a VCU test of a derived GM variety is to show there is no difference in VCU compared to the original variety, with the exception of the modification. The VCU therefore compares the varieties based on characteristics that are relevant for the value for cultivation and use of a variety.

The relevant characteristics of forage maize are:

- Emergence (Plant population)
- Development
- Flowering (on a limited number of sites – in relation to emergence and development)
- Plant length (on a limited number of sites – in relation to the previous characteristics)
- Lodging
- Common smut
- Fusarium
- Fresh weight, dry matter weight and dry matter content
- VEM/kg dm
- Starch content
- Digestibility

These characteristics are established according to the methods described in the standard protocol.

Any distinction between the varieties is established using a Student t-statistic (two-tailed test – at 5 % probability level).
The coefficient of variation (CV) of the dry matter content and the dry matter yield in the various trials is in the order of magnitude of 3 to 6 %. As a guideline, a CV below 4 indicates a valid trial. If a CV is above 6, the trial is rejected in principle. If a CV is between 4 and 6, the validity of the trial will be discussed in the meeting between the Plant Variety Board and the Trials Coordinator. A total of 14-22 replicates are necessary to establish a significant difference of 5 % for the characteristics concerned.
Earlier studies on the reliability of variety testing reveal that the relative gain in reliability decreases with more than 3 replicates per trial. Therefore, at least 5 trials in 3 replicates are necessary to achieve an optimal result. To be on the safe side, the trials should be sown in 4 replicates, so the minimum number of replicates can most probably be arrived at, even if one site is discarded.

Maximum size of individual trials

The current trials in regular tests comprise 240 plots. This is the maximum number that is feasibly achievable in practice. Assuming these trials are sown in the same way as the trials of conventional varieties, for a trial consisting of 4 replicates this means:

Maximum number of varieties:	60 varieties
Divided over GM and original varieties:	35 GMs and 25 original varieties

A trial must consist of at least 10 varieties. If there are less than 10 varieties, a selection of varieties from the Recommended list will be added (A or N varieties). The rest of the trial is performed according to the standard protocol.

Special requirements for the trial

The seed quality can greatly influence comparison of the varieties from start. The seed lots must therefore be of comparable quality with a field emergence rate of at least 80 %. The varieties are treated using standard methods. This also applies to weed control treatments.

2.2.4. Examining derived non-GM varieties of forage maize

Derived non-GM varieties can be tested according to the standard protocol and in regular trials.

Derived non-GM varieties
If a variety has completed the second year of testing and whereby a new non-GM characteristic compared with the original variety has been added to the derived variety (e.g. herbicide resistance), the testing can be limited to the extent to which the derived variety is comparable to the original variety in terms of its VCU characteristics. It is assumed that year interactions do not play a role in this comparison, meaning the testing can be completed in one year. This comparative test examines whether the derived variety differs from the original variety, assuming the null hypothesis that both varieties are identical.

VCU testing of the added characteristic depends on the characteristic. Details of these tests will be established on an *ad hoc* basis. A DUS report in which the modification in question has been tested and included (performed by an organisation such as GEVES) can demonstrate the modification concerned.

Admission of derived non-GM varieties to the regular trials
Applications for derived non-GMs of varieties that have completed at least 2 years of VCU testing can be considered. The original variety and the derived non-GM variety will be included in the regular trials. A derived non-GM variety, of which the original variety is not in the Dutch National List, but has been included in the EU Common Catalogue, is considered to be an independent variety.

To limit the size of the regular trials, admission to the trials may be limited in the following way:

- derived varieties based on existing varieties from the Recommended List will be given priority.
- derived varieties based on varieties which are in the third year of testing can enter the trials as long as the maximum trial capacity is not exceeded.
- in the case of varieties that do not continue in the regular trials (National List), admission to the trials is only possible if places are still available.

For inclusion in the National List, the derived variety is tested for one year. The null hypothesis is tested (with the exception of the added characteristic) based on the results after one year. After one year of testing, the derived variety can be included in the National List if the derived variety is identical to the original variety. The derived variety can be re-tested if there is a reason for the deviation which is not caused by the variety itself (e.g. germination power/ emergence). Insufficient reliability of the trial or doubt concerning the validity of the null hypothesis for one or more characteristics may be a reason to test a derived variety for a second year. If a positive decision on VCU is arrived at, but Plant Breeders' Rights have not yet been granted (i.c. unfinished DUS test), the derived variety does not need to be re-tested. When Plant Breeders' Rights are granted, the derived variety will then be included in the National List.

The examination
The distinctness of a variety is primarily determined using the UPOV characteristics that are established during the DUS testing (DUS - Distinctness, Uniformity and Stability). These characteristics are mainly morphological. For derived varieties, the modification (e.g. herbicide tolerance) is often added as the distinctive characteristic. The DUS report shows whether the derived variety is distinct for this characteristic and that all the other characteristics are identical to those of the original variety.
The purpose of a VCU test of a derived variety is to show there is no difference in VCU compared to the original variety, with the exception of the modification. The VCU therefore compares the varieties based on characteristics that are relevant for the value for cultivation and use of a variety.

The relevant characteristics of forage maize are:

- Emergence (Plant population)
- Development
- Flowering (on a limited number of sites – in relation to emergence and development)
- Plant length (on a limited number of sites – in relation to the previous characteristics)
- Lodging
- Common smut
- Fusarium
- Fresh weight, dry matter weight and dry matter content
- VEM/kg dm
- Starch content
- Digestibility

These characteristics are established according to the methods described in the standard protocol. Any distinction between the varieties is established using a Student t-statistic (two-tailed test – at 5 % probability level)

2.3. Varieties to be tested

A total of maximum 120 varieties per trial are sown (first, second and third year trials and the A and N1 and N0 varieties from the latest Recommended List). Certain standard varieties that have been tested already for several years can skip a year.
In consultation with the Trials Coordinator, each breeder can submit applications for varieties for the first year trials, stating the earliness group of the variety. If the number of varieties within a single group is too high, Plantum should initially be consulted to try and limit the numbers to the scope of the examination. If this consultation fails to result in a solution, each breeder will be asked to withdraw 1 application (or more if necessary).

2.4. Trial layout, Trial operations and Trial husbandry

Fields used for the trials must be as regular as possible. High and dry or wet and low fields are undesirable. Ploughed pasture is usually unsuitable for trials in the first years after ploughing. No trials should preferably have taken place for at least the past two years on the field concerned. In case of drained fields, the trial lanes must run parallel to the drains and the plots should be drilled across the direction of the drains. Treatments and cultivations must be done in the direction of the trial lanes as much as possible. Furthermore, the agronomy should follow best local practice of an average Dutch arable farm. This also holds for the preparation of the seedbed, fertilisation and for weed control.
The trials must not be treated using glyfosaat, due to possible damage (e.g. as a result of vaporisation) caused by this product. The trial must be irrigated if there are indications of disproportional effects in development and flowering of varieties, causing non-representative deviations between varieties. The Technical Committee of Plantum is consulted if irrigation is not possible. Irrigation is optional after flowering if necessary to safeguard the validity of the trial.

Sowing time must comply with local practice. Generally the trials will be sown between April 10 and May 15.

The trials are sown at final planting distance. From 2023, the target population is 95.000 plants/ha for the very early/early varieties and 90.000 plants/ha for the mid-early/mid-late varieties. To achieve a uniform plant population, the trials must be drilled at final planting distance plus 7% additional seed. Varieties with a high emergence rate are thinned to maximum 100.000 plants/ha for very early/early varieties and maximum 95.000 plants/ha for mid-early/mid-late varieties.

It is important to choose the time of thinning carefully. If the plants are thinned while they are too small, there is a high risk that many plants will be broken above the growing point so that they will re-grow at a later stage. If the plants are thinned too late, competition may occur in the row which leads to excessive damage to the root system of the remaining plants.

It is also important not just to thin the central rows (row 3 and 4) but also to accurately thin rows two and five. Rows one and six can be drilled directly at the final planting distance.

Very early/early plots and varieties with a plant population of less than 85.000 plants/ha and mid-early/mid-late varieties with a plant population of less than 81.000 plants/ha are discarded. In this approach, all very early/early varieties will have a plant population between 85.000 and 100.000 plants/ha and all mid-early/mid-late varieties will have a plant population between 81.000 and 95.000 plants/ha. The minimum number of trials of a variety should be 3 out of 7 and the minimum number of plots should 6 out of 14.

No other comparative maize variety trials must be sown on fields used for VCU. Any other comparative variety trials must have a separate entrance. Non-applicants are not permitted to visit the trials.

2.5. Trial log

The identity of the varieties will not be announced publicly during the VCU testing (to avoid undesirable visits to the trials). Applicants will only be given the codes of their own varieties and the A and N varieties from the Recommended List.

3. Observations and measurements during the growing season (forage maize)

In variety trials of forage maize, the following observations and measurements are performed. All replicates must be assessed, even though only the two central rows are used per plot. When scores are given, a high score indicates a favourable assessment of the characteristic involved. In principle, the varieties are scored using "9" for the best variety and "1" for the worst variety in the trial concerned.

All those involved can report any problematic trials to the chairman of the Technical Committee of Maize at Plantum NL, or to the secretariat of Plantum NL. If problems are noted (problem trials) a committee consisting of applicants, experts from the Plant Variety Board and the Trials Coordinator will visit the problem trial. They will advise, or take a decision, to either approve or reject the trial in question. During this visit, Plantum NL must be represented by the chairman of the Technical Committee of Maize as well as by two Plantum representatives from the VCU Working Group on Forage and Grain Maize. Other applicants may join the group.

In case of lodging at a later stage (around harvest) the Trials Coordinator should inform the Plant Variety Board and the chairman of the Technical Committee of Maize of Plantum, who can decide then whether it makes sense to harvest the trial. If it is decided to harvest such trial indeed, the trial should be harvested within one week.

3.1. Plant population

The plant population at each trial is assessed after emergence.
The emergence percentage is based on the amount of seed sown per plot. Plots where emergence deviates by more than 10% from the target plant population of 9.5 plants per m^2 after thinning must be discarded. If the cause is poor seed quality, a variety can be withdrawn from testing before the crop starts to flower. This also applies to varieties that have already been approved for marketing. There should preferably be two plots per variety per trial, but if a plot is discarded (in a trial with a cv < 4) the trial can still be considered valid.
If the number of plants is too low, the distribution of plants within the row is usually also poor.

If in this case more than 20% of the plots are discarded, the entire trial is rejected.
If individual plots are discarded, these plots must be re-seeded in good time.

3.2. Earliness of ground cover

Realising the highest possible yield requires a crop with early ground cover. The seed quality sometimes greatly influences the earliness of ground cover. The variety itself also plays a clear role. Varieties that develop quickly achieve full ground cover sooner than slower developing varieties.
The scores for the earliness of ground cover are estimated in the period around mid-June. Rapid ground cover is understood to mean the extent of ground coverage as well as the extent of leaf mass area. This observation is based on the central 4 rows in a plot.

3.3. Sensitivity to cold in the early summer (optional)

Low temperatures can influence crop growth. In some years, periods of cold weather can still occur in June. This causes certain varieties to exhibit severe yellowing. Depending on the duration and intensity of the cold weather, certain cold-sensitive varieties may show yields lower by a few percent in these years.
The sensitivity to cold of the varieties is rated as a score. The extent of yellowing of the youngest leaves is the main factor that determines the order of categorisation. This yellowing in response to cold weather in June should not be confused with the yellowing that sometimes occurs in a much earlier stage shortly after emergence (early vigour). To establish the sensitivity to cold of the varieties on average a quarter of the fifth leaf must be visible on the plants. This observation is based on the central 4 rows in a plot.
The ability to rate this characteristic demands a high degree of alertness. The yellowing often disappears within one or a few days as the temperature begins to rise.

3.4. Earliness of female flowering

At a less mature stage, and therefore at low dry matter contents, there is a good relationship between the time of flowering and the dry matter content. This correlation decreases as the dry matter content increases. Late flowering varieties have to catch up on early flowering varieties with regard to the dry matter content. This is usually achieved in years with early flowering and favourable conditions for ripening.
However, in conditions where reaching a high dry matter content is difficult, late flowering varieties often have a disappointing dry matter content.

The earliness of female flowering is determined on two trials by regularly counting the number of flowering plants in the two central rows per plot. The plant is considered to be flowering if the silks are just visible (0.5 cm). This method is used to determine the day on which 50% of the plants have visible silks (median female flowering date). Once this day is identified, the plants on the plot concerned no longer need to be counted. The median female flowering date is expressed per plot as the number of days after sowing.

On average, very early flowering varieties flower approximately:
94 days after sowing at a cumulative degree days (CDD) calculation of 150 in May (base temperature 6°C);
86 days after sowing at a CDD calculation of 200 in May;
78 days after sowing at a CDD calculation of 250 in May.
The medium-early varieties flower on average eight days later.

3.5. Drought (optional observation)

Irrigation is not common practice in variety trials of forage maize.
Observing drought symptoms (per plot) can sometimes be helpful for a correct judgment and analysis of the trial data. Observation of symptoms of drought can therefore be registered in the trial log. In periods of drought, the response of the varieties can be recorded once a week by rating the response as a score. A score of "9" indicates "no influence of drought" and "1" indicates "severe influence of drought", relative to the extent of curled or dead leaves. Only the 4 central rows of a plot are used for observation.
The external, visible response of varieties to drought often varies widely. The upper leaves of certain varieties normally stay green and lush for a long time, while their lower leaves quickly start to senesce in periods of drought. There are also varieties with leaves that all stay relatively green; however, the leaves on these varieties will start to curl strongly at an early stage.
The influence of drought on yields greatly depends on the time and duration of the drought period. If the drought is brief, the varieties that respond quickly may lag relatively behind in yield, however during prolonged periods of drought the quick protection response of these varieties may sometimes mean that they perform relatively better than varieties that initially used more moisture.

It is therefore important to record the response of varieties to drought at different times. For a correct interpretation of the data from the various trials, the score must be accompanied by an indication of the duration of the drought shown as the date on which the first symptoms of drought became visible and the date on which sufficient rainfall was recorded again.

3.6. Tillering (optional observation)

The extent of tillering is not only influenced by the plant population and the growing conditions, but is also variety-dependent. One or more side shoots start to sprout next to the main shoot. This development is usually detrimental to the earliness of the plant and the relative size of the ear. Side shoots longer than 50 cm are considered as tillers. The percentage of tillering plants is calculated after all tillers have been counted. The extent of tillering can also be estimated visually and expressed as a score. This observation is based on the central 4 rows in a plot.

3.7. Plant length and ear height

Plant length and ear height are observed on two sites. The plant length indicates the variety type. The ear height is important in less frequent cases, whereby autumn lodging is caused through weak stems. The green stems break or buckle (not caused by stem rot), at around one metre above ground level, often caused by strong winds. This type of lodging mainly occurs in varieties where ears grow high or very high up the stem.
Plant length is measured per plot and expressed in centimetres. The plant length in the plot is determined in the two central rows. A telescopic measuring stick with horizontal bar can be used to gain a good estimation of plant length. The length is measured from ground level to the tip of the tassel. The average height of the ear can also be estimated using the calibration marks on the lower part of the measuring stick. The height of the node of the highest positioned ear should be used.
The easiest way to perform this observation is to use two people. One person uses the measuring stick among the plants on the plot while the second person stands on the pathway and gives instructions on how to adjust the measuring stick. The second half of August is the best time for measurements. All varieties will have reached their final length by this time and no problems with stalk rot or lodging will usually be apparent.

3.8. Summer lodging

Explanatory statement	Weak roots can sometimes cause plants to lodge around the time of flowering. Plants usually recover from summer lodging. This does however sometimes result in characteristic “curved stalks” that leave a longer stubble at harvest Varieties that show summer lodging are assessed by estimating the number of lodged plants and scoring on a scale of 2-9.
observation:	Score per plot in rows 2-5, within 7 days after lodging Summer lodging is considered to be plants bent over from the roots at an angle greater than 45° from the vertical
score per plot:	Assessment on a 2 to 9 scale most severely affected variety 2, mildest affected variety 9
score per trial:	If distributed evenly over the trial Average of the scores per plot
annual score:	Average of the scores per trial With 30% or more of the varieties > 2% lodging: I trial suffices With 15-30% of the varieties > 2% lodging: 3 trials suffice (2 trials with grain maize) With 10-15% of the varieties > 2% lodging: 5 trials suffice (3 trials with grain maize), whereby the same varieties show lodging in the various trials Trials with less than 10% of the varieties >2% lodging are not included in the annual score. The annual average of the standard varieties must be in line with their scores in the Recommended List.
Multi-year score:	Average of the annual scores At least 3 trials in at least 2 years
Recommended List score	Convert multi-year score based on existing Recommended List scores

3.9. Lodging

Explanatory statement	Due to capacity losses at harvest, more than 5% of lodged plants is generally considered to be a problem. The stability of the plants is observed as close as possible before harvesting. When counting the incidence of lodging, take care not to include any plants that have lodged caused by stalk rot only. Plants that show clear signs of stalk and root weakness and have been infected with stalk rot are included in the count for both characteristics. The extent of lodging can also be estimated visually on a scale of 1-9, with the absolute extent of lodging being indicated by counting plots with severe lodging. With visual estimation, lodging caused by stalk rot must also be separated from other causes of lodging.
	Plants are counted as lodged if: a. they are bent over from the roots at an angle greater than 45° from the vertical; b. show "curved stalks" that re-grow upright more than 20 cm next to the row; c. (green) plants that are broken or buckled without being infected by stalk rot.
observation:	percentage per plot counted in rows 2- 5
score per plot:	If more than 2% of the plants per plot is lodged Assessed on a scale from 2 (highest %) to 9 (lowest %)
score per trial:	If distributed evenly over the trial Average of the scores per plot

annual score:	Average of the scores per trial With 30% or more of the varieties > 2% lodging: I trial suffices With 15-30% of the varieties > 2% lodging: 3 trials suffice (2 trials with grain maize) With 10-15% of the varieties > 2% lodging: 5 trials suffice (3 trials suffice with grain maize) whereby the same varieties show lodging in the various trials
	Trials with less than 10% of the varieties >2% lodging are never included in the annual score. The annual average of the standard varieties must be in line with their scores in the Recommended List.
Multi-year score:	Average of the annual scores At least 3 trials in at least 2 years
Recommended List score	Convert multi-year score based on existing Recommended List scores

3.10. Stalk rot

Explanatory statement	Stalk rot (caused by Fusarium spp.) mainly occurs in plants with declining vitality. Maturity, drought, frost or serious lodging can exacerbate the infection. The symptoms of the disease are a decaying base of the stalk, drooping ears and buckling of decaying stalks that often lodge in different directions. With dry matter contents below ± 28%, stalk rot will not usually cause major problems. The point on the stalk where fusarium infection appears can vary greatly from year to year. In some years, the lower node will mainly decay, while in other years the plants will tend to buckle at or between the second and third node on the stalk seen from the base. There are also years in which the stalks will only be mildly infected but a lot of drooping ears will be seen due to decaying ear shanks. Sometimes plants may die within a few days. One of the first symptoms is usually dull greyish-green discolouration of the leaves.
	The extent of infection by stalk rot is determined just before harvest. All plants with decayed nodes or internodes are counted. To determine the extent of infection, all plants are pushed to one side using some force at a height of approx. 80 cm above ground level. Plants with decayed stalks will buckle. Sometimes green (usually thin) stalks will buckle too. However, the difference with stalk rot is fairly plain to see. The greener colour of the stalk, the sharp snap when the stalk buckles as opposed to the "dull" sound heard when a decayed stalk collapses and the leakage of sap clearly differentiate these plants from those infected by stalk rot. Any decayed plants that have already collapsed must also be counted. The number of infected, decayed plants is expressed as a percentage of the plot population.
observation:	percentage per plot counted in rows 2 and 5 after plants have been pushed
score per plot:	If more than 2% infection per plot: assessed from 2 (highest %) to 9 (lowest %) corrected for earliness - forage maize only
score per trial:	If distributed evenly over the trial Average of the scores per plot
annual score:	Average of the scores per trial With 30% or more of the varieties > 2% stalk rot: I trial suffices With 15-30% of the varieties > 2% stalk rot: 3 trials suffice (2 trials with grain maize). With 10-15% of the varieties > 2% stalk rot: 5 trials suffice (3 trials with grain maize), whereby the same varieties show lodging in the various trials
	Trials with less than 10% of the varieties >2% stalk rot are never included in the annual score. The annual average of the standard varieties must be in line with their scores in the Recommended List. Trials harvested at a time when the average dry matter content of the 4 standard varieties, which are used to determine the moment of harvest (see appendix), is more than 38% are not used for stalk rot determination if the average percentage of stalk rot of the A and N listed varieties is above 10%

Multi-year score:	Average of the annual scores At least 3 trials in at least 2 years
Recommended List score	Convert multi-year score based on existing Recommended List scores

3.11. Common smut

Explanatory statement	Maize plants can become infected by common smut (*Ustilago maydis*) at every stage of development, as soon as the pathogen invades meristematic tissue. Primary infection in spring originates from the soil. Injuries due to frit fly, hail or mechanical cultivation can create a port of entry for common smut infection. The disease is prevalent in warm summers. Infection can sometimes manifest in patches. Infected plants are counted just before harvest. Plants with common smut in the ear as well as plants with any symptom of common smut on the stalks should both be counted as infected (with grain maize only observe and count common smut in the ear). If the differences are evident, preferably record ear and stalk infections separately.
observation:	percentage per plot counted in rows 2- 5
score per plot:	If more than 1% infection per plot: from 2 (highest %) to 9 (lowest %)
score per trial:	If distributed evenly over the trial Average of the scores per plot
annual score:	Average of the scores per trial With 30% or more of the varieties > 1% common smut: I trial suffices With 15-30% of the varieties > 1% common smut: 3 trials suffice (2 trials with grain maize) With 10-15% of the varieties > 1% common smut: 5 trials suffice (3 trials with grain maize) whereby the same varieties show common smut in the various trials.
	Trials with less than 10% of the varieties >1% common smut are never included in the annual score. The annual average of the standard varieties must be in line with their scores in the Recommended List.
Multi-year score:	Average of the annual scores At least 3 trials in at least 2 years
Recommended List score	Convert multi-year score based on existing scores in the Recommended List

3.12. Helminthosporium

Explanatory statement	Along the edges, or in headlands, highly susceptible varieties (very early and medium) are included to assess if there is a sufficient level of infection.
	Make observations at several moments during the season, depending on the moment the first infection was noticed. Observe every two weeks, from the moment of flowering, or from the moment infection was noticed (if the susceptible standard variety shows sufficient symptoms of infection). Continue observations until harvest, provided there is enough green foliage. Include all lesions (specks and spots) caused by *Helminthosporium* in one observation and note the extent to which each infection was caused by each fungus.

observation: 9 8.5 8 7.5 7 6 5 4 3 2	score (on a 2-9 scale) per plot in rows 2- 5 no spots 1-2 spots per plot up to 5 spots up to 10 spots per plot > 10 spots per plot > 20 spots per plot spots on half of the plants spots on every plant 25% of crop dead 50% or more of crop dead Half points on the scale of 2-9 may be used if whole points do not correctly reflect the actual observation.
score per plot:	Only include observations if at least 25% of the varieties in the trial are "sufficiently" infected. The definition of "sufficient" is left to the expertise of the Observer alias Data Handling Operator. The progress of the infection over time is summarised using the AUDPC (Area Under Disease Progress Curve). The final score is calculated based on this technique.
score per trial:	With even distribution over the trial Average of the scores per plot
annual score	Average of the scores per trial Only include trials where at least 25% of the varieties in the trial are, on average, "sufficiently" infected.
Multi-year score	Average of the annual scores At least 3 trials in at least 2 years
Recommended List score	Convert multi-year score based on existing scores in the Recommended List.

3.13. Green snap

Explanatory statement	Green snap refers to stalk breakage, often on a stalk node. The plant has completely broken away from the remaining stubble, so there is generally no longer any connection between the two parts of the plant. Breakage with the snap higher than two nodes or more above the ear is not included. Observe before flowering (otherwise it will be recorded as final lodging)
observation:	percentage per plot counted in rows 2- 5
score per plot:	if more than 2% per plot on a scale from 2 (highest %) to 9 (lowest %)
score per trial:	If distributed evenly over the trial Average of the scores per plot
annual score:	Average of the scores per trial With 30% or more of the varieties > 2% green snap: I trial suffices. With 15% or more of the varieties > 2% green snap: 3 trials suffice (2 trials with grain maize). With 10% of the varieties > 2% green snap: 5 trials suffice (3 trials with grain maize).
Multi-year score:	Average of the annual scores At least 3 trials in at least 2 years
Recommended List score	Convert multi-year observation score into score to be used in the Recommended List *[this issue is* still *being debated among stakeholders]*

3.14. Eyespot

Explanatory statement	Observe every two weeks from the moment the infection was noticed. Continue observations until harvest, provided there is enough green foliage left.
observation: 9 8.5 8 7.5 7 6 5 4 3 2	score per plot in rows 2- 5 Score on a scale from 2-9 depending on the % of infected leaf area, most severely affected variety 2, mildest affected variety 9 no spots some spots on top leaf some spots on upper 2-3 leaves numerous spots on top leaf numerous spots on 2-3 top leaves spots on upper 25% of plant spots on upper 50% of plant top leaf dead upper 25% of crop dead 50% or more of crop dead Half points on the scale of 2-9 may be used if whole points do not correctly reflect the actual observation
score per plot:	Only include trials where at least 25% of the varieties in the trial are, on average “sufficiently” infected. The definition of “sufficient” is left to the expertise of the Observer alias Data Handling Operator. The progress of the infection over time is summarised using the AUDPC (Area Under Disease Progress Curve). The final score is calculated based on this technique.
score per trial:	with even distribution over the trial Average of the scores per plot
annual score:	Average of the scores per trial Only include trials where at least 25% of the varieties in the trial are, on average, “sufficiently” infected.
Multi-year score:	Average of the annual scores At least 3 trials in at least 2 years
Recommended List score	Convert multi-year observation score into score to be used in the Recommended List *[this issue is* still *being debated among stakeholders]*

3.15. Maize Head Smut

Varieties to be tested on the specific Maize Head Smut trials are compared with a susceptible standard variety and with a maximum of 5 best standard varieties (i.e. varieties with less than 1% of infection). The level of infection of the trial (or year) is established based on the susceptible standard variety.

All varieties from the second year of VCU testing are included in the two Maize Head Smut trials and subsequently tested for 3 years. Fields used for these trials must be (heavily) infested as regular as possible. Varieties are clustered in separate blocks per crop (forage maize or grain maize) and per group of earliness (forage maize). Varieties are sown at final planting distance, with a target population of 10 plants per m^2, in three replicates and plots consisting of 2 rows of 9 metres length.
Trials should be planted before 15 May. The number of plants is established per plot after emergence. In years with a low natural disease pressure irrigation can be considered.
The number of infected plants is counted around 1 September. Plants with symptoms of infections in the cob or in the tassel are counted as infected plants. The percentage of infection is calculated per plot. This percentage is analysed further into an annual percentage and a multi-year percentage.

Varieties in the second year of VCU testing which, after one year of Head Smut testing, have a real chance to be included on the positive list after 2 years of Head Smut testing, are tested for Head Smut again together with the third year of VCU testing. Varieties without prospect are not tested again to avoid unnecessary costs.

3.16. Other pests/diseases (optional)

Possibly observe at several times during the season, depending on the moment of infection. Score on a 2-9 scale depending on the % of infection, most severely affected variety 2, mildest affected variety 9. Only observe if infection is distributed evenly over the trial (sometimes only a small patch/corner is infected). Only include observation if 25% of the varieties in the trial are “sufficiently” infected. The definition of “sufficient” is left to the expertise of the observer.

4. Harvesting forage maize

4.1. Time of harvest

Taken as a multi-year average, the optimal time to harvest forage maize is at the grain firm dough stage and a 34-36 % dry matter content (DM%) in the whole plant. To determine the moment of harvest, four standards are designated in each group (see appendix) which are sown in discard plots at the border ends of the trial. During the ripening phase, these varieties are regularly sampled and analysed to determine the DM%. The progressive development of the dry matter over time is a guideline for the optimal moment of harvest, with a focus on the following points:

- The desirable range within which the average of the four standards must fall is 34-36% DM.
- If harvested before 10 October the average DM% of these four standards must be at least 32% and maximum 38%, otherwise this trial site will be excluded from further data analysis (with the exception of exceptional circumstances and *force majeure).*
- If a plot is harvested with a DM% below 32% or above 38%, the Plant Variety Board will determine to which extent the average result of that year is distorted by a "too early" or "too late" harvest. The correlation of the trial in question to the other trials will be leading, with particular attention being paid to the characteristics yield and feed value.
  Fusarium (stalk rot) observations of these trials are not taken into account..
- After 10 October harvest must be planned as soon as possible according to the expertise of

the Trials Organiser.

From a practical perspective, it is advisable to drill the rest of the field with a very early/early variety. Clear agreements regarding the harvest must also be made with the Trial Operator. The ripening must be closely monitored on each trial site. In various years, the dry matter content can suddenly increase very sharply towards the end of the growing season so the initial harvest planning may have to be altered to avoid harvesting maize with a too high DM content.

4.2. Determination of the yield

The plots are harvested using a one- or two-row precision chop machine. To determine the plot fresh yield, the total chopped product from the two central rows is weighed. State the harvested plot weight and also state the net plot area of the harvested rows. The entire trial should preferably be harvested in a single day. If circumstances prevent this, harvesting activities should stop as far as possible on the boundary between two replicates, or possibly on the edge of a sub-block. All plots must be cut to a height of 10 cm. The coefficient of variation (CV) of the dry matter content and the dry matter yield in the various trials is in the order of magnitude of 4 - 5 % (in years with favourable conditions). As a guideline, at a CV below 4 the trial is considered valid. At a CV above 6, the trial is considered invalid and rejected in principle. If a CV is between 4 and 6, the validity of the trial will be discussed in the meeting between the Plant Variety Board and the Trials Coordinator.

4.3. Sampling

To determine the various characteristics, a sample of the total chopped product is taken per plot. Taking a good, representative sample of the chopped forage maize is difficult, as the product is heterogeneous, and may have already started to separate in the cyclone of the chopper. There is a great risk, particularly with high DM contents, that the sample will not contain the correct proportion of stalk and ear. A good mechanical sampling method is preferable. The entire flow of chopped product must be sampled per plot. The size of the sample is approximately 1 kg of fresh mass product. Mechanical sampling is standard for the trials.

Manual sampling may be necessary as an emergency action. In that case, one person must take the samples for the entire trial. If several people take the samples, significant differences may occur among the samples mutually, due to differences in the method of sampling.
In the case of manual sampling, taking a large sample of 15 kg from below the cyclone first is recommended. This sample is then spread out flat on a table. Smaller samples are taken from various segments of the material to create the final sample size of at least 1000 g.

The sample should be weighed immediately after sampling. The samples must be dried on the same day as sampling (e.g. air dried on a drying floor) without being stored in the meantime. If logistics do not permit this, the samples must be taken to cold storage as soon as possible. Leaving the samples in the field in direct sunlight must absolutely be avoided. Samples that are also used for digestibility must not be dried at a temperature above 70 °C.

For further details on sampling and drying, see:
Deutsches Maiskomitee e.V., AG Züchtung, 1996. Richtlinien für die Probenahme, Probenaufbereitung und Probentrocknung von Silomaisganzpflanzen für Qualitätsuntersuchungen mit der Nah-Infrarot-Reflektionsspektroskopie (NIRS).

4.4. Dry matter content

To determine the dry matter content, the samples must be processed as soon as possible to avoid losses caused by respiration. Respiration must be halted as soon as possible by drying the samples. Samples can start heating, particularly if the sample is dried as a compact mass. If immediate analysis is not possible, the samples must be taken to a cold store as soon as possible. The dry matter content is determined for each separate plot on all the trials (according to method in appendix 2). The complete sample (see 4.3) must be dried. The earliness of a variety is determined using the (multi-year) average DM content of all trials.

4.5. Digestibility

Digestibility (digestibility coefficient of organic matter) is determined using near-infrared spectroscopy analysis (NIRS) (see Internal Report of the Institute for Livestock Feeding and Nutrition Research, IVVO Report no. 177 for a description of the method). To calculate the VEM (feed units of lactation) the ash content is also determined by ashing.
See Appendix 2 for VEM calculation.
In principle, NIRS determination is performed on all trials. Digestibility is determined for each separate plot. After the dry matter content (incl. residual moisture determination, see appendix 2) has been determined, the samples must be ground in their entirety and mixed before they are divided into sub-samples for the digestibility determination. All the samples from the same trial must be tested for digestibility in a single run.

4.6. Starch content

When the digestibility of samples from trials is determined, the starch content must also be determined. The starch content is determined using near-infrared spectroscopy analysis (NIRS) as used by BLGG (Farm Laboratory for Soil and Crop Research – now) for applied research (see appendix 2).
The starch content gives greater insight into the nature and composition of the crop, which also enables better assessment of the value of digestibility figures.

5. Examination of varieties of Grain Maize and CCM (Corn Cob Mix)

The same system is used for grain maize and CCM as for forage maize: As the observations overlap, grain maize and CCM are assessed in the same trial. CCM is therefore examined in the same way as grain maize. The varieties suitable for grain maize are usually also suitable for CCM.

5.1. Trial seed

All seed submitted for the trials must be disinfected with a fungicide (Redigo M). The seed used in the trials is distributed by the Trials Organiser.

First year official trials
The applicant must submit 11 bags each containing 800 seeds of each variety admitted to the trials to the Trials Organiser no later than 15 March. The number of bags equals the number of plots plus two extra bags (for storage).

Second/third year trials and subsequent trials
The applicant must submit 11 bags each containing 800 seeds of the second and third year variety to the Trials Organiser no later than 15 March. 11 bags each containing 800 seeds are also necessary for the standard varieties (A and N varieties from the latest Recommended List). The follow-up testing of the Recommended List varieties is performed using samples from commercial seed lots supplied directly by the breeders.

If (due to circumstances) no seed can be submitted on 15 March, the closing date for submission may be delayed until 1 April, provided this request for postponement is announced at the breeders' meeting.

5.2. Trial design

5.2.1. Official trials

The official trials are performed on four sites with two replicates per site.
Six rows are sown per plot with a distance of 75 cm between the rows. The rows must be at least 6 metres long, with the pathways kept as narrow as technically possible.
All pathways must be of identical width.
Sufficient numbers of guard rows must be sown around the trial.
All trials are carried out in complete replicates. These replicates (blocks) are sub-divided into sub-blocks of 5 or 6 plots.
The Trials Organiser sets up the trial plans. The varieties of each replicate should preferably be grown in a single lane. In case replicates need to be split, due to the local farming conditions, varieties must not be removed from their original sub-block.
Due to potential competition between the plots, only the two central rows should be used for observations and assessments.
The sites should be spread as far as possible over the main growing regions. The sites should be spread as follows:

Region	Number of sites
Southern sandy soil	2
Eastern sandy soil	2

5.2.2. Examining genetically modified varieties (GMs) of grain maize and CCM

VCU testing of genetically modified varieties (GMs) depends on the way in which the GM variety was created. Refer to EU-directive 2001/18 for a definition of derived and independent genetically modified organisms.

At the moment, a decision has been made not to test (derived) GM varieties in the regular trials. If a modified variety has not (yet) been granted commercial authorisation in the EU, trials may take place under an authorisation for the deliberate release of GMOs for any other purpose than for placing on the market (Part B release), if necessary with a permit of one or more of the applicants.

Derived GM varieties
If a GM variety is derived from an existing hybrid variety, which has completed at least the second year of trials in the Netherlands- and whereby a new characteristic has been added to the original variety through genetic modification - the testing can be limited to the extent to which the derived variety is comparable to the original variety in terms of its VCU characteristics. It is assumed that year interactions do not play a role in this comparison, meaning the testing can be completed in one year.
This comparative test examines whether the derived variety differs from the original variety, assuming the null hypothesis that both varieties are identical. As these derived GM varieties cannot be included in the regular trials, separate trials must be sown for the purpose of this comparison.

Independent GM varieties
These GMs (created independently to an existing hybrid variety) are tested in the same way as conventional (unmodified) varieties. Derived GMs of varieties that are not in the Dutch National List are also included in this category. However, similar to varieties included in the EU Common Catalogue, these varieties can enter the official trials directly. The testing is performed in accordance with the standard protocol and generally lasts 2 years for the National List and 3 years for the Recommended List. As these independent GM varieties cannot be included in the regular trials, separate trials must be sown for this purpose.

Further details will be provided when applications are received for these GM varieties. This chapter of the VCU protocol for maize therefore only discusses the examination of derived GM varieties.

VCU testing of the characteristic added through modification depends on the type of modification. Details of these tests will be established on an *ad hoc* basis at such time as an application for a new type of modification is received. A DUS report in which the modification in question has been tested and included (performed by an organisation such as GEVES) can demonstrate the modification concerned.

<u>Admission of derived GM varieties to the trials</u>

Applications for GM varieties derived of varieties that have completed at least 2 years of VCU trials can be considered.

To limit the size of these trials, admission to the trials may be limited in the following way:

- GMs based on existing varieties from the Recommended List will be given priority
- GMs based on varieties which are in the third year of testing can enter the trials as long as the maximum trial capacity is not exceeded
- In the case of varieties that are only included in the National List, admission to the trials is only possible if places are still available.

For inclusion in the National List, the GM variety is tested for one year. The null hypothesis is tested based on the results after one year (with the exception of the added characteristic). After one year of testing, the variety can be included in the National List if the GM variety is identical to the original variety. The derived GM variety can be re-tested if there is a reason for the deviation from the null hypothesis which is not caused by the GM itself (e.g. germination power / emergence). Insufficient reliability of the trial or doubt concerning the validity of the null hypothesis for one or more characteristics may be a reason to test a derived variety for a second year. If a positive decision on VCU is arrived at, but Plant Breeders' Rights have not yet been granted (due to unfinished DUS testing, for example), the derived variety does not need to be re-tested. When Plant Breeders' Rights are granted, the derived GM variety will then be included in the National List.

The examination

The distinctness of a variety is primarily determined by the UPOV characteristics that are established during the DUS testing (DUS - Distinctness, Uniformity and Stability). These characteristics are mainly morphological. For derived GM varieties, the modification (e.g. herbicide tolerance) is often added as the distinctive characteristic. The DUS report shows whether the derived GM variety is distinct for this characteristic and that all the other characteristics are identical to those of the original variety.
The purpose of a VCU test of a derived GM variety is to show there is no difference in VCU compared to the original variety, with the exception of the modification. The VCU therefore compares the varieties based on characteristics that are relevant for the value for cultivation and use of a variety.

The relevant characteristics of grain maize and CCM are:

- Emergence (Plant population)
- Earliness of ground cover
- Flowering (in relation to emergence and development)
- Plant Length (in relation to previous characteristics)
- Lodging
- Fusarium
- Harvestability (as a result of lodging and fusarium)
- Common smut
- Grain ripeness
- Fresh weight, dry matter weight and dry matter content of the grain
- Grain yield (at 16 % moisture)

These characteristics are established according to the methods described in the standard protocol.

Any distinction between the varieties is established using a Student t-statistic (two-tailed test - at 5 % probability level).
The coefficient of variation (CV) of the dry matter content and the dry matter yield in the various trials is in the order of magnitude of 3 to 6 %. As a guideline, a CV below 4 indicates a valid trial. If a CV is above 6, the trial is rejected in principle. If a CV is between 4 and 6, the validity of the trial will be discussed in the meeting between the Plant Variety Board and the Trials Coordinator. A total of 14-22 replicates are necessary to establish a significant difference of 5 % for the characteristics concerned.
Earlier studies on the reliability of variety testing reveal that the relative gain in reliability decreases with more than 3 replicates per trial. For an optimal result and to ensure the trial aligns with the extent of conventional trials, at least 4 trials in 3 replicates are necessary.

Maximum size of individual trials

The current trials of the conventional varieties of grain maize comprise a maximum of 40 varieties. Assuming these trials are sown in the same way as the trials of conventional varieties, the maximum feasible size of the trial is approximately 80 plots. For a trial consisting of 3 replicates this means:

Maximum number of varieties:	27 varieties (grain maize or CCM)
divided over GM and original varieties:	14 GMs and 13 original varieties

A trial must consist of at least 10 varieties. If there are less than 10 varieties, a selection of varieties from the Recommended List will be added (A or N varieties). The rest of the trial is performed according to the standard protocol.

Special requirements for the trial

The seed quality can greatly influence comparison of the varieties from the start. The seed lots must therefore be of comparable quality with a field emergence rate of at least 80 %. The varieties are treated using standard methods. This also applies to weed control treatments.

Special requirements for the trial of derived GM varieties

The seed quality can greatly influence comparison of the varieties from the start. The seed lots must therefore be of comparable quality with a field emergence rate of at least 80 %.
The varieties are treated using standard methods. This also applies to weed control treatments.

5.2.3. Examining derived non-GM varieties of grain maize and CCM

Derived non-GM varieties can be tested according to the standard protocol and in regular trials.

Derived non-GM varieties
If a variety has completed the second year of a testing and whereby a new non-GM characteristic compared with the original variety has been added to the derived variety (e.g. herbicide resistance) the testing can be limited to the extent to which the derived variety is comparable to the original variety in terms of its VCU characteristics.

It is assumed that year interactions do not play a role in this comparison, meaning the testing can be completed in one year. This comparative test examines whether the derived variety differs from the original variety, assuming the null hypothesis that both varieties are identical.

VCU testing of the added characteristic depends on the characteristic. Details of these tests will be established on an *ad hoc* basis. A DUS report in which the modification in question has been tested and included (performed by an organisation such as GEVES) can demonstrate the modification concerned.

Admission of derived non-GM varieties to the regular trials

Applications for derived non-GMs of varieties that have completed at least 2 years of VCU testing can be considered. The original variety and the derived non-GM variety will be included in the regular trials. Derived non-GM varieties of which the original variety is not in the Dutch National List, but has been included in the EU Common Catalogue, will be considered as independent varieties.

To limit the size of the regular trials, admission to the trials may be limited in the following way:
- derived varieties based on existing varieties from the Recommended List will be given priority
- derived varieties based on varieties which are in the third year of testing can enter the trials as long as the maximum trial capacity is not exceeded
- in the case of varieties that do not continue in the regular trials (National List), admission to the trials is only possible if places are still available.

For inclusion in the National List, the derived variety is tested for one year. The null hypothesis is tested based on the results after one year (with the exception of the added characteristic). After one year of testing, the derived variety can be included in the National List if the derived variety is identical to the original variety. The derived variety can be re-tested if there is a reason for the deviation which is not caused by the variety itself (e.g. germination power / emergence). Insufficient reliability of the trial or doubt concerning the validity of the null hypothesis for one or more characteristics may be a reason to test a derived variety for a second year. If a positive decision on VCU is arrived at, but Plant Breeders' Rights have not yet been granted (due to unfinished DUS testing, for example), the derived variety does not need to be re-tested. When Plant Breeders' Rights are granted, the derived variety will then be included in the National List.

The examination

The distinctness of a variety is primarily determined by the UPOV characteristics that are established during the DUS testing (DUS - Distinctness, Uniformity and Stability). These characteristics are mainly morphological. For derived varieties, the modification (e.g. herbicide tolerance) is often added as the distinctive characteristic. The DUS report shows whether the derived variety is distinct for this characteristic and that all the other characteristics are identical to those of the original variety.
The purpose of a VCU test of a derived variety is to show there is no difference in VCU compared to the original variety, with the exception of the modification. The VCU therefore compares the varieties based on characteristics that are relevant for the value for cultivation and use of a variety.

The relevant characteristics of grain maize and CCM are:

- Emergence (Plant population)
- Earliness of ground cover
- Flowering (in relation to emergence and development)
- Plant Length (in relation to previous characteristics)
- Lodging
- Fusarium
- Harvestability (as a result of lodging and fusarium)
- Common smut
- Grain Ripeness
- Fresh weight, dry matter weight and dry matter content of the grain
- Grain yield (at 16 % moisture)

These characteristics are established according to the methods described in the standard protocol. Any distinction between the varieties is established using a Student t-statistic (two-tailed test - at 5 % probability level).

5.3. Varieties to be tested

The official trials comprise a maximum of 120 new varieties, including standard varieties. In addition to new applications being tested, all the A (generally recommended) and N (newly recommended) varieties of the Recommended List are included in the trials.

5.4. Trial layout, Trial operations and Trial husbandry

Fields used for the trials must be as regular as possible. High, dry or wet, low fields are undesirable. Due to early sowing and late harvest, good accessibility of machinery to the fields should be ensured both early and late in the season. No trials should preferably have taken place for at least the past two years on the field concerned. In case of drained fields, the lanes must run parallel to the drains and the plots must be drilled across the direction of the drains. Treatments and cultivations must be done in the direction of the trial lanes as much as possible. Furthermore, the agronomy should follow best local practice of an average Dutch arable farm. This also holds for the preparation of the seedbed, fertilisation and for weed control.
Irrigation is only permitted if necessary to safeguard the validity of the trial. Sowing times must comply with local practice. The trials should preferably be sown between 20 April and mid-May.

From 2015 onwards, grain maize is sown with a precision drill at final planting distance.
From 2022, the trials are thinned after emergence to a maximum of 9 plants per m^2. To achieve a uniform plant population, the trials must be drilled at final planting distance plus 7% additional seed (approximately 91.500 seeds per ha).
Varieties with a 98% emergence rate or more are thinned to a maximum of 90.000 plants/ha.
It is important to choose the time of thinning carefully. If the plants are thinned while they are too small, there is a high risk that many plants will be broken above the growing point so that they will re-grow at a later stage. If the plants are thinned too late, competition may occur in the row which leads to excessive damage to the root system of the remaining plants.
It is also important not just to thin the central rows (row 3 and 4) but also to accurately thin rows two and five. Rows one and six can be drilled directly at the final planting distance.
Varieties with a plant population of less than 80.000 plants/ha are discarded. In this approach, all varieties will have a plant population between 80.000 and 90.000 plants per ha.

The desirable number of plants is 85.000 plants per hectare.

- Average emergence in recent years has been 95%, with a minimum of 92% in 2010.
- The seed rate is 92,500 seeds/ha (92% of 92,500 = 85.000).

6. Observations and measurements during the growing season (grain maize)

In variety trials of grain maize, the following observations and measurements are performed. All replicates must be assessed, but only observations of the two central rows are used per plot. When scores are given, a high score indicates a favourable assessment of the characteristic involved. In principle, the varieties are ranked using "9" for the best variety and "1" for the worst variety on the trial concerned.
Reference is made for certain observation to the protocol for forage maize in the previous chapters where the observations concerned have already been described in full.

6.1. Plant population

Each trial is assessed for emergence.
The emergence percentage is based on the amount of seed sown per plot. Plots with fewer than 8.0 plants/m^2, must be discarded from the trial. If the number of plants is fewer than 8.3 plants/m^2 caused by poor seed quality, the variety can be withdrawn from testing before flowering. This also applies to varieties that have already been approved for marketing. There should preferably be two plots per variety per trial, but if a plot is discarded (in a trial with a CV < 4) the variety can be retained and the trial can still be considered valid. There must be at least 3 sites and at least 5 plots in total per variety, except in exceptional circumstances. A variety may only miss one year of results if circumstances make it impossible to generate results for that year. If the number of plants is too low, the distribution of plants within the row is usually also poor. If in this case more than 20% of the plots are discarded, the entire trial is rejected.
If individual plots are discarded, these plots must be re-seeded in good time.

6.2. Earliness of ground cover

See 3.2 forage maize

6.3. Sensitivity to cold in early summer

See 3.3 forage maize

6.4. Earliness of female flowering

The earliness of female flowering needs to be observed on two trials. Earliness is determined by regularly counting the number of flowering plants per plot. A plant is considered to be flowering if the silks are just visible (± 0.5 cm). In this way the day is determined on which silks are visible on 50% of the plants (median female flowering date). Once this date is known, the plants on the plot concerned do not have to be counted any more. The median female flowering date is expressed per plot as the number of days after sowing.

6.5. Drought (optional observation)

Grain maize is general very sensitive to drought. Irrigation is not common practice in variety trials of grain maize. Observing drought symptoms (per plot) can sometimes be helpful for a correct judgment and analysis of the trial data. Observation of symptoms of drought can therefore be registered in the trial log. In periods of drought, the response of the varieties can be recorded once a week by rating the response as a score. A score of "9" indicates "no influence of drought" and "1" indicates "severe influence of drought", relative to the extent of curled or dead leaves. The external, visible response of varieties to drought often varies widely. The upper leaves of certain varieties normally stay green and lush for a long time, while their lower leaves quickly start to senesce in periods of drought. There are also varieties with leaves that all stay relatively green; however, the leaves on these varieties will start to curl strongly at an early stage.

The influence of drought on yields greatly depends on the time and duration of the drought period. If the drought is brief, the varieties that respond quickly may lag relatively behind in yield, however during prolonged periods of drought the quick protection response of these varieties may sometimes mean that they perform relatively better than varieties that initially used more moisture. It is therefore important to record the response of varieties to drought at different times. For a correct interpretation of the data from the various trials, the score must be accompanied by an indication of the duration of the drought shown as the date on which the first symptoms of drought became visible and the date on which sufficient rainfall was recorded again.

6.6. Tillering (optional observation)

The extent of tillering is not only determined by the plant population and the growing conditions, but is also variety-dependent. One or more side shoots start to sprout next to the main shoot. This development is usually detrimental to the earliness of the plant and the relative size of the ear. Side shoots longer than 50 cm are considered as tillers. The percentage of tillering plants is calculated after all tillers have been counted. The extent of tillering can also be estimated visually and expressed as a score.

6.7. Plant length and ear height

See 3.7 for forage maize.

Plant length and ear height only need to be observed on one trial site.

6.8. Summer lodging

See 3.8 for forage maize

6.9. Lodging

See 3.9 for forage maize

6.10. Fusarium stalk rot and ear rot

Explanatory statement	Stalk rot (caused by Fusarium spp.) mainly occurs in plants with declining vitality. Maturity, drought, frost or serious lodging can exacerbate the infection. The symptoms of the disease are a decaying base of the stalk, drooping ears and buckling of decaying stalks that often lodge in different directions. With dry matter contents below ± 28%, stalk rot will not usually cause major problems. The point on the stalk where fusarium infection appears can vary greatly from year to year. In some years, the lower node will mainly decay, while in other years the plants will tend to buckle at or between the second and third node on the stalk seen from the base. There are also years in which the stalks will only be mildly infected but a lot of drooping ears will be seen due to decaying ear shanks. Sometimes plants may die within a few days. One of the first symptoms is usually dull greyish-green discolouration of the leaves.
	The extent of infection by stalk rot is determined just before harvest. All plants with decayed nodes or internodes are counted. To determine the extent of infection, all plants are pushed to one side using some force at a height of approx. 80 cm above ground level. Plants with decayed stalks will buckle. Sometimes green (usually thin) stalks will buckle too. However, the difference with stalk rot is fairly plain to see. The greener colour of the stalk, the sharp snap when the stalk buckles as opposed to the "dull" sound heard when a decayed stalk collapses and the leakage of sap clearly differentiate these plants from those infected by stalk rot. Any decayed plants that have already collapsed must also be counted. The number of infected, decayed plants is expressed as a percentage of the plot population.
observation:	percentage per plot counted in rows 2 and 5 after plants have been pushed
score per plot:	If more than 2% infection per plot: assessed from 2 (highest %) to 9 (lowest %) corrected for earliness - forage maize only
score per trial:	If distributed evenly over the trial Average of the scores per plot

annual score:	Average of the scores per trial With 30% or more of the varieties > 2% stalk rot: I trial suffices With 15-30% of the varieties > 2% stalk rot: 3 trials suffice (2 trials with grain maize). With 10-15% of the varieties > 2% stalk rot: 5 trials suffice (3 trials with grain maize), whereby the same varieties show lodging in the various trials
	Trials with less than 10% of the varieties >2% stalk rot are never included in the annual score. The annual average of the standard varieties must be in line with their scores in the Recommended List.
Multi-year score:	Average of the annual scores At least 3 trials in at least 2 years
Recommended List score	Convert multi-year score based on existing Recommended List scores

6.11. Harvestability

Grain maize and CCM are harvested late, which means that many forms of lodging can occur, which, contrary to forage maize, cannot be avoided (by harvesting earlier). This risk is indicated by the harvestability characteristic.
Harvestability is a compound characteristic of lodging and stalk rot and covers all lodged plants irrespective of the cause.

6.12. Common smut

See 3.11 for forage maize

6.13. Helminthosporium

See 3.12 for forage maize

6.14. Green snap

See 3.13 for forage maize

6.15. Eyespot

See 3.14 for forage maize

6.16. Maize Head Smut

See 3.15 for forage maize

6.17. Other pests/diseases (optional)

See 3.16 for forage maize

7. Harvest of grain maize

7.1. Time of harvest

The trials are harvested as grain maize. The optimal time of harvest for grain maize is when the kernels are ripe and the ear has the highest possible dry matter content. At present very early varieties that are, in principle, suitable as grain maize, are combined in one trial with later varieties with less or no suitability as grain maize. Harvesting takes place at the end of October. All trials must be harvested before 8 November: all trials still unharvested at that point must be harvested then, even if the target dry matter content has not yet been reached.

7.2. Yield determination: grain yield

The trials are harvested using a combine adapted for use on trials. If such a machine is unavailable, the trial is harvested manually.
Only the two central rows are harvested per plot.
State the harvested plot weight and also state the net plot area of the harvested rows.
The entire trial should preferably be harvested in a single day. If circumstances prevent this, harvesting activities should stop as far as possible on the boundary between two replicates, or possibly on the edge of a sub-block.
If the plot is harvested manually, the husks around the ear are removed.
With manual threshing, after all the harvested ears have been weighed, a sample of 20 ears can be threshed per plot. The total grain yield is calculated based on the weight of this sample.
A plot thresher is used to thresh the ears. The plot fresh grain yield is then determined.
With mechanical harvesting, depending on the system, the plot fresh grain yield is determined immediately at harvest.
A sample weighing at least 200 g is taken to determine the dry matter content of the grains. The grain yield is converted at a later stage into a grain yield with 16 % moisture.
The coefficient of variation (CV) of the dry matter content and grain yield in the various trials is in the order of magnitude of 4-5 % (in years with favourable conditions). As a guideline, at a CV below 4 the trial is considered valid. At a CV above 6, the trial is considered invalid and rejected in principle. If a CV is between 4 and 6, the validity of the trial will be discussed in the meeting between the Plant Variety Board and the Trials Coordinator.

7.3. Dry matter content of the grain

The dry matter content of the grains is determined by drying the grains at 103 °C for 48 hours.
The dry matter content of the grains is expressed as a percentage of the fresh product.
The dry matter content is determined of all the plots of all trials.

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Annex 2 Determinations and data handling

Where applicable, reference is made to the applicable NEN regulations and EU directives or to the publication describing the method.

Cumulative degree days

This is a figure that characterises the average temperature over a certain period of time. Cumulative degree days (CDDs) is defined as the sum of the mean daily temperatures (average of daily maximum and minimum temperatures) above a certain base temperature of 6°C.

Calculation daily average: (Tmax+Tmin)/2 + 6

Dry matter content (DM %)

Air-dry matter.
The sample to be weighed must be large enough to ensure that approx. 150 to 200 g of air-dry matter remains in order to perform the required determination (digestibility, starch content, crude ash content). The sample is dried at 70°C. The sample is re-weighed after conditioning. The total drying time depends on the moisture content and the nature of the material (NEN 3328).

Residual Moisture

A weighed amount of ground, air-dry material is heated at 103°C for four hours.
The reduction in weight is residual moisture.
The absolute dry matter = air-dry matter minus residual moisture (NEN 3332, PB L 279/71).
The dry matter content is expressed as a percentage of the fresh weight.

Crude ash content (CA)

An amount of weighed, ground, air-dry material is ashed at 550°C for three hours, after which time the residue is weighed. (NEN 3329, PB L 155/71).
CA is expressed in grams per 1000 g dry matter.

Starch content determination (NIRS analysis)

NEN standard 3574 applies to the determination of the starch content using
Near-infrared spectroscopy.
Starch content is expressed in grams per 1000 g dry matter.

Digestibility coefficient of organic matter (DCOM)

NIRS analysis (calibrated on Tilley and Terry method).
The DCOM is estimated based on the NIR spectra of a set of calibration samples, which are analysed according to the reference methods.
The method is described in Internal Report IVVO, no. 177.
(IVVO = Institute for Livestock Feeding and Nutrition Research – now part of Wageningen UR).
The DCOM is expressed as a percentage of the organic matter.

VEM calculation

The nutritional value is expressed in VEM - feed units of lactation - the Dutch parameter indicating the net energy for lactation per 1000 g dry matter. One kVEM approximates to the net energy value of 1 kg barley.

According to the Manual of Calculating the Feeding Value of Roughages (*Centraal Veevoeder Bureau*) VEM is calculated as follows:

OM	=	1000 – CA
DOM	=	OM * DCOM/100
ME	=	3.7 * DOM
GE	=	4650 – (4.650 * CA)
Q	=	100 ME/GE
VEM	=	(0.00142q + 0.27376) * ME

OM	=	organic matter
CA	=	crude ash
DOM	=	digestible organic matter
DCOM	=	digestibility coefficient of organic matter
ME	=	metabolisable energy
GE	=	gross energy
Q	=	% ME in GE (metabolisability of gross energy)
VEM	=	feed units of lactation

Earliness classification of forage maize and indexation level Recommended List 2023-2024

To monitor the limit between very early/early and medium early/medium late, two medium early standard varieties are sown in the very early/early group, and two early standard varieties in the medium early/medium late group. The following varieties have been designated for this purpose:

Earliness group	**Standard variety**	**Standard variety**
Very early/early	Privat	Farmodena
Medium early/medium late	Genialis KWS	P83224

Recalculation limit values:

The limit values are recalculated according to the methods used to calculate the earliness groupings from 2001 to 2011: the relative values are corrected every year depending on the year effect and the new varieties included in the relative value defining 100 = level.

- The A and N varieties in the very early and early group on the Recommended List 2012 determine which relative value the limit value of 34.4635% in the very early/early group is allocated and therefore also the relative value of the extreme lower limit of 34.4635% - 0.25% = 34.7135%. At the end of 2016, a decision was made to increase this lower limit by 0.3% (absolute) commencing with the Recommended List 2018.
- The A and N varieties in the medium early / medium late group of the Recommended List 2012 determine which relative value the limit value 34.4635% in the medium early/ medium late group is allocated and therefore also the extreme upper limit of 34.4635% + 0.75% = 35.2135%.

Based on the four identical standards that are sown in both groups (two from the very early/early and two from the medium early/medium late group) the recalculations above (lower limit from very early/early and upper limit of medium early/medium late) will be examined annually to see whether they correspond mutually and with the original limit value The protocol will be modified if necessary.

Harvest reference varieties:

To determine the moment of harvest, the following varieties are sown (on the discard plots):

Earliness group	**Ref. var**	**Ref. var**	**Ref. var**	**Ref. var**
very early/ early	Privat	Farmodena	LG 31.205	Papageno
medium early/medium late	Genialis KWS	DKC3323	EC Gisella	P83224

Forage maize Early - Correction factor earliness limit from 2024 to 2025, for Recommended List 2026

Based on A/N varieties of Rec. List 2025, the 6-year average in 2024 will be compared with 6-year average in 2025.

Level difference will be corrected.

A/N varieties RL 2025 - very early / early group

	dm%2024	100=2024	rel.dm%		dm%2025	Corr fact.		2024abs			2025abs	rel		
LG31211	38,8558	37,4763	103,681		101,9668938		LG31211	38,8558		LG31211	39,29233333	102,7555033	A	
LG31205	38,6955		103,253		103,7697772		LG31205	38,6955		LG31205	39,27683333	102,7149684	A	
LG31214	37,3955		99,784		98,06544971		LG31214	37,3955		LG31214	37,5825	98,28402575	A	
Papageno	39,4897		105,372		102,997177		Papageno	39,4897		Papageno	40,2345	105,2194142	A	
Benco	38,0097		101,423		97,74527708		Benco	38,0097		Benco	38,58266667	100,8996156	A	
Emeleen	38,1683		101,847		94,00696097		Emeleen	38,1683		Emeleen	38,40016667	100,42235	A	
KWSJohaninio	38,0207		101,453		101,3304091		KWSJohaninio	38,0207		KWSJohaninio	38,56219346	100,846075	A	
LG31206	38,8817		103,750		99,5518701		LG31206	38,8817		LG31206	39,28533333	102,7371972	A	
Revelation	36,4935		97,378		91,82764959		Revelation	36,4935		Revelation	37,03363559	96,84866079	N2	
Cheerful	36,1765		96,532		105,7006097		Cheerful	36,1765		Cheerful	36,56083559	95,61221598	N2	
Clipperton	35,74219426		95,373		101,2587153		Clipperton	35,74219426		Clipperton	35,81432424	93,6599738	N1	
					95,86371771			37,8117			38,2387			
					101,9201906									
					99,15423719									
					101,2188831									
					103,6221818									
										100=	38,2387	100		
			100,895		100,000	0,99113								

New limit to assess correct earliness grouping of Rec. List 2026

Multiply relative limit value Rec. List 2025 by 0.99113

								+0.3			2020	2021	2022	2023	2024	2025
	RL2012	RL2013	RL2014	RL2015	RL2016	RL2017	RL2018	RL2018	RL2019	RL2020	RL2021	RL2022	RL2023	RL2024	RL2025	RL2026
100=	36,003	36,38	36,25	36,07	35,56	36,49	37,09	37,09	37,27	37,18	36,364	36,849	36,898	37,428	37,476	38,2387
grensabs	34,464	34,23	34,28	34,25	33,72	34,48	34,83	35,13	35,25	35,25	34,764	34,821	34,873	35,088	35,124	35,521
grensrel	95,724	94,09	94,57	94,95	94,85	94,50	93,92	94,73	94,59	94,82	95,601	94,498	94,513	93,749	93,723	92,89157575
grens -0.25%		32,73	32,78	32,75	32,22	32,98		33,63	34,50	34,50	34,014	34,071	34,123	34,338	34,874	35,2705
from RL2025								From 2018 (RL 2019) absolute limit – 0.75							From 2024 absolute limit	

Forage maize late - Correction factor earliness limit from 2024 to 2025, for Recommended List 2026

Based on A/N varieties Rec. List 2025, the 6-year average in 2024 will be compared with 6-year average in 2025.

Level difference will be corrected.

A/N-varieties Rec. List 2025 - medium early/ medium late group

Status RL2025	dm%2024		100=2024	rel dm%		dm%2025		A-NrassenRL2025				abs2025	rel
A	Farmoritz	35,1	36,040	97,5		96			abs2024		Farmoritz	35,0	96
A	Farmueller	36,0		100,0		100		Farmoritz	35,1		Farmueller	36,5	100
A	ECGisella	34,8		96,4		96		Farmueller	36,0		ECGisella	35,2	96
A	Greatful	37,0		102,7		102		ECGisella	34,8		Greatful	37,4	102
A	P8153	37,1		102,8		103		Greatful	37,0		P8153	37,6	103
A	Glutexo	36,3		100,6		100		P8153	37,1		Glutexo	36,4	100
A	Gwendoleen	37,3		103,6		103		Glutexo	36,3		Gwendoleen	37,6	103
N2	P8317	35,6		98,9		99		Gwendoleen	37,3		P8317	36,2	99
N1	KWSEditio	36,5		101,4		101		P8317	35,6		KWSEditio	36,8	101
N1	P82703	35,7		99,0		100		KWSEditio	36,5		P82703	36,4	100
N1	P83224	37,0		102,5		102		P82703	35,7		P83224	37,3	102
N1	DKC3323	38,0		105,3		105		P83224	37,0		DKC3323	38,3	105
N1	SYArax	36,2		100,6		101		DKC3323	38,0		SYArax	36,9	101
N1	SYRemus	36,1		100,3		100		SYArax	36,2		SYRemus	36,4	100
N1	SYOpale	37,5		104,2		103		SYRemus	36,1		SYOpale	37,6	103
N1	Nashorn	33,7		93,6		92		SYOpale	37,5		Nashorn	33,6	92
								Nashorn	33,7				
											100=	36,565	100,000
				100,588		100,000	0,99415						

New limit to assess correct earliness grouping of Rec. List 2026

Multiply relative limit value Rec. List 2025 by 0,99415

From 2018 (RL2019) marge is +0.75%/ from 2024 marge +0.25%/ in 2025 abs - 0.5

														2024	2025	abs-0.5
	RL2012	RL2013	RL2014	RL2015	RL2016	RL2017	RL2018	RL2019	RL2020	RL2021	RL2022	RL2023	RL2024	RL2025	RL2026	RL2026
100=	36,003	34,358	34,579	34,485	34,605	35,436	36,553	36,749	36,325	36,367	36,568	36,401	36,737	36,040	36,565	36,565
grensabs	34,464	34,601	35,253	35,675	35,653	36,651	37,675	38,111	38,391	38,613	38,747	38,618	38,611	38,431	38,763	38,263
grensrel	95,724	100,707	101,949	103,451	103,028	103,428	103,069	103,707	105,685	106,178	105,958	106,089	105,101	106,636	106,012	104,6439225
grens+0.25%		36,101	36,753	37,175	37,153	38,151	39,175	38,861	39,141	39,363	39,497	39,368	39,361	38,681	39,013	38,513
							From 2018 (RL 2019) absolute limit + 0.75							From 2024 absolute limit -0.25		