PROTOCOL FOR THE EXAMINATION OF VALUE FOR CULTIVATION AND USE OF

POTATO VARIETIES

In The Netherlands

2024

Raad voor plantenrassen (Rvp)
Plant Variety Board

March 2024

Contents

1. Introduction

VCU testing of potato varieties comprises the following items:

- yield trials (including maturity and cooking type).
- virus testing to determine the resistance to PVY_{NTN}.
- field trial to determine the resistance to Foliage Late Blight.
- determination of the Solanine GlycoAlkaloid (SGA) content

The yield trials will be performed by the applicants. The other trials will be performed centrally (i.e. all varieties in the same trial) by NAK Services.

The virus and *Phytophthora* trials must include six standard varieties per characteristic that is to be observed in the relevant resistance trial, in addition to the varieties submitted for the relevant target use (starch or ware potatoes).
The glycoalkaloid content determination trial must include one standard variety, in addition to the varieties submitted for the relevant target use (starch or ware potatoes).
In relation to the multi-year analysis of the results, the standard varieties shall be determined in mutual consultation between the Trials Organiser and the Plant Variety Board.

Testing for resistance to potato cyst nematodes (PCN) and potato wart disease are the responsibility of the Netherlands Food and Consumer Products Safety Authority (NVWA) and are described in separate protocols issued by the NVWA. PCN and potato wart disease are not part of the VCU.

Only applications submitted to the Plant Variety Board before 15 December (see www.raadvoorplantenrassen.nl) are eligible for official VCU testing and trials.

True Potato Seed

True Potato Seed (TPS) hybrids are tested for VCU from 2023. TPS varieties and vegetatively propagated varieties are tested in the same trials. Within trials TPS hybrids are tested on more plots than vegetatively propagated varieties due to the potential genetic diversity within a TPS hybrid.
VCU testing of TPS hybrids is performed on the basis of first generation planting material harvested by the breeder from plants which have been grown from true potato seeds. The way in which TPS hybrids are integrated in the existing trials is described in Annex 1.

2. Planting material for the trial

The planting material required for the trials performed centrally (virus test, late blight and SGA) will be ordered and received by the Trials Coordinator/Trials Organiser (NAK Services) before being distributed to the various trial sites.

The seed tubers for the official applications is provided annually directly by the applicant. The seed tubers must be delivered to NAK Services in jute sacks before 1 February. NAK Services is responsible for ordering the standard varieties from the representing trading companies, stating the quantity of tubers required and the required tuber sizes. Naktuinbouw (the service officially responsible for DUS testing) will check and authenticate the identity of the seed tubers supplied.

Quality of the planting material

The seed tubers must satisfy the conditions of high quality seed – class S or SE (in accordance with NAK Inspection Regulations).

Virus-free planting material is a prerequisite for virus tests. For the other tests, a virus infection of maximum 1 % is considered acceptable (total for Potato Leaf Roll Virus, PVY, PVX, PVS and PVA).

The seed tubers must be accompanied by a Plant Passport (which declares it is free from brown rot, among others). Results of varieties that appear to have been infected afterwards, will be entirely discarded.

3. Yield determination

Yield trials will be performed independently by the breeders themselves.
A summary of the results of these trials must be submitted with the application to the Plant Variety Board using the VCU technical questionnaire (see: www.raadvoorplantenrassen.nl).

Observations and determinations

- tuber yield
- specific gravity (dry matter content)
- maturity

Cooking type.

For ware potatoes only. The breeder submits the cooking type
(based on the internationally recognised EAPR scale from A to D).

4. Resistance tests

Separate tests are performed to determine resistance to PVY_{NTN} and Phytophthora. These tests are performed under field conditions.

4.1 Testing for resistance to Potato Virus Y_{NTN}

PVY is a non-persistent virus transmitted by aphids. There are various strains (Y_{NTN}, Y_N, Y_C and Y_O) which cannot be serologically distinguished, but which do respond with an individual characteristic symptom which may vary per variety (mosaic, wrinkling, stipple streak, etc.).

Resistance to PVY_{NTN} will be tested in the field in a “neighbouring plant infection test", a test in which virus-free plants are infected naturally (aphid-borne transmission) by neighbouring plants that have been inoculated with a virus strain of known origin. Physically isolating the trial plot prevents the introduction of other strains. The percentage of infected plants is tested serologically.

Trial design
Randomised incomplete block design (4 complete blocks sub-divided into incomplete sub-blocks of 8 - 10 plots).
Number of years: minimum of 2
Number of sites: 2
Number of replicates (blocks): 4
Number of plants per plot (per replicate): 4
Seed tubers of the material to be tested: virus-free, tuber size 45 - 55 mm
Source of infection: a tolerant variety (on which the Y_{NTN} virus can multiply sufficiently and one that is resistant to the other viruses) inoculated with the Y_{NTN} strain. The material used as infector plants must be renewed at least every two years. An ELISA test is used to check for the presence of the correct virus strain and for the absence of other viruses.
In each replicate, a row of four plants should be planted per variety (a b c and d), across the direction of the ridges, with an infector plant at the end of the row.
This way each row (plot) is limited at one end by an infector plant (see trial scheme below).
Planting space 40 cm within the ridges; ridge distance 75 cm.

variety 2	->	a	b	c	d	*	d	c	b	a	<-	variety 3
variety 1	->	a	b	c	d	*	d	c	b	a	<-	variety 4
		^	^	^	^	^	^	^	^	^	=	direction of ridge
						*					=	infector row

The trial can be protected by surrounding it with one or more discard rows.
The virus is transmitted naturally by aphids.

Standard varieties:
A minimum of six standard varieties must be included in the trial. The standard varieties should as far as possible cover the range from susceptible (1) up to and including resistant (9). The standard varieties shall be determined in mutual consultation between the Trials Coordinator and the Plant Variety Board. The following standard varieties are used in 2024: Agria, Alouette, Arizona, Bintje, Hansa, Jazzy, Mondial and Maritiema.

Origin of trial material

The seed tubers are supplied by the applicant in both years. The applicant supplies virus-free planting material (of S or SE quality). After emergence, the trial is checked for secondary infections. If the presence of secondary infection of plants is established, the results of this variety will be rejected. Moreover, these plants will be removed from the trial.

Trial site and time of planting:

Planting time: two to four weeks later than local practice, in order to avoid differences arising from maturity (e.g. differences in resistance due to ageing of the plants) at the moment of infection. Moreover, the infector plants are pre-sprouted (contrary to the candidate varieties) to compensate for any delayed vigour. The trial should be located, as far as possible, in an area with a potentially high aphid pressure and with a low risk of infection from external sources. The trial should receive normal applications of fertiliser, weed control treatments etc. To encourage the spread of aphids and to screen the trial for secondary infections, the trial should be visually inspected once or twice during the season before the crop canopy closes. The aphid pressure on site is established using insect traps.

Harvest:

The plants in the trial are lifted in September, based on data of flights of aphids or aphid infection in farmer's fields. Severe infections must be avoided. At lifting, each plant must be sampled individually. Sample size: 3 tubers per plant, from psychically different stems. Each plot is sampled separately, i.e. 12 tubers per plot. In 4 replicates; i.e. 48 tubers per variety. Harvested tubers are stored at 10-15 ^{o}C.

Analysis

In early January, after dormancy breaking (by immersion in Gibberelline - 2 ppm for 20 minutes), an eye plug (preferably from the main shoot on the top of the tuber) is planted in the greenhouse. After approximately six weeks, each plant is individually inspected for the presence of the Y_{NTN} virus using an ELISA test based on a polyclonal antiserum (Clark and Adams, 1977), performed in accordance with the NAK manual (ELISA version 1993 - 1). The varieties are analysed for virus per replicate.

Result

The number of positive results compared with the total number of harvested tubers per plot (i.e. the percentage of infected tubers per plot). The percentage of infected tubers is converted into a score on a scale of 1–9 using linear regression based on the score of the standard varieties.

Literature

Clark, M.F. and Adams, A.N., 1977. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. Journal of General Virology 34: 475 - 483.

NAK, 1993. Elisa manual version 1993 - 1.

4.2 Test for resistance to Foliage Late Blight (*Phytophthora infestans)*

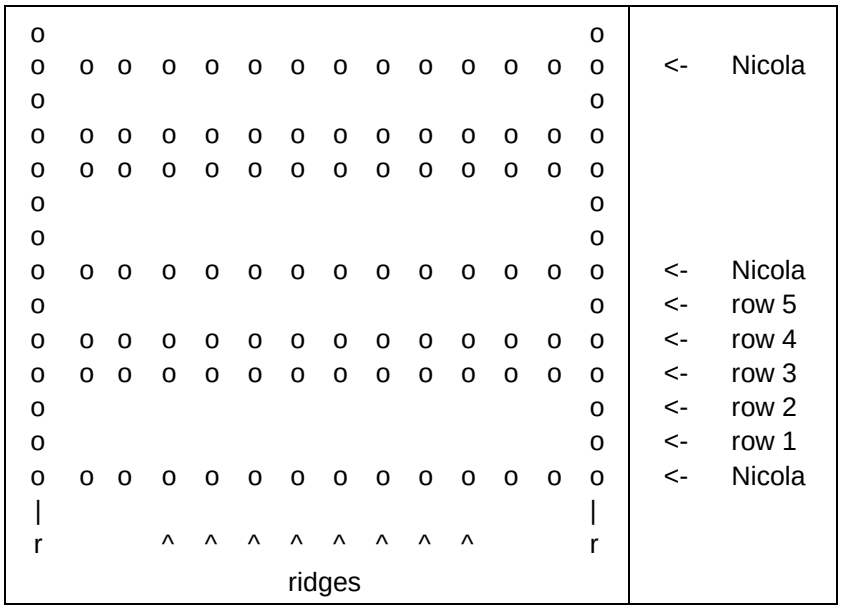
The test is performed in the field using artificial inoculation. The progression of the epidemic is assessed at regular intervals.

Trial design

Randomised incomplete block design (complete blocks sub-divided into incomplete sub-blocks).
Number of replicates (blocks): 3
Number of sites: 1 (as far as possible, beyond the range of important potato growing areas)
Number of years: minimum of 2 years.
Number of plants per plot (per replicate): 6
The trial is organised into strips of 14 ridges (with a pathway equal to 4 ridges between the strips), see the figure of a strip below.
A late and moderately resistant variety (r) is planted in both outer ridges of a strip - e.g. Pimpernel or Irene. Rows run across the direction of the ridges.
Every sixth row is planted with the moderately susceptible variety Nicola, which is used as an infector plant for a gradual increase of the infection and to sustain the epidemic.
Every first, second and fifth row is empty to be used as pathways for observation purposes.
In between these rows are two rows containing the varieties to be tested in plots of 3x2 plants (3 ridges wide, 2 plants deep, see figure below - see also Colon and Budding, 1988).
The trial is planted in early May.

Standard varieties

A minimum of 6 standard varieties should be included in the trial. The standard varieties cover the range from susceptible to resistant, whereby Bintje is always included as the default standard.
The other standard varieties shall be determined in mutual consultation between the Trials Coordinator and the Plant Variety Board. The following standard varieties are used in 2023: Bintje, Eigenheimer, Aventra, Nicola, Mondial, Markies and Avamond.



Inoculation

The entire trial is inoculated with a suspension of zoospores and zoosporangia, derived from a concentration of the order of 15,000 zoosporangia per ml. Inoculation takes place on plants with a closed canopy (in July). Prior to inoculation, the crops (and the soil) are irrigated, after which the inoculum is sprayed (misted with uniform and constant spray pressure) onto the plants at dusk. The inoculum originates from Wageningen University & Research. An R-gene complex isolate is used for inoculation (IPO-complex 1.2.3.4.5.6.7.10.11 isolate number IPO 82001).

The trial is irrigated mechanically (mornings/evenings, 3 to 5 sessions per hour, depending on weather conditions) and observed 6-10 times at intervals of 3-4 days.

Observations:

The percentage of infected foliage is estimated.

% infected leaf area (severity)	Symptoms
0	no visible infection.
0.01	1 lesion per plot.
0.02	2 lesions per plot.
0.05	5 lesions per plot.
0.1	6 - 10 lesions per plot.
0.5	2 - 5 lesions per plant.
1	6 - 10 lesions per plant.
5	up to an average of 20 lesions per plant.
10	10 % leaf area of the plot is necrotic. Plant appears healthy, but on closer inspection lesions are clearly visible.
25	25 % leaf area of the plot is necrotic.
50	50 % leaf area of the plot is necrotic.
75	75 % leaf area of the plot is necrotic. Plot appears green with brown blotches. Lower layers of leaves are necrotic
90	90 % leaf area of the plot is necrotic. Plot appears brown-green. Only the top leaves are green. Multiple stem lesions visible.
97.5	97.5 % leaf area of the plot is necrotic. Plot appears brown. Only some of the top leaves are still green. Stems have multiple lesions or are already necrotic.
100	all leaves and stems are necrotic.

The percentage of infected leaf is converted into a score on a scale of 1-9 using linear regression based on the score of the standard varieties.

Literature

Colon, L.T. and D.J. Budding, 1988. Resistance to late blight (*Phytophthora infestans*) in ten wild *Solanum* species. Euphytica 37: 77 - 86.

Fry, W.E., 1978. Quantification of general resistance of potato cultivars and fungicide effects for integrated control of potato late blight. Phytopathology 68: 1650 - 1655.

5. Determination of the Solanine Glycoalkaloid content (SGA)

Solanine Glycoalkaloids (SGA) are natural toxins that occur in various parts of the potato plant. In potatoes α-solanine, α-chaconine and β-chaconine account together for approximately 95 % of the total glycoalkaloid content.

As from listing 2015, the maximum permitted content in ware potatoes is equal to the multi-year average content in the Innovator variety. As from listing 2012, the maximum permitted content in starch potatoes is equal to the multi-year average content in the Aventra variety. A minimum of two years of testing is necessary to determine the multi-year average content (see 5.4).

5.1 Trial design

Two trials (starch and ware potatoes each) are set up for the purpose of sampling. These sampling trials are for the purpose of determining the SGA content only.

The trials are planted singly (i.e. without replication) on different soil types per target usage: on clay and sandy soil for ware potatoes, reclaimed peat soil and sandy soil for starch potatoes. Starch and ware potatoes can also be combined on one site with sandy soil. The net size of the plots is a minimum of 10 plants. These plots are surrounded by a discard row. Factors that influence the SGA content – such as drought stress, soil structure problems, pests and diseases - must be avoided as far as possible. Ridges must be formed as well as possible; soil loss from the ridges – caused by heavy rainfall – may possibly cause green tubers, meaning the ridges must be repaired in-situ. The trials must be regularly inspected for any disruptive factors during the season.
Samples are also taken from the standard varieties Innovator and Aventra on all sites. Standard varieties are important indicators of abnormal conditions. On trials that test ware potatoes, Innovator is planted in quadruplicate. On trials that test only starch potato varieties, Aventra is planted in quadruplicate. Starch potato varieties that show a high SGA content in the first year of testing, should be placed adjacent to Aventra in the second year of testing. Ware potato varieties that show a high SGA content in the first year of testing, should be placed adjacent to Innovator in the second year of testing.

5.2 Sampling

The samples consist of 8 kg (fresh weight) of mature potatoes (no green tubers), with a tuber size of 35 mm and above. As the SGA content increases if tubers are exposed to light, the tubers must be stored in the dark by placing them in non-translucent paper bags immediately after lifting. The samples must be supplied unwashed (due to the risk of rotting during storage). The samples must be correctly labelled, on and in the bag. Site and date of lifting must be stated at delivery. The samples must be stored at a temperature of 10-15 ^{o}C (not lower).

5.3 Methodology

The samples are analysed individually using an HPLC method, as described by Houben and Brunt (1994). The SGA content is established in mg per 100 g fresh weight.

5.4 Statistical analysis of SGA results

Transformation of the measured contents.
As the variance of the measured SGA contents is generally proportional to the average, the measurements must be transformed before the variance analysis can be performed.
The data is analysed on a logarithmic scale.

Summary of individual trials over years
The results are analysed per target usage (ware or starch). Per target usage, just two determinations per variety are performed per year. An analysis performed within the year based on these two determinations does not usually generate any relevant statistical data: the results are therefore analysed directly over the last 20 years. The over-year analysis may give rise to analysis of interaction and location effects within years, in order to establish the validity of the trial in the over-year data analysis.

Only data of varieties with four or more observations is included in the analysis.
Data of other varieties is excluded.

The incomplete data-sets are analysed using the REML algorithm, whereby VARIETY is applied as a fixed factor and YEAR applied as a random factor.
This generates variance components for YEAR (V_{year}) and RESIDUAL ($V_{residual}$).

Calculation of the multi-year average
The average SGA values are retransformed into real values using the formula:

$$SGA = e^{\text{Variety average} + (V_{year} + V_{residual}) / 2}$$

Literature
Houben, R.J. and Brunt, K., 1994. Determination of glycoalkaloids in potato tubers by reversed-phase high-performance liquid chromatography. J. of Chromatography A, 661: 169-174.

6. Standards for listing on the National List of the Netherlands

The minimum standards for listing were established in a memorandum by the Plant Variety Board on 14 September 2018. This memorandum also explains how these standards were created (see: www.raadvoorplantenrassen.nl). The following minimum standards apply for potatoes:

SGA
The maximum permitted SGA content for ware potatoes is equal to the multi-year average content of Innovator.
The maximum permitted SGA content for starch potatoes is equal to the multi-year average content of Aventra.

If, after two years of testing, data from only three instead of four trials is available, ware potatoes may be eligible for listing provided the SGA content is 5.46 mg/100 g fresh weight lower than the multi-year average content of Innovator. The following applies to starch potatoes that may be eligible for listing provided the SGA content is 9.42 mg/100 g fresh weight lower than the multi-year average content of Aventra.

Virus diseases
Resistance to PVY_{NTN} must not be lower than rounded 5.

If, due to circumstances, from one or from both years of testing only one trial of PVY_{NTN} is available, the PVY_{NTN} score (after two years of testing) must not be lower than rounded 5.5.
In such case of insufficient test results, varieties that fail to meet this minimum standard can continue to be tested in a third year.

The criteria for listing are summarised in the table below.

Criteria for National Listing at various numbers of successful trials				
		Number of successful trials after two or more years of testing		
		4 or more trials	3 trials	2 trials (in 2 different years)
SGA	Ware	< content of Innovator[1)]	< content of Innovator - 5,46[2)]	One year extra needed
	Starch	< content of Aventra[1)]	< content of Aventra - 9,42[2)]	One year extra needed
Y-virus	all varieties	> 4,75	> 5,25[3)]	> 5,25[3)]
[1)] Moving multi-year average content				
[2)] Possibility to continue the testing in an additional year until 4 successful test results have been reached and the criteria for listing apply: i.e. < content of Innovator, < content of Aventra respectively				
[3)] Possibility to continue the testing in an additional year until 4 successful test results have been reached and the criterion for listing applies: i.e. > 4,75				

Annex 1 True Potato Seed (TPS) hybrid

In 2024 True Potato Seed (TPS) hybrids are tested on the same trials as those used for VCU testing of vegetatively propagated varieties. VCU testing of TPS hybrids is performed on the following trials:

- Two trials to determine the resistance to PVY_{NTN}
- One trial to determine the resistance to Foliage Late Blight
- Two trials to determine the Solanine Glycoalkaloid content (SGA)

Planting material

330 tubers of a TPS variety must be submitted to NAK (Services). These tubers must have been harvested from 330 seedlings (1 tuber from each seedling). A minimum of 60 plants is tested, to guarantee that the full genetic diversity of a hybrid is taken into account.

Testing for resistance to PVY_{NTN}

In the virus trials as described in paragraph 4.1, a TPS hybrid is planted on four plots in each replicate. Each plot has the same size as a plot of a vegetatively propagated variety, i.e. four plants. Hence, a total of 64 plants of a TPS hybrid are tested in a trial. At lifting, each plant must be sampled individually. Sample size: 3 tubers per plant, from psychically different stems. Each plot is sampled separately, i.e. 12 tubers per plot. That is total of 192 tubers of each hybrid being tested in a trial.

Testing for resistance to Foliage Late Blight

In the trial as described in paragraph 4.2, a TPS hybrid is planted on 4 plots in each replicate. Each plot has the same size as a plot of a vegetatively propagated variety, i.e. six plants. Hence, a total of 72 plants of a TPS hybrid is tested in this trial. The observations on the percentage of infected foliage are made in the same way as described in paragraph 4.2. In case of clear differences in the degree of infection between plants within a plot, an additional observation needs to be done by recording the percentage of necrotic leaf area of each plant.

Determination of the Solanine Glycoalkaloid content.

In the trials as described in paragraph 5, each TPS hybrid is planted on 6 plots. Each plot has the same size as a plot of a vegetatively propagated variety, i.e. 10 plants. At lifting, a sample of 8 kg per plot is taken in the same way as it is taken in a plot of a vegetatively propagated variety.

Hence, a total of 60 plants (from 6 TPS-plots) of a TPS hybrid is tested in a trial.

Depending on the level of SGA content in the first year of testing the Plant Variety Board can decide that a supplemental SGA-determination needs to be done per plant in the second year of testing. For that purpose, 1 or 2 tubers per plant of each TPS-plot need to be harvested separately, prior to the lifting of the 8 kg sample. The SGA content of these separate tubers is determined only from the TPS-plot (or plots) with the highest SGA content in the 8 kg sample.

Determination of the yield

The yield is determined by the breeder on trials performed on clay and sandy soils. The specific gravity, maturity and cooking type are also determined on these trials. The TPS hybrid is tested on the basis of planting material harvested from plants which have been grown from true potato seeds.

Annex 2 Contact details.

Plant Variety Board (Rvp) / Naktuinbouw

Postbus 40
2370 AA Roelofarendsveen, NL

Visitors address:
Sotaweg 22
2371 GD Roelofarendsveen, NL

j.r.vd.schoot@naktuinbouw.nl
m.hoffman@naktuinbouw.nl
www.naktuinbouw.nl
www.raadvoorplantenrassen.nl

NAK Services B.V. (Trials Coordinator / Trials Organiser)

Postbus 1115
8300 BC Emmeloord, NL

Visitors address NAK office
Randweg 14
Emmeloord, NL

Visitors address NAK testing station:
Johannes Postweg 1
Tollebeek, NL

www.nak.nl
r.vanhulzen@nak.nl