PROTOCOL FOR THE EXAMINATION OF VALUE FOR CULTIVATION AND USE OF POTATO VARIETIES

2017

Raad voor plantenrassen (Rvp)
Plant Variety Board

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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} and PVX.
- field trial to determine the resistance to Foliage Late Blight.
- determination of the Solanine GlycoAlkaloid content (SGA)

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 three standard varieties, 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 Trial Operator 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.

2. Planting material for the trial

The planting material required for the trials performed centrally (virus tests, late blight and SGA) will be ordered and received by the Trial Coordinator/Trial 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 I 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 leafroll 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 on their own material. 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 viruses 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
Incomplete randomised 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.
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 Trial Coordinator and the Plant Variety Board. The following standard varieties are used: Astarte, Bintje, Desirée, Doré, 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 Potato Virus X

Potato virus X (PVX) shows a mottled interveinal mosaic and is transmitted mechanically to healthy plants by contact. For example, the virus is transmitted by humans, or by the wind, or by infected objects, e.g. machinery driving through the crop rows. In the Netherlands, at least four groups of strains have been described (based on their resistance genes). Strains from group 3 are the most common.

Resistance to PVX will be tested in the field in a "neighbouring plant infection test", a test in which virus-free plants are brought into contact with neighbouring plants that have been inoculated with X-virus. The percentage of newly infected plants is tested serologically.

Trial design:
Incomplete randomised block design (4 complete blocks sub-divided into incomplete 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 X-virus can multiply sufficiently and one that is resistant to the other viruses) inoculated with a strain from the X3 group originating from Plant Research International. 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. A row of infector plants is planted in every other row, also across the direction of the ridges.

Infected row	->	*	*	*	*	*	*	*	*	<- Infected row
Variety 4	->	a	b	c	d	d	c	b	a	<- Variety 5
Variety 3	->	a	b	c	d	d	c	b	a	<- Variety 6
Infected row	->	*	*	*	*	*	*	*	*	<- Infected row
Variety 2	->	a	b	c	d	d	c	b	a	<- Variety 7
Variety 1	->	a	b	c	d	d	c	b	a	<- Variety 8
Infected row	->	*	*	*	*	*	*	*	*	<- Infected row
direction of cultivation		^	^	^	^	^	^	^	^	= ridge direction

The trial can be protected by surrounding it with one or more discard rows.
The rows (of a single variety) must be planted across the direction of cultivation to encourage transmission by (mechanical) contact: in an advanced growth-stage the crop is treated using a harrow passing over the tops of the plants (once to twice).

Standard varieties:
A minimum of 6 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 Trial Coordinator and the Plant Variety Board. The following standard varieties are used: Astarte, Bintje, Desirée, Diamant, Eigenheimer, Saturna 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.

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 infector plants must be at the same height as the surrounding plants. Normal planting space, fertiliser, etc.

Harvest:
The plants in the trial are lifted in September. At lifting, each plant must be sampled individually. Sample size: 3 tubers per plant, preferably from psychically different stems. Each plot is sampled separately, i.e. 12 tubers per plot. In 4 replicates; i.e. 48 tubers per variety.

Analysis
The harvested tubers are stored at room temperature.
Each tuber is inspected individually for the presence of X-virus in the skin 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.

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

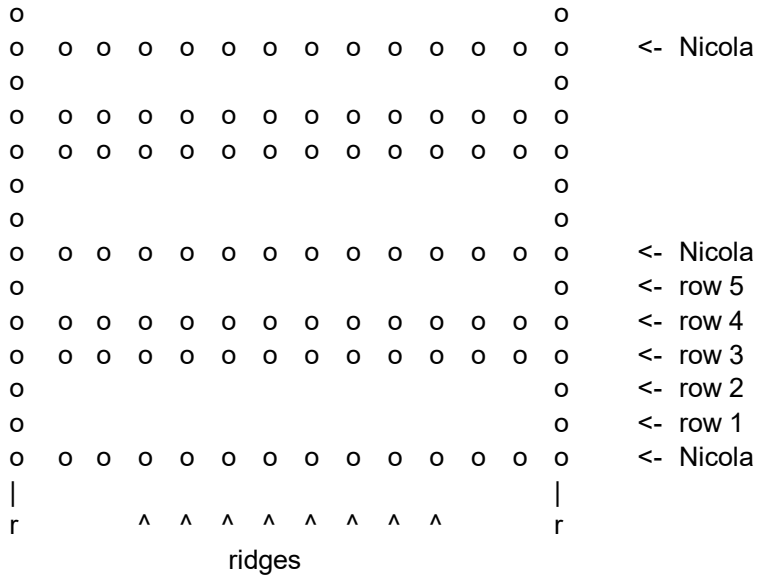
The test is performed in the field using artificial inoculation. The progression of the epidemic is assessed at regular intervals.

Trial design

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).
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 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 Trial Coordinator and the Plant Variety Board. The following standard varieties are used: Bintje, Eigenheimer, Irene, Karnico, Nicola, Mondial and Markies.



Inoculation

The entire plot 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 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 Diamant, Innovator and Aventra on all sites. Standard varieties are important indicators of abnormal conditions. All three standard varieties are planted in duplicate on the trials for ware varieties. This also applies to trials that test both ware potato and starch potato varieties. On trials that test only starch potato varieties, Aventra is planted in duplicate and Diamant and Innovator singly (without replication). 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 °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 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.
Due to modifications in 1985 to the sampling method, only data dating from 1985 and later is included in the analysis. 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_{yea}) and REST (V_{rest}).

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

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

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 18 January 2008. 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 PVX may not be lower than 4, provided resistance to PVY_{NTN} is a minimum of 5.
And/or:
Resistance to PVY_{NTN} may not be lower than 4, provided resistance to PVX is a minimum of 5.

From 2016, it will be possible to take an interim decision regarding listing in January based on one year of PVY_{NTN} testing and two years of PVX testing. The following criteria apply:

> If the score for PVX is 5 or higher after two years of testing, the PVY_{NTN} score (after one year) must be a minimum of 5.5;
> If the score for PVX is 4 after two years of testing, the PVY_{NTN} score (after one year) must be a minimum of 6.5

As soon as the 2nd year score for PVY_{NTN} is known, a decision will be taken on the varieties that fail to meet the interim criteria in January.

If, due to circumstances, only one year of PVX scores are available, it is possible to accept varieties for listing, provided two years of PVY_{NTN} scores are known and the following criteria are complied with:

> If the score for PVY_{NTN} is 5 or higher after two years of testing, the PVX score (after one year) must be a minimum of 5.5
> If the score for PVY_{NTN} is 4 after two years of testing, the PVX score (after one year) must be a minimum of 6.5

A decision can be taken for varieties that fail to meet these criteria, provided two years of PVX scores are available.

Annex Contact details.

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