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**METHOD OF TESTING THE VALUE OF A VARIETY
FOR CULTIVATION AND USE (VCU)**

GRAIN MAIZE

(PARS-VCU/2/1)

Name of the method: The method of testing the value of a variety for cultivation and use (VCU) – grain maize

Method code: PARS-VCU/2/1

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Method of testing the value of a variety for cultivation and use (VCU) – grain maize (PARS-VCU/2/1), adopted by a resolution of the director of the Phytosanitary Administration of the Republic of Slovenia, No.: 3431-32/2008/1 of 28 March 2008.

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**METHOD OF TESTING THE VALUE OF A VARIETY FOR CULTIVATION
AND USE (VCU) – GRAIN MAIZE
(PARS-VCU/2/1)**

1 GENERAL PART

1.1 Scope and Objectives

This method shall specify the method of testing the value for cultivation and use of the hybrids of grain maize (hereinafter referred to as: VCU) with a view to their entry in the national variety list of the Republic of Slovenia (hereinafter referred to as: NVL).

This method shall lay down the procedures for establishing qualitative and quantitative characteristics of the examined hybrid by means of observation, measuring and laboratory analysis, with a view to assessing the value of such hybrid for cultivation and use in comparison to standard hybrids, pursuant to Article 43 of the Agricultural Seeds and Propagating Material Act (ZSMKR-UPB1; Official Gazette of the Republic of Slovenia, No. 25/05) and Article 7 of the Rules on the Procedure for Acceptance of a Variety in the National Catalogue of Varieties and on Keeping of the National Catalogue of Varieties (Official Gazette of the Republic of Slovenia, No. 102/05, 64/06, 66/07 and 90/07).

This method shall apply also for special testing of the hybrids of grain maize (hereinafter referred to as: STV) with the aim to prepare the descriptive list of varieties (hereinafter referred to as: DLV), pursuant to Article 60 of the Agricultural Seeds and Propagating Material Act.

1.2 Performers of Testing

VCU and STV testing of the hybrids of grain maize shall be carried out by the performer of testing appointed by the Phytosanitary Administration of the Republic of Slovenia (hereinafter referred to as: Administration). Individual VCU or STV test phases (varietal trials at a certain location, assessments and measurements, or laboratory and other tests) may be, pursuant to the decision on appointment, carried out in cooperation with sub-contractors.

1.3 Varieties to be Included in the Testing

VCU testing of the hybrids of grain maize shall be carried out for the hybrids in respect of which applications for official testing of varieties for the entry in the NVL have been lodged with the Administration. Inclusion of maize hybrids in the STV under this method shall follow the STV annual program, adopted by the Administration.

1.4 Time Limits for Application, Delivery of Seeds and Sample Size

The following time limits shall be followed when lodging an application for entry of the hybrids of grain maize in the NVL, and when providing a seed sample for VCU testing or STV of the hybrids of maize:

Deadline for application	Deadline for delivery of seeds	Quantity (No. of grains)
10. 3.	1. 4.	10.000

The seeds for VCU testing shall be provided free of charge by the applicant of the variety, and for STV of the hybrids of maize by the applicant of the variety for inclusion in the STV (applicant of the variety, person responsible for the maintenance of the variety, or another interested supplier). Considering the quality and health condition, the seeds shall comply with minimum requirements laid down in the Rules on the Marketing of Cereal Seed (Official Gazette of the Republic of Slovenia, No. 100/05 and 94/06). The seed sample shall be disinfected by means of a fungicide and designated by a breeder's designation or a proposed varietal denomination.

1.5 Technical Data on the Hybrid

The applicant shall attach the application for VCU testing of maize hybrids by a completed technical questionnaire. Technical questionnaire shall state data on the hybrid (e.g. FAO maturity class) necessary for trial design and selection of standard hybrid. The applicant shall also indicate other possible special features of the hybrid (e.g. sensitivity to a certain active substance, etc.).

1.6 Duration of Testing

VCU testing for the entry of maize hybrids in the NVL shall take 2 years, and STV of the maize hybrids shall take 3 years, in so far as the hybrid is not subject to the entry procedure in the NVL. STV in respect of the hybrids which are subject to the entry procedure in the NVL shall take one additional year.

VCU testing of maize hybrids for entry in the NVL may be, upon the proposal by the varietal commission for maize (hereinafter referred to as: varietal commission), extended for 1 year or in total to three-year testing. Such extension may be a result of unfavourable weather conditions in a certain testing year, and when the varietal commission can not, on the basis of two-year results of testing, take a reliable decision on a suitable VCU of the hybrid. The applicant shall be informed in advance on the proposal for the extension of testing. In such case, costs of the testing shall be born by the applicant.

When STV of maize is extended for 1 year, the costs of carrying out the tests shall be born by the performers of testing.

1.7 Test Location

VCU testing of maize hybrids and STV of maize from FAO maturity classes 100 – 400 shall be performed in the territory of the north-eastern Slovenia (NE) at 2 locations, in the territory of the central Slovenia (CS) at 1 location, and in the territory of the south-eastern Slovenia (SE) at 1 location. Testing of the hybrids from maturity classes 500 – 700 shall be performed in the territory of the western Slovenia (WS) at 2 locations.

Test locations shall be published annually by the Administration on its website <http://www.furs.si/> before the testing is carried out.

1.8 Standard Hybrids

A hybrid shall be designated as standard hybrid which meets the following criteria:

- its cultivation is distributed;
- maturity classes which include hybrids with intermediate and dent-like type of grain must include standard hybrids with a suitable form of grain;
- the length of its growing season must comply with the indicative time of maturity of the tested hybrid;

- when the hybrid is included in the testing, the results regarding the yield quantity or quality, resistance to diseases and harmful organisms, or other characteristics which have a significant impact on the value of a certain variety for cultivation or use, must be over-average.

Each hybrid which is applied for the entry procedure of a variety in the NVL shall have, considering data in the technical questionnaire, at least one standard hybrid designated as a comparison in the VCU testing. If the application includes special requirements in respect of testing of the hybrid, or points out special characteristics of the tested hybrid, an additional standard hybrid can be designated for VCU testing of such hybrid. During the testing period of an individual hybrid, standard hybrids to which the tested hybrid is compared shall not be subject to any changes, save in exceptional cases upon proposal by the varietal commission.

Standard hybrids shall be confirmed by the varietal commission on the proposal by the performer of testing. The current list of standard hybrids to serve as a comparison for individual maize hybrids in VCU testing or STV shall be published in the official journal of the Administration and on its website <http://www.furs.si/>.

1.9 Costs of Testing

The costs of VCU testing shall be laid down in the Rules on the Procedure for Acceptance of a Variety in the National Variety List and on Keeping the National Variety List (Official Gazette of the Republic of Slovenia, No. 102/05, 64/06 and 66/07). The costs that occur during VCU testing of the hybrid as a result of special or additional requirements of the applicant shall be paid by the applicant directly to the performer of the testing of the variety on the basis of an invoice.

The method of covering the costs of STV of maize hybrids is laid down in the STV program.

2 PERFORMANCE OF THE TRIAL

2.1 Choosing and Preparing the Land

The land shall be uniform, free of micro depressions and of the smallest possible angle of inclination. The whole surface shall be covered with the same preceding crop.

The basic and pre-sowing land treatment shall comply with the Technological Guidelines for Integrated Production of Field Crops which is published annually by the Ministry of Agriculture, Forestry and Food. Portions of fertilizers for basic fertilisation shall be adjusted to the nutrient stock in the soil on the basis of a preliminary analysis of the soil (pH, P₂O₅, K₂O, humus).

2.2 Trial Layout

Prior to the testing, all hybrids to be included in the trial (tested and standard hybrids) shall be classified with regard to maturity classes (FAO 100 - 700) and the form of grain (from flint type to dent type and all intermediate types). All hybrids shall be codified.

The trial shall be set according to the randomised block design in four repetitions. The whole variety trial at one location shall be sown on the same day. The sowing shall be performed within an optimal time term by means of a special seed drill. The foreseen date of harvest shall be between 20 April and 9 May, and the depth of sowing between 4-6 cm. The trial shall be always sown transversally to the direction of ploughing.

The trial design at a certain location is indicated in the table below.

Length of basic plot	7 m
Width of basic plot	2.8 m
Surface of basic plot	19,6 m ²
Distance between plots	70 cm
Spacing between rows	70 cm
Number of rows	4
Number of rows under consideration	2 (inner)
Surface of plot under consideration	9,8 m ²
Distance between plots	1.4 – 2.0 m

2.2.1 Sowing Density

Sowing density is adapted to the duration of the growing season of hybrids and growing conditions of test sites. The sowing density shall be at least 30% greater than the planned sowing density at harvesting. The planned sowing densities after thinning are shown in the table below.

FAO maturity class	Sowing density at harvesting (plants/ha)			Number of plants in a row (7 m)		
	Central and SE Slovenia	NE heavy soil	NE light soil	Central and SE Slovenia	NE heavy soil	NE light soil
100	95.918	95.918	89.796	47	47	44
200	89.796	89.796	85.714	44	44	42
300	85.714	85.714	79.592	42	42	39
400	79.592	79.592	75.510	39	39	37
	Locations in the Western Slovenia					
500	73.469			36		
600	67.347			33		
700	61.225			30		

2.3 Care of the Trial

Land cultivation, protection against weeds and harmful organisms, side dressing, and other technological measures carried out on the trial plot shall comply with the Technological Guidelines for Integrated Production of Field Crops for the current year and with the guidelines to good agricultural practice.

2.3.1 Thinning

Thinning of plants is aimed at achieving the planned sowing density at harvest. Thinning shall be carried out during the development phase of 6-8 leaves, whereby the soil moisture should be such as to prevent breaking or tearing of plants during uprooting. Prior to the thinning of the rows under consideration, the plants in the row shall be counted and the surplus plants shall be eliminated by means of a special tool or manually. The eliminated plants shall be removed from the trial plot so as to prevent their re-rooting. About 10 days after the thinning, sowing density shall be re-checked and the redundant and damaged plants shall be removed, if necessary. The first and second rows of the plot which are not considered in the trial may be thinned without counting however their density should be similar to that in the rows under consideration.

2.4 Harvesting

Harvesting shall be carried out when a variety achieves technological ripeness. The yield from inner and edge rows under consideration shall be harvested separately. Harvesting shall be carried out by means of machinery.

3 MONITORING, SAMPLING AND MEASUREMENTS DURING GROWTH

3.1 Recording of Trial Data

3.1.1 Weather conditions during the trial

Average temperatures (in °C) and precipitation (in mm) shall be recorded during the trial. Multiannual average values from the nearest meteorological station shall be given as a comparison.

3.1.2 Sowing Date

3.1.3 Assessment Dates of Health Condition, Observation of Phenophases

3.1.4 Harvest Date

3.1.5 Technology

Carrying out the protection against weeds and harmful organisms, thinning, fertilizing, and other technological measures – e.g. date of harvest/measure; type and quantity of the PPP or fertilizer used.

3.2 Observation and Recording of Phenophases

Observation of phenophases (according to the BBCH scale)

Development phase	Code	Description
Emergence	09	75% of emerged plants – rows are clearly visible.
The middle of tasseling	55	Top of the tassel can be seen at 2/3 of plants.
The middle of silking	63	Silk can be seen at the top of the corn cone at 2/3 of plants.

3.3 Observation of Other Agronomic Features

During the trial, the characteristics indicated in the table below shall be observed and recorded.

Characteristics observed	Description
Tendency to tillering	Each basic plot in the phase of maize height over 50 cm shall be graded once on a sample of 20 successive plants inside the rows under consideration. Grades shall be from 1 to 9, where 1 means 0-1 branched-out plants and 9 more than 16 branched-out plants The table with the grades is indicated in Annex 1 which is the integral part of this method.
Height of plants	The height of plants shall be determined prior to harvesting at two levels, as follows: a) up to the top of the tassel On each plot, the height of plants from the ground to the top of the tassel shall be measures on a sample of 5 plants. The average values shall be entered in the observation booklet. b) to the base of the cone On each plot, the height of plants from the ground to the base of cones shall be measures on a sample of 5 plants. The average values shall be

	entered in the observation booklet.
Broken plants	The number of broken plants shall be established on each plot before harvest during the phase of technological ripeness of grains, when the moisture of the grains is below 35%. Only the plants shall be considered as broken which are broken under the cone. The table indicating the grades is given in Annex 2.
Lodged plants	The number of lodged plants shall be established before harvesting during the phase of technological ripeness of grains, when the grain moisture is below 35%. The plants shall be considered as lodged whose stems are bent for more than 30° from the vertical line. The cause for lodging shall also be stated (e.g. strong wind, shower, hail, etc.). The table indicating the estimations is given in Annex 2.
Solidity or type of grain	Solidity of grain shall be assessed in full ripeness on a sample of 6 cones from the rows not considered in the trial. The estimation shall be visual, and the hybrids shall be compared with standard hybrids whose type of grain is adjusted to international grades. Classification of grain types is indicated in Annex 3.

3.4 Assessment of Health Condition

During growth, the occurrence of diseases and harmful organisms shall be monitored on trial plots for all hybrids included in the trial, whereby at least 2 monitorings are to be performed from the phenophase of heading until technological ripeness. The list of more important diseases and harmful organisms of maize, the methods of monitoring health condition, and the criteria for evaluating the occurrence of diseases are laid down in Annex 4. In the case of the occurrence of other diseases and harmful organisms, such occurrence shall be recorder and intensity of the attack shall be estimated correspondingly on the basis of the share of the attacked plants.

3.5. Determining the Number of Plants Before Harvest

The number of plants shall be established in inner rows under consideration on each trial plot before harvesting by means of counting, whereby the plants emerged by branching-out are not considered.

4 LABORATORY ANALYSIS AND OTHER MEASUREMENTS AND OBSERVATIONS UPON AND AFTER THE HARVEST

4.1 Yield Determination

The yield shall be determined by determining grain mass in kg/plot, by weighting directly after harvest the whole sample of one repetition separately for each repetition. The grain mass shall be determined with a precision of 0.05 kg.

The yield shall be expressed as the grain mass with the moisture of 14% which is calculated on the basis of moisture content in grains and grain mass by repetitions.

4.2 Other Measurements, Assessments and Observations

Quality analysis of the maize grain shall be determined as indicated in the table below.

Analysis	Method of analysis or standard
Moisture content in grains	ISO 711:1997

5 DATA PROCESSING AND EVALUATION OF RESULTS

5.1 Validity of the Trial

Validity of the trial shall be determined on the basis of 2 factors, as follows:

- correctness of performance during the growing season,
- statistical analysis of grain yield.

5.1.1 Correctness of Performance

In the case of a trial affected by abiotic factors (e.g. hail, strong wind, drought), the whole testing on a certain location for the current year may be discarded. Elimination of the results for an individual location shall be decided by the varietal commission.

5.1.2 Statistical Analysis

When performing statistical evaluation of the grain yield which is one of the indicators of correctness of the trial performed, the following criteria shall apply:

- if the KV is $\leq 25\%$, the results are accepted,
- if the KV is $> 25\%$, the results are not accepted.

In the case of a high value of the coefficient of variation, an explanation shall be necessary.

5.2 Statistical Treatment of Results

The following test results shall be treated:

- grain yield.

Annual results on the grain yield shall be treated by means of the statistical method of the analysis of variance (Anova), and the relevant differences are to be detected by means of Duncan test of multiple comparisons (or LSD test) with a confidence interval of 95%.

Multiannual results of the grain yield and of other characteristics shall be treated according to the index system. It is a connected system of trial layouts with a greater number of standard hybrids and evaluation method of multiannual results, which both enable a direct comparison of economically significant characteristics of maize hybrids irrespective of the period of their testing. With a view to an easier and more reliable evaluation of test results, individual economically important characteristics may be assigned coefficients with regard to the significance of such characteristics in our growing conditions. The coefficients shall be assigned by the varietal commission.

On the basis of the treated test results, an opinion shall be given in respect of an individual hybrid subject to the entry procedure in the national variety list, concerning the suitability for cultivation in certain ecological conditions.

6 REPORTING AND COMMUNICATION OF RESULTS

Data are displayed annually for each location. After the testing is completed, data shall be evaluated at the level of the hybrid and displayed in the report which must include:

- description of test locations,
- list of tested hybrids (marked with codes and registration numbers) including standard hybrids,

- description of performance of the trial,
- statistically evaluated results

Considering the purpose of the testing, the results shall be classified as:

- the results on testing of the VCU of hybrid maize,
- the results for STV of hybrid maize.

The performer of the testing shall draw up interim (annual) and final reports of VCU testing of hybrids for the needs of varietal commission or STV and deliver these to the Administration. The results of the testing of maize hybrids shall be submitted by the end of November of the current year.

ANNEX 1

- Assessments marks for the characteristic tendency to tillering

Assessment mark	No. of branched-out plants
1	0 - 1
2	2 - 3
3	4 - 5
4	6 - 7
5	8 - 9
6	10 - 11
7	12 - 13
8	14 - 15
9	> 16

ANNEX 2

- Assessment marks for the resistance to breaking and lodging, and to cone diseases

In trials, assessment marks for the resistance to breaking and lodging of plants and to cone diseases are determined in per cents and transformed into assessment marks as indicated in the below table.

Share (%)	Assessment mark
0,0-0,5	1
0,6-1,0	2
1,1-2,0	3
2,1-3,0	4
3,1-5,0	5
5,1-7,0	6
7,1-9,0	7
9,1- 11,0	8
>11,1	9

ANNEX 3

- Classification of the types of maize grains

Mark	Significance
T	flint type
Tz	flint type with very insignificant dent expression
TZ	mixed type with expressed solidity
ZT	mixed type with significant dent expression
Zt	dent type with a very low expression of solidity
Z	dent type

ANNEX 4

- Assessment of infestation by plant diseases and harmful organisms

Assessing an infestation by plant diseases and harmful organisms shall include the organisms indicated in the below table.

Diseases and harmful organisms	Description
Corn borer (<i>Ostrinia nubilalis</i>)	The number of plants in the rows under consideration on which a caterpillar of the corn borer is present (the most visible injuries are breaking-over of tassels and of the upper part of the stem) shall be counted upon harvest. For late hybrids (FAO 500-700) the number of cones with visible injuries by caterpillars shall be counted in the rows under consideration. The table showing evaluation of the intensity of the attack of the pest is provided in the continuation of Annex 4.
Angoumois grain moth (<i>Sitotroga cerealella</i>)	The number of cones in the rows under consideration with visible injuries caused by caterpillars shall be counted upon harvest. The table showing evaluation of the intensity of the attack of the pest is provided in the continuation of Annex 4.
Corn smut (<i>Ustilago maydis</i>)	The number of plants in the rows under consideration on which corn smut is present (the most frequent on male and female inflorescences) shall be counted.
Leaf blight on maize (<i>Helminthosporium turticum</i>)	It shall be estimated in the full wax ripening with grades 1-9, whereby 1 indicates the condition free of any symptoms, and 9 a heavy attack of the disease. The basic classification scheme of the attack is provided in the continuation of Annex 4.
Stem infection <i>Fusarium</i> (<i>F. culmorum</i> , <i>F. graminearum</i> , <i>F. moniliforme</i>).	In the event of a severe attack they cause an early dying out of plants or forced ripening. The intensity of infestation shall be evaluated immediately before harvest, so that in one row under consideration 30 successive plants are subjected to aside pressure and the share of the plants broken thereby is determined. The table showing evaluation of the intensity of the attack of the pest is provided in the continuation of Annex 4.
Infestation of cones with fungi from the genus <i>Fusarium</i> (<i>F. culmorum</i> , <i>F. graminearum</i> , <i>F. moniliforme</i>).	All cones and the cones with symptoms (usually white reddish coating on grains) shall be counted upon manual harvesting. The intensity of infestation shall be expressed in per cents and subsequently transformed into assessment marks. The table for the transformation of the share is provided in Annex 2.

- **Basic classification scheme**

Assessment mark	Meaning
1	no symptoms of disease or injuries
2	very few symptoms of disease or injuries
3	few symptoms of disease or injuries
4	few to medium symptoms of disease or injuries
5	medium symptoms of disease or injuries
6	medium to strong symptoms of disease or injuries
7	strong symptoms of disease or injuries
8	strong to very strong symptoms of disease or injuries
9	very strong symptoms of disease or injuries

- **Intensity of stem infestation with fungal diseases**

Assessment mark	Intensity if attack
1	up to 7% of infested plants
2	8 – 17%
3	18 – 27%
4	28 – 37%
5	38 – 47%
6	48 – 57%
7	58 – 67%
8	68 – 77%
9	more than 77%

- **Intensity of the corn borer and Angoumois grain moth attack**

Assessment mark	Intensity of attack
1	up to 7% of attacked plants/cones
2	8 – 17%
3	18 – 27%
4	28 – 37%
5	38 – 47%
6	48 – 57%
7	58 – 67%
8	68 – 77%
9	more than 77%