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(FGBU "VNIIKR")

VALIDATION ACCORDING TO PM 7/98 METHOD OF EXTRACTION AND ISOLATION OF *STENOCARPELLA MAYDIS* FROM CORN SEEDS

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Maisons-Alfort (FR) - 2019

Information about the target organism:
Stalk tor of maize or leaf spot of maize (official Russian name)
Stenocarpella maydis (Berkeley) Sutton (scientific name)
Stenocarpella maydis (Berkeley) Sutton - Teleomorphic stage
Diplodia maydis (Berkeley) Saccardo - anamorphic stage

Taxonomy:
Fungi – Ascomycetes
Class – Dothideomycetes
Order – Botryosphaeriales
Family – Botryosphaeriaceae
Genus – Stenocarpella

Characteristic substrates of the target organism (affected crops):
Maize seeds (Zea mays L)

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STO VNIIKR 3.008−2011
Methods for detection and identification of *STENOCARPELLA MAYDIS (BERKELEY) SUTTON* and *STENOCARPELLA MACROSPORA (EARLE) SUTTON*.

Detection of Stenocarpella maydis in corn seeds by method of isolation in the wet chamber.

Analytical sensitivity
Repeatability
Reproducibility

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Determination of analytical sensitivity

Specialist: Senior researcher mycologist **Kuznetsova A.**
Date and time: 30.05.2018
Description of the variants (samples):
1) K-negative control (400 seeds without target)
2) 1:399 (1 infected seed to 399 seeds without target)
3) 2:398 (2 infected seeds to 398 seeds without target)
4) 4:396 (4 infected seeds to 396 seeds without target)
5) 8:392 (8 infected seeds to 392 seeds without target)

Corn seed variants selected for analysis were washed under running water for 10-15 minutes. The seeds were then superficially sterilized by immersion in ethyl alcohol for 1-2 minutes, followed by washing in distilled water (for 3-5 minutes).
The prepared material was placed in Petri dishes on moistened filter paper and in parallel on artificial culture media (2% potato glucose agar). 320 seeds of each variant were arranged into 16 square cups (20 seeds each) and 80 remaining seeds into 11 cups with culture medium, i.e. 1:4. The cups were incubated for 7 days at a temperature of 20 oC, with daily examination after two days of aging at the specified mode. In order to speed up the formation of fruit bodies, the samples were kept under variable light conditions (12 hours in the dark, 12 hours in the light). We started to look through the variants on the 2nd day.

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Item No.	Sample code	Target organism content	Obtained result
1.	1	1:399	+
2.	2	1:399	+
3.	3	1:399	+
4.	4	1:399	+
5.	5	2:398	+
6.	6	2:398	+
7.	7	2:398	+
8.	8	2:398	+
9.	9	4:396	+
10.	10	4:396	+
11.	11	4:396	+
12.	12	4:396	+
13.	13	8:392	+
14.	14	8:392	+
15.	15	8:392	+
16.	16	8:392	+
17.	K-	-	-
18.	K-	-	-
19.	K-	-	-
20.	K-	-	-

AS value: 1:399 (1 infected seed to 399 seeds without target)

The studies carried out to determine the analytical sensitivity showed 100% detection of the *Stenocarpella maydis* pathogen in all variants of the experiment.

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Determination of repeatability
Specialist: Senior researcher mycologist **Kuznetsova A.**
Date and time: 08.06.2018
Description of the variants (samples):
1) K - Negative control
2) 1:399 (1 infected seed to 399 seeds without target)
3) 2:398 (2 infected seeds to 398 seeds without target)

Item No.	Sample code	Target organism content	Obtained result
1.	1	1:399	+
2.	2	1:399	+
3.	3	1:399	+
4.	4	1:399	+
5.	5	2:398	+
6.	6	2:398	+
7.	7	2:398	+
8.	8	2:398	+
9.	K-	-	-
10.	K-	-	-
11.	K-	-	-
12.	K-	-	-

Repeatability value: 100%

The studies carried out to determine the repeatability of showed 100% detection of the *Stenocarpella maydis* pathogen in all variants of the experiment

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Determination of reproducibility
Specialist: Head of the mycology laboratory **Surina T.**
Date and time: 6.07.18
Description of the variants (samples):
1) K - Negative control
2) 1:399 (1 infected seed to 399 seeds without target)
3) 2:398 (2 infected seeds to 398 seeds without target)

Item No.	Sample code	Target organism content	Obtained result
1.	1	1:399	+
2.	2	1:399	+
3.	3	1:399	+
4.	4	1:399	+
5.	5	2:398	+
6.	6	2:398	+
7.	7	2:398	+
8.	8	2:398	+
9.	K-	-	-
10.	K-	-	-
11.	K-	-	-
12.	K-	-	-

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Determination of reproducibility
Specialist: Senior researcher mycologist **Dudchenko I.**
Date and time: 18.10.18
Description of the variants (samples):
1) K - Negative control
2) 1:399 (1 infected seed to 399 seeds without target)
3) 2:398 (2 infected seeds to 398 seeds without target)

Item No.	Sample code	Target organism content	Obtained result
1.	1	1:399	+
2.	2	1:399	+
3.	3	1:399	+
4.	4	1:399	+
5.	5	2:398	+
6.	6	2:398	+
7.	7	2:398	+
8.	8	2:398	+
9.	K-	-	-
10.	K-	-	-
11.	K-	-	-
12.	K-	-	-

Reproducibility value:100%
The studies carried out to determine the reproducibility showed 100% detection of the *Stenocarpella maydis* pathogen in all variants of the experiment.

Summary

Criteria	Value	Assessment*
Analytical sensitivity	100% in the sample1:399 (1 infected seed for 399 seeds without target organism)	corresponds
Repeatability	100%	corresponds
Reproducibility	100%	corresponds

Conclusion on the applicability of the method:

The biological method of isolating *Stenocarpella maydis (Berkeley) Sutton* (target organism) from maize seed material (substrate) is characterized by high analytical sensitivity, provides a high level of repeatability and reproducibility of the results and may be recommended for laboratory testing to detect Stenocarpella maydis (Berkeley) Sutton in maize seeds.

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Thanks for attention

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