UPOV
International Union for the Protection of New Varieties of Plants

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PARTIAL REVISION OF THE TEST GUIDELINES FOR VEGETABLE MARROW, SQUASH

Document prepared by experts from France

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1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Vegetable Marrow, Squash (document TG/119/4 Corr. 2).

2. The Technical Working Party for Vegetables (TWV), at its fifty-third session, held in Seoul, Republic of Korea, from May 20 to May 24, 2019, agreed that the Test Guidelines for Vegetable Marrow, Squash (document TG/119/4 Corr. 2) be partially revised to add new Characteristics "Resistance to *Zucchini yellow mosaic virus*" and "Resistance to *Watermelon mosaic virus*" (see document TWV/53/14 Rev. "Report", paragraph 91 and Annex III).

3. The following changes are proposed:

(a) Addition of new Characteristic 82 "Resistance to *Zucchini yellow mosaic virus* (ZYMV)" at the end of the Table of Characteristics
(b) Addition of an explanation Ad. 82. "Resistance to *Zucchini yellow mosaic virus* (ZYMV)" in Chapter 8.2 "Explanations for individual characteristics"
(c) Addition of new Characteristic Characteristic 83 "Resistance to *Watermelon mosaic virus* (WMV)" at the end of the Table of Characteristics
(d) Addition of an explanation Ad. 83 "Resistance to *Watermelon mosaic virus* (WMV)" in Chapter 8.2 "Explanations for individual characteristics"

Proposal to add new Characteristic 82 "Resistance to Zucchini yellow mosaic virus (ZYMV)" at the end of the Table of Characteristics

	English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
82.	**Resistance to *Zucchini yellow mosaic virus* (ZYMV)**	**Resistance au *Zucchini yellow mosaic virus* (ZYMV)**	**Resistenz gegen *Zucchini yellow mosaic virus* (ZYMV)**	**Resistencia a *Zucchini yellow mosaic virus* (ZYMV)**		
	susceptible	sensible	anfällig	sensible	Cora	1
	moderatly resistant	modérément résistante	mäßig resistent	moderadamente resistente	Mirza	3
	resistant	résistante	resistent	resistente	Mikonos	5

Proposal to add explanation Ad. 82. "Resistance to *Zucchini yellow mosaic virus* (ZYMV)" in Chapter 8.2 "Explanations for individual characteristics"

Ad. 82: Resistance to *Zucchini yellow mosaic virus* (ZYMV)

1.	Pathogen	*Zucchini yellow mosaic virus* (ZYMV)
2.	Quarantine status	No
3.	Host species	*Cucurbita pepo* L.
4.	Source of inoculum	GEVES[1] (FR)
5.	Isolate	e.g. strain E9
6.	Establishment isolate identity	-
7.	Establishment pathogenicity	Symptoms on susceptible squash variety
8.	Multiplication inoculum	
8.1	Multiplication medium	Living plant
8.2	Multiplication variety	e.g. Cora
8.3	Plant stage at inoculation	-
8.4	Inoculation medium	-
8.5	Inoculation method	-
8.6	Harvest of inoculum	-
8.7	Check of harvested inoculum	-
8.8	Shelflife/viability inoculum	-
9.	Format of the test	
9.1	Number of plants per genotype	At least 20
9.2	Number of replicates	At least 2
9.3	Control varieties	susceptible : Cora moderatly resistant: Mirza resistant: Mikonos Up to now, no complete resistance is identified. The two intermediate and resistant controls are necessary to validate the agressiveness of the test.
9.4	Test design	add non inoculated plants
9.5	Test facility	Climatic room or greenhouse
9.6	Temperature	e.g. 22°C or 24°C/18°C
9.7	Light	12h-16h
9.8	Season	
9.9	Special measures	-
10.	Inoculation	
10.1	Preparation inoculum	1 g leaf with symptoms with 4 mL of PBS with carborundum (400 mg) and activated carbon (400 mg) or similar buffer, homogenize
10.2	Quantification inoculum	-
10.3	Plant stage at inoculation	First expanded leaf
10.4	Inoculation method	Rubbing with virus suspension
10.5	First observation	14 days post-inoculation
10.6	Second observation	-
10.7	Final observations	21 days post-inoculation
11.	Observations	
11.1	Method	Visual observation

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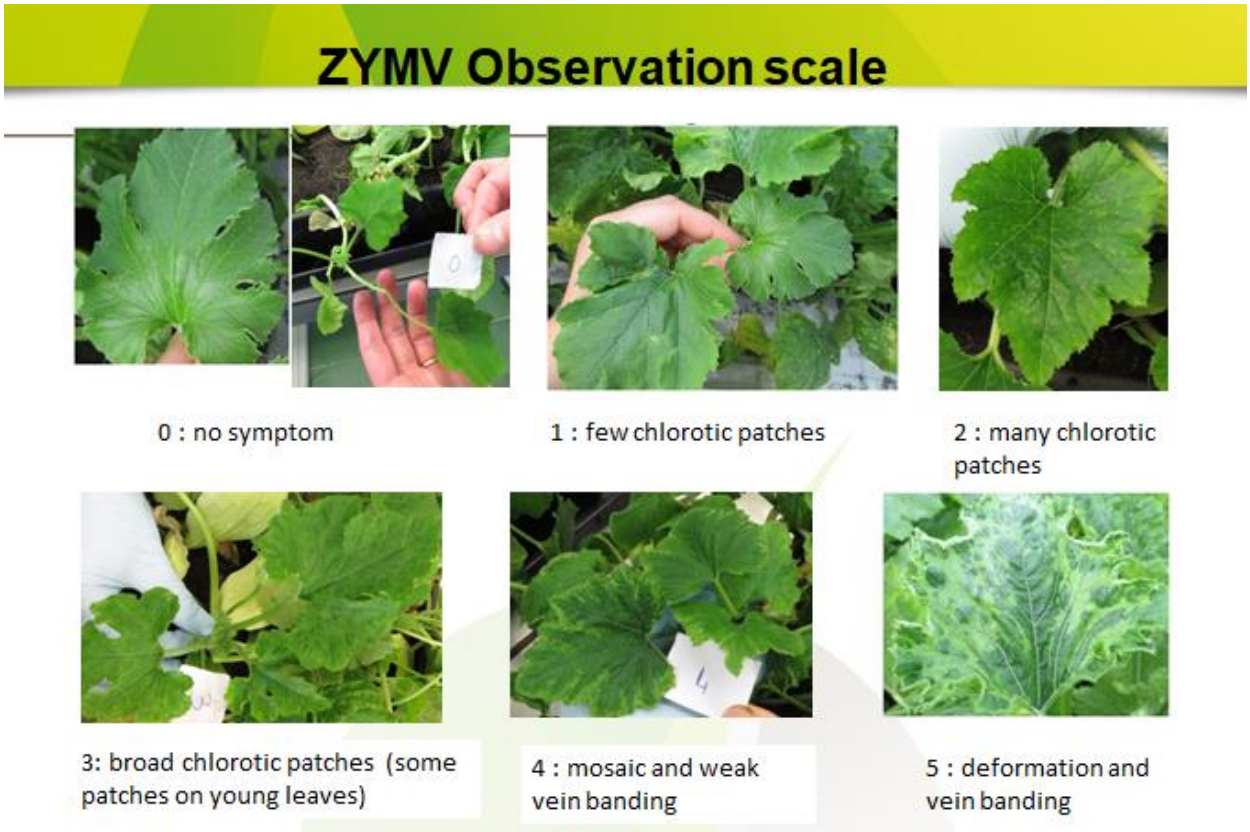
11.2	Observation scale	Class 0: no symptoms Class 1: few chlorotic *patches* *Class 2: many chlorotic patches* *Class 3: large chlorotic areas (some patches on young leaves)* *Class 4: mosaic and weak vein banding* Class 5: deformation and vein banding
11.3	Validation of test	Results should be compared with results of controls and are depending of the aggressiveness of the test and the distribution of the plants over the categories. The analysis of raw data of the couple ZYMV / Squash is planned in the Pathostat tool (free statistic analysis dedicated to quantitative disease resistances) https://pathostat.geves.fr
11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	Classes 0, 1 are commonly judged as resistant – Note 5 Classes 2, 3 are commonly judged as moderately resistant – Note 3 Classes 4 and 5 are commonly judged as susceptible – Note 1
13.	Critical control points	Recommended dates of notation should be adapted depending on expression of symptoms on controls. Environmental conditions can have an effect on the expression of symptoms over time. In this case a second notation could be necessary.



Proposal to add new Characteristic Characteristic 83 “Resistance to *Watermelon mosaic virus* (WMV)” at the end of the Table of Characteristics

	English	français	Deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
83.	**Resistance to *Watermelon mosaic virus* (WMV)**	**Resistance au *Watermelon mosaic virus* (WMV)**	**Resistenz gegen *Watermelon mosaic virus* (WMV)**	**Resistencia a *Watermelon mosaic virus* (WMV)**		
	susceptible	sensible	anfällig	sensible	Cora	1
	moderatly resistant	modérément résistante	mäßig resistent	moderadamente resistente	Sofia	3
	resistant	résistante	resistent	resistente	Mikonos, Syros	4

Proposal to add explanation Ad. 83 “Resistance to *Watermelon mosaic virus* (WMV)” in Chapter 8.2 “Explanations for individual characteristics”

Ad. 83: Resistance to *Watermelon mosaic virus* (WMV)

Proposed new wording

1.	Pathogen	*Watermelon mosaic virus* (WMV)
2.	Quarantine status	No
3.	Host species	*Cucurbita pepo* L.
4.	Source of inoculum	GEVES[2] (FR)
5.	Isolate	e.g. strain LL1A
6.	Establishment isolate identity	-
7.	Establishment pathogenicity	Symptoms on susceptible squash variety
8.	Multiplication inoculum	
8.1	Multiplication medium	Living plant
8.2	Multiplication variety	e.g. Cora
8.3	Plant stage at inoculation	-
8.4	Inoculation medium	-
8.5	Inoculation method	-
8.6	Harvest of inoculum	-
8.7	Check of harvested inoculum	-
8.8	Shelflife/viability inoculum	-
9.	Format of the test	
9.1	Number of plants per genotype	At least 20
9.2	Number of replicates	At least 2
9.3	Control varieties	susceptible: Cora moderately resistant: Sofia (moderate resistant control of lower level) moderately resistant: Mikonos or Syros (moderate resistant controls of higher level) Up to now, no complete resistance is identified. The two levels of intermediate resistant controls are necessary to validate the agressiveness of the test.
9.4	Test design	add non inoculated plants
9.5	Test facility	Climatic room or greenhouse
9.6	Temperature	e.g. 22°C or 24°C/18°C

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9.7	Light	12h-16h
9.8	Season	
9.9	Special measures	-
10.	Inoculation	
10.1	Preparation inoculum	1 g leaf with symptoms with 4mL of PBS with carborundum (400mg) and activated carbon (400mg) or similar buffer, homogenize
10.2	Quantification inoculum	-
10.3	Plant stage at inoculation	First expanded leave
10.4	Inoculation method	Rubbing with virus suspension
10.5	First observation	14 days post-inoculation
10.6	Second observation	-
10.7	Final observations	21 days post-inoculation
11.	Observations	
11.1	Method	Visual observation
11.2	Observation scale	Class 0: no symptoms Class 1: few chlorotic patches Class 2: many chlorotic patches Class 3: large chlorotic areas (some patches on young leaves) Class 4: mosaic, weak vein banding Class 5: deformation and vein banding
11.3	Validation of test	Results should be compared with results of controls and are depending of the aggressiveness of the test and the distribution of the plants over the categories. *The analysis of raw data of the couple WMV / Squash is planned in the Pathostat tool (free statistic analysis dedicated to quantitative disease resistances)* *https://pathostat.geves.fr*
11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	Classes 0, 1, 2 are commonly judged as resistant – Note 4 Classes 2, 3, 4 are commonly judged as intermediate resistant – Note 3 Classes 4 and 5 are commonly judged as susceptible – Note 1
13.	Critical control points	Recommended dates of notation should be adapted depending on expression of symptoms on controls. Environmental conditions can have an effect on the expression of symptoms over time. In this case a second notation could be necessary.

WMV Observation scale

0 : no symptom

1 : few chlorotic patches

2 : many chlorotic patches

3: broad chlorotic patches (some patches on young leaves)

4 : mosaic and weak vein banding

5 : deformation and vein banding

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