UPOV

International Union for the Protection of New Varieties of Plants

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Technical Working Party for Vegetables

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PARTIAL REVISION OF THE TEST GUIDELINES FOR VEGETABLE MARROW, SQUASH

Document prepared by the Office of the Union

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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-fifth session, hosted by Turkey and organized by electronic means, from May 3 to 7, 2021, agreed that the Test Guidelines for Vegetable Marrow, Squash (document TG/119/4 Corr. 2) be partially revised for the addition of new Characteristics "Resistance to *Zucchini yellow mosaic virus* (ZYMV)" and "Resistance to *Watermelon mosaic virus* (WMV)" (see document TWV/55/16 "Report", 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 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"

4. The proposed changes are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

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. **(+)**	**VS**	**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)**		
QN		susceptible	sensible	anfällig	sensible	Cora	1
		susceptible to intermediate resistant	sensible à résistant à un niveau intermédiaire	anfällig bis mittel resistent	sensible a resistencia intermedia	-	2*
		intermediate resistant	résistant à un niveau intermédiaire	mittel resistent	resistencia intermedia	Mirza	3
		intermediate resistant to resistant	résistant à un niveau intermédiaire à résistant	mittel resistent bis resistent	resistencia intermedia a resistente	-	4*
		highly resistant	hautement résistant	hochresistent	altamente resistente	Mikonos	5
							(*= restricted use suggested of this level. See Ad. 82, 12.)

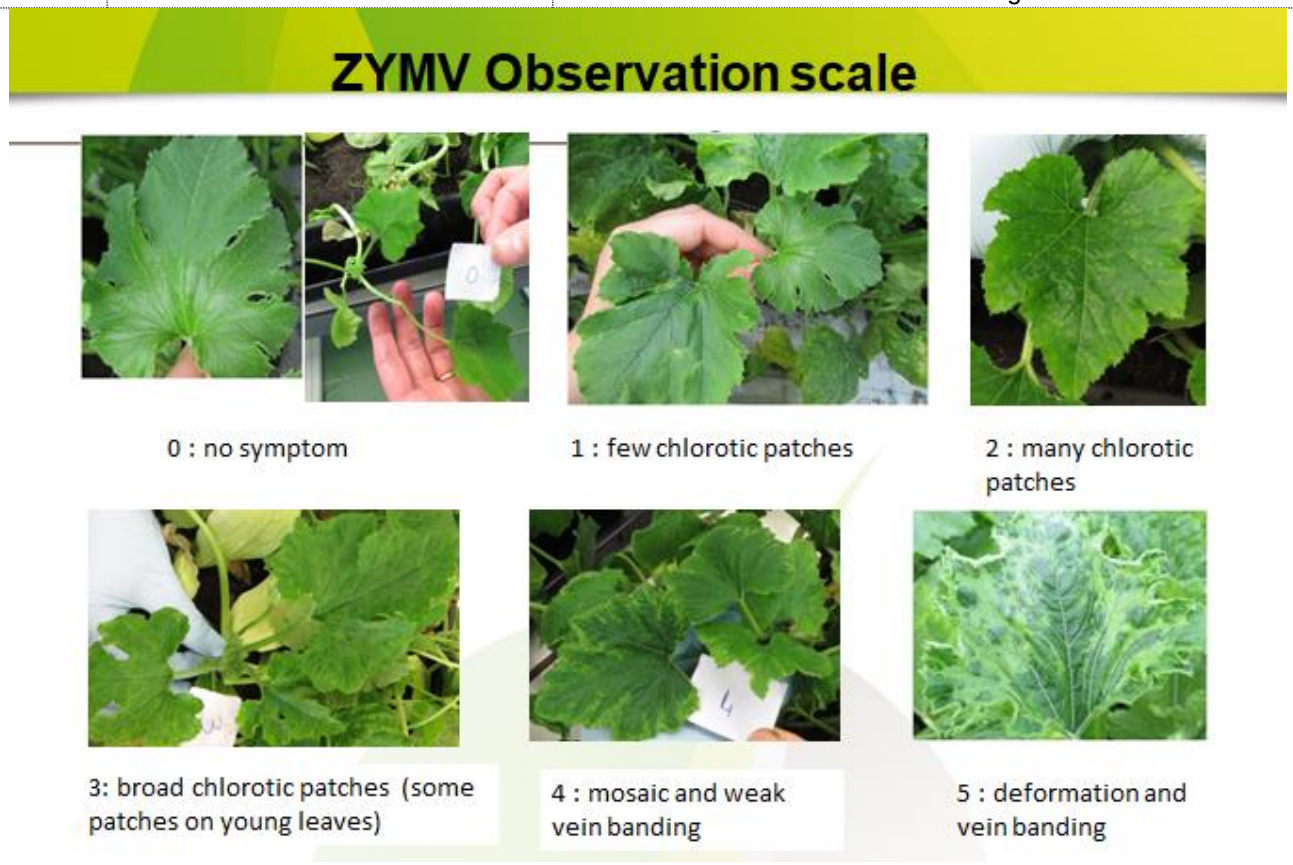
Proposed addition of an explanation Ad. 82 “Resistance to *Zucchini yellow mosaic virus* (ZYMV)” in Chapter 8.2 “Explanations for individual characteristics”

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

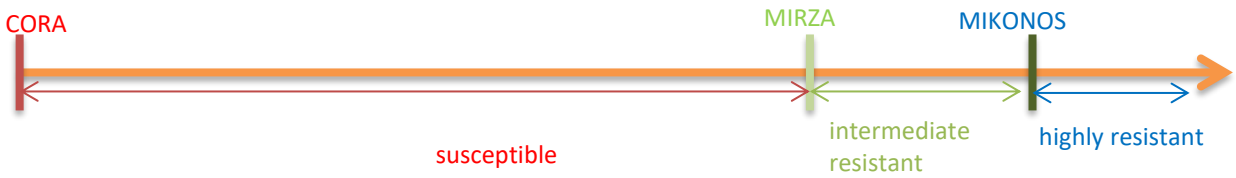
1.	Pathogen	*Zucchini yellow mosaic virus* (ZYMV)
2.	Quarantine status	No
3.	Host species	*Cucurbita pepo* L.
4.	Source of inoculum	GEVES (FR)[1]
5.	Isolate	e.g. strain E9 = MAT/REF/06-08-02-02 [1]
6.	Establishment isolate identity	-
7.	Establishment pathogenicity	Symptoms on susceptible squash variety
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	Shelf life/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

[1] matref@geves.fr

9.3	Control varieties	• susceptible: Cora • intermediate resistant: Mirza (low threshold of intermediate resistance level): • highly resistant: Mikonos (low threshold of high resistance level):
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	keep glasshouse free of aphids
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 cotyledons 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 and weak vein banding Class 5: deformation and vein banding



Courtesy of GEVES-SNES

11.3	Validation of test	The highly resistant control (Mikonos), the intermediate resistant control (Mirza) and the susceptible control (Cora) are necessary to validate the aggressiveness of test. Results should be compared with results of controls, based on disease index AND distribution of plants over the classes.
11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	- Note 1: Classes 4 and 5 are predominantly observed on susceptible plants. - Note 3: Classes 2, 3 are predominantly observed on intermediate resistant plants. - Note 5: Classes 0, 1 are predominantly observed on highly resistant plants. Notes 2 and 4 exist, but no control for these levels are commonly validated yet. In the framework of harmonisation of the produced descriptions for this new quantitative characteristic, we suggest concentrating the UPOV used notes to the notes 1, 3, and 5 only. A variety with a lower resistance than Mirza (note 3) will be described note 1. A variety with a lower resistance than Mikonos (note 5), will be described note 3. An additional statistical analysis can be used to finalize the pathologist's raw observation to the assessment of uniformity, and relative position regarding the controls results.
		
13.	Critical control points	Recommended dates of notation should be adapted depending on expression of symptoms on controls. Environmental conditions can influence the expression of symptoms over time. In this case a second notation could be necessary. Aphids may transmit ZYMV as well as other viruses that may contaminate the ZYMV strain. Test should be in aphid-free compartment.

Proposed addition of new 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. **(+)**	**VS**	**Resistance to *Watermelon mosaic virus* (WMV)**	**Resistance au *Watermelon mosaic virus* (WMV)**	**Resistenz gegen *Watermelon mosaic virus* (WMV)**	**Resistencia a *Watermelon mosaic virus* (WMV)**		
QN		susceptible	sensible	anfällig	sensible	Cora	1
		susceptible to intermediate resistant	sensible à résistant à un niveau intermédiaire	anfällig bis mittel resistent	sensible a resistencia intermedia	-	2*
		intermediate resistant	résistant à un niveau intermédiaire	mittel resistent	resistencia intermedia	Sofia	3
		intermediate resistant to resistant	résistant à un niveau intermédiaire à résistant	mittel resistent bis resistent	resistencia intermedia a resistente	Mikonos, Syros	4
		highly resistant	hautement résistant	hochresistent	altamente resistente	-	5*
						(*= restricted use suggested of this level. See Ad. 83, 12.)	

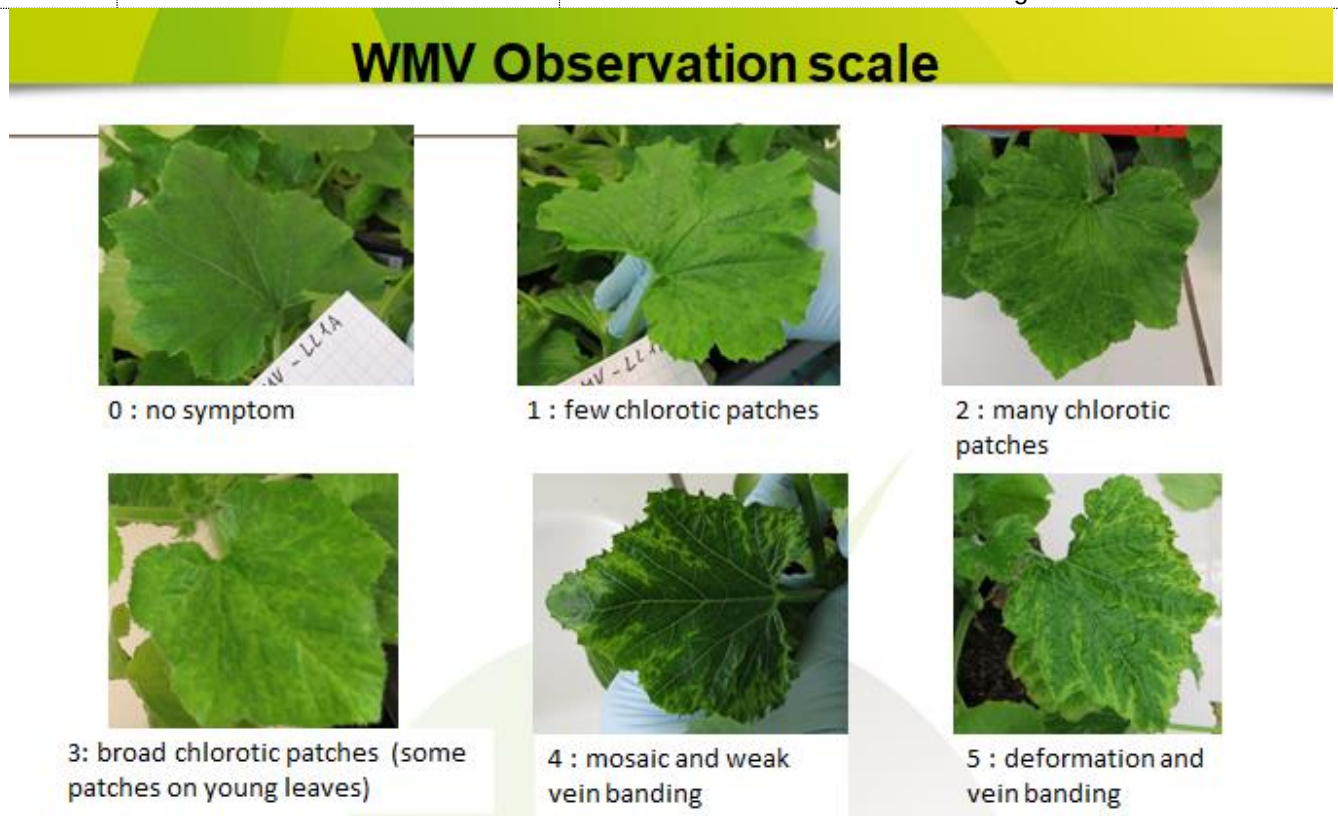
Proposed addition of an explanation Ad. 83 “Resistance to *Watermelon mosaic virus* (WMV)” in Chapter 8.2 “Explanations for individual characteristics”

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

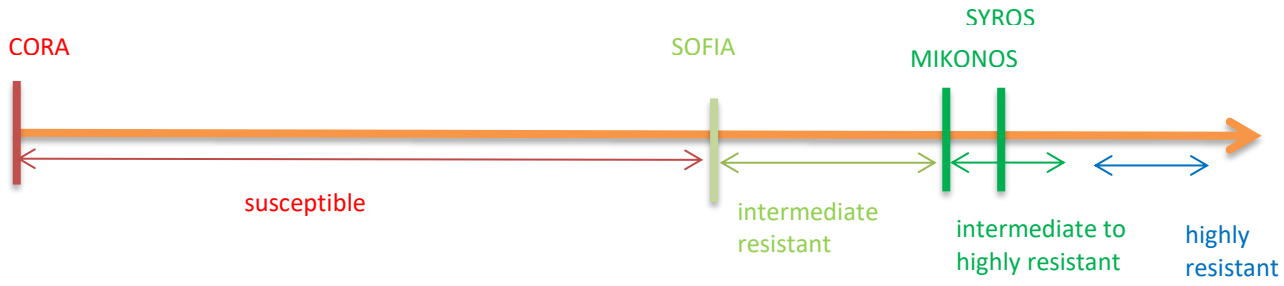
1.	Pathogen	*Watermelon mosaic virus* (WMV)
2.	Quarantine status	No
3.	Host species	*Cucurbita pepo* L.
4.	Source of inoculum	GEVES (FR)[2]
5.	Isolate	e.g., strain LL1A = MAT/REF/06-09-01 [2]
6.	Establishment isolate identity	-
7.	Establishment pathogenicity	Symptoms on susceptible squash variety
8.	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	Shelf life/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

[2] matref@geves.fr

9.3	Control varieties	• susceptible: Cora • intermediate resistant - Sofia (low threshold level) - Mikonos, Syros = intermediate resistant to highly resistant (intermediate resistant controls of higher level
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	Keep glasshouse free of aphids
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 cotyledons 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



Courtesy of GEVES-SNES

11.3	Validation of test	The controls for two levels of intermediate resistance and the susceptible control are necessary to validate the aggressiveness of the test. Results should be compared with the results of controls, based on disease index AND distribution of plants over the classes.
11.4	Off-types	-
12.	Interpretation of data in terms of UPOV characteristic states	- Note 1: Classes 4 and 5 are predominantly observed on susceptible plants. - Note 3: Classes 2, 3, 4 are predominantly observed on intermediate resistant plants. - Note 4: Classes 0, 1, 2, 3 are predominantly observed on plants with a higher level of intermediate resistance (intermediate to resistant level). Up to now, no complete resistance is identified against this virus. It is the reason why no example variety is provided to illustrate the Note 5. Note 2 exists, but no control for this level is commonly validated yet. In the framework of harmonisation of the produced descriptions for this new quantitative characteristic, we suggest concentrating the UPOV used notes to the notes 1, 3, and 4 only. A variety with a lower of resistance than Sofia (note 3), will be described note 1. A variety with a lower resistance than Mikonos or-Syros (note 4), will be described as note 3. An additional statistical analysis can be used to finalize the pathologist's raw observation to the assessment of uniformity, and relative position regarding the controls results.
		
13.	Critical control points	Recommended dates of notation should be adapted depending on expression of symptoms on controls. Environmental conditions can influence the expression of symptoms over time. In this case a second notation could be necessary. Aphids may transmit WMV as well as other viruses that may contaminate the WMV strain. Test should be in aphid-free compartment.

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