

Technical Working Party for Vegetables

TWV/53/7

Fifty-Third Session

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PARTIAL REVISION OF THE TEST GUIDELINES FOR TOMATO

*Document prepared by experts from the Netherlands**Disclaimer: this document does not represent UPOV policies or guidance*

1. The purpose of this document is to present a proposal for a partial revision of the Test Guidelines for Tomato (document TG/44/11 Rev.).
2. The Technical Working Party for Vegetables (TWV), at its fifty-second session, held in Beijing, China, from September 17 to 21, 2018, agreed that the Test Guidelines for Tomato (document TG/44/11 Rev.) be partially revised for Characteristics 48 “Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)” and 53 “Resistance to *Pyrenochaeta lycopersici* (PI)” (see document TWV/52/20 “Report”, Annex V).
3. The TWV, at its fifty-second session, considered documents TWV/52/11 and TWV/52/19 and agreed that Characteristic and Ad. 48 “Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)” be excluded from the partial revision presented in document TWV/52/11 “Matters to be resolved concerning Test Guidelines adopted by the Technical Committee: partial revision of the test guidelines for tomato” as research was ongoing and agreed that this characteristic should be reconsidered by the TWV at its fifty-third session (see document TWV/52/20 “Report”, paragraph 62).
4. The following changes are proposed:
 - (a) To change the example varieties and to change the denomination of the races of Characteristics 48.1, 48.2 and 48.3 “Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)”;
 - (b) To change the explanation Ad. 48 by adding an alternative method to observe the resistance and by minor changes in the current method in Chapter 8.2 “Explanations for individual characteristics”;
 - (c) To change the example varieties of Characteristic 53 “Resistance to *Pyrenochaeta lycopersici* (PI)”;
 - (d) To change the explanation Ad. 53 in Chapter 8.2 “Explanations for individual characteristics”
5. The proposed changes are presented below in highlight and underline (insertion) and ~~strikethrough~~ (deletion).

Proposal to change the example varieties and to change the denomination of the races of Characteristics 48.1, 48.2 and 48.3 "Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)"

Current wording

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
48. (+)	VG Resistance to <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Résistance à <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Resistenz gegen <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Resistencia a <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)		
48.1 (*)	VG – Race 0 (ex 1)	– Pathotype 0 (ex 1)	– Pathotyp 0 (ex 1)	– Raza 0 (ex 1)		
QL	absent	absente	fehlend	ausente	Marmande verte	1
	present	présente	vorhanden	presente	Anabel, Marporum, Marsol	9
48.2 (*)	VG – Race 1 (ex 2)	– Pathotype 1 (ex 2)	– Pathotyp 1 (ex 2)	– Raza 1 (ex 2)		
QL	absent	absente	fehlend	ausente	Marmande verte	1
	present	présente	vorhanden	presente	Motelle, Walter	9
48.3	VG – Race 2 (ex 3)	– Pathotype 2 (ex 3)	– Pathotyp 2 (ex 3)	– Raza 2 (ex 3)		
QL	absent	absente	fehlend	ausente	Marmande verte, Motelle	1
	present	présente	vorhanden	presente	Alliance, Florida, Ivanhoé, Tributes	9

Proposed new wording

	English	français	deutsch	español	Example Varieties Exemples Beispielssorten Variedades ejemplo	Note/ Nota
48. (+)	VG Resistance to <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Résistance à <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Resistenz gegen <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)	Resistencia a <i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i> (Fol)		
48.1 (*)	VG – Race 0 (ex 1) <u>0EU/1US</u>	– Pathotype 0 (ex 1) <u>0EU/1US</u>	– Pathotyp 0 (ex 1) <u>0EU/1US</u>	– Raza 0 (ex 1) <u>0 EU/1US</u>		
QL	absent	absente	fehlend	ausente	Marmande verte, <u>MoneyMaker</u>	1
	present	présente	vorhanden	presente	Anabel, Marporum, Marsol, <u>Motelle, Tradiro</u>	9
48.2 (*)	VG – Race 1 (ex 2) <u>1EU/2US</u>	– Pathotype 1 (ex 2) <u>1EU/2US</u>	– Pathotyp 1 (ex 2) <u>1EU/2US</u>	– Raza 1 (ex 2) <u>1EU/2US</u>		
QL	absent	absente	fehlend	ausente	Marmande verte, <u>Marporum, MoneyMaker</u>	1
	present	présente	vorhanden	presente	Motelle, <u>Walter Tradiro</u>	9
48.3	VG – Race 2 (ex 3) <u>2EU/3US</u>	– Pathotype 2 (ex 3) <u>2EU/3US</u>	– Pathotyp 2 (ex 3) <u>2EU/3US</u>	– Raza 2 (ex 3) <u>2EU/3US</u>		
QL	absent	absente	fehlend	ausente	Marmande verte, Motelle	1
	present	présente	vorhanden	presente	Alliance, Florida, <u>Ivanhoé Ivanhoé</u> , Tributes	9

Proposal to change the explanation Ad. 48 by adding an alternative method to observe the resistance and by minor changes in the current method in Chapter 8.2 “Explanations for individual characteristics”

Current wording

Ad. 48: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)

1. Pathogen *Fusarium oxysporum* f. sp. *lycopersici*
 3. Host species..... *Solanum lycopersicum*
 4. Source of inoculum..... Naktuinbouw¹ (NL) and GEVES² (FR)
 5. Isolate Race 0 (ex 1) (e.g. strains Orange 71 or PRI 20698 or Fol 071 1 (ex 2) (e.g. strains 4152 or PRI40698 or RAF 70 and 2 (ex 3) individual strains may vary in pathogenicity
 6. Establishment isolate identity..... use differential varieties (see 9.3)
 7. Establishment pathogenicity..... on susceptible tomato varieties
 8. Multiplication inoculum
 8.1 Multiplication medium Potato Dextrose Agar, Medium “S” of Messiaen
 8.4 Inoculation medium water for scraping agar plates or Czapek-Dox culture medium (7 d-old aerated culture)
 8.6 Harvest of inoculum..... filter through double muslin cloth
 8.7 Check of harvested inoculum..... spore count; adjust to 10⁶ per ml
 8.8 Shelf-life/viability inoculum 4-8 h, keep cool to prevent spore germination
 9. Format of the test
 9.1 Number of plants per genotype..... at least 20 plants
 9.2 Number of replicates 1 replicate
 9.3 Control varieties for the test with race 0 (ex 1)
 Susceptible..... Marmande, Marmande verte, Resal
 Resistant for race 0 only..... Marporum, Larissa, “Marporum x Marmande verte”, Marsol, Anabel
 Resistant for race 0 and 1..... Motelle, Gourmet, Mohawk
 Control varieties for the test with race 1 (ex 2)
 Susceptible..... Marmande verte, Cherry Belle, Roma
 Resistant for race 0 only Marporum, Ranco
 Resistant for race 0 and 1..... Tradiro, Odisea
 Remark: Ranco is slightly less resistant than Tradiro
 Control varieties for the test with race 2 (ex 3)
 Susceptible for race 0, 1 and 2 Marmande verte, Motelle, Marporum
 Resistant for race 0, 1 and 2..... Tributes, Murdoch, Marmande verte x Florida
 9.4 Test design..... >20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
 9.5 Test facility glasshouse or climate room
 9.6 Temperature..... 24-28°C (severe test, with mild isolate)
 20-24°C (mild test, with severe isolate)
 9.7 Light 12 hours per day or longer
 9.8 Season all seasons
 9.9 Special measures slightly acidic peat soil is optimal; keep soil humid but avoid water stress
 10. Inoculation
 10.1 Preparation inoculums aerated Messiaen or PDA or Agar Medium S of Messiaen or Czapek Dox culture or scraping of plates
 10.2 Quantification inoculums..... pore count, adjust to 10⁶ spores per ml, lower concentration for a very aggressive isolate
 10.3 Plant stage at inoculation..... 10-18 d, cotyledon to first leaf
 10.4 Inoculation method roots and hypocotyls are immersed in spore suspension for 5-15 min; trimming of roots is an option
 10.7 Final observations 14-21 days after inoculation
 11. Observations
 11.1 Method visual
 11.2 Observation scale..... symptoms: growth retardation, wilting, yellowing, vessel browning extending above cotyledon
 11.3 Validation of test..... evaluation of variety resistance should be calibrated with results of resistant and susceptible controls. Standards near borderline R/S will help to compare between labs.
 12. Interpretation of test results in comparison with control varieties
 absent..... [1] severe symptoms
 present..... [9] mild or no symptoms
 13. Critical control points
 Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature.

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Proposed new wording

Ad. 48: Resistance to *Fusarium oxysporum* f. sp. *lycopersici* (Fol)

Resistance to race 0EU/1US and race 1EU/2US to be tested in a bio-assay (method i) or in a DNA marker test (method ii), if appropriate. Resistance to race 2EU/3US to be tested in a bio-assay (method i).

(i) Bio-assay

1.	Pathogen	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>
3.	Host species	<i>Solanum lycopersicum</i>
4.	Source of inoculum	Naktuinbouw ³ (NL), GEVES ⁴ (FR) or INIA ⁵ (ES)
5.	Isolate	race 0 (ex 1) 0EU/1US (e.g. strains Orange 71 or PRI 20698 or Fol 071) race 1 (ex 2) 1EU/2US (e.g. strains 4152 or PRI40698 or RAF 70) race 2 (ex 3) 2EU/3US (e.g. strain Fol029) individual strains may vary in pathogenicity
6.	Establishment isolate identity	use differential varieties (see 9.3)
7.	Establishment pathogenicity	on susceptible tomato varieties
8.	Multiplication inoculum	
8.1	Multiplication medium	Potato Dextrose Agar, Medium "S" of Messiaen
8.4	Inoculation medium	water for scraping agar plates or Czapek-Dox culture medium (7 d-old aerated culture)
8.6	Harvest of inoculum	filter through double muslin cloth
8.7	Check of harvested inoculum	spore count; adjust to 10 ⁶ per ml
8.8	Shelflife/viability inoculum	4-8 h, keep cool to prevent spore germination
9.	Format of the test	
9.1	Number of plants per genotype	at least 20 plants
9.2	Number of replicates	1 replicate
9.3.1	Control varieties for the test with race 0 (ex 1) 0EU/1US	
	Susceptible	Marmande, Marmande verte, Resal, <u>Money maker</u>
	Resistant for race 0 only	Marporum, Larissa, "Marporum x Marmande verte", <u>Marsol</u> , <u>Anabel</u> , <u>Motelle</u> , <u>Gourmet</u> , <u>Mohawk</u> , <u>Tradiro</u>
	Resistant for race 0 and 1	<u>Motelle</u> , <u>Gourmet</u> , <u>Mohawk</u>
9.3.2	Control varieties for the test with race 1 (ex 2) 1EU/2US	
	Susceptible	Marmande verte, Cherry Belle, Roma, <u>Marporum</u> , <u>Ranco</u> , <u>Money maker</u>
	Resistant for race 0 only	<u>Marporum</u> , <u>Ranco</u>
	Resistant for race 0 and 1	Tradiro, Odisea, " <u>Motelle x Marmande verte</u> ", <u>Motelle</u>
9.3.3	Control varieties for the test with race 2 (ex 3) 2EU/3US	
	Susceptible for race 0, 1 and 2	Marmande verte, Motelle, Marporum
	Resistant for race 0, 1 and 2	Tributes, Murdoch, "Marmande verte x Florida", <u>Alliance</u> , <u>Florida</u> , <u>Ivanhoe</u>
9.4	Test design	>20 plants; e.g. 35 seeds for 24 plants, including 2 blanks
9.5	Test facility	glasshouse or climate room
9.6	Temperature	24-28°C (severe test, with mild isolate) 20-24°C (mild test, with severe isolate)

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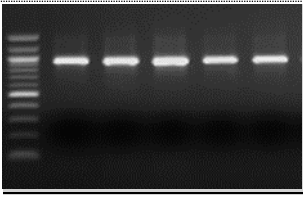
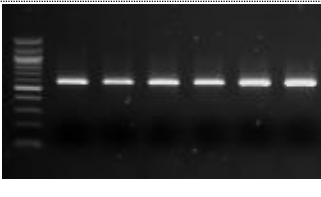
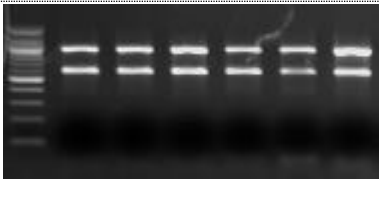
9.7	Light	12 hours per day or longer
9.8	Season	all seasons
9.9	Special measures	slightly acidic peat soil is optimal; keep soil humid but avoid water stress
10.	Inoculation	
10.1	Preparation inoculum	aerated Messiaen or PDA or Agar Medium S of Messiaen or Czapek Dox culture or scraping of plates
10.2	Quantification inoculum	spore count, adjust to 10^6 spores per ml, lower concentration for a very aggressive isolate
10.3	Plant stage at inoculation	10-18 d, cotyledon to first leaf
10.4	Inoculation method	roots and hypocotyls are immersed in spore suspension for 5-15 min; trimming of roots is an option
10.7	Final observations	14-21 days after inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	symptoms: growth retardation, wilting, yellowing, vessel browning extending above cotyledon
11.3	Validation of test	evaluation of variety resistance should be calibrated with results of resistant and susceptible controls. Standards near borderline R/S will help to compare between labs.
12.	Interpretation of test results in comparison with control varieties	
	absent	[1] severe symptoms
	present	[9] mild or no symptoms
13.	Critical control points	Test results may vary slightly in inoculum pressure due to differences in isolate, spore concentration, soil humidity and temperature.

(ii) DNA marker test

Dominant resistance gene I2 is always associated with resistance to both race 0EU/1US and race 1EU/2US. The presence or absence of the resistance allele can be detected by the co-dominant marker as described in this method.

1.	<u>Pathogen</u>	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>
2.	<u>Functional gene</u>	<u>I2</u>
3.	<u>Primers</u>	
3.1	<u>Allele for susceptibility</u>	<u>Z1063-i2-F 5'-GTT TGA CAG CTT GGT TTT GT-3'</u> <u>Z1063-i2-R 5'-CTC AAA CTC ACC ATC ATT GA-3'</u>
3.2	<u>Allele for resistance</u>	<u>TFusF1 5'-CTG AAA CTC TCC GTA TTT C-3'</u> <u>TFusRR1 5'-CGA AGA GTG ATT GGA GAT-3'</u>
4.	<u>Format of the test</u>	
4.1	<u>Number of plants per genotype</u>	<u>at least 20 plants</u>
4.2	<u>Control varieties</u>	<u>homozygous allele for susceptibility present: Marmande verte, Marporum, Moneymaker</u> <u>homozygous allele for resistance present: Motelle, Tradiro</u> <u>add blanks as negative PCR control</u>
5.	<u>Preparation</u>	
5.1	<u>Preparation DNA</u>	<u>harvest per individual plant a part of a young leaf. Isolate total DNA of each individual plant separately with a standard DNA isolation protocol (CTAB/SDS based). Re-suspend in 100 μl T₁₀E_{0.1} or another suitable buffer. Dilute total DNA to 1/10 (H₂O) to obtain a DNA concentration between 1-10 ng/μl.</u>

5.2	<u>Preparation PCR</u>	<p>for example, use 3 µl of each diluted DNA sample into individuals PCR reactions. prepare the PCR master mix, 20µl reaction volume, for example:</p> <ul style="list-style-type: none"> • 3 µl of 10x diluted DNA • 2,5 µl of 10x reaction buffer • 2 mM MgCl₂ • 0.1 µM of resistance primers each • 0.2 µM of susceptible primers each • 200 µM of each of the four dNTPs • 1 unit of Taq DNA polymerase
6.	<u>PCR conditions</u>	<p>for example:</p> <ol style="list-style-type: none"> 1. initial denaturation step at 94°C for 3 minutes 2. 35 cycles at 94°C for 1 minute, 56°C for 1 minute, and 72°C for 2 minutes 3. final extension step of 72°C for 10 minutes
7.	<u>Observations</u>	
7.1	<u>Method</u>	visual, after detection on gel together with a suitable ladder
7.2	<u>Observation scale</u>	

		
<p>amplicon of 940bp only homozygous susceptible allele present</p>	<p>amplicon of 600bp only homozygous resistant allele present</p>	<p>amplicons of 940bp and 600bp susceptible and resistant allele present: heterozygous resistant</p>

7.3	<u>Validation of test</u>	control varieties should give the expected band(s).
8.	<u>Interpretation of test results</u>	
	<u>48.1 Resistance to race 0EU/1US</u>	
	<u>absent</u>	[1] can not be concluded from the DNA-test, a bio-assay should be performed.
	<u>present</u>	[9] homozygous resistant or heterozygous resistant in DNA marker test. In case the DNA marker test result does not confirm the morphological declaration in the TQ, a bio-assay should be performed to observe whether the variety is resistant e.g. on another mechanism like gene I2 without I.
	<u>48.2 Resistance to race 1EU/2US</u>	
	<u>absent</u>	[1] homozygous susceptible in DNA marker test
	<u>present</u>	[9] homozygous or heterozygous resistant in DNA marker test. In case the DNA marker test result does not confirm the morphological declaration in the TQ, a bio-assay should be performed to observe whether the variety is resistant e.g. on another mechanism like gene I3.

Proposal to change the example varieties of Characteristic 53 "Resistance to *Pyrenochaeta lycopersici* (PI)"

Current wording

53. (+)	VG	Resistance to <i>Pyrenochaeta lycopersici</i> (PI)	Résistance à <i>Pyrenochaeta lycopersici</i> (PI)	Resistenz gegen <i>Pyrenochaeta lycopersici</i> (PI)	Resistencia a <i>Pyrenochaeta lycopersici</i> (PI)		
QL		absent	absente	fehlend	ausente	Montfavet H 63.5	1
		present	présente	vorhanden	presente	Kyndia, Moboglan, Pyrella	9

Proposed new wording

53. (+)	VG	Resistance to <i>Pyrenochaeta lycopersici</i> (PI)	Résistance à <i>Pyrenochaeta lycopersici</i> (PI)	Resistenz gegen <i>Pyrenochaeta lycopersici</i> (PI)	Resistencia a <i>Pyrenochaeta lycopersici</i> (PI)		
QL		absent	absente	fehlend	ausente	Montfavet H 63.5 <u>Marmande verte</u>	1
		present	présente	vorhanden	presente	Kyndia, Moboglan, Pyrella <u>Garance</u>	9

Proposal to change the explanation Ad. 53 in Chapter 8.2 "Explanations for individual characteristics"*Current wording*Ad. 53: Resistance to *Pyrenochaeta lycopersici* (PI)

1. Pathogen *Pyrenochaeta lycopersici*
3. Host species *Solanum lycopersicum*
4. Source of inoculum -
5. Isolate -
7. Establishment pathogenicity biotest
8. Multiplication inoculum
 - 8.1 Multiplication medium V8 Agar
 - 8.2 Multiplication variety susceptible tomato variety
 - 8.3 Plant stage at inoculation seed
 - 8.4 Inoculation medium mixture of soil, e.g. (70%), sand (20%) and inoculum (10.1) (10%) or soil mixed with diseased roots cut to small pieces
 - 8.5 Inoculation method sowing, or transplanting at fruit maturity
 - 8.6 Harvest of inoculum diseased roots are harvested after 2-4 months
 - 8.7 Check of harvested inoculum visual inspection of lesions on roots
 - 8.8 Shelf-life/viability inoculum the fungus will not die quickly, but may lose its pathogenicity within a week after isolation on an agar medium
9. Format of the test
 - 9.1 Number of plants per genotype 20 plants
 - 9.2 Number of replicates 1 replicate
 - 9.3 Control varieties
 - Susceptible Montfavet H 63.5
 - Resistant Kyndia, Moboglan, Pyrella
 - 9.5 Test facility greenhouse or climate cell
 - 9.6 Temperature day 24°C, night 14°C
 - 9.7 Light 12 h minimum
10. Inoculation
 - 10.1 Preparation inoculum e.g. double-autoclaved mixture of soil with 10% oatmeal added e.g. Incubate for 10-14 d at 20°C with occasional, repeated turning
 - 10.3 Plant stage at inoculation 6 weeks
 - 10.4 Inoculation method transplanting into mixture of soil, sand and inoculum (8.4) or soil mixed with diseased roots cut to small pieces or naturally infected soil
 - 10.7 Final observations 6-8 weeks after transplanting (flowering plant)
11. Observations
 - 11.1 Method visual
 - 11.2 Observation scale symptoms: brown lesions on roots
 - 11.3 Validation of test evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12. Interpretation of test results in comparison with control varieties

absent [1]	symptoms
present [9]	no symptoms
13. Critical control points:
The fungus loses its pathogenicity quickly after isolation on an agar medium. It is advisable to keep the isolate alive on living plants.

Proposed new wording

Ad. 53: Resistance to *Pyrenochaeta lycopersici* (PI)

1.	Pathogen	<i>Pyrenochaeta lycopersici</i>
2.	Quarantine status	No
3.	Host species	<i>Solanum lycopersicum</i>
4.	Source of inoculum	GEVES ⁶ (FR)
5.	Isolate	e.g. strain PI 21
6.	Establishment isolate identity	On susceptible plant
8.	Multiplication inoculum	
8.1	Multiplication medium	Messiaen agar or synthetic medium
8.4	Inoculation medium	Autoclaved grains (e.g. barley)
8.5	Inoculation method	Mix of contaminated grains (e.g. 1 kg) with inoculum (e.g. medium from 2 Petri dishes with mycelium)
8.6	Harvest of inoculum	After 3 weeks
9.	Format of the test	
9.1	Number of plants per genotype	At least 20
9.2	Number of replicates	1 replicate
9.3	Control varieties	Susceptible : Marmande verte Resistant : Garance and (<i>S. lycopersicum</i> x <i>habrochaites</i>) Emperador
9.4	Test design	add non inoculated plants
9.5	Test facility	Greenhouse or climatic chamber
9.6	Temperature	20°C
9.7	Light	At least 12h
10.	Inoculation	
10.1	Preparation inoculum	Homogenize the contaminated grains
10.3	Plant stage at inoculation	3-4 leaf stage
10.4	Inoculation method	Transplanting of plantlets in a mixture of soil (e.g. 3750 ml of soil with 750 ml of inoculum)
10.7	Final observations	40 days post inoculation
11.	Observations	
11.1	Method	visual
11.2	Observation scale	Class 0: no necrotic lesion on roots Class 1: few small and uncoloured necrotic lesions Class 2: some brown necrotic lesions clearly visible (less than half the surface of the pivot) Class 3: several brown necrotic lesions clearly visible (more than half the surface of the pivot) Class 4: complete necrosis or destruction of the pivot
11.3	Validation of test	Evaluation of variety resistance should be calibrated with results of resistant and susceptible controls
12.	Interpretation of data in terms of UPOV characteristic states	Any variety judged to be of the same resistance level or higher than Garance is judged as resistant. Classes 0, 1 and 2 are commonly judged as resistant – Note 9 Classes 3 and 4 are commonly judged as susceptible – Note 1

[End of document]

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