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INTERNATIONAL UNION FOR THE PROTECTION OF NEW VARIETIES OF PLANTS
Geneva

TECHNICAL WORKING PARTY FOR VEGETABLES

Forty-Sixth Session
near the city of Venlo, Netherlands, June 11 to 15, 2012

PARTIAL REVISION OF THE TEST GUIDELINES FOR LETTUCE
(DOCUMENT TG/13/10 REV.)

Document prepared by experts from Japan

1 The Technical Working Party for Vegetables (TWV), at its forty-fifth session, held in Monterey, California, United States of America, from July 25 to 29, 2011 agreed to propose a partial revision of the Test Guidelines for Lettuce concerning resistance to *Fusarium* and to lettuce big-vein disease.

2 It is proposed to make a partial revision of the Test Guidelines for Lettuce (document TG/13/10 Rev.) in order to include:

(a) Resistance to *Fusarium oxysporum* f.sp*. lactucae* Race 1 and 2
(b) Resistance to lettuce big-vein disease (*Olpidium brassicae* carrying Mirafiori Lettuce Big-Vein Virus(MLBVV) and/or Lettuce Big-Vein associated Virus)

3 Annex I to this document contains the proposed partial revisions of the Test Guidelines for Lettuce.

[Annex I follows]

ANNEX

PROPOSED PARTIAL REVISION OF THE TEST GUIDELINES FOR LETTUCE
(DOCUMENT TG/13/11)

new 42. **(+)**	**MS/ VG**	**Resistance to *Fusarium oxysporum f.sp. lactucae***		
42.1	**MS/ VG**	**-Race 1**		
QL		susceptible	Patriot , Salinas	1
		resistant	Costa Rica No.4 Romasol'	9
42.2	**MS/ VG**	**-Race 2**		
QL		susceptible	Patriot	1
		resistant	Banchu red fire	9
43. **(+)**	**MS/ VG**	**Resistance to lettuce big-vein disease (*Olpidium brassicae* carrying Mirafiori Lettuce Virus and/or Lettuce Big-Vein Associated Virus)**		
QN		susceptible	Cisco	3
		moderately resistant	Logic	5
		highly resistant	Antore 411	7

Ad 42. Resistance to Fusarium oxysporum f.sp. lactucae (Fol)

* 1. Pathogen	Fusarium oxysporum f.sp. lactucae
2. Quarantine status	EPPO alert list
* 3. Host species	Lactuca sativa L.
* 4. Source of inoculum	NIAS Genebank, INRAN, Naktuinbouw, GEVES
* 5. Isolate	Fol : 1 and Fol : 2
6. Establishment isolate identity	Use microscope and inoculation to lettuce susceptible standard
7. Establishment pathogenicity	Use lettuce susceptible standard
8. Multiplication inoculum	
8.1 Multiplication medium	Inoculation by sowing on contaminated soil: Wheat bran-soil medium. Inoculation by soaking seedlings: on synthetic liquid medium (e.g. Potatoes Dextrose Broth)
8.2 Multiplication variety	
8.3 Plant stage at inoculation	See 10.3
8.4 Inoculation medium	
8.5 Inoculation method	See 10.4
8.6 Harvest of inoculum	Inoculation by sowing on contaminated soil: 7-10 day-old culture Inoculation by soaking seedlings: 15 days
8.7 Check of harvested inoculum	
8.8 Shelf life/viability inoculum	
9. Format of the test	
* 9.1 Number of plants per genotype	20 plants
* 9.2 Number of replicates	
* 9.3 Control varieties	
Susceptible	Patriot
Resistant to Fol:1	Costa Rica No.4, Romasol
Resistant to Fol:2	Banchu red fire
* 9.4 Test design	Include control varieties
9.5 Test facility	Greenhouse or climate room
9.6 Temperature	20-28 ^{o}C
9.7 Light	Under natural day length
9.8 Season	
9.9 Special measures	
10. Inoculation	
10.1 Preparation inoculum	Inoculation by sowing on contaminated soil: Wheat bran-soil medium culture are mixed with sterilized soil Inoculation by soaking seedlings: soaking of roots and of hypocotyls axis for 5 to 15 min in the inoculums suspension and transplantation of inoculated plantlets in soil
10.2 Quantification inoculum	Inoculation by sowing on contaminated soil: soil: culture =20: 1 Inoculation by soaking seedlings: spores are harvested and adjusted to 10^{7} sp/mL
*10.3 Plant stage at inoculation	Inoculation by sowing on contaminated soil: seeds stimulated to emerge Inoculation by soaking seedlings: cotyledons
*10.4 Inoculation method	Two methods can be use for inoculation : by sowing seeds to contaminated soil or by soaking seedlings
10.5 First observation	After 7- 10 days from inoculation
10.6 Second observation	14 days from inoculation
*10.7 Final observations	20-25 days after inoculation (sowing or soaking)
11. Observations	

*11.1 Method	Visual and/or counting number of plants with symptom
*11.2 Observation scale	Inoculation by sowing on contaminated soil: Symptoms: stunting, wilting, dead plant As reference calculate of Disease Severity Index (DSI) and Disease Incidence(DI) 0: healthy 1: slightly stunting, growing reduction 2: severely stunting 3: die DSI = (0A + 1B + 2C + 3D) / (A + B + C + D) *A ~ D: number of plants of each category DI = (0A + 1B + 2C + 3D) *100/ ((A + B + C + D)*3) Inoculation by soaking seedlings: Symptoms: growth reduction and brown vessels above cotyledons, dead plant
*11.3 Validation of test	Analysis of results should be calibrated with results of controls
11.4 Off-types	
*12. Interpretation of data	Inoculation by sowing on contaminated soil: Susceptible: severely stunting, wilting, dead plant (DSI :Relative evaluation to DSI of example variety) (Race1:DI Value is higher than 10%) Resistant: no stunting, no wilting (DSI :Relative evaluation to DSI of example variety), (Race1:DI Value is lower than 10%) Inoculation by soaking seedlings: susceptible: growth reduction and brown vessels above cotyledons, dead plant Resistant: no growth reduction and no brown vessels above cotyledons
13. Critical control points:	Avoid rotting seeds Plant stage at inoculation: inoculation of 30 day old plants can change the results from susceptible to resistant

Race 1 and 2:

NIAS: National Institute of Agrobiological Sciences
2-1-2, Kannondai, Tsukuba, Ibaraki,305-8602, Japan
Tel: +81-29(838)7406, fax: +81-29(838)7408, E-mail: genebank@nias.affrc.go.jp
http://www.gene.affrc.go.jp/about_en.php

Only Race 1:

INRAN: National Research Institute for Food and Nutrition
Loc. Corno d'Oro SS 18, km 77.70 – 84091 Battipaglia (SA) ITALY
Tel: +39 0828 309484, fax +39 0828 302382, E-mail: r.bravi@ense.it
http://www.ense.it

Naktuinbouw, Sotaweg 22, 2370 AA Roelofarendsveen. THE NETHERLANDS
Email: info@Naktuinbouw.nl

GEVES : Groupe d'Etude et de contrôle des Variétés Et des Semences

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Ad 43. Resistance to lettuce big-vein disease

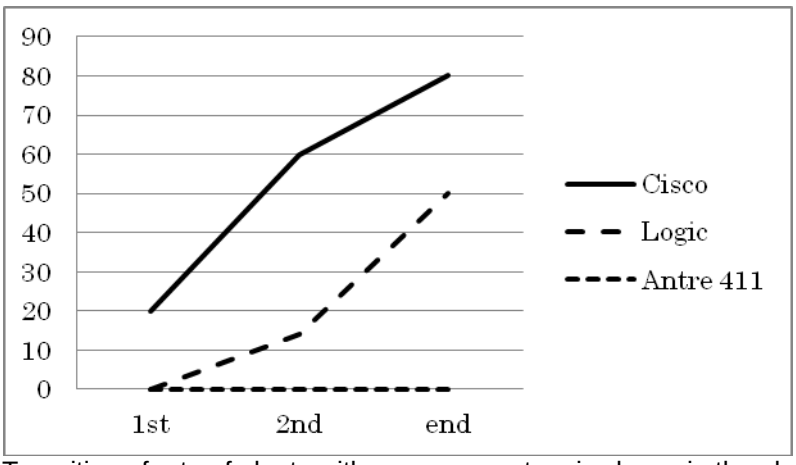
* 1. Pathogen	*Olpidium brassicae* carrying Mirafiori Lettuce Big-Vein Virus and/or Lettuce Big-Vein Associated Virus
2. Quarantine status	
* 3. Host species	Lactuca sativa L.
* 4. Source of inoculum	-
* 5. Isolate	Non-resistance breaking
6. Establishment isolate identity	Use lettuce susceptible standard
7. Establishment pathogenicity	Use lettuce susceptible standard
8. Multiplication inoculum	
8.1 Multiplication medium	Living plant
8.2 Multiplication variety	'Cisco' or other susceptible varieties
8.3 Plant stage at inoculation	See 10.3
8.4 Inoculation medium	-
8.5 Inoculation method	See 10.4
8.6 Harvest of inoculum	Root systems are collected without soil
8.7 Check of harvested inoculum	Visual check for symptom of leaves or ELISA
8.8 Shelf life/viability inoculum	One day
9. Format of the test	
* 9.1 Number of plants per genotype	20 plants
* 9.2 Number of replicates	
* 9.3 Control varieties	
Susceptible	'Cisco'
Moderately resistant	'Logic'
Resistant	'Antore 411'
* 9.4 Test design	Include control varieties
9.5 Test facility	Greenhouse or climate room
9.6 Temperature	Not over 20 ^{o}C
9.7 Light	Natural day length
9.8 Season	-
9.9 Special measures	-
10. Inoculation	
10.1 Preparation inoculum	Homogenized one g of roots from plants show severe symptom with 60ml distilled water
10.2 Quantification inoculum	10 ml per plant
*10.3 Plant stage at inoculation	Three to four true leaves are emerged
*10.4 Inoculation method	Root suspensions are poured onto the base of young plants
10.5 First observation	30 – 40 days after inoculation ('Cisco' begins to show the symptom)
10.6 Second observation	15 days after first observation
*10.7 Final observations	60 – 70 days after inoculation (30 days after first observation)
11. Observations	
*11.1 Method	
*11.2 Observation scale	Counting the number of plants with symptom
*11.3 Validation of test	on standards
11.4 Off-types	Resistant varieties may have a few plants with symptom

*12. Interpretation of data

[3] About 50 % of plants show the symptom at second observation. Almost plants show the symptom at the end of test.

[5] Few plants show the symptom at second observation. About 50 % of plants show the symptom at the end of test.

[7] Almost plants show no or slightly mild symptom until the end of test.



Transition of rate of plants with severe symptom is shown in the glaph.

13. Critical control points: Avoid rotting roots; high temperature causes masking of symptom.

Literature references

· Y. Kawazu, et. Al. A transgenic Lettuce line with resistance to both Lettuce Big-Vein associated virus and Mirafiori Lettuce Virus (J. Amer. Soc. Hort. Sci. 131(6):760-763.2006

· Shoji KOBAYASHI, Shinji NISHIGUTI, Tetsuji OSIMOTO and Masanobu KATO. Searching for Resistant Varieties to Lettuce Big Vein Disease *(Bull. Hyogo Pre. Tech. Cent. Agr. Forest. Fish*. (*Agricuture*)53, 17-23 (2005)

[End of Annex and of document]