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Geneva

TECHNICAL WORKING PARTY FOR VEGETABLES

Fiftieth Session

Brno, Czech Republic, June 27 to July 1, 2016

ADDENDUM TO

USE OF DISEASE AND INSECT RESISTANCE CHARACTERISTICS IN DUS EXAMINATION

Document prepared by the Office of the Union

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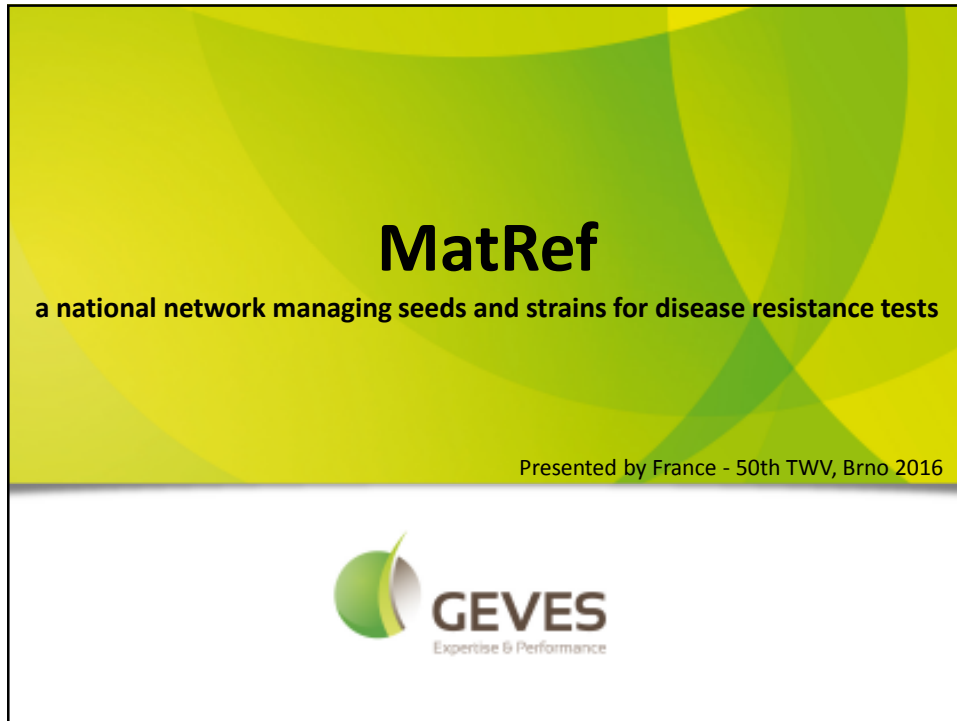
This document contains presentations to be made at the fiftieth session of the Technical Working Party for Vegetables (TWV), as follows:

- Annex I: “MatRef: a national network managing seeds and strains for disease resistance tests”, by an expert from France;
- Annex II: “Harmonization of resistance tests to diseases for DUS testing: Harmores 2”, by an expert from the Community Plant Variety Office of the European Union (CPVO)

[Annexes follow]


ANNEX I

MATREF - A NATIONAL NETWORK MANAGING SEEDS AND STRAINS FOR DISEASE RESISTANCE TESTS
BY AN EXPERT FROM FRANCE



MatRef
a national network managing seeds and strains for disease resistance tests

Presented by France - 50th TWV, Brno 2016



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Expertise & Performance



GEVES missions

- Conduct studies for
 - Registration
 - Plant breeder's rights
 - Seed certification
- Training
- Seed companies monitoring
- Methodological research
- Expertise




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Use of disease resistance tests

- For registration or protection :
 - For DUS (mainly vegetables)
 - Majority of lab tests, carried out by SNES
 - Some tests carried out by INRA
 - Some tests carried out by seed companies (blind test)
 - For VCUS (other species)
 - Majority of field tests, carried out by SEV (network)
 - Lab tests carried out by SNES
 - Some tests carried out by INRA
- For seed companies, phytosanitary companies...



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...but sometime we are facing discrepancies

Applicant's claim



Official observation



Requirements for resistance tests

We respect these 3 requirements for resistance tests

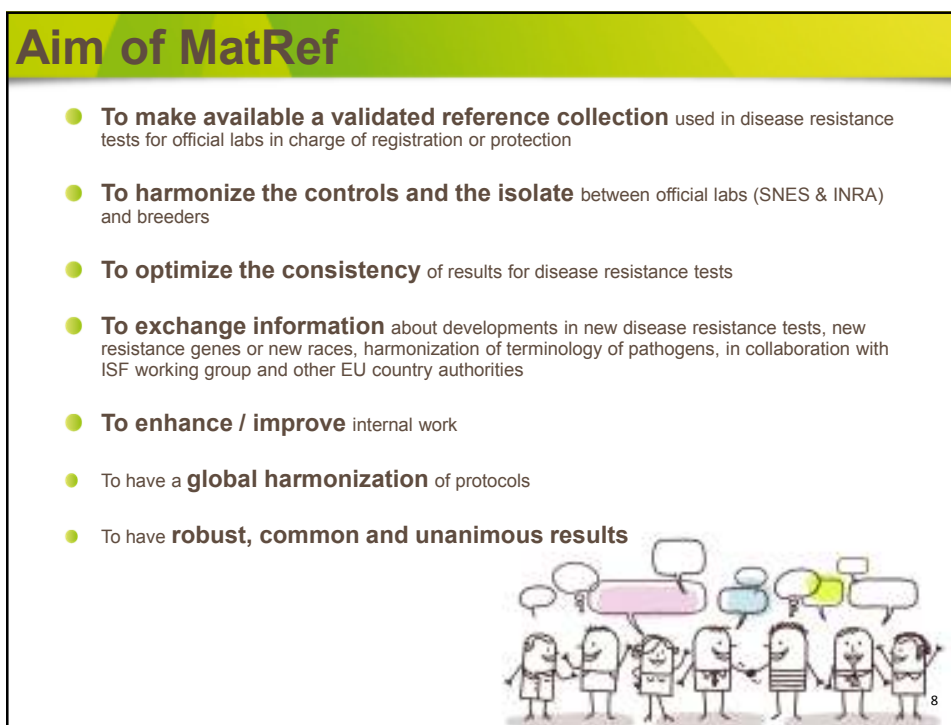
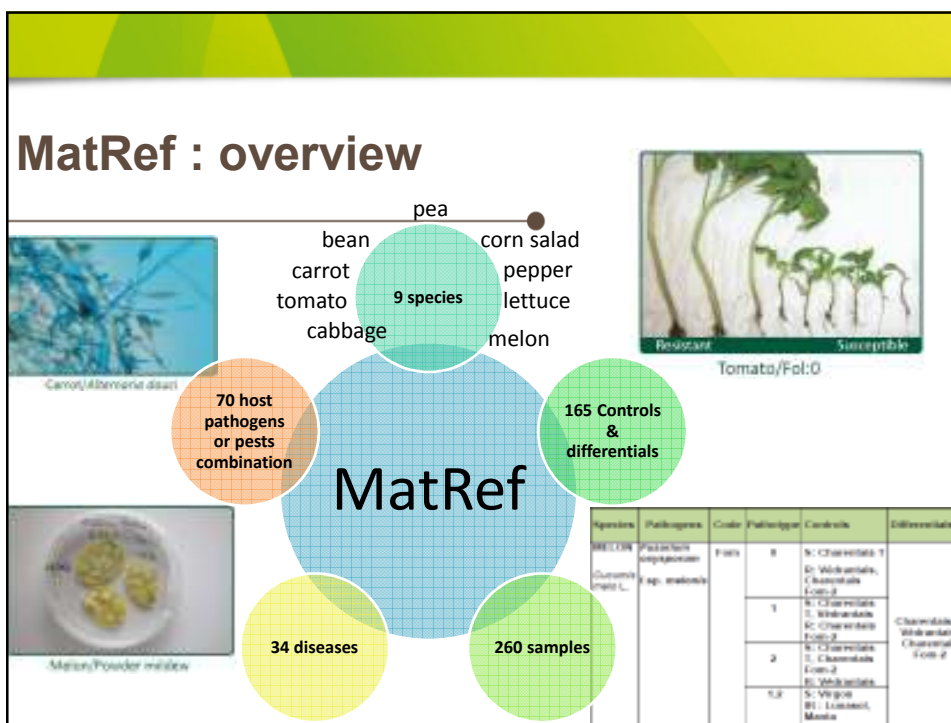
- Controls (S, IR, R) → validated and available
- Differentials → validated and available
- Reference strains → validated, available, stable and representative of natural conditions



What is MatRef ?

- A network created in 2002
 - Public partners:
 - National regulatory authority : **GEVES, coordinator**
 - Research institute : **INRA Avignon**
 - Private partners: seed companies
- Managing **MATERIAL** of **REFERENCE**
 - **Controls**: susceptible, resistant cultivars
 - **Differential hosts**: for identification of races
 - **Pests and pathogen strains**





Activities of MatRef

- **Task-sharing between network-members :**

*database management,
seeds multiplication and storage,
control of germination, of seed health and behaviour in relation
to the pathogen
strains maintenance
distribution of seeds and strains*



Activities of MatRef

- **Providing of a collection of reference material, managed by database**

- **Seed material**

- GEVES or seed companies multiply
- GEVES validates resistance / susceptibility behavior , but also germination and sanitary aspect
- GEVES stores in an active bank at 4°C and back up at -18 °C

- **Isolates**

- GEVES validates aggressiveness and virulence on controls and differentials
- GEVES and INRA (official laboratories) maintain as an active bank and store as long term back-up in 2 secure locations

Activities of MatRef

● Seed health tests : quarantine & quality

To be able to distribute these differentials and controls to breeders and official labs, seed must be guaranteed free of disease, with at least , the list of quarantine disease

Species	16 pathogens
Carrot	<i>Xanthomonas hortorum</i> pv. <i>carotae</i>
Bean	<i>Pseudomonas savastanoi</i> pv. <i>phaseolica</i> <i>Pseudomonas syringae</i> pv. <i>syringae</i> <i>Xanthomonas axonopodis</i> pv. <i>phaseolii</i> (quarantine)
Lettuce	LMV (except for <i>Bremia</i>)
Melon*	<i>Acidovorax avenae</i> subsp. <i>citrullii</i> Squash mosaic virus
Pepper	TMV PMMoV <i>Xanthomonas vesicatoria</i> & <i>X. axonopodis</i> pv. <i>vesicatoria</i> (quarantine)
Pea	<i>Pseudomonas syringae</i> pv. <i>pisii</i> Pea seed-borne mosaic virus **
Tomato	<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> (quarantine) Pepino Mosaic Virus (quarantine) ToMV <i>Xanthomonas vesicatoria</i> & <i>X. axonopodis</i> pv. <i>vesicatoria</i> (quarantine)

* if regenerated out of France

** not compulsory



MATREF storage and distribution



Else carried out to detect LMV

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Activities of MatRef

● Distribution of reference material :

- Free of charge for MatRef members
- Available for non-members , with a financial contribution and a restricted seed quantity

List of MatRef material and prices : www.geves.fr

Products & services / MATREF Network

Contact : matref@geves.fr

Seeds

Species (200 seeds/genotype)	Price
Carot, Cornsalad	€ 31.50
Bean, Lettuce, Pea	€ 42.00
Melon	€ 53.00
Pepper, Tomato	€ 63.00
Complete pack of <i>Bremia</i> differentials	€ 242.00

Strain

Pathotype	Price
1 <i>Bremia</i> race	€ 56.00
Nr:0	€ 74.00
<i>Podosphaera xanthii</i>	€ 74.00



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Activities of MatRef

These collections (strains and seeds) are now being used at

→ a national level by official French laboratories (GEVES and INRA), breeders (Matref members and non-members), research laboratories, schools and universities, ...

→ an international level by Matref members-breeders and Matref non-members-organization: GEVES counterparts (Naktuinbouw-NL, CeRSAA-IT,...) , research laboratories (CPPSI-USA, Research Institute of Plant Production -SK,...) seed companies, schools and universities, ...

Involvement of MatRef in research projects



Harmores : Harmonization of resistance tests to diseases for DUS testing

European projects set up and managed by GEVES in collaboration with partners

Harmores (2004-2006)

Bean

BCMV/BCMV
Colletotrichum lindemuthianum
Pseudomonas savastanoi pv *phaseolica*

Tomato

Fol race 0 et race 1
Verticillium dahliae
ToMV 0

Harmores 2 (2012-2014)

Lettuce

Bremia lactucae

Pea

Fop race 1
Ascochyta pisi race C

Pepper

TMV:0
PMMoV:1.2
PMMoV:1.2.3
PVY:0

GEVES organized 2 WS

- Lettuce: 04/11/2014 , 23 part.
- Pea: 05/11/2014, 6 part.

Harmores 3 (2016-2019)

Tomato

Fusarium oxysporum f.sp. *lycopersici* Race 0
(ex 1) and Race 1 (ex 2)
Meloidogyne incognita

Pea

Erysiphe pisi

Melon

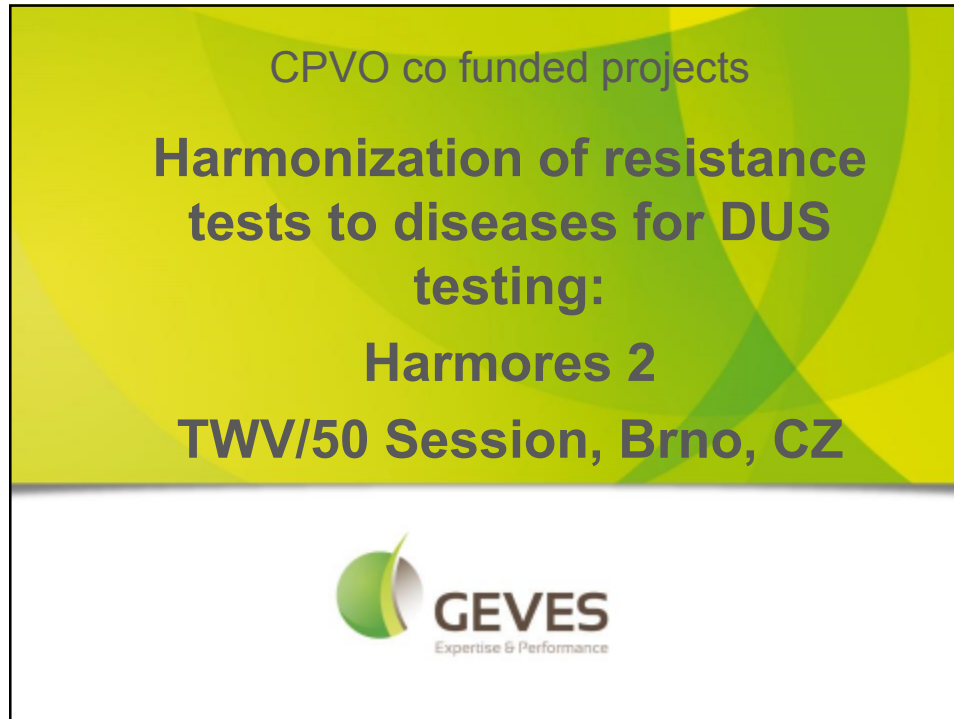
Fusarium oxysporum f.sp. *melonis* race 1.2
Fusarium oxysporum f.sp. *melonis* race 2
(+ validation with also Fom:0 and 1)
Podosphaera xanthii

6 WS planned during the 3 years
(organization : GEVES)

CASDAR - CTPS Characterization of virulence of *Podosphaera xanthii*, causing powdery mildew of melon, and development of a race codification system



HARMONIZATION OF RESISTANCE TESTS TO DISEASES FOR DUS TESTING: HARMORES 2
BY AN EXPERT FROM THE COMMUNITY PLANT VARIETY OFFICE OF THE EUROPEAN UNION




CPVO co funded projects

Harmonization of resistance tests to diseases for DUS testing:

Harmores 2

TWV/50 Session, Brno, CZ

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Harmores 2 Goal/deliverable Results Conclusion

- Three year project: 2012-2015
- 7 EOs and 5 ESA members involved
- Aims: harmonize, at the European level, resistance tests to seven vegetable diseases:
 - *Bremia lactucae*/lettuce,
 - *Fusarium oxysporum* f. sp. *pisi* race 1/pea,
 - *Ascochyta pisi* race C/pea,
 - TMV: 0/pepper,
 - PMMoV: 1.2/pepper,
 - PMMoV:1.2.3/pepper,
 - PVY: 0/pepper

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Harmores 2 Goal/deliverable Results Conclusion

Harmonize DUS resistance protocols:

- ↪ better coherence of results between countries
- ↪ between declarations of breeders and official tests,
- ↪ better definition and exchange of reference material
- ✓ updated bibliography on the selected host/pathogen combinations
- ✓ available reference isolates with maintainers laboratories
- ✓ available reference resistant and susceptible controls
- ✓ optimised culture conditions for all the studied pathogens
- ✓ optimised test conditions
- ✓ harmonised protocols to be proposed to CPVO

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Harmores 2 Goal/deliverable Results Conclusion

Phase 1 • *Description and comparison of the existing tests*

Phase 2 • *Selection of common reference material*

Phase 3 • *Harmonisation of protocols*


Phase 4 • *Validation of harmonized protocols*


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Harmores 2	Goal/deliverable	Results	Conclusion	
• Pepper				
Nb of controls	TMV: 0	Tested: 10	Validated: 6	Selected: 5
	PMMoV: 1.2	12	8	5
	PMMoV: 1.2.3	10	8	4
	PVY: 0	10	7	6
Nb of strains	TMV: 0	Tested: 5	Validated: 2	Selected: 1
	PMMoV: 1.2	5	2	1
	PMMoV: 1.2.3	4	1	1
	PVY: 0	4	1	1
Nb of stage of inoculation	TMV: 0	Tested: 2	Validated: 2	Selected: 2
	PMMoV: 1.2	2	1	1
	PMMoV: 1.2.3	2	1	1
	PVY: 0	2	1	1


TMV → 1 strain not validated as TMV: 0







TMV : comparison of strains








Harmores 2	Goal/deliverable	Results	Conclusion	
• Lettuce				
Nb of substrate	BI: 24	Tested: 4	Validated: 3	Selected: 3
	BI: 26	4	3	3
Nb of notation scales	BI: 24	Tested: 3	Validated: 1	Selected: 1
	BI: 26	3	1	1




Bremia : comparison of substrates




Harmores 2	Goal/deliverable	Results	Conclusion
<p>• Lettuce</p> <ul style="list-style-type: none"> ➤ A workshop was planned on the 4th November 2014 to harmonize the different official notation scales: CPVO, UPOV and IBEB. ➤ Objectif: <ul style="list-style-type: none"> ✓ to draw the line between resistance and susceptibility around light or sparse sporulation, ✓ to harmonize a common notation scale and interpretation of observed symptoms. ➤ 25 people from national examination offices and ESA representatives of 7 European countries participated. ➤ 21 “difficult to judge” varieties, on 3 substrates with 2 races <p>↪ Common notation scale and interpretation rule</p> <p style="text-align: center;">  GEVES Groupe d'Étude et de contrôle des Variétés Et des Semences </p>			

Harmores 2	Goal/deliverable	Results	Conclusion		
RESISTANT	 No sporulation + no necrosis	 No sporulation + necrosis	 Weak sporulation (much less than susceptible control) + necrosis	 Weak sporulation less than susceptible control not evolving between second and third notation + necrosis	 In some cases very sparse sporulation can occur (without necrosis) and does not evolve between 2 nd and 3 rd notation
	SUSCEPTIBLE	 Reduced sporulation (compared to susceptible control) without necrosis	 Normal sporulation without necrosis	OTHER CASE	

Harmores 2				
Goal/deliverable		Results	Conclusion	
• Pea				
		Tested	Validated	Selected
Number of controls	<i>Ascochyta pisi</i>	5	5	5
	<i>Fusarium oxysporum</i> f. sp. <i>pisii</i>	10	3	3
		Tested	Validated	Selected
Number of strains	<i>Ascochyta pisi</i>	2	2	2
	<i>Fusarium oxysporum</i> f. sp. <i>pisii</i>	3	1	1
		Tested	Validated	Selected
Number of stage of inoculation	<i>Ascochyta pisi</i>	2	1	1
	<i>Fusarium oxysporum</i> f. sp. <i>pisii</i>	2	2	2
		Tested	Validated	Selected
Number of notation scales	<i>Ascochyta pisi</i>	2	1	1
	<i>Fusarium oxysporum</i> f. sp. <i>pisii</i>	2	1	1


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Harmores 2				
Goal/deliverable		Results	Conclusion	
• Pea				
<ul style="list-style-type: none"> ➤ A workshop was planned 5th November 2014 to describe a harmonized notation scale of <i>Fusarium oxysporum</i> f. sp. <i>pisii</i> ➤ Objectif: <ul style="list-style-type: none"> ✓ Harmonization of a common notation scale ✓ Definition of an interpretation rule ➤ 5 people from national examination offices from 4 European countries participated. ➤ Comparison on a panel of two methods of inoculation and two notation scale 				
<p>➤ Common notation scale and interpretation rule</p>				


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Harmores 2 Goal/deliverable Results Conclusion

- **Harmonized rules of decision**
 - ↳ Proposal for *Ascochyta pisi*: Interpretation of varieties with same level of resistance than Nina and/or Rondo and Madonna as resistant

Kelvedon Wonder Crecerelle Rondo Madonna Nina

Susceptible Resistant

- ↳ Proposal for *Fusarium oxysporum* f. sp. *pisi*: Interpretation of varieties with same level of resistance than New Era and/or Nina as resistant

Bartavelle New Era Nina

Susceptible Resistant

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Harmores 2 Goal/deliverable Results Conclusion

- **Exemple of results for *Fusarium oxysporum* f. sp. *pisi***

Seed sample code	number of plants			Interpretation	Nb plants R	Nb plants S
	Note 0	Note 1	Note 2			
Sample N° 4			26	S		26
Sample N° 21		26		R	26	
Sample N° 41	26			R	26	
Sample N° 49		26		R	26	
Sample N° 57		26		R	26	
Sample N° 79			26	S		26
Sample N° 87			26	S		26
Bartavelle (S control)			26	S		26
New Era (IR control)		26		R	26	
Nina (R control)	26			R	26	

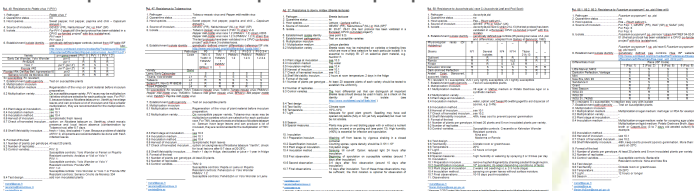

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Harmores 2 Goal/deliverable Results Conclusion

- **Reference material**
 - Should be available:
 - ✓ Maintainers of isolates defined and indicated in protocols
 - Mention in each protocol “this protocol has been validated on...”
 - MTA shared, to be harmonized
 - A general rule for maintenance of isolates and controls established:
 - ✓ Availability in the network of maintainers
 - ✓ If outside of maintainers network, forward the request to original provider of the isolate
 - ✓ Control by maintainers of isolates on differentials and control varieties twice every 5 years

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Harmores 2 Goal/deliverable Results Conclusion

- **Dissemination**
 - 5 updated robust protocols proposed to CPVO
 - Presentation to the new notation scale to IBEB
 - Presentation of a poster on harmonized lettuce notation scale during the Eucarpia Leafy Vegetables congress in April 2015
 - Proposition of revision of the Lettuce UPOV guideline
 - Planned communication at general Eucarpia congress in 2016.

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Thank you for your attention

Pictures: GEVES



WWW.GEVES.FR



[End of Annex II and of document]